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Molecular and systems mechanisms of memory consolidation and storage

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Abstract

Until recently, memory consolidation and storage had been traditionally viewed as a permissive process derived from learning-activated molecular signaling cascades which include activations of the NMDA receptors, CaMKII, PKC, PKA and other kinases, new protein synthesis and CREB-mediated gene expression, and subsequent structural modifications at certain synapses. However, the time-scale of such a cascade is incompatible with the timescale of systems-level memory consolidation. Furthermore, increasing evidence suggests that synaptic proteins and structures are not stationary, but rather are highly dynamical and subjected to metabolic turnovers which would cause drift in synaptic efficacy and subsequently unstable neural circuits. Recent experiments using inducible gene- or protein-knockout techniques reveal that post-learning NMDA receptor and CaMKII reactivations are required for the systems-level consolidation of both hippocampal-dependent and hippocampal-independent memories. Furthermore, the reactivations of the NMDA receptors are also necessary for the long-term storage of old memories in the neural circuits. Therefore, the NMDA receptor reactivation-mediated synaptic reentry reinforcement (SRR) process may represent the unifying cellular mechanism in linking the consolidation and storage of long-term memories from the molecular level to the systems-level.

Keywords: Memory; Learning; Memory consolidation; Memory storage; Synaptic reentry reinforcement (SRR); NMDA receptor; CaMKII; Hippocampus; Fear conditioning; Water maze; Conditioned taste aversion; Cortex; CREB; Conditional gene knockout; Inducible protein knockout; Memory reactivation

Contents

Abbreviations: SRR, synaptic reentry reinforcement; MTL, medial temporal lobe; PFC, prefrontal cortex; APV, 2-amino-5-phosphonovalerate; MDA, multiple discriminant analysis; NMDA, *N*-methyl-D-asparate; CaMKII, calcium calmodulin dependent protein kinase II; PKA, protein kinase A; PKC, protein kinase C; CREB, cAMP responsive element binding protein

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1. Introduction

The long-term memory process can be generally divided into four distinct stages: learning, consolidation, storage and retrieval. Over the past century, researchers have made great efforts to understand the molecular and cellular mechanisms underlying the memory formation (Muller and Pilzecker, 1900; Dudai, 2004; Lechner et al., 1999; McGaugh, 2000; Frey, 2001; Sara, 2000; Nadel and Bohbot, 2001; Tsien, 2000a; Debiec et al., 2002). Studies of amnesiac patients and experimental animals have demonstrated an important role for the hippocampus in consolidating the labile short-term memory into a more stable long-term memory (Scoville and Milner, 1957; Jarrard, 1993; Squire and Alvarez, 1995; McGaugh, 2000; Wittenberg and Tsien, 2002; Squire et al., 2004). Upon the completion of hippocampal-dependent consolidation, these memories are thought to be transferred to and stored in the cortex without significant hippocampal contribution (Zola-Morgan and Squire, 1990; Kim and Fanselow, 1992; Nadel and Moscovitch, 1997; Bontempi et al., 1999; Frankland et al., 2001; Wiltgen et al., 2004). In this review, we will first examine the neurological and anatomical evidence for the role of the hippocampus and cortex in the long-term memory process and then discuss the unifying molecular and systems mechanisms underlying memory consolidation and storage in the mammalian brain.

2. Memory consolidation and storage

2.1. Gradual consolidation of memories over time

In 1900, Müller and Pilzecker reported that the formation of stable memory is disrupted by new stimuli shortly after the first learning (Muller and Pilzecker, 1900). This has led to the notion that memories are highly labile in their initial stage, and then become more stable over time—a process termed memory consolidation (Cohen and Eichenbaum, 1993). Later on, extensive studies have confirmed that the newly formed memories were susceptible to a variety of post-learning (minutes to half hour) manipulations such as electroconvulsive shock, protein synthesis inhibitor or hypothermia treatment. Moreover, the disruptive effects of these post-learning manipulations decrease as the time interval between the acquisition and the intervention increases. Intensive research in the past several decades suggests that this type of memory consolidation, occurring within minutes to hours after initial learning, may reflect the ongoing changes in the intracellular signaling pathways and new protein synthesis and gene expression by which subsequent modifications in synaptic properties and structures are produced (McGaugh, 2000; Kandel, 2001; Dudai, 2004).

2.2. Hippocampal involvement in memory consolidation

It has been long known from neurological studies of patients with region-selective damage or lesion that another type of memory consolidation occurs at a much slower time scale. This type of memory consolidation, now often termed as the systems-level consolidation, can take weeks, months or even years to be accomplished. In the 1950s, a patient known as H.M. was treated for his severe epilepsy by a bilateral removal of the medial temporal lobe (MTL). While the surgery successfully relieved his debilitating seizures, he was left with profound amnesia (Scoville and Milner, 1957). With the removal of the MTL, H.M. exhibited anterograde amnesia, a loss of ability to acquire new memories of people, events, and places. This class of memory is known as declarative memory, which can be further divided into episodic memory (memory of events that have specific spatial and temporal context) and semantic memory (memory of general knowledge, facts and concepts). Moreover, H.M. suffered from retrograde amnesia (loss of memory of past events) although his retrograde amnesia was not complete. For example, H.M. lost his more recent memories of events that happened to him months and year(s) before his surgery, but he seemed to retain well his memories of events dated back about 11 years before the surgery (Corkin, 2002). This crucial observation becomes the first evidence for the involvement of MTL in the consolidation of long-term memory. Subsequent studies of patients with MTL-related lesions demonstrated similar levels of retrograde amnesia (Squire and Alvarez, 1995; Squire et al., 2001, 2004). It appears that the length of the gradient is generally correlated with the extent of the MTL damage. For instance, the damage restricted to the CA1 region of the hippocampus leads to more limited retrograde amnesia (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996), whereas patients with more extensive MTL damage have more extended retrograde amnesia (Squire and Alvarez, 1995; Squire et al., 2001, 2004). Thus, those reports suggest that the hippocampus and its related structures are still actively engaged in the consolidation process even weeks, months, and year(s) after initial memory formation. Such a slower consolidation process has been postulated to reflect the systems-level memory consolidation that perhaps involves

gradual reorganization of the brain circuits and perhaps transfer of recent memory to some cortical areas for the permanent memory storage.

While the human patient studies have provided key clues about the memory consolidation, animal models have become the favored path to study the relationship between the retrograde amnesia and molecular, cellular and anatomical mechanisms. Research from the past several decades indicates that the disruption of hippocampal structure affects recent memories preferentially (Zola-Morgan and Squire, 1990; Kim and Fanselow, 1992; Kim et al., 1995; Anagnostaras et al., 1999), whereas damage in neocortex affects more remote memories (Frankland and Bontempi, 2005; Squire and Alvarez, 1995; Squire et al., 2001). Thus, the general consensus is that the hippocampus plays a time-limited role in consolidating labile new memory into more stable long-term memory (Scoville and Milner, 1957; Squire et al., 1989; Jarrard, 1993; Squire and Alvarez, 1995; Zola-Morgan and Squire, 1990; Wittenberg and Tsien, 2002). Upon the completion of hippocampal-dependent consolidation, these memories are eventually stored in the cortex without significant hippocampal contribution (Bontempi et al., 1999; Frankland et al., 2001; Nadel and Moscovitch, 1997; Zola-Morgan and Squire, 1990; Dudai, 2004).

2.3. Cortical involvement in memory consolidation and storage

Memory consolidation and storage have not been well defined as to how those two events can be temporally separated. It has often been interpreted that neocortical damage-induced impairment in long-term memory suggests the disruption in remote memory storage (Corodimas and LeDoux, 1995; Graham and Hodges, 1997; Squire et al., 2001). For example, a series of lesion studies has reported that the neocortex such as the perirhinal cortex, postrhinal cortex and prefrontal cortex can produce impairment in memory consolidation/storage (Rosen et al., 1992; Suzuki, 1996; Bucci et al., 2000; Frankland et al., 2004; Maviel et al., 2004; Burwell et al., 2004).

It is particularly interesting to note the role of the prefrontal cortex. The prefrontal cortex consists of several highly interconnected regions, including the anterior cingulate, prelimbic and infralimbic cortex. These regions have high connections with sensory, motor and limbic cortex reciprocally, which enable them to integrate information from a large number of different sources (Miller, 1996). The ability of the prefrontal cortex to integrate information from different cortical modules might mirror that of the hippocampus to integrate information from the distributed cortical modules (McClelland et al., 1995; Squire and Alvarez, 1995).

In the cortical memory consolidation scheme, the new memories are initially processed in hippocampal–cortical loops by which the hippocampus drives the co-activation of various distributed cortical modules. As the direct connectivity between the different cortical modules strengthens, the role of the hippocampus in integrating information gradually transfers to the prefrontal cortex. With the gradual strengthening of the cortical–cortical connection, the memories become rather stable, and can function in the absence of the hippocampus.

Some recent experiments have provided experimental support for this notion (Frankland et al., 2004; Maviel et al., 2004; Takehara et al., 2003). For example, lesions of the medial PFC (including the anterior cingulate cortex and prelimbic cortex), made 4 weeks after training, produced significant deficits in trace fear conditioning but had little effect when made day(s) after learning. In contrast, a lesion of the hippocampus made 1 day after training caused significant impairment in retrieval, but had no effect when made weeks after learning (Takehara et al., 2003). Studies using (^{14}C)2-deoxyglucose uptake also report that retrieval of recent spatial memories seemed to generate more 2-deoxyglucose uptake in the hippocampus, whereas remote memories produced more uptake in the neocortex (Bontempi et al., 1999). The expression of immediate early gene (IEG) also follows similar patterns (Frankland et al., 2004; Maviel et al., 2004). For example, robust IEG expression was found in the hippocampus when recent contextual fear memories were retrieved. However, retrievals of remote contextual fear memories induced preferential expression of IEG in the neocortical area. Therefore, emerging evidence collectively suggests that cortical regions are also involved in memory consolidation in addition to the potential storage sites of remote memories in the brain.

2.4. Role of hippocampal–cortical interactions during memory consolidation

Marr was the first to propose a model to address the hippocampal–neurocortical interaction during memory consolidation (Marr, 1970, 1971). He suggested that the hippocampus rapidly stores the memory traces that are then transferred to the cortex for subsequent reorganization. Marr further proposed that the transfer process may depend on playback of waking patterns during sleep. McClleland and his colleagues extended the above idea and suggested that gradual incorporation of memories into the neocortex is critical for the discovery of generalities and the ultimate formation of knowledge structure (McClelland et al., 1995). Their computation modeling shows that rapid incorporation of new information would otherwise lead to interference of stored information. They suggest that this might be the reason why the cortical consolidation is such a slow and gradual process, during which the hippocampus can serve as a temporary link between cortical memories. It has been further postulated that this gradual consolidation of cortical memories is dependent on the reactivations of the hippocampus (Alvarez and Squire, 1994; Sutherland and McNaughton, 2000; Wittenberg et al., 2002; Wittenberg and Tsien, 2002). Under this scheme, reactivation of the hippocampus acts as a coincidence-regenerator that can provide the coherent drive to reactivate various cortical regions. This can lead to gradual strengthening of the cortical–cortical connectivity for permanent storage (Wittenberg and Tsien, 2002). Once those cortical connections

become strongly consolidated, long-term memories can remain stable even in the absence of hippocampus. In the literature those cortically stored long-term memories are often referred as remote memories.

It is noteworthy to mention that there are still several important questions that have not been resolved: (1) Are the time courses for consolidation of various forms of memories the same or different? (2) If one can distinguish the cortical consolidation process from the cortical storage process, how is it done? (3) Where are the exact sites for cortical consolidation and storage? (4) What happens to the consolidated memory traces left behind in the hippocampus? (5) Finally, what are the molecular and cellular mechanisms underlying memory process that can explain those phenomena observed from both lesion experiments and computation modeling?

3. Molecular and systems mechanism underlying memory consolidation and storage

3.1. Molecular mechanisms of synaptic plasticity

The search for the molecular and cellular mechanisms underlying learning and memory has made much progress in the past two decades by using long-term potentiation (LTP) as an experimental model. It has been firmly established that the NMDA receptor is a crucial molecular switch for the induction of LTP (Fig. 1) (Wigstrom and Gustafsson, 1985; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Tsien, 2000a). By developing and applying conditional gene knockout techniques (Tsien et al., 1996a), researchers have demonstrated that the NMDA receptor indeed serves as a cellular coincidence detector for memory formation (Tsien et al., 1996b; Rampon et al., 2000; Tang et al., 1999; Huerta et al., 2000; Tsien, 2000a). While opening the NMDA receptors is crucial for coincidence-detection, the sensitivity and robustness of coincidence-detection is believed to be determined by the opening time-duration, the peak amplitude, and proper intracellular signal transduction (Tsien, 2000b).

Among many downstream signaling molecules of the NMDA receptor pathway, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has supplied the most convincing evidence for being a key mediator in regulating the early phase expression of LTP (Malinow et al., 1989; Silva et al., 1992a; Lisman et al., 2002) and memory formation (Silva et al., 1992b; Mayford et al., 1995; Giese et al., 1998). Ca^{2+} entry through the NMDA receptors promotes binding of calcium/calmodulin to CaMKII, which causes physical translocation of CaMKII to post-synaptic density zones (PSD) by binding to the C-terminus of the NMDA receptor NR2B subunits at synapses (Fig. 1) (Strack and Colbran, 1998; Shen and Meyer, 1999; Shen et al., 2000; Bayer et al., 2001). It is believed that the activated CaMKII at the PSD zone is responsible for potentiating synapses, probably by causing synaptic insertion of AMPA receptors and/or increasing their single channel conductance (Nicoll and Malenka, 1999). Several other kinases, such as PKC and MAP kinase, may also be involved in the expression of LTP (Sweatt, 1999). This phosphorylation-dependent modification

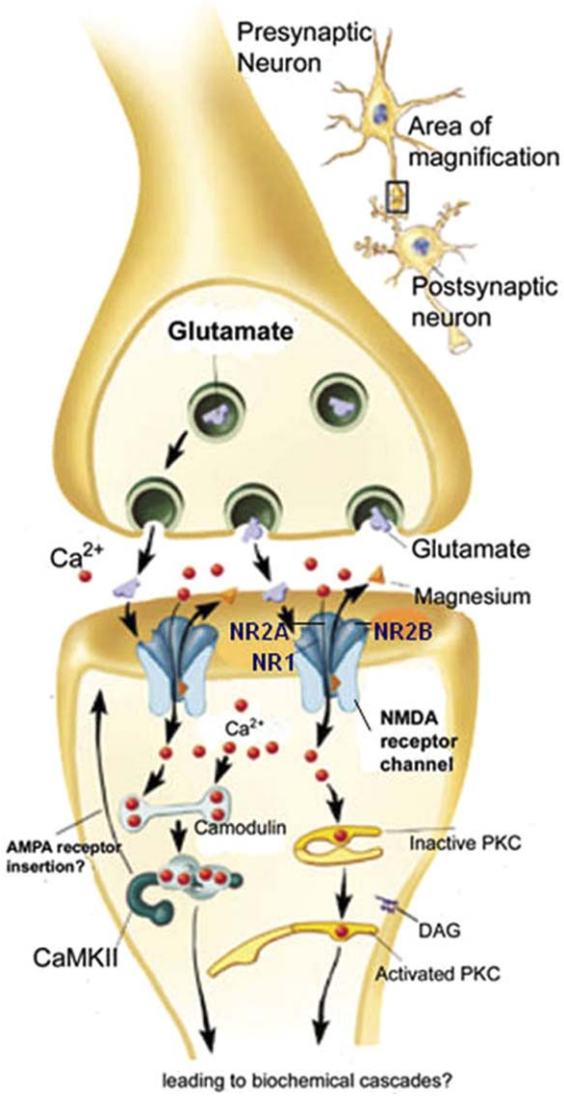

Fig. 1. The key molecules in regulating synaptic plasticity. A synapse between the presynaptic and postsynaptic neurons is illustrated. The glutamate released from presynaptic terminal activates both AMPA and NMDA receptors. While the AMPA receptor is responsible for basal synaptic transmission, the NMDA receptor acts like the volume controller regulating the efficacy of synaptic transmission. Synaptic transmission is enhanced if the NMDA receptor detects the co-activity of the presynaptic (release and binding of glutamate) and postsynaptic neuron (enough depolarization to expel Mg^{2+} from the channel pore). When such a coincidence event occurs, the NMDA receptor is activated, which opens the channel pore and allows Na^+ and Ca^{2+} to rush in and K^+ to rush out. The influx of Ca^{2+} then activates biochemical cascades that eventually strengthen the synapse. It is believed that some of these kinases bind directly to the C-terminus of the NR2B subunit, allowing efficient signal detection and amplification.

of synaptic potentiation is believed to be capable of supporting LTP for 1–3 h. This period is termed the early phase of LTP.

For maintaining synaptic potentiation beyond the initial 3 h, protein kinase A (PKA) and ERK pathways may be involved (Abel et al., 1997; Wang et al., 2004; Hayashi et al., 2004; Chen et al., 2005). This longer-term maintenance of LTP is termed late-phase LTP, first described by Frey and her colleagues (Frey et al., 1988), and appears to require new protein synthesis and perhaps CREB (cAMP response element-binding protein)

activation-mediated gene expression (Kandel, 2001). However, the role of CREB in the later-phase LTP and long-term memory has been significantly discounted by more stringent genetic analyses (Balschun et al., 2003; Perazzona et al., 2004). For example, the conditional knockout mice with forebrain-specific deletion of CREB have completely normal LTP and performed normally in the Morris-water maze and fear conditioning (Balschun et al., 2003). Such critical experiments have cast a severe doubt about the role of CREB in long-term plasticity and memory formation.

If gene expressions were ultimately involved in laying down structural changes needed for long-term synaptic plasticity, complex morphological specialization and the modifications of a large number of synapses would mean that newly synthesized proteins have to be selectively transported to these activated synapses without altering the function of all other synapses in the activated cell. One way to deal with this issue is that synaptic plasticity may be partially mediated via local production of new proteins only at specific subsets of synapses or individual spines (Steward and Schuman, 2001). Another way is that the activated synapses may create some types of ''synaptic tagging'' signals by which the newly synthesized proteins can find their ways to the supposed sites (Frey and Morris, 1998). So far the molecular identity of ''synaptic tagging'' has yet to be identified.

3.2. Single molecular cascade hypothesis for memory consolidation

Although the exact molecular basis underlying long-term plasticity is not fully understood, there has been a general belief that structural plasticity underlies the consolidation and storage of long-term memories in the brain. Such structural modifications of synapses is a permissive product from the activation of the NMDA receptor which initiates a molecular signaling cascade that includes αCaMKII, PKC, PKA kinase activations, new protein synthesis, and gene expression such as Arc, tPA, and BDNF, etc. (Fig. 2). The essence of this ''single cascade hypothesis'' is that once the signaling cascade is initiated by learning, the NMDA receptor is no longer needed because synaptic consolidation should be the innate end-point upon the completion of the molecular cascade (Kandel, 2001).

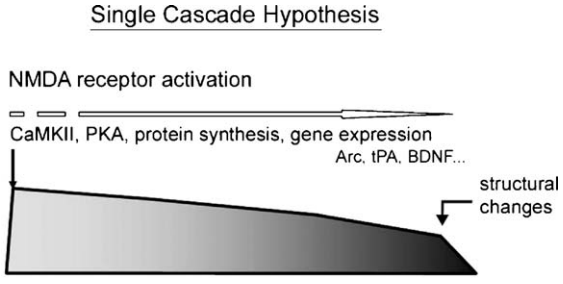

Fig. 2. ''A single cascade hypothesis'' for memory consolidation and storage. The traditional view of memory formation is represented by the ''single cascade hypothesis.'' Learning activates NMDA receptor and various kinases such as CaMKII and PKA, followed with new protein synthesis, and gene expression. This molecular cascade has been postulated to lead structural changes underlying long-term memory consolidation and storage, but faces many problems such as unmatched time courses with systems-level consolidation as well as the failure to consider the metabolic turnovers of synaptic proteins, etc.

While the single cascade hypothesis is attractive for its simplistic description of molecular events, several considerations reveal that the single cascade hypothesis is incompatible for the actual memory consolidation occurring in the mammalian brain. First, the molecular signaling cascade triggered by learning within the hippocampus is usually completed within several hours or a day, whereas hippocampus-mediated consolidation of long-term memories occurs over a timescale of one or more weeks in rodents (Anagnostaras et al., 1999; Kim and Fanselow, 1992; Zola-Morgan and Squire, 1990) and months and even years in humans (Kritchevsky and Squire, 1989; Squire et al., 1989; Haist et al., 2001). Thus, the time-scales of those two phenomena do not match with each other. Second, although protein synthesis inhibitors seem to produce impairment in both the late-phase LTP and long-term memory deficits (which were often tested within 24 h after training), spontaneous recovery or reminders-induced recovery of memory over a time course of weeks has been reported in animals which were initially thought to be amnesic (Quartermain et al., 1972; Squire and Barondes, 1972; Van Abeelen et al., 1973; Miller and Springer, 1974). Third, synaptic structures in the adult brain are not stationary and synaptic receptors and proteins have recently been shown to undergo metabolic turnovers (Shimizu et al., 2000). For example, Shimizu and his colleagues have demonstrated that pre-made synaptic NMDA receptors were completely degraded in about 5 days in the brain of freely behaving mice after the NR1 subunit was inducibly knocked out (Shimizu et al., 2000). Such routine metabolic turnovers of synaptic receptors raise the fundamental question of how synaptic efficacy can be precisely maintained without suffering from the accumulative drift in face of molecular turnovers of synaptic machinery (Wittenberg and Tsien, 2002).

3.3. NMDA receptor-reactivations are required for consolidating memory traces

If the single molecular cascade hypothesis cannot explain how long-term memory is consolidated in the memory circuits, what would be the alternative mechanism(s) that would account for both the molecular and systems-level consolidation of long-term memory? Recently, researchers have initiated a set of experiments to determine whether the reactivation of NMDA receptors is required for memory consolidation (Wittenberg and Tsien, 2002). The role of NMDA receptor-dependent synaptic plasticity in memory consolidation can be explored using either pharmacological inhibitors or molecular genetics. But as discussed below, pharmacological approaches come with inherent problems, thus, are not desirable for molecular analyses of long-term memory consolidation mechanisms.

The NMDA receptors are known to be heteromeric complexes consisting of NR1 and various NR2 subunits (NR2A, NR2B, NR2C and NR2B) (Nakanishi, 1992; Hollmann and Heinemann, 1994). The NR1 subunit is essential for ion selectivity and agonist binding of the NMDA receptors, whereas the NR2 subunits are mainly responsible for regulating

channel gating and Mg^{2+} dependency (Monyer et al., 1992). The combination of NR1 and different NR2 subunits conveys functional diversity and unique properties in electrophysiology and pharmacology (Monyer et al., 1992).

Such different molecular compositions of the NMDA receptor complex in different brain regions can greatly compromise the interpretation of experimental results obtained with NMDA antagonists. For example, post-learning chronic infusion of an NMDA antagonist, 2-amino-5-phosphonovalerate (APV), into the dorsal hippocampus has been reported to be ineffective (only one dose was tried) in blocking the consolidation of long-term spatial memory (16-day retention) using the Atlantis water maze paradigm (Morris, 2003). The authors interpreted their results as the evidence for lack of the role of the NMDA receptor in memory consolidation. However, such claims need to be guarded with great caution: First, the Atlantis water maze paradigm involves repetitive multi-training sections for many days, thus, significant inter-trial consolidation during those learning days have already taken place before APV infusion. Second, in that particular experiment, behavioral variability of wild-type control groups from one experiment to another greatly undermined the authors' claims (Morris, 2003). Third, delivery of APV (either via ventricular route or the local infusion of the drug into the dorsal hippocampus) is unlikely to produce uniform blockade of NMDA receptor function in the entire hippocampus. For example, the banana-shaped hippocampal structure is not an easy place for drug injection; it would require multi-site injection at both the dorsal and ventral portions, rather than the dorsal-only injection. Fourth, while APV blocks NR2A or NR2B-type of NMDA receptors, it also inhibits NR2C or NR2D type of the NMDA receptor which is involved in basic synaptic transmissions. Fifth, chronic diffusion of APV in the brain produces variable concentrations from region to region, thus, it would create treacherous scenarios in which the toxicity in some regions intermingles with the incomplete inhibition of the NMDA receptors in other areas. This may explain why NMDA antagonists produce strong variable side effects including sensorimotor disturbances from animal to animal (Salt, 1986; Cain et al., 1996; Saucier et al., 1996). Finally, chronic exposure of neurons to APV has been shown to up-regulate NMDA receptors including NR2B subunits (Follesa and Ticku, 1996), thus, an unintended consequence is that chronic APV infusion in the brain up-regulates NMDA receptor sensitivity and function, which may even improve synaptic plasticity under certain circumstances (Villarreal et al., 2002). Thus, post-learning chronic infusion of the NMDA receptor into the brain is so problematic that it greatly undermines its effectiveness in examining the role of the NMDA receptor in memory consolidation or storage.

To circumvent this pharmacological limitation, Shimizu and his colleagues have developed the third-generation gene knockout technique and created the inducible, reversible and CA1-region specific NR1 knock-out mice (iCA1-KO) by combining the tTA and Cre/loxP recombination system to examine the involvement of the NMDA receptor in memory consolidation (Shimizu et al., 2000). Since it has been observed that the deletion of NR1 subunit using this Cre transgenic line seems to spread to the other forebrain regions such as the cortex at older ages (>3–5-month old), those researchers exclusively used young adult mice (4.5–7.5 weeks of age) in which NMDA receptor knockout was highly restricted to the CA1 region of the hippocampus. Furthermore, they designed two behavioral paradigms for the analysis of memory consolidation, which include the single foot-shock fear conditioning paradigm and a modified hidden-platform water maze which has fewer learning trials. The one-trial fear conditioning can eliminate any inter-trial consolidation, whereas the modified water maze can somewhat minimize the inter-trial memory consolidation. These genetic experiments have convincingly demonstrated for the first time that the reactivation of the CA1 NMDA receptor during the immediate post-learning week(s) is required for the consolidation of long-term memory. For example, Shimizu et al. have demonstrated that the deletion of the CA1 NMDA receptor in the first two post-learning weeks severely impaired the consolidation of 1-month-old contextual fear memory (Fig. 3, Experiment 1). Interestingly, no memory deficit was detected when the knockout of CA1 NMDA receptor occurred in the fourth post-learning weeks (Fig. 3, Experiment 2) (Shimizu et al., 2000). The CA1 restricted knockout is also evident since the NR1 knockout had no effect on the consolidation of hippocampal-independent cued fear memories. In consideration of the fact that the CA1 NMDA receptor is not involved in basal synaptic transmission, Shimizu et al. has postulated that NMDA-reactivations would initiate the repeated synaptic reentry reinforcement (SRR) process to consolidate the newly acquired memory trace at the synaptic level.

The requirement of reactivations of the NMDA receptor pathways for memory consolidation has been further tested by post-learning manipulation of α-Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII), a major downstream molecule in the NMDAR mediated cascade (Malenka and Nicoll, 1999; Malinow et al., 1989; Giese et al., 1998; Lisman et al., 2002; Silva et al., 1992a,b; Mayford et al., 1995). To examine the role of αCaMKII in memory consolidation, one also needs to employ an inducible knockout approach. As demonstrated, inducible gene knockout techniques are powerful for molecular and temporal analysis of biological processes. However, because the inactivation event occurs at the DNA level, manifestation of any phenotype depends on the turnover rate of the existing protein, which may take days depending on the turnover rate of the proteins. Therefore, it would be highly desirable to develop new types of techniques that can direct the knockout event at the protein level, rather than at the DNA level, for achieving almost instantaneous effects.

By integrating convergent protein engineering and rational inhibitor design, Tsien and his colleagues have developed an *in vivo* conditional protein knockout technology (Wang et al., 2003). This method is based on the creation of a specific interaction interface (bump-and-hole) between a modified protein domain and sensitized inhibitors. By introducing this bump-and-hole system into genetically modified mice (Fig. 3B), the researchers were able to switch on or off the transgenic αCaMKII kinase activity rapidly during various

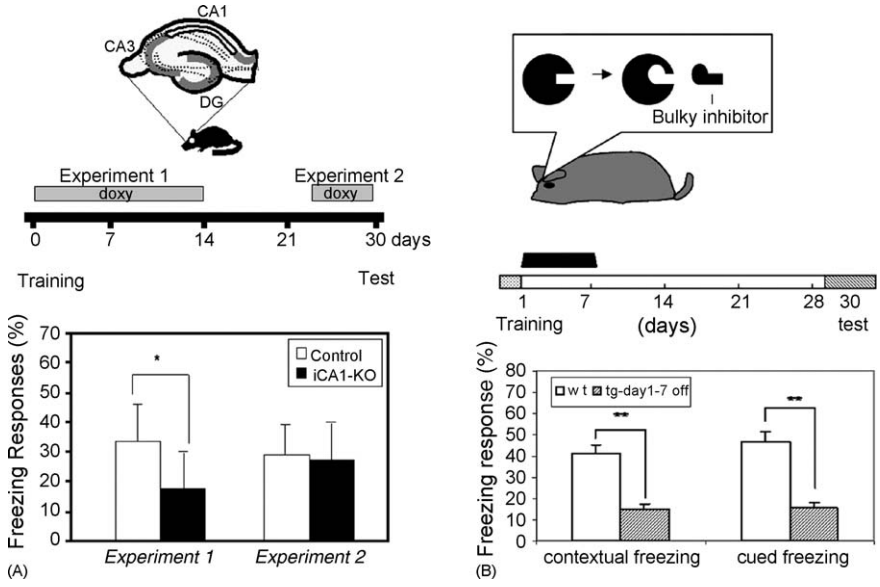

Fig. 3. Reactivations of the NMDA receptor and CaMKII are required for memory consolidation. (A) The inducible knockout of the CA1 NMDA receptor in mouse hippocampus in the initial weeks post-training leads impairments in 1-month-old hippocampal memories (contextual fear memory shown here in Experiment 1). Whereas inducible knockout of the CA1 NMDA receptor at late stage, before retrieval does not impair memory recall (Experiment 2). The figure adopted from Shimizu et al. (2000). (B) The inducible manipulation of αCaMKII activity in the forebrain during the first post-training week also impaired the consolidation of long-term contextual and cued fear memories. The cartoon above illustrates the bump-and-hole based chemical genetic method which a rationally designed bulky inhibitor can specifically inhibit the transgenic αCaMKII in which a hidden cavity inside of ATP-binding pocket was created by targeted mutagenesis. The figure adopted from Wang et al. (2003).

temporal processes for the analysis of memory consolidation (Wang et al., 2003). The systematic temporal manipulation of CaMKII activity during the memory consolidation period suggests that the first post-learning week is the critical time-window during which changes in CaMKII activity level disrupt the consolidation of 1-month-old fear memories (Fig. 3B). Therefore, both inducible CA1-specific NMDA receptor knockout and inducible manipulation of forebrain CaMKII activity levels strongly illustrate that memory consolidation is an active and dynamic molecular process that requires multiple rounds of reactivations of the NMDA receptor and αCaMKII.

The consistent requirement of the NMDA receptor in the two distinct temporal stages, namely, memory acquisition (learning) and memory consolidation, raises the question as to whether mild performance deficits observed in the CA3-specific NR1 knockout mice in the partial cue retrieval test of the water maze can be truly interpreted as the recall deficits (Nakazawa et al., 2002). Since the knockout was not inducible, a more likely explanation might be that the retrieval deficits actually reflect the incomplete pattern formation and binding during the learning and consolidation. In support of this alternative explanation, acute pharmacological infusion of the NMDA blockers right before retention tests has not reported significant effects on the retrieval of previously acquired memories. Thus, one needs to generate inducible knockout mice to examine the role of the CA3 NMDA receptor in recall.

3.4. Synaptic reentry reinforcement (SRR) hypothesis for memory consolidation

The discovery of the requirement of NMDA receptor reactivations for memory consolidation has led to an alternative hypothesis known as synaptic reentry reinforcement (SRR) (Shimizu et al., 2000; Wittenberg and Tsien, 2002). The SRR hypothesis posits that memory consolidation needs multiple rounds of NMDAR-mediated synaptic modifications to reinforce the synaptic changes as a cellular means to counteract the synaptic efficacy drift resulting from metabolic turnovers of synaptic receptors (Fig. 4). More important, the SRR process serves as a cellular mechanism for the hippocampus to transfer and convert new short-term memories to the cortex for permanent storage over the time course of week(s) (Shimizu et al., 2000). During the post-learning consolidation period (days and weeks), the hippocampus could act as a coincidence regenerator for activating cortical neurons (Fig. 5). This would allow cortical neurons previously corresponding to the different sensory modules to be reactivated simultaneously, consequently strengthening the connections between those cortical neurons in a gradual manner.

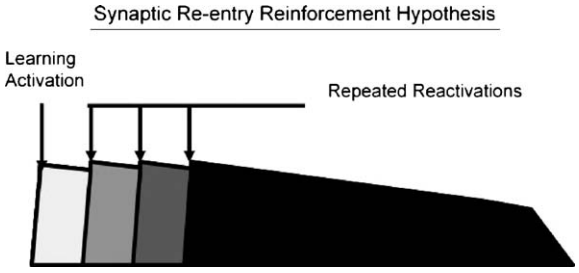

Fig. 4. The ‘‘synaptic reentry reinforcement (SRR) hypothesis’’ for memory consolidation. To achieve synaptic consolidation, repeated reactivations of the NMDA receptor are required for converting the short-term memory into long-term memory. The SRR is capable of overcoming the accumulative drift in synaptic efficacy as resulting from routine metabolic turnovers of synaptic proteins.

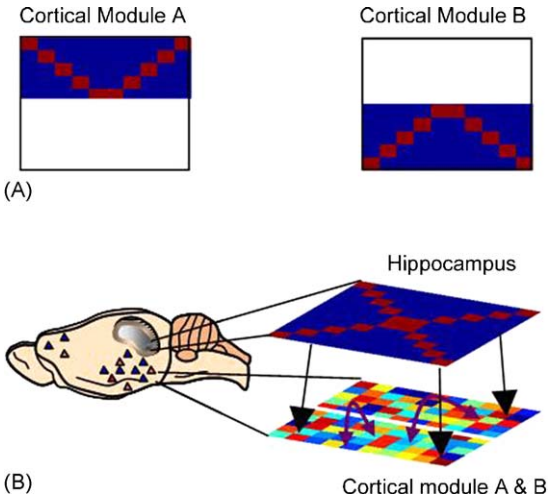

Fig. 5. Coherent SRR process in both the hippocampus and cortex during memory consolidation. SRR within the hippocampus is required in order to provide coordinated output to drive cortical memory consolidation. (A) During learning cortical modules A and B are activated, and provide input driving hippocampal neurons. (B) During consolidation, the hippocampus (in grey banana-shaped region) reactivates and further strengthens the stored memory trace by SRR. Coherent hippocampal reactivation provides coordinated reactivation of cortical modules, resulting in the SRR-based strengthening of synaptic efficacies primarily between cortical modules A and B, as well as within each module. Colored triangles represent cortical neurons undergoing repeated reactivations for achieving consolidation and storage. The figure adopted from Wittenberg et al. (2002).

The hypothesis that the SRR process can account for both molecular and systems-level memory consolidation has been further elaborated by the computational modeling using the simulation of a recurrent network consisting of 2500 neurons (Wittenberg et al., 2002). The computational analysis shows that in the presence of repeated NMDAR-mediated synaptic modification, SRR is fully capable of consolidating long-term memory traces. On the contrast, in the absence of SRR, synaptic efficacy cannot be stably consolidated and preserved; consequently, the memory traces gradually become unreliable, thereby undermining long-term storage of information in the brain.

In theory, the SRR-mediated off-line strengthening of synaptic connections requires only pair-wise reactivation between two activated neurons. Thus, this synaptic reactivation feature does not necessarily involve the reactivation of memory at the cognitive level. When and how does SRR occur? What triggers the SRR process and when does SRR usually take place? One conceivable triggering mechanism could be conscious recall initiating the SRR process. This is consistent with common experience, where the more frequently a particular memory is recalled, the better and longer it will be remembered.

Another triggering mechanism could be the spontaneous reactivations of the brain networks in the awake state. By using a 96-channel array capable of simultaneously recording the activity patterns of as many as 260 individual neurons in the mouse hippocampus during various episodic events (Lin et al., 2006a), Lin et al. have found that the mnemonic episodes triggered firing changes in a subset of CA1 neurons in both startle-type and environment-dependent manner (Lin et al., 2005). The application of the multiple-discriminant analysis (MDA) and hierarchical clustering methods has led to the discovery of memory-encoding units and their organizational structure (Lin et al., 2005, 2006b). It also allowed, for the first time, the visualization and monitoring of network-level memory patterns and their spontaneous, immediate reactivations in the awake-behaving state (Lin et al., 2005). Interestingly, those reactivated trace trajectories are geometrically similar to the encoding traces during learning and occurred with variable intervals and frequency (Fig. 6).

Similarly, SRR could also be achieved during sleep because the sleep state is known to produce high levels of synchronized neuronal activity across many brain regions. For example, Datta and his colleagues showed that after learning trials, rats spent more time in both REM sleep and the transitional state between slow-wave sleep and REM sleep, associated in the density of pontine-waves that were generated in the pons by a group of cells firing as high as 500 Hz (Datta, 2000). More interestingly, those P-wave generating cells send their efferent projects to the hippocampus, amygdala, and other cortical structures known to be involved in memory processing, thereby providing a high-frequency input to activate the NMDA receptor-mediated signaling pathway in those forebrain regions (Datta, 2006). Consistent with this anatomical observation, McNaughton and his colleagues have used correlation-based analysis to show the heightened firing correlation between overlapping place cells in the CA1 during sleep (Wilson and McNaughton, 1994; Sutherland and McNaughton, 2000). It is important to mention that consolidation of synaptic traces during sleep only requires pair-wise co-reactivation between the connected neurons to maintain their existing synaptic efficacy.

3.5. Clearance of outdated memory traces in the hippocampus—is adult dentate neurogenesis a means for memory clearance?

What happens to memory traces left behind in the hippocampus after long-term memories have been successfully stored in the cortex? It has been estimated that the hippocampus may have limited storage capacity. For instance, there are roughly only 200,000–300,000 CA3 and 300,000–400,000 CA1 pyramidal cells, and 700,000–1,000,000 granule cells in the rodent hippocampus. It is conceivable that continued accumulation of outdated memory traces in the hippocampus could overload the system over time, eventually disabling the ability of hippocampal functioning in memory consolidation. So how might the hippocampus deal with this overloading problem?

The possible answer comes unexpectedly from genetic experiments originally aimed at investigating the role of the presenilin-1 gene, whose mutations are responsible for more than 95% of the familial early onset Alzheimer's disease (Price and Sisodia, 1998; Hardy, 1997). This type of Alzheimer's disease is known to be the most aggressive form that can cause severe memory loss and dementia in patients as early as in their 30 s (George-Hyslop, 2000). Recent studies have shown that

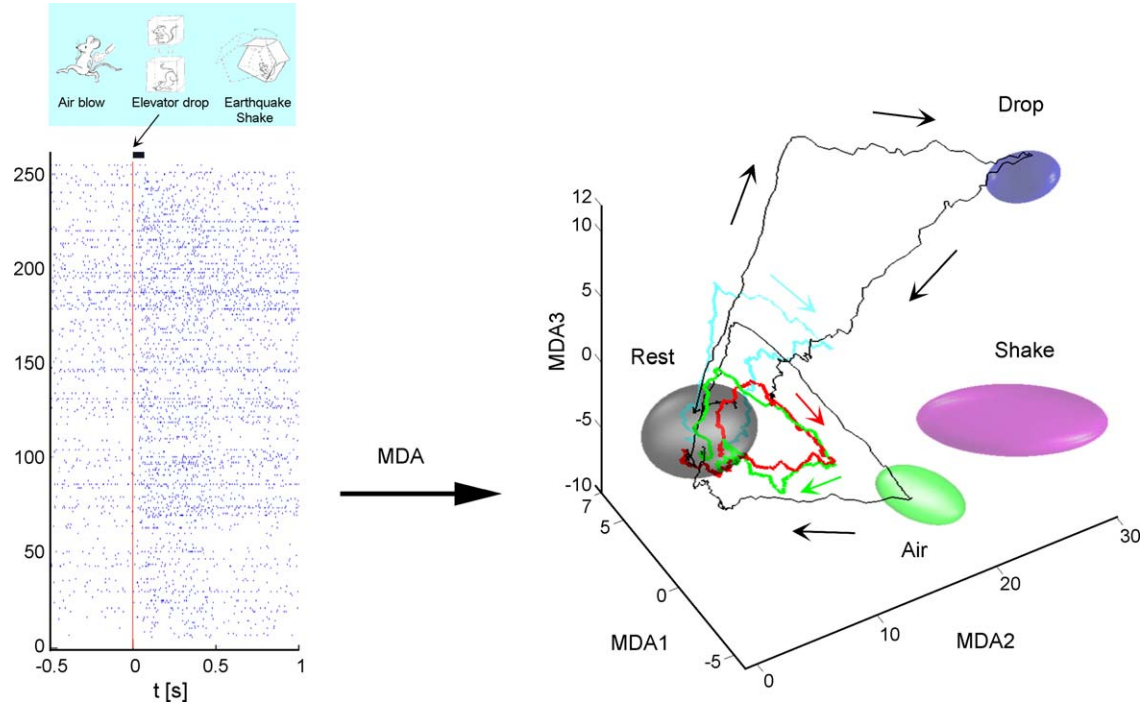

Fig. 6. Visualization of network-level memory encoding patterns and their reactivations in the hippocampus during the awake state. The activity of 260 simultaneously recorded single neural units (listed in *Y*-axis of the left side panel) from a mouse brain during a period of 0.5 s prior to and 1 s after the occurrence of startling episodes ($t = 0$ marked with vertical red line) has been reduced into a three-dimensional encoding subspace by MDA method. In this encoding subspace (each axis, MDA1, 2 and 3 are vectors which provides the best pattern separation), the distinct ensemble memory patterns during the resting state (yellow ellipsoid), air-blow (green ellipsoid), drop (blue ellipsoid) and earthquake (magenta ellipsoid) epochs can be easily visualized. Dynamical monitoring of memory formation (black trajectories) and reactivations (red or green trajectories) are shown as triangular lines. This MDA-based decoding method has unprecedented sensitivity, it can detect the spontaneous reactivations of newly formed memory traces, represented by dynamical trajectories with the similar geometric shapes but smaller amplitudes, occurring causally at intervals arranging from several seconds to minutes after the actual event. The figure adopted from Lin et al. (2006a,b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

forebrain-specific knockout of the presenilin-1 gene results in a pronounced deficiency in enrichment-induced adult neurogenesis in the dentate gyrus (Feng et al., 2001). It has been revealed that deficient neurogenesis in the dentate gyrus is associated with impaired clearance of outdated memory traces from the hippocampus. This unexpected finding has led to a hypothesis that adult neurogenesis in the dentate gyrus plays a crucial role in periodic clearance of outdated hippocampal memory traces after cortical memory consolidation, thereby ensuring that the hippocampus is continuously able to process new memories (Feng et al., 2001). On the other hand, chronic abnormal clearance process in the hippocampal system, caused by a presenilin-mediated neurogenesis deficiency, may lead to memory disorders as observed in Alzheimer' patients.

This proposed ''neurogenesis-memory clearance hypothesis'' is attractive because addition and removal of adult-born neurons in local hippocampal network could gradually destabilize the stored memory traces. Moreover, since the adult neurogenesis occurs in the dentate gyrus which is located in the upstream point of the hippocampus, its destabilization effect is likely to be amplified throughout the hippocampal tri-synaptic circuits. Interestingly, these newborn neurons are short-lived, typically with a life-span of 3 weeks in rodents (Cameron et al., 1993; Hastings and Gould, 1999), which seems to coincide with the time-duration for the hippocampal engagement in memory consolidation.

3.6. SRR is also crucial for the consolidation of hippocampal-independent memories

For clinical–neurological reasons, most consolidation experiments have been focused on the investigation of consolidation of declarative memories because they are sensitive to the damage to the hippocampus. On the other hand, it is known that non-declarative memory can still be formed and converted to long-term memory in a hippocampal-independent manner. Historically, the consolidation of long-term non-declarative memories has not received the same level of attention and, as a result, the molecular mechanisms underlying the consolidation of non-declarative memories remain relatively unexplored.

Conditioned taste aversion (CTA) is a valuable memory paradigm for the study of the formation of long-term non-declarative memory in the mammalian brain (Hogan, 1973). When an animal encounters a novel taste and later experiences nausea, it develops an extreme aversion to the novel taste. This behavior has been shown to be conserved among many species, ranging from humans to rats, most likely due to its importance for survival. CTA occurs when an animal encounters a novel taste which is followed by malaise approximately 1.5–3 h later (Hogan, 1973; Yamamoto et al., 1994; Schafe et al., 1995; Bures et al., 1998). The formation of CTA memory is known to involve cortical areas such as the insular cortex and other

subcortical regions (Braun et al., 1972; Bermudez-Rattoni and McGaugh, 1991; Bures et al., 1998). CTA can produce a robust long-term memory and is usually learned in a single trial thus is suitable for the detailed analysis of various temporal stages of memory process (Bures et al., 1998).

Cui et al. employed inducible gene knockout technique to investigate the role of the cortical NMDA receptor during the various temporal stages of conditioned taste aversion memories (Cui et al., 2005). They have shown that temporally restricted knockout of the cortical NMDA receptor during either the learning or the post-learning consolidation stage, but not during the retrieval stage, causes severe performance deficits in the 1-month taste memory retention tests. More interesting, they have demonstrated that the consolidation and storage of the long-term non-declarative taste memories requires cortical NMDA receptor reactivations (Cui et al., 2005). Thus, the dynamical engagement of the NMDA receptor during the post-learning stage suggests that NMDA receptor reactivation-mediated SRR is also crucial for achieving consolidation and storage of non-declarative memories in the brain. Currently it is not clear when the consolidation of taste memory ends and the storage process begins. This, in a way, also reflects the history of memory research in which those two processes have never really been precisely defined. In the literature, the conventional definition of memory consolidation often refers to the consolidation as the hippocampal-dependent process, whereas the storage refers to the state of memory once becoming independent of the hippocampus. So far, there is no good definition when it comes to the hippocampal independent non-declarative memory.

3.7. SRR for the stable storage of remote memories in the brain

Although long-term memory is known to consist of several distinct temporal stages, the vast majority of experiments to date have been focused on the analysis of learning and consolidation. Little attention has been directed towards understanding the molecular process underlying the storage of remote memories in the cortex (Wiltgen et al., 2004). It is generally believed that long-lasting memory is stored in the form of structural synaptic modifications triggered by original learning. Such structural changes, once laid down, have been assumed to confer the long-term stability of stored memories. The observation that molecular and structural machineries at the synapse undergo routine metabolic turnover (Shimizu et al., 2000), an intrinsic process likely independent of whether memory is in the dormant or active form, also raises the fundamental question as to how the memory remains stable over time.

To investigate how the brain preserves its delicate synaptic efficacies over a long period of time, Cui et al. generated inducible and reversible knockout mice in which the NMDA receptor can be temporarily switched off in the cortical regions during the memory storage stage (Cui et al., 2004). It has been reported that the NMDA receptor, especially the NR2B-containing NMDA receptor in the prefrontal cortex is involved in the formation of long-term fear memory (Zhao et al., 2005). To increase the certainty that the molecular manipulation occurred during the storage stage, Cui et al. designed a series of experiments in which the NMDA receptor was temporarily disabled 6 months after the original training. Since this 6-month duration corresponds to approximately one-fourth of a mouse's life expectancy, the researchers reasoned that memory consolidation should be completed in 6 months after learning occurs. Thus, the ''inducible-knockout-6-month-after-learning'' paradigm should allow our temporal analysis of NMDA receptor function restricted to the storage, not consolidation, phase of remote memories.

Specifically, Cui et al. have demonstrated that the 9-month retention of both contextual and cued fear memories in the inducible and forebrain-specific NR1 gene knockout (iFB-KO) mice is profoundly impaired by administering doxycycline (dox), a tetracycline analog capable of diffusing into the brain and switching off tTA-mediated transgene NR1/GFP expression for 30 days beginning 6 months after fear conditioning but ending 2 months before memory retrieval (Fig. 6) (Cui et al., 2004). In a subsequent set of memory tests including novel object recognition and a new (second) contextual fear conditioning following the completion of the 9-month retention tests, these mice exhibited normal capacity to learn, retain and retrieve new memories. Therefore, the observed 9-month-retention deficits are likely to reflect the disruption of the storage process rather than the disturbance of recall or performance capability.

Furthermore, untreated iFB-KO mice learned and retained 9-month-old fear memories as effectively as untreated control mice, suggesting that observed deficits in the dox-treated iFB-KO mice were not simply caused by genotypic alterations. Equally important, the control mice receiving the same 30-day-dox-treatment showed normal retention of 9-month contextual and cued fear memories, indicating that dox feeding alone did not produce detectable side effects on learning behavior. In addition, long-term dox treatment (1 month) does not alter nociceptive responses as measured by the amount of current necessary to elicit flinching/running, jumping, and vocalizing in mice (Shimizu et al., 2000). Finally, as shown by those authors, the 30-day-dox-treatment in iFB-KO mice did not alter their performance in the open-field test and rota-rod test, indicating normal cerebellar coordination and locomotor function.

The use of both contextual and cued fear conditioning has also allowed the researchers to simultaneously investigate the storage mechanisms underlying neurologically different types of memories: namely, the hippocampal-dependent (contextual) and hippocampal-independent (cued) fear memories (Phillips and LeDoux, 1992; Maren, 2001). Similar to the disruption of both types of fear learning by disabling the NMDA receptor function, it seems that the prolonged knockout of the NMDA receptor in the forebrain during the storage stage results in profound storage deficits for both contextual and cued fear memories (Fig. 7) (Cui et al., 2004). Therefore, those studies suggest that NMDA receptor-mediated SRR process is also critical for the storage of remote memories in the brain, regardless whether memories were formed initially in the hippocampal-dependent or hippocampal-independent manner.

The role of αCaMKII in cortical storage has also been implicated by Silva and his colleagues during the analysis of

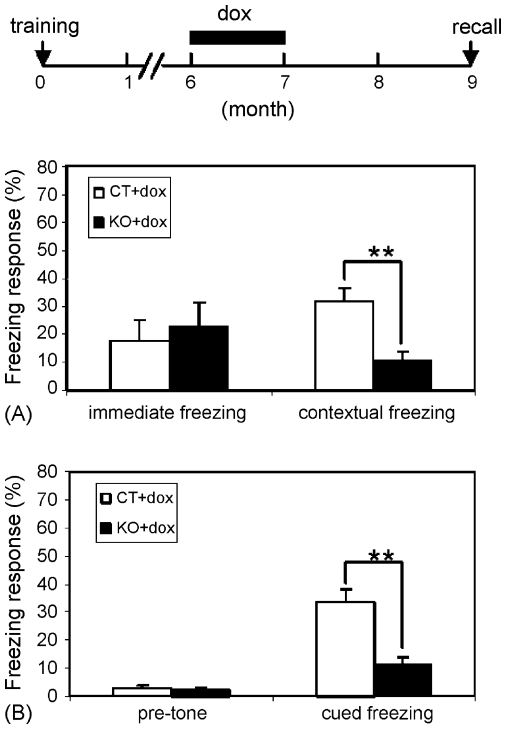

Fig. 7. Periodic reactivations of the NMDA receptor are required for the long-term storage of remote memory in the brain. The forebrain-specific NR1 knockout induced by 30-day-dox-treatment during the 7th month disrupts the storage of the 9-month-old remote contextual and cued fear memories. (A) Contextual learning in these two groups of mice during initial training was the same as shown by similar amount of immediately freezing. However, the retention of 9-month-contextual fear memory was significantly impaired in dox-treated inducible knockout mice in comparison to that of dox-treated control mice. (B) A similar storage deficit was also observed in dox-treated inducible knockout mice as revealed by tone-elicited cued memory retention test. The data adopted from Cui et al. (2004).

global knockout mice carrying an αCaMKII heterozygous null mutation (Frankland et al., 2001). It was shown that this mutation is correlated with selective impairment in the formation of remote hippocampus-dependent memory (tested 10–50 days post-training). This impairment coincides well with electrophysiological data which show normal hippocampal LTP, but impaired cortical LTP (Frankland et al., 2001). Therefore, emerging molecular and genetic analyses suggest that the storage of remote memory requires the dynamic engagement of the NMDA receptor and its downstream signaling pathway.

4. Conclusion

Until recently, the traditional view has been that memory consolidation and storage are a permissive process as the result of learning-activated molecular signaling cascades such as activations of receptors and kinases, new protein synthesis and CREB-mediated gene expression, and subsequent structural modifications at certain synapses. A major new development in the field is the realization that synaptic proteins are not stationary, but rather highly dynamical and routinely undergo metabolic turnovers. Such metabolic turnovers of synaptic machinery can cause accumulative drift in synaptic efficacy, thereby unstable storage of memory traces in the neural circuits. The latest sets of molecular genetic experiments have revealed that post-learning NMDA receptor reactivations are required for the consolidation of both hippocampal-dependent and hippocampal-independent memories. Furthermore, the reactivations of the NMDA receptors are also necessary for the stable storage of remote memories and long-term stability of the brain circuitry. Therefore, the NMDA receptor reactivation-mediated synaptic reentry reinforcement (SRR) process serves as a crucial cellular mechanism in linking the consolidation and storage of long-term memories from the molecular level to the systems-level.

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