Glutamate receptor mediated bi-directional plasticity is responsible for reconsolidation of fear memories

Subhrajit Bhattacharya

A dissertation submitted to the Graduate Faculty of
Auburn University
In partial fulfillment of the
Requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
December 12, 2015

Keywords: Memory Reconsolidation, Glutamate Receptors, Hippocampus, Long Term Potentiation, Fear Conditioning, Retrieval

Approved by

Vishnu Suppiramaniam, Chair, Professor of Drug Discovery and Development
Martha Escobar, Associate Professor of Psychology
David Riese, Associate Dean of Research, Professor of Drug Discovery and Development
Muralikrishnan Dhanasekharan, Associate Professor of Drug Discovery and Development
Rajesh Amin, Assistant Professor of Drug Discovery and Development
Jianzhong Shen, Associate Professor of Drug Discovery and Development
Bruce Smith, Professor of Pathobiology, School of Veterinary Medicine

Abstract

Retrieval of a memory appears to render it unstable until the memory is once again re-stabilized or *reconsolidated*. Although the occurrence and consequences of reconsolidation have received much attention in recent years, the specific mechanisms that underlie the process of reconsolidation have not been fully described. Here, we present the first electrophysiological model of the synaptic plasticity changes underlying the different stages of reconsolidation of a conditioned fear memory. In this model, retrieval of a fear memory results in immediate but transient alterations in synaptic plasticity, mediated by modified expression of the glutamate receptor subunits GluA1, GluA2, and GluN2B. Retrieval of a memory results in an immediate impairment in Long Term Potentiation (LTP), which recovers 6 h following memory retrieval. Conversely, memory retrieval results in an immediate enhancement of Long Term Deppression (LTD), which decreases with time. These changes in plasticity are accompanied by increased immediate expression of GluN2B and decreased expression of GluA1/2 receptor subunits. Recovery of LTP and LTD correlates with progressive normalization in GluN2B expression (return to pre-retrieval levels), and subsequent overexpression of GluA2 receptor subunits (above pre-retrieval levels). The contribution of each receptor was confirmed by interfering with receptor expression at the postsynaptic sites. Blocking GluA2 endocytosis restored LTP and attenuated LTD during the initial portion of the reconsolidation period, while antagonism of GluN2B attenuated LTD. These findings suggest that altered glutamate receptor expression controls different forms of synaptic plasticity during reconsolidation.

Acknowledgements

The author would like to express his gratitude to almighty God for guiding him through the entire work spiritually and showing him the right path throughout his tenure as a PhD candidate. This work would not have been possible without God's help and blessings. The author would also like to express his heartfelt gratitude to his major professor Dr. Vishnu Suppiramaniam for his support, mentorship, flexibility, encouragement and personal care during the course of this study. The author would concurrently like express utmost thankfulness towards Dr. Martha Escobar for her unrelenting help and support. Without her extreme enthusiastic participation and help this work would not have been as satisfactory as it is. The author would like to mention a special note of thanks to Dr. David Riese for his support towards the dissertation work. The author is also grateful to Dr. Bruce Smith, Dr. Muralikrishnan Dhanasekaran, Dr. Jianzhong Shen, and Dr. Rajesh Amin, the committee members of this dissertation for their constructive inputs. The author would like to extend his gratitude to Dr. Kodeeswaran Parameswaran for the excellent training provided on various electrophysiological techniques. While Dr. Kodeeswaran was in Auburn, he was available at all times for help. The author is also grateful towards Dr. Manal Ali Buabeid, Dr. Engy Rahman, Dr. Manuj Ahuja, Dwipayan Bhattacharya, Jenna Bloemer, Ahmad Alhowail, Fatema Aljedani and Brian Lee for their help in successfully carrying out experiments. The author would also like to thank his family members who provided moral and spiritual support during the pursuit of this academic goal. In this regard the author would like to thank his parents Mr. and Mrs. Bhattacharya, wife Swagata for their continuous encouragement and support. Finally, but not least, the author would like to thank former and current colleagues in Dr. Suppiramaniam's lab for their support.

Table of Contents

Table of Figures

List of Tables

Chapter I

Introduction

1.1. Memory Formation:

Memory formation in mammals is a complex phenomenon which involves a series of interconnected and interdependent physiological, anatomical as well as biochemical steps. The process involves different areas of the brain which function in a coherent manner to produce a series of events that constitutes what is called memory formation. Each area has its own important role to play in the entire process, which results in the formation of new memory traces (the so-called memory 'engrams,' formed by de-novo protein synthesis) which, after becoming stable, are processed for long term storage. There are different types of memory, all distinct based on the information they contain. If we specifically discuss declarative (explicit memory, e.g. memorizing words) and propositional memories (non-declarative memory, e.g. learning to ride a bike, Squire et. al, 2004, 2005, 2007, 2011, 2012, 2013, 2014 and 2015, and Tulving, 2003), their processing in the brain relates to sub regions of the medial temporal lobe. The region under consideration is generally referred to as the hippocampus (Greek word for sea horse because of its shape). Memory regions are extremely interconnected and positioned in close proximity of each other, with connections to other areas of the brain such as the cortex, amygdala and the cerebellar formation.

1.2. The Hippocampus:

The hippocampus is one of the most significant and major sub-structures of the mammalian brain that is involved with formation, processing and storage of memory, as well as the main are for processing of contextual and spatial information (Verfaelli et. al, 2015 and

Eichenbaum et. al, 1999). The structure lies anatomically within the medial temporal lobe and is a part of the limbic system. The cerebral cortex forms the roof of the hippocampus and is deeply innervated by afferent and efferent fibers from the hippocampus (Tromp et. Al, 2015 and Gray et. Al, 2015). Evolutionarily, the hippocampus is considered to be an extension of the contours of the cerebral cortex (Shomrat et. Al, 2015 and Bartsch et. Al, 2015). The hippocampus is a laminar structure that consists of microscopically distinct packed areas of pyramidal cells. The hippocampus is divided in sub-regions based on synaptic connections within the structure (Fig. 1A), namely the Dentate Gyrus (DG), Subiculum, Entorhinal cortex (EC), and the Schaffer Collateral Pathway (SC pathway), which is further subdivided into the CA1, CA2, CA3 and CA4 areas (Harris et. Al, 1992). Longitudinally, the hippocampus is divided into dorsal and ventral regions. This general structure is conserved across a wide variety of animals, suggesting the importance of this particular laminar arrangement in the formation and storage of memory.. The hippocampus is present in the two brain hemispheres, with the two halves of the structure being joined at the stem area by hippocampal commissures that traverse the central division under the anterior corpus callosum. The EC (Entorhinal Cortex) is cocooned within the parahippocampal gyrus and is connected with the cerebral cortex in a very complex manner (briefly depicted in Fig. 1B). The EC receives afferent neuronal appendages from several other nuclei like the medial septal nucleus, anterior nuclear complex and nucleus reuniens of the thalamus, supramammillary nucleus of the hypothalamus, raphe nuclei and locus coeruleus in the brainstem (Lewis and Shute, 1975). The major pathway out of the EC is known as the perforant pathway as its neuronal outgrowths puncture the subiculum while projecting from the pyramidal cells in layer II. These projections ultimately send their inputs to the granule cells in the DG and sparsely innervate the CA3/CA1 collateral bundles. The internal sub regions of the hippocampus have

extensively connected circuitry that helps process the flow of information during memory formation. (Burgess and Bavers, 2010, Novick et. Al, 2009 and Krause-Utz et. al, 2014). The Dentate Gyrus (DG) forms an intermediate connecting region between peripheral areas of the hippocampus and the SC pathways.. The DG is composed of three different types of cells (granule, molecular and polymorphic), and comprises a significant part of the Mossy Fiber pathway (Kemperman et. al, 2015, Nakahara et. al, 2015, McAvoy et. al, 2015, Hansen et. al, 2015, Yu et. al, 2014 and Llorens-Martin et. al, 2015). The DG is an an important structure because of its role in inhibitory networks mediated by interneurons that project on to the SC CA3/CA1 region (Li et. al, 2015, Kann, 2015, Wadiche and Wadiche, 2015, Chancey et. al, 2014, Tsai et. al, 2012 and Markwardt et. al, 2009).

The hippocampus plays an extremely important role as a determinant in the ultimate fate of a newly formed memory (Kim et. al, 2015, Zhang and Jacobs, 2015 and Rosatto et. al, 2015). Furthermore, retrieval of a memory appears to involve the hippocampus (Wiltgen et al., 2010) and restabilization of memory after retrieval also requires hippocampal activity (Rao-Ruiz et al., 2012). The present project investigates how retrieval of a newly formed contextual memory depends on hippocampal activity related to its destabilization and subsequent stabilization, in a process known as *memory reconsolidation*.

1.3. The Hippocampus and Memory:

The exact role of hippocampus in memory formation is still not clear. However, de novo protein synthesis in the SC CA3/CA1 pathway appears to be particularly important in case of new memory formation (Fado et. al, 2015, Dubue et. al, 2015, Suga et. al, 2015, Furini et. al, 2015, Fioriti et. al, 2015, Jarome et. al, 2014). Hence, it is generally agreed that the hippocampus plays an important role in contextual learning and memorization of learned events for long term

usage (Wojtowicz et. al, 2015, Uchida et. al, 2015, Leser et. al, 2015, Bailey et. al, 2015, giese et. al, 2015). Both the Morris Water Maze tasks (a test for spatial memory analysis, Morris et al., 1982, Devan and White, 1999; Pouzet et al., 1999) and the contextual fear conditioning task (Phillips and LeDoux, 1995; Maren and Fanselow, 1997; Bannerman et al., 2001) are sensitive to disrupted hippocampal function. The major excitatory neurotransmitter system present in the hippocampus is the glutamate and its target receptors that are responsible for memory encoding (Orzeł-Gryglewska et al., 2015, Mather et al., 2015). We will discuss the neurotransmitters and their receptors in detail in the successive sections of this chapter. The process of memory formation in the hippocampus involves formation of fragile traces when learning tasks are undertaken. These traces are formed and generated by complex and controlled de novo protein synthesis (Phillips and LeDoux, 1995), and specific post translational modification of existing proteins, particularly phosphorylation and dephosphorylation (Ogawa et al., 2015, Guida et al. 2012, Hinnebusch 2015 and Marquez et al., 2015). Receptor trafficking and endocytosis are equally important in process new memory formation (Rao-Ruiz et a., 2015 and 2011). Once a trace is formed after performing a task, the trace progressively becomes stable over a period that can range from 5-6 h to several days (Monfils et al., 2014, 2013 and 2011). This process is called *memory consolidation*.

The present project investigates contextual memory the consolidation of which, is highly dependent on the hippocampus (in conjunction with other structures such as the amygdala). It was previously thought that, once memory traces are formed in brain regions including the hippocampus, they are stable and not prone to any further changes. However in the last decade, it has been found that once consolidated, memories are still malleable to changes under certain circumstances. If the memory is recalled in an extremely similar environment (having the same

context, e.g. smell, sound, light), the memory trace can become fragile or unstable once again. This process of recalling the memory trace is known as *retrieval*. After retrieval (post-retrieval), the unstable memory must be stabilized once again. This process is referred to as *reconsolidation*. At the present time, it is known that the hippocampus and amygdala play an important role in reconsolidation of contextual fear memories; however, the specific changes that lead to reconsolidation in the hippocampus have been less well described than those taking place in the amygdala. Better describing the role of hippocampal function during reconsolidation of memory is one of the principal questions that will be investigated in Chapters III-V. In the following chapter we will discuss the processes of consolidation and reconsolidation in detail, particularly in relation to memories associated with fear and the role played by glutamate receptors.

1.4. Glutamate receptor system:

Glutamate is the major excitatory neurotransmitter in the vertebrate central nervous system. Glutamate receptors are crucial for synaptic plasticity mechanisms including LTP (Fonseca et al. 2006; Schmitt et al. 2005) and LTD (Bear & Abraham 1996). LTP and LTD are relatively long-lasting increases in synaptic strength (average change in the voltage or current of the post-synaptic neuron), which can be induced across synapses depending on various stimulation parameters (like High Frequency or Low Frequency stimulus). LTP and LTD are widely accepted cellular models of memory formation (Collingridge et al 2010; Lynch 2004). The two major glutamate receptors, N-Methyl-D-Aspartate receptors (NMDARs) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPARs) are involved in the induction and maintenance of both LTP and LTD (Carroll & Zukin 2002; Schmitt et al. 2005; Yashiro & Philpot 2008).

Recent advances in molecular biology have made it possible to study the structure of both, AMPARs and NMDARs. Overall, the glutamate receptors are composed of an agonist-binding domain and three transmembrane domains: a cytoplasm facing loop domain, an N-terminal domain (NTD), and a cytoplasmic C-terminal domain (CTD, Fig 1.2 and 1.3) (Mayer and Armstrong, 2004). It was only in the mid-nineties that the detailed structure of the glutamate receptors was resolved. Later it was shown that the S1S2 units constituted the agonist binding core of the AMPARs and NMDARs (Stern-Bach et al., 1994; Paas et al., 1996; Swanson et al., 1997; Foucaud et al., 2003).

The NMDARs are more complex in their structure, heteromerism and subunit stoichiometry. NMDARs have Mg2+ binding sites that can act as an ion-channel blocker. An important non-competitive antagonist for NMDARs is the drug Ifenprodil, which binds to the glutamate binding site of the receptor (Masuko et al., 1999; Paoletti et al., 2000; Zheng et al., 2001; Perin-Dureau et al., 2002). Importantly, agonist and antagonist binding is more complex in NMDARs compared to AMPARs because NMDARs require simultaneous binding of glycine along with glutamate for the receptors to be activated. Two obligatory GluN1s are required for receptor activation because they have the Glycine binding sites. It was initially suggested that receptor stoichiometry only allowed GluN1/GluN2A or GluN1/GluN2B to exist. However, it appears that more than 50% of the NMDARs are actually heterotrimers formed of two GluN1s and a mixture of GluN2A/B (Kasper et al., 2010). This newly found receptor population data is still under intense investigation. Ifenprodil has been found to be selective towards GluN2B subtypes (Honjie et al., 2014). The affinity of Ifenprodil for GluN2B NMDARs was used in the present research to antagonize the GluN2B receptor subtype with minimal effects on the GluN2A receptor subtype. Note that after this project was completed, a newer subclass of drugs,

the TCN-201 series (Honjie et al., 2014), has been developed with a partial agonism for the Glycine binding site in the GluN2 heteromer, with resulting higher selectivity towards the GluN2B subunit than Ifenprodil; subsequent research will benefit from the higher specificity of this novel antagonist..

The complex nature of NMDARs has led to them being called “coincidence detectors”, given that these receptors function only when there is a surge of glutamate released from the presynaptic neurons, alongside the presence of glycine, an important co-activator of the receptor heteromers. NMDARs need depolarization mediated by AMPAR activation, for the removal of the voltage-gated Mg2++ blockade. The above mentioned properties of NMDARs are particularly important for synaptic plasticity and memory reconsolidation (Huganir et al., 2012).

The S1/S2 amino acid sequences are blocked by introduction of two semi transmembrane portions which along with the pore helix structure and the loop structure, make the size defining ion-entry domain (Kuner et al., 1996; Kuner et al., 2001; Panchenko et al., 2001; Wang et al., 2002). The pore is wide enough to barely allow Na+ and K+ ions to pass through, and the pore loops in GluRs are flexible torsionally to permit movement of Na+/K+ ions. The CTDs of glutamate receptors vary in length, with the CTD of NMDRs being comparatively larger than that of AMPARs. The CTDs bind to several cytoskeletal proteins and are deemed important for receptor trafficking and endocytosis (Scannevin and Huganir, 2000; Sheng et al., 2001). The CTD is of particular importance to the present research because we use a C-terminal tail-mimicking peptide that is tagged with the TAT-delivery system to block receptor endocytosis.

1.5 Glutamate receptor system and synaptic plasticity:

Glutamate receptors are thought to play an important role in reconsolidation through their effect on synaptic plasticity. In this section we will describe their role in plasticity. Synaptic plasticity is a process responsible for long lasting changes in synaptic efficacy (Fung and Lau, 1989; Malinow and Malenka, 2002; MacDonald et al., 2006; Genoux and Montgomery, 2007), which are presumed to be the cellular substrate of learning and memory (Alkon and Nelson, 1990; Kandel, 1997). AMPARs and NMDARs appear to play equally important roles in different forms of synaptic plasticity The two forms of plasticity patterns that have been widely studied in context of the mammalian nervous system are LTP and LTD. The fact that a short period of synaptic stimulation (of high- or low-frequency) can initiate persistent and long lasting changes of synaptic transmission expressed for several hours and often longer makes LTP and LTD prime candidates for memory research(Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973). Over the past three decades, extensive work in the field of electrophysiology and the system-level

dissection of LTP (high frequency stimulation) and LTD (low frequency stimulation) has reinforced the view that these two forms of plasticity are extremely relevant for the understanding of memory formation (Zoghbi et al., 2000; Martin et al., 2003). However, there are conflicting views on the cellular mechanisms involved in memory formation. One view highlights the role of postsynaptic exocytosis; infusing postsynaptic hippocampal neurons with toxins capable of specifically perturbing membrane fusion blocked LTP (Lledo et al., 1998). Another study in cultured neurons identified a particular kind of receptor exocytosis dependent on activation of Calcium Calmoduline Kinase II (CaMKII) (Maletic-Savatic et al., 1998), an enzyme that plays a critical role in LTP and LTD expression (Lisman et al., 1997). These studies suggest that AMPAR trafficking to synapses are controlled via complex mechanisms.

LTD has been considered to be a phenomenon that opposes LTP and has been mostly associated with depression of synapses. Nonetheless, LTD is not detrimental to memory formation. LTD and LTP are both needed for proper formation and processing of memory traces. A fine-tuned and delicate balance of LTP and LTD (directionality of synaptic strengthening) determines the strength of memory formation (Huganir et al., 2010).

The frequency and intensity of stimulation determines whether a synapse expresses LTP or LTD. LTD causes a decrease in the percentage of surface AMPARs, but does not have any effect on the distribution of synaptic NMDARs (Man et. al., 2000). *In vivo* studies have revealed that expression of LTD in hippocampus causes a decrease in the number of AMPARSs providing further evidence for the role of AMPAR endocytosis in plasticity (Heynen et al., 2000). Depletion of synaptic AMPARs during LTD involves their clathrin coated pit-mediated endocytosis and subsequent degradation (Man et. al., 2000). Consistent with this observation, loading CA1 pyramidal neurons with a peptide that disrupts the function of dynamin (a molecule

that helps receptor encapsulation in endocytotic vesicles) blocks LTD. (Luscher et al., 1999; Wang et al., 2002). These results were the first direct demonstration that two forms of LTD that previously were thought to be mechanistically distinct (i.e. cerebellar and NMDA receptor dependent LTD in the hippocampus) appear to share a common mechanism of expression and maintenance (Ebrahim et al., 2000; Lin et al., 2000).

Memory formation includes the process of consolidation, but memory content may be altered with each retrieval episode due to the process of reconsolidation. Considering the important role that LTP and LTD play in memory consolidation, one could assume that these two cellular processes play an equally important role in the process of reconsolidation. However the role of LTP- and LTD-mediated plasticity in reconsolidation has been largely ignored. Although some studies have assessed synaptic activity at some point during the reconsolidation period, it is also possible that plasticity changes during the sequential process of destabilization, use, and restabilization of the retrieved memory. LTP and LTD are dependent on glutamate receptor expression and function; thus, relationship between glutamate receptor expression and function during reconsolidation and the resultant pattern of LTP/LTD should also be elucidated to fully understand synaptic changes through this memory process. My research work attempts to accomplish this by investigating the time-dependent plasticity mechanisms underlying reconsolidation of fear memory, using as a strategy the thorough investigation of LTP, LTD, and glutamate receptor expression during the reconsolidation process.

1.6. **Legends:**

Figure 1.1. A. Diagrammatic representation of different hippocampal pathways; Neurons in the SC pathway run parallel in laminar formations forming cell bodies in the CA1 region away from

the CA3 area. Other neuronal pathways include appendages from DG to CA3 and CA1 to the subiculum (DG: Dentate Gyrus, CA: Schafer Collateral, Sb: Subiculum, PP: Perforant Pathway, EC: Entorhinal Cortex, M: Medial, L: Lower: adapted from University of Bristol, UK, Center for Synaptic Plasticity). **B**. Diagrammatic representation of the anatomical position of the hippocampus (adapted from Bright Focus Foundation teaching material, 2000).

Figure 1.2. Generalized structure of glutamate receptor showing glutamate binding extracellular domain, glycine rich flip flop domain, and the LVIP conserved domain. Number 2 shows the re-entrant loop, characteristics of glutamate receptor family (Parameswaran et al., 2012).

Figure 1.3. Structural representation of GluN1-2B diheteromer showing the beta strands and helices (top), the Ifenprodil (GluN2B selective antagonist) binding site (middle) and torsional stoichiometry (bottom, adapted from Karakas et. al., Nature, 2011). Ifenprodil is a commonly used drug to block GluN2B receptor activity. GluN2B exists as heteromers with GluN1.

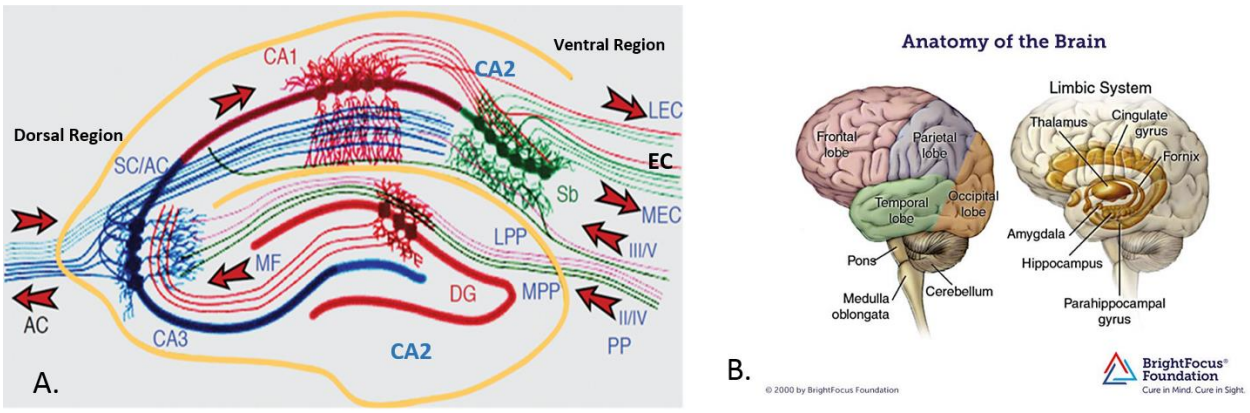
Ventral Region
Dorsal Region
CA1
CA2
LEC
EC
SC/AC
Sb
MEC
LPP
III/V
MF
DG
MPP
II/IV
AC
CA3
CA2
PP
A.
Anatomy of the Brain
Limbic System
Frontal lobe
Parietal lobe
Temporal lobe
Occipital lobe
Pons
Medulla oblongata
Cerebellum
Thalamus
Cingulate gyrus
Fornix
Amygdala
Hippocampus
Parahippocampal gyrus
B.
© 2000 by BrightFocus Foundation
BrightFocus® Foundation
Cure in Mind. Cure in Sight.

Figure 1.1

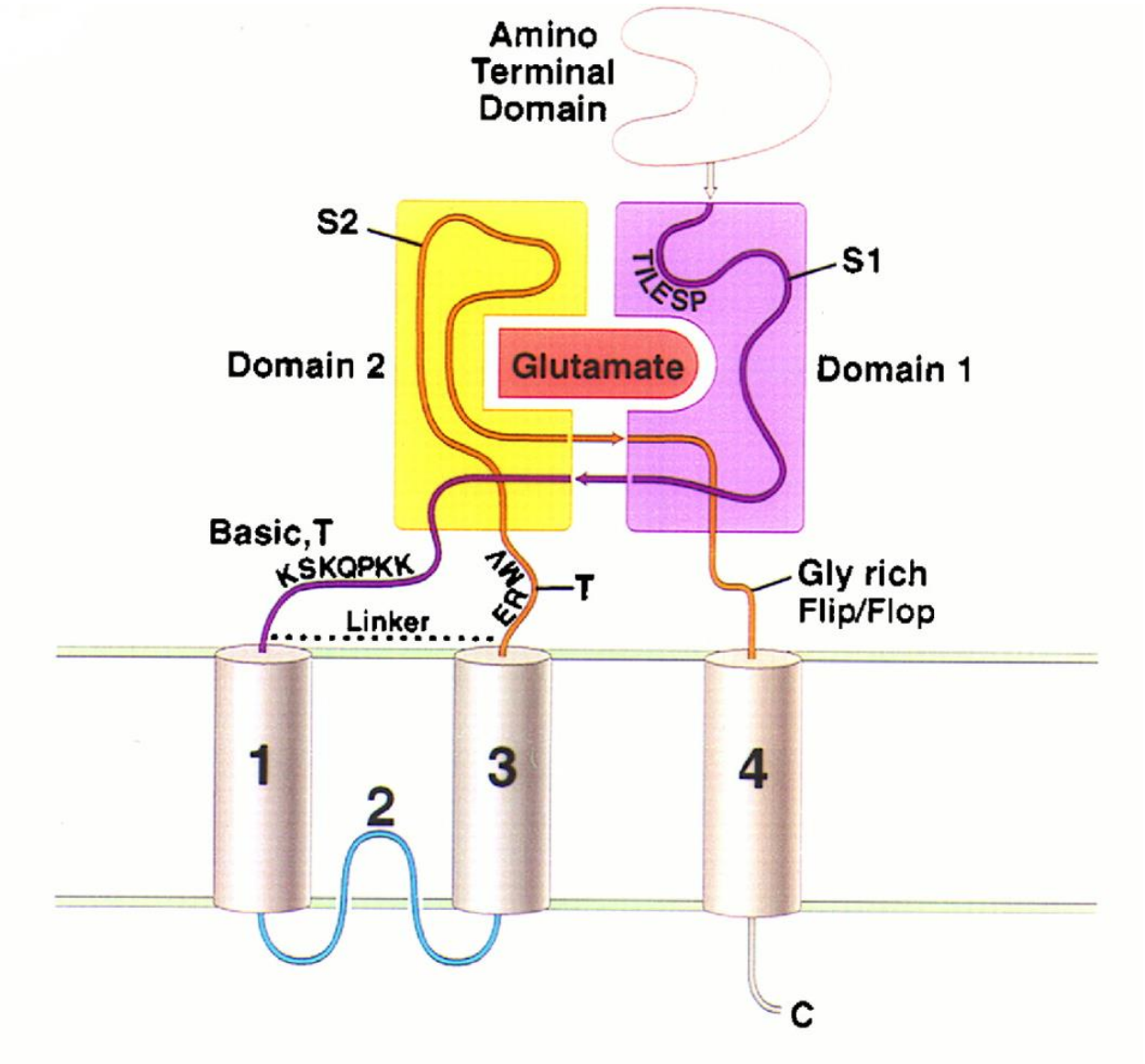
Amino
Terminal
Domain
S2
S1
TILESP
Domain 2
Glutamate
Domain 1
Basic,T
KSKQPKK
EMV
T
Linker
Gly rich
Flip/Flop
1
2
3
4
C

Figure 1.2

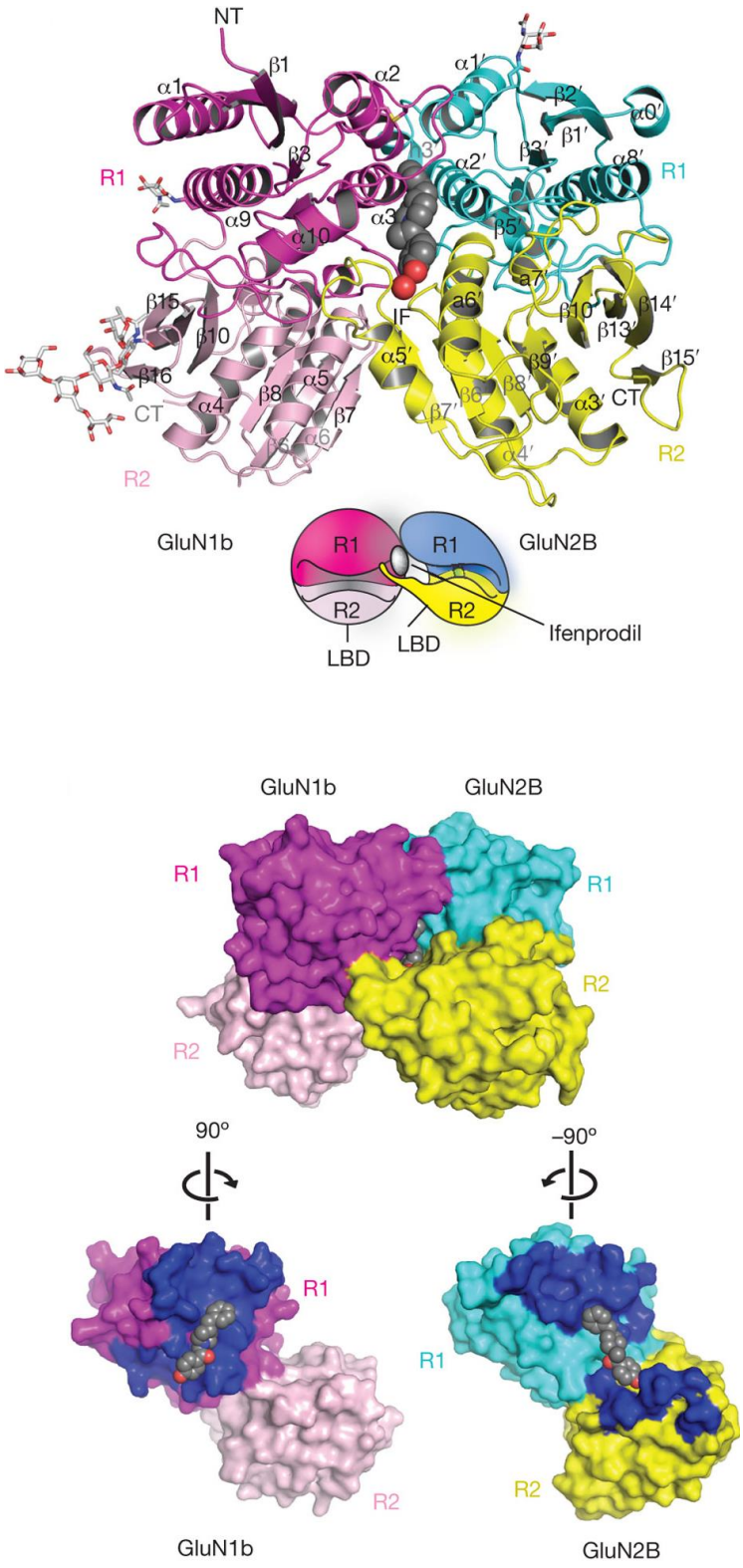
NT
β1
α1
α2
α1′
β2′
α0′
β1′
β3′
α8′
R1
α2′
R1
α9
α10
α3
β5′
α7′
β15
β10
IF
α6′
β10′
β14′
β13′
α5′
β9′
β15′
β16
α5
β8′
β8′
β6′
CT
α4
β7
β7′
α3′
CT
α6
α4′
R2
R2
GluN1b
R1
R1
GluN2B
R2
R2
Ifenprodil
LBD
LBD
GluN1b
GluN2B
R1
R1
R2
R2
90°
−90°
R1
R1
R2
R2
GluN1b
GluN2B

Figure 1.3

Chapter II

Consolidation and Reconsolidation

2.1. **Consolidation of fear memory**

Memory consolidation is a complex cascade of physiological and cellular events that tend to stabilize a memory trace after its initial acquisition. Researchers have deciphered two principle components of the conceptual term consolidation: synaptic consolidation which is equivalent to late LTP or L-LTP occurring within few hours of the initiation of the learning process, and systems consolidation, where hippocampus dependent memories or for that matter any form of newly acquired memory becomes independent of its primary storage site over a longer period of time. To further elaborate, immediately after learning, memories are labile and subject to interference and trauma. Later they are stabilized, such that they are no longer prone to the same disrupting events. This process (which will be discussed in more detail below) involves cellular and molecular events that alter synaptic efficacy, as well as prolonged systems level cross-talk between hippocampus, cerebral cortex and the amygdala(Fig. 2.1 and 2.2). A little more than a century has passed since Műller and Pilzecker hypothesized that consolidation takes place through preservation of memory (Rodriguez-Ortiz and Bermúdez-Rattoni, 2007). Their pioneering work shows the path for research on time-dependent involvement of neural systems networks and molecular processing which ultimately leads to stabilization of weak traces with the goal of forming uninterruptable and long-lasting memory. However, once memories are consolidated, they can be destabilized once again, and the memory must undergo a process similar to the original consolidation to have the memory reconsolidated. The focus of the present research is this reconsolidation process, specifically, the role of LTP/LTD and glutamate receptors.

2.2. Reconsolidation:

Misanin et al. (1968) observed that a retrieved memory was subject to disruption if electroconvulsive shock was administered shortly after memory retrieval, an effect that was previously thought to be limited to memories that were undergoing reconsolidation. This finding went largely unnoticed for decades, but in recent years there has been an increased interest in the processes of reconsolidation (Auber et al., 2013 and Tronson et al., 2007). *Reconsolidation* refers to the process of re-stabilization of recently retrieved memories, including destabilization and restabilization of such memories. Although similar to the process of consolidation, the two have clearly distinct characteristics. To outline the differences underlying the processes of consolidation and reconsolidation, it is easier to delineate the physiological and functional characteristics of each individual process separately. Hebb and Gerard proposed dual-trace theories of memory suggesting that stabilization of reverberating neural activity underlying short-term memory, produces long-term memory (Auber et al., 2013). The process by which memories get consolidated is disrupted by amnesic agents like the protein-synthesis inhibitors, Anisomycin (Rodriguez-Ortiz et al., 2008). These inhibitors do not prevent learning of novel tasks but disrupts training related memory, indicating that acquisition and consolidation of memories are fairly independent but sequential processes, at the physiological, anatomical and cellular levels (Rodriguez-Ortiz et al., 2008). Whether these memories are sequentially linked or they act completely independent of each other is still a matter of intense research. The time dependent characteristics of reconsolidation might be a direct consequence of the fact that the physiological and molecular apparatus working to consolidate memories, is itself time consuming (Fig. 2.3, Rao-Ruiz et al., 2012). Functionally the process of consolidation is meant to stabilize memory. But the argument that reconsolidation is time dependent process can be

refuted with examples of short term and other working memories, which take far less time to form (Kumaran, 2008). Physiological evidence suggests that slow consolidation of memories serve as an adaptive function enabling endogenous processes stimulated by experience to manipulate the strength of memory.

2.3. Physiological and biochemical characteristics of Consolidation and Reconsolidation:

Standard consolidation theories formulated prior to the 1960s characterized consolidation as a one-time event, after which a memory is impermeable to subsequent disruption. This concept was challenged by studies reporting that presentation of reminder cue made a completely consolidated aged memory labile once again in experimental conditions (e.g., Misanin et al., 1968). In early 2000s, Nader and colleagues showed that a conditioned stimulus-alone-reminder presented long after consolidation was complete, reengaged the "temporal susceptibility" of the memory (Nader et al., 2000 and 2010). Functionally, this is the difference between consolidation and reconsolidation: Consolidation is the initial memory processing while reconsolidation is the recall of that earlier stored memory. The general consensus at this time is that retrieval of a memory makes the original memory trace labile, and storage of that memory for future use requires that it is stabilized (reconsolidated). Importantly, a labile memory should be susceptible to interference or disruption, just as a new memory prior to consolidation. In spite of the broad consensus on the generality of reconsolidation, several studies have failed to find that amnestic agents can alter labile memories if applied during the reconsolidation period. This suggests that there are boundary conditions in reconsolidation; for example, the age and strength of the memory, whether new information is introduced during the labile period, the kind of memory that is active at the time of amnestic intervention, and whether the retrieval produces new learning, all determine whether the memory gets reconsolidated. Consolidation is largely

independent of these parameters. Failure of amnesic agents to disrupt labile memories can also be attributed to procedural factors including different experimental paradigms, lack of motivation, and stimulus parameters (Auber et al., 2012). There may also be competing phenomena that take place in the same preparation. For example, in Pavlovian conditioning studies (in which a cue is paired with a shock and the cue is later presented to reactivate a memory of the shock) presentation of the cue alone can have two opposite effects, it can act as a reminder to engage the original memory trace or it can generate engramming of new and competing memory traces in which the cue is no longer followed by shock (i.e., extinction; Pavlov, 1927). In this situation, the outcome of the amnestic treatment can differ depending on which state was dominant during the treatment (Fig 2.3 and 2. 4, Auber et al., 2012).

During the early stages of memory consolidation (synaptic consolidation), both the early and late phases of, LTP allow synapses to increase strength of communication (Rao-Ruiz et al., 2012). As new experience gathers, the brain creates more and more connections and re-wires itself by rerouting its organization. Similar experiences utilize similar neural networks; thus an enduring pattern is engraved and neural messages are encrypted, shunting along the path of least resistance (Brahman, 2008). Sleep, particularly slow wave or deep sleep, is thought to improve consolidation (and reconsolidation) of memory. Activation patterns in a sleeping brain suggest that newly acquired memories are consolidated during sleep through reactivation and rehearsal (Siegel, 2001). Physiologically the process of consolidation in the mammalian brain involves increased plasticity. Several studies with one trial-tasks and shock have shown different receptor, enzyme, protein expression patterns and LTP/LTD profiles are intricately involved in the process of consolidation (Besnard et al., 2012).

To shed some light on how region specific physiology affects consolidation of fear memories, some insight into neuroanatomical nature of consolidation is warranted. Experience activates time dependent cellular storage in various sub-regions of the brain. Initiation of memory consolidation and learning experience simultaneously stimulate the Basolateral Amygdala and the adrenal gland (Fig 2.1). The adrenal gland which is an important constituent of the hypothalamic-pituitary-adrenal axis stimulates glucocorticoid and epinephrine release. This in turn has its modulatory effect on norepinephrine release from the afferent neurons to the Basolateral Amygdala and the efferent process is from the Basolateral Amygdala to other regions of the brain. The Basolateral Amygdala in turn has connectivity with the Neocortex, Hippocampus, Caudate Nucleus and Peduncular structures as well as the Cerebellum. The pituitary axis independently modulates functioning of the Basolateral Amygdala through its stimulation. A complex interplay of these structures continuously refines and redefines the process of consolidation during the phase of memory stabilization.

The above mentioned physiological effects of reconsolidation are caused by several downstream intracellular molecules. The biochemical signature of reconsolidation is currently under vigorous investigation. Several researchers have found conflicting trends in AMPAR and NMDAR downstream signaling during reconsolidation of fear memory (Fig. 2.2 and 2.3 shows role of glutamate receptor and its signaling in reconsolidation). Studies have found different downstream signaling molecules, acting in both convergent and divergent manner during reconsolidation of fear memories (Auber et. al, 2011). The primary reason, as suggested by many researchers in the field, might be subtle and minor differences between experimental procedures such as intensity of shock, time interval, time of recording, choice of contex, the way shock and context has been paired and even the room temperature (Tronson et. al., 2013). However a

complete understanding of signaling processes and their relation to altered synaptic plasticity during reconsolidation is currently unavailable.

Most of the receptors under consideration are ion channel receptors. Their effect is carried out by opening of the ion channel pore, entry of different charged ions that ultimately change the internal milieu of the cell. But in addition there are metabotropic receptors like the mGluA1, which act through second messenger system. Recent research has shown that CaMKII levels (in response to Calcium entry) and its active form p-CaMKII vary as time progresses after training session. There is an upsurge of CaMKII levels along with that of cGMP immediately after training (Kang et al., 2001) but, as time progresses, their levels drop below control levels. Even though there is evidence of levels of downstream signaling molecules increasing immediately after retrieval, there is hardly any evidence of such dramatic rise of receptor expression immediately after training begins (at the same time point after training). As time progresses, kinase levels decrease. Consistent with this kinase activity, phosphorylation of GluA1 receptor at Ser831 increases immediately after retrieval in the amygdala, in cued fear conditioning (i.e., when a signal for shock is given during training). To date, receptor phosphorylation profiles during reconsolidation have not been investigated in the hippocampus or with contextual fear conditioning models.

Xing Liu et. al. (2013), showed pERK levels increase right after (10 min) retrieval session was over and slowly decreased after 1 h. They further showed that this mediation of ERK phosphorylation was through an endocytosis biased signaling. A similar trend was observed in pRSK and pELF4B patterns after retrieval. Consistent with these observations, members of the Ras/Raf/MEK family show a similar trend as time progresses during consolidation. However, the signal transduction mechanism during the later points of post-retrieval period is somewhat

different to what is seen immediately after reconsolidation begins. Sudden increase in expression is followed by decrease in expression and phosphorylation at the 1 h time point, followed by a subsequent increase in activity towards the end of the reconsolidation window (which extends for approximately 6h in rodents). This second phase of increased phosphorylation is then followed by gradual normalization outside the reconsolidation window. A similar trend is seen cAMP and CREB levels (LeDoux et al, 2013).

The trend described above (as happens during reconsolidation of fear memory in the amygdala and to an extent in the hippocampus) is very different from what is seen in fear memory formation or during consolidation. During fear memory consolidation, release of glutamate causes opening of AMPARs and NMDARs and entry of Calcium ions into the post synaptic neuron. Calcium entry causes calcium induced calcium release and opening of voltage and ligand gated calcium channels. Downstream of AMPAR and NMDAR, Calcium entry further modulates Gap and GEF/Rho activity leading to a conversion of GDP-Rho to GTP-Rho. Simultaneously Integrins and their kinases like Integrin Linked Kinases (ILK), cause activation of the CDC42/Rac/GAP/GEP pathway. This in turn causes Calcium mediated CREB activation and subsequent RNA synthesis. On the contrary, Rac-GDP gets converted to Rac-GTP. Rac-GTP and Rho-GTP together affect ROCK/LIGK pathway leading to inactivation of cofilin movement (Schafe et al., 2000). This leads to altered cellular morphology and spine formation is decreased through changed action potential profiles. However, there is very little evidence available to link these changes to any receptor expression patterns during reconsolidation of contextual fear memory in the hippocampus. Hence a primary objective of future research is to establish a complete understanding of these downstream signaling mechanisms during contextual fear memory reconsolidation and link the findings to newer models synaptic plasticity post-retrieval.

It is important to note that reconsolidation is a novel field of study. Specific information on receptors expression and function, their downstream signaling and how they affect memory strength and content is not known. Our research for the first time proposes a complete model of time dependent synaptic plasticity and receptor expression during reconsolidation. Future work can be directed towards more in-depth dissection of downstream signaling mechanisms involved and their functional correlates.

2.4. **Conclusion:**

To conclude, in this chapter we have presented a comprehensive view of memory formation, the different areas of brain that are involved in the process, and different steps involved in memory processing. We have extensively described different areas of the brain that are involved in maintaining, processing and storing explicit and implicit memories for long term usage. To reiterate, these regions are mainly the different areas of the hippocampus, the cortex and the lateral amygdala. In course of our discussion we have described that glutamate receptors (AMPA and NMDA subtypes) play an important role in formation of new memories. These receptors have complex structures and stoichiometry which can vastly affect their functionality. Trafficking of the different glutamate receptor subunits play an important role in determining their surface expression. Subsequently we have described in detail the process of consolidation and reconsolidation. To summarize consolidation is the initial process that compasses memory formation after learning. Reconsolidation on the other hand, is a consecutive process of restabilizing the memory once it becomes fragile upon recall which occurs over the course of about 6-7 h; it is during this ‘reconsolidation window’ that the memory is prone to disruption or manipulation. The reconsolidation window is of particular importance to this project, because it provides us with an opportunity to manipulate the memory either to selectively weaken it in case

of maladaptive memories (e.g., fear memories that lead to stress, anxiety and trauma) or strengthen it in case of more adaptive memories (e.g., information that increases survival). Our model is based on the assumption that memory traces are labile during the initial period of reconsolidation, and as time progresses the traces become stable. We aim to study the role of glutamate receptors and LTP/LTD during the reconsolidation window. We believe that receptor expression will have a direct effect on synaptic plasticity. We also aim to investigate the relationship between protein synthesis mediated reconsolidation and glutamate receptor expression pattern post retrieval. The available literature suggests that glutamate receptors play a complex role in the process of reconsolidation. These receptors communicate to the internal milieu of the cell through a complex network of downstream signaling kinases, scaffolding proteins and transcription factors that act as the ultimate effectors of the process. In the next chapter we will discuss in further details about the current state of knowledge on the role of these receptors in reconsolidation of memory and their consequences in synaptic plasticity. We will also show how biphasic waves of synaptic AMPARs and overexpression of NMDARs are important for reconsolidation. Our research will also further support the assumption that receptor mediated bidirectional plasticity is the key to reconsolidation of fear memory in the hippocampus.

2.5. **Legends:**

Figure 2.1. Figure shows how different regions of the brain process different memory forms. This memory in turn gets reactivated upon retrieval and then reconsolidated over hours. The reconsolidated memory can then be updated to weaken it or recover spontaneously over weeks to months (adapted from LaBar and Cabeza, Nature review, 2006).

Fig 2.2. Diagrammatic representation of excitatory neurotransmission in the synapse. The neurotransmitter involved is glutamate, which binds to the postsynaptic AMPA and NMDA receptors. These receptors are in turn attached to intracellular scaffolding proteins like the Postsynaptic Density (PSD) complex that plays a role in their trafficking (Modified from Genoux, et al, Auckland, NZ).

Fig 2.3. Diagrammatic representation of role of calcium permeable AMPAR expression during different phases of retrieval induced reconsolidation. The figure also shows the role of AMPAR endocytosis blockade in amygdala. Use of D-AP5 (NMDAR blocker) and TAT-3Y compounds (GluA2 endocytosis blocker) confirm their role in progression of memory reconsolidation (from Ingie Hong et al. PNAS 2013).

Fig 2.4. Simple experimental model showing different fates of memory reconsolidation post retrieval. Freezing used as an overt expression of fear is used to understand the role of GluA2 manipulation using peptide delivery mediated methods (from Tronson et. al. Nature Neuroscience, 2011).

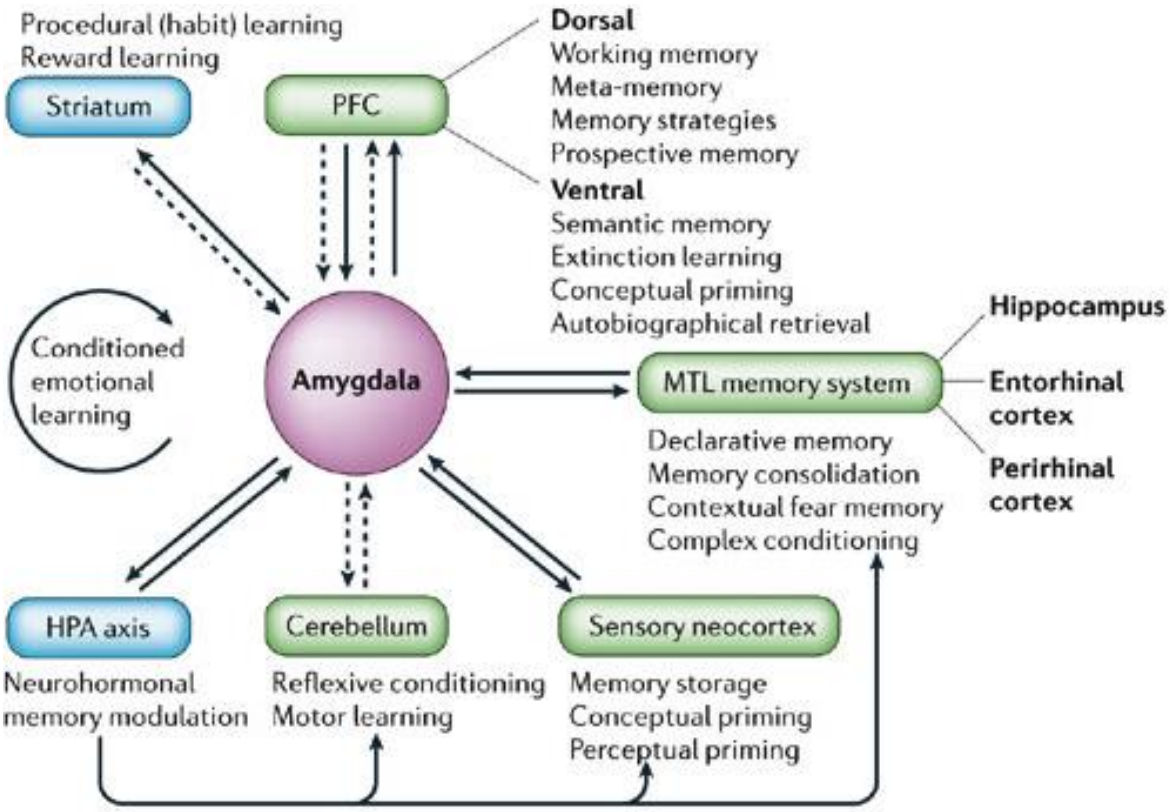
Procedural (habit) learning
Reward learning
Striatum
PFC
Dorsal
Working memory
Meta-memory
Memory strategies
Prospective memory
Ventral
Semantic memory
Extinction learning
Conceptual priming
Autobiographical retrieval
Conditioned emotional learning
Amygdala
MTL memory system
Hippocampus
Entorhinal cortex
Perirhinal cortex
Declarative memory
Memory consolidation
Contextual fear memory
Complex conditioning
HPA axis
Cerebellum
Sensory neocortex
Neurohormonal memory modulation
Reflexive conditioning
Motor learning
Memory storage
Conceptual priming
Perceptual priming

Figure 2.1

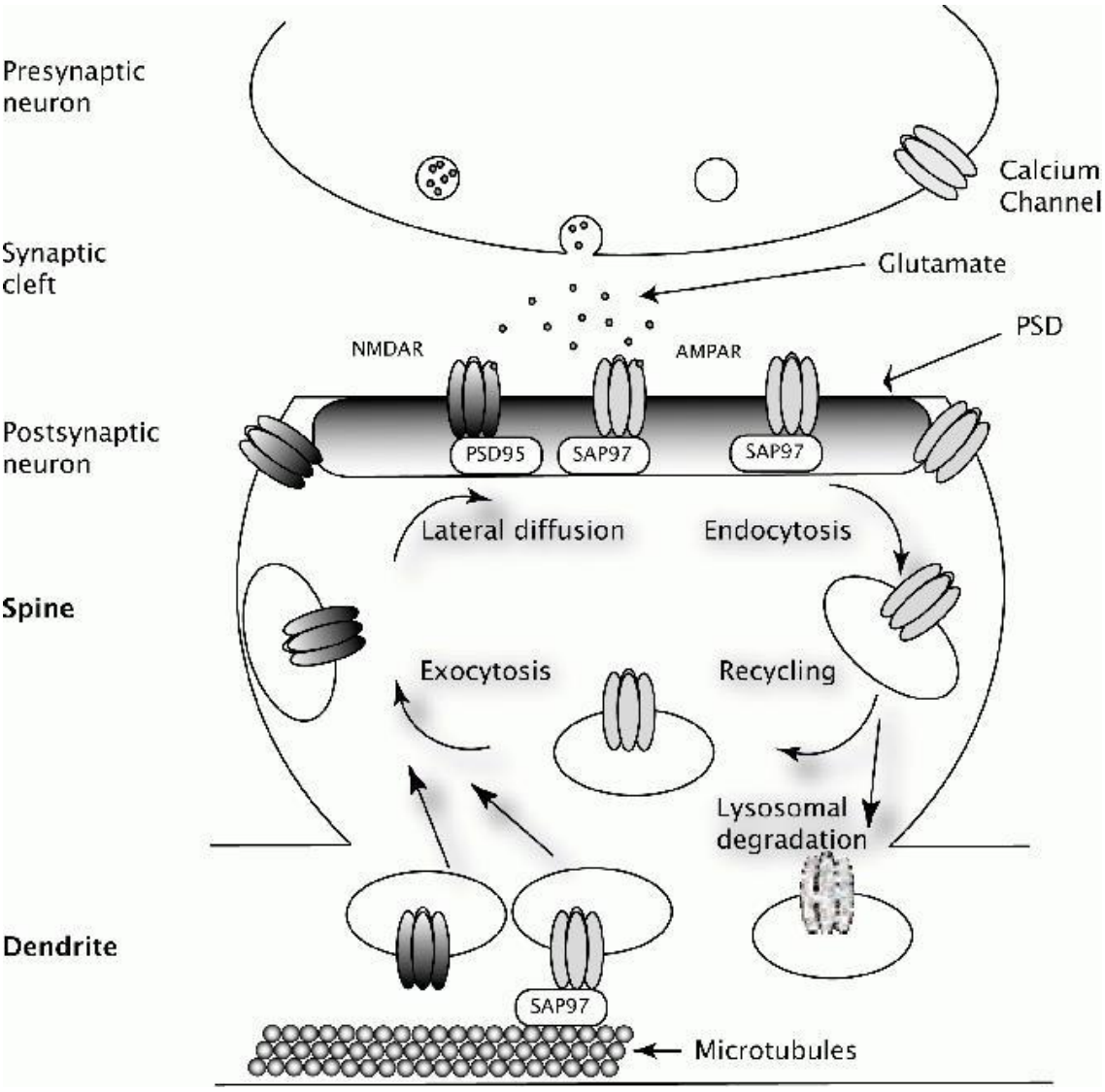
Presynaptic neuron
Synaptic cleft
Postsynaptic neuron
Spine
Dendrite
Calcium Channel
Glutamate
PSD
NMDAR
AMPAR
PSD95
SAP97
SAP97
Lateral diffusion
Endocytosis
Exocytosis
Recycling
Lysosomal degradation
SAP97
Microtubules

Figure 2.2

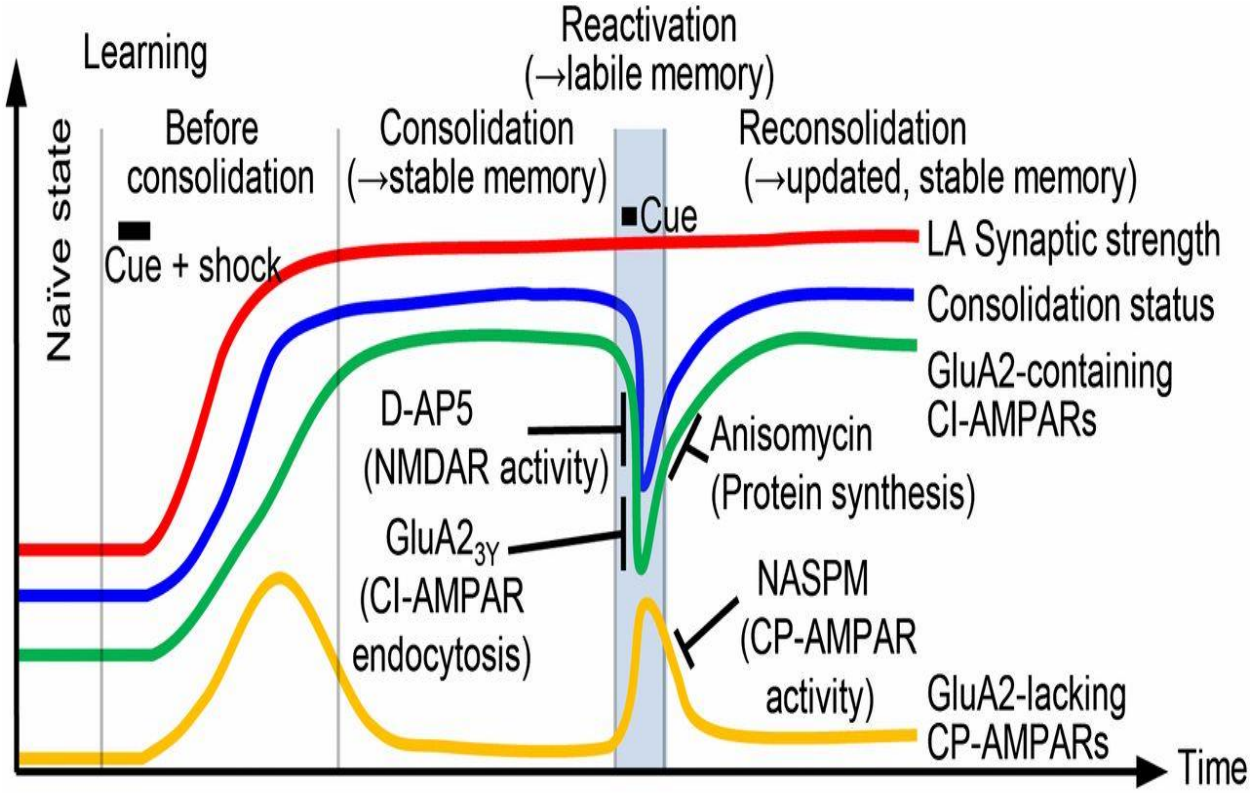
Learning
Reactivation
(→labile memory)
Naïve state
Before
consolidation
Consolidation
(→stable memory)
Reconsolidation
(→updated, stable memory)
Cue
Cue + shock
LA Synaptic strength
Consolidation status
GluA2-containing
CI-AMPARs
D-AP5
(NMDAR activity)
Anisomycin
(Protein synthesis)
GluA2$_{3Y}$
(CI-AMPAR
endocytosis)
NASPM
(CP-AMPAR
activity)
GluA2-lacking
CP-AMPARs
Time

Figure 2.3

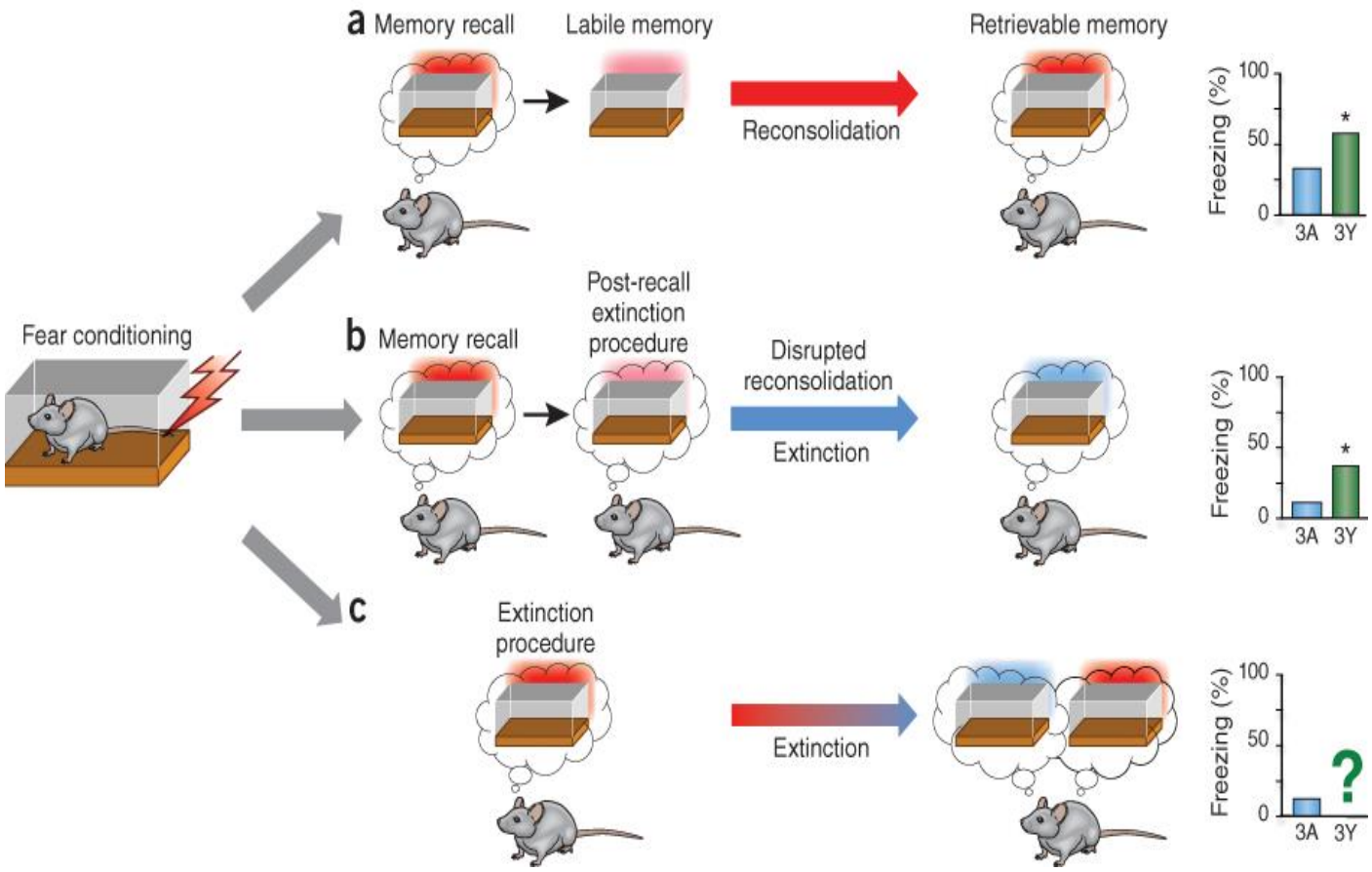
a Memory recall
Labile memory
Retrievable memory
Reconsolidation
Freezing (%)
100
50
0
3A 3Y
*
Fear conditioning
b Memory recall
Post-recall extinction procedure
Disrupted reconsolidation
Extinction
c
Extinction procedure
Extinction
?

Figure 2.4

2.6. **References:**

Alkon DL, Nelson TJ (1990) Specificity of molecular changes in neurons involved in memory storage. Faseb J 4:1567-1576

Auber A, Tedesco V, Jones CE, Monfils MH, Chiamulera C (2013) Post-retrieval extinction as reconsolidation interference: methodological issues or boundary conditions? Psychopharmacology; 226:631-47

Balfour DJ (1991) The influence of stress on psychopharmacological responses to nicotine. Br J Addict 86:489-493

Bayley PJ, Frascino JC, Squire LR (2005) Robust habit learning in the absence of awareness and independent of the medial temporal lobe. Nature. Jul 28;436(7050):550-3

Bartsch T, Döhring J, Reuter S, Finke C, Rohr A, Brauer H, Deuschl G, Jansen O (2015) Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. J Cereb Blood Flow Metab. Jun 17 (7052):556-60

Bailey CH, Kandel ER, Harris KM (2015) Structural Components of Synaptic Plasticity and Memory Consolidation. Cold Spring Harb Perspect Biol. Jul 1;7(7):a021758

Bannerman DM, Yee BK, Lemaire M, Jarrard L, Iversen SD, Rawlins JN, Good MA (2001) Contextual fear conditioning is disrupted by lesions of the subcortical, but not entorhinal, connections to the hippocampus. Exp Brain Res 141:304-311

Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25:515-532

Bard L, Groc L (2011) Glutamate receptor dynamics and protein interaction: lessons from the NMDA receptor. Mol Cell Neurosci Dec;48(4):298-307

Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D (2007) Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology Jan;52(1):60-70

Bashir ZI, Alford S, Davies SN, Randall AD, Collingridge GL (1991) Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature. Jan 10;349(6305):156-8

Bear MF, Abraham WC (1996) Long-term depression in hippocampus. Annu Rev Neurosci 19:437-62

Ben Mamou C, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci Oct;9(10):1237-9

Besnard A, Caboche J, Laroche S (2012) Reconsolidation of memory: a decade of debate. Prog Neurobiol. Oct;99(1):61-80

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature Jan 7;361(6407):31-9

Buller AL, Monaghan DT (1997) Pharmacological heterogeneity of NMDA receptors: characterization of NR1a/NR2D heteromers expressed in Xenopus oocytes. Eur J Pharmacol Feb 5;320(1):87-94

Burgess GC, Braver TS (2010) Neural mechanisms of interference control in working memory: effects of interference expectancy and fluid intelligence. PLoS One. Sep 20;5(9):e12861

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the performant path. J Physiol 232:331-356

Bliss TV, Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol 232:357-374

Brahman CR (2008), Local protein synthesis, actin dynamics, and LTP consolidation, Current Op NeuroBiol, Vol-18-5:524-31

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci 2:454-460

Carroll RC, Zukin RS (2002) NMDA-receptor trafficking and targeting: implications for synaptic transmission and plasticity Trends Neurosci Nov;25(11):571-7

Rodriguez-Ortiz and Federico Bermúdez-Rattoni (2007), Memory Reconsolidation or Updating Consolidation? Neural Plasticity and Memory: From Genes to Brain Imaging Chapter 11

Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science Nov 19;330(6007):1108-12

Chancey JH, Poulsen DJ, Wadiche JI, Overstreet-Wadiche L (2014) Hilar mossy cells provide the first glutamatergic synapses to adult-born dentate granule cells. J Neurosci. Feb 5;34(6):2349-54

Collingridge GL, Peineau S, Howland JG, Wang YT (2010) Long-term depression in the CNS. Nat Rev Neurosci. Jul;11(7):459-73

Clarke PB, Pert A (1985) Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348:355-358

Corrigall WA, Coen KM (1991) Selective dopamine antagonists reduce nicotine self-administration.
Psychopharmacology (Berl) 104:171-176

Corrigall WA, Coen KM, Adamson KL (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res
653:278-284

Corrigall WA, Franklin KB, Coen KM, Clarke PB (1992) The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology (Berl) 107:285-289

Dalton GL, Wu DC, Wang YT, Floresco SB, Phillips AG (2012) NMDA GluN2A and GluN2B receptors play separate roles in the induction of LTP and LTD in the amygdala and in the acquisition and extinction of conditioned fear. Neuropharmacology Feb;62(2):797-806

Devan BD, White NM (1999) Parallel information processing in the dorsal striatum: relation to hippocampal function. J Neurosci 19:2789-2798

Dias C, Wang YT, Phillips AG (2012) Facilitated extinction of morphine conditioned place preference with Tat-GluA2(3Y) interference peptide. Behav Brain Res Aug 1;233(2):389-97

Dudai Y (1996) Consolidation: fragility on the road to the engram. Neuron Sep;17(3):367-70

Dubue JD, McKinney TL, Treit D, Dickson CT (2015) Intrahippocampal Anisomycin Impairs Spatial Performance on the Morris Water Maze. J Neurosci. Aug 5;35(31):11118-24

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61

Egebjerg J, Heinemann SF (1993) Ca2+ permeability of unedited and edited versions of the kainate selective glutamate receptor GluR6. Proc Natl Acad Sci USA 90:755-759

Eichenbaum H, Dudchenko P, Wood E, Shapiro M (1999) The hippocampus, memory, and place cells: is it spatial memory or a memory space?, Neuron, 5;3(38):118-24

Einarsson EÖ, Pors J, Nader K (2015) Systems reconsolidation reveals a selective role for the anterior cingulate cortex in generalized contextual fear memory expression. Neuropsychopharmacology Jan;40(2):480-7

Faraone SV, Doyle AE (2000) Genetic influences on attention deficit hyperactivity disorder. Curr Psychiatry Rep 2:143-146

Fadó R, Soto D, Miñano-Molina AJ, Pozo M, Carrasco P, Yefimenko N, Rodríguez-Álvarez J, Casals N (2015) Novel regulation of the synthesis of AMPA receptor subunit GluA1 by carnitine palmitoyltransferase 1C (CPT1C) in the hippocampus. J Biol Chem. 46: 33-39

Fioriti L, Myers C, Huang YY, Li X, Stephan JS, Trifilieff P, Colnaghi L, Kosmidis S, Drisaldi B, Pavlopoulos E, Kandel ER (2015) The Persistence of Hippocampal-Based Memory Requires Protein Synthesis Mediated by the Prion-like Protein CPEB3. Neuron. Jun 17;86(6):1433-48

Feng B, Raghavachari S, Lisman J (2011) Quantitative estimates of the cytoplasmic, PSD, and NMDAR-bound pools of CaMKII in dendritic spines. Brain Res Oct 24;1419:46-52

Fonseca R, Nägerl UV, Bonhoeffer T (2006) Neuronal activity determines the protein synthesis dependence of long-term potentiation. Nat Neurosci Apr;9(4):478-80

Foucaud B, Laube B, Schemm R, Kreimeyer A, Goeldner M, Betz H (2003) Structural model of the N-methyl-D-aspartate receptor glycine site probed by site-directed chemical coupling. J Biol Chem 278:24011-24017

Furini CR, Myskiw Jde C, Schmidt BE, Zinn CG, Peixoto PB, Pereira LD, Izquierdo I (2015) The relationship between protein synthesis and protein degradation in object recognition memory. Behav Brain Res. Nov 1;294:17-24

Garris PA, Kilpatrick M, Bunin MA, Michael D, Walker QD, Wightman RM (1999) Dissociation of dopamine release in the nucleus accumbens from intracranial selfstimulation, Nature 398:67-69

Genoux D, Montgomery JM (2007) Glutamate receptor plasticity at excitatory synapses in the brain. Clin Exp Pharmacol Physiol 34:1058-1063

Giese KP, Aziz W, Kraev I, Stewart MG (2015) Generation of multi-innervated dendritic spines as a novel mechanism of long-term memory formation. Neurobiol Learn Mem. Oct;124:48-51

Gomperts SN (1996) Clustering membrane proteins: It's all coming together with the PSD-95/SAP90 protein family. Cell Mar 8;84(5):659-62

Gray DT, Barnes CA (2015) Distinguishing adaptive plasticity from vulnerability in the aging hippocampus. Neuroscience. Aug 6. pii: S0306-4522(15)00712-5

Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D (2006) NMDA receptor surface mobility depends on NR2A-2B subunits. Proc Natl Acad Sci U S A Dec 5;103(49):18769-74

Groc L, Bard L, Choquet D (2009) Surface trafficking of N-methyl-D-aspartate receptors: physiological and pathological perspectives. Neuroscience Jan 12;158(1):4-18

Guida F, Luongo L, Marmo F, Romano R, Iannotta M, Napolitano F, Belardo C, Marabese I, D'Aniello A, De Gregorio D, Rossi F, Piscitelli F, Lattanzi R, de Bartolomeis A, Usiello A, Di Marzo V, de Novellis V, Maione S (2015) Palmitoylethanolamide reduces pain-related behaviors and restores glutamatergic synapses homeostasis in the medial prefrontal cortex of neuropathic mice. Mol Brain. Aug 12;8:47

Hansen N, Manahan-Vaughan D (2015) Hippocampal long-term potentiation that is elicited by perforant path stimulation or that occurs in conjunction with spatial learning is tightly controlled by beta-adrenoreceptors and the locus coeruleus. Hippocampus. Feb 27

Harris KM, Jensen FE, Tsao B (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci. Jul;12(7):2685-705

Hinnebusch AG (2015) Cell biology. Blocking stress response for better memory? Science. May 29;348(6238):967-8

Halt AR, Dallapiazza RF, Zhou Y, Stein IS, Qian H, Juntti S, Wojcik S, Brose N, Silva AJ, Hell JW (2012) CaMKII binding to GluN2B is critical during memory consolidation. EMBO J Mar 7;31(5):1203-16

Hammond MS, Sims C, Parameshwaran K, Suppiramaniam V, Schachner M, Dityatev A (2006) Neural cell adhesion molecule-associated polysialic acid inhibits NR2B-containing N-methyl-D-aspartate receptors and prevents glutamate-induced cell death.J Biol Chem Nov 17;281(46):34859-69

Heynen AJ, Quinlan EM, Bae DC, Bear MF (2000) Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. Neuron 28:527-536

Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, Monaghan DT, Sacktor TC (2000) Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. J Neurosci Jun 15;20(12):RC81

Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028-1031

Jarome TJ, Helmstetter FJ (2014) Protein degradation and protein synthesis in long-term memory formation. Front Mol Neurosci. Jun 26;7:61

Jeneson A, Squire LR (2011) Working memory, long-term memory, and medial temporal lobe function. Learn Mem. Dec 16;19(1):15-25

Kandel ER (1997) Genes, synapses, and long-term memory. J Cell Physiol 173:124-125

Kang H, Sun LD, Atkins SM, Tonegawa S (2001) An Important Role of Neural Activity-Dependent CaMKIV Signaling in the Consolidation of Long-Term Memory. Cell, Volume 106, Issue 6:Pages 771–783

Kempermann G, Song H, Gage FH (2015) Neurogenesis in the Adult Hippocampus. Cold Spring Harb Perspect Med. Jul;5(7):a018812

Kuner T, Beck C, Sakmann B, Seeburg PH (2001) Channel-lining residues of the AMPA receptor M2 segment: structural environment of the Q/R site and identification of the selectivity filter. J Neurosci 21:4162-4172

Kim DH, Moon EY, Yi JH, Lee HE, Park SJ, Ryu YK, Kim HC, Lee S, Ryu JH (2015) Peptide fragment of thymosin β4 increases hippocampal neurogenesis and facilitates spatial memory. Neuroscience. Sep 9. pii: S0306-4522(15)00827-1

Kim S, Dede AJ, Hopkins RO, Squire LR (2015) Memory, scene construction, and the human hippocampus. Proc Natl Acad Sci U S A. Apr 14;112(15):4767-72

Kida S (2014) Mechanisms for regulation of fear conditioning and memory]. Nihon Shinkei Seishin Yakurigaku Zasshi Nov;34(5-6):117-25

Kim J, Song B, Hong I, Kim J, Lee J, Park S, Eom JY, Lee CJ, Lee S, Choi S (2010) Reactivation of fear memory renders consolidated amygdala synapses labile. J Neurosci Jul 14;30(28):9631-40

Kim JI, Lee HR, Sim SE, Baek J, Yu NK, Choi JH, Ko HG, Lee YS, Park SW, Kwak C, Ahn SJ, Choi SY, Kim H, Kim KH, Backx PH, Bradley CA, Kim E, Jang DJ, Lee K, Kim SJ, Zhuo M, Collingridge GL, Kaang BK (2011) PI3Kγ is required for NMDA receptor-dependent long-term depression and behavioral flexibility. Nat Neurosci Oct 23;14(11):1447-54

Kim R, Moki R, Kida S (2011) Molecular mechanisms for the destabilization and restabilization of reactivated spatial memory in the Morris water maze. Mol BRAIN Feb 11;4:9

Kochlamazashvili G, Senkov O, Grebenyuk S, Robinson C, Xiao MF, Stummeyer K, Gerardy-Schahn R, Engel AK, Feig L, Semyanov A, Suppiramaniam V, Schachner M, Dityatev A (2010) Neural cell adhesion molecule-associated polysialic acid regulates synaptic plasticity and learning by restraining the signaling through GluN2B-containing NMDA receptors. J Neurosci Mar 17;30(11):4171-83

Kochlamazashvili G, Bukalo O, Senkov O, Salmen B, Gerardy-Schahn R, Engel AK, Schachner M, Dityatev A (2012) Restoration of synaptic plasticity and learning in young and aged NCAM-deficient mice by enhancing neurotransmission mediated by GluN2A-containing NMDA receptors. J Neurosci Feb 15;32(7):2263-75

Krawczyk MC, BLAKE MG, Baratti CM, Romano A, Boccia MM, Feld M (2015) Memory reconsolidation of an inhibitory avoidance task in mice involves cytosolic ERK2 bidirectional modulation. Neuroscience Mar 17;294:227-237

Krause-Utz A, Elzinga BM, Oei NY, Paret C, Niedtfeld I, Spinhoven P, Bohus M, Schmahl C (2014) Amygdala and Dorsal Anterior Cingulate Connectivity during an Emotional Working Memory Task in Borderline Personality Disorder Patients with Interpersonal Trauma History. Front Hum Neurosci. Oct 28;8:848

Krzystanek M, Bogus K, Pałasz A, Krzystanek E, Worthington JJ, Wiaderkiewicz R (2015) Effects of long-term treatment with the neuroleptics haloperidol, clozapine and olanzapine on immunoexpression of NMDA receptor subunits NR1, NR2A and NR2B in the rat hippocampus. Pharmacol Rep. Oct;67(5):965-969

Kwapis JL, Jarome TJ, Schiff JC, Helmstetter FJ (2011) Memory consolidation in both trace and delay fear conditioning is disrupted by intra-amygdala infusion of the protein synthesis inhibitor anisomycin. Learn Mem Oct 25;18(11):728-32

Kuner T, Wollmuth LP, Karlin A, Seeburg PH, Sakmann B (1996) Structure of the NMDA receptor channel M2 segment inferred from the accessibility of substituted cysteines. Neuron 17:343-352

Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, Morikawa K (2000) Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. Nature 407:971-977

Kuusinen A, Abele R, Madden DR, Keinanen K (1999) Oligomerization and ligandbinding properties of the ectodomain of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit GluRD. J Biol Chem 274:28937-28943

Kumaran D, (2008) Short-Term Memory and the Human Hippocampus, JNeurosci 28(15): 3837-3838

Lau CG, Zukin RS (2007) NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci 8:413-426

Lewis PR, Shute CC (1967) The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. Brain. Sep;90(3):521-40

Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, Wang YT, Sheng M (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nat Neurosci 3:1282-1290

Li JT, Zhao YY, Wang HL, Wang XD, Su YA, Si TM (2015) Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory-inhibitory balance in rat hippocampus. Neuroscience. Sep 6. pii: S0306-4522(15)00813-1

Lisman J, Malenka RC, Nicoll RA, Malinow R (1997) Learning mechanisms: the case for CaM-KII. Science 276:2001-2002

Lledo PM, Zhang X, Sudhof TC, Malenka RC, Nicoll RA (1998) Postsynaptic membrane fusion and long-term potentiation. Science 279:399-403

Llorens-Martín M, Jurado-Arjona J, Avila J, Hernández F (2015) Novel connection between newborn granule neurons and the hippocampal CA2 field. Exp Neurol. Jan;263:285-92

Louis M, Clarke PB (1998) Effect of ventral tegmental 6-hydroxydopamine lesions on the locomotor stimulant action of nicotine in rats. Neuropharmacology 37:1503-1513

Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA (1999) Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron 24:649-658

Lynch MA (2004) Long-term potentiation and memory. Physiol Rev Jan;84(1):87-136

Mac Callum PE, Hebert M, Adamec RE, Blundell J (2014) Systemic inhibition of mTOR kinase via rapamycin disrupts consolidation and reconsolidation of auditory fear memory. Neurobiol Learn Mem Jul;112:176-85

MacDonald JF, Jackson MF, Beazely MA (2006) Hippocampal long-term synaptic plasticity and signal amplification of NMDA receptors. Crit Rev Neurobiol 18:71-84

Markwardt SJ, Wadiche JI, Overstreet-Wadiche LS (2009) Input-specific GABAergic signaling to newborn neurons in adult dentate gyrus. J Neurosci. Dec 2;29(48):15063-72

Marquez Y, Höpfler M, Ayatollahi Z, Barta A, Kalyna M (2015) Unmasking alternative splicing inside protein-coding exons defines exitrons and their role in proteome plasticity. Genome Res. Jul;25(7):995-100

Mather M, Clewett D, Sakaki M, Harley CW (2015) Norepinephrine ignites local hot spots of neuronal excitation: How arousal amplifies selectivity in perception and memory. Behav Brain Sci. Jul 1:1-100

Maletic-Savatic M, Koothan T, Malinow R (1998) Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part II: mediation by calcium/calmodulindependent protein kinase II. J Neurosci 18:6814-6821

McAvoy K, Russo C, Kim S, Rankin G, Sahay A. Fluoxetine induces input-specific hippocampal dendritic spine remodeling along the septotemporal axis in adulthood and middle age. Hippocampus. 2015 Apr 7. doi: 10.1002/hipo.22464

Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. Annu Rev Neurosci 25:103-126

Mathie A, Cull-Candy SG, Colquhoun D (1991) Conductance and kinetic properties of single nicotinic acetylcholine receptor channels in rat sympathetic neurones. J Physiol 439:717-750

Mayer ML, Armstrong N (2004) Structure and function of glutamate receptor ion channels. Annu Rev Physiol 66:161-181

Masuko T, Kashiwagi K, Kuno T, Nguyen ND, Pahk AJ, Fukuchi J, Igarashi K, Williams K (1999) A regulatory domain (R1-R2) in the amino terminus of the N-methyl-Daspartate receptor: effects of spermine, protons, and ifenprodil, and structural similarity to bacterial leucine/isoleucine/valine binding protein. Mol Pharmacol 55:957-969

Matsuo N, Reijmers L, Mayford M (2008) Spine-type-specific recruitment of newly synthesized AMPA receptors with learning Science Feb 22;319(5866):1104-7

McGaugh JL (2000) Memory--a century of consolidation. Science Jan 14;287(5451):248-51

Milton AL, Merlo E, Ratano P, Gregory BL, Dumbreck JK, Everitt BJ (2013) Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. J Neurosci Jan 16;33(3):1109-15

Mereu G, Yoon KW, Boi V, Gessa GL, Naes L, Westfall TC (1987) Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. Eur J Pharmacol 141:395-399

Maren S, Fanselow MS (1997) Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. Neurobiol Learn Mem 67:142-149

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science May 3;160(3827):554-5

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron Mar;12(3):529-40

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science May 22;256(5060):1217-21

Monyer H, Seeburg PH, Wisden W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. Neuron 6:799-810

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683

Museo E, Wise RA (1995) Cytisine-induced behavioral activation: delineation of neuroanatomical locus of action. Brain Res 670:257-263

Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Ann N Y Acad Sci Mar;1191:27-41

Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. Nat Rev Neurosci Mar;10(3):224-34

Nader K, Schafe GE, LeDoux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature Aug 17;406(6797):722-6

Nakahara S, Miyake S, Tajinda K, Ito H. Mossy fiber mis-pathfinding and semaphorin reduction in the hippocampus of α-CaMKII hKO mice. Neurosci Lett. 2015 Jun 26;598:47-51

Novick JM, Kan IP, Trueswell JC, Thompson-Schill SL. A case for conflict across multiple domains: memory and language impairments following damage to ventrolateral prefrontal cortex. Cogn Neuropsychol. 2009 Sep;26(6):527-67

Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci Apr 1;16(7):2157-63

O'Neill MF, Dourish CT, Iversen SD (1991) Evidence for an involvement of D1 and D2 dopamine receptors in mediating nicotine-induced hyperactivity in rats Psychopharmacology (Berl) 104:343-350

Ohno K, Pettigrew KD, Rapoport SI (1979) Local cerebral blood flow in the conscious rat as measured with 14C-antipyrine, 14C-iodoantipyrine and 3H-nicotine. Stroke 10:62-67

Ogawa M, Sawaguchi S, Kamemura K, Okajima T (2015) Intracellular and extracellular O-linked N-acetylglucosamine in the nervous system. Exp Neurol. Aug 14. pii: S0014-4886(15)30070-4

Omvik P (1996) How smoking affects blood pressure. Blood Press 5:71-77

Olshavsky ME, Song BJ, Powell DJ, Jones CE, Monfils MH, Lee HJ (2013) Updating appetitive memory during reconsolidation window: critical role of cue-directed behavior and amygdala central nucleus. Front Behav Neurosci. Dec 9;7:186

Olshavsky ME, Jones CE, Lee HJ, Monfils MH (2013) Appetitive behavioral traits and stimulus intensity influence maintenance of conditioned fear. Front Behav Neurosci. Dec 2;7:179

Orzeł-Gryglewska J, Matulewicz P, Jurkowlaniec E (2015) Brainstem system of hippocampal theta induction: The role of the ventral tegmental area. Synapse. Nov;69(11):553-75

Ortega LA, Glueck AC, Papini MR (2014) Anisomycin Disrupts Consummatory Behavior after Incentive Downshift via Conditioned Taste Aversion. International Journal of Psychology and Psychological Therapy 14, 1, 71-84

Paas Y, Eisenstein M, Medevielle F, Teichberg VI, Devillers-Thiery A (1996) Identification of the amino acid subsets accounting for the ligand binding specificity of a glutamate receptor. Neuron 17:979-990

Palombo DJ, Keane MM, Verfaellie M (2015) How does the hippocampus shape decisions? Neurobiol Learn Mem. Aug 19;125:93-97

Parameshwaran K, Buabeid MA, Bhattacharya S, Uthayathas S, Kariharan T, Dhanasekaran M, Suppiramaniam V (2013) Long term alterations in synaptic physiology, expression of β2 nicotinic receptors and ERK1/2 signaling in the hippocampus of rats with prenatal nicotine exposure. Neurobiol Learn Mem Nov;106:102-11

Pavlov PI (2010) Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Ann Neurosci Jul;17(3):136-41

Petralia RS, Wang YX, Hua F, Yi Z, Zhou A, Ge L, Stephenson FA, Wenthold RJ (2010) Organization of NMDA receptors at extrasynaptic locations. Neuroscience Apr 28;167(1):68-87

Panchenko VA, Glasser CR, Mayer ML (2001) Structural similarities between glutamate receptor channels and K(+) channels examined by scanning mutagenesis. J Gen Physiol 117:345-360

Paoletti P, Perin-Dureau F, Fayyazuddin A, Le Goff A, Callebaut I, Neyton J (2000) Molecular organization of a zinc binding n-terminal modulatory domain in a NMDA receptor subunit. Neuron 28:911-925

Perin-Dureau F, Rachline J, Neyton J, Paoletti P (2002) Mapping the binding site of the neuroprotectant ifenprodil on NMDA receptors. J Neurosci 22:5955-5965

Phillips RG, LeDoux JE (1995) Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning. J Neurosci 15:5308-5315

Phillips RG and LeDoux JS (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience V106 No.2-274-285

Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ (2005) The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron Sep 15;47(6):845-57

Przybyslawski J, Roullet P, Sara SJ (1999) Attenuation of emotional and nonemotional memories after their reactivation: role of beta adrenergic receptors. J Neurosci Aug 1;19(15):6623-8

Picciotto MR, Zoli M (2008) Neuroprotection via nAChRs: the role of nAChRs in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Front Biosci 13:492-504

Pouzet B, Welzl H, Gubler MK, Broersen L, Veenman CL, Feldon J, Rawlins JN, Yee BK (1999) The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat. Eur J Neurosci 11:123-140

Rao-Ruiz P, Carney KE, Pandya N, van der Loo RJ, Verheijen MH, van Nierop P, Smit AB, Spijker S (2015) Time-dependent changes in the mouse hippocampal synaptic membrane proteome after contextual fear conditioning. Hippocampus Feb 23

Rao-Ruiz P, Rotaru DC, van der Loo RJ, Mansvelder HD, Stiedl O, Smit AB, Spijker S (2011) Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear. Nat Neurosci Sep 11;14(10):1302-8

Rescorla RA (2004). Spontaneous recovery. Learn Mem Sep-Oct;11(5):501-9

Rescorla RA (2001) Retraining of extinguished Pavlovian stimuli. J Exp Psychol Anim Behav Process Apr;27(2):115-24

Rodriguez-Ortiz CJ, Garcia-DeLaTorre P, Benavidez E, Ballesteros MA, Bermudez-Rattoni F (2008) Intrahippocampal anisomycin infusions disrupt previously consolidated spatial memory only when memory is updated. Neurobiol Learn Mem Mar;89(3):352-9

Rossato JI, Köhler CA, Radiske A, Bevilaqua LR, Cammarota M (2015) Inactivation of the dorsal hippocampus or the medial prefrontal cortex impairs retrieval but has differential effect on spatial memory reconsolidation. Neurobiol Learn Mem. Sep 5;125:146-151

Schiller D, Kanen JW, LeDoux JE, Monfils MH, Phelps EA (2013) Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. Proc Natl Acad Sci U S A. Dec 10;110(50):20040-5

Rose MP, McGlynn FD (1997) Toward a standard experiment for studying post-treatment return of fear. J Anxiety Disord May-Jun;11(3):263-77

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science Apr 1;308(5718):83-8

Romanelli MN, Gratteri P, Guandalini L, Martini E, Bonaccini C, Gualtieri F (2007) Central Nicotinic Receptors: Structure, Function, Ligands, and Therapeutic Potential. ChemMedChem 2:746-767

Sack JS, Saper MA, Quiocho FA (1989) Periplasmic binding protein structure and function. Refined X-ray structures of the leucine/isoleucine/valine-binding protein and its complex with leucine. J Mol Biol 206:171-191

Scannevin RH, Huganir RL (2000) Postsynaptic organization and regulation of excitatory synapses. Nat Rev Neurosci 1:133-141

Sanz-Clemente A, Gray JA, Ogilvie KA, Nicoll RA, Roche KW (2013) Activated CaMKII couples GluN2B and casein kinase 2 to control synaptic NMDA receptors. Cell Rep Mar 28;3(3):607-14

Schafe GE, Nader K, Blair HT, LeDoux JE (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci Sep;24(9):540-6

Schiller D, Monfils MH, Raio CM, Johnson DC, LeDoux JE, Phelps EA (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature Jan 7;463(7277):49-53

Schmitt WB, Sprengel R, Mack V, Draft RW, Seeburg PH, Deacon RM, Rawlins JN, Bannerman DM (2005) Restoration of spatial working memory by genetic rescue of GluR-A-deficient mice. Nat Neurosci Mar;8(3):270-2

Shipton OA, Paulsen O (2013) GluN2A and GluN2B subunit-containing NMDA receptors in hippocampal plasticity. Philos Trans R Soc Lond B Biol Sci Dec 2;369(1633):20130163

Simon CM, Hepburn I, Chen W, De Schutter E (2014)The role of dendritic spine morphology in the compartmentalization and delivery of surface receptors. J Comput Neurosci Jun;36(3):483-97

Sorg BA, Todd RP, Slaker M, Churchill L (2015) Anisomycin in the medial prefrontal cortex reduces reconsolidation of cocaine-associated memories in the rat self-administration model. Neuropharmacology May;92:25-33

Shomrat T, Turchetti-Maia AL, Stern-Mentch N, Basil JA, Hochner B (2015) The vertical lobe of cephalopods: an attractive brain structure for understanding the evolution of advanced learning and memory systems. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. Sep;201(9):947-56

Shute CC, Lewis PR. Cholinergic pathways. Pharmacol Ther B. 1975;1(1):79-87

Siegel JM (2008) The REM-sleep memory consolidation hypothesis. Science. Vol. 294 no. 5544 pp. 1058-1063

Smith C, Squire LR. Declarative memory, awareness, and transitive inference. J Neurosci. 2005 Nov 2;25(44):10138-46

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci May 19;24(20):4787-95

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward.

Science 275:1593-1599

Smith CN, Jeneson A, Frascino JC, Kirwan CB, Hopkins RO, Squire LR (2014) When recognition memory is independent of hippocampal function. Proc Natl Acad Sci U S A. Jul 8;111(27):9935-40

Smith CN, Urgolites ZJ, Hopkins RO, Squire LR (2014) Comparison of explicit and incidental learning strategies in memory-impaired patients. Proc Natl Acad Sci U S A. Jan 7;111(1):475-9

Squire LR, Dede AJ (2015) Conscious and unconscious memory systems. Cold Spring Harb Perspect Biol. Mar 2;7(3):a021667

Suga K, Saito A, Mishima T, Akagawa K (2015) ER and Golgi stresses increase ER-Golgi SNARE Syntaxin5: Implications for organelle stress and βAPP processing. Neurosci Lett. Sep 14;604:30-5

Sommer B, Keinanen K, Verdoorn TA, Wisden W, Burnashev N, Herb A, Kohler M, Takagi T, Sakmann B, Seeburg PH (1990) Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS. Science 249:1580-1585

Stern-Bach Y, Bettler B, Hartley M, Sheppard PO, O'Hara PJ, Heinemann SF (1994) Agonist selectivity of glutamate receptors is specified by two domains structurally related to bacterial amino acid-binding proteins. Neuron 13:1345-1357

Swanson GT, Gereau RWt, Green T, Heinemann SF (1997) Identification of amino acid residues that control functional behavior in GluR5 and GluR6 kainate receptors, Neuron 19:913-926

Tedesco V, Roquet RF, DeMis J, Chiamulera C, Monfils MH (2014) Extinction, applied after retrieval of auditory fear memory, selectively increases zinc-finger protein 268 and phosphorylated ribosomal protein S6 expression in prefrontal cortex and lateral amygdala.Neurobiol Learn Mem. Nov;115:78-85

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci Apr;8(4):262-75

Tromp D, Dufour A, Lithfous S, Pebayle T, Després O (2015) Episodic memory in normal aging and Alzheimer disease: Insights from imaging and behavioral studies. Ageing Res Rev. Aug 28. pii: S1568-1637(15)30019-2

Tsai MC, Tanaka K, Overstreet-Wadiche L, Wadiche JI (2012) Neuronal glutamate transporters regulate glial excitatory transmission. J Neurosci. Feb 1;32(5):1528-35

Tulving E (2002) Episodic memory: from mind to brain. Annu Rev Psychol.;53:1-25

Uchida S, Shumyatsky GP (2015) Deceivingly dynamic: Learning-dependent changes in stathmin and microtubules. Neurobiol Learn Mem. Oct;124:52-61

Wadiche JI, Overstreet-Wadiche L (2015) New neurons don't talk back. Neuron. Jan 7;85(1):3-5. doi: 10.1016/j.neuron.2014.12.047

Waldeck-Weiermair M, Jean-Quartier C, Rost R, Khan MJ, Vishnu N, Bondarenko AI, Imamura H, Malli R, Graier WF (2011) Leucine zipper EF hand-containing transmembrane protein 1 (Letm1) and uncoupling proteins 2 and 3 (UCP2/3) contribute to two distinct mitochondrial Ca2+ uptake pathways. J Biol Chem. Aug 12;286(32):28444-55

Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B (1991) Structural determinants of ion flow through recombinant glutamate receptor channels. Science 252:1715-1718

Wang SH, de Oliveira Alvares L, Nader K (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nat Neurosci Jul;12(7):905-12

Wiltgen BJ, Zhou M, Cai Y, Balaji J, Karlsson MG, Parivash SN, Li W, Silva AJ (2010) The hippocampus plays a selective role in the retrieval of detailed contextual memories Curr Biol 20(15):1336-44

Wyllie DJ, Livesey MR, Hardingham GE (2013) Influence of GluN2 subunit identity on NMDA receptor function. Neuropharmacology Nov;74:4-17

Washburn MS, Numberger M, Zhang S, Dingledine R (1997) Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. J Neurosci 17:9393-9406

Wójtowicz T, Brzdąk P, Mozrzymas JW (2015) Diverse impact of acute and long-term extracellular proteolytic activity on plasticity of neuronal excitability. Front Cell Neurosci. Aug 10;9:313

Yu G, Song D, Berger TW (2014) Implementation of the excitatory entorhinal-dentate-CA3 topography in a large-scale computational model of the rat hippocampus. Conf Proc IEEE Eng Med Biol Soc. 2014:6581-4

Zhang H, Jacobs J (2015) Traveling Theta Waves in the Human Hippocampus. J Neurosci. Sep 9;35(36):12477-87

Zheng F, Erreger K, Low CM, Banke T, Lee CJ, Conn PJ, Traynelis SF (2001) Allosteric interaction between the amino terminal domain and the ligand binding domain of NR2A. Nat Neurosci 4:894-901

Zoghbi HY, Gage FH, Choi DW (2000) Neurobiology of disease. Curr Opin Neurobiol 10:655-660

Zelikowsky M, Bissiere S, Hast TA, Bennett RZ, Abdipranoto A, Vissel B, Fanselow MS (2013) Prefrontal microcircuit underlies contextual learning after hippocampal loss. Proc Natl Acad Sci U S A Jun 11;110(24):9938-43

Zelikowsky M, Hast TA, Bennett RZ, Merjanian M, Nocera NA, Ponnusamy R, Fanselow MS (2013) Cholinergic blockade frees fear extinction from its contextual dependency. Biol Psychiatry. 2013 Feb 15;73(4):345-52

Chapter III

Introduction to research work

3.1. **Introduction:**

New memories are in a labile state until they are *consolidated* (i.e., stabilized) over time (Dudai, 1996; Mc Gaugh, 2000). Although consolidated memories were initially thought to be permanent, recent evidence suggests retrieval of consolidated memories returns them to a transient labile state, after which they are *reconsolidated* (Kida, 2014; Kim et al., 2010, 2011; Misanin et al., 1968; Nader et al., 2000; Nader and Hardt, 2009; Nader et al., 2010). Reconsolidation occurs over a period of approximately 6h in rodents (Krawczyk et al., 2015; Nader and Hardt 2009; Nader et al., 2000; Przybylawski et al., 1999; Suzuki et al., 200), and procedures that disrupt the reconsolidation process, (e.g., protein synthesis inhibitors, such as anisomycin) result in amnesia of retrieved information (Rodriguez-Ortiz et al., 2008). Furthermore, new information acquired during the reconsolidation period can permanently alter (or update) retrieved memories (Monfils et al., 2009; Rose and McGlynn, 1997; Zelikowsky et al., 2013, 2013).

Much is known about the cellular processes underlying reconsolidation, specifically the involvement of protein synthesis, early gene expression, and glutamate receptor trafficking in the hippocampus (Matsuo et al., 2008; Rao-Ruiz et al., 2011; Rumpel et al., 2005; Schafe et al., 2001). However, the time course of these processes during reconsolidation and their effects on synaptic function are not well understood. Rao-Ruiz et al. (2011) suggested that memory retrieval results in time-dependent changes in glutamate receptor expression and function in the hippocampus. Specifically, retrieval of memory appears to trigger a biphasic wave of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receprtors). Rao-Ruiz et al.

observed that synaptic levels of the GluA1, GluA2, and GluA3 subunits of AMPA receptors were reduced 1h after memory retrieval. Four hours after memory retrieval (during reconsolidation) GluA1 expression was normalized, and 7h after retrieval (after reconsolidation), GluA2 expression was increased. The N-Methyl-D-Aspartate receptors (NMDA receptors) also appear to be crucial for memory reconsolidation, playing a relevant role in memory destabilization (GluN2B-NMDA receptors) and restabilization (GluN2A-NMDA receptors; Milton et al., 2013). The effect of this pattern of AMPA and NMDA receptor activity on synaptic plasticity during reconsolidation is still not clear. Glutamate receptors play a crucial role in synaptic strengthening, including the processes of long-term potentiation (LTP; Fonseca et al., 2006; Schmitt et al., 2005) and long-term depression (LTD; Bear and Abraham, 1996). LTP/LTD are established cellular models of changes in synaptic plasticity (Collingridge et al., 2010; Lynch, 2004); however, little is known about changes in such plasticity during the reconsolidation period. LTP after retrieval appears to be insensitive to protein synthesis inhibitors unless re-stimulated (Fonseca et al., 2006), and some memory "updating" manipulations lead to decreased expression of AMPA receptors and increased expression of NMDARs (Clem and Huganir, 2010), both of which are also observed during LTD.

From this limited information, it can be hypothesized that memory retrieval induces sensitive periods of plasticity, the outcome of which should be dependent on stimulation occurring during the reconsolidation period. The purpose of this research was to investigate the changes in synaptic plasticity that occur during memory reconsolidation in the rodent hippocampus, as well as the relationship between these plasticity changes and glutamate receptor expression. This relationship was confirmed by manipulating glutamate receptor expression (antagonism of GluN2B and inhibition of GluA2 endocytosis) during the initial portion of the

reconsolidation period. This strategy should provide insight on the mechanisms underlying synaptic plasticity changes during memory reconsolidation.

Chapter IV

Materials and Methods

4.1. **Fear conditioning and behavioral assessment of fear memory**

The subjects were outbred, male, Sprague-Dawley rats (2-4 months of age, Charles River Laboratories, Wilmington, MA). Animals were housed in pairs in a vivarium maintained at a constant temperature of 22.5°C, and a 12h:12h light:dark cycle (lights on at 6:00 am). All live animal procedures were approved by the Auburn University Animal Care and Use Committee (IACUC), and animals were euthanized (in a CO2 chamber) in accordance with the American Veterinary Medical Association (AVMA) Panel on Euthanasia regulations.

4.2. **Reconsolidation**

All behavioral manipulations were conducted in standard rat operant chambers (MED Associates, St. Albans City, VT), housed in sound-isolation cubicles. The chambers' grid floors could be electrified to deliver a foot-shock. The walls of the chambers were made of clear polypropylene, covered with a white screen. A speaker could be used to produce a background sound (click train, approx. 73 dB A-scale), and a house light was used to dimly illuminate the chamber. The distinctiveness of the chamber was enhanced with a scent cue (1 ml of undiluted PineSol^{TM}) placed in a small plastic cup outside the animal enclosure.

Animals were trained with a *conditioned freezing* protocol. All animals were acclimated to the chamber (hereon, the *context*) the day prior to initiation of fear conditioning. During fear conditioning, animals in the Retrieval (Rtv) group were placed in the context, and 180s later, a 2-s, 0.75-mA foot-shock was delivered. Animals were removed from the context 30s after foot-shock delivery. Control animals received the same treatment, but experienced no shock in the context; thus, although a memory of having been in the context should be activated, this memory

should not be associative. A further set of animals received conditioning in a context different from the test context (white coverings, background sound, and odor cue were not present); thus, these animals had experience with shock but not associated to the target context. The data from these latter group of subjects were contrasted against those of the Control group to determine the validity of the Control. During the retrieval session (24h later), animals were returned to the training context and exposed to all contextual (i.e., environmental) stimuli, but not shock, for 180s. This exposure constituted the retrieval manipulation. Animals recalling shock delivery 24h prior were expected to display freezing, a species-specific defensive behavior to impending threat. Freezing behavior was defined as the absence of all movements, except those related to respiration. Freezing behavior during the retrieval session was scored by at least 2 individual observers, blind to the condition to which each animal had been assigned. The 180-s test period was divided into 36 X 5-s intervals, and animal behavior was quantified by assigning a score of freezing or no freezing to each interval. After the retrieval session, subjects were randomly divided into 3 groups and euthanized after 1, 4, or 6h (yielding the Rtv-1h, Rtv-4h, and Rtv-6h conditions, respectively). Control subjects were also euthanized at 1, 4, or 6h. There were no differences between control subjects based on time to euthanasia, and the data from all control subjects were pooled together for data analysis. Animals that received conditioning in a second context were euthanized at the same time points as the previous two groups. Once again, differences were not observed based on time to euthanasia, and the data from these subjects was pooled together for data analyses yielding the Context condition.

4.3. **Tissue homogenization**

Immediately following euthanasia, rat brains were dissected, and whole hippocampi and cerebella were separated with continuous washing in ice-cold PBS (P4417-100TAB, 5 tabs in 1

L DI Water, pH = 7.4, Sigma Aldrich, St. Louis, MO) and stored at -80°C for protein extraction. Brain tissue from 5 animals in each condition was homogenized in Cell Lysis Buffer (9803-10X, Cell Signaling, Danvers, MA) containing 1X PMSF (made from a 200X solution, P7626, Sigma Aldrich, St. Louis, MO) and pooled together for analyses. Protein estimation was conducted using BSA standards (Thermo Fisher Scientific, Waltham, MA) for input control before beginning Immunoprecipitation experiments. Each experiment was replicated three times, and 300 µg of total protein was used in each replicate.

4.4. **PSD-95 fraction pull down assays**

Post Synaptic Density (PSD 95) is a major scaffolding protein responsible for postsynaptic glutamate receptor expression. PSD-95 fractions were pulled down using Pure-Proteome A/G Magnetic Beads (Cat No# LSKAMAGAG10, Millipore, Billerica, MA) and vendor-supplied direct IP protocol (http://www.emdmillipore.com/US/en/product/PureProteome%E2%84%A2-Magnetic-Beads, MM_NF-C77625). Briefly, 40 µl magnetic beads were washed with 500 µl of 1X IMP buffer (pH = 7.4) and incubated with 10 µl PSD-95 primary antibody (1:10, Santa-Cruz cat# Dallas, TX), used to capture and precipitate PSD fraction from the tissue homogenate. The immmunoprecipitated fraction was purified by washing it several times with 1X IMP buffer (pH=7.4). Finally, beads were boiled in 50 µl Laemmli Buffer at 80°C and quantified for protein content (BSA, Thermo Scientific, Waltham, MA) before being loaded in equal amounts (50 µl) for western blots.

4.5. **Immunoblotting**

Immmunoprecipitated PSD-95 fraction was immunoblotted with PSD-95 rabbit primary antibody (1:1000, Santa-Cruz, Dallas, TX). Equal amounts of samples were loaded on to an SDS PAGE gel to probe for the presence of PSD-95, as well as its with GluA1, GluA2, GluN2A and

GluN2B receptor subunits using rabbit primary antibodies (1:1000, Cell Signaling, Danvers, MA). All blots were probed with Dy-Light conjugated 550 anti-rabbit secondary or HRP conjugated antibody (Catalog # 84541, 1:10000, Thermo Scientific, Waltham, MA) using a Fuji FLA 5100 scanner, Nikon Film Scanner or an Alpha Innotech Image system.

4.6. mRNA isolation and Quantitative PCR

Total RNA was isolated from hippocampal tissue samples (pooled from 5 animals per group) using Trizol reagent, and following protocol from Life Technologies (Carlsbad, CA). RNA concentration was quantified using Nanodrop (Thermo Scientific, Waltham, MA). Approximately 1 µg of RNA was used for Reverse Transcription PCR (RT PCR), using Biorad iScript cDNA Kit (Hercules, CA) and following manufacturer's protocol to convert mRNA to cDNA. Approximately 100 ng of mRNA was used for quantitative PCR (qPCR) assay using Biorad SYBR green mix (Hercules, CA). Three qPCR cycles were used to measure the mRNA transcript (1) 95°C/.3min, (2) 95°C/.05min, 60°C/.1min, repeated 40X, and (3) 60°C/.1min, repeated 71X. The primers were designed using the standard IDT primer design software. The list of primer sequences used were as follows. For GluN2B, the forward sequence was 5' TCTGTCCAC CATTCCTGTTCCCAT 3', and the reverse sequence was 5' AAAGCCTCGCTCA AAGTGAATCGC 3'. For GluA2, the forward sequence was 5' CGGGTAGGGATGGTTCAGTTT 3', and the reverse sequence was 5' TGGCTACCTCCAAATTGTCGAT 3'. For GluA1, the forward sequence was CAACAATCACAGGAACA TGCGGCT 3', and the reverse sequence was 5' TGGAGAACTGG GAACAGAAA CGGT 3'. Finally, for GAPDH, the forward sequence was5' TGTGATGGGTGTGAAC CACGAGAA 3', and the reverse sequence was 5' CATGAGCCCT TCCACAA TGCCAAA 3'.

4.7. **Systemic Injection of Drugs and Behavioral Assessment**

Receptor levels were manipulated to determine their role on the observed synaptic plasticity patterns recorded during the reconsolidation period. The GluA2 endocytosis blocker HIV TAT-fused GluA2-derived C-terminal peptide (TAT-GluA2-3Y, peptide sequence: YGRKKRRQR RRYKEGYNVYG, Ana Spec, Freemont, CA, Cat# 64429, http://www.anaspec.com/products/product.asp?id=51747) was dissolved in autoclaved water and administered intravenously (i.v.) via the lateral tail vein at a concentration of 1.5nM/gm (Dias et al., 2012). The efficacy of the peptide was assessed using a scrambled control peptide, in which the Tyrosine residues were mutated with Alanines (TAT-GluA2-3A; peptide sequence: YGRKKRRQRRRAKEGANVAG, Ana Spec, Freemont, CA, Cat# 64984, http://www.anaspec.com/products/product.asp?id=53474). The GluN2B specific antagonist Ifenprodil (ab120111, Abcam, San Francisco, CA) was administered intraperitoneally (i.p.) at a dose of 20 mg/kg and dissolved in DMSO (Clem and Huganir, 2010). A group of subjects scheduled for behavioral experiments received either autoclaved water (i.v.) or DMSO (i.p.) to provide a vehicle control. All drugs or vehicle were administered 30 min prior to the retrieval manipulation.

The effects of blocking GluA2 endocytosis and antagonizing GluN2B on memory retrieval were assessed behaviorally as follows. Animals received conditioning as described above. On the retrieval day, they received their scheduled drug or vehicle 30 min prior to retrieval and, 5 min following retrieval, they received a single i.p. dose of Anisomycin (50 mg/kg, s.c., Sigma Aldrich, St. Louis, MO; Ortega et. Al., 2014, Sorg et al., 2015; Mac Callum et al., 2014; Kwapis et al., 2011) or vehicle (DMSO, Sigma Aldrich, St. Louis, MO). Animals were

then returned to their home cages and memory of the conditioning experience was again assessed after a 3d retention interval. Anisomycin is a protein synthesis inhibitor known to disrupt memory reconsolidation on memories destabilized by retrieval (Lee et al., 2008; Rao-Ruiz et al., 2011; Suzuki et al., 2004), resulting in disruption of recall of the memory reactivated prior to its administration. Thus, animals receiving Anisomycin were expected to exhibit less fear at the 3 d test (representing failure to remember the shock experience). However, fear was expected in animals in which alterations of the expected pattern of receptor expression during the initial part of reconsolidation was prevented by administration of Ifenprodil (antagonism of GluN2B should prevent memory destabilization) or TAT-GluA2-3Y (blocking GluA2 endocytosis should maintain synaptic GluA2 subunit expression during the initial portion of the reconsolidation period).

4.8. **Preparation of hippocampal slices**

Transverse hippocampal slices (350uM) were sectioned using a Vibrotome series 1000 or Leica VT-1200S (Parameshwaran et al., 2007). Briefly, slices were sectioned while submerged in high sucrose cutting solution (in mM: 85 NaCl, 2.5 KCl, 4 $MgSO_4$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 ascorbate, and 2 kynurenic acid) maintained at 0-4C. After sectioning was completed, the slices were incubated for 1hr in artificial Cerebrospinal Fluid (aCSF in mM: 119 NaCl, 2.5 KCl, 1.3 MgSO4, 2.5 CaCl2, 1 NaH2PO4, 26 NaHCO3 and 11 dextrose). All solutions were bubbled with 95%CO2/5%O2 carbogen. Details of solution composition and procedures are described elsewhere (Parameshwaran et al., 2007, 2013).

4.9. **Long Term Potentiation and Long Term Depression**

Following incubation, electrophysiological recordings were performed in a submerged recording chambers with continuous perfusion with aCSF (2-3ml/min) bubbled with

95%CO2/5%O2 carbogen, maintained at room temperature (25C). Field excitatory postsynaptic potentials (fEPSPs) from Schaffer Collateral pathways SC-CA1 synapses with a glass pipette filled with aCSF (2-4MΩ). Stimulating pulses were applied at Schaffer collaterals via a bipolar stimulating electrode positioned 300 µm closer to CA3 subfield than recording electrode. Frequency of test stimulation was 0.33Hz (every 20 sec). After placing stimulating and recording electrodes in the CA3 and CA1 regions of the Schaffer Collateral (SC) commissural pathway of the hippocampal slices, stimulus intensity was lowered to the point where the field Excitatory Post-Synaptic Potential disappeared (fEPSP) completely disappeared leaving the stimulus artifact intact. For stimulus response curves current intensity was altered from 0-300µA at steps of 25µA. For LTP and LTD experiments, baseline was recorded at 50% of amplitude at which initial population spike appeared. LTP was induced after 10 min of stable baseline recording using Theta Burst Stimulation protocol (10 bursts of stimuli, each of four pulses at 100 Hz, 200 ms, and 20 s intervals between individual TBS), and recording was continued till 50-60 min post TBS. Separately, fEPSP was recorded for 50-60 min without inducing any protocol to verify that baseline was stable (for both LTP and LTD recordings). LTD was induced using two low frequency stimuli (LFS: 900 pulses at 1 Hz) delivered at an interval of 10 min and preceded by 15 min of stable baseline. Stimulation intensity while recording baseline, in between two trains of LFS, and immediately after induction were set to 30% of maximal fEPSPs. The stimulation intensity was set to 50% when 1 Hz LFS trains were delivered. Within and Between Train Analysis (WTF and BTF respectively) and Slow Component Decay Analysis (SCDA) were performed using amplitudes from Theta Burst Stimulation induction period. Consecutive pulse amplitudes (in different bursts or sweeps) in WTF or BTF analysis were normalized with the first pulse amplitude of the trains used. For SCDA, amplitude of pulses 80-180 s after first pulse were

used for 10 bursts and 5 sweeps (slow component, fourth pulse in each burst) and expressed as percentage of the GluA mediated first fast pulse amplitude of the first train. (Kochlamazashvili et al., 2014).

4.10. **Statistical Analysis**

All data were analyzed using analyses of variance (ANOVA), either as one-way analyses or factorial analyses as detailed in the results section. All significant omnibus tests were followed by Tukey post hoc comparisons. Nonsignificant effects are reported where appropriate.

Chapter V

Results

5.1. **Post-retrieval temporal changes in freezing behavior**

Figure 5.1A presents the experimental model used and the points of intervention post-retrieval. Animals in the Rtv condition, which experienced shock in the test context, exhibited more freezing than animals in the Control condition (n=9), which experienced the test context but no shock during the conditioning day, $F_{1,74} = 41.08$, $p < .001$. Freezing levels in the Control group were indistinguishable from freezing levels in the group that received conditioning in a different context (No-Rtv group, n=9), $F_{1,31} < 1$, confirming the validity of our Control treatment (Figure 5.1B). Animals were euthanized 1, 4, or 6h after retrieval and freezing was assessed. As expected, the differences in amount of freezing observed based on whether or not subjects had experienced shock during conditioning, $F_{1,70} = 40.74$, $p < .001$, were not related to the group (1h, 4h, or 6h, n=14-18 per time point) to which the subject was assigned after the retrieval trial, $F_{2,70} = 1.16$, and there was no interaction between these two factors. $F_{2,70} < 1$.

5.2. **Temporal changes in synaptic GluA1, GluA2, and GluN2B receptor expression during reconsolidation**

Biphasic waves of synaptic GluA1 and GluA2 subunits mediate recall of memory at 1, 4 and 6 h post retrieval in the dorsal hippocampus (Rao-Ruiz et al., 2011, 2015). However, synaptic receptor expression can be assessed through multiple procedures, including surface receptor quantification, synaptosomal studies and PSD-95 interaction. Since synaptic receptors are activated more significantly when they interact with the scaffolding-PSD complex and PSD-associated receptor expression levels may vary during the reconsolidation process, hippocampal

synaptic glutamate receptor expression was assessed by pulling down PSD-95 fraction 1, 4 and 6 h after retrieval (Conditions Rtv-1h, Rtv-4h, and Rtv-6h). With this strategy, surface expression levels exclude receptors from the extrasynaptic zones, (Feng et al., 2011; Hammond et al., 2006; Hrabetova et al., 2000; Kochlamazashvili et al., 2010; Petralia et al., 2010; Simon et al., 2014). PSD-95 fractions from the hippocampus were immunoprecipitated and immunoblotted for GluA1, GluA2, GluN2A and GluN2B receptor subunits in the Rtv-1, Rtv-4, and Rtv-6 conditions. Our data suggest that reconsolidation of memory causes altered interaction of AMPA and NMDA receptor subunits with PSD-95 in a pattern that is specific to the hippocampus but not other areas (e.g., the cerebellum; Figure 5.2). These data were compared against no shock control animals (the Control condition) at equivalent points after retrieval. To provide control for the region-specific nature of observed changes in receptor interaction, samples were also obtained from the cerebellum. Immunoprecipitation of PSD-95 and associated GluA1, GluA2, and GluN2B subunits showed marked alterations throughout the reconsolidation period in the hippocampus, whereas no significant changes were observed in cerebellum (Figure 5.2A, IgG control shown in Figure 5.2B). In comparison to the Control, GluA1 receptor interaction with PSD-95 changed as reconsolidation progressed, $F_{3,9} = 3.90$, $p < .05$. As compared to the Control, GluA1 expression was decreased in Rtv-1h, $p < .05$. However, expression increased to become equivalent to Control in Rtv-4h and Rtv-6h, ps $> .33$ (Figure 5.2D, n=5, immunoblotted 3-4 times). GluA2 receptor expression was similarly impaired through reconsolidation, $F_{3,8} = 54.83$, $p < .001$. GluA2 receptor expression in Rtv-1h was below Control, Rtv-4h, and Rtv-6h levels, ps $< .05$, .05, and .001, respectively. GluA2 expression was equivalent to Control in Rtv-4h, $p = .98$, and above Control levels in Rtv-6h, $p < .001$ (Figure 5.2C, n=5, immunoblotted 3-4 times). This pattern of AMPA receptor expression suggests that synaptic plasticity might be altered

during the initial period of memory reconsolidation. Normal levels of GluN2B receptors are also critical for stabilization of glutamatergic synapses in the hippocampus (Kim et al., 2011), and their interaction with PSD-95 was altered through the reconsolidation period, $F_{3,8} = 5.09$, $p < .05$. Indeed, GluN2B interaction with PSD-95 increased in the hippocampus in Rtv-1h, $p < .05$, was marginally higher than Control in Rtv-4h, $p = .06$, and returned to Control levels in Rtv-6h, $p > .26$ (Figure 5.2F, n=5, immunoblotted 3-4 times). In contrast, GluN2A receptor interaction with PSD-95 was not altered through the reconsolidation period, $F_{3,8} = 1.63$, $p > .25$ (Figure 5.2E, n=5, immunoblotted 3-4 times). Importantly, none of these changes (patterns of change) were observed in the immunoblotting experiments performed with homogenates from the cerebellum (Figure 5.2A). Thus, our data suggest that retrieval of a fear memory induces distinct patterns of GluA1, GluA2, and GluN2B receptor interaction with PSD-95 in the hippocampus.

5.3. **Basal synaptic transmission is altered during the reconsolidation period**

The activity of GluA1 and GluA2 receptors is essential for proper synaptic transmission; thus, it is likely that altered synaptic interaction of these receptor subunits with PSD-95 leads to altered synaptic transmission during reconsolidation. Stimulus-response experiments were conducted at 1, 4, and 6h after retrieval to assess how basal synaptic transmission is affected due to the observed alterations in glutamate receptor expression. All groups were sensitive to changes in stimulus intensity, $F_{6,48} = 66.59$, $p < .001$, and intensity interacted with time since retrieval, $F_{18,48} = 2.24$, $p < .05$ (Figure 5.3, n=3). This interaction reflects the observation that basal synaptic transmission was impaired in Rtv-1h compared to the remaining groups, which did not differ from each other, suggesting that synaptic communication is compromised in the early stages of reconsolidation.

5.4. **Synaptic plasticity follows the biphasic wave of Glutamate receptor expression observed during reconsolidation**

The interaction of post-synaptic GluA1, GluA2, and GluN2B receptors with the PSD-95 scaffolding complex is important for their activity and stabilization at the surface of the neurons. Inhibition of this interaction can lead to altered synaptic physiology and plasticity (Gomperts, 1996; Niethammer et al., 1996). In our model, the interaction between glutamate receptors and the PSD complex changed as a function of time since retrieval, and we anticipated that synaptic plasticity would change accordingly. Changes in LTP in the SC CA3-CA1 pathway of the hippocampus were investigated at 1-2, 4-5, and 6-7 h after retrieval of the conditioned fear memory, measuring 55-60 min post induction using TBS, and compared against Control and No-Rtv. The average fEPSP slope, computed as a percentage of the baseline, differed based on time since memory retrieval, $F_{4,23} = 28.78$, $p < .001$. Post-hoc analyses confirmed that animals in the Rtv-1h group exhibited impaired LTP (<20%) as compared to all other groups, ps < .005 (Figures 5.4A and 5.4C, n=5). LTP recovered over time; the Rtv-4h group did not differ from Control and No-Rtv groups (>50%), ps > .10; however, Rtv-4h still differed from the Rtv-6h group, $p < .001$. Indeed, the Rtv-6h group exhibited higher maintenance of LTP (>80%; Figures 5.4A and 5.4C) than all other groups, ps < .005. This pattern of time-dependent recovery of LTP reflects the biphasic wave of GluA2 receptor expression observed, in which GluA2 receptor interaction with PSD-95 increased to a level above control 6h after retrieval.

Overexpression of GluN2B receptors is implicated in induction of LTD and hence altered synaptic plasticity (Dalton et al., 2012; Huganir et al., 2010; Shipton and Paulsen, 2013). GluN2B receptor interaction with PSD-95 increased during the initial reconsolidation period (Figure 5.2A, 5.2F). LTP was significantly altered during the reconsolidation period, but the

observed LTP patterns do not provide information on whether such plasticity changes are unidirectional or bidirectional. Thus, low frequency-mediated LTD was induced in hippocampal slices to test the nature of plasticity during reconsolidation. This protocol revealed changes in LTD as a function of time since retrieval, $F_{4,17} = 17.05$, $p < .001$ (Figures 5.4B and 5.4D, n=5). Tukey post-hoc comparisons revealed a long-term reduction of the fEPSP slope in the Rtv-1h group (>30%), as compared to the Control (<30%), No-Rtv (<30%) and Rtv-6h (approx. 10%) groups, *p*s < .05. fEPSP slopes gradually approached control levels 4h after retrieval (Rtv-4h did not differ from Rtv-1h, Control, or No-Rtv, *p*s > .17) and were well below control levels 6h after retrieval (Rtv-6h differed from all groups, *p*s < .05).

The observed time-dependent, biphasic wave of glutamate receptor expression resulted in consistent changes in LTP and LTD expression during the reconsolidation period. The decreased LTP and enhanced LTD observed shortly after memory retrieval suggest that the initial portion of the reconsolidation period is favorable for destabilization of memory and, possibly, unfavorable for new memory formation. As time progresses, recovery of LTP and reduction of LTD suggest that later portions of the reconsolidation period are favorable for stabilization of memory and new memory formation. Thus, altered synaptic plasticity (as reflected by the observed changes in LTP and LTD) may play an important role in memory modification and weakening during memory reconsolidation.

The LTP induction protocol used in our studies in CA1 synapses uses NMDA receptors during potentiation (Bashir et al., 1991; Carroll et al., 2002; Parameshwaran et al., 2012). Depolarization of the postsynaptic membrane is dependent on GluN2A receptor activity and is required to remove the voltage-dependent Mg^{2+} block of NMDA receptors, which is critical because the induction and expression of LTP depends on the strength of depolarization phase

during TBS (Bliss and Collingridge, 1993). LTP was impaired and GluN2B interaction with PSD-95 increased in Rtv-1h, but it was not clear whether strength of depolarization of the post synaptic membrane was altered. The amplitude of fEPSP of the first pulses of each burst within the TBS sweep was not altered significantly based on time since memory retrieval, $F_{3,8} < 1$, $p > .75$. Although fEPSP amplitude changed across bursts, $F_{8,88} = 10.23$, $p < .001$, these changes were not associated with time since memory retrieval, $F_{24,88} = 1.08$, $p > .38$ (Figures 5.5A and 5.5B, n=3). However, when the amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline, there was an effect of time since memory retrieval, $F_{3,9} = 7.73$, $p < .05$, and this effect was independent of sweep number, $F_{9,27} = 1.49$, $p > .20$ (Figure 5.5D, n=3). Potentiation in the Rtv-6h group was higher than in all other groups, $Fs_{1,9} > 6.17$, $ps < .05$, reflecting the observation that, although there were no significant differences in within train analysis, depolarization increased in the Rtv-6h group during the latter sweeps of TBS. Possibly, this increased depolarization during TBS contributes to the high level of LTP observed in the Rtv-6h group.

During the initial period of the induction phase, fEPSP amplitudes might have contributions from AMPA receptor-mediated fast responses. However, late (slow) components of the induction phase are mainly GluN2A driven (Kochlamazashvili et al., 2014). We observed that GluN2B interaction increases in the PSD-95-associated fraction of neurons in the hippocampus. To understand the contributions of GluN2A receptor subunits during potentiation, and hence maintenance of LTP (Bartlett et al., 2007), we analyzed the fEPSP amplitudes of slow components of potentiation (Buller et al., 1997; Hrabetova et al., 2000; Monyer et al., 1992, 1994; Wyllie et al., 2013). fEPSP amplitudes compared 80-180 s into the potentiation phase revealed a decrease across bursts within each sweep, $F_{9,81} = 5.78$, $p < .001$ (Figure 5.5C, n=3).

Furthermore, fEPSP amplitudes exhibited a gradual shift toward higher amplitudes across sweeps, $F_{4,36} = 9.42$, $p < .001$. However, there were no shifts in amplitude based on time since memory retrieval even though the Rtv-6h group was comparably higher in potentiation than all other groups, $F_{3,9} < 1$, $p > .85$ (all interactions fell short of statistical significance, all Fs < 1, all ps > .70). Overall, during the depolarization period (within the TBS protocol), there were no differences based on time since memory retrieval. This finding further suggests that GluN2A receptors did not directly contribute to the altered plasticity observed 1-6h after memory retrieval in our study.

5.5. **Transcription of glutamate receptors follow altered wave of surface expression during reconsolidation**

Surface expression and active population (associated with PSD-95) of GluA1, GluA2, and GluN2B receptors change during the reconsolidation period after memory retrieval in rats. This change can be due to altered trafficking and/or transcription; thus, we investigated mRNA transcription levels of GluA1, GluA2, and GluN2B receptors in the hippocampus. Quantitative PCR analysis shows a wave of transcription similar to expression patterns of GluA1, GluA2 and GluN2B receptors during the reconsolidation period. GluA1 and GluA2 receptor mRNA levels remained the same shortly after memory retrieval (Rtv-1h) and decreased from control levels as reconsolidation progressed (Rtv-4h). In the Rtv-6h group, GluA2 mRNA expression was higher than control levels, consistent with the observed expression of receptors (Table 1). However, despite of lower than control levels of GluA1 receptor interaction with PSD-95, mRNA expression at Rtv-1h was slightly higher than controls. These findings suggest that the initial down-regulation of surface expression of GluA1 and GluA2 receptors, their initial impaired interaction with PSD-95, their gradual recovery through the reconsolidation period, and

overexpression of GluA2 receptors during the final portion of reconsolidation might be a result of an initial decrease and subsequent normalization of receptor transcription. GluN2B transcription was higher in Rtv-1h than Rtv-4h and Rtv-6h which was in turn higher than control levels; Table 1). This observation is consistent with our observed increased GluN2B interaction with PSD-95 during reconsolidation, and suggest that surface expression and interaction of GluA1, GluA2, and GluN2B receptors are altered in two distinct waves during reconsolidation.

5.6. **Blocking GluN2B expression and GluA2 endocytosis attenuates the effects of Anisomycin**

GluA2 and GluN2B receptors appear to play a critical role on synaptic plasticity through the reconsolidation process. Thus, the effects of manipulating these receptor levels were assessed in terms of memory protection from the amnestic effects of the protein synthesis inhibitor Anisomycin, which if administered shortly after memory retrieval, decreases recall of the retrieved memory. However, if destabilization of memory is prevented, Anisomycin should have little impact on subsequent memory recall. One day after contextual fear conditioning, animals received either Anisomycin (Ani) or vehicle (Veh) 5 min after the memory retrieval session. For all animals, the retrieval session was preceded by administration of either GluA2 endocytosis blocker TAT-GluA2-3Y (TAT) or the GluN2 selective antagonist Ifenprodil (Ifenp) (Figure 6A, n=9). This resulted in four groups of subjects: Veh+Veh, Veh+Ani, TAT+Ani, and Ifenp+Ani (each group name represents the treatment they received 30 min prior to memory retrieval + the treatment they received 5 min following memory retrieval). Conditioned freezing was assessed again 3d later to ensure complete elimination of all antagonists.

There were differences in memory recall at the 3d test based upon the treatment received, $F_{3,17}$ = 3.88, p = .05. Planned pairwise comparisons revealed that Anisomycin effectively disrupted memory of the conditioning event, with the Veh+Ani group exhibiting less conditioned freezing than the Veh+Veh group, $F_{1,17}$ = 4.71, p < .05. Administration of TAT-GluA2-3Y and Ifenprodil ameliorated the amnestic effect of Anisomycin, making these groups equivalent to the Veh+Veh group, ps > .50, and different from the Veh+Ani group, Fs$_{1,17}$ = 9.37 and 7.26, ps < .01 and .05, respectively (Figure 5.6B, n=9).

5.7. **GluN2B receptor blockade alters LTD 1h after memory retrieval**

Our data and results from other laboratories strongly indicate that a retrieval-induced wave of AMPA and NMDA receptors is a likely physiological substrate for the lability of memory during reconsolidation. To investigate this assumption, we investigated whether manipulating receptor levels in our reconsolidation model would lead to altered LTP and LTD. Higher levels of GluN2B receptors are implicated in induction and maintenance of LTD; thus, we used the GluN2B selective antagonist Ifenprodil to better understand the role of GluN2B on synaptic plasticity during reconsolidation in an LTD induction experiment. Ifenprodil was administered 30 min prior to memory retrieval, and LTD was assessed 1h after retrieval. The main effect on LTD of administering the drug was significant, $F_{2,10}$ = 89.68, p < .001. Post-hoc Tukey tests revealed that LTD was decreased (>1% above baseline) compared to Control and Rtv-1h levels, ps < .001 (Figures 5.7A and 5.7B, n=5), and approached baseline during the maintenance phase. This confirms our earlier conclusion that GluN2B plays an important role in the depression of synaptic activity (i.e., enhanced LTD) triggered by memory retrieval and that GluN2B antagonism decreases LTD and, hence, prevents the destabilization of synapses that would be otherwise produced by memory retrieval. To measure whether antagonism of GluN2B

resulted in altered strength of post synaptic membrane depolarization, we analyzed fEPSP amplitudes within TBS components by comparing Ifenprodil vs. no drug controls assessed 1h after memory retrieval. fEPSP amplitude of the first pulses of each burst within the TBS sweep was not altered significantly by administration of Ifenprodil, all *F*s < 1.55, all *p*s > .48 (Figures 5.8A and 5.8B, n=3). This was also the case when the amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline, all *F*s < 1.83, all *p*s > .19 (Figure 5.8D, n=5). Analysis of the slow pulse of each burst revealed that fEPSP amplitudes decreased across bursts within each sweep, $F_{9,36} = 4.12$, $p < .005$, and exhibited a gradual shift toward higher amplitudes across sweeps, $F_{4,16} = 3.33$, $p < .05$. However, there were no shifts in amplitude based on drug treatment, and drug treatment did not interact with any of the other factors, all *F*s < 1.50, all *p*s > .28 (Figure 5.8C, n=3).

5.8. **Controlled Inhibition of GluA1 endocytosis increases LTP and decreases LTD during reconsolidation**

Memory retrieval appears to trigger a biphasic wave of GluA2 receptors in the hippocampus, and blockade of GluA2 endocytosis leads to memories that appear insensitive to manipulations during the reconsolidation period (Rao-Ruiz et al., 2011, 2015). Controlled GluA2 receptor endocytosis blockade increases miniature AMPA receptor-currents in reconsolidation in a mice 1h after retrieval (Rao-Ruiz et al., 2011), suggesting that such blockade increases potentiation in synapses. However, the physiological relevance of such intervention in terms of synaptic plasticity is not known. To investigate effect of GluA2 receptor manipulation on altered LTP and LTD (bidirectional plasticity) shortly after memory recall, we administered either TAT-GluA2-3Y (TAT-3Y, active peptide) or TAT-GluA2-3A (TAT-3A, scrambled control peptide) 30 min before retrieval. This should result in disrupted endocytosis of synaptic GluA2 receptors

during the initial portion of the reconsolidation period (Dias et al., 2012). LTP was assessed 1 and 4h after retrieval (Rtv-1h and Rtv-4h). LTP levels were dependent on time since retrieval, $F_{1,23}$ = 22.45, p < .001, and were also dependent on the compound administered (TAT-3Y, >40%; TAT-3A <20%; or no drug Rtv-1h, <20%), $F_{2,23}$ = 16.15, p < .001. More importantly, these two factors interacted to determine LTP levels, $F_{2,23}$ = 8.52, p < .005. Post-hoc Tukey tests revealed that LTP levels were significantly increased by TAT-GluA2-3Y 1h after retrieval, as compared to both Rtv-1hand TAT-GluA2-3A groups, ps < .001; there was, however, no change in LTP 4h after retrieval (>30% for TAT-3A and TAT-3Y), ps > .86 (Figures 5.9A and 5.9C, n>3). Other laboratories (e.g., Rao-Ruiz et al., 2011) have reported that disruption of GluA2 endocytosis after retrieval failed to alter miniature AMPAR currents after approximately 4h (Rao-Ruiz et al., 2011), suggesting that an initial decrease in GluA2 receptors is critical for the occurrence of reconsolidation. Our data further suggest that controlled inhibition of GluA2 endocytosis leads to improved LTP in the SC-CA1/CA3 commissural pathways during fear memory reconsolidation. We further investigated the effect of GluA2 endocytosis blockade on LTD expression. Blockade of GluA2 receptor endocytosis by TAT-GluA2-3Y attenuated LTD 1h after memory retrieval (<5%), $F_{2,9}$ = 91.07, p < .001, as compared to both TAT-GluA2-3A (<30%) and no drug Rtv-1h (<30%), ps < .001 (Figures 5.9B and 5.9D, n>3). Thus, interruption of the retrieval-induced wave of GluA2 receptors interferes with the otherwise occurring patterns of LTP and LTD, and could have important implications for memory destabilization after retrieval.

To further analyze whether blocking GluA2 endocytosis has an effect on membrane depolarization (via GluN2A expression) we performed train analyses after administration of the TAT-GluA2-3Y and TAT-GluA2-3A 1 and 4h after memory retrieval. fEPSP amplitude of the

first pulses of each burst changed across the first sweep, $F_{8,136} = 10.80$, $p < .001$, and based on time since memory retrieval, $F_{8,136} = 2.65$, $p < .01$. Furthermore, there was a 3-way interaction with drug administered, $F_{16,136} = 2.41$, $p < .005$ (all other Fs < 2.74, all ps > .10). This interaction was likely due to decreased amplitudes observed during the initial sweeps in the TAT-3A condition 4h after retrieval, which normalized to control levels in later sweeps (Figures 5.10A and 5.10B, n=3). When the amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline, no differences were observed across bursts or sweeps, all Fs < 1.83, all ps > .19 (Figure 5.10D, n=3). Analysis of the fourth slow pulse of each burst revealed that fEPSP amplitudes decreased across bursts within each sweep, $F_{9,36} = 4.12$, $p < .005$, and exhibited a gradual shift toward higher amplitudes across sweeps, $F_{4,16} = 3.33$, $p < .05$. However, there were no shifts in amplitude based on drug treatment, and drug treatment did not interact with any of the other factors, all Fs < 1.50, all ps > .28 (Figure 5.10C, n=3).

5.9. Figure legends:

Figure 5.1. A. Schematic design and timing of events. During conditioning, a 3-min context exposure (represented by small rectangles above the timeline) was (Retrieval and NoRetrieval) or was not (Control) paired with delivery of electric footshock (lightning symbol). Memory of the conditioning trial was assessed as percent time freezing in the conditioning context (Retrieval and Control) or a novel context (NoRetrieval) 24h later in a second 3-min session. Animals were then euthanized for biochemical and electrophysiological experiments 1, 4, or 6h after retrieval (upward arrows). Tick marks represent 30-min intervals. The shaded area represents the progression of reconsolidation, from highest destabilization of memory (darker portion) to memory restabilization (lighter portion). **B.** Freezing scores expressed as percent freezing during retrieval of memory of the conditioning experience, which involved pairings of the context with footshock (Retrieval, Rtv, *n*s = 14-18), exposure to the context (Control, *n*s = 9), or conditioning in a context different from the context of Retrieval (NoRtv, *n*s =9). Freezing was observed only in the Rtv condition, $F_{1,\ 74} = 41.08$. Asterisks (** represents $p<0.01$) represent post-hoc comparisons (Tukey's test) against control.

Figure 5.2. Immunoblotting of precipitated PSD-95 fraction shows the biphasic pattern of interaction of the target receptors at the postsynaptic density. **A**. Immunoblots indicate altered synaptic receptor interaction with PSD-95 at different time points in Hippocampus (area of interest) and Cerebellum (control area to show that the changes are not global) compared to control groups. **B**. Control and IgG samples were probed with PSD-95 specific antibody, used as negative control. Numbers on left side of each panel indicate the molecular weights of each probed protein. Each group had an *n*=5 (pooled together), and was ran on SDS-PAGE gel >3

times. Samples were loaded in different orders each time to avoid bias. **C-F.** Percent ratio of expressed protein (GluA2, GluA1, GluN2A, and GluN2B for Panels C, D, E, and F, respectively) *vs*. PSD-95. All data are presented as mean ± SEM. Asterisks (*) represent differences from Control, hashtags (#) represent differences from Rtv-6h. * = $p < .05$, ** = $p < .01$, *** = $p < .005$, **** = $p < .001$ (equivalent representation of statistical significance for comparisons against Rtv-6h).

Figure 5.3. Basal synaptic transmission recorded from CA3-CA1 pathway of the hippocampus was impaired shortly after retrieval (Rtv-1h), and was normalized to Control levels at 4 and 6h after retrieval (Rtv-4h and Rtv-6h). Slopes were calculated from fEPSPs generated from *n*= 3 animals for each group. Data are presented as mean ± SEM (brackets). A Group X Stimulus Intensity ANOVA revealed a main effect of stimulus intensity, $F_{6,48} = 66.59$, $p < .001$, and an interaction, $F_{18,48} = 2.24$, $p < .05$. Traces shown are calibrated at a 5mV/50ms scale.

Figure 5.4. LTP and LTD from CA3-CA1 pathway of the hippocampus during reconsolidation. **A.** Compared to Controls receiving no shock during conditioning (which did not differ from subjects receiving shock in a context different from the context of retrieval, No-Rtv), TBS-induced LTP was impaired 1h after retrieval (Rtv-1h, *n*=5), returned to control levels 4h after retrieval (Rtv-4h, *n*=5), and was expressed significantly above controls 6h after retrieval (Rtv-6h, *n*=5). Representative traces were collected before and after LTP induction (within first 5 min). Baseline was recorded for 55-60 min without induction to verify stability of fEPSP slope recording (n=8). **B.** Dual-LFS induced LTD was increased 1h after retrieval (Rtv-1h, *n*=5), returned to control levels 4h after retrieval (Ret-4h, *n*=5), and decreased to near baseline levels

6h after retrieval (Rtv-6h, n=5). Representative traces were obtained as described for LTP. Recordings were conducted 55-60 min post induction. **C and D.** Normalized fEPSP slopes obtained from the last 5 min of recording for LTP and LTD, respectively. All data are presented as mean ± SEM. Asterisks (*) represent differences from control, hashtags (#) differences from Rtv-6h, and crosses (+) differences from No-Rtv. * = $p < .05$, ** = $p < .01$, *** = $p < .005$, **** = $p < .001$ (equivalent representation of statistical significance for comparisons against Rtv-6h and No-Rtv).

Figure 5.5. A. Traces are a representation of TBS sweep (top), the control (middle) and Rtv-6h (bottom) groups. **B.** Within Train Facilitation. TBS sweep was not altered significantly based on time since memory retrieval, $F_{3,8} < 1$, $p > .75$. fEPSP amplitude changed across bursts, $F_{8,88} = 10.23$, $p < .001$, but these changes were not associated with time since memory retrieval, $F_{24,88} = 1.08$, $p > .38$. **C.** Slow component decay assay. fEPSP amplitudes decreased across bursts within each sweep, $F_{9,81} = 5.78$, $p < .001$, but there was no interaction among groups (SEMs omitted for clarity). **D.** Amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline. There was a main effect of time since memory retrieval, $F_{3,9} = 7.73$, $p < .05$, and this effect was independent of sweep number, $F_{9,27} = 1.49$, $p > .20$. There was higher potentiation in the Rtv-6h group, compared to all other groups, $Fs_{1,9} > 6.17$, $ps < .05$. Groups Rtv-1h and Rtv-4h did not differ from controls. Data are presented as mean ± SEM (brackets), and all data points represent an n=3.

Figure 5.6. A. Schematic design and timing of events. During conditioning, a 3-min context exposure (represented by small rectangles above the timeline) was paired with delivery of

electric footshock (lightning symbol). Memory retrieval was achieved by exposing the animals to the conditioning context 24h later in a second 3-min session. Animals received vehicle (Veh), TAT-GluA2-3Y (TAT), or Ifenprodil 30 min prior to retrieval, and vehicle or Anisomycin (Ani) 5 min after retrieval (*n*s = 9). Memory was again assessed 3 d later. **B.** Shows drug treatment determining freezing scores during memory assessment, $p = .05$. Anisomycin resulted in decreased retrieval of the conditioning memory (Veh+Ani *vs.* Veh+Veh, $p < .05$), showing Anisomycin's amnesic effect. TAT-GluA2-3Y and Ifenprodil ameliorated the amnestic effect of Anisomycin, making these groups equivalent to the Veh+Veh group, *p*s > .50, and different from the Veh+Ani group, *p*s < .01 and .05, respectively. Data are presented as mean ± SEM.* = $p < .05$, ** = $p < .01$ (equivalent representation of statistical significance for comparisons against Veh+Ani group).

Figure 5.7. LTD recorded from CA3-CA1 pathway of the hippocampus 1h after memory retrieval after Ifenprodil treatment (Ifenprodil-1h) or no drug treatment (Rtv-1h). **A.** No significant overall differences were noted in the depolarization envelope, but dual-LFS induced LTD was decreased to baseline levels after Ifenprodil treatment (*n*s =5). Representative traces were collected before and after LTD induction (within first 5 min). Recordings were conducted 55-60 min post induction. Normalized fEPSP slopes obtained from the last 5 min of recording and confirmed the effect of Ifenprodil on LTD, $p < .001$. The Ifenprodil group differed from both the Rtv-1h and Control groups, Tukey post-hoc *p*s < .005. All data are presented as mean ± SEM. (*) asterisks represent differences from Control, (#) hashtags represent differences from Ifenprodil-1h. *** = $p < .005$, **** = $p < .001$ (equivalent representation of statistical significance for comparisons against Rtv-Ifenprodil-1h).

Figure 5.8. A. Traces are a representation of TBS sweep (top), the Rtv-1h (middle) and Ifenprodil (bottom) groups. **B.** Within Train Facilitation. TBS sweep was not altered significantly based on administration of Ifenprodil, all *p*s > .48. **C.** Slow component decay assay. fEPSP amplitudes decreased across bursts within each sweep, $p < .005$, and there was a gradual shift toward higher amplitudes across sweeps, $p < .05$. However, there were no shifts in amplitude based on drug status, and drug status did not interact with any of the other factors, all *p*s > .28. **D.** Amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline, and they were equivalent across groups, all *p*s > .19. Data are presented as mean ± SEM (brackets), and all data points represent $n=3$.

Figure 5.9. LTP and LTD recorded from CA3-CA1 pathway of the hippocampus 1h after memory retrieval after inhibition of GluA2 endocytosis or control treatment. **A.** As compared to Rtv-1h, TBS-induced normalized fEPSP increased in the TAT-3Y 1h group; however, LTP was near Rtv-4h levels in the TAT-3Y 4h (Rtv-1h and Rtv-4h or an inactive, scrambled peptide [TAT-3A 1h and TAT-3Y 4h] were used as controls in this experiment). Representative traces were collected before and after LTP induction (within first 5 min), smooth traces are before induction, while rough traces are after induction. **B.** Dual-LFS induced LTD was at Rtv-1h levels 1h after administration of TAT-3A and decreased to baseline levels after administration of TAT-3Y. Representative traces were obtained as described for LTP. Recordings were conducted 55-60 min post induction. **C and D.** Normalized fEPSP slopes obtained from the last 5 min of recording for LTP and LTD, respectively. LTP level was determined by time since retrieval, and compound (TAT-3Y, TAT-3A, or no drug Rtv-1h control). Importantly, these factors recorded

from CA3-CA1 pathway of the hippocampus 1h after memory retrieval to determine LTP levels, ***$p < .005$ All data are presented as mean ± SEM (SEMs were omitted in **A** for clarity). For all measures, $n>3$. (*) asterisks represent differences from Rtv-1h, (#) hashtags represent differences from TAT-3Y. *** = $p < .005$ (equivalent representation of statistical significance for comparisons against Rtv-1h and TAT-3Y).

Figure 5.10. A. Traces show representation of TBS sweep (top) and the TAT-3Y 1h (middle) and TAT-3A 1h (bottom) groups. **B.** within Train Facilitation. fEPSP amplitude of the first pulse of each burst changed across the first sweep, $p < .001$, and based on time since memory retrieval, $p < .01$, interacted with drug administered, $p < .005$**C.** Slow component decay assay. fEPSP amplitudes decreased across bursts within each sweep, $p < .005$, with gradual shift toward higher amplitudes across sweeps, $p < .05$, but no shifts in amplitude based on drug status, nor interactions between drug status and any other factor, ps > .28. Amplitudes of the first fEPSP elicited in each TBS sweep were normalized using the first amplitude of the first sweep as baseline, with no significant effects or interactions; all ps > .19. Data are presented as means ± SEM (brackets). For all data points, n=3.

Table 5.1. Delta-Delta Ct values and their standard deviations along with Fold change values for the three target genes, GluN2B, GluA1 and GluA2, as expressed 1, 4 and 6h after retrieval (Groups Rtv-1h, Rtv-4h, and Rtv-6h, respectively, ns = 5). GluN2B mRNA increased significantly in Rtv-1h and subsequently decreased to control levels. Contrarily, GluA1 and GluA2 mRNA levels decreased in Rtv-1h, and GluA2 overexpressed slightly in Rtv-6h. Negative values in Delta-Delta Ct indicate increased mRNA levels, while positive values indicate

decreased mRNA levels. Standard deviations below 1 are considered significant. Fold change values higher than 1 (control level) indicate increase in mRNA levels for the respective groups at the specific time points, while a value lower than 1 indicates decreased mRNA levels. q-PCR experiments were conducted 4-6 times with GAPDH loading control. Brackets represent standard deviations.

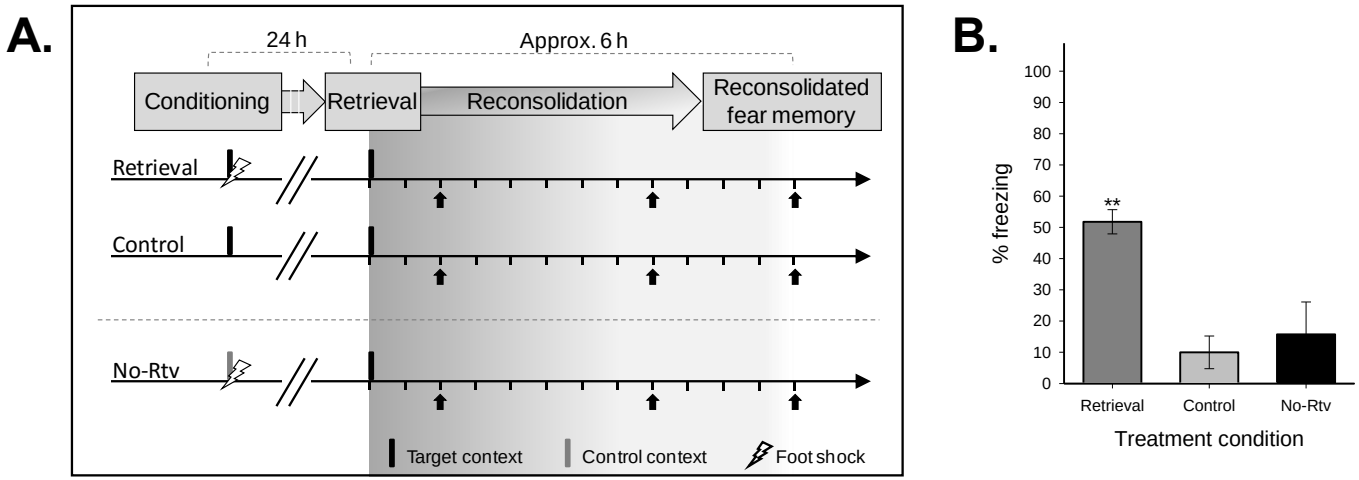
A.
24 h
Approx. 6 h
Conditioning
Retrieval
Reconsolidation
Reconsolidated fear memory
Retrieval
Control
No-Rtv
Target context
Control context
Foot shock
B.
% freezing
100
90
80
70
60
50
40
30
20
10
0
**
Retrieval
Control
No-Rtv
Treatment condition

Figure 5.1

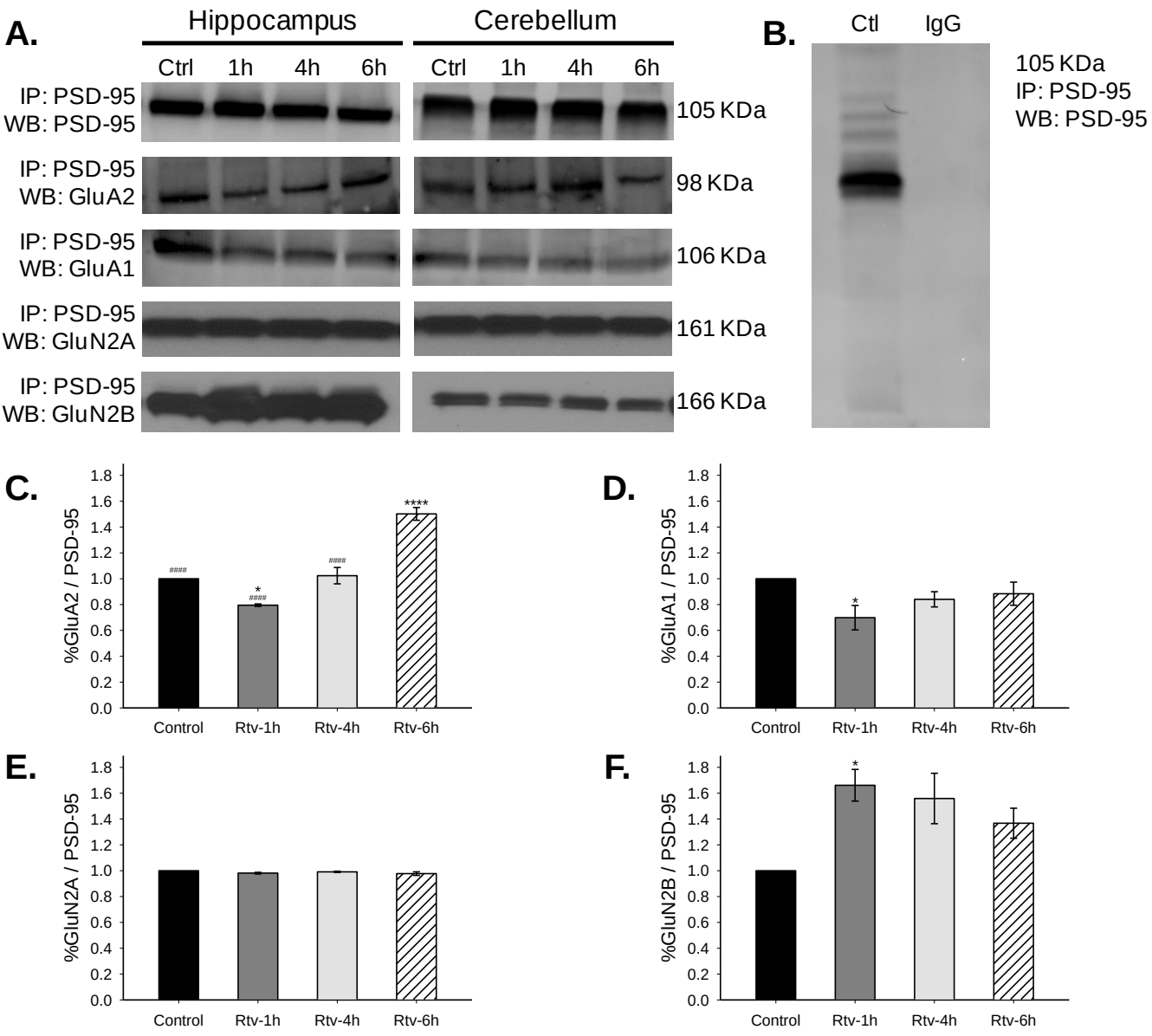
A.
Hippocampus
Cerebellum
Ctrl
1h
4h
6h
Ctrl
1h
4h
6h
IP: PSD-95
WB: PSD-95
105 KDa
IP: PSD-95
WB: GluA2
98 KDa
IP: PSD-95
WB: GluA1
106 KDa
IP: PSD-95
WB: GluN2A
161 KDa
IP: PSD-95
WB: GluN2B
166 KDa
B.
Ctl
IgG
105 KDa
IP: PSD-95
WB: PSD-95
C.
%GluA2 / PSD-95
D.
%GluA1 / PSD-95
E.
%GluN2A / PSD-95
F.
%GluN2B / PSD-95
Control
Rtv-1h
Rtv-4h
Rtv-6h

Figure 5.2

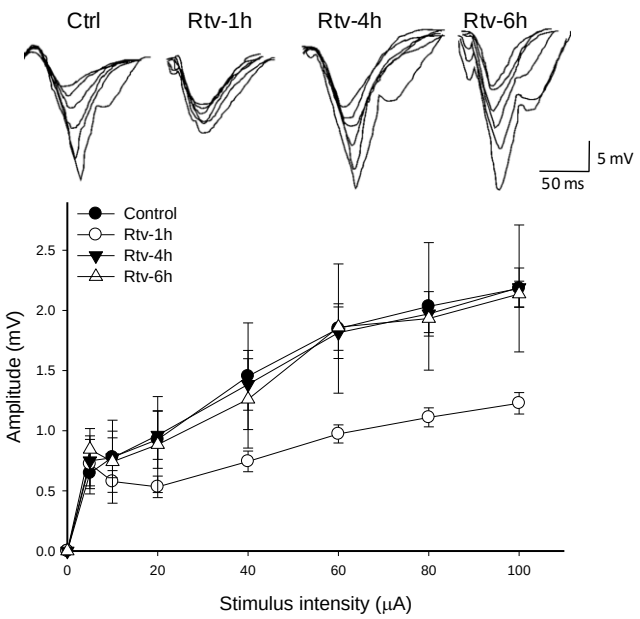
Ctrl
Rtv-1h
Rtv-4h
Rtv-6h
5 mV
50 ms
Control
Rtv-1h
Rtv-4h
Rtv-6h
Amplitude (mV)
0.0
0.5
1.0
1.5
2.0
2.5
0
20
40
60
80
100
Stimulus intensity (μA)

Figure 5.3

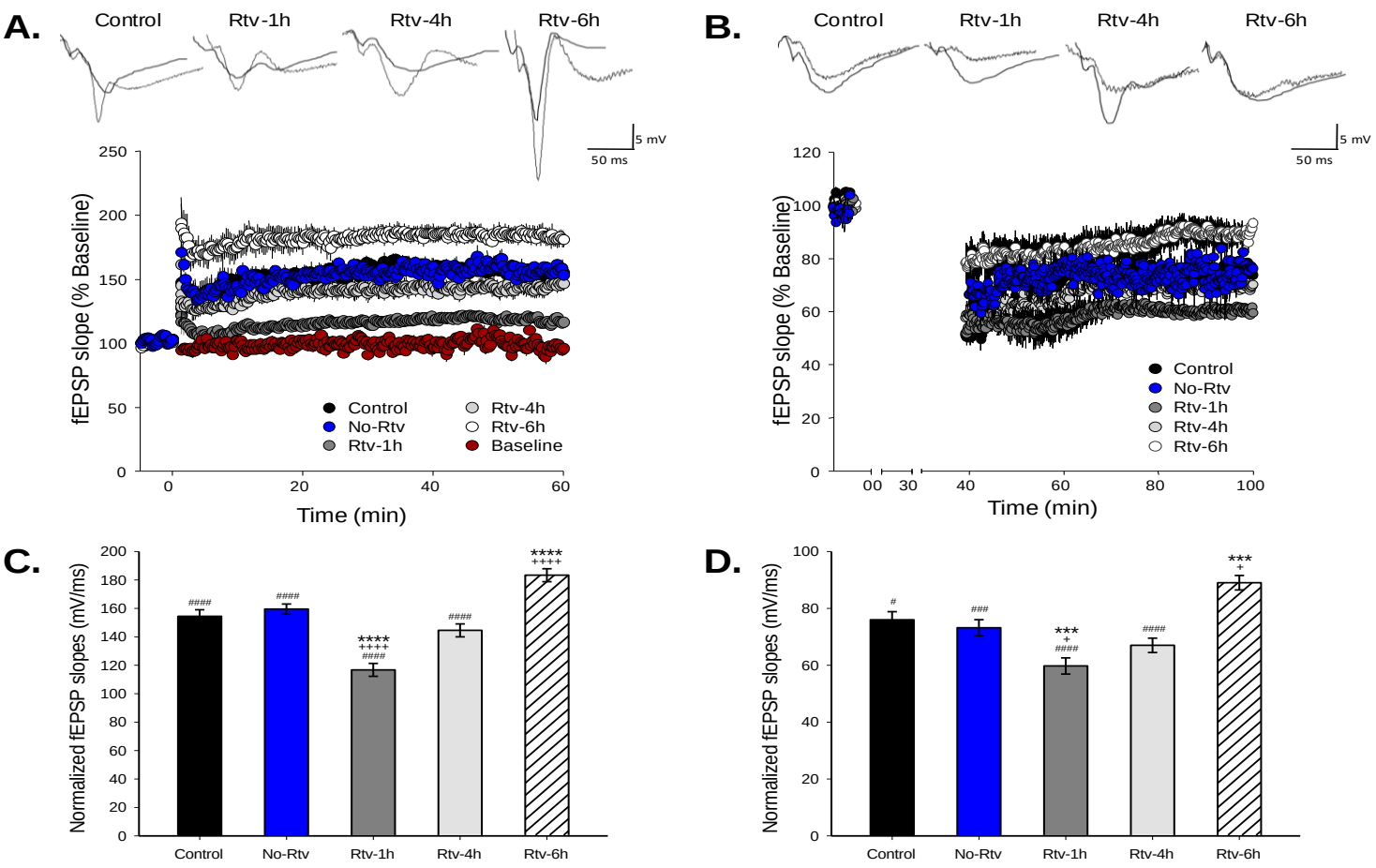
A.
Control
Rtv-1h
Rtv-4h
Rtv-6h
5 mV
50 ms
fEPSP slope (% Baseline)
Time (min)
Control
No-Rtv
Rtv-1h
Rtv-4h
Rtv-6h
Baseline
B.
Control
Rtv-1h
Rtv-4h
Rtv-6h
5 mV
50 ms
fEPSP slope (% Baseline)
Time (min)
Control
No-Rtv
Rtv-1h
Rtv-4h
Rtv-6h
C.
Normalized fEPSP slopes (mV/ms)
Control
No-Rtv
Rtv-1h
Rtv-4h
Rtv-6h
D.
Normalized fEPSP slopes (mV/ms)
Control
No-Rtv
Rtv-1h
Rtv-4h
Rtv-6h

Figure 5.4

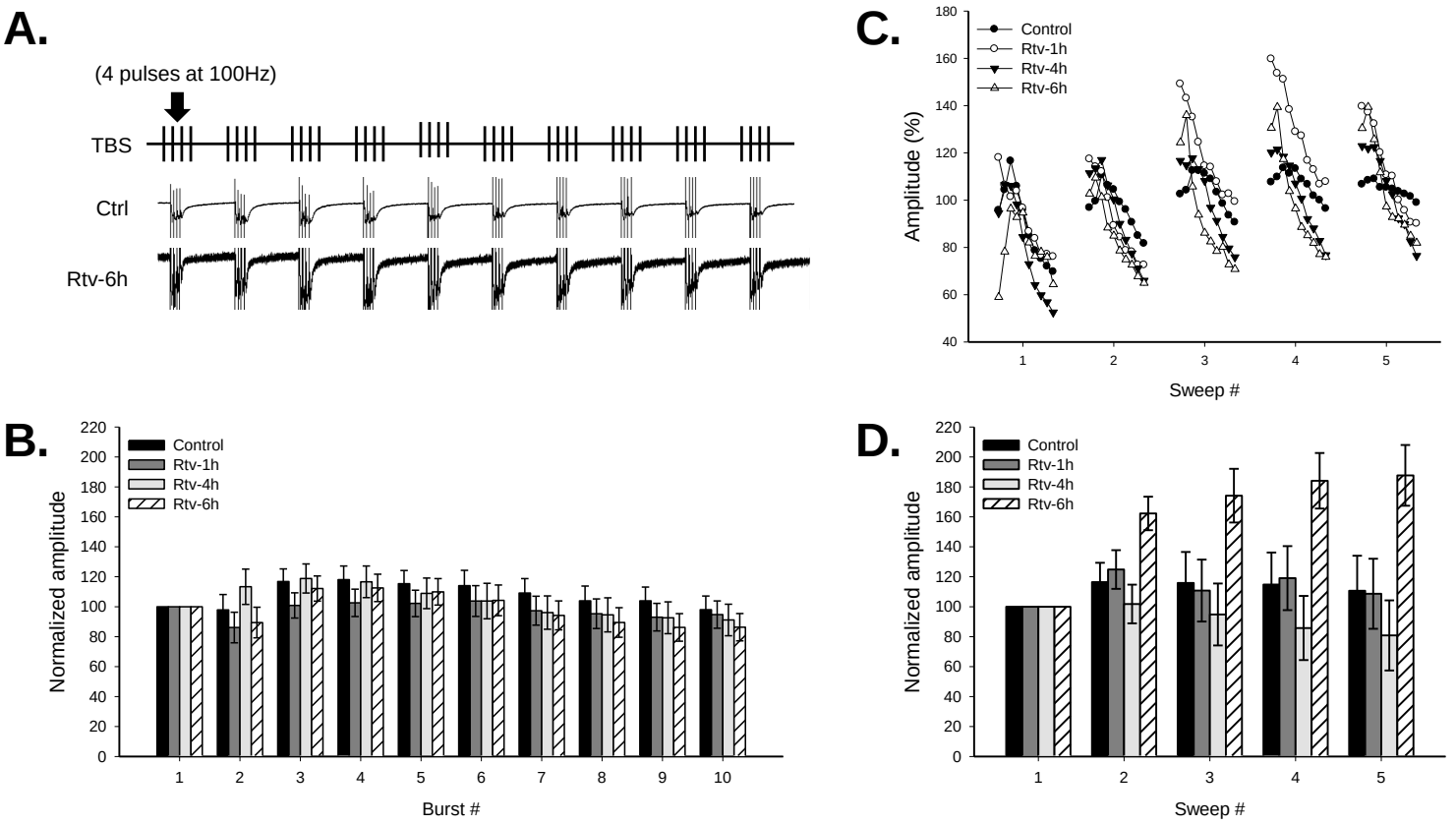
A.
(4 pulses at 100Hz)
TBS
Ctrl
Rtv-6h
C.
Control
Rtv-1h
Rtv-4h
Rtv-6h
Amplitude (%)
Sweep #
B.
Normalized amplitude
Burst #
D.
Normalized amplitude
Sweep #

Figure 5.5

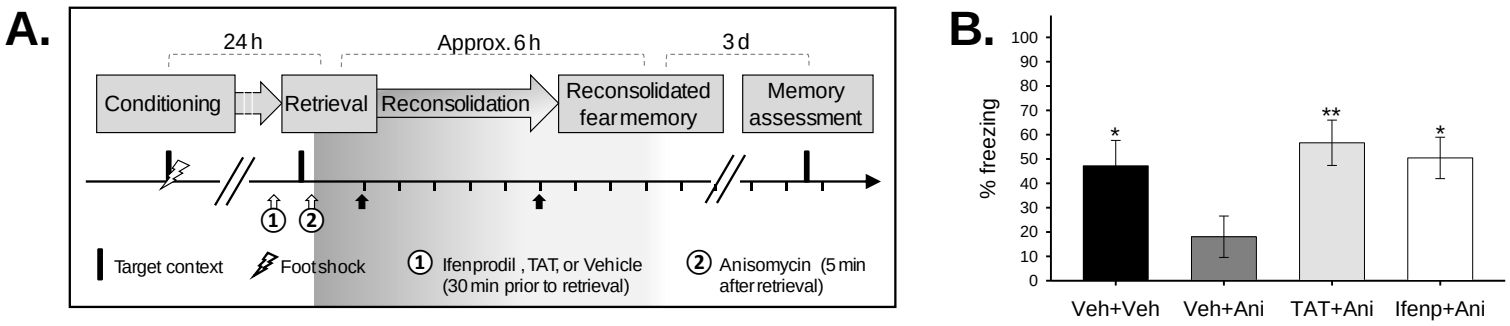
A.
24 h
Approx. 6 h
3 d
Conditioning
Retrieval
Reconsolidation
Reconsolidated fear memory
Memory assessment
1
2
Target context
Foot shock
1 Ifenprodil , TAT, or Vehicle (30 min prior to retrieval)
2 Anisomycin (5 min after retrieval)
B.
% freezing
100
90
80
70
60
50
40
30
20
10
0
*
**
*
Veh+Veh
Veh+Ani
TAT+Ani
Ifenp+Ani

Figure 5.6

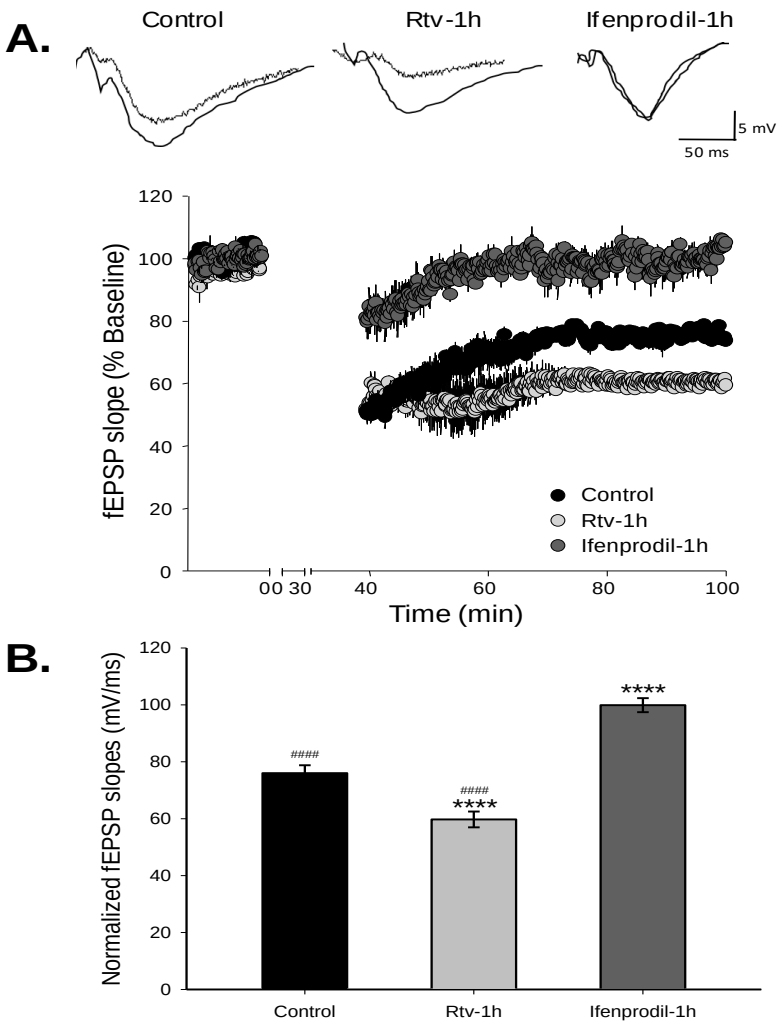
A.
Control
Rtv-1h
Ifenprodil-1h
5 mV
50 ms
fEPSP slope (% Baseline)
120
100
80
60
40
20
0
00 30
40
60
80
100
Time (min)
Control
Rtv-1h
Ifenprodil-1h
B.
Normalized fEPSP slopes (mV/ms)
####
####
****
****
Control
Rtv-1h
Ifenprodil-1h

Figure 5.7

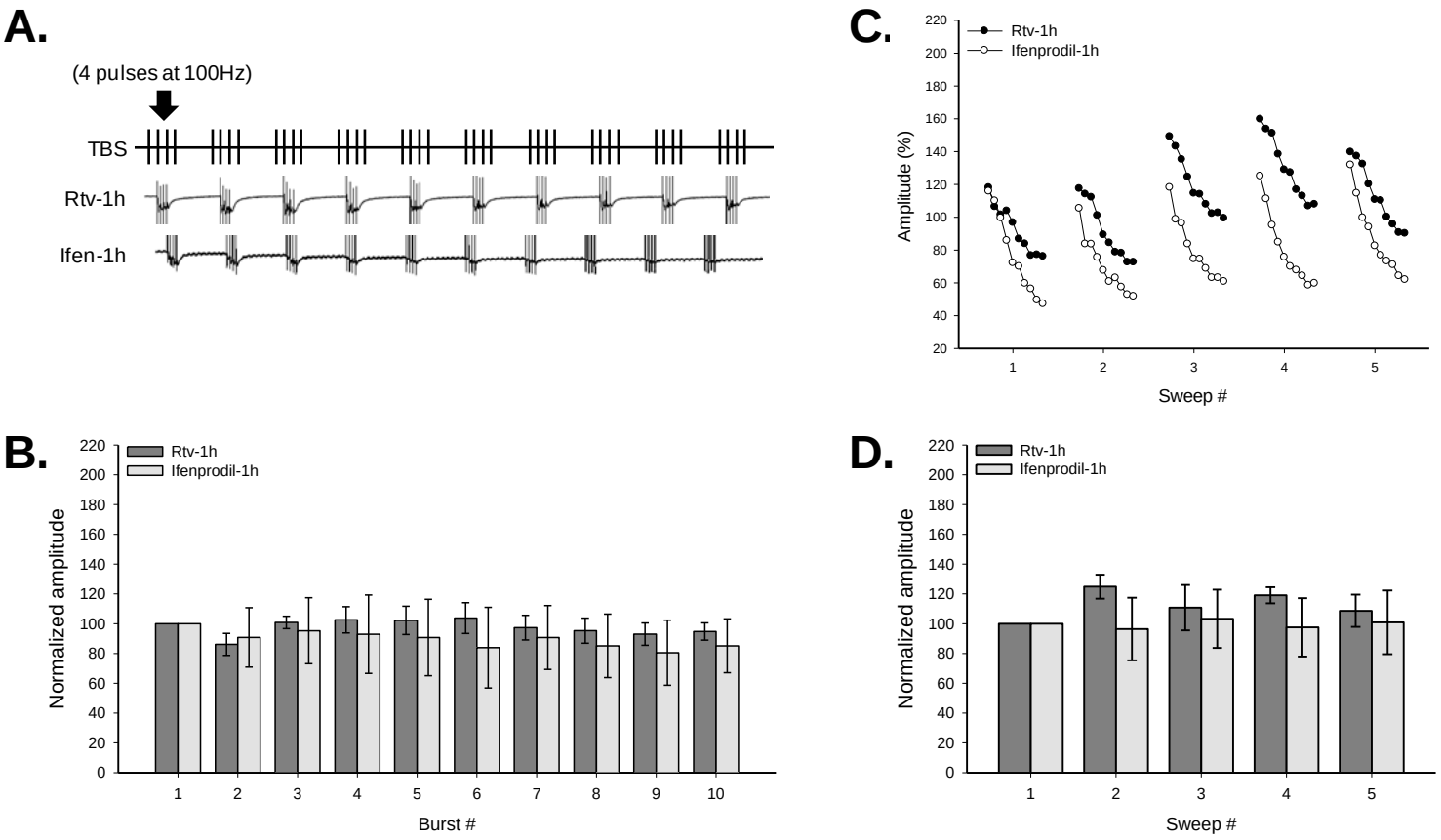
A.
(4 pulses at 100Hz)
TBS
Rtv-1h
Ifen-1h
C.
Rtv-1h
Ifenprodil-1h
Amplitude (%)
Sweep #
B.
Rtv-1h
Ifenprodil-1h
Normalized amplitude
Burst #
D.
Rtv-1h
Ifenprodil-1h
Normalized amplitude
Sweep #

Figure 5.8

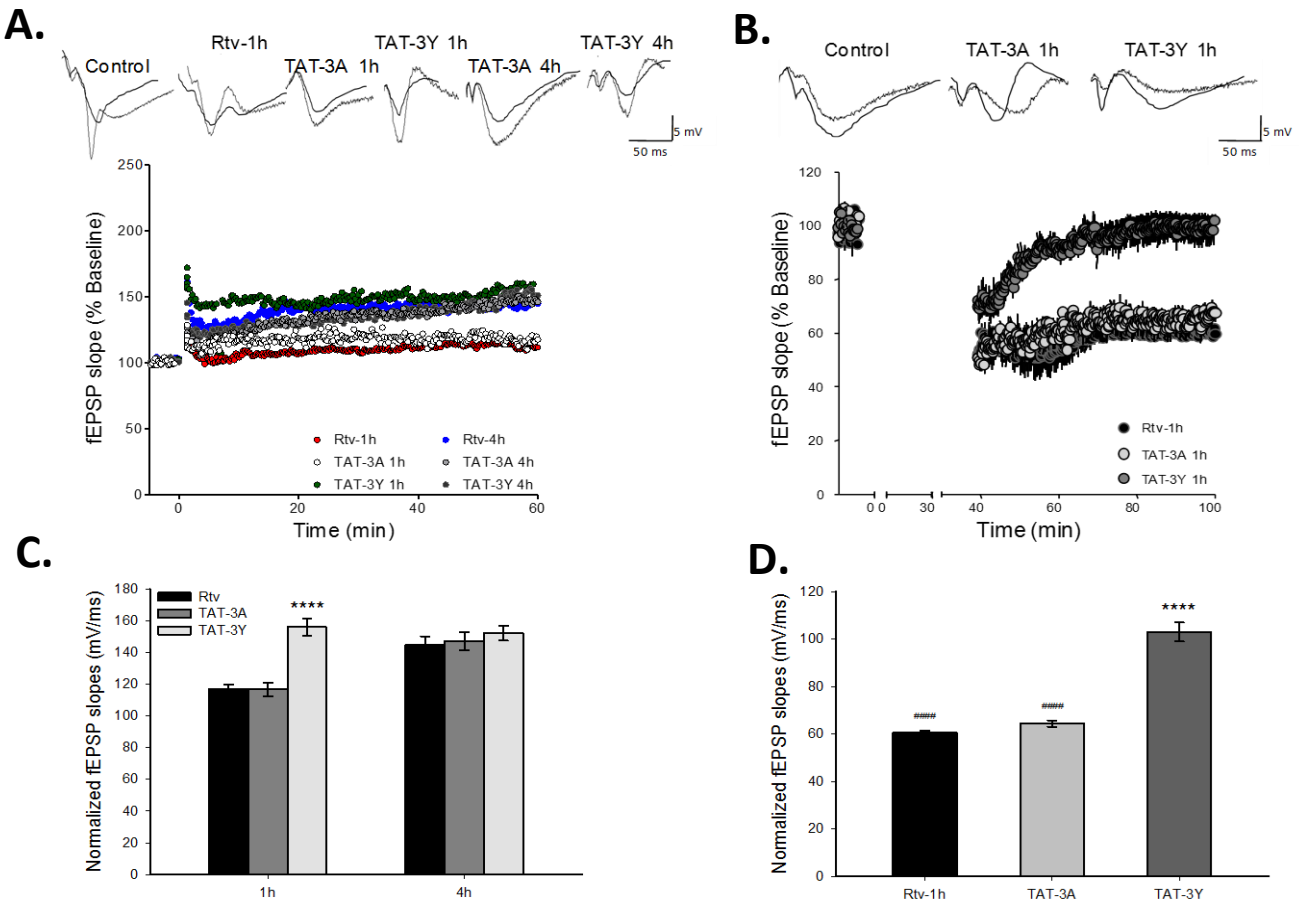
A.
Control
Rtv-1h
TAT-3A 1h
TAT-3Y 1h
TAT-3A 4h
TAT-3Y 4h
5 mV
50 ms
fEPSP slope (% Baseline)
Time (min)
Rtv-1h
Rtv-4h
TAT-3A 1h
TAT-3A 4h
TAT-3Y 1h
TAT-3Y 4h
B.
Control
TAT-3A 1h
TAT-3Y 1h
5 mV
50 ms
fEPSP slope (% Baseline)
Time (min)
Rtv-1h
TAT-3A 1h
TAT-3Y 1h
C.
Rtv
TAT-3A
TAT-3Y
Normalized fEPSP slopes (mV/ms)
****
1h
4h
D.
Normalized fEPSP slopes (mV/ms)
####
####
****
Rtv-1h
TAT-3A
TAT-3Y

Figure 5.9

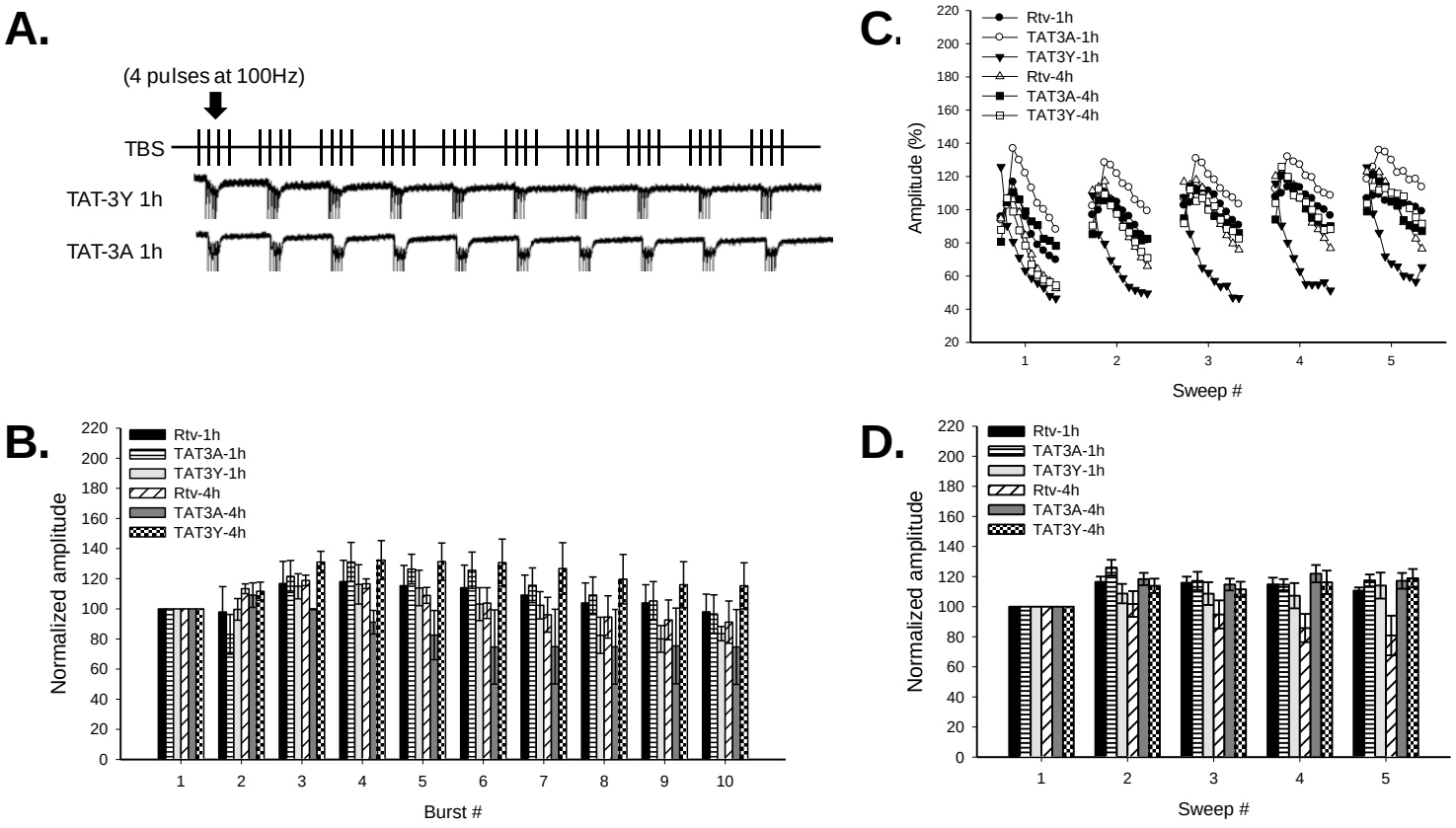

Figure 5.10

	Delta-Delta Ct			Fold Change			Standard Dev (±)		
	GluN2B	**GluA1**	**GluA2**	**GluN2B**	**GluA1**	**GluA2**	**GluN2B**	**GluA1**	**GluA2**
Retrieval 1h	-4.44	-0.11	-0.38	21.71	1.07	1.30	0.58	0.49	0.71
Retrieval 4h	-1.07	0.14	0.26	2.09	0.9	0.83	0.64	0.60	0.53
Retrieval 6h	-2.44	-0.94	-1.17	5.42	1.91	2.25	0.64	0.48	0.49

Table 5.1

Chapter VI

Discussion

Some of the cellular processes underlying reconsolidation have been described, specifically the involvement of protein synthesis and receptor trafficking at glutamatergic synapses in the hippocampus (Matsuo et al., 2008; Rumpel et al., 2005; Schafe et al., 2001). The present study used a contextual fear conditioning preparation in which rats were exposed to a novel environment paired with a fear-inducing stimulus. Memory of this experience was retrieved by placing the animal back in the conditioning context and measuring fear levels. Contextual fear conditioning is highly dependent on hippocampal function (Phillips and LeDeoux, 1992) and constitutes an ideal model of hippocampal learning to investigate memory reconsolidation. Following retrieval of conditioned fear memory, we analyzed the role of GluA1, GluA2, and GluN2B receptor-mediated hippocampal bidirectional plasticity (LTP/LTD) during reconsolidation (a period of approx. 6h following memory retrieval, Rao-Ruiz et al., 2011). This analysis resulted for the first time in a novel model of time-based synaptic plasticity changes during the reconsolidation period. Based on this model, we also provide the first demonstration of modulatory effects of an endocytosis blocker (TAT-GluA2-3Y) and GluN2B antagonist (Ifenprodil) on LTP/LTD during reconsolidation of a conditioned fear memory.

Our receptor expression study suggests that there are distinct stages during the reconsolidation process, each characterized by a specific pattern of receptor interaction with PSD-95. During the initial stage (in our study, 1h after retrieval), GluA1/2 expression was impaired, and GluN2B was higher than controls in the hippocampus, but these effects were not observed in the cerebellum. Moreover, no changes were observed during the equivalent time period on GluN2A receptor expression in the hippocampus. This suggests that decreased

synaptic expression of GluA1/2 and increased expression of GluN2B in the hippocampus underlies the altered balance of plasticity observed at the onset of reconsolidation. GluA1 receptor interaction with PSD-95 normalized to control levels later in the reconsolidation process (4-6h after retrieval), but GluA2 receptors followed a different pattern of recovery. GluA2 receptors gradually normalized (4h after retrieval) and then overexpressed as the memory became restabilized (6h after retrieval). Thus, although GluA1 and GluN2B alterations appear critical for the onset of reconsolidation, a biphasic wave of GluA2 activity appears critical for the progression of reconsolidation (Rao-Ruiz et al., 2011).

Synaptic receptor levels during reconsolidation appear to be at least partially modulated by altered transcription. GluA1 mRNA expression remained at control levels 1h after retrieval and decreased below control levels 4h after retrieval. However. GluA1 mRNA levels were higher than control 6h after retrieval, although the synaptic levels of GluA1 receptors remained below control. GluA2 mRNA levels were at control levels shortly after memory retrieval (1h), fell below control levels 4h after retrieval, and steadily increased to exceed control levels 6h after retrieval. This pattern of mRNA expression suggests that receptor expression levels are at least partially determined by changes in receptor transcription during reconsolidation instead or in addition to impaired trafficking (Rao-Ruiz et al., 2011). Unlike GluA1/2, GluN2B mRNA levels were significantly higher than the controls 1, 4, and 6h after memory retrieval. This suggests a different time course for transcriptional changes for GluA1/2, and GluN2B receptors during reconsolidation. Possibly, a direct positive feedback mechanism between receptor expression and mRNA synthesis might be responsible for such an effect.

Synaptic plasticity is expressed in two main forms, LTP and LTD. A controlled balance between these two different forms of plasticity is considered crucial for short- and long-term memory storage. The present study shows that this balance is also crucial in memory reconsolidation, with LTP and LTD showing opposing but equally calibrated patterns through memory stabilization and restabilization periods, and these patterns being closely related to glutamate receptor activity. Our underlying assumption is that retrieval induces a sensitive period of synaptic plasticity. This assumption is partially based on reports that LTP after retrieval is not sensitive to protein synthesis inhibitors unless re-stimulation occurs during the maintenance phase (Fonseca et al., 2006). The present study suggests that impaired LTP is a physiological marker of the initiation of memory reconsolidation (Rtv-1h), and the reconsolidation process is accompanied by a gradual normalization (Rtv-4h) and overexpression (Rtv-6h) of LTP. These changes in LTP expression are accompanied by an initial increase in LTD expression (Rtv-1h), followed by a gradual normalization (Rtv-4h) and reduction (Rtv-6h) of LTD. All observed plasticity changes are consistent with changes in glutamate receptor expression. GluA1/2 receptor expression, which was decreased shortly after retrieval, is involved in induction and maintenance of LTP, whereas GluN2B receptor overexpression, which was observed shortly after retrieval, favors induction of LTD. GluN2A receptor activity did to appear to be related to retrieval-induced plasticity changes, since these changes were not accompanied by any significant alteration in GluN2A expression or membrane depolarization properties in our train facilitation studies (Kochlamazashvili et al., 2012).

If GluA2 and GluN2B activity is crucial for the occurrence of LTP/LTD, selective disruption of these two processes should have profound effects on memory reconsolidation. Retrieval-induced endocytosis of GluA2 receptors was blocked with TAT-GluA2-3Y, whereas

GluN2B activity was reduced with the subtype selective antagonist Ifenprodil. Controlled blockade of GluA2 endocytosis and antagonism of GluN2B receptors prevented the initial memory destabilization that characterizes the onset of reconsolidation, effectively reducing the amnesic effects of Anisomycin, a protein synthesis inhibitor known to disrupt reconsolidation of recently retrieved memories. These behavioral observations were the result of TAT-GluA2-3Y abolishing the retrieval-induced deficits in LTP otherwise observed 1h after memory retrieval, and both TAT-GluA2-3Y and Ifenprodil reducing LTD to control levels (for TAT-GluA2-3Y) or baseline levels (for Ifenprodil). The short half-life of both the compounds (TAT peptide-90 min and Ifenprodil-30 min) further suggest that the effect they show on LTP/LTD is due to their ability to interfere with initial receptor expression patterns rather than a lingering effects of the drugs.

The occurrence of memory destabilization as a consequence of retrieval has been known for years (*cf.* Misanin et al., 1968). More recently, there has been a renewed interest in reconsolidation processes because of the possibility that the content of a retrieved memory can be altered by new information acquired while the memory is unstable. For example, Monfils et al. (2009; and Rao-Ruiz et al., 2011; Schiller et al., 2010) reported that treatments attenuating fear conditioning (i.e., extinction, Pavlov, 1927) administered shortly after memory retrieval can dramatically decrease the likelihood of fear relapsing at a later time (fear relapse is a ubiquitous behavioral observation after extinction due to the passage of time or *spontaneous recovery*; Pavlov, 1927; Rescorla, 2004). The applications of such an approach are clear, since updating of anxiety-producing memories with a new memory devoid of anxiety could provide a powerful tool to address anxiety disorder (e.g., phobias) and trauma-related disorders (e.g., post-traumatic stress disorder [PTSD]). However, the utility of this approach is limited. Although the process of

reconsolidation has received great attention (Besnard et al., 2010; Nader and Einarsson, 2010, 2015; Tronson and Taylor, 2007), it is still unclear whether memory updating during the reconsolidation period changes the to-be reconsolidated memory, or results in the formation of new memories with common retrieval links (Tronson and Taylor, 2007). Furthermore, updating manipulations are not effective in all cases (Auber et al., 2013). A full characterization of the physiological processes that underlie memory reconsolidation can provide a framework upon which such manipulations can be more successful. The present study provides a thorough description of the synaptic plasticity changes that occur during the reconsolidation period, along with a conceptualization of the mechanisms that lead to such changes, providing the first unified model of glutamate receptor expression and synaptic plasticity across the different stages of the reconsolidation period.

Our findings are consistent with data from other laboratories (Rao-Ruiz et al., 2011), and suggest that the process of reconsolidation is characterized by bidirectional synaptic plasticity changes predominantly determined by postsynaptic mechanisms. They also show how depolarization of synapses change over time during memory reconsolidation, in processes driven by altered waves of glutamate receptor expression in the Schaffer collateral hippocampal synapses. These changes in the balance of LTP and LTD may reflect a natural way for hippocampal synapses to control various components of the to-be-reconsolidated memory, such as content or strength. Our data further suggest the specificity of hippocampus in reconsolidation. However, given the importance of amygdala in fear memory reconsolidation, investigating system level interactions between the amygdala and hippocampus in our current model is a future direction worth cultivating. A detailed understanding of the processes triggered by memory retrieval is an important first step to fully characterize the process of memory

reconsolidation and potentially lead to development of therapeutics that increase or prevent destabilization, modification, or restabilization of memory after retrieval (reconsolidation model, Figure 6.1).

6.1. Innovation:

The main innovation of the work described in the subsequent chapter of this dissertation is that it is based on a novel, time-dependent, model of receptor expression and synaptic activity during the reconsolidation period, and will extend this model to provide a characterization of the synaptic plasticity changes that underlie memory updating in future. The expectation is that retrieval of previously acquired memories is a consequence of the immediate events that trigger depression of synaptic plasticity after memory recall. Disruption of immediate memory destabilization (i.e., maintenance of high levels of synaptic plasticity) should prevent the occurrence of memory updating in this preparation. The main innovation of this project is that it is based on a novel, time-dependent, model of receptor expression and synaptic activity during the reconsolidation period, and will extend this model to provide a characterization of the synaptic plasticity changes that underlie memory updating. The expectation is that updating of previously acquired memories is a consequence of the immediate events that trigger depression of synaptic plasticity after memory retrieval. Disruption of immediate memory destabilization (i.e., maintenance of high levels of synaptic plasticity) should prevent the occurrence of memory updating in this preparation.

Pathological fear and anxiety can be addressed through pharmacological and behavioral interventions, but the risk of relapse is very high. The public health relevance of the research work shown in this dissertation is that it will provide both a framework to determine when

behavioral interventions will be more successful in permanently modifying fear memories, as well as information regarding which biological mechanisms should be targeted in pharmacological interventions in order to maximize their success.

6.2. **Legend:**

Figure 6.1. Model for bidirectional synaptic plasticity (LTP and LTD), during memory reconsolidation of a conditioned fear memory, and their correlation with receptor expression and trafficking. Shortly after retrieval (at our 1h assessment), there is overexpression of the GluN2B NMDA receptors in the active zone of the neurons accompanied by a downregulation of GluA1 and endocytosis of GluA2 AMPA receptors. This pattern of receptor activity leads to an increase in LTD and decrease in LTP. As reconsolidation progresses (at our 4h assessment), GluA1 and GluA2 receptor expression approaches control levels, leading to a normalization of LTP and LTD. As reconsolidation comes to an end (at our 6h assessment), LTP is overexpressed while LTD decreases to baseline levels. GluN2B NMDA receptor interaction with PSD-95 remains higher than controls and GluA1 AMPA receptors remain at a control levels on the surface, while GluA2 AMPA receptors are overexpressed. This effect is possibly a delayed reaction to the initial downregulation of the particular receptor subtype during the destabilizing period of reconsolidation. Antagonizing GluN2B subtypes shortly after retrieval leads to attenuation of LTD. Controlled inhibition of endocytosis of GluA2 AMPA receptors lead to increased LTP and decreased LTD shortly (1h) after retrieval, but had no effects later during the reconsolidation period (4h after retrieval). These observations suggest that LTP and LTD mechanisms determine synaptic plasticity during memory reconsolidation, and these effects are mediated by glutamate receptor activity. Manipulating the target receptors during the initial period of reconsolidation may provide a specific approach to further understand and dissect the process.

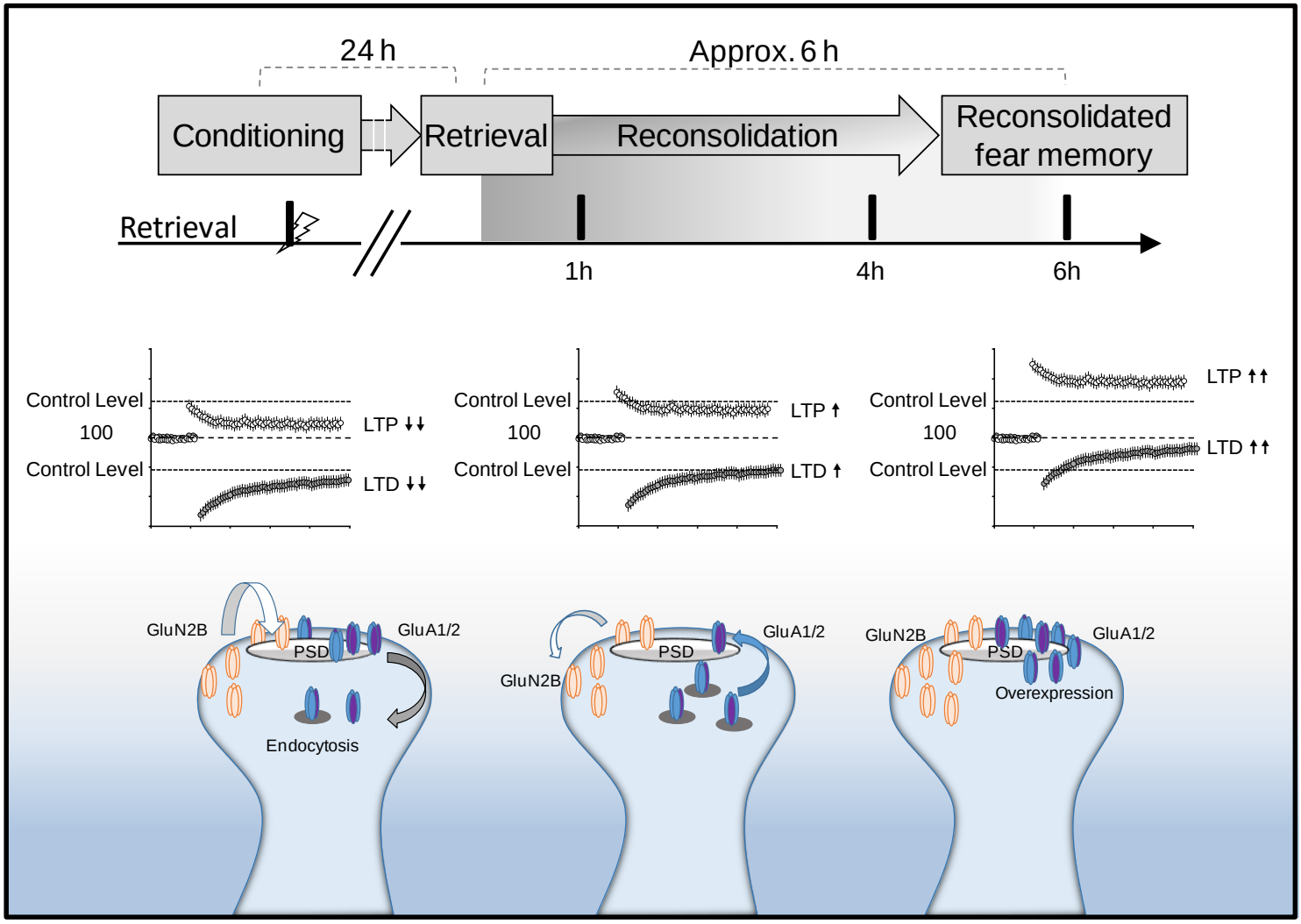
24 h
Approx. 6 h
Conditioning
Retrieval
Reconsolidation
Reconsolidated fear memory
Retrieval
1h
4h
6h
Control Level
100
Control Level
LTP ↓↓
LTD ↓↓
Control Level
100
Control Level
LTP ↑
LTD ↑
Control Level
100
Control Level
LTP ↑↑
LTD ↑↑
GluN2B
GluA1/2
PSD
Endocytosis
GluN2B
GluA1/2
PSD
GluN2B
GluA1/2
PSD
Overexpression

Figure 6.1

6.3. **References:**

Auber A, Tedesco V, Jones CE, Monfils MH, Chiamulera C (2013) Post-retrieval extinction as reconsolidation interference: methodological issues or boundary conditions? Psychopharmacology; 226:631-47

Bard L, Groc L (2011) Glutamate receptor dynamics and protein interaction: lessons from the NMDA receptor. Mol Cell Neurosci Dec;48(4):298-307

Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D (2007) Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology Jan;52(1):60-70

Bashir ZI, Alford S, Davies SN, Randall AD, Collingridge GL (1991) Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature. Jan 10;349(6305):156-8

Bear MF, Abraham WC (1996) Long-term depression in hippocampus. Annu Rev Neurosci 19:437-62

Ben Mamou C, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci Oct;9(10):1237-9

Besnard A, Caboche J, Laroche S (2012) Reconsolidation of memory: a decade of debate. Prog Neurobiol. Oct;99(1):61-80

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature Jan 7;361(6407):31-9

Buller AL, Monaghan DT (1997) Pharmacological heterogeneity of NMDA receptors: characterization of NR1a/NR2D heteromers expressed in Xenopus oocytes. Eur J Pharmacol Feb 5;320(1):87-94

Carroll RC, Zukin RS (2002) NMDA-receptor trafficking and targeting: implications for synaptic transmission and plasticity Trends Neurosci Nov;25(11):571-7

Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science Nov 19;330(6007):1108-12

Collingridge GL, Peineau S, Howland JG, Wang YT (2010) Long-term depression in the CNS. Nat Rev Neurosci. Jul;11(7):459-73

Dalton GL, Wu DC, Wang YT, Floresco SB, Phillips AG (2012) NMDA GluN2A and GluN2B receptors play separate roles in the induction of LTP and LTD in the amygdala and in the acquisition and extinction of conditioned fear. Neuropharmacology Feb;62(2):797-806

Dias C, Wang YT, Phillips AG (2012) Facilitated extinction of morphine conditioned place preference with Tat-GluA2(3Y) interference peptide. Behav Brain Res Aug 1;233(2):389-97

Dudai Y (1996) Consolidation: fragility on the road to the engram. Neuron Sep;17(3):367-70

Einarsson EÖ, Pors J, Nader K (2015) Systems reconsolidation reveals a selective role for the anterior cingulate cortex in generalized contextual fear memory expression. Neuropsychopharmacology Jan;40(2):480-7

Feng B, Raghavachari S, Lisman J (2011) Quantitative estimates of the cytoplasmic, PSD, and NMDAR-bound pools of CaMKII in dendritic spines. Brain Res Oct 24;1419:46-52

Fonseca R, Nägerl UV, Bonhoeffer T (2006) Neuronal activity determines the protein synthesis dependence of long-term potentiation. Nat Neurosci Apr;9(4):478-80

Gomperts SN (1996) Clustering membrane proteins: It's all coming together with the PSD-95/SAP90 protein family. Cell Mar 8;84(5):659-62

Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D (2006) NMDA receptor surface mobility depends on NR2A-2B subunits. Proc Natl Acad Sci U S A Dec 5;103(49):18769-74

Groc L, Bard L, Choquet D (2009) Surface trafficking of N-methyl-D-aspartate receptors: physiological and pathological perspectives. Neuroscience Jan 12;158(1):4-18

Halt AR, Dallapiazza RF, Zhou Y, Stein IS, Qian H, Juntti S, Wojcik S, Brose N, Silva AJ, Hell JW (2012) CaMKII binding to GluN2B is critical during memory consolidation. EMBO J Mar 7;31(5):1203-16

Hammond MS, Sims C, Parameshwaran K, Suppiramaniam V, Schachner M, Dityatev A (2006) Neural cell adhesion molecule-associated polysialic acid inhibits NR2B-containing N-

methyl-D-aspartate receptors and prevents glutamate-induced cell death.J Biol Chem Nov 17;281(46):34859-69

Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, Monaghan DT, Sacktor TC (2000) Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. J Neurosci Jun 15;20(12):RC81

Kida S (2014) Mechanisms for regulation of fear conditioning and memory]. Nihon Shinkei Seishin Yakurigaku Zasshi Nov;34(5-6):117-25

KIM J, Song B, Hong I, Kim J, Lee J, Park S, Eom JY, Lee CJ, Lee S, Choi S (2010) Reactivation of fear memory renders consolidated amygdala synapses labile. J Neurosci Jul 14;30(28):9631-40

Kim JI, Lee HR, Sim SE, Baek J, Yu NK, Choi JH, Ko HG, Lee YS, Park SW, Kwak C, Ahn SJ, Choi SY, Kim H, Kim KH, Backx PH, Bradley CA, Kim E, Jang DJ, Lee K, Kim SJ, Zhuo M, Collingridge GL, Kaang BK (2011) PI3Kγ is required for NMDA receptor-dependent long-term depression and behavioral flexibility. Nat Neurosci Oct 23;14(11):1447-54

Kim R, Moki R, Kida S (2011) Molecular mechanisms for the destabilization and restabilization of reactivated spatial memory in the Morris water maze. Mol BRAIN Feb 11;4:9

Kochlamazashvili G, Senkov O, Grebenyuk S, Robinson C, Xiao MF, Stummeyer K, Gerardy-Schahn R, Engel AK, Feig L, Semyanov A, Suppiramaniam V, Schachner M, Dityatev A (2010) Neural cell adhesion molecule-associated polysialic acid regulates synaptic plasticity and

learning by restraining the signaling through GluN2B-containing NMDA receptors. J Neurosci Mar 17;30(11):4171-83

Kochlamazashvili G, Bukalo O, Senkov O, Salmen B, Gerardy-Schahn R, Engel AK, Schachner M, Dityatev A (2012) Restoration of synaptic plasticity and learning in young and aged NCAM-deficient mice by enhancing neurotransmission mediated by GluN2A-containing NMDA receptors. J Neurosci Feb 15;32(7):2263-75

Krawczyk MC, BLAKE MG, Baratti CM, Romano A, Boccia MM, Feld M (2015) Memory reconsolidation of an inhibitory avoidance task in mice involves cytosolic ERK2 bidirectional modulation. Neuroscience Mar 17;294:227-237

Kwapis JL, Jarome TJ, Schiff JC, Helmstetter FJ (2011) Memory consolidation in both trace and delay fear conditioning is disrupted by intra-amygdala infusion of the protein synthesis inhibitor anisomycin. Learn Mem Oct 25;18(11):728-32

Lynch MA (2004) Long-term potentiation and memory. Physiol Rev Jan;84(1):87-136

Mac Callum PE, Hebert M, Adamec RE, Blundell J (2014) Systemic inhibition of mTOR kinase via rapamycin disrupts consolidation and reconsolidation of auditory fear memory. Neurobiol Learn Mem Jul;112:176-85

Matsuo N, Reijmers L, Mayford M (2008) Spine-type-specific recruitment of newly synthesized AMPA receptors with learning Science Feb 22;319(5866):1104-7

McGaugh JL (2000) Memory--a century of consolidation. Science Jan 14;287(5451):248-51

Milton AL, Merlo E, Ratano P, Gregory BL, Dumbreck JK, Everitt BJ (2013) Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. J Neurosci Jan 16;33(3):1109-15

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science May 3;160(3827):554-5

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron Mar;12(3):529-40

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg PH (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science May 22;256(5060):1217-21

Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Ann N Y Acad Sci Mar;1191:27-41

Nader K, Hardt O (2009) A single standard for memory: the case for reconsolidation. Nat Rev Neurosci Mar;10(3):224-34

Nader K, Schafe GE, LeDoux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature Aug 17;406(6797):722-6

Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci Apr 1;16(7):2157-63

Ortega LA, Glueck AC, Papini MR (2014) Anisomycin Disrupts Consummatory Behavior after Incentive Downshift via Conditioned Taste Aversion. International Journal of Psychology and Psychological Therapy 14, 1, 71-84

Parameshwaran K, Buabeid MA, Bhattacharya S, Uthayathas S, Kariharan T, Dhanasekaran M, Suppiramaniam V (2013) Long term alterations in synaptic physiology, expression of β2 nicotinic receptors and ERK1/2 signaling in the hippocampus of rats with prenatal nicotine exposure. Neurobiol Learn Mem Nov;106:102-11

Pavlov PI (2010) Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Ann Neurosci Jul;17(3):136-41

Petralia RS, Wang YX, Hua F, Yi Z, Zhou A, Ge L, Stephenson FA, Wenthold RJ (2010) Organization of NMDA receptors at extrasynaptic locations. Neuroscience Apr 28;167(1):68-87

Phillips RG and LeDoux JS (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience V106 No.2-274-285

Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ (2005) The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. Neuron Sep 15;47(6):845-57

Przybyslawski J, Roullet P, Sara SJ (1999) Attenuation of emotional and nonemotional memories after their reactivation: role of beta adrenergic receptors. J Neurosci Aug 1;19(15):6623-8

Rao-Ruiz P, Carney KE, Pandya N, van der Loo RJ, Verheijen MH, van Nierop P, Smit AB, Spijker S (2015) Time-dependent changes in the mouse hippocampal synaptic membrane proteome after contextual fear conditioning. Hippocampus Feb 23

Rao-Ruiz P, Rotaru DC, van der Loo RJ, Mansvelder HD, Stiedl O, Smit AB, Spijker S (2011) Retrieval-specific endocytosis of GluA2-AMPARs underlies adaptive reconsolidation of contextual fear. Nat Neurosci Sep 11;14(10):1302-8

Rescorla RA (2004). Spontaneous recovery. Learn Mem Sep-Oct;11(5):501-9

Rescorla RA (2001) Retraining of extinguished Pavlovian stimuli. J Exp Psychol Anim Behav Process Apr;27(2):115-24

Rodriguez-Ortiz CJ, Garcia-DeLaTorre P, Benavidez E, Ballesteros MA, Bermudez-Rattoni F (2008) Intrahippocampal anisomycin infusions disrupt previously consolidated spatial memory only when memory is updated. Neurobiol Learn Mem Mar;89(3):352-9

Rose MP, McGlynn FD (1997) Toward a standard experiment for studying post-treatment return of fear. J Anxiety Disord May-Jun;11(3):263-77

Rumpel S, LeDoux J, Zador A, Malinow R (2005) Postsynaptic receptor trafficking underlying a form of associative learning. Science Apr 1;308(5718):83-8

Sanz-Clemente A, Gray JA, Ogilvie KA, Nicoll RA, Roche KW (2013) Activated CaMKII couples GluN2B and casein kinase 2 to control synaptic NMDA receptors. Cell Rep Mar 28;3(3):607-14

Schafe GE, Nader K, Blair HT, LeDoux JE (2001) Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. Trends Neurosci Sep;24(9):540-6

Schiller D, Monfils MH, Raio CM, Johnson DC, LeDoux JE, Phelps EA (2010) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature Jan 7;463(7277):49-53

Schmitt WB, Sprengel R, Mack V, Draft RW, Seeburg PH, Deacon RM, Rawlins JN, Bannerman DM (2005) Restoration of spatial working memory by genetic rescue of GluR-A-deficient mice. Nat Neurosci Mar;8(3):270-2

Shipton OA, Paulsen O (2013) GluN2A and GluN2B subunit-containing NMDA receptors in hippocampal plasticity. Philos Trans R Soc Lond B Biol Sci Dec 2;369(1633):20130163

Simon CM, Hepburn I, Chen W, De Schutter E (2014)The role of dendritic spine morphology in the compartmentalization and delivery of surface receptors. J Comput Neurosci Jun;36(3):483-97

Sorg BA, Todd RP, Slaker M, Churchill L (2015) Anisomycin in the medial prefrontal cortex reduces reconsolidation of cocaine-associated memories in the rat self-administration model. Neuropharmacology May;92:25-33

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci May 19;24(20):4787-95

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci Apr;8(4):262-75

Wang SH, de Oliveira Alvares L, Nader K (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nat Neurosci Jul;12(7):905-12

Wyllie DJ, Livesey MR, Hardingham GE (2013) Influence of GluN2 subunit identity on NMDA receptor function. Neuropharmacology Nov;74:4-17

Zelikowsky M, Bissiere S, Hast TA, Bennett RZ, Abdipranoto A, Vissel B, Fanselow MS (2013) Prefrontal microcircuit underlies contextual learning after hippocampal loss. Proc Natl Acad Sci U S A Jun 11;110(24):9938-43

Zelikowsky M, Hast TA, Bennett RZ, Merjanian M, Nocera NA, Ponnusamy R, Fanselow MS (2013) Cholinergic blockade frees fear extinction from its contextual dependency. Biol Psychiatry. 2013 Feb 15;73(4):345-52