

UNDERSTANDING THE NEUROBIOLOGICAL MECHANISMS OF LEARNING AND MEMORY: CELLULAR, MOLECULAR AND GENE REGULATION IMPLICATED IN SYNAPTIC PLASTICITY AND LONG-TERM POTENTIATION. PART IVB

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SUMMARY

Extensive cellular and behavioral studies have led to the postulation that memories are encoded by changes in synaptic strength between neurons, as demonstrated by the correlation between long-term changes in animal behavior and long-term changes in neuronal connections underlying a specific behavior in invertebrate animals, or even in vertebrate animals, where cellular models of synaptic plasticity using genetic approaches, such as *Long-Term Potentiation* (LTP) and *Long-Term Depression* (LTD), have been shown to depend on long-term changes in synaptic activity implicated in behavioral learning and memory. Long-term memory (LTM) is crucial for animal's survival and thus represents a mechanism that underlies fundamental neurobiological events in the nervous system of vertebrate and non-vertebrate species including the human. Long-term changes in synaptic connectivity as well as long-term behavioral changes (both activities that underlie several of the properties of LTM and are used as a parameter to explain the long-lasting enhancement of neuronal function after a stimulus) have been demonstrated to rely on signals that initially occur in the cell body. LTP is a form of synaptic plasticity widely accepted as a cellular model for stabilization of synapses in neurobiological phenomena such as development and learning and memory. Much of the experimental work concerning LTP in learning has been focused on the NMDA receptor dependent forms of LTP. But several questions have arisen regarding if LTP equals memory. If LTP has a real role in memory, a more appropriate hypothesis should be stated by postulating that activity-dependent synaptic plasticity and multiple forms of memory known to exist, share a common core; that is, the synaptic plasticity and memory hypothesis states that *activity dependent synaptic plasticity is induced at appropriate synapses during memory formation*. Synaptic plasticity is a physiological phenomenon that induces specific patterns of neural activity sustained by chemical and molecular mechanisms, that gives rise to changes in

synaptic efficacy and neural excitability that long outlast the events that trigger them. Based on the various properties of synaptic plasticity discovered, LTP may be proposed as a suitable neuronal mechanism for the development of several memory systems, including initial encoding and storage of memory traces and initial phases of trace consolidation over time. Such memory processing made up by LTP or LTD most probably occur as a network specific process, making LTP a universal mechanism for encoding and storage of memory traces and, what gets encoded, is part of a network property rather than mechanisms working at individual synapses. For example, the type of information processed at the hippocampus is quite different from the information processed by the amygdala, and such information should remain if the mechanisms of plasticity operating in each brain area are conserved. Decades of research have demonstrated that LTP in the hippocampus is induced by synaptic activity and that cytoplasmic membrane-bound molecule(s) are required to transduce extracellular signals mediated by receptor-activation into activation of intracellular signaling processes. Most of these processes depend on intracellular calcium activity, and thereby on calcium-dependent mechanisms that are recruited for LTP induction and expression. For instance, NMDA receptors have been shown to be essential for initiation of LTP, but the expression of this phenomenon is brought about primarily by AMPA receptors. Induction of LTP in CA1 hippocampal region has been shown to depend on increases of intracellular calcium and activation of specific calcium-dependent molecules such as the calcium/calmodulin-dependent protein kinase (CaMKII), whose cell expression is confined predominantly at postsynaptic densities. Moreover, long-term expression of LTP requires protein synthesis, where transient signals will be linked to activation of specific genes that ultimately will determine growth and remodeling of potential active synapses. Different types of synapses may express and use a different set of molecules mediating activation of intracellular signaling pathways to initiate and maintain synaptic

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plasticity. Several studies have demonstrated that neuronal modifications of neurotransmitter receptors or membrane-receptor subunits at postsynaptic densities represent one of the neuronal mechanisms by which neurons regulate their synaptic strength. For instance, it has been demonstrated that neuronal dendrites are able to regulate their own transmembrane receptor synthesis in response to external stimuli (i.e., GluR2 subunit of AMPA receptor) and such molecular mechanisms posed important implications in the understanding of how individual synapses are selectively strengthened. In addition, recent experiments have demonstrated that specific intracellular signaling molecules (i.e., neuronal Synaptic GTPase-activating protein or SynGAP) are selectively expressed and enriched at excitatory synapses. Interesting enough result the evidences that demonstrate that different subsets of protein kinases (MAPKs, SAPKs, MAPKAKs, p38MAPK, etc.) and intracellular signaling pathways activate transcription factors (AP-1 complex, CREB) that regulate the expression of different immediate early genes (IEG) that are crucial for neuronal development, glutamate receptor trafficking to specific synapses and for LTP induction. Much of the neurochemical and molecular changes that occurred in synaptic plasticity, may be well associated with dynamic morphological changes in spine synapses as suggested to participate in the development and consolidation of LTP. In addition, glial cells, known to participate in the excitatory neurotransmission in the CNS, besides their conceptualized cellular function as elements for structural support and homeostasis, may play an important role in synaptic plasticity and thereby, may regulate the information processed in the brain. As hippocampal LTP has been the target of intensive molecular genetic analysis, several studies have demonstrated that LTP is altered when particular single genes are knocked out or overexpressed in null mutant mice or transgenic mice. Such studies have led to the amazing observation that variations in LTP exist within natural inbred mouse strains.

Key words: Long-term potentiation, synapses, synaptic plasticity, NMDA receptor, AMPA receptor, protein kinase A, hippocampus, amygdale.

RESUMEN

Extensos estudios celulares y conductuales han llevado a la postulación de que la memoria es codificada por cambios en la fuerza sináptica entre las neuronas, como se ha demostrado por medio de la correlación entre los cambios a largo plazo en la conducta de los animales y en las conexiones neuronales que generan una conducta específica en animales invertebrados o vertebrados; en la que los modelos celulares de plasticidad sináptica, usando aproximaciones genéticas como el fenómeno de potenciación de largo plazo (LTP) o el fenómeno de la depresión de largo plazo (LTD), han demostrado que dependen de cambios a largo plazo en la actividad sináptica implicada en las conductas de aprendizaje y memoria. La memoria de largo plazo (LTM) es crucial para la supervivencia de los animales y representa un mecanismo fundamental para los eventos neurobiológicos en el sistema nervioso de las especies de los vertebrados e invertebrados, incluyendo la humana. Los cambios a largo plazo en la conectividad sináptica, así como los cambios conductuales de largo plazo (ambas actividades son responsables de varias propiedades que caracterizan el fenómeno de LTM y se usan como parámetros funcionales para explicar el aumento de la actividad neuronal dependiente

de estímulos) han demostrado que las señales ocurren inicialmente en el cuerpo celular. El fenómeno biológico de LTP es una forma de plasticidad sináptica ampliamente aceptada como un modelo celular que promueve la estabilización de sinapsis activas y que participan en eventos neurobiológicos como el desarrollo, el aprendizaje y la memoria. Una gran mayoría de los trabajos experimentales concernientes al fenómeno biológico del LTP en el aprendizaje, se ha enfocado en la actividad funcional de los receptores glutamatérgicos, tipo NMDA. No obstante, muchas preguntas han surgido con respecto a si el fenómeno de LTP es equivalente a la función de memoria, esto es, si el fenómeno de LTP juega un papel real y preponderante en la función de la memoria. Entonces, una hipótesis apropiada debería establecer el postulado de que el fenómeno de LTP como la actividad dependiente de los eventos de plasticidad sináptica y de múltiples formas de memoria que existen, compartan un denominador común; lo que a su vez permite postular la hipótesis que sugiere que la actividad dependiente de la plasticidad sináptica es inducida en una sinapsis particular y específica durante la formación de aprendizaje y consolidación de la memoria. La plasticidad sináptica es un fenómeno fisiológico que induce patrones específicos de actividad neuronal, sostenidos por mecanismos químicos y moleculares, que dan origen a cambios en la eficiencia sináptica y en la excitabilidad neuronal que perdura por más tiempo que los eventos que los originan. Con base en algunas propiedades de plasticidad sináptica recientemente estudiadas y documentadas, el fenómeno de LTP puede ser propuesto como un mecanismo neuronal para el desarrollo de algunos sistemas de memoria, que incluye la codificación inicial, el almacenamiento de la memoria y las primeras fases de la consolidación de la misma. Si el procesamiento funcional de la memoria es mediado por el fenómeno de LTP o LTD, muy probablemente ocurre como un proceso específico, dentro de una red de circuitos neuronales, situando al fenómeno de LTP como un mecanismo universal para la codificación y almacenaje de la memoria. Asimismo, la codificación sería parte de una propiedad de red neuronal más que de un mecanismo neuronal de contactos sinápticos individuales. Por ejemplo, el tipo de información procesada en el hipocampo es muy diferente de la información procesada por la amígdala y esta información puede permanecer si el mecanismo de plasticidad que opera en cada región del cerebro se conserva con el tiempo. Décadas de investigación han demostrado que el fenómeno de LTP en el hipocampo es inducido por la actividad sináptica y por moléculas citoplasmáticas unidas a la membrana, que son requeridas para traducir las señales extracelulares mediadas por la activación del receptor dentro de la activación de procesos de señalización intracelular. La mayoría de estos procesos depende de los movimientos del calcio intracelular, y de este modo, los mecanismos dependientes de calcio son necesarios para la inducción y la expresión de este fenómeno celular. En este contexto, se ha demostrado que los receptores glutamatérgicos, tipo NMDA, son esenciales para la iniciación del fenómeno de LTP; sin embargo, la expresión de este fenómeno requiere de la participación de los subtipos de receptores glutamatérgicos, AMPA. Mas aún, se ha demostrado que la inducción del fenómeno de LTP en la región hipocampal CA1 depende de los aumentos intracelulares de calcio, así como de la subsecuente activación de moléculas proteicas-calcio-dependientes, tal como lo representa la proteína quinasa dependiente de calcio, calmodulina (CaMKII). La expresión de esta proteína quinasa-dependiente de calcio en la neurona ha sido ampliamente demostrada en las densidades postsinápticas (PSD). Por otra parte, la expresión a largo plazo del fenómeno de LTP requiere de la

síntesis de proteínas, en la que las señales transitorias pueden estar ligadas a la activación de genes específicos que determinarán, en última instancia, el crecimiento y remodelación de sinapsis potencialmente activas. Diversos tipos de sinapsis pueden expresar y hacer uso de diversos grupos de moléculas proteicas que participan en la activación de diferentes vías de señalamiento intracelular y que, por igual, son responsables de las fases iniciales y de sostenimiento de los eventos de plasticidad sináptica. Varios estudios han demostrado que las modificaciones neuronales de los receptores específicos de unión de alta afinidad de diferentes neurotransmisores, o de las subunidades proteicas que componen estos receptores membranales en las densidades postsinápticas (PSD), representan uno de los mecanismos celulares por los cuales las neuronas regulan su actividad de reforzamiento sináptico. Por ejemplo, se ha demostrado que las dendritas neuronales pueden regular su propia síntesis de receptores proteicos membranales en respuesta a estímulos externos (por ejemplo, la subunidad GluR2 del receptor glutamatérgico, AMPA) y tales mecanismos moleculares implican importantes planteamientos en la comprensión de cómo las sinapsis individuales se consolidan selectivamente. Mas aún, recientes experimentos han demostrado que las moléculas que participan en vías de señalamiento intracelular (v.g., la proteína sináptica neuronal con actividad de GTPasa, denominada como SynGAP) están selectivamente expresadas y enriquecidas en las neuronas que median respuestas sinápticas excitatorias. Estos estudios han demostrado que diversos subgrupos de proteínas kinasas (v.g., MAPKs, SAPKs, MAPKAKs, p38MAPK, etc.) implicadas en la activación de diversas vías de señalamiento intracelular son reponsables de la actividad funcional de distintos factores de transcripción (v.g., complejo AP-1, C-Fos, Jun, CREB, etc.) los que a su vez regulan la expresión de múltiples genes de expresión temprana [*intermediate early genes* (IEG), por sus siglas en inglés] que son cruciales para el desarrollo neuronal, para la regulación del transporte vesicular de receptores glutamatérgicos a sinapsis específicas, así como para la inducción del fenómeno de LTP. Gran parte de los cambios neuroquímicos y moleculares que ocurren en los eventos de plasticidad sináptica, se puede asociar con cambios morfo-celulares dinámicos en las espinas sinápticas, tal como han demostrado diversos estudios durante el desarrollo y la consolidación del fenómeno de LTP. Además, aunque diversos trabajos experimentales han demostrado la participación de las células gliales en la neurotransmisión excitatoria en el SNC, estas células, además de ejercer una función celular ampliamente conceptualizada, como elementos de soporte estructural y de homeostasis, poseen un papel crucial en los eventos de plasticidad sináptica, de tal forma que también regulan la información procesada en el cerebro de los mamíferos, incluyendo los sistemas neuronales de especies de invertebrados. No obstante, el fenómeno de LTP en el hipocampo ha sido el blanco de mayor intensidad del estudio, y en particular en el análisis genético molecular. A este respecto, varios estudios han demostrado que el fenómeno de LTP está alterado cuando los genes particulares son inahabilitados permanentemente (*knockout*) o temporalmente (*knockdown*) en su expresión funcional y/o sobre-expresados en ratones mutantes nulos o en ratones transgénicos. Estos estudios han llevado a observaciones interesantes que demuestran que dentro de diferentes cepas naturales del ratón existen variaciones naturales en la expresión del fenómeno de LTP.

Palabras clave: Potenciación a largo plazo, sinapsis, plasticidad sináptica, receptor NMDA, receptor AMPA, proteína kinasa A, hipocampo, amígdala.

IV. LTP IN HIPPOCAMPUS AND AMYGDALA

Decades of research have demonstrated that LTP in the hippocampus is induced by synaptic activity and that cytoplasmic membrane-bound molecule(s) are required to transduce extracellular signals mediated by receptor-activation into activation of intracellular signaling processes (Chapman et al. 2003). Most of these processes have been demonstrated to depend on intracellular calcium, and therefore, calcium-dependent mechanisms will be eventually recruited for LTP induction and expression (Lynch et al., 1983). In addition, as long-term expression of LTP requires protein synthesis, transient signals will be linked to activation of specific genes that ultimately will determine growth and remodeling of potential active synapses (Chapman et al., 2003; Frey et al. 1997). Thus, different types of synapses may express and use a different set of molecules mediating activation of intracellular signaling pathways for initiating and maintaining synaptic plasticity. In this context, several studies have demonstrated that LTP expression in the CA1 field and basolateral amygdala evidences, are quite different in the steps that involve extracellular signal transduction to gene expression (Chapman et al., 2003). Moreover, despite of the extensive studies that have shown that the intracellular activated Ca^{2+} signaling mechanisms are totally dependent upon receptor activation and Ca^{2+} influx through specific voltage-dependent-calcium channels, several recent evidences showed that intracellular Ca^{2+} released from sarcoplasmic reticulum in cerebellar Purkinje cells and in spines protruding from hippocampal CA1 pyramidal neurons is significantly important in the Ca^{2+} dependent mechanisms operating in LTP and LTD (Parekh, 2001). For instance, these Ca^{2+} stores were shown to be functionally important preventing the expression of LTD, when these intracellular calcium stores were completely depleted, through store-operated Ca^{2+} channels. Moreover, as dendritic spines from hippocampal CA1 neurons are highly dynamic structures, several experiments have shown that spine density and length change in the presence of AMPA receptor antagonism are mediated by botulinum toxin. These set of results suggests that activation of postsynaptic AMPA receptors following spontaneous release of glutamate is necessary to maintain dendritic spines in CA1 pyramidal cells (Parekh, 2001). Such changes in spine morphology could depend on the alteration and redistribution of intracellular Ca^{2+} stores, and such Ca^{2+} changes should reflect importantly in the expression of LTP and LTD (Parekh, 2001). As explained in previous articles (Leff et al., 2002), one of the main properties of LTP is the axonal-input

specificity, so that only activated synapses become potentiated, while neighboring naive synapses remain unchanged (Harris, 1995).

Several studies have demonstrated that spread of LTP can occur from potentiated synapses to neighboring neurons that were still not activated to induce LTP (Harris, 1995). One model that has been offered to explain the spread of LTP to neighboring pyramidal cells in the hippocampal CA1 area is based on the concept that the signal that is initiated at the potentiated postsynaptic neuron spreads intracellularly through the input axons for a limited distance to activate other cells that are associated with the same axons. Another hypothesis offered on the interneuronal spread of LTP is that LTP spread occurs via the diffusion of a retrograde messenger beyond activated synapses onto neighboring synapses on other neurons (Harris, 1995). Based on these experimental hypotheses, several studies have proposed an alternative model that considers that a retrograde signal is restricted to the activated synapses of hippocampal neurons, and thus, LTP spread is initiated at multiple-synapse boutons (MSBs) of activated inputs that synapse on the potentiated neuron and neighboring cells as well (Harris, 1995). In such a context, several experimental findings have shown that potentiation is spread via the axonal input that formed synapses on dendrites onto the activated CA1 pyramidal neurons and into close neighboring cells, within a limited area of 150 μm from the potentiated cell (Harris, 1995). Based on the wide agreement as to LTP induction occurring postsynaptically, LTP spread to neighboring cells via the input axons must use a retrograde diffusion messenger to signal presynaptic axons when LTP is expressed. Several candidates that could operate as retrograde signals have been offered, ranging from mechanical signaling molecules [via proteins that span the synaptic cleft, such as the integrin-type molecules, neural cell adhesion proteins, and peptides that block integrins, that have been shown to disrupt the stabilization of LTP (Harris, 1995)] to diffusible molecules, such as nitric oxide, carbon monoxide, arachidonic acid and platelet-activating factor, molecules that have been identified to enhance either synaptic potentiation or synaptic depression (box 1). Whether these molecules act individually or in concert is not yet clear, but recently novel molecules have been demonstrated to be specifically implicated in LTP induction and maintenance in CA1 pyramidal cells in the hippocampus (see below) (Harris, 1995).

a) Hippocampus. Several studies have demonstrated and reported that blocking NMDA receptors by application of specific antagonists (e.g., AP5, CPP) prevents induction of LTP of Schaffer collateral

synapses onto CA1 pyramidal neurons (Collingridge et al., 1983; Harris et al., 1984) induced by a variety of stimuli (e.g., 100 Hz /1 sec; theta burst; pairing of depolarized postsynaptic cell with low frequency stimulation) (Bliss et al., 1993). Moreover, deficit in LTP induction in CA1 field was demonstrated after inducing targeted deletion of the mice NMDA membrane-receptor subunits (Tsien et al., 1996). For example, when knockout of the mice NR1 subunit of the glutamate receptor is restricted to CA1 region of the hippocampus, NMDA-mediated synaptic currents and LTP induction are abolished (Tsien et al., 1996). However, transgenic mice expressing a mutant NMDA-receptor at the NR1 protein subunit (a polypeptide domain strictly necessary for expression of the functional receptor) using conventional gene targeting results in neonatal death (Forrest et al., 1994).

Despite of the several studies that have shown the implication of voltage-gated calcium channels (VGCCs) in LTP induction (Chapman et al., 2003) their participation has been shown to be detectable only upon application of strong tetanic stimulation (Grover & Teyler, 1995; 1990). Moreover, calcium-dependent activation of downstream signaling pathways involved in LTP induction and maintenance has been shown to be recruited by either NMDA-receptor-gated and VGCCs. For instance, some studies have shown that Ca^{2+} influx entering through VGCCs activate specifically Protein Kinase C (PKC) (Cavus & Teyler, 1996), while others have demonstrated the activation of the calcium-calmodulin-dependant-protein kinase II pathway (an enzyme implicated in the regulation of several intracellular processes in the receptor-activation of intracellular signaling pathways) in LTP in CA1 region (Giese et al., 1998). Interestingly enough, is the observation that the mechanisms that participate in the induction of LTP at CA1 and CA3 differ significantly (Chapman et al., 2003; Nicoll & Malenka, 1995). CA3 pyramidal cells receive inputs from excitatory synapses from recurrent collaterals from CA3 efferent axons; from associational/commissural inputs and mossy fibers from dentate gyrus granule cells, impinging on the apical dendrites of these cells. In this context, it has been demonstrated that associational/commissural afferents behave like Schaffer collateral inputs to CA1 field in which LTP induction is blocked by NMDA-receptor antagonists (Harris & Cotman, 1986). Nonetheless, induction and expression of LTP in mossy fibers impinging on CA3 region of the hippocampus behave quite differently mainly due to the fact that NMDA antagonists do not have any effect in abolishing LTP induction in this hippocampal region (Weisskopf & Nicoll, 1995). Although the release of glutamate from mossy fibers is able to bind NMDA

receptors, thus enhancing its activation, it does not seem to participate in mossy fiber LTP induction. These data led researchers to conceive that postsynaptic depolarization and Ca^{2+} influx is required for mossy fiber LTP induction, and that LTP expression at these synapses should be driven by a significant presynaptic component (Yeckel et al., 1999; Chapman et al., 2003).

b) LTP variation in hippocampal regions between inbred mouse strains. As hippocampal LTP has been the target of intensive molecular genetic analysis, several studies have demonstrated that LTP is altered when particular single genes are knocked out or overexpressed in null mutant mice or transgenic mice (Gerlai, 2001). But it is quite amazing to observe that natural variation in LTP exists in natural inbred mouse strains (Nguyen et al., 2000). Experiments that used the hippocampal slice preparation have demonstrated the robust expression of LTP in the CB7B/6J or B6 mice while other strains such as the CBA(CBA/J); DBA (DBA/2J) and 129 (129/SVEms/J) strains express different degrees and suffer from different sorts of abnormalities in LTP. For instance, theta burst stimulation induces a strong LTP in B6 but much weaker LTP in 129 strains. Furthermore, LTP in DBA and CBA strains decayed significantly faster than in B6 strain, in one sort of experiments and based on another LTP protocol, tetanic stimulation induced a dramatic increase in LTP in both B6 and 129 strains, with complete absence of potentiation in DBA and CBA strains (Gerlai, 2001).

These results suggest that hippocampal neurons of some mouse strains may be optimally tuned up to particular temporal patterns of synaptic activity as authors explain, but conversely, to the observed strain differences in LTP expression; hippocampal LTD (activity dependent reduction of synaptic transmission) seems to be unaltered in the four mouse strains. Moreover, neurons from these strains exhibit similar patterns of membrane excitability; membrane input resistance and synaptically evoked glutamatergic postsynaptic currents. These observations, based on the strain differences in LTP, posit the hypothesis of the presence of altered molecular mechanisms regulating with synaptic strengthening and not due to altered properties of the neuronal membrane as described (Nguyen et al., 2000). One particular mechanism implicated in the strain LTP variation may be the cAMP-PKA intracellular signaling pathway (Nguyen et al., 2000; Gerlai, 2001). Several implications have been suggested to explain the variation of LTP between mice strain. One of them explains that variation in synaptic strengthening has either existed in wild mice from where inbred strains derived, and/or as alternate hypothesis; changes in synaptic

strengthening might have occurred in the presence of random mutations during inbreeding. Second, based on the differences observed in behavior among inbred strains, it should be possible to search and correlate between alterations of synaptic plasticity and behavioral expression using quantitative and genetic techniques (Gerlai, 2001). Third, LTP exists in multiple forms of synaptic plasticity processes, so that these processes might be influenced by genetic variations between inbred strains (Gerlai, 2001). Inbred strains with impaired LTP might be useful to test potential LTP by enhancing the effects of genes or drugs (Nguyen et al., 2000; Gerlai et al., 1999), while strains expressing robust LTP might be useful to search the molecular mechanisms that impair synaptic plasticity (Gerlai, 2001).

c) Amygdala. The basolateral region of the amygdala (BLA) has been shown to play a crucial role in the consolidation of newly created memories as well as in learning and memory processing and consolidation, including LTP (for a comprehensive review, on this topic, see McGaugh, 2002). NMDA-receptor activation has been shown to be crucial for LTP induction at least in some hippocampal subregions, but in amygdala the important role of the NMDA-receptor has raised controversial issues, due to the inconsistent effects of LTP blockage by NMDA receptor antagonists (Chapman et al., 2003). This discrepancy results from the controversial issues that posit that the amygdala should express at least a variety of morphological and electrophysiological subtypes of synapses in a similar fashion to the hippocampus, segregated in different nuclei (Chapman et al., 2003). Initial demonstrations that NMDA antagonist AP5 blocked LTP in the *basolateral amygdala* in response to afferent cortical input stimulation in the external capsule was observed only at antagonist concentrations that affected synaptic responses evoked at low frequencies (Chapman & Bellavance, 1992), but doses used to blocked LTP in CA1 were without effect in LTP and even, in baseline synaptic transmission.

These results have posit the hypothesis expressed by some authors that a significant percentage of LTP expressed in the lateral and basolateral amygdala is NMDA-independent (Huang & Kandel, 1998; Weisskopf & LeDoux, 1999), while others support that LTP in the same amygdaloid areas are NMDA-dependent, that is, LTP is blocked by NMDA antagonists (Rammes et al., 2000). In support to this discrepancy, some authors (Weisskopf & LeDoux, 1999) have demonstrated that the activation of NMDA receptors by endogenous release of glutamate from activated cortical and thalamic inputs impinging into

lateral amygdala (LA) neurons produced different cell responses, thus contributing in a different manner to the LTP induction in these areas (Chapman et al., 2003). The lack of a complete pharmacological block of LTP by AP5 suggested that VGCCs may be importantly involved in LTP induction in BLA. Application of intracellular recording procedures and experimental protocols used to pair direct depolarization with stimulation of thalamo-amygdala neuronal efferents, made it possible to demonstrate that thalamic inputs into the LA produced potentiation that was associative and occurred only on the activated synaptic inputs (Chapman et al., 2003; Weisskopf & LeDoux, 1999). LTP induction was not blocked by either AP5 or by MK-801 (a noncompetitive antagonist NMDA) but by the L-type channel blocker, nifedipine or by the intracellular Ca^{2+} chelator, BAPTA. Moreover, induction of LTP resulted to be completely blocked after administration of combined receptors and VGCC antagonists, 50 μM of D-AP5 and 30 μM nimodipine, respectively. Each antagonist applied alone, did not show a significant blocking effect on LTP (Ramsay et al., 2001). These data show at least that both NMDA receptors and VGCCs contribute to LTP, induced either with tetanic stimulation or TBS (Chapman et al., 2003).

In another context, several studies have demonstrated PKA-dependent of synaptic plasticity in the amygdala (Huang & Kandel, 1998; Maren, 2001; Rammes et al., 2000) in a similar fashion as shown in hippocampal synapses for early and late phases of LTP (Abel et al., 1997; Nguyen & Kandel, 1996; Frey et al., 1993; Huang et al., 1994). Moreover, emotional learning results to be impaired after inhibition of PKA within this brain region (Goosens et al., 2000; Schafe & LeDoux, 2000). In addition to these results, different studies have demonstrated that amygdala plays a crucial role in the acquisition and extinction of fear memory (Maren, 1999; Nader et al., 2000; Lu et al., 2001). Moreover, the activation of different subset of protein kinases (such as calcium/calmodulin kinase II, cAMP-dependent protein kinase A and MAPK) (see below) required in long-term memory formation is crucial for acquisition of fear memory as well (Impey, 1998; Schafe & LeDoux, 2000; Josselyn et al., 2001). In such a context, it has been shown recently that acquisition of fear is associated with activation of phosphatidylinositol 3-kinase (PI-3 kinase) and its downstream molecular target Akt in rat amygdala (Lin et al., 2001). For extinction of memory, recent studies have shown that calcineurin plays a key signal in extinction of fear memory, demonstrating that while fear-training induced the phosphorylation of specific substrates in the rat amygdala, fear extinction trials produced a

significant reduction of such phosphorylated substrates, associated with an increase in enzymatic activity of calcineurin and phosphatase activity (Lin et al., 2003). Furthermore, application of calcineurin inhibitors prevented extinction-induced protein dephosphorylation as well as extinction of fear memory. Thus, it seems that calcineurin, as explained by authors (Lin et al., 2003), creates a negative-feed-loop to suppress phosphorylation of protein substrates, thereby weakening the original acquired memory.

d) Calcium signaling in the hippocampus and amygdala. LTP in CA1 hippocampal region has been shown to depend on increases of intracellular calcium, and thus events that follow induction of LTP will involve calcium dependent molecules (Chapman et al., 2003). One of such candidates is the calcium/calmodulin-dependent protein kinase (CaMKII) whose cell expression is confined predominantly at postsynaptic densities, and whose activation depends on the presence of intracellular high calcium concentrations (Kennedy & Greengard, 1981). This enzyme with the ability to autophosphorylate itself, thereby reduces its own dependence on high concentrations of calcium, a functional property required for a molecule to convert transient signals into a long-lasting change in synaptic function (Lisman et al., 1997). This phosphorylating enzyme has been implicated in the early stages of LTP induction as demonstrated by local application of peptide inhibitors of CaMKII into CA1 pyramidal neurons, resulting in a complete block of LTP induction neurons as does, for the deletion of the CaMKII gene (Silva et al., 1992). And, in a similar fashion, with transgenic homozygous mutant animals expressing an altered form of the CaMKII, where a single amino acid substitution at the phosphorylation site of the enzyme (CaMKII^{T286A}) has been induced by DNA recombinant technology (Giese et al., 1998), thereby leaving the enzyme without autophosphorylation activity. Thus, these results confirm that autophosphorylation activity is an important molecular-dependent activity for LTP induction (Chapman et al., 2003).

Theta burst-stimuli applied to brain slices of T286A mutant aCaMKII mice at CA1 region of hippocampus demonstrated an almost complete block of LTP, but not in LA-BLA synapses of different slice preparations of the same animal, that showed a reduced magnitude of LTP by approximately 20% (residual LTP) (Chapman et al., 2003). These results implicate that CaMKII participates in LTP induction in the BLA, whose contribution in hippocampus is less notorious, and that alternate intracellular mechanisms in BLA compensate for the functional loss of aCaMKII (CaMKII^{T286A}) (Chapman et al., 2003). Moreover,

parallel studies have demonstrated that the residual LTP in the mutant mice was insensitive to NMDA competitive antagonist AP5 (Giese et al., 1998). Thus, these data supporting the reduced activity of aCaMKII in NMDA-receptor-dependent activity and CaMKII mediating LTP in the amygdala (Chapman et al., 2003) shows that the residual LTP found in BLA might have a VGCC component, that mediate induction of LTP, in which NMDA antagonist would not display a pharmacological effect.

V. GLIAL CELLS AND LTP

Glial cells play an active role in excitatory neurotransmission in the CNS, besides of their traditional function regarded as elements for structural support and homeostasis (Araque et al., 2001). Based on this evidence, it has been demonstrated that these cells release glutamate in response to physiological increases of intracellular calcium, evoking an important increase of glutamatergic current in neighboring neurons (Parpura and Haydon, 2000). Moreover, these cells express different subtypes of neurotransmitter receptors, to which they respond by generating slow calcium currents. In addition, recent findings demonstrate that glial cells (oligodendrocyte precursor cells) receive glutamatergic inputs from hippocampal pyramidal cells (Parpura and Haydon, 2000). Overall, these findings support the hypothesis that the back and forth signaling between glial and neuron, play an important role in synaptic plasticity and thus in the information processed in the brain (Nishiyama et al., 2002). This novel notion can be supported by recent findings that demonstrate that mutant mice lacking glial fibrillary acidic protein–GFAP (an intermediate filament specific to astrocytes) express an enhanced LTP in hippocampal CA1 neurons and a decreased LTD in cerebellum associated with impaired eye–blink conditioning (McCall et al., 1996; Shibuki et al., 1996). At the molecular level, several reports have shown that the astrocyte calcium-binding protein, S100B, a member of the S100 protein family, is released from glial cells, suggesting an extracellular function, as a trophic factor. Moreover, this protein is also involved in cell functions, such as cell growth, cell structure, energy metabolism, and calcium homeostasis (Nishiyama et al., 2002; Zimmer et al., 1995).

Recent findings have demonstrated that transgenic mutant mice overexpressing human S100B exhibit impaired LTP and spatial learning and constitutive overexpression of this calcium-binding protein might cause neuronal damage (Gerlai et al., 1995; Reeves et al., 1994; Whitaker-Azmitia et al., 1997). Conversely,

homozygous transgenic mutant mice, that completely lacked the mRNA that encodes the S100B protein product, show a strengthened synaptic plasticity, expressing an enhanced LTP in the Schaffer collateral-CA1 pyramidal cell synapse in the adult hippocampus, which is known to be mediated by the NMDA-glutamate receptor (Kato et al., 1991). In addition, these mutant mice express an enhanced spatial working memory and enhanced contextual fear memory as compared to wild type when subjected to Morris water-maze tasks and to conditioned electric foot shock paradigms, respectively (Nishiyama et al., 2002). In addition to the observed results, hippocampal slices perfused with exogenous recombinant S100B protein (1 µg/ml)/ACSF solution, reversed the enhanced response of LTP in the mutant mice to those of wild-type control slices (Nishiyama et al., 2002). These results show clearly that S100B protein modulates synaptic plasticity mediated through the extracellular activity of the secreted protein from local glial cells as described by authors and based on several experiments performed (Nishiyama et al., 2002). In such a context, S100B protein content has been shown to change in hippocampus during LTP, and its detected activity has been shown to be increased in membrane fractions versus water soluble fractions 1 h after tetanization (Popov et al., 1988). This set of results has raised the interesting hypothesis that secretion of S100B protein from hippocampal astrocytes binds to a specific neuronal membrane receptor and activates intracellular signaling cascades that eventually result in changes in synaptic plasticity (Nishiyama et al., 2002). One recent receptor for the secreted glial protein is the EN-RAGE receptor, a newly identified protein receptor that has been shown to be highly expressed in lungs and adult human CNS (Sasaki et al., 2001). Whether this receptor or structurally related receptors are implicated in the underlying synaptic plasticity events mediated by S100B protein is still elusive, as well as the intracellular signaling cascade driven by protein binding and receptor activation (Nishiyama et al., 2002). Overall, these results put forward the importance of the glial-to-neuron signaling, showing that the glial calcium-binding protein S100B modulates synaptic plasticity in neurons and might regulate hippocampal-dependent learning and memory processes in the brain of mammals (Nishiyama et al., 2002).

Glutamate release from astrocytes participates in synaptic plasticity as well as in brain intercellular signaling. Astrocytes express ion channel receptors namely defined as P2X₇, that are activated by ATP and several other ligands that provide a route for excitatory amino acid release from astrocytes, such as glutamate as D-aspartate, mediated by activation and P2X₇ large-

channel opening (Duan et al., 2003). Moreover, recent experimental works using transgenic mice [that contain the GFAP promoter-controlled enhanced green fluorescent protein (EGFP) expression] and application of patch-clamp recordings and single-cell reverse transcription-PCR techniques, have shown the coexistence of distinct independent populations of cell types that express astrocytes-specific markers (GFAP promoter-regulated EGFP expression and S100 β /GFAP immunoreactivity) within the developing mouse hippocampus that differentially express AMPA-type glutamate receptors and glutamate transporters in an heterogeneity of cells (Matthias et al., 2003). Other experimental works, using RT- and immunofluorescence techniques in cultured neonate rat hippocampal astrocytes demonstrated the functional expression of the metabotropic glutamate receptor subtype, mGluR1, and the ionotropic glutamate receptor subtypes iGluR1, and iGluR4, in these cells, associated with the expression of a Ca²⁺-activated K⁺-channel subtype (Kca β 4 subunit) as detected by RT-PCR and Northern-blot analysis. Activation of mGluR1 by ligand agonists, L-glutamate and quisqualate, demonstrated to increase two types of K⁺-channel currents that were blocked by specific mGluR1 antagonists and not by iGluR antagonists. Activation of these K⁺ ion channel currents by mGluR agonist were shown to be attenuated by pertussis toxin, by inhibition of phospholipase C and cytochrome P450 arachidonate epoxygenase. These data demonstrated that astrocytes functionally express two types of K_{Ca} channels that are gated by activation of g-protein couple metabotropic glutamate receptor functionally linked to PLC and cytochrome P450 arachidonate epoxygenase (Gebremedhin et al., 2003).

VI. PROTEIN SYNTHESIS AND LTP

One crucial property of many synapses in the brain is the ability to modulate the efficacy of transmission in response to incoming signals. Changes occurring in the efficiency of synaptic transmission are crucial for neuronal processes such as learning and memory (Spier, 2001). Experimental works have demonstrated that modifications of the density or subunit composition of neurotransmitter receptors in the postsynaptic (dendritic) membranes represents one of the neuronal mechanisms by which neurons regulate their synaptic strength. Still, the molecular and neurochemical mechanisms not proved completely, in the context of whether transcriptions of presynaptic and/or postsynaptic genes are required for maintenance of late-phase LTP (Nayak et al., 1998), are becoming elucidated. In this context, synthesis and targeting of

receptor proteins to dendritic areas were thought initially to be functionally dependent of the cell body (e.g., synthesis, packaging, sorting and transportation to dendritic membranes), but recent experiments have shown that receptors are synthesized inside dendrites, inserted into membranes, and such process is pharmacologically sensitive as shown to occur in mechanically isolated dendrites (Spier, 2001). Using isolated dendrites from hippocampal neurons and exposed to liposomal transfection of mRNAs encoding either the N- or C-terminal Myc-tagged glutamate receptor (GluR2) into dendrites, investigators demonstrated the successful synthesis of membrane receptors in several dendrites (Kacharina et al., 2000). The demonstration that the cMyc epitope of the N-terminally tagged GluR2 was fully inserted into membranes, facing the extra cellular space, shows that this subunit of AMPA glutamate receptors was successfully integrated into dendritic membranes, showing that functional expression of this membrane receptor subtype requires the correct translocation of the protein subunit of AMPA receptors. Moreover, this set of experiments demonstrated that GluR2 synthesis was dramatically increased by DHPG (a metabotropic glutamate receptor agonist) in several dendrites, while its absence produced low to negligible receptor expression and extracellular translocation of this protein subunit (Spier, 2001). These results show that neuronal dendrites are able to regulate their own transmembrane receptor synthesis in response to external stimuli, and such molecular mechanisms posed important implications for the understanding of different neuronal processes, as in how individual synapses are selectively strengthened, by altering the receptor concentration in response to presynaptic input (Spier, 2001).

Interestingly enough, such novel mechanisms can be implicated in the maintenance of the late-phase of LTP (3-5 hrs) after high frequency stimulation (HFS) of presynaptic elements, known to be dependent of transcription of specific mRNAs, protein synthesis, and cyclic-AMP-dependent protein kinase (PKA) (Frey et al., 1988; Frey et al., 1993; Nguyen et al., 1994). In such a context, post-translation events are known to mediate the early maintenance phase of LTP, while transcription and translation dependent events mediate events that underlie the late maintenance phase (Huang et al., 1994; Huang & Kandel, 1998; Bennet, 2000). Such a division of early and late phases of LTP maintenance has been demonstrated for the intracellular mechanisms that underlie the Schaffer collateral/commissural input to the CA1 area (Frey et al., 1988) and the perforant pathway input to dentate granule cells in the hippocampus (Krug et al., 1984; Otani & Abraham,

1989). These afferent pathways differ from the LTP at the Mossy fiber (MF) input to CA3 pyramidal cells, in the lack of dependence on the NMDA receptor activation for LTP induction (Harris and Cotman, 1986), and are apparently dependent on presynaptic mechanisms for LTP maintenance and expression (Xiang et al., 1994; Weisskopf & Nicoll, 1995; Lopez-Garcia, 1998). Moreover, recent experiments have demonstrated that LTP maintenance at MF input to CA3 pyramidal cells depends on protein synthesis and mRNA transcription soon after LTP induction, which differs from LTP maintenance at non-MF synapses (Calixto et al., 2003), and provides evidence that maintenance of the early phase of MF-LTP (immediately after induction) requires RNA and protein synthesis as shown for its sensitivity to protein inhibitors, such as emetine (20 μ M) or cycloheximide (60 μ M) and RNA synthesis inhibitors, such as D- Actinomycin (25 μ M)(Calixto et al., 2003), and requires intact signaling between presynaptic granule cell soma and MF boutons, as shown from the lack of the cyclic cAMP-PKA activation to increase MF-EPSPs (30 minutes after MF potentiation) induce by the adenylyl cyclase activator; forskolin (100 μ M) in addition to the nonspecific inhibitor of cAMP/cGMP phosphodiesterase; IBMX (50 μ M) in the presence of protein synthesis inhibitors (Calixto et al., 2003). These results differ from LTP obtained in non-MF synapses, in that, the early phase of LTP in Commissural/Association input to CA3 pyramidal cells (C/A-LTP) and CA1 region, is insensitive to protein synthesis inhibitors, which support the non-requirement of novel proteins in this form of LTP (Krug et al., 1984; Nguyen et al., 1994; Calixto et al., 2003). Moreover, the blockade of protein synthesis and/or RNA synthesis in MF LTP resulted to be selectively inhibited in the early maintenance phase of MF LTP, but not in the induction process (Calixto et al., 2003). In such a context, several studies have demonstrated that train stimulation of afferent fibers may induce local protein synthesis in dendrites of postsynaptic cell (Job and Eberwine, 2001; Smith et al., 2001; Steward and Worley, 2002), as shown for the expression and insertion of AMPA receptors (Malinow and Malenka, 2002) expression of cadherins and related cytoskeleton proteins (Kim and Lisman, 1999; Yamagata et al., 1999; Krucker et al., 2000). In addition, recent experiments have demonstrated that pituitary adenylyl cyclase activating polypeptide (PACAP) type 1-receptor (PAC-1), a G-protein-coupled receptor that activates adenylyl-cyclase-protein kinase A (PKA) signal transduction pathway (Christophone, 1993) that is abundantly expressed in different areas of the brain [i.e., neocortex, limbic system, brainstem, including the hippocampus, whose expression is restricted to the

granule cells of the dentate gyrus and the PAC-1 protein, is localized presynaptically in hippocampal mossy fiber terminals)(Hashimoto et al., 1996; Otto et al., 1999], binds with high affinity PACAP with regard to its orthologous-related vasoactive intestinal peptide (VIP) (Shivers et al., 1991) and plays a crucial role in neuronal differentiation and synaptic plasticity (Arimura, 1998), may have a crucial role in MF-LTP and associative learning in the hippocampus, after demonstrating that transgenic mutant mice strains lacking this PACAP-PAC-1 receptor subtype, show a deficit in contextual fear condition, while amygdala-dependent cued fear conditioning events remained intact (Otto et al., 2003). Moreover, mutant mice expressing this deficit in hippocampus-dependent associative learning was associated with an impairment in MF-LTP, because the expression of PAC-1 receptor is restricted to MF terminals (Otto et al., 2003).

In addition, recent experiments, using rat hippocampal CA1 mini-slices, in which cell bodies of presynaptic Schaffer collateral/commissural fibers were removed (see schematic diagram of structural neuronal array of hippocampus in Leff et al., 2002) using a multi-electrode which potentiates a much larger fraction of CA1 synapses, rather than a single electrode, showed that transcription of presynaptic genes is not necessary for maintenance of late-phase of LTP, but rather a transcription of postsynaptic genes may be required and implicated in the maintenance of late phases of LTP (Nayak et al., 1998), based on the assumption that late-phase of LTP is absent in dendrites excised from CA1 pyramidal somata (Frey et al., 1989). These experiments showed that the AMPA receptor is the main ionotropic receptor that responds to synaptically released glutamate in the hippocampus, and the synthesis of their protein subunits (GluR1 and GluR2/R3) is increased three hours after LTP induction, as observed to be enriched in plasma membranes, and blocked by PKA antagonist and transcription as well (Nayak et al., 1998). Moreover, presynaptic proteins, such as synapsin I or NMDA receptor subunits (NR2A and NR2B), were not found to be increased or changed after achieving late phase LTP, showing that only AMPA receptor subunits were largely modified during this stage (Nayak et al., 1998). These studies could posit the hypothesis that postsynaptic increase and expression of AMPA receptor subunits in late phase of LTP in the hippocampus may be locally synthesized in dendrites, whose increase is fairly dependent on transcription and PKA activation (Nayak et al., 1998). Conversely, these changes observed for the increased postsynaptic expression of AMPA receptors during the late-phase LTP in the rat hippocampus may differ from other forms of long-lasting plasticity in Aplysia

and *Drosophila*, in which transcription of presynaptic genes seems to be predominantly essential for the expression of these plastic events in the neural systems of these species (Nayak et al., 1998). In addition, recent experiments have demonstrated that the distribution of the syntaxin mRNA (a protein that regulates vesicle docking and fusion for neurotransmitter release) in the soma of sensory neurons (SNs) of *Aplysia* maintained in cell culture, is affected by synapse formation, synapse stabilization and long-term facilitation (LTF), induced by 5-HT (Hu et al., 2003). These studies have shown that distribution of syntaxin mRNA not only regulates the expression and axonal transport of the protein, which accumulates at the axon hillock of SNs during the initial phase of synapse formation. After synaptic stabilization, both mRNA and protein result to be targeted away from the axon hillock as detected by the reduced number of axonally transported syntaxin granules (Hu et al., 2003). Evoked LTF induced by repeated application of 5-HT, resulted in a dramatic increase of mRNA and protein accumulation as well as of syntaxin granules at the axon hillock, an event that was blocked with anisomycin and KT5720 (a protein kinase A inhibitor), that abolished the accumulation of the mRNA and protein, but increased syntaxin granules and SN axons. These results demonstrate that target interaction and activation of the intracellular protein kinase A signaling pathway mediated by 5-HT regulate the expression of syntaxin and its packaging for transport into axons by altering the distribution of its mRNA in the soma (Hu et al., 2003).

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