A PRION LIKE-PROTEIN, PROTEIN KINASE MZETA 
AND MEMORY MAINTENANCE

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ABSTRACT

Molecular studies of both declarative and non-declarative memory in Aplysia californica, lymaea stagnalis and hippocampal slices implicate experience-dependent changes of synaptic structure and strength as the fundamental basis of memory storage and maintenance. The essential outcome of these changes in synaptic structure and strength is our ability to remember what we are thought. Remembrance is of critical importance. In disease conditions like Alzheimer’s there is lack of the ability to recreate the past. From this perspective, memory literally is the glue that binds our mental life, the scaffolding that holds our personal history and that makes it possible to change throughout life. What causes memory persistence after labile phase of memory is not yet fully known. Elegant discoveries have explained why labile memory phase could persist over time into long term memory phase. Synaptic connections are not fixed but become modified by learning. These modifications in synaptic structure and strength persist and become the fundamental component of memory storage after learning. Learning-induced changes in behavioural performance are the result of a fundamental physiological phenomenon. The fundamental physiological phenomenon is neuronal plasticity. In the process of neuronal plasticity, we review only the emerging aspect of the roles of prion like-protein, neuronal astrocyte and protein kinase Mzeta (PKMζ) in memory maintenance.

Keywords: Memory Maintenance, NMDARs and AMPARs, CPEB, Neuronal Lacate and Protein Kinase Mzeta.

INTRODUCTION

Memory defines the ability to retain, store and recall events. Memory maintenance is the process of keeping optimally these events. For instance, the beautiful nature of Sussex genomic center and its Medical School are examples of explicit or declarative memory. Memories such as these are stored very well in the brain for recall of details later in life. Apart from these explicit or declarative memories another type of memory is implicit or non-declarative memory. In this latter type of memory, motor skills and other type of tasks are done through performance with no conscious recall of past experience. For instance riding a bicycle and driving a car. Studies suggest that experience-dependent changes of synaptic strength, growth, structure and fundamental mechanism are ways of which these memories are encoded, processed and stored within the brain (Hawkins et al., 2006; Bailey et al., 2004; and Beckinschtein et al., 2010). In these processes of initial memory formation and consolidation, memory basically exists in forms. These forms may include; short term memory (STM), intermediate memory (IM) and Long term memory (LTM) (Beckinschtein et al., 2010). There is also early and late LTM. Memories are maintained because, if all these memories are formed by similar molecular process, then what accounts for these types of basic memory? LTM is the type of memory that lasts for numerous hours and years (Beckinschtein et al., 2010). Several amount of information regarding the participation of many biological molecules, pathways and brain areas in LTM formation.
exist (Bekinschtein et al., 2010). The feature that preserves this ‘long’ in long term memory perpetuation within duration of time demand critical understanding. A key process of this feature is consolidation, where genes are expressed, new proteins synthesized and synaptic connections strengthened. Before these consolidation events is posttranslational modification of proteins. Recent molecular mechanisms have been proposed which ensure this ‘long’ in LTM which provides the bases for understanding and appreciating how memory could last for durations of time (Si et al., 2010; Si et al., 2003; Chae et al., 2010; Suzuki et al., 2011 and Bezzi and Volterra, 2011). Despite the above information, understanding how pieces of information from our external environs are encoded and processed for storage in our brain leading to memory formation still poses one of the greatest challenges. Here, we review some of the emerging aspect of the roles of prion like-protein, neuronal astrocyte and protein kinase Mzeta (PKMζ) in memory maintenance.

**Astrocytes and Memory Persistence**

Lactate is an intermediate metabolic product of glucose. The demand is intense in the muscles during exercise when energy production through anaerobic respiration is insufficient. Recent studies have reported the role of astrocytes in memory (Suzuki et al., 2011 and Brooks, 2009; Figure 1). Halassa et al., 2009 reported astrocyte adenosine change in sleep homeostasis and cognitive consequences of sleep loss. Memory maintenance is a product of long lasting changes occurring following structural changes in synapses. Very central and vital to the maintenance process is the work of N-methyl D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). NMDARs initiate synaptic plasticity while regulated AMPARs trafficking to and from the synapse is a major component of synaptic plasticity in memory formation. It appears amazing that a brain cell, astrocyte could be participating in the formation of this memory (Henneberger et al., 2010; Santello and Volterra, 2010). Henneberger et al., (2010) demonstrated that increase in astrocyte Ca\(^{2+}\) induces the release of molecules needed for synaptic memory formation (Figure 1). The result of their work showed that introduction of Ca\(^{2+}\) blocker prevented increase in intracellular Ca\(^{2+}\) and abolished initiation of LTF at the surrounding synapse (bottom panel of figure 1). Therefore, the labile phase of memory

**Figure 1. Hippocampal Neuronal Astrocyte.** This illustrates an elegant data from whole-cell recording procedure following potentiation by High frequency stimulation (HFS) of a presynaptic bouton. Vice versa, two neuronal astrocyte cells of the Schaffer collateral-CA1 of the hippocampus were involved; one (bottom) clamped that is its Ca\(^{2+}\) kept relatively constant using Ca\(^{2+}\) buffer, the other (top) control that is when the neuronal astrocyte cell was loaded with D-serine using pipette. Keeping the neuronal astrocyte Ca\(^{2+}\) concentration constant in the formal abolished enhancement of long term potentiation. When D-serine was added in the latter it restored enhancement of LTP in an NMDA dependent fashion. Following HFS, increased Ca\(^{2+}\)concentration did not allow D-serine to activation NMDA receptors in the formal but does in the latter, thus the enhanced LTP. (Henneberger et al., 2010).
Figure 2  Astrocyte-Neuronal Lactate: This succinctly explains top-down approach where models (vertebrate and invertebrate) are trained and after training the molecular mechanism of memory and learning elucidated e.g. in robust paradigm of classical conditioning. Hippocampus is very essential in memory consolidation. A) is a rat hippocampus following training task. B) is the biochemical changes and molecular involvement at CA1 synapses (brown colour) and the astrocytes (blue colour) of the rat hippocampus. Following learning in the rat hippocampus, lactate is formed as an intermediary product of glycogen. This lactate is transported by lactate transporters-MCT4 into the neuron. The availability of the lactate in the neurons helps in kinases activation, and enhanced other processes necessary for formation of LTM (Paola and Andrea 2011, in Suzuki et al., 2011).

is not even enhanced not to talk of the lasting phase. D-serine can bind with NMDAR-glycine. The binding of D-serine with NMDAR-glycine activates NMDARs channel to open when glutamate binds (top panel of figure 1). This binding process importantly demands attention because it appears to be part of the major process of NMDARs activation through membrane depolarization. Previous studies (Diamond, 2006 and Shleper et al., 2005) of the astrocyte cell culture and hippocampal slices suggest that astrocytes can release D-serine via Ca\(^{2+}\)-dependent exocytosis and therefore astrocytes are the possible source of the D-serine that activate NMDARs.

Extracellular lactate concentration in rat hippocampus increased immediately after engaging in learning task (Suzuki et al., 2011 and Bezzi and Volterra, 2011). This increase in astrocyte neuronal-lactate may be critically fundamental for the establishment of the long term memory after learning task (Figure 2). This is a crucial role of lactate, a byproduct of glycogen not glucose. Lactate has different effect from glucose in two ways: lactate depends on monocarboxylate transporters (MCTs) while glucose does not and lactate has signaling function unlike glucose (Bezzi and Volterra, 2011). Blocking the expression of astrocyte transporter (MCT4) but not (MCT2) caused memory loss. This was restored by addition of L-lactate (Suzuki et al., 2011). This could imply that L-lactate is transported from astrocyte into neurons and not vice versa. This lead to their conclusion that astrocyte neuronal-lactate is required for long term memory establishment. Figure 2 further explains these findings. Intriguingly, Suzuki and colleagues reported that cAMP response element binding protein (pCREB), the most influential material for gene expression and activity-dependent gene Arc expression for long term synaptic plasticity, synaptic structural changes and consequent memory formation rely on astrocyte neuron lactate transporter-MCT4 (Figure 2). Furthermore, it posited that lactate constitutes the energy source used by activated neurons during protein synthesis, breakdown and activation of signaling pathways necessary for the regulation of gene expression characterizing LTM. This demands more essential studies to elucidate lactate transport pathways, lactate transporters and their specific roles.

The Role of Prion Like-Proteins

Prions are a special class of proteins best known as the causative agent of neurodegenerative disease (trans-
Prion protein can adopt an alternative self-perpetuating conformation that resembles any of the established processes of memory induction. For instance, is the conversion of ApCPEB from monomeric to multimeric enhanced by application of stimulus (given repeated pulses of 5-HT)? To investigate whether or not ApCPEB of yeast has similar behavior in neurons, Si et al., (2010) over-expressed enhanced green fluorescence protein (EGFP) - tagged ApCPEB in sensory neurons of Aplysia. Consistent with what was observed in the yeast, deletion of N-terminal 252 amino acids reduced puncta appearance of ApCPEB. It has been stated earlier, protein synthesis is critically essential for the formation of both STM and LTM. Inhibition of protein synthesis by protein inhibitors example rapamycin and emetine does not allow for memory persistence. This occurs within a critical time window. Determination of the critical time window within which synthesized protein can be propelled to enhance other processes of activity-dependent synaptic changes ensuring memory persistence is essential. Knowing what propels the synthesized protein in ensuring this process is as well essential. The propellant could be the ApCPEB multimeric form. To understand the critical time course of long lasting synaptic plasticity in Aplysia cell culture (Miniaci et al., 2008) investigated 5-HT- induced increase in activity-dependent synaptic structure durability at the sensory-motor neuron. In their experiment, they applied a protein inhibitor, emetine (100um) at different time intervals of 24, 48 or 72h after bath application of five pulses of 5-HT to the sensory-motor neuron culture. At 24h, the protein inhibitor blocked protein synthesis. At 48h the level of the blockage was reduced. Then at 72h it was noticed that emetine had no interference with the maintenance of protein synthesis after 5-HT training. They therefore concluded that the labile state of memory is time-dependent after which it assumes a state that is more difficult to perturb with a help of a propellant i.e ApCPEB multimeric form. What regulates this protein synthesis (Si et al., 2003) has been found in the Aplysia neuron-specific isoform of CPEB, an RNA binding protein that promotes polyadenylation and translation activation. The ApCPEB play important role in persistence of LTF (Miniaci et al., 2008 and Si et al., 2003). To identify the critical time window for the requirement of CPEB to propel synthesized protein to produce an enduring activity-dependent synaptic changes in structure and strength and to compare this with that of protein synthesis using emetine, ApCPEB antisense oligonucleotide was perfused locally to covalently couple with 11 amino acid peptide of the sensory- motor neuron culture (Miniaci et al., 2008). Consistent with Si et al., 2003 the critical local time window was similar to the result of emetine during protein synthesis. It therefore became evident that it is the ApCPEB multimeric form, - the higher molecular weight atypical isoform but not the dominant and self-perpetuating form is required to persist long term facilitation.

Prion protein can adopt an alternative self-perpetuating conformation that causes this disease (Heinrich and Lindquist, 2010 and Si et al., 2003). Despite this negative reputation Si et al., (2003) reported that Aplysia cytoplasmic polyadenylation element binding protein (CPEB), a prion like-protein has important and very essential role in the memory. These proteins can exist in at least two distinct conformational forms, one of which is the dominant and self-perpetuating form (Si et al., 2010). Molecular mechanism that contributes to memory storage has been studied in details in the monosynaptic connections between sensory and motor neurons of the gill withdrawal reflex of Aplysia both in the intact animal and in the cell culture (Miniaci et al., 2004). The molecular mechanisms of long term facilitation (LTF) and synaptic growth is likely distinct from those of LTM perpetuation or maintenance (Bailey et al., 2004; Miniaci et al., 2008). Although increased synaptic strength could be found during short and long term memories, LTM differs from the short term memory in at least two different ways: structural changes and gene transcription (Bailey et al., 2004;). In Aplysia sensory – motor neuron synapses of gill withdrawal reflex, local protein synthesis are critical for two distinct functions (Bailey et al., 2004; Miniaci et al., 2008 and Si et al., 2010). These critical roles are: synthesis of retrograde signals that travel from synapse to the cell body to activate transcription processes and final synthesis of synaptic structures that stabilize the functional and structural changes at the synapse. The action of a protein inhibitor, rapamycin in blocking translation of synaptic mRNA in growth process, seems not to interfere with retrograde signals but blocks stabilizing synaptic structures (Casadio et al., 1999). Preventing stabilization of changes in synaptic structures does not produce LTM. This begs the question where in rapamycin is the stabilizing region that blocks enhancement of synaptic structures. In an effort to discover the stabilizing region for rapamycin, cytoplasmic polyadenylation element binding protein (CPEB) was made in Aplysia (Si et al., 2003). Cytoplasmic polyadenylation element (CPE)-binding protein (CPEB) binds to CPE containing mRNA on their 3’ untranslated region (Chae et al., 2010). CPEB has crucial role in perpetuating the functional and morphology of long lasting synaptic conformations that characterize learning and memory (Chae et al., 2010 and Heinricha and Lindquist, 2010). CPEB was first discovered in oocytes (Hake and Richter, 1994) and secondly in the hippocampal neurons (Wu et al., 1998).

Prion like-protein exists in two forms; the monomeric and the multimeric forms. Prion like-protein can be converted in yeast from monomeric form to multimeric form. This conversion may be essential for memory persistence and therefore demands to be tested in neurons. This assumption generated some interesting questions of whether or not the conversion process...
**Protein Kinase Mzeta ζ (PKMζ)**

The most physiological aspect of memory is long term potentiation. The mechanisms underlying potentiation could be grouped into two: induction and maintenance (Sacktor, 2008). Many kinases participate in induction processes but not in maintenance (Serrano et al., 2005), these kinase are: CAMKII, protein kinase A, protein kinase C (PKC) and mitogen activated protein kinase (MAP) (Sweat et al., 1999). PKC isoforms are grouped into three forms: conventional, novel and atypical. Each is made of one polypeptide of N-terminal regulating domain and a C-terminal catalytic domain joined by a hinge region. Second messenger activates PKC by binding to the regulatory domain, targeting the enzyme to the membrane and initiating structural changes that release autoinhibition (Hernandez et al., 2003). PKMζ is an atypical isoform type of PKC. It is produced from an alternate gene product ζ RNA instead from PKCζ. Thus, it is called atypical form of PKC (Hernandez et al., 2003). Apart from this proteolytic mechanisms, PKMζ is synthesized from brain mRNA (Hernandez et al., 2003).

The molecular mechanism of initiation of potentiation by other kinases is transient apart from the one by PKC isofoms, PKMζ that persists (Serrano et al., 2005) its actions longer. The regulatory domain of the other PKC isoforms possesses binding regions for second messenger and pseudosubstrate sequence that inhibits the catalytic domain during the process of their activity. In contrast, PKMζ has separate catalytic domain quite distinct from those of other PKC isoforms. More so, this atypical PKC isoform, PKMζ lacks inhibition from the pseudosubstrate (Serranto et al., 2005). Lacking the regulatory domain autoinhibition this catalytic domain is constitutively and thus persistently active (Hernandez et al., 2003) to function.

The Step by step roles of NMDA and AMPA receptors in for instance the hebbian forms of Plasticity is essential to understand the critical role of PKMζ in memory maintenance. To begin with, NMDARs initiate synaptic plasticity (Figure 1, top panel). Transient activation of NMDARs is followed by increase in the post-synaptic calcium concentration and then activation of intracellular Ca-dependent machinery that mediates the enhancement of AMPA receptor function. This would then produce increase in synaptic strength and the LTP. Before the LTP, interestingly, AMPA receptor is made up of subunits (GluR1-4). By way of phosphorylation, PKMζ acts through GluR2 subunit to persistently shuttle AMPA receptors to the postsynaptic terminal, thus increasing the number of AMPA receptors at the postsynaptic terminal. This increase of AMPA receptors at the postsynaptic terminal produced synaptic potentiation (Sacktor, 2011), thus the LTP which is the physiological form of memory maintenance.

**CONCLUSION**

Principally two basic mechanisms of memory maintenance have been described in declarative and non-declarative memories. The first mechanism is in STM where memory last from minutes to hours. Here, there are changes in the strength of pre-existing synaptic structures following posttranslational modification of pre-existing proteins. The second mechanism is in LTM where memory last from hours to years. This involves the synthesis of new proteins, gene expression and increase in strength of synaptic connection and structure. The knowledge about the particular roles of a prion like-protein, neuronal astrocyte and protein kinase mzeta in memory maintenance is absolutely necessary. It is remarkable to note that astrocyte-neuronal lactate transport is essential for long-term synaptic plasticity, long-term memory, and their underlying molecular and synaptic changes. This could be by way of import of neurotransmitter substances to the pre and post synaptic terminals in a process that may involve ATP release. This is a signaling process that demands robust investigation. PKMζ is a self-active atypical isoform of PKC. PKMζ is capable of enhancing synaptic transmission during long-term potentiation (LTP) maintenance. PKMζ has absolute effect on the trafficking of AMPARs by shuttling them to the postsynaptic compartment. This shuttling event potentiates synaptic transmission and persistently increases their numbers at the postsynaptic structure. The shuttling of AMPARs to the postsynaptic terminal therefore perpetuates memory maintenance. CPEB may be critically important to the maintenance of memory storage perhaps in two ways. It could be that CPEB stabilizes the labile form of synaptic growth by directing the local synthesis of cytoskeletal components N-actin of the presynaptic compartment through mRNAs that encode structural proteins. A possible process of mRNAs involvement could be through polyadenylation. The next could be by regulating molecules necessary for synaptic growth and maturation. Put together, this study highlights the contributions of prion like-protein, neuronal astrocyte and PKMζ in memory maintenance. These molecules act at the level of structural changes in neuronal plasticity making them critically essential for memory maintenance. The holistic view of this study proposes a causal relationship between: prion like-protein, neuronal astrocyte and PKMζ at the level of enhancing activity-dependent synaptic changes in strength and structure to enhance memory maintenance. This calls for further robust research investigations to elucidate the exert roles of prion like-protein, neuronal astrocyte and PKMζ in memory maintenance.
REFERENCES


