UNDERSTANDING THE NEUROBIOLOGICAL MECHANISMS OF LEARNING AND MEMORY: MEMORY SYSTEMS OF THE BRAIN, LONG TERM POTENTIATION, AND SYNAPTIC PLASTICITY. PART III A

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SUMMARY

Recent theories postulate that memory can be divided into multiple brain memory systems. Although memory systems depend on the function of variables based on the level of analysis and are subserved by different neural substrates, current definitions of memory systems have categorized them as psychological and biological entities. Under such context, the studies of different memory systems have shown that complex interactions take place during performance of any memory task. Such interactions among multiple memory systems are based on dynamic interactive independent neural networks which make possible the better understanding of how memory systems work in the brain of mammals. Both behavioral and electrophysiological studies over the last decades demonstrate that learning and memory are encoded through activity dependent changes of the strength of synaptic connections between neurons, as experimentally demonstrated by Long-Term Potentiation (LTP) in mammalian synapses. LTP is a form of synaptic plasticity, and is considered as an accepted cellular model for stabilization of synapses involved in the expression of several neurobiological phenomena. Most of the understanding of the neurochemical, pharmacological, and molecular mechanisms involved in LTP induction, expression, and maintenance, have been demonstrated through the involvement of glutamate neurotransmission system, as well as through the different glutamate receptor subtypes, known to be expressed widely in different neural networks of the brain of mammals.

Key words: Glutamate receptors, N-Methyl-D-Aspartate, Metabotropic receptors.

RESUMEN

El fenómeno de la memoria se define como un proceso de adquisición, almacenamiento y recuperación de información. En términos operacionales, el fenómeno de la memoria se infiere como un evento neurobiológico resultado de alteraciones en el comportamiento del sujeto, causado por experiencias previas no dependientes de modificaciones de los órganos efectores sensoriales. En este contexto, algunas teorías recientes postulan que la memoria puede dividirse en múltiples sistemas de memoria funcional en el cerebro de los mamíferos. Si bien estos sistemas de memoria funcional dependen de múltiples variables sujetas al grado de análisis del experimentador, asimismo están regulados por diferentes circuitos neuronales enlazados entre sí. Las definiciones más recientes de estos sistemas de memoria funcional postulan que estos sistemas se enmarcan ya sea como entidades psicológicas, al considerar que los sistemas de memoria operan como módulos especializados que poseen tanto la capacidad de procesar diferentes tipos de información como de realizar tareas operacionales y almacenar información en lapsos cortos o largos, o sea como entidades biológicas, si se define que los sistemas de memoria operan mediante circuitos neuronales y conexiones neurales complejas que, en conjunto, permiten operar un tipo particular de información y procesar el almacenamiento de información dentro del mismo circuito neuronal u otro distinto. Por ejemplo, el lóbulo temporal medial (LTM) que contiene la estructura nerviosa del hipocampo y sus interconexiones con los diferentes campos corticales resultan ser cruciales para estructurar y consolidar la memoria de tipo declarativo. En este contexto, diversos trabajos en el campo de la neuropsicología y la neurobiología de la memoria han mostrado que los diferentes sistemas de memoria operan según complejas interacciones durante la ejecución de tareas de aprendizaje y memorización en el cerebro de

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los mamíferos. Estas interacciones entre los múltiples sistemas de memoria funcional dependen de complejas interacciones dinámicas de sistemas o sustratos neuronales independientes que posibilitan una mejor comprensión de la forma en que trabaja nuestra memoria en el cerebro. Además, diversos trabajos experimentales de naturaleza tanto conductual como electrofisiológica en las últimas décadas demuestran que tanto el aprendizaje como la memoria se codifican mediante cambios dependientes de la actividad entre las conexiones nerviosas, tal como comprobó el descubrimiento del fenómeno de Potenciación de Largo Plazo (LTP, por sus siglas en inglés) en las sinapsis de las neuronas del hipocampo en el cerebro de los mamíferos. El fenómeno de LTP, considerado una forma de expresión de plasticidad sináptica, también se ve como un modelo celular que favorece la estabilidad de la actividad sináptica y su expresión en múltiples eventos neurobiológicos. En este contexto, diversos estudios del fenómeno de LTP tanto *in vitro* como *in vivo*, con diferentes métodos experimentales y de registro, han demostrado que el fenómeno de LTP ocurre en múltiples regiones del cerebro, como son la neocorteza, la amígdala y en estructuras que conforman el sistema nervioso periférico de los mamíferos. Más aún, estudios recientes muestran que el fenómeno de LTP puede inducirse en tejidos neurales de animales invertebrados, como ocurre en la unión neuromuscular y en sinapsis específicas de diferentes estructuras nerviosas del cerebro de los artrópodos. En su gran mayoría, los eventos neuroquímicos, neurofarmacológicos y moleculares que participan en la inducción, mantenimiento y expresión del fenómeno de LTP se basan en la actividad de un sistema de transmisión particular, como es el sistema de neurotransmisión glutamatérgica mediado a través de la activación de diferentes subtipos de receptores glutamatérgicos, como los receptores ionotrópicos tipo NMDA (NMDA glutamate receptors, por sus siglas en inglés), los receptores ionotrópicos tipo no-NMDA (Non-NMDA glutamate receptors, por sus siglas en inglés) y los receptores metabotrópicos que se encuentran ampliamente distribuidos y se expresan de manera funcional en diferentes circuitos neuronales y sinapsis en el SNC de los mamíferos.

Palabras clave: Receptores de glutamato, N-metil-D-aspartato, receptores metabotrópicos.

MEMORY SYSTEMS OF THE BRAIN

Memory can be defined as a function of many variables depending on the levels of analysis (Kim and Baxter, 2001). Memory has been typically attributed to a process of information acquisition, storage, and retrieval, but in operational terms, memory has been inferred from alterations in behavior that are caused by some prior experience not dependent on the modifications of the responsivity of sensory effector organs (Rescorla, 1988). Moreover, memory systems can be grouped either as a psychological, considering that memory systems work as specialized modules that process particular kinds of information, perform particular operations and store information over a short and long-term period basis, or as a biological entity, if defined that memory systems work on network structures by way of complex interconnections, which operate together on a particular type of information, processing the storage of the information within the same network structure or some other (see Kim and Baxter, 2001). For instance, the medial temporal lobe (MTL) that confines the hipoccampal structure and interconnected cortical areas, seems to be crucial for the declarative memory (Squire, 1987). Based on that both psychological and biological conceptions of memory systems represent same entities "screened" at different levels of analysis, one entity or domain may validate the other one (Kim and Baxter, 2001). At the experimental level, both entities of memory systems are examined as individual systems in relative isolation and then investigated at multiple levels of analysis (e.g., behavioral, neuropsychological, functional imaging, electrophysiological, including neurochemical, molecular and genetics) (Kim and Baxter, 2001). Besides the conceptualization that different models of memory are defined as independent systems, it is generally assumed that all memory systems functionally interact between each other. For instance, the amygdala, a neural structure implicated in modulating emotional memory, has the capability to modulate several kinds of memories (Le Doux, 2000; MacGaugh, 2000; see Pitkänen et al., 1997). Moreover, the more complex the interaction between neural structures involved in a particular memory system, higher implications can be assumed for a memory system theory, as postulated from a simple possibility that the degree to which a system is involved in a task might be relatively determined by the degree of involvement of another system, or the more elaborate possibility that the nature of information processing of one system might be completely altered by the involvement of another system (see Kim and Baxter, 2001). Based on behavioral studies, it has been hypothesized that different kinds of interaction between different memory systems might occur. As an hypothetical model of memory interaction based on mathematical modeling, it has been hypothesized that

hipoccampal memory interacting with other network systems might acquire information represented as associative strength, either independently, synergistically, or competitively (see Kim and Baxter, 2001, for detailed information). Thus, when memory systems (such as the hippocampal formation) act independently, only one system is able to acquire information (acquires associative strength) under a learning experience or situation. For instance, if hippocampal memory system is required to acquire a certain kind of learning information, lesions to this structure might obliterate the learning acquisition, while lesions to other systems will not influence the process of learning in the former memory system (Kim and Baxter, 2001). Synergistic interactions between memory systems functionally characterizes that at least two-memory systems are required for the acquirement of some degree of information. Thus, lesions impinge on either one of the memory systems, which might result in an impairment of the learning processing or complete deficit or absent of acquisition of information, when both memory systems or neural structures confining them are exposed to combined injuries (Kim and Baxter, 2001). Moreover, competitive interactions allows one of the memory system to acquire high levels of information (over normal levels) if other memory system results to be damaged; nevertheless, in such model, the intact memory system will acquire much more of the information, that potentially will be distributed between both systems (see Kim and Baxter, 2001, for more descriptive details of such interacting models of memory systems in the brain). Although much of the conceptualized framework have emerged from lesion studies performed in experimental animals, complex results might be expected to occur from the interaction of such memory systems (Kim and Baxter, 2001) as will be described below. For instance, in the classical (Pavlovian) eye-blink conditioning, it has been demonstrated that the cerebellum is essential for mammals to learn a relationship between conditioned (CS) and unconditioned stimuli (US) (Kim and Thompson, 1997; McCormick et al., 1982). This simple form of associative learning seems to engage several brain structures, which presumably are involved in the performance of different aspects of the conditioning response (Thompson and Kim, 1996). For example, hippocampal neurons exhibit unit-firing patterns that emulate the amplitude time-course events of the conditioned response during delay eye-blink conditioning, favoring the idea that the hipoccampal formation is implicated in the development of such reflex response, even though this neural structure is not required for the development of such delay conditioning response. Thus, besides the implication of the hippocampus in the delayed eye-blink conditioning response, several studies have demonstrated that other conditioning-related events altered the hipoccampal physiology in experimental animals. Thus, it is possible to argue that during conditioning-related response, hipoccampal neurons process information in a similar context as the formation of the CS-US association in the cerebellum, interacting or interfering with this association process, in such a way, that an apparent competition between both neural structures seems to occur in standard delay conditioning (Kim and Baxter, 2001). Several experimental evidences support this view, as shown that manipulations that altered hipoccampal physiology facilitate acquisition of delay eye-blink conditioning (see in Kim and Baxter, 2001, table 1). Moreover, experimental evidences have demonstrated that although both cerebellum and hippocampus are essential for acquisition and development of eye-blink conditioning response, a minor alteration to the conditioning procedure eventually alters the interaction between both neural structures, such as when a brief time interval separates the CS and US (Solomon et al., 1986). These results demonstrate that multiple memory systems are engaged in the development of even simple classical conditioning tasks (Kim and Baxter, 2001).

Several experimental works have shown also that interactions between hipoccampal and extra hipoccampal memory systems occurs when rats are subjected to spatial memory tasks, as exemplified by interaction of hipoccampal-based spatial and caudate-based cue memory systems used for fixed-localization of visible platform in water maze task (McDonald and White, 1994). [Severing hipoccampal afferents or efferents (e. g., fornix) impair used spatial information and facilitate cue information to find the platform (McDonald and White, 1994) as well as in amygdala-dependent conditioned cue preference task (McDonald and White, 1995)]. Similar results have shown to occur after stress-induced impairments in hipoccampal LTP (Kim et al.,

2001), and infusion of glutamate into the hippocampus or the caudate induced an hipoccampal dependent strategy to persist in contrast to a caudate-dependent response strategy (Packard, 1999). These findings supports the idea that multiple memory systems in the brain are recruited in normal learning tasks and behavioral performance, where each structure–relationship memory system encodes different patterns of the learned task (Thompson and Kim, 1996; Kim and Baxter, 2001). Such behavioral conducted experiments revealed that hipoccampal memory systems interfere with the natural operation of other memory systems —whether this interactions are competitive or synergistic depends on the structure-relationship memory system used to consolidate a behavioral task response. For instance, neocortical areas and hipoccampal formation interactions might operate synergistically based on that sensory information conveyed by cortical efferents into the hippocampus subserves spatial cognition (Aggleton et al, 2000; Kim and Baxter, 2001).

LONG-TERM POTENTIATION

One of the crucial functional properties of the brain is memory. Memory is part of our daily functions of our lives, which allow us to accomplish numerous tasks, such as recalling personal experiences, learning facts and knowledge about our environment, as well as recognizing people, objects, and even acquiring skills and habits (Eichenbaum et al, 1999). Memory is not a unitary monolithic entity, reflecting a single faculty of the mind and brain. Several converging evidences from psychology and neuroscience have emerged concerning the existence of multiple memory systems than can be dissociated from one another (Eichenbaum et al, 1999). Moreover, the concept that the brain uses multiple memory systems (see below) comes from recent physiological and behavioral experimental evidences, but most of the historical background that led to this criteria emerged from several philosophers, thinkers, and neurologists from the 19th century; such as Gall, founder of the phrenological movement, who focused on the notion that each specialized faculty of the mind is concerned with particular contents (from a complete review see Zola-Morgan, 1995; Gall, 1835); De Biran, who distinguished three kinds of memory such as a representative memory, a mechanical memory and a sensitive memory (see Maine de Biran, 1929); the french psychologist T. Ribot (1881), who viewed that brain contains memory centers specialized to handled different kinds of information, such as the cortical auditory, visual, and motor centers, handling each one different forms of memory (for a complete review see Eichenbaum et al, 1999). Over the past 20th century, different disciplines in the neuroscience field have focused on the learning and memory processing in the brain. As such, several hypothesis proposed that learning and memory could be encoded via activity dependent changes in the strength of synaptic connections between neurons, initially postulated by D. Hebb, 1949 (Hebbian postulate), who advanced the concepts that underlie the conditions that cause synapses to change, which resulted essential for the experiments that demonstrated the mechanisms of LTP (Beggs et al., 1999).

The first interesting evidence showing that mammalian synapses could set specific type of modifications came at the beginning of the 70´s, where Bliss and Lomo demonstrated LTP (long-term potentiation) in anesthetized rabbits (Bliss and Lomo, 1973). In this very well known classical paper, these authors reported that brief, high frequency stimulation of the perforant pathway input to the dentate gyrus of the hippocampus produced a long lasting enhancement of the extracellularly recorded field potential (Bliss and Lomo, 1973; Beggs et al., 1999), and that non anesthetized animals showed LTP enduring for weeks and even months. LTP is a form of synaptic plasticity widely accepted as a cellular model for stabilization of synapses in neurobiological phenomena such as development including learning and memory (Harris, 1995). Although initially LTP was reported in the mammalian hipoccampal formation, several LTP studies done both *in vivo* and *in vitro* in different types of synapses, employing different methods and recording approaches, have led to the demonstration that LTP occurs not only in neocortical regions and subcortical nuclei of the brain (e. g., amygdala) of mammals, but also in the peripheral nervous system of non mammalian species –for instance in the arthropod neuromuscular junction and in different localized synapses of the invertebrate brain system (Beggs et al., 1999). Many of the experimental work in LTP have used brain slices of the hipoccampal

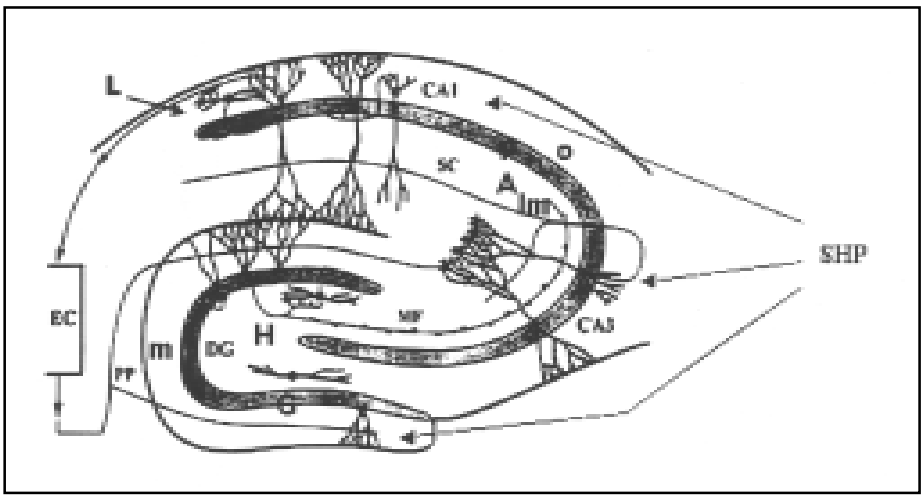

Figure 1. Structure and organization of the hipoccampal formation.- The hipoccampal formation is located in the posteromedial border of the hemispheres. It extends from the rostromedially located septum to the ventrolaterally located amygdaloid area. Although the hipoccampal region is structurally divided into the area dentate and the hippocampus proper, the hipoccampal formation includes the subicular region, retrospenial area and the entorhinal cortex. From the hipoccampal structure depicted above, the major hipoccampal neuronal circuit is described as follows: the entorhinal cortex **(EC)** sends the perforant pathway fibers (PP) to the granule cells of the dentate gyrus **(DG)** as well as to the pyramidal cells of the CA3 region. Major axonal output of the DG-granule cells **(G)**, the mossy fibers **(MF)**, make synaptic contacts with principal neurons of the CA3 field. While CA3 pyramidal cells project recurrent axon fibers into the same CA3 region, they also emit axon fibers into CA1 hipoccampal subfield through the Schaffer collaterals **(SC)**, where CA1 region sends projecting axons back to the **EC** and to the medial septal complex (not shown). Both DG-granule cells, CA1 and **CA3** pyramidal cells receive cholinergic afferents through the septo-hypocampal pathway **(SHP)**. Most of the principal neurons confined in each hipoccampal region are arranged in a dense continue layer; the stratum pyramidale **(p)** containing the pyramidal cell layer divides the CA1 and CA3 subfields into the stratum laconosum **(L)**/stratum oriens layer **(o),** which contains the basal dendrites of the pyramidal cells; the alveus/stratum radiatum **(A)**/stratum lacunosum moleculare **(lm)** contains the apical pyramidal dendrites, as shown in the figure. Several classes of neurons have been shown to be confined in the different cell layers that structure the hippocampal formation. Thus, confined in the dentate gyrus **(DG)** of the hilus fasciae dentate **(H)** are gabaergic polymorphic cells, mossy cells, chandelier cells, granule, basket, and molecular **(m)** associated pathway cells; interneurons have been described in the Stratum radiatum/stratum oriens layer; chandelier and pyramidal cells, in the CA3 hipoccampal subfield; interneurons in the lacunosum-moleculare layer; and both chandelier, pyramidal and basket cells, in the CA1 hipoccampal subfield (for more details see Walaas, 1983)(figure adapted from Ascoli et al., 1998, and modified by author of the present article).

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formation, due to the intrinsic circuitry is a relatively simple structure, where major cells within the laminar organization in this brain area remains intact in transverse slides (Dingledine, 1984; Barrionuevo and Brown, 1983). Such tissue preparations have resulted to be useful to obtain both extracellular and intracellular recordings as well (Beggs et al., 1999). Moreover, one of the most common synapse studied during LTP formation and maintenance in the mammalian brain, is the Schaffer collateral/commissural input to the pyramidal cells in the CA1 region within the hipoccampal formation (see figure 1) (Beggs et al., 1999). Nonetheless, several studies have been focused on the circuitry formed by the mossy fiber input arising from the granule cells of the dentate gyrus to the pyramidal neurons of the CA3 region in this same brain region (Beggs et al., 1999). For example, LTP can be induced after presynaptic tetanic stimulation of mossy fibers with short train of stimuli (e. g., 10 trains at 100 Hz) in transverse brain slices of the hipoccampal formation, while postsynaptic cell was depolarized under current clamp conditions. Under such experimental procedures defined as "paired-pulse" paradigm used to reveal "paired-pulse" facilitation (PPF) in the mossy fiber/CA3 circuitry, these showed potentiation of the excitatory postsynaptic current (EPSC) amplitude recorded in response to the application of set of paired-pulses. Thus, LTP induction at least in these *in vitro* preparations of the hipoccampal formation was observed to last for an hour or more (Barrionuevo et al., 1986; Xiang et al., 1994), with almost no decrement of the EPSC amplitude, but *in vivo* studies have reported longer periods of weeks or even months to obtain similar electrophysiological recording responses (Beggs et al., 1999).

Most of the synapses in the hipoccampal

formation that have shown to exhibit LTP are characterized by several "classical properties" (cooperativity, associativity, input sensitivity, and spatiotemporal specificity) (Brown et al., 1990; Bliss et al., 1993) which are out of scope to describe them in this review (for more information see Beggs et al., 1999). They reflect different expressions of the same underlying mechanisms responsible for the induction of different forms of LTP. Briefly, LTP induction in the SCh/com synaptic input to pyramidal neurons in the CA1 region of the hippocampus requires strong high-frequency stimulation (tetanic). However it does not need weak stimulation to recruit enough axons (cooperativity) to induce LTP, neither the association between a weak stimulation of an afferent input (e. g., Schaffer collateral/commisure input to CA1 or the perforant pathway input to dentate gyrus of hipoccampal formation) nor a strong stimulation of same afferent inputs for LTP to be induced —i. e., LTP can be induced only if a weak input (small number of stimulated afferents recruited), is associated with a strong input (large number of stimulated afferents recruited) and this response is restricted to the afferent inputs receiving tetanic stimulation (input specificity) at the same time (spatial and temporal specificity) (Beggs et al., 1999). Although most of the research focused in LTP requires explanations of how this process occurs physiologically in the CNS system, including the underlying neurochemical and molecular mechanisms involved in, conventionally LTP has been divided in three correlated mechanisms defined as induction, expression, and maintenance, describing each step, the initial events that trigger the specific plastic synaptic modifications in neural pathways, the expression of the final synaptic enhancement, and the enduring over time of the enhancement of the synaptic strength defined as maintenance (Beggs et al., 1999).

Much of the understanding of the neurochemical, pharmacological, and molecular mechanisms involved in LTP induction, and that ultimately leads to LTP expression and maintenance, has been through the study of the glutamate neurotransmission system and their receptors. By far, most of the neurotransmission system shown to participate in LTP formation involve glutamate as preferential neurotransmitter, although exceptions have been recently reported (Beggs et al., 1999). Therefore, in order to understand the neurochemical and molecular events occurring during LTP it is crucial to relate LTP with glutamate and their receptors as well (Beggs et al, 1999).

a) Glutamate receptors subtypes. Glutamate receptors (GluRs) known to be widely distributed in the nervous system are responsible for mediating major excitatory

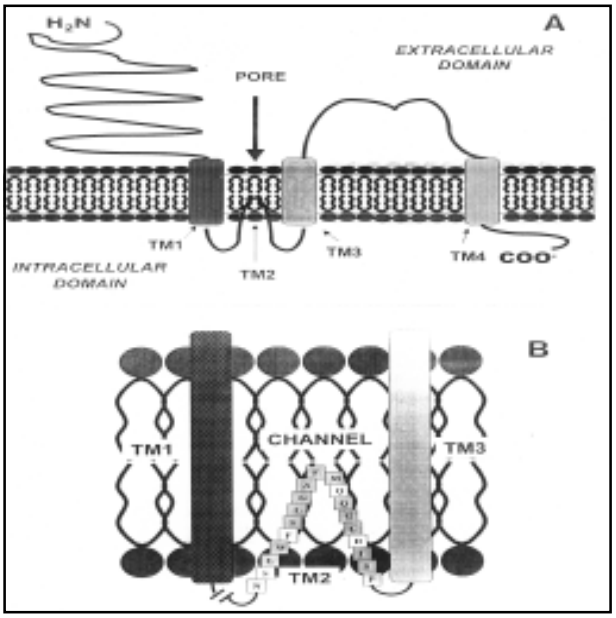

Figure 2. - Schematic representation of a structural model of a Non-NMDA (ionotropic) glutamate receptor. (A) Shows the structural conformation of one of the subunits of a typical ionotropic glutamate receptor conformed by four hydro-phobic membrane- spanning domains (TM1-TM4), as evidenced by recent molecular and biochemical studies. The TM2 membrane-spanning segment forms a "hook" that does not transverse the membrane completely, and extends right back into the cytoplasm, sharing some similarities with the P segment (pore forming domain) of the voltage-activated K^+ channels. Both NH2-terminus extending from the TM1 segment and the loop formed between the TM3 and TM4 membrane-spanning segments may be critical extra-cellular domains for ligand binding as well as proper activation of the channel–receptor. COOH terminus- extending to the intracellular domain may serve as a regulatory domain whose function is still elusive. (B) Depicts an enlarged area of the predicted structural model of the TM2 membrane spanning segment of the glutamate receptor subunit, GluR3. Both TM1 and TM3 drawn as cylinders flanked the TM2 region. This region may vary in length among ionotropic glutamate receptors as shown by the break in the TM2 loop. The amino acid sequence of this region is highlighted in order to show that the glutamine residue **(Q)** determines the permeability of calcium ions through the pore or ion channel. This amino acid vary among the different glutamate receptor subtypes, as produced by the edition of the mRNA. For instance, in NMDA receptors the asparagine residue (E) at the same position of the (Q) residue defines the interaction of Mg2+ ions within this polypeptide segment, enhancing the blocking of the voltage-dependent channel (see figure 3 and text). Moreover, the highlighted serine (S) and phenylalanine (F) amino acids depicted in white have been shown to be conserved in all Non-NMDA glutamate receptor family. (Figure and most of legend has been adapted from Waxham, 1999, and modified by author of the present publication.)

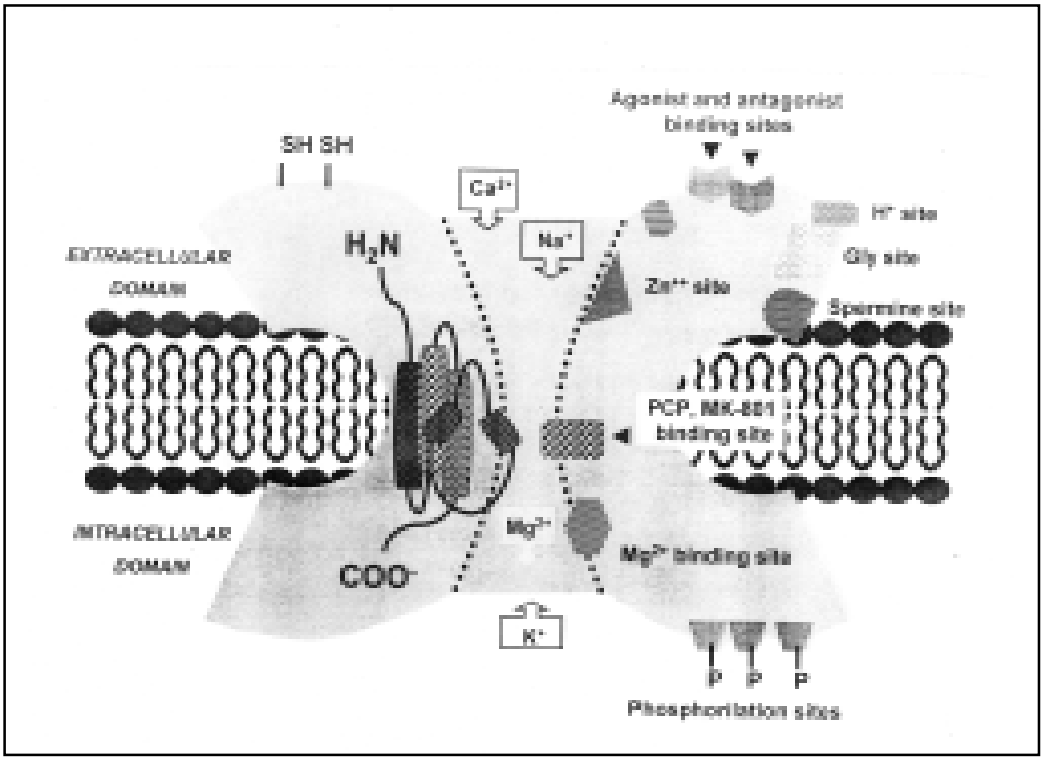

Figure 3. Schematic representation of the structure of the glutamate NMDA receptor. NMDA receptors comprise a family of ligand-gated voltage-dependent ion channels, structurally conformed by four membrane-spanning segments, as shown for Non-NMDA glutamate receptors, with the NH2-terminus extending to the extracellular domain and the COOH-terminus extending to the intracellular domain as well. The figure depicts several binding sites for both glutamate and ligand agonists and antagonists as shown, as well as for different binding sites for several regulatory molecules (see text). Mg2+ binding sites as well as binding sites for hallucinogenic compounds, MK-801 and PCP, are localized inside the channel that effectively produce a voltage-dependent block for Ca2+ permeation and other ion influx as well. (Figure has been adapted from Waxham, 1999, and modified by author of the present article.)

synaptic transmission in the brain and spinal cord (Waxham, 1999). Most of the neural systems where LTP has been identified used glutamate as neurotransmitter (Beggs et al., 1999). Initial pharmacological studies performed early in the 70´s suggested that glutamate receptors were not homogenous in the CNS, as they could be distinguished and separated in different glutamate receptors subtypes after demonstrating that different synthetic agonists, namely N-methyl-D-aspartate or *NMDA*; α-amino-3-hydroxy-5-methyl-isoxazoleproprionic acid or *AMPA*; *kainate* and *quisqualate,* in the presence of competitive antagonists were able to bind distinctively different types of membrane receptors (Watkins et al., 1990). These agonists (used extensively to characterize the glutamate receptor family) led to the initial identification of *ionotropic* (membrane bound protein complexes that combine to form an ion permeable pore or ion channel through the membrane) *and metabotropic glutamate receptors* (receptors composed by a single transmembrane polypeptide chain containing an extracellular binding domain for the preferential ligand-agonist and an intracellular binding domain that couples and activates upon receptor activation, GTP-binding proteins or G proteins (Waxham, 1999). For instance, the pharmacological agonist quisqualate is unique in that it shows a binding profile to both ionotropic and metabotropic glutamate receptor subtypes (Hollmann and Heinemann, 1994). In same context, ionotropic glutamate receptors [see figure 2 (a, b), and figure 3] were defined as either NMDA or non-NMDA receptors subtypes, depending on the capability of the NMDA agonist to bind with high affinity and selectively to such receptors subtypes (Watkins et al., 1990; Hollmann and Heinemann, 1994, Waxham 1999).

b) Non-NMDA glutamate receptors and LTP. Introduction of DNA recombinant technology widely used for molecular cloning of receptors made feasible to identify and isolate initially a cDNA that encoded a protein with

a molecular mass of 99.8 kDa, after its transfection and expression in Xenopus oocytes, producing a functional and stable glutamate-activated channel, named GluR-K-1 (Hollman et al., 1989). Similar reports describing the isolation and functional expression of a new family of glutamate receptor subunits (termed Glu-R1-Glu-R4) demonstrated that the Non-NMDA-glutamate receptor form a native pentameric complex in the brain, structured with four predicted membrane-spanning segments (TM1-TM4) (figure 2a) and a large extracellular domain expressing a total molecular mass of around 600 kDa (Boulter et al., 1990; Keinanen et al., 1990; Nakanishi et al., 1990; Blackstone et al., 1992; Wenthold et al., 1992). Thus, these results demonstrated that the molecular complex of this receptor is twice the molecular size of the reported for the nAChR (see Waxham , 1999). Moreover, functional expression of these cloned cDNAs encoding such glutamate receptors showed that they were capable to produce inward currents after its functional and stable expression either in oocytes or Hek-293 cells and after application of Non-NMDA agonist AMPA or Kainate. Similar studies showed that both GluR1 and GluR3 subunits when expressed alone or in combination in these cells produced functionally channels with large inward currents exhibiting channels permeable exclusively to the calcium ion, situation that did not occur when co-expression was performed in the presence of the GluR2 subunit (Hollmann and Heinemann, 1994). Therefore, the expression of this glutamate receptor in the presence of the GluR2 subunit induced channels impermeable to Ca^{2+} (Hollmann and Heinemann, 1994). This structure-activity relationship studies of the Non-NMDA glutamate receptor led to the hypothetical proposition that two types of receptors were expressed in neurons, as several electrophysiological studies showed in embryonic hipoccampal neurons describing the presence of a glutamate receptor impermeable to Ca^{2+}, and another one permeable to the same ion (Hollmann and Heinemann, 1994). Actually, it has been shown that glutamate receptors have the ability to acquire different properties producing different intracellular responses depending on the structural conformation of their protein subunits expressed (Waxham, 1999). For instance, several studies have demonstrated that these receptors are able to switch from a Ca^{2+} impermeable to a Ca^{2+} permeable channel through the exchange of a single amino acid (Arg→Gln) on the TM2 loop of the GluR-2 subunit, or replacing of Arg-to-Gly at GluR3 and GluR4 subunits, a conversion that allows a fast rate of receptor recovery from desensitized state (see figure 2b)(Lomeli et al., 1994). This molecular event depends on the molecular mechanism in which the mRNAs of different GluR subunits are edited and spliced inside the neuron, enhancing the functional expression of glutamate receptor subtypes (see Waxham, 1999, for more details). In this context, molecular studies on the analysis of the mRNAs encoding the different GluR subunits have shown that each subunit can be expressed in one of two splice variants, as defined in terms as flip and flop (Sommer et al., 1990). For example, this flip and flop variants are represented by small segments that will determine the nature of the TM4 transmembrane domain in all four GluR subunits favoring the expression of a GluR-receptor channel with different properties. These flip-flop expressing versions in glutamate receptors are widely expressed in the brain with some exceptions (e. g., CA3 pyramidal cells in rat hippocampus contain GluRs deficient in flop-version-modules, while CA1 pyramidal and dentate granule cells express GluRs with high abundant flop-version-modules) (Waxham, 1999). For instance, it has been shown that glutamate receptors expressing the "flop-version-modules" exhibit greater magnitudes of desensitization after application of glutamate, and express steady-state currents than receptors expressing the "flip-version-modules" (e. g., CA3 and dentate granule cells) (Waxham, 1999).

Concerning the implication of non NMDA receptors subtypes in LTP induction, several studies have demonstrated that the widely accepted classical Hebbian or NMDA-R dependent form of LTP can be only applicable to certain synapses under specific experimental conditions. As explained above, some synapses forming the mossy-fiber synaptic input to CA3 pyramidal cells or the SCh/com inputs to CA1 pyramidal cells in the hippocampus exhibit a NMDA-indepen-

dent forms of LTP induction, besides the demonstration that same synapses respond to the classical Hebbian form of LTP mediated by NMDA receptors (Johnston et al., 1992). In such context, high frequency tetanic stimulation of hipoccampal synapses was able to induce LTP in the presence of the competitive antagonist DL-APV. Tetanic stimulation was able to release glutamate from presynaptic terminals and competitively unblocked the binding of competitive antagonist when applied in high concentration (200 mM)(Grover and Teyler, 1995). Although the onset of the NMDA-R-independent LTP resulted to be slow (20-30 min), this form of LTP showed input specificity (see below) and could be prevented by L-type calcium channel blockers (e. g., nifedipine)(Grover and Teyler, 1995). These results, in addition to others, have demonstrated that NMDA-R-independent form of LTP is not only restricted to the CA1 or CA3 hipoccampal region but also exists in several neocortical areas (Beggs et al., 1999). Based on these experimental results, is possible to suggest that both NMDA-R-dependent and NMDA-R independent forms of LTP could be potentially co-expressed in same brain regions, where different types of synaptic inputs and/or same inputs could be recruited, impinging on same postsynaptic neuron. Given such potential possibilities, one could tentatively suggest that NMDA-R antagonists (APV) may not be expected to block all forms of LTP when applied in behavioral studies (see above)(Beggs et al., 1999).

c) *Ligand-gated ion channels coupled to NMDA glutamate receptors and its role in LTP.* NMDA receptors have been shown to be involved partially in several neurobiological functions such as neural development, learning and memory, as well as neuronal damage induced by brain injury. The significance between the functional expressions of this ionotropic receptor subtype to neuron function is mainly due to several functional properties of this receptor (Waxham, 1999). Under such context, one property, known as *associativity*, defines that a sequence of events must first occur in order to allow Ca^{2+} ions to permeate through the channel-membrane receptor (second property). Thus, as an initial step, the binding of glutamate will alter its receptor conformation as to facilitate a membrane depolarization state, and then produce a calcium ions influx through the receptor-channels. This natural behavior of this receptor is merely due because at the physiological membrane resting potential, this receptor shows a Mg^{2+}-dependent blockage (see figure 3) (Ascher & Novak, 1988). The intracellular increase of calcium, brought by receptor-channel Ca^{2+} influx, produce an activation of several neuron processes that ultimately modify the properties of the neuron (Waxham, 1999). Is worth to note that high levels of Ca^{2+} might be toxic to neurons, thus, hyperactivity of NMDA receptors have been postulated to promote a variety of neurodegenerative disorders (Waxham, 1999). Furthermore, pharmacological studies have demonstrated that although the specific ligand agonist for this receptor is NMDA (see above); glutamate is one order of magnitude more potent for activating this receptor. In addition, further studies have demonstrated that potent antagonists of such receptor-channels, such as the hallucinogenic compound phencyclidine (PCP) and the non competitive antagonist MK-801, effectively block the NMDA-receptor-ion channel when the receptor is in an open-state to allow access to intra-channel-binding sites (open-channel blockers) and prevent NMDA-R dependent LTP induction (Waxham, 1999, Beggs et al., 1999). Moreover, such antagonists result to be trapped when channel is closed, and therefore they are difficult to be washed out from cell or tissue preparations (see figure 3).

Molecular characterization of the primary structure of the NMDA receptor showed that the deduced amino acid sequence from the first isolated cDNA, which encodes one subunit of this receptor (named NMDAR1), indicates a protein with a molecular mass of 97 kDa, contains at least four identified transmembrane domains (Moriyoshi et al., 1991), and suggests that five individual subunits conform the complexity of the NMDA receptor. These results demonstrate, at least from the molecular and structural view, that this receptor displays a similar identity to other entities conforming the large GluR family (Waxham, 1999). Moreover, molecular studies have shown that at least eight splice variants are formed from the NMDAR1 subunit, producing a range of functional properties of the expressed receptors (Hollmann and Heinemann, 1994).

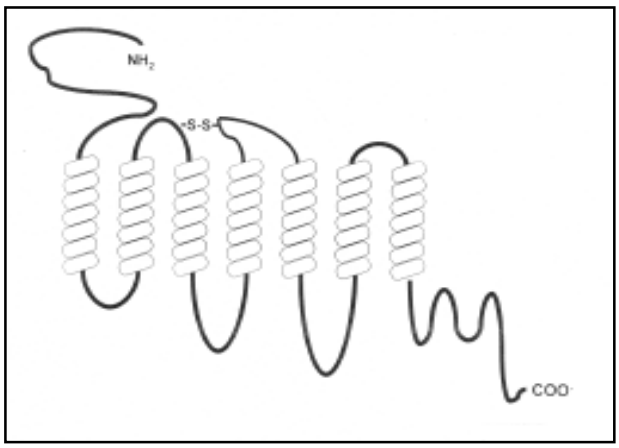

Figure 4. – Schematic representation of the structure of meta-botropic (glutamate) receptor. Metabotropic glutamate receptors are comprised within a large family of G-coupled protein receptors (over 250 cloned members) containing seven membrane-spanning (α-helical) domains that share structural homology with several other well-characterized metabotropic receptors. The figure depicts a structural model of a metabotropic glutamate receptor (mGluR) consisting of seven transmembrane segments (TM1-TM7) which traverse the membrane lipid bilayer, giving rise to three extracelular loops and the initial NH2-termini domain, and three intracellular loops and the COOH terminal segment. Specific amino acids codified within the TM3, TM5, and TM5 membrane are crucial for binding of ligand agonists (not shown). A disulfide bond **(S-S)** between two cysteine residues is localized between the 2nd and 3rd extracellular loops, favoring the structural stabilization of the protein receptor. In a similar context, specific group of amino acids codified at the 3rd and the COOH terminal tail are known to be important for the functional coupling of G proteins to the ligand-bound conformation state of the membrane receptor. (See text for details)(Figure has been adapted from Waxham, 1999, and modified by author

Recently, four new protein subunits of this receptor subtype have been identified and cloned (NMDAR2A-2D), with the difference that these protein subunits do not structurally conform a membrane-receptor channel when expressed by themselves, unless they coexpressed with NMDAR1 receptors (Kutsuwada et al., 1992; Meguro et al., 1992). Functional studies revealed that these receptors are relevant for modulating receptor activity when mixed as heteromeric forms with NMDAR1 (Waxham, 1999; Monyer et al., 1992). From the molecular characterization of the cloned cDNA of the NMDAR2 subunit, it was revealed that the C-terminus of this subunit is relatively large as compared to the NMDAR1 receptor subunit (C-terminus) suggesting that this domain possibly interacts with other intracellular proteins, potentially used to target this receptor subunit to specific domains of the neuron (Ehlers et al., 1995; Komau et al., 1995). The biophysical properties of the NMDA receptor have been shown to be complex, because different levels of single-channel conductances can be registered after combining different protein-receptor subunits. Moreover, neurochemical and biophysical studies have shown that Ca^{2+} influx through the NMDA receptor induce the binding of Ca^{2+}-calmodulin, producing a significant decrease in ion influx (Ehlers et al., 1996). Studies performed to reveal the anatomical distribution of these protein-receptor subunits have shown the neural expression of these receptors in restricted areas of the brain when compared to NMDAR1 (with exception of the NMDAR2A, which display a widely distribution throughout the brain) (Waxham, 1999).

NMDA receptors have been shown to be involved in LTP induction at specific hippocampal synapses. Due that NMDA receptors are permeable to Ca^{2+}, postsynaptic Ca^{2+} has been shown to play a crucial role for induction of the NMDA receptor-dependent form of LTP. Channel-receptor permeability depends on several presynaptic and postsynaptic conditions that ultimately will activate the channel-receptor opening. First, channel opening requires that glutamate (or any ligand agonists) released by presynaptic activity (presynaptic condition) binds to NMDA binding site, localized in the extracellular domain of the channel-receptor (Beggs et al., 1999). Second, as Mg^{2+} physiologically blocks the channel-receptor at the usual resting membrane potential, induced depolarization of the postsynaptic membrane where NMDA-receptors are located will unblock the ionic channel, and the enhanced ionic conductance through the channel will allow Ca^{2+} permeability only when presynaptic release of glutamate is paired with postsynaptic depolarization (postsynaptic condition)(figure 3).

Several studies have shown that ionotropic NMDA-R and AMPA-R receptors have different biophysical properties that make them unique in their role of induction of specific forms of LTP. For instance, AMPA-receptors do not exhibit a voltage-dependent blocking effect by magnesium ions, and the ionic conductance mediated by this channel-receptor is actually voltage independent. Not surprising, it could be assumed that glutamate could activate both ionic-channel receptors

(NMDA and AMPA) co-localized in same dendritic spines (Beggs et al., 1999; Magee & Johnston, 1997). In such context, one would be able to scope that sequence of concurring events defined by presynaptic and post-synaptic activity, mediated by an initial significant amount of presynaptic glutamate released, binding of same neurotransmitter to channel-receptor ligand agonist-binding sites at postsynaptic membrane, and release of Mg^{2+} block by concurrent postsynaptic depolarization, will enhance Ca^{2+} inflow into dendritic spines confined in the postsynaptic neurons (Beggs et al., 1999). Thus, based on computational models of NMDA-receptor behavior in LTP, (Holmes and Levy, 1990) Ca^{2+} influx into dendritic spines and the resultant increased of intracellular concentration of Ca^{2+} in critical regions of same dendritic spines (close to NMDA-R), will activate calcium dependent enzymes (e. g., CAM-kinase II) that play a crucial role in LTP induction (Beggs et al., 1999). Furthermore, correlation between molecular events can be visualized with the physical properties of LTP (see above); for instance, active synapses releasing glutamate will result in an initial binding of the neurotransmitter to NMDA-R, which will cause a Ca^{2+} influx into dendritic spines on postsynaptic neurons, only when the synaptic input is strong enough to cause a depolarization on the postsynaptic membrane, resulting in an input specific LTP. Moreover, calcium permeability into post-synaptic neurons will relieve the blocking effect of Mg^{2+}, enhancing another property of LTP, defined as *cooperativity* (see above, Beggs et al., 1999). Although depolarization itself is mostly mediated by voltage-independent-AMPA-R, activity of a weak input will not be enough to depolarize the postsynaptic membrane and to unblock the binding of Mg^{2+} from the channel-receptor. Therefore, a strong synaptic input on same postsynaptic cell will then be necessary to relieve the Mg^{2+} blocking effect (Beggs et al., 1999). Depolarization of postsynaptic cell by both synaptic inputs will result in two other properties of LTP, such as, *associativity and spatio-temporality specificity* (Beggs et al., 1999).

Computational models based on the gating properties of the NMDA-R have been developed and used to evaluate much of what has been already investigated, regarding the properties of NMDA-receptor dependent form of LTP. Computer analysis of formal models of this neurobiological process have revealed that NMDA-R alone is not sufficient to account for the classical properties of LTP, due to the intrinsic characteristics of the dendritic spines that play a main role in LTP induction, as well as the activity of second messenger signals mediated by changes of intracellular concentration of calcium ions ($[Ca^{2+}]i$) known to perform a number of functions that allow the functional operation of this neural mechanism (for details see Beggs et al., 1999, Zador et al., 1990; Holmes and Levy, 1990; Martin et al., 2000). Moreover, several studies using computer simulations, have shown that propagation of antidromically action potentials mediated by Na^{+} ions (spikes generated in the soma and back-propagating into dendrites) have little effect, unless antidromic Na^{+}-dependent-spikes activate voltage gated calcium channels (VGCCs), which will cause a pronounced effect in inducing or at least will effectively participate in LTP induction in active synapses (Beggs et al., 1999).

d) Metabotropic glutamate receptors and its implication in LTP expression. Metabotropic glutamate receptors (mGluR) are G-protein-coupled membrane receptors, structurally conformed by seven transmembrane-peptide domains which share minimal homology with other well characterized metabotropic receptors (e. g., muscarinic Ach receptors, adrenergic receptors, dopamine receptors, purinergic receptors, serotonin receptors, GABA-B, as well as the super family comprising the large opioid and non opioid peptide receptors)(Waxham, 1999; Deutch & Roth, 1999). Metabotropic glutamate receptors have been shown to be widely distributed in the CNS and functionally expressed at both presynaptic and post-synaptic neurons (Waxham, 1999). In general, metabotropic receptors are G-coupled protein receptors structurally conformed by a conserved single polypeptide structure, which consist of a seven trans-membrane-spanning α-helical domains or hydrophobic peptide segments (TM1-TM7), where each TM domain is connected with both extracellular and intracellular loops (as shown in figure 4) (Strader et al., 1995; Mizobe et al., 1996; Kobilka et al., 1992). In addition, both N-terminus and C-terminus of

the receptor polypeptide extend to the extracellular and intracellular space, respectively (Waxham, 1999). Several studies concerning proposed models for metabotropic receptors have assumed that binding of ligand-agonists to receptor-binding sites (which in the case for the mGLuR, the binding site for glutamate resides at the N-terminal extracellular domain) induced an inactive to an active conformational change of such receptors which enhance the coupling of Gs/Go proteins at the third intracellular loop and the C-terminus of same polypeptide chain (see Waxham, 1999, for more details). Moreover, molecular studies have confirmed that coupling of G-proteins to metabotropic receptors increases the binding affinity of such receptors for ligand agonists (Waxham, 1999). Molecular studies using DNA recombination technology have identified and characterized eight different mGLuRs, which overall comprise a large family of heterogeneous protein receptors which vary in size (from 854-1179 amino acids), where the N- and C-terminus domains are unusually large as compared with several other identified and molecular characterized G-protein-coupled membrane receptors (Waxham, 1999). Moreover, same studies have shown, for instance, that class I mGluRs subtypes (mGLuR1 and mGLuR5) activate G-protein-coupled to phospholipase C (PLC) and phospholipase A_2, as demonstrated after stably transfection and functional expression of both mGluRs subtypes cDNAs into eukaryotic cells. Activation of PLC induces the enzymatic breakdown of membrane phospholipids to produce dyacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) (Beggs et al., 1999; Bashir et al., 1993; Aramori, et al., 1992). While DAG released will be responsible for modulating channel activity after functional activation of protein kinase C (PKC); IP_3 will induce the release of Ca^{2+} from intracellular stores. Such metabolic process does not lead to a fast increase of Ca^{2+} concentration, as does opening of membrane voltage-gated calcium channels (Beggs et al., 1999; Bashir et al., 1993). Conversely, Class II mGluRs subtypes, represented by mGluR2 and mGluR3, are G-protein-coupled receptors that mediate inhibition of the intracellular signaling cascade pathway formed by adenylate cyclase-cAMP and several phosphorilating proteins (e. g., PKA, PKC) (Beggs et al., 1999) which will result in a significant decreased of cAMP and reduced activity of several intracellular processes.

In addition, activation of mGluR1 has been implicated with long-term depression in the cerebellum, as well as in synaptic plasticity events in many areas of the brain (Waxham, 1999). Concerning the role of such mGluRs in long-term potentiation in the hippocampus, several studies have demonstrated the implication of mGluR1 in LTP induction in the Sch/com input to the hippocampal CA1 region (Waxman, 1999; Bashir et al., 1993). These experiments have shown that LTP induction could be blocked by application of the mGluR-antagonist, α-methyl-4-carboxy-phenylglycine (MCPG), in hippocampal synapses that were not exposed to previous high frequency stimulation (HFS), but not in those that received prior exposure to same HFS. Such results led to hypothetical postulation that mGluR may act as a "molecular switch" (Beggs et al., 1999; Bortolotto et al., 1994) that need to be activated for LTP induction. Although extensive experimental work has been performed to enroll mGluRs in LTP induction, several issues concerning the implication of the different mGluRs in the different process or events taking place in LTP (e.g., LTP induction, expression and maintenance) still remains elusive. Moreover, one important aspect that still needs to inquire for refers to the molecular mechanisms involved in the neural distribution and quantitative cell expression of each of the mGLuRs subtypes if implicated in LTP. Finally, according to their brain distribution and particular cellular expression of these glutamate receptors subtypes, the next search would then be focused in the roles each receptor display in neuronal function.

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