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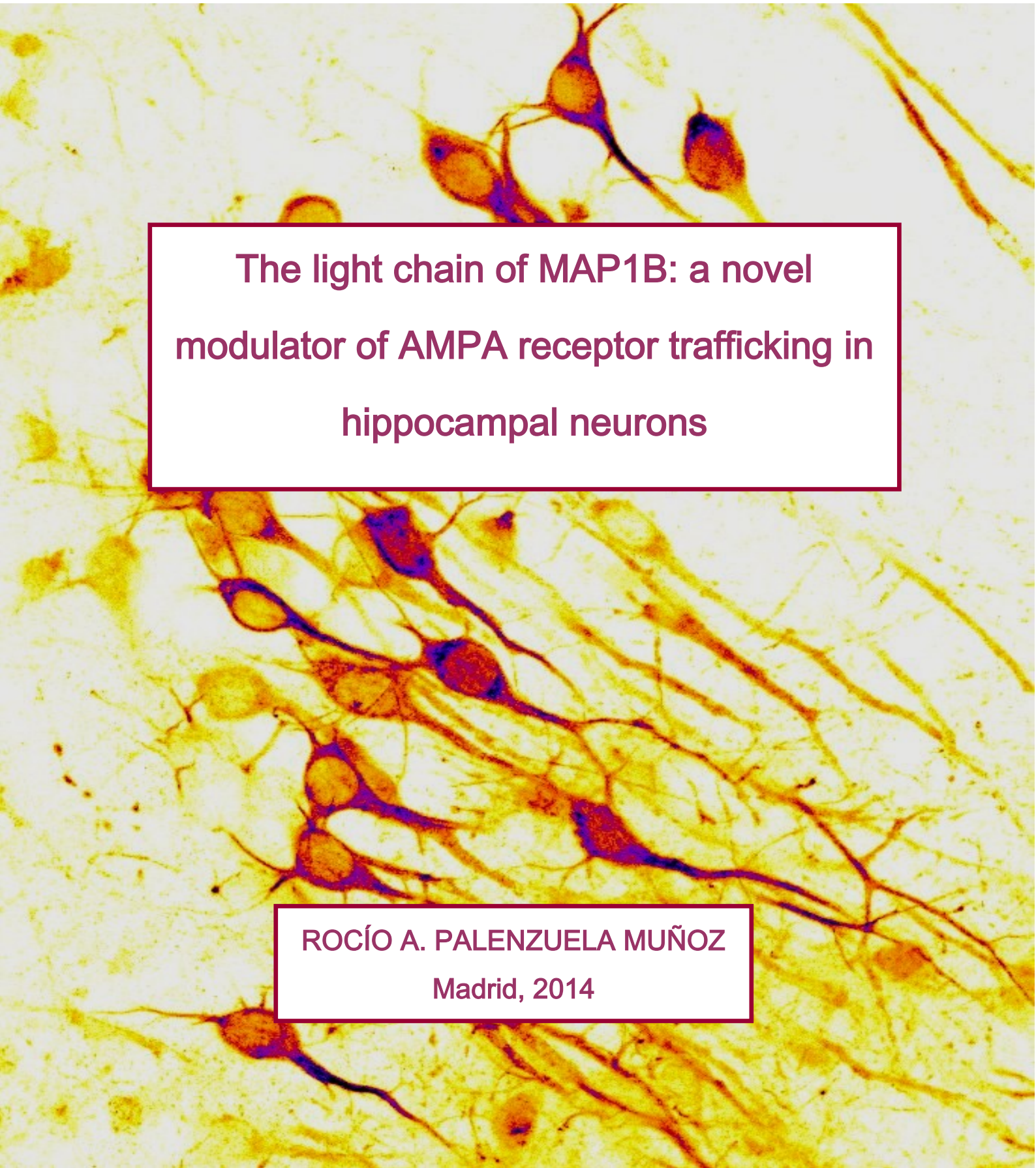

The light chain of MAP1B: a novel modulator of AMPA receptor trafficking in hippocampal neurons

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Madrid, 2014

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FACULTAD DE CIENCIAS

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The light chain of MAP1B: a novel modulator of AMPA receptor trafficking in hippocampal neurons

Memoria presentada por Rocío A. Palenzuela Muñoz, Licenciada en Farmacia, para optar al grado de Doctor en Bioquímica, Biología Molecular, Biomedicina y Biotecnología (Biociencias Moleculares) por la Universidad Autónoma de Madrid.

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La realización de esta tesis doctoral ha sido posible gracias a la concesión de una Ayuda Predoctoral de Formación en Investigación en Salud (PFIS) del Instituto de Salud Carlos III (FI10/00446). Su desarrollo ha tenido lugar en el laboratorio del Dr. José Antonio Esteban García, en el Centro de Biología Molecular "Severo Ochoa" (CBMSO), centro mixto del Consejo Superior de Investigaciones Científicas (CSIC) y la Universidad Autónoma de Madrid (UAM). Esta tesis doctoral ha sido dirigida por el Dr. José Antonio Esteban García y co-dirigida por la Dra. Marion Benoist.

El Dr. José Antonio Esteban García, Profesor de Investigación del Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) y director de esta Tesis Doctoral, y la Dra. Marion Benoist, investigadora en el Instituto de Neurobiología del Mediterráneo (INMED), Marsella (Francia), y co-directora de esta Tesis Doctoral,

Hacen constar:

Que el trabajo descrito en la presente memoria, titulado "The light chain of MAP1B: a novel modulator of AMPA receptor trafficking in hippocampal neurons", ha sido realizado por Rocío A. Palenzuela Muñoz bajo su dirección y supervisión, dentro del programa de Doctorado en Biociencias Moleculares del Departamento de Biología Molecular de la Universidad Autónoma de Madrid. Por reunir los requisitos de rigor científico, innovación y correcta aplicación metodológica, director y co-directora dan su Visto Bueno a la presentación de dicha Tesis Doctoral.

Madrid, a 30 de mayo de 2014

Fdo: Dr. José Antonio Esteban García

Fdo: Dra. Marion Benoist

AGRADECIMIENTOS.

Lo bueno, si breve, dos veces bueno.

Gracias a mis directores de tesis, los Drs. José A. Esteban y Marion Benoist. Por todo lo que me han enseñado y por su dedicación y disponibilidad a lo largo de estos casi cinco años. Gracias por su exigencia, que me ha ayudado a mejorar poco a poco. Gracias por enseñarme qué es el rigor científico y por ayudarme a adquirir un profundo sentido crítico. Gracias por transmitirme su pasión por la ciencia. Aunque la vida me lleve por otros derroteros, la formación que he adquirido con y gracias a ellos, a nivel profesional y personal, me acompañará siempre.

Gracias a todos los miembros del "Esteban Lab", presentes y pasados, por prestarme su ayuda y brindarme sus consejos. Gracias a María M. por su apoyo y su amistad.

Gracias a los miembros presentes y pasados del Servicio de Microscopía Óptica y Confocal del CBMSO por su amabilidad, sus expertos consejos, y el tiempo dedicado a ayudarme en mis experimentos. Gracias al personal de los distintos servicios del CBMSO por su calidad humana y por levantarme una sonrisa al final de la jornada.

Gracias a mis buenos amigos, los de toda la vida y los más recientes, por su cariño y apoyo incondicional.

Gracias a mi familia, por su apoyo sin fisuras y su fe en mí. Gracias por su confianza y su aliento. Gracias por transmitirme los valores que hoy considero míos y la independencia de pensamiento que tanto valoro. Gracias por enseñarme a seguir trabajando y a no rendirme, sean cuales sean las circunstancias.

Y gracias a Juan Carlos, por acompañarme de la mano en este viaje y por responder a los problemas y las dudas con infatigable optimismo y buen humor. Sin ti, nunca habría llegado hasta aquí.

"[Las neuronas son] células de formas delicadas y elegantes, las misteriosas mariposas del alma, cuyo batir de alas quién sabe si esclarecerá algún día el secreto de la vida mental."

Santiago Ramón y Cajal.

RESUMEN.

En el cerebro, la eficacia de la transmisión sináptica depende en gran medida del control dinámico ejercido sobre la adición a las sinapsis y la eliminación de las mismas de ciertas clases de receptores de neurotransmisores. Los receptores de glutamato de tipo AMPA (AMPARs) median, de forma mayoritaria, la transmisión sináptica excitatoria en el sistema nervioso central de los mamíferos. De hecho, la regulación de su tráfico intracelular constituye uno de los mecanismos principales por los cuales se modulan los fenómenos de plasticidad sináptica en las sinapsis hipocampales. En este trabajo nos hemos propuesto explorar la función de MAP1B, una proteína asociada a microtúbulos, en la regulación del tráfico de AMPARs en las neuronas piramidales CA1 de hipocampo.

Mediante una combinación de herramientas moleculares, electrofisiología y microscopía confocal, hemos revelado una nueva función de la cadena ligera de MAP1B (MAP1B-LC) como elemento regulador del transporte intracelular de una población específica de AMPARs. Hemos podido determinar que la sobre-expresión de MAP1B-LC resulta en una reducción neta de la fracción móvil en dendritas de los AMPARs constituidos por la subunidad GluA2, y como consecuencia, en una acumulación disminuida en espinas, sin que los receptores formados por la subunidad GluA1 se vean afectados. En efecto, hemos podido comprobar que es la población endógena de receptores GluA2-GluA3 la que se ve afectada específicamente cuando se sobre-expresa MAP1B-LC, ya que su transporte constitutivo hacia las sinapsis, y por tanto, la transmisión sináptica basal, se ven reducidos en presencia de niveles incrementados de MAP1B-LC. Por otra parte, hemos demostrado que la distribución a lo largo de las dendritas de GRIP1, una proteína de ensamblaje que interacciona específicamente con las subunidades GluA2 y GluA3 y también con MAP1B-LC, se ve así mismo alterada como consecuencia de la sobre-expresión de MAP1B-LC. Así, por medio de mutantes de deleción de MAP1B-LC, hemos podido concluir que la unión de MAP1B-LC a GRIP1 junto con su interacción con los microtúbulos es esencial para regular la expresión en superficie y la presencia en las sinapsis de la población GluA2-GluA3 de AMPARs, y por consiguiente, su contribución a la transmisión sináptica basal en neuronas hipocampales CA1.

Es importante destacar que el modelo que proponemos asigna, por primera vez, un significado funcional a la interacción entre MAP1B-LC y GRIP1.

SUMMARY.

The strength of synaptic transmission in the brain relays largely on the controlled addition and removal of neurotransmitter receptors to and from synapses. AMPA-type glutamate receptors (AMPARs) mediate the vast majority of excitatory transmission in the mammalian central nervous system. Their regulated trafficking has been proposed to be one of the major mechanisms underlying the expression of synaptic plasticity at hippocampal synapses. In this work, we have explored the potential role of a microtubule-associated protein, MAP1B, in the fine-tuning of AMPAR trafficking in CA1 hippocampal neurons.

Using a combination of molecular tools, electrophysiology and confocal microscopy, we reveal a novel role of the light chain of MAP1B (MAP1B-LC) as a key player in the subcellular sorting of a specific population of AMPARs. We demonstrate that MAP1B-LC over-expression results in a net reduction of the mobile population in dendrites and their accumulation in spines of recombinant GluA2 AMPARs, whereas the dendritic trafficking and delivery to spines of recombinant GluA1 AMPARs is unaltered. Indeed, we show that MAP1B-LC targets specifically the endogenous GluA2-GluA3 population of AMPARs, as their constitutive cycling toward synapses is impaired upon MAP1B-LC over-expression and consequently, basal synaptic transmission is decreased. We also demonstrate that the dendritic targeting of GRIP1, a specific interactor of GluA2/GluA3 subunits that also binds MAP1B-LC, is altered in the presence of enhanced levels of MAP1B-LC. Using deletion mutants of MAP1B-LC, we conclude that MAP1B-LC binding to GRIP1 together with its ability to interact with microtubules is essential to regulate the surface expression and presence at synapses of the GluA2-GluA3 population of AMPARs, and consequently, the degree to which they contribute to basal synaptic transmission in CA1 hippocampal neurons.

Importantly, the model we propose assigns a functional meaning to the interaction between MAP1B-LC and GRIP1 for the first time.

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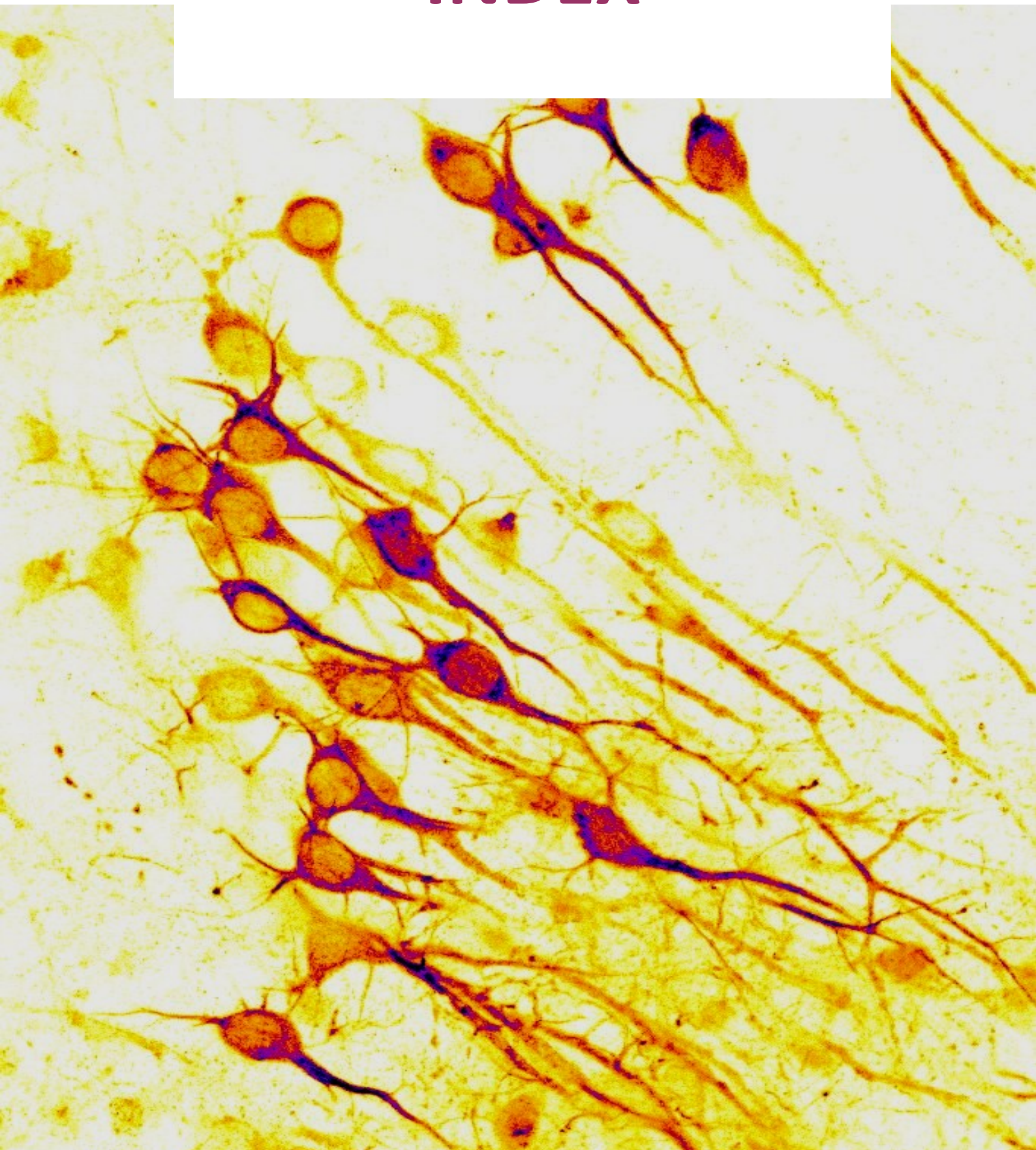

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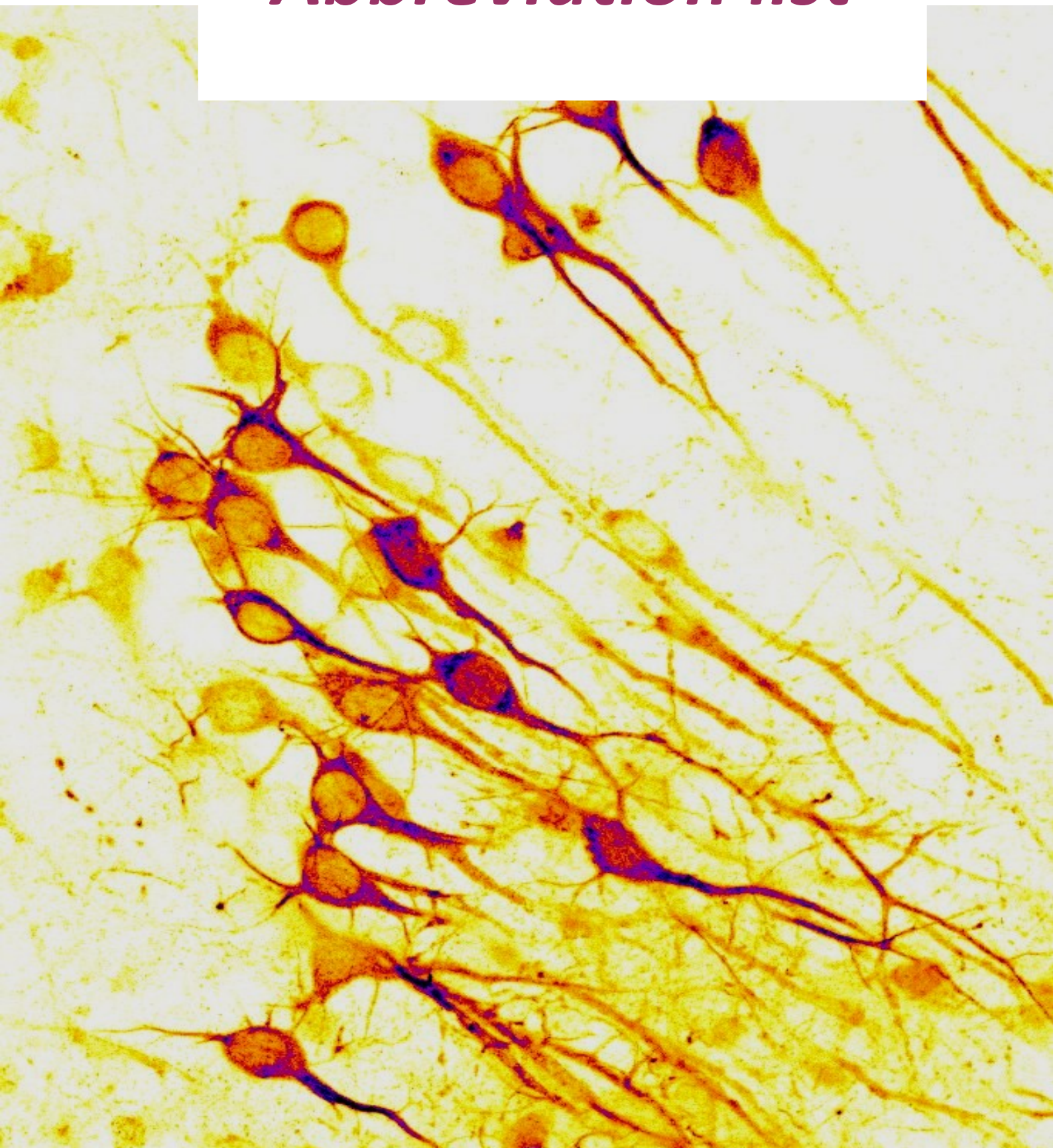
Abbreviation list



Abbreviation list.

ABD: actin-binding domain.

ABP: AMPA-type glutamate receptors binding protein.

ACSF: artificial cerebro-spinal fluid

AMPA: α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid.

AMPAR: AMPA-type glutamate receptors.

APV: (DL)-2-amino-5-phosphopentanoic acid.

BHK: baby hamster kidney.

BSA: bovine serum albumin.

CA1: *Cornu ammon* 1 (subfield of hippocampus).

CA3: *Cornu ammon* 3 (subfield of hippocampus).

CamKII: calcium/calmodulin-dependent kinase II.

Cdc2: cell division cycle 2 protein kinase.

Cdk5: cyclin-dependent kinase 5.

CMV: cytomegalovirus.

DH-BB: defective helper, deleted between BspMII and BamHI.

DHPG: (S)-3,5-dihydroxyphenylglycine.

DIV: days *in vitro*.

DMEM: Dulbecco´s modified Eagle´s medium.

DTT: dithiothreitol.

ECL: Immobilon Western Chemiluminescent HRP Substrate.

EEA-1: early endosomal antigen 1.

EPSP: excitatory postsynaptic potentials.

FBS: fetal bovine serum.

FMRP: Fragile X mental retardation protein.

FRAP: Fluorescence Recovery After Photobleaching.

FXS: Fragile X syndrome.

GABA: gamma-aminobutyric acid.

GAN: Giant Axon Neuropathy.

GFP: green fluorescent protein.

GRIP1/2: glutamate receptor interacting protein 1/2.

GTPase: guanosine triphosphatase.

INF: infected cell.

IP: immunoprecipitation.

IPTG: isopropyl-β-D-1-thiogalactopyranoside.

JNK: c-Jun N-terminal kinase.

KA: kainate receptors.

LB: Luria-Bertani liquid medium.

LTD: long-term depression.

LTP: long-term potentiation.

mA: milliamps.

MAP: microtubule-associated protein.

MAP1B: microtubule-associated protein 1B.

MAP1B-HC: heavy chain of MAP1B.

MAP1B-LC: light chain of MAP1B.

MAPK: mitogen-activated protein kinases.

MBD: microtubule-binding domain.

MEM: minimum essential medium.

mGluR: metabotropic glutamate receptor.

MKLP1: mitotic kinesin-like protein.

MRI: magnetic resonance imaging.

MT: microtubule(s).

NMDA: N-methyl-D-aspartate.

NMDAR: NMDA-type glutamate receptors.

NSF: N-ethylmaleimide sensitive fusion protein

PA: photoactivation.

PAGE: polyacrylamide gel electrophoresis.

PAK1: p21 activated kinase.

PBD: Rac1 binding domain of PAK1.

PDZ: abbreviation from PSD-95 (postsynaptic density protein of 95 kDa molecular weight), DlgA (*Drosophila* discs-large protein) and ZO-1 (protein of epithelial tight junctions).

Pep2m: peptide 2m.

PFA: paraformaldehyde.

PI3K: phosphoinositide 3-kinase.

PICK1: protein interacting with C Kinase 1.

PKA: protein kinase A.

PKC: protein kinase C.

PP1: protein phosphatase 1.

PP2B: calcineurin protein phosphatase.

PTMs: post-translational modifications of tubulin.

PVDF: poly(vinylidene fluoride).

PVP: polyvinylpyrrolidone.

RBD: RhoA binding domain of Rhotekin.

RFP: red fluorescent protein.

SDS: sodium dodecyl sulfate.

shRNA: short hairpin RNA.

TARPs: transmembrane AMPA receptor regulatory proteins.

TBS: Tris-buffered saline.

TfR: transferrin receptor.

TM: transmembrane.

TRANSF: transfected cell.

UNB: unbound.

UNINF: uninfected cell.

UNTRANSF: untransfected cell.

Abbreviation list (Spanish).

ABP: proteína de unión a receptores de glutamato de tipo AMPA.

AMPA: ácido α-amino-3-hidroxi-5-metil-isoxazol-propiónico.

AMPAR: receptores de glutamato de tipo AMPA.

CA1: *Cornu ammon* 1 (subcampo del hipocampo).

CA3: *Cornu ammon* 3 (subcampo del hipocampo).

CamKII: calcio calmodulina quinasa II.

Cdc2: proteína quinasa cdc2 (ciclo de división celular).

Cdk5: ciclina dependiente de quinasa 5.

DHPG: (S)-3,5-dihidroxifenilglicina.

EPSP: potencial postsináptico excitatorio.

FMRP: proteína del retraso mental X-frágil.

FXS: Síndrome del X frágil.

GAN: Neuropatía Axonal Gigante.

GRIP1: proteína de interacción con receptores de glutamato 1.

GTPasa: trifosfatasa de guanosina.

JNK: quinasa c-jun N-terminal.

LTD: depresión a largo plazo.

LTP: potenciación a largo plazo.

MAP: proteína asociada a microtúbulos.

MAP1B: proteína asociada a microtúbulos 1B.

MAP1B-HC: cadena pesada de MAP1B.

MAP1B-LC: cadena ligera de MAP1B.

MAPK: proteínas quinasas activadas por mitógenos.

mGluR: receptores metabotrópicos de glutamato.

MRI: imagen por resonancia magnética.

MT: microtúbulos.

NSF: proteína de fusión sensible a N-etilmaleimida.

PDZ: abreviatura derivada de: PSD-95 (proteína de la densidad postsináptica de 95 kDa de peso molecular), DlgA (proteína de los discos imaginales de la larva de Drosophila) y ZO-1 (proteína de las uniones estrechas epiteliales).

PI3K: quinasa de los 3-fosfoinosítidos.

PICK1: proteína de interacción con la quinasa C 1.

PKA: proteína quinasa A.

PKC: proteína quinasa C.

PP1: proteína fosfatasa 1.

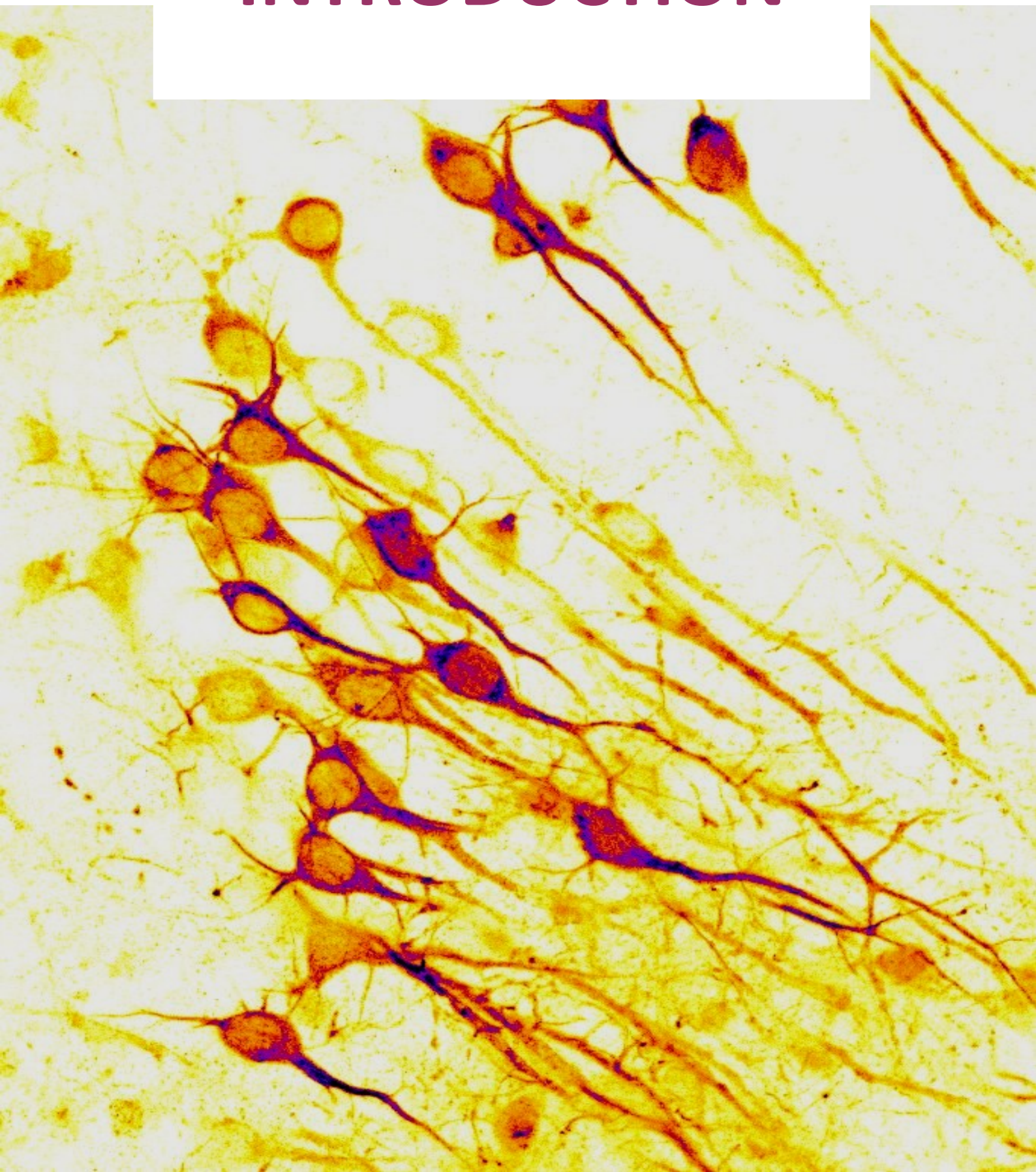
PP2B: proteína fosfatasa calcineurina.

TARPs: proteínas transmembrana reguladoras de receptores AMPA.

TM: transmembrana.

TfR: receptor de transferrina.

INTRODUCTION



1. The hippocampus: a model for synaptic plasticity.

Higher functions such as sleep, cognition, emotion, language and memory are encoded by specific regions of the brain. One of these areas is the hippocampus, a horse-shoe shaped structure located in the medial temporal lobe underneath the cortical surface. From an historical perspective, the hippocampus has been the structure in which many of the general principles of modern neuroscience have been studied and established. Furthermore, it has been the preferred neuronal network to study the best characterized form of neuronal plasticity, synaptic plasticity (Andersen 2007).

It is widely accepted nowadays that the hippocampus is involved in the formation and/or retrieval of some forms of memory. This notion arose from the early work of Scoville and Milner in 1957. They first presented the case of a patient (HM) who had severe anterograde amnesia following bilateral medial temporal lobe resection. After the following examination of other patients with milder amnesia, they concluded that memory impairments in patients were observed whenever the hippocampus was damaged bilaterally (Milner 1972).

Thereafter, the extensive research conducted mainly in rodents led to the conclusion that the hippocampus is required specifically for spatial navigation and spatial memory (O'Keefe and Nadel 1978; Burgess et al. 2002). Much of the evidence has come from the observation of *place cells* and lesion studies combined with spatial memory tasks in these animals.

The identification in hippocampus of *place cells*, those neurons that selectively increase their firing rate only when the animal occupies a well-defined, small patch of the environment, rarely firing outside this region, gave rise to the idea that the hippocampus functions as a spatial map (O'Keefe and Dostrovsky 1971). Pioneer studies in rodents confirmed impaired spatial navigation due to hippocampal lesions (Morris et al. 1982). Subsequent research based also on hippocampal damage revealed that the hippocampus is required for scene or context-specific object memories, as hippocampal lesions erase the memory for the spatial layout of a context where an object was recently experienced (Good 2002). Functional MRI studies supported the idea that the hippocampus is indeed required for spatial navigation (Maguire et al. 1998; Maguire et al. 1999) also in humans.

Given the complexity of the processes entailing memory formation, it is evident that the proper function of many other brain areas apart from hippocampus underlies the ability to learn and remember; however, the solid body of evidence pointing to the involvement of the

hippocampus in information storage and retrieval has encouraged many investigators over the last decades to choose this structure as a model to study the generation of memory (Purves 2004; Andersen 2007).

But what is the cellular substrate of memory and learning? Memory and learning are extremely convoluted processes that rely on the ability of the plastic brain to adapt to environmental variations. Therefore, neuronal plasticity, the ability of the brain to be shaped by experience, underlies the acquisition and consolidation of new memories. Synaptic plasticity is the most representative example of neuronal plasticity. Synaptic plasticity can be defined as a persistent or transient alteration of transmission efficiency at a neuronal synapse in response to intrinsic or extrinsic signals. Evidence for synaptic plasticity in the mammalian nervous system is largely widespread. Although short-term forms of synaptic plasticity also occur, long-lasting forms are plausible substrates for more permanent changes in behavior. Because of their duration, these forms of synaptic plasticity are widely believed to be the cellular correlates of learning and memory.

2. Hippocampal pyramidal neurons.

As previously mentioned, most of the progress in understanding the molecular mechanisms underlying synaptic plasticity has emerged from *ex vivo* studies using slices of living hippocampus. The particular arrangement of neurons allows the hippocampus to be sectioned such that most of the relevant circuitry is left intact. In such preparations, the cell bodies of neurons lie in a single densely packed layer that is readily apparent. This layer is divided into several distinct subfields, the major ones being CA1 and CA3. “CA” stands for *Cornu Ammon*, the Latin translation for Ammon´s horn, the ram´s horn that resembles the shape of the hippocampus (figure 1A).

CA3 and CA1 neurons in hippocampus are referred to as “pyramidal” because they are characterized by the pyramidal shape of their soma, from which a unique axon and several dendrites emerge (figure 1B). The lone axon of each pyramidal neuron typically emanates from the base of the soma and branches profusely, making many excitatory glutamatergic synaptic contacts along its length. Critical to the function of pyramidal neurons is how they respond to synaptic inputs to produce an action potential that excites their postsynaptic targets (Spruston 2008).

On its part, the dendritic tree of a pyramidal neuron has two distinct domains: the basal and the apical dendrites, which descend from the base and the apex of the soma, respectively. Basal dendrites are relatively short in pyramidal neurons; usually, several oblique apical dendrites emanate from one main apical dendrite at various angles. The dendrites of pyramidal neurons are profusely covered by dendritic spines that constitute the postsynaptic site for most excitatory glutamatergic synapses (Spruston 2008).

The apical dendrites of pyramidal cells in the CA1 subfield form a thick band (the *stratum radiatum*) where they receive synapses from Schaffer collaterals, the axons of pyramidal cells in the CA3 region. The Schaffer collaterals form a homogeneous pathway that can be easily activated to study synaptic transmission and plasticity.

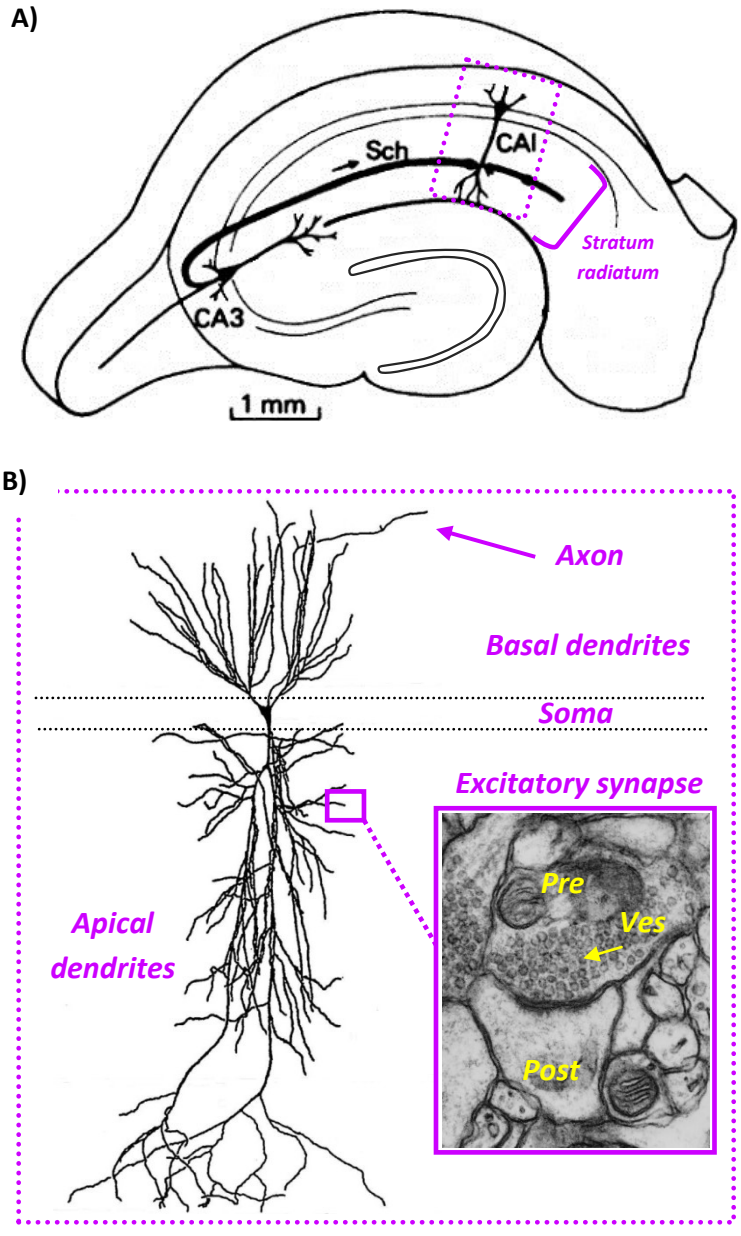


Figure 1. Pyramidal neurons in the hippocampus. A) Neuronal network in a hippocampal slice. The CA3 pyramidal cells project via the Schaffer collaterals ("Sch") to the CA1 pyramidal cells. The apical dendrites of CA1 neurons form the *stratum radiatum*. **B)** Detail of a pyramidal neuron from the CA1 subfield of hippocampus. CA3-CA1 synapses are excitatory synapses, like the one shown in the electron microscopy image on the right. In a chemical synapse, neuronal communication relays on neurotransmitters (glutamate in the case of excitatory synapses). The neurotransmitter is stored in synaptic vesicles ("Ves") in the presynaptic terminal ("Pre"). Upon depolarization, it is released to the synaptic cleft; the activation of specific receptors at the postsynaptic element ("Post") enables the generation of an electrical signal that guarantees the flow of information. Adapted from Ishizuka et al. 1995 and Wedding and Stevens 2009.

3. Glutamatergic synaptic transmission.

The main excitatory neurotransmitter in the hippocampus, and elsewhere in the mammalian central nervous system (CNS), is glutamate. The glutamate that is released from the presynaptic terminal upon depolarization activates several types of receptors at the postsynaptic membrane. Glutamate receptors can be divided into two functionally distinct categories: ionotropic ligand-gated ion channels and metabotropic glutamate receptors (mGluRs), which mediate their effects via coupling to G-protein second messenger systems (Simeone et al. 2004).

Ionotropic ligand-gated ion channels were named after the specific agonists able to activate them in a relatively selective fashion: α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors (KA). AMPA and NMDA receptors are the members of this family directly involved in the generation and expression of synaptic plasticity of excitatory transmission. Their different roles are notably determined by their composition and structural particularities.

- **AMPA receptors (AMPARs):** they are composed of four subunits (GluA1-GluA4) assembled as dimers of dimers. Each subunit contains an extracellular N terminus, four hydrophobic domains (TM1-4), and an intracellular C terminus. The N terminus is expressed on the exterior surface of the neuron, and contains the ligand-binding core; the TM1, TM3 and TM4 regions are all transmembrane spanning domains, whereas TM2 forms a hairpin loop on the intracellular side of the cell membrane (Traynelis et al. 2010). The intracellular C terminus of AMPARs has been shown to be the interaction site for a range of different proteins, many of which are involved in the trafficking of the receptor and in synaptic plasticity (Malinow and Malenka 2002; Henley 2003). AMPARs occur at almost all excitatory synapses in the hippocampus and all subtypes gate Na^+ ions; on the contrary, the entry of Ca^{2+} ions through AMPARs depends on subunit composition. The RNA coding for the GluA2 subunit is edited at the 607 position (Q607R); GluA2(R)-containing AMPARs have low permeability to Ca^{2+} ions (Burnashev et al. 1992; Swanson et al. 1997), and show no inward rectification but linear or slightly outward rectification (Verdoorn et al. 1991; Dingledine et al. 1992).

- **NMDA receptors (NMDARs):** NMDARs are composed of four subunits belonging to three different categories: GluN1, GluN2 and GluN3. They function as heteromeric assemblies in which typically GluN1 subunits associate with GluN2 subunits or a combination of GluN2 and GluN3 subunits. Similar to AMPARs, NMDARs subunits consist of four discrete modules: the

extracellular N-terminus contains the agonist-binding domain; the transmembrane domain is composed of three transmembrane helices plus a pore loop that lines the ion selectivity filter; and an intracellular C terminus, particularly long in the case of NMDARs, which is involved in receptor trafficking, anchoring and coupling to signaling molecules. Several remarkable properties distinguish NMDARs from other ionotropic receptors: 1) the ion channel is subject to a voltage-dependent block by Mg^{2+} that is relieved upon depolarization of the postsynaptic terminal; 2) NMDAR channels are highly permeable to Ca^{2+}, whose influx via NMDARs plays a central role in long-term synaptic plasticity; 3) their activation requires the presence not only of glutamate, but also of a co-agonist (glycine or D-serine) (Traynelis et al. 2010).

However, not only ionotropic glutamate receptors are involved in synaptic plasticity. Certain forms of long-term synaptic plasticity require the activation of metabotropic glutamate receptors (mGluRs). Unlike ionotropic glutamate receptors, mGluRs contain seven transmembrane segments and are coupled to nucleotide-binding G proteins, which mediate most of their actions. Quite differently from the well characterized role of AMPARs and NMDARs in synaptic transmission, the physiological roles of mGluRs are not fully understood yet. The activation of postsynaptic group I receptors (mGluR1 and 5) leads to cell depolarization and increased cell firing and so, increases in neuronal excitability and activation of specific signaling pathways (Niswender and Conn 2010). In contrast, presynaptic group II (mGluR 2, 3) and group III (mGluR 4, 6, 7, 8) mGluRs inhibit neurotransmitter release (Niswender and Conn 2010).

4. The fine tuning of glutamatergic synaptic transmission: AMPAR trafficking.

The modulation of synaptic strength, both during neuronal development and experience-conditioned plasticity, depends intimately on the regulated trafficking of AMPARs (Esteban 2003). AMPARs are not static entities at synapses, but display a highly dynamic behavior (Shepherd and Huganir 2007; Henley et al. 2011). Indeed, the strength of synaptic transmission relays at least partly on the addition and/or removal of AMPARs in and out of synapses. Thus, to ensure proper neuronal communication, the number and synaptic localization of AMPA receptors is subject to a strict control in neurons.

To maintain such function-specific subcellular distribution of AMPARs, neurons have a variety of trafficking proteins that mediate their intracellular targeting, retention and removal at their destination sites. The most prevalent type of protein-protein interaction underlying intracellular trafficking is the one established between a short amino acid motif typically

present at the C-terminal end of the trafficked protein (AMPARs in this case) and a PDZ domain of its interactor. The PDZ abbreviation is derived from three proteins originally identified to contain this approximately 90-amino-acid structural motif: PSD-95 (postsynaptic density protein of 95 kDa molecular weight), DlgA (*Drosophila* discs-large protein) and ZO-1 (protein of epithelial tight junctions) (Sheng and Sala 2001). AMPARs establish PDZ interactions through group I and group II PDZ domains, depending on the specific subunit involved in the interaction (Barry and Ziff 2002; Malinow and Malenka 2002; Song and Huganir 2002; Bredt and Nicoll 2003).

The regulation of the intracellular sorting of AMPARs can occur at several subcellular locations and states of activity in neurons:

- The trafficking of AMPARs from dendrites to spines is differentially modulated in basal transmission and during the induction of patterns of activity that trigger synaptic plasticity. Moreover, it depends greatly on subunit composition, mainly due to the subunit specificity of the PDZ interactions established between AMPARs and their binding partners.
- On the other hand, before reaching their dendritic destination, AMPARs have to be transported from the cell body, where they are mostly synthesized, to the spines vicinity along dendrites. This long-range transport is microtubule-dependent and performed by specific motor proteins and their adaptors (Hirokawa and Takemura 2005; Kapitein and Hoogenraad 2011).

4.1. Maintenance of basal transmission versus synaptic plasticity.

AMPARs function as hetero-oligomers composed of different combinations of four subunits, GluA1 to GluA4. GluA4 is mostly expressed early in postnatal development (Zhu et al. 2000). In adult hippocampus, two major complexes of AMPA receptors have been described: those containing GluA1 and GluA2 subunits, and the GluA2-GluA3 oligomers (Wenthold et al. 1996).

These two distinct populations of AMPARs contribute to synaptic transmission differently. GluA2-GluA3 AMPA receptors maintain synaptic strength by cycling continuously in and out of synapses; the so called “constitutive pathway” is thus responsible for a continuous addition and removal of synaptic AMPARs. On the contrary, the delivery into synapses of the GluA1-GluA2 population requires the induction of neuronal activity (Passafaro et al. 2001; Shi et al. 2001). This differential trafficking seems to be largely controlled by the specific interactions established between the carboxy-terminal domain of the GluA1 subunit (long tail, group I PDZ

domains) and GluA2-GluA3 subunits (short tail, group II PDZ domains) with PDZ domain containing-proteins.

According to this scenario, a model in which the local insertion and removal of AMPARs from the synapse is governed by two distinct regulatory mechanisms has been proposed (Hayashi et al. 2000; Malinow et al. 2000). The "constitutive pathway" (GluA2-GluA3 receptors) would allow the maintenance of synaptic strength in the face of protein turnover, acting in a relatively fast manner (half-time of minutes). On the contrary, the "regulated pathway" (GluA1-GluA2 receptors) would act transiently upon the induction of plasticity leading to the long-lasting enhancement of synaptic strength known as long-term potentiation or LTP. The regulated pathway would be thus responsible for the formation of memories, whereas the constitutive pathway would be responsible for their maintenance. This model implies that AMPAR subunit composition dictates the availability of receptors for delivery to or removal from synapses through the constitutive or the regulated pathways.

4.2. Synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD).

LTP and LTD, long-term, activity-dependent changes in synaptic function, are thought to underlie the formation of memories (Bliss and Collingridge 1993; Bear 1996; Kemp and Manahan-Vaughan 2007). The molecular mechanisms that account for both processes have been, therefore, the subject of intense investigation during the last 25 years.

Long-term potentiation is a long-lasting increase in synaptic strength produced by specific patterns of synaptic activity in the CNS. A long-lasting decrease in synaptic strength is known as long-term depression.

4.2.1 Long-term potentiation.

LTP was first described by Bliss and Lomo (Bliss and Lomo 1973) in the rabbit hippocampus. They found that repetitive stimulation of the perforant path fibers resulted in the potentiation of the response recorded from granule cells in the dentate gyrus lasting between 30 minutes and 10 hours. Thenceforth, however, LTP has been most thoroughly studied at excitatory synapses in the rodent hippocampus. Specifically, much of the work on LTP has focused on the synaptic connections between the Schaffer collaterals and CA1 pyramidal cells. Electrical stimulation of Schaffer collaterals generates excitatory postsynaptic potentials (EPSPs) in the postsynaptic CA1 cells. If the Schaffer collaterals are stimulated at a low frequency, the

amplitude of the postsynaptic EPSPs remains constant. However, a brief, high-frequency train of stimuli causes LTP, which is evident as a long-lasting increase in EPSPs amplitude (figure 2).

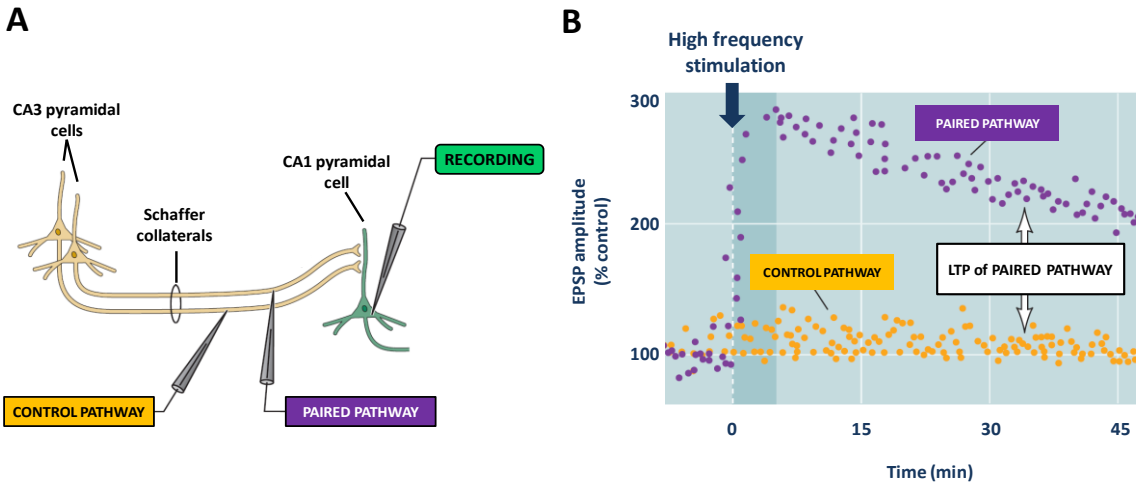


Figure 2. Long-term potentiation of Schaffer collaterals-CA1 synapses. A) Schaffer collaterals (axons given off by CA3 pyramidal cells that project to CA1 area) are stimulated with a stimulating electrode at high frequency ("paired pathway"). The synaptic response from the corresponding CA1 pyramidal cell is registered with a recording electrode ("recording"). The "control pathway" activates a separate population of Schaffer collaterals that are not subject to high frequency stimulation, thereby acting as control. **B)** Time course of changes in the amplitude of excitatory postsynaptic potentials (EPSP) evoked by stimulation of paired and control pathways. A stable and prolonged potentiation results from the high frequency stimulation of the paired pathway. Synaptic responses corresponding to the control pathway are unchanged. Adapted from Purves 2004.

Regarding its molecular basis, the long-term potentiation of synaptic efficacy is a consequence of increases in synaptic AMPAR function (Kauer et al. 1988; Muller and Lynch 1988; Davies et al. 1989; Isaac et al. 1995; Liao et al. 1995; Durand et al. 1996) that depend on NMDA receptor transient activation (Bliss and Collingridge 1993). AMPAR function might be enhanced through changes in the number or composition of receptors at synapses and/or changes in their properties, such an increase in conductance (Benke et al. 1998; Derkach et al. 1999).

The incorporation into synapses of new AMPARs has been demonstrated as a principal mechanism underlying LTP. As previously mentioned, subunit specificity in the delivery of AMPARs from dendrites to spines after the induction of LTP has been corroborated. It was first shown that over-expressed GluA1-GFP was driven to dendritic spines only after the induction of LTP in hippocampal slices, and that this redistribution was dependent on NMDAR-activation (Shi et al. 1999). In a subsequent study, it was demonstrated that GluA1-containing receptors were inserted into synapses upon LTP induction in a process dependent on a PDZ interaction established through the GluA1 C-terminal domain (Hayashi et al. 2000). Afterwards, direct

evidence of the role of GluA1 in LTP and in specific forms of learning came from mice that lack the GluA1 subunit (Zamanillo et al. 1999; Reisel et al. 2002).

The signaling pathways activated as a consequence of LTP induction will be discussed below.

4.2.2 Long-term depression.

If activity-dependent plasticity operated only to enhance synaptic weights, saturation of synaptic efficacy would eventually occur. A neural net composed of synapses whose synaptic weights were maximal would be unable to acquire new memories. An activity-driven mechanism to allow erasure, or depotentiation, of LTP would therefore guarantee the computational flexibility of the network. Besides, if an additional mechanism, independent of LTP, permitted activity-dependent LTD from baseline values of synaptic efficacy, the flexibility of the system and its storage capacity would be further enhanced.

Hippocampal long-term depression was first described in Schaffer collaterals-CA1 synapses (Dunwiddie and Lynch 1978). This work provided evidence that, in addition to LTP, hippocampal synapses could undergo long-term, activity-dependent reductions in synaptic efficacy. Dudek and Bear (Dudek and Bear 1992) demonstrated that LTD could be electrically induced by prolonged trains of low frequency stimulation (figure 3), and that this induction was dependent on NMDAR activation. The initial debate about whether the induction of LTD in the CA1 area was NMDAR-dependent, as shown by Dudek and Bear, or mGluR-dependent (Bashir and Collingridge 1994) was ended thanks to the work of Oliet et al. (Oliet et al. 1997) showing that it was possible to obtain either result by manipulating the induction protocol, thus confirming the existence of two independent forms of LTD. In the CA1 area of the hippocampus, mGluR-dependent LTD is reliably induced by exposure to the group 1 agonist DHPG (Palmer et al. 1997) or by low-frequency trains of pairs of pulses at an appropriate inter-pulse interval (Kemp et al. 2000).

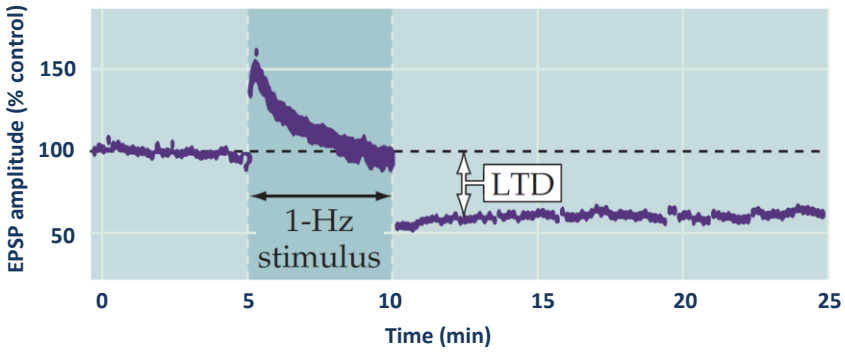


Figure 3. Long-term depression of hippocampal synapses. Low frequency (1 pulse per second) stimulation of Schaffer collaterals ensues prolonged and stable depression of excitatory postsynaptic potentials (EPSP) recorded from CA1 neurons. Adapted from Purves 2004.

The molecular mechanisms responsible for the expression of LTD have been extensively studied, particularly in the case of NMDAR-dependent LTD. The induction and expression of NMDAR-dependent LTD are postsynaptic whereas the expression of mGluR-LTD appears to involve both presynaptic and postsynaptic components. In any case, the activity-dependent and regulated endocytosis of AMPARs from synapses is the event that leads to the long-lasting depression of synaptic strength. In contrast with the subunit-specific pathways for receptor delivery and LTP, it is much less clear which AMPAR subpopulations are targeted by the regulated pathways driving LTD in hippocampal neurons. In GluA2, GluA3 double knock-out mice, basal synaptic transmission is severely impaired but LTD is completely normal, suggesting that GluA1-containing AMPARs are subject to regulated removal (Meng et al. 2003). On the other hand, disrupting the function of PDZ domain-containing proteins specifically interacting with the GluA2 subunit, like GRIP1/2 and PICK1 (discussed below), has been shown to prevent the expression of LTD (Daw et al. 2000), indicating that GluA2 may be essential for this form of synaptic plasticity, too. In conclusion, it is possible that, in contrast to LTP, the regulated removal of AMPARs during LTD affects all subpopulations of AMPARs (Lee et al. 2002).

4.2.3 Signaling pathways underlying synaptic plasticity.

It is well established nowadays that the opening of NMDARs and the concomitant entry of Ca^{2+} ions into the postsynaptic terminal are the events triggering the regulated addition and removal of AMPARs at synaptic sites. Multiple signaling cascades are thought to be activated by this rise in postsynaptic calcium, and it is probable that complex interactions between different signaling pathways determine either a net increase or decrease of synaptic AMPARs.

AMPAR phosphorylation plays a crucial role in regulating synaptic plasticity. In the case of LTP, there is strong evidence that the opening of NMDARs generates a sufficient increase in calcium concentration in the dendritic spine to activate calcium/calmodulin-dependent kinase II (CamKII), which is found at very high concentrations in spines and which is clearly required for LTP (Lisman et al. 2002). CamKII directly phosphorylates GluA1 at Ser 831 (Mammen et al. 1997; Barria et al. 1997a; Barria et al. 1997b) during LTP (Lee et al. 2000) increasing AMPAR conductance (Benke et al. 1998), another postsynaptic mechanism that contributes to at least the early phase of LTP. In addition, the increase in CamKII activity contributes to the insertion of AMPARs in the postsynaptic membrane (Ehlers 2000).

Although CamKII is well accepted to be one major requisite trigger for LTP, the signaling cascades underlying the induction and maintenance of LTP are not completely understood yet. More recent findings have shown that CamKII activates the small guanosine triphosphatase (GTPase) Ras (Chen et al. 1998), which would in turn lead to synaptic delivery of AMPARs via activation of its downstream effectors mitogen-activated protein kinases (MAPK) and/or phosphatidylinositol 3-kinase (PI3K) (Seger and Krebs 1995; Zhu et al. 2002). In fact, it has been demonstrated that the activity of PI3K and the availability of its phosphorylation product, phosphoinositide-3,4,5-trisphosphate (PIP3), are required for the delivery of new AMPARs into synapses in response to NMDAR activation (Man et al. 2003) and for the maintenance of AMPAR clustering on the synaptic membrane (Arendt et al. 2010).

The PKA signaling pathway is also involved in the regulation of synaptic plasticity. In particular, phosphorylation of GluA1 by PKA is required for AMPAR synaptic delivery (Lee et al. 2000; Esteban et al. 2003) and controls also the recycling of receptors between the plasma membrane and endosomal compartments (Ehlers 2000). However, it seems that PKA phosphorylation of GluA1 is necessary but not sufficient to trigger the regulated delivery of AMPARs (Esteban et al. 2003), and that the activation of the above mentioned cascade (CamKII-Ras-MAPK) is concomitantly required for AMPAR delivery during LTP. More recently, tyrosine kinases, acting on NMDARs and enhancing their function, have also been implicated in the induction of long-term potentiation (Salter and Kalia 2004) (figure 4).

If LTP involves the activation of various kinases and LTD represents the inverse of LTP, a logical hypothesis would be that LTD requires preferentially the activation of protein phosphatases. Indeed, an early model proposed that NMDAR-dependent LTD depends on the calcium/calmodulin-dependent protein phosphatase calcineurin (PP2B) as well as on protein phosphatase 1 (PP1) (Lisman 1989). Some excellent works have provided strong evidence of the involvement of these two phosphatases in LTD, perhaps by influencing the phosphorylation state of AMPARs (Mulkey et al. 1993; Mulkey et al. 1994; Carroll et al. 2001).

In any case, it is well established that the regulation of the phosphorylation state of AMPARs is crucial not only for LTP but also for LTD expression. During hippocampal LTD, the PKA site on GluA1, Ser 845, is dephosphorylated, whereas LTD induction in previously potentiated synapses leads to dephosphorylation of the CamKII site, Ser 831 (Lee et al. 2000). Mice that have these two sites mutated exhibit major deficits in LTD and AMPAR internalization induced by NMDAR activation (Lee et al. 2003). The mechanism by which the phosphorylated state of GluA1 is translated into AMPAR internalization is currently unknown, but the differential

regulation of proteins interacting with AMPARs might be involved. In addition, it is fair to say that not only AMPARs but also phosphoinositides are subject to dephosphorylation during LTD. Analogous to the connection between PIP3 formation and synaptic potentiation, PIP3 turnover by the lipid phosphatase PTEN has been linked to synaptic depression (Wang et al. 2006; Jurado et al. 2010).

Contrary to the original conception of LTD relying on the activation of phosphatases, more recent investigations have demonstrated that several kinases do also play a role in LTD expression and maintenance. It has been shown that the removal of AMPARs during LTD correlates with the phosphorylation of the GluA2 subunit by Protein Kinase C (PKC) (Daw et al. 2000; Kim et al. 2001). The most accepted model for this regulated removal involves the preferential interaction of unphosphorylated GluA2 with the PDZ domain-containing proteins GRIP1/ABP (see below), which would favor the stabilization of receptors at synapses. After phosphorylation of GluA2 at Ser 880 by PKC, GluA2 would dissociate from GRIP1/ABP and bind PICK1 (see below), which would facilitate the removal of AMPARs from synapses. Another signaling pathway more recently proposed to be involved in LTD is the MAPK signaling pathway, given that Rap (a specific activator of p38 MAPK) increased activity results in the occlusion of LTD (Zhu et al. 2002). Surprisingly, autonomous CamKII has been shown lately to be required for NMDAR-dependent LTD in hippocampus as well, apparently through the phosphorylation of GluA1 at Ser 567 (Coultrap et al. 2014).

As described, the signaling pathways underlying LTD are considerably complicated (figure 4), and clearly more work needs to be done to clarify their precise contribution to the regulated endocytosis of AMPARs. Furthermore, many other signaling pathways are required to orchestrate the intracellular disposal of AMPARs once they have been endocytosed after the induction of LTD. Indeed, to prevent AMPARs from returning to the plasma membrane so that depression is maintained, AMPARs might also need to be degraded via lysosomal or proteasomal pathways, which are as well subject to a convoluted regulation. Indeed, it has been recently shown that the balance between receptor recycling (from recycling endosomes to the postsynaptic compartment, in a process depending on Rab11 activation) and degradation (Rab7-dependent trafficking towards lysosomes) determines the extent of synaptic depression upon LTD induction (Fernandez-Monreal et al. 2012).

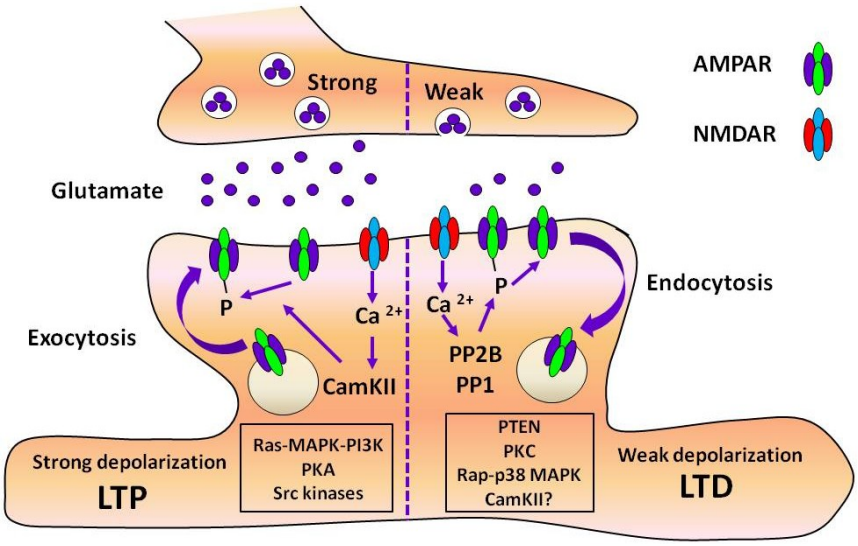


Figure 4. Postsynaptic expression mechanisms of LTP and LTD. Left, strong activity of the presynaptic neuron paired with strong depolarization of the postsynaptic element triggers LTP in part via CamKII, receptor phosphorylation and exocytosis. Apart from CamKII, several kinases (inset) have been shown more recently to be involved in LTP, too. **Right,** weak activity of the presynaptic neuron leads to modest depolarization and modest calcium influx through NMDA receptors. This preferentially activates phosphatases that dephosphorylate AMPA receptors, thus favoring receptor endocytosis. Other proteins (inset) have been proposed to mediate the molecular mechanisms triggering AMPAR endocytosis during LTD as well. Adapted from Luscher and Malenka 2012.

4.3. AMPAR interacting proteins.

4.3.1. N-Ethylmaleimide-Sensitive Fusion protein (NSF). NSF plays a key role in membrane fusion events such as synaptic vesicle exocytosis (Rothman 1994). The interaction of NSF with the C-terminus of the GluA2 subunit of AMPARs has been described to be a crucial factor in the regulation of AMPAR surface expression. Disruption of the interaction between NSF and GluA2 results in a fairly rapid decrease of the amplitude of synaptic currents, which suggests a loss of synaptic AMPARs (Nishimune et al. 1998; Luscher et al. 1999; Noel et al. 1999).

4.3.2. Glutamate Receptor Interacting Protein 1 (GRIP1), AMPAR Binding Protein (ABP, also called GRIP2) and Protein Interacting with C kinase 1 (PICK1). GRIP1 and ABP (or GRIP2) are multi-PDZ domain proteins that bind to GluA2 and GluA3 subunits of the AMPA receptor. GRIP1 binds to GluA2 and GluA3 subunits but not to GluA1 or GluA4 (Dong et al. 1997). ABP/GRIP2 is a protein closely related to GRIP1 (Dong et al. 1999). GRIP1 contains 7 PDZ domains whereas ABP/GRIP2 exists in two isoforms with 6 and 7 PDZ domains, respectively. The shorter isoform binds to GluA2/GluA3 subunits (Srivastava et al. 1998) and is functionally indistinguishable from GRIP1.

The functions of GRIP1/2 appear to be many and varied, and controversy in the field still exists. Some studies addressing GRIP1/2 function in AMPAR trafficking are consistent with the idea of GluA2 association with GRIP1/2 being essential for maintaining AMPARs at synapses, perhaps by limiting their endocytic rate (Osten et al. 2000). The binding of GRIP1/2 to GluA2/GluA3 has also been proposed to stabilize AMPARs in an intracellular pool preventing their reinsertion into the synaptic plasma membrane after LTD (Daw et al. 2000). On the contrary, more recent publications suggest that GRIP1/2 might be facilitating the recycling back to the plasma membrane of the previously endocytosed AMPARs (Mao et al. 2010).

On its part, PICK1 has been demonstrated to interact with GluA2/GluA3 AMPARs subunits, too, (Dev et al. 1999; Xia et al. 1999), via their extreme C-terminal PDZ-binding motifs. As GRIP1/2, it has been proposed to play multiple roles in neurons including the regulation of AMPAR synaptic insertion and subunit composition at synaptic sites (Daw et al. 2000; Terashima et al. 2004).

Interestingly, the binding of GluA2/GluA3 to GRIP1/2 is in dynamic equilibrium with PICK1 due to the phosphorylation status of Ser880 (Matsuda et al. 1999; Chung et al. 2000). It may well function as a mechanism to coordinate GRIP1/2 and PICK1 in their regulation of AMPAR trafficking, both in basal conditions and during LTD (Hanley 2008).

4.3.3. Synapse-Associated Protein 97 (SAP97) and Protein 4.1. SAP97 is another multi-domain structural protein that interacts with AMPA receptors, but via the GluA1 subunit (Leonard et al. 1998). The trafficking function of SAP97 appears to be similar for GluA1 as that of GRIP1/2 for GluA2. SAP97 has been proposed to play a role in the delivery of GluA1-containing AMPARs to dendritic spines on the basis of the observation that SAP97 is directed to spines under the control of CamKII phosphorylation (Mauceri et al. 2004).

Protein 4.1R is a cytoskeletal protein first identified in erythrocytes. Protein 4.1 neuronal homologues, 4.1N and 4.1G, have been shown to interact with the GluA1 subunit of AMPARs (Shen et al. 2000) and are believed to play a role linking the GluA1 subunit to the actin cytoskeleton and favoring its stabilization at the postsynaptic membrane.

4.3.4. Transmembrane AMPA receptor regulatory proteins (TARPs). TARPs, a family of small transmembrane AMPA receptor regulatory proteins including stargazing, are also worth mentioning. They have recently emerged as primary AMPAR auxiliary subunits that control both AMPA receptor trafficking and anchorage at the synapse, and channel gating (Ziff 2007; Jackson and Nicoll 2011).

4.4. Microtubule-dependent transport of AMPARs along dendrites.

AMPARs are mostly synthesized in the cell body. Thus, they need to be transported long distances along dendrites to reach their synaptic targets at dendritic spines; this process has been demonstrated to occur in a microtubule-dependent manner. Apart from ensuring the maintenance of cell shape, the microtubule cytoskeleton running along dendritic shafts provide tracks along which membranous organelles embedded in vesicles can be transported. This active mode of transport is effectively powered by microtubule-associated motor proteins of the kinesin and dynein superfamilies (Goldstein and Yang 2000; Hirokawa and Takemura 2005).

The mechanisms governing cargo specificity and directionality of transport towards axons or dendrites are not fully understood yet. However, adaptor proteins linking cargo and molecular motors are good candidates to mediate such specificity. GRIP1 has been shown to interact directly with the heavy chain of conventional kinesin (Setou et al. 2002). As mentioned above, GRIP1 interacts with GluA2/GluA3 subunits of AMPARs, and therefore, it may function to link AMPARs to the microtubule cytoskeleton and molecular motors. The GRIP1-GluA2 complex has also been reported to associate with liprin-α. This interaction seems to be critical for AMPAR trafficking as a liprin-α mutant unable to bind GRIP1 disrupts the surface expression of AMPARs in hippocampal neurons (Wyszynski et al. 2002). Interestingly, liprin-α interacts with a kinesin family member, KIF1, and AMPARs can be immunoprecipitated with KIF1 from brain lysates (Shin et al. 2003).

On the other hand, dendritic spines are mostly devoid of microtubules (MTs) and characterized by their rich actin cytoskeleton, which is responsible for orchestrating the structural changes underlying plasticity (Matus 2000). Therefore, AMPARs transported along dendritic microtubule tracks need to be transferred to the actin-based cytoskeleton in spines at some point to reach synapses. The molecular mechanisms underlying this transition are currently unknown, but protein 4.1 has been proposed as a reasonable candidate. It is worth noting, however, that recent reports have evidenced the transient entry of dynamic microtubules into dendritic spines (Jaworski et al. 2009), too.

5. Microtubules in neurons. Structural microtubule-associated proteins (MAPs).

Microtubules play central roles in neurons. They are critical to establish neuronal polarity and morphology during development and to maintain cell architecture in mature neurons. They

provide a structural basis for intracellular transport in axons and dendrites. In addition, they act as scaffolds for signaling molecules (Gundersen and Cook 1999; Davies and Morris 2004; Conde and Caceres 2009).

A characteristic property of microtubules is their ability to undergo cycles of rapid growth and disassembly. This behavior of microtubules, observed both *in vitro* and *in vivo*, has been referred to as "dynamic instability". The dynamics of microtubules as well as their interactions with other cellular components are regulated by microtubule-binding proteins.

Microtubule-associated proteins (MAPs) were the first microtubule-binding proteins to be identified. They favor tubulin assembly into microtubules and remain attached to the microtubule surface, resulting in microtubule stabilization. (Davies and Morris 2004; Conde and Caceres 2009). One of these microtubule-associated proteins is microtubule-associated protein 1B or MAP1B.

6. Microtubule-associated protein 1B (MAP1B): physiology and pathology.

6.1 MAP1B molecular particularities.

MAP1B is a large protein with an apparent molecular weight of 320 kDa (Noble et al. 1989). It is composed of 2464 amino acids. MAP1B is encoded as a polyprotein precursor that is subsequently cleaved into a heavy chain (MAP1B-HC) and a light chain (MAP1B-LC) (Hammarback et al. 1991), the cleavage site being located near amino acid sequence 2100 (figure 5). MAP1B-LC associates non-covalently with the N-terminal region of the heavy chain to form a protein complex.

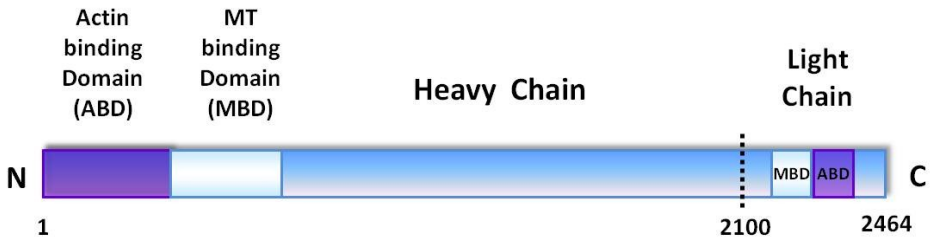


Figure 5. Full length MAP1B. MAP1B is composed of 2464 aminoacids. Actin-binding domains (ABD) localize in the N-terminal domain of MAP1B-HC (1-517) and at the MAP1B-LC (2336-2459). The microtubule-binding domain of MAP1B-HC (517-848) is characterized by 21 KKEK repeats near the N terminus; another MBD is localized at the MAP1B-LC (2210-2336). Adapted from Riederer 2007.

The heavy chain of MAP1B contains a microtubule-binding site near the N-terminus (Noble et al. 1989) and an actin-binding site (Cueille et al. 2007); the light chain of MAP1B includes both

a microtubule-binding domain and an actin-binding domain as well (Zauner et al. 1992; Togel et al. 1998). As both chains of MAP1B are able to bind microtubules, it has been suggested that MAP1B may act as a microtubule cross-linker (Hammarback et al. 1991; Zauner et al. 1992; Pedrotti et al. 1996a).

In homogenates from postnatal rat brain, MAP1B-LC is found in a 6:1 to 8:1 molar ratio to MAP1B-HC (Mei et al. 2000). A greater half-life of MAP1B-LC has been proposed to explain such stoichiometry, given that both chains are synthesized at a 1:1 ratio (Mei et al. 2000). As MAP1B-LC exists in excess over MAP1B-HC *in vivo*, it has been suggested that MAP1B-LC might have additional functions outside of the complex with the heavy chain (Mei et al. 2000). In a study addressing possible independent functions of the light chain when it is not complexed by the heavy chain, a model has been proposed in which the heavy chain of MAP1B might act as the regulatory subunit of the MAP1B complex to control light chain activity (Togel et al. 1998). Indeed, one of the main observations of this study is that, although both the heavy chain and the light chain of MAP1B contain a microtubule-binding domain, the strongest microtubule stabilizing activity corresponds to the light chain by far. Interestingly, MAP1B-LC binding to microtubules confers them a characteristic wavy appearance and unusual stability against the action of depolymerizing agents (Togel et al. 1998; Noiges et al. 2002) that is distinctive of the light chain of MAP1B comparing to other MAPs, like MAP2.

6.2 MAP1B function.

MAP1B is the first MAP to be expressed during neuronal development (Tucker et al. 1989). It is present in axon, soma and dendrites (Matus and Riederer 1986; Tucker et al. 1989). It is specially abundant in developing axons, and its expression declines with age (Schoenfeld et al. 1989); yet, MAP1B presence in somatodendritic compartments has been corroborated in adult brain (Kawakami et al. 2003; Peng et al. 2004; Collins et al. 2005; Tortosa et al. 2011).

MAP1B has been classically studied as a critical modulator of axogenesis through its interaction with the microtubule cytoskeleton (Gonzalez-Billault et al. 2004). According to the classical model of neuronal polarization (Kirschner and Mitchison 1986a; Kirschner and Mitchison 1986b), axon formation is related to dramatic changes in the organization and dynamics of the microtubule cytoskeleton in a specific region of a neuron; therefore, an important factor influencing this process is the existence of a protein that acts as a microtubule stabilizer, like MAP1B. There is strong evidence supporting the role of MAP1B in axon formation and elongation during development (Gonzalez-Billault et al. 2004; Montenegro-Venegas et al.

2010); in fact, independent groups have reported a severe impairment of brain development as a result of the targeted disruption of the MAP1B gene (Edelmann et al. 1996; Takei et al. 1997; Gonzalez-Billault et al. 2000; Meixner et al. 2000; Takei et al. 2000).

In addition, the crosstalk between microtubules and actin in growth cones has been revealed as a critical factor to enable axonal outgrowth during neuronal development. Because of its ability to interact not only with microtubules but also with actin filaments, MAP1B has been proposed as a mediator of such crosstalk. This hypothesis is supported by the observation that MAP1B modulates Rac1, Cdc42 and RhoA activities, small GTPases involved in the fine tuning of the actin cytoskeleton required during axonal outgrowth, elongation and branching (Montenegro-Venegas et al. 2010). Interestingly, the ability of MAP1B to regulate Rac1 activity through the binding of Tiam1 (Rac1 guanosine nucleotide exchange factor) has been reported to reside in the light chain, not in the heavy chain (Henriquez et al. 2012).

More recently, novel crucial roles for MAP1B in the postsynaptic compartment have been proposed in relation with its ability to regulate small GTPases, and therefore, modulate the actin cytoskeleton. Apart from being essential during axogenesis, it has been shown to be required for proper dendritic spine morphogenesis, too (Tortosa et al. 2011). Furthermore, results from our group have also revealed that MAP1B function is essential during synaptic plasticity in mature neurons, by providing Rac1 activation during the regulated removal of AMPA receptors after LTD induction (Benoist et al. 2013). In fact, previous reports had indicated an increase in MAP1B protein levels after treatment of cultured neurons with the specific mGluRs agonist DHPG, which induces AMPAR endocytosis, strengthening the notion that MAP1B might act as a critical regulator of the trafficking of AMPARs during synaptic plasticity paradigms (Davidkova and Carroll 2007).

It has been proposed, too, that MAP1B may function as a scaffold protein. This role would be mainly determined by the fact that MAP1B is able to interact with many different proteins, either directly or via tubulin or actin. In addition, these interactions might be different for the heavy chain and the light chain (Riederer 2007).

Interestingly, MAP1B has also been implicated in the subcellular targeting of different types of receptors and ion channels, by mediating their interaction with the microtubule cytoskeleton. MAP1B has been shown to regulate the synaptic localization of ionotropic GABA receptors at retinal synapses through the binding of GABAc ρ1 subunit (Hanley et al. 1999); based on its interaction with the NR3A subunit, MAP1B has been suggested to regulate the trafficking of

NMDARs, too (Eriksson et al. 2010). Concerning specifically the light chain of MAP1B, it has been characterized as a binding partner of the voltage gated sodium channel Na_v 1.6, facilitating its trafficking to the neuronal cell surface (O'Brien et al. 2012). Two subtypes of serotonin receptors have been described to interact directly with the light chain of MAP1B: in the case of the 5-HT_6 serotonin receptor, its interaction with MAP1B-LC promotes its expression in the cell surface (Kim et al. 2014); on the contrary, the surface expression of the serotonin receptor $5HT_{3A}$ is decreased as a result of MAP1B-LC binding (Sun et al. 2008). Similarly, over-expression of MAP1B-LC produces a decreased surface expression of the N-type Ca^{2+} channel $Ca_v2.2$, apparently by acting as a scaffold protein to increase the UBE2L3-mediated ubiquitination of the channel by simultaneously binding the channel and the enzyme (Gandini et al. 2014). Especially relevant in relation with the trafficking of AMPARs is the reported interaction between MAP1B-LC and GRIP1, which has been described to happen via a non-PDZ domain of GRIP1 (Seog 2004; Davidkova and Carroll 2007). The functional relevance of this interaction has remained unraveled so far.

It is important to note that MAP1B function is modulated by phosphorylation (Gonzalez-Billault et al. 2004; Riederer 2007). Two phosphorylation modes have been described for MAP1B; they are independently regulated during brain development and have different subcellular distributions in neurons. Mode I phosphorylation is catalyzed by proline-dependent kinases (such as cdc2, glycogen synthase kinase 3, cdk5 and JNK), and is present in outgrowing axons (with an increasing gradient of phosphorylated MAP1B towards the growth cone); a Casein Kinase II (CKII)-dependent mode or mode II phosphorylation can be identified in both axons and dendrites and remains into adulthood (Diaz-Nido et al. 1988; Ulloa et al. 1993a; Avila et al. 1994; Gonzalez-Billault et al. 2004). Protein phosphatases contribute to regulate total phosphorylation levels of MAP1B as well; thus, mode I phosphorylation is regulated by protein phosphatases PP2A and PP2B (Ulloa et al. 1993c; Gong et al. 2000) and mode II is regulated by PP1 and PP2A (Ulloa et al. 1993c; Gong et al. 2000).

6.3 MAP1B in pathology.

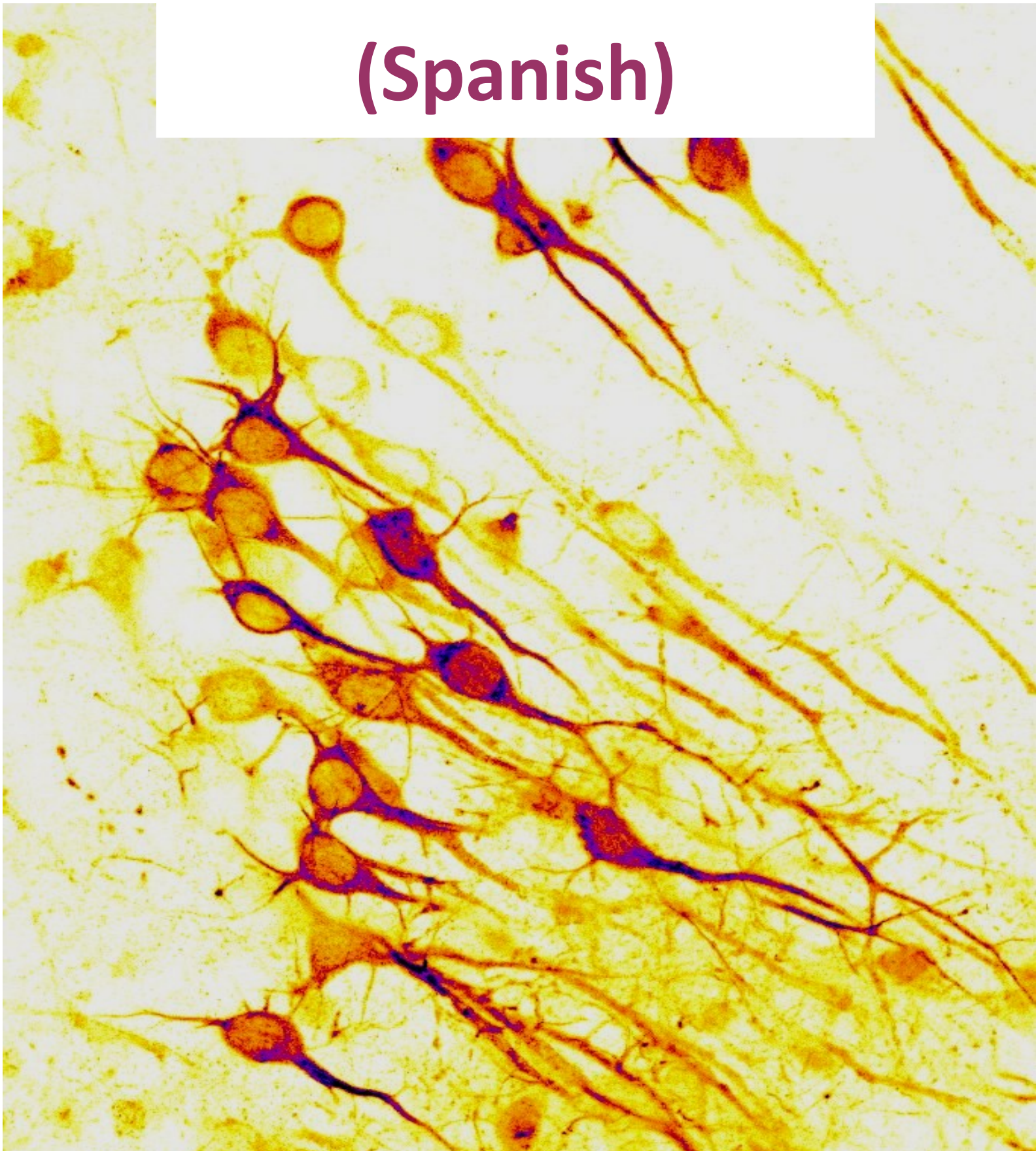
The strongest evidence of MAP1B involvement in pathology has been gathered for Fragile X syndrome (FXS) and Giant Axon Neuropathy (GAN).

FXS is the most common cause of inherited mental retardation and results from the absence of the fragile X mental retardation protein (FMRP). FMRP controls the translation of several mRNAs, including that of MAP1B. The lack of FMRP results in the aberrantly elevated

expression of MAP1B, which is linked to abnormally increased microtubule stability. Anomalous microtubule dynamics due to the lack of FMRP have been suggested as a conceivable underlying factor for the pathogenesis of fragile X mental retardation (Lu et al. 2004). Curiously, delayed dendritic spine development is a hallmark of the disease in patients.

GAN is an autosomal recessive disorder caused by mutations in gigaxonin, a protein that links microtubules and intermediate filaments and has been shown to interact with the light chain of MAP1B (Ding et al. 2002). GAN is characterized cytopathologically by cytoskeletal abnormalities. The clinical relevance of this interaction has been demonstrated in patients (showing axonal degeneration and neuronal death) with two specific mutations in gigaxonin that prevented gigaxonin-MAP1B interaction (Ding et al. 2002).

INTRODUCTION (Spanish)



1. El hipocampo como modelo para estudiar la plasticidad sináptica.

En el cerebro, las funciones superiores como el sueño, la cognición, la emoción, el lenguaje y la memoria se codifican en regiones específicas. El hipocampo, una estructura con forma de herradura situada en el lóbulo medial temporal bajo la superficie cortical, es una de estas áreas. Desde un punto de vista histórico, el hipocampo ha sido la estructura en la que se han estudiado y establecido muchos de los principios generales de la neurociencia moderna. Además, ha sido la red neuronal preferida para el estudio de la forma de plasticidad neuronal mejor caracterizada, la plasticidad sináptica (Andersen 2007).

Hoy en día se sabe que el hipocampo está involucrado en la formación y/o recuperación de ciertas formas de memoria. Scoville y Milner fueron los primeros en sugerir la relación entre hipocampo y memoria en 1957, al publicar el caso de un paciente (HM) que presentaba una amnesia severa tras la resección bilateral del lóbulo temporal medial. Tras examinar otros pacientes con amnesia moderada, los autores concluyeron que la lesión bilateral de hipocampo conducía a pérdidas irreversibles de memoria (Milner 1972).

Desde entonces, la intensa investigación llevada a cabo principalmente con roedores permitió llegar a la conclusión de que el hipocampo es necesario específicamente para la memoria y la navegación espaciales (O'Keefe and Nadel 1978; Burgess et al. 2002). La mayoría de las evidencias en este sentido proceden de la observación de *células de ubicación* en roedores, y de estudios basados en la lesión de hipocampo combinados con tareas de memoria espacial también en estos animales.

La identificación en el hipocampo de *células de ubicación*, aquellas neuronas que aumentan selectivamente su tasa de disparo sólo cuando el animal está ocupando una pequeña zona bien definida del entorno, dio lugar a la idea de que el hipocampo funciona como un mapa espacial (O'Keefe and Dostrovsky 1971). Estudios pioneros con roedores permitieron confirmar que, en efecto, las lesiones hipocampales conducen a una deficiente navegación espacial (Morris et al. 1982). Investigaciones posteriores basadas también en la generación de daño en el hipocampo revelaron que esta estructura es necesaria para la adquisición de memorias específicas de escena o contexto, dado que las lesiones hipocampales borran la memoria de la disposición espacial de un contexto en el que se ha experimentado recientemente un objeto (Good 2002). Estudios de imagen por resonancia magnética (MRI) permitieron confirmar que el hipocampo es necesario para la navegación espacial (Maguire et al. 1998; Maguire et al. 1999), también en seres humanos.

Dada la extraordinaria complejidad de los procesos que subyacen a la formación de la memoria, es evidente que se requiere el funcionamiento apropiado de muchas otras áreas cerebrales además del hipocampo para tener la habilidad de aprender y recordar; sin embargo, la sólida evidencia que apunta a la implicación del hipocampo en el almacenamiento de información y recuperación de la misma ha llevado a muchos investigadores a lo largo de las últimas décadas a escoger esta estructura como modelo para estudiar la generación de la memoria (Purves 2004; Andersen 2007).

Pero, ¿cuál es el sustrato celular de la memoria y el aprendizaje? Ambos procesos dependen íntimamente de la capacidad plástica del cerebro para adaptarse a cambios en el entorno. Por lo tanto, la plasticidad neuronal, entendida como la habilidad del cerebro de ser modelado por la experiencia, subyace a la adquisición y consolidación de nuevas memorias. El ejemplo más representativo de plasticidad neuronal es la plasticidad sináptica. La plasticidad sináptica se define como una alteración persistente o transitoria en la eficacia de la transmisión sináptica en respuesta a señales intrínsecas o extrínsecas. Aunque las formas de plasticidad sináptica de corta duración también existen, son las formas de larga duración las que constituyen el sustrato para los cambios permanentes en el comportamiento. A causa de su duración, se cree que estas formas de plasticidad sináptica son de hecho los correlatos celulares de los procesos de aprendizaje y memoria.

2. Neuronas piramidales del hipocampo.

Como se ha mencionado previamente, la mayor parte del progreso alcanzado en la comprensión de los mecanismos moleculares que subyacen a los procesos de plasticidad sináptica procede de estudios *ex vivo* con rodajas de hipocampo. La disposición particular de las neuronas en esta estructura permite que el hipocampo pueda ser seccionado de forma que la mayor parte del circuito neuronal más relevante quede intacto. En tales preparaciones, los cuerpos celulares de las neuronas, que se organizan en una única capa densamente empaquetada, se identifican con facilidad. Esta capa se divide a su vez en varios campos, siendo los principales CA1 y CA3. “CA” procede de la traducción del latín *Cornu Ammon*, el asta de Amón, el cuerno de carnero cuya forma se parece a la del hipocampo (figura 1A).

El soma o cuerpo celular de las neuronas CA3 y CA1 del hipocampo tiene forma piramidal, por lo que se las conoce como neuronas piramidales; de este cuerpo celular emergen un axón único y varias dendritas (figura 1B). El axón único de cada neurona piramidal parte típicamente

de la base del soma y se ramifica profusamente, estableciendo múltiples contactos sinápticos glutamatérgicos excitatorios a lo largo de todo su recorrido (Spruston 2008).

Por su parte, el árbol dendrítico de una neuronal piramidal presenta dos dominios bien diferenciados: las dendritas basales, que descienden de la base del soma y son relativamente cortas, y las dendritas apicales, que parten del ápex. Generalmente existe una dendrita apical principal de la que emanan varias dendritas apicales oblicuas desde varios ángulos. Las dendritas de las neuronas piramidales aparecen cubiertas extensamente por espinas dendríticas, donde se ubican la mayoría de las sinapsis excitatorias glutamatérgicas (Spruston 2008).

Las dendritas apicales de las neuronas piramidales del campo CA1 constituyen una banda gruesa (el *stratum radiatum*) donde reciben las sinapsis de las colaterales de Schaffer, los axones de las células piramidales de la región CA3. Las colaterales de Schaffer forman una vía homogénea que se puede activar fácilmente para estudiar la transmisión sináptica y los fenómenos de plasticidad sináptica.

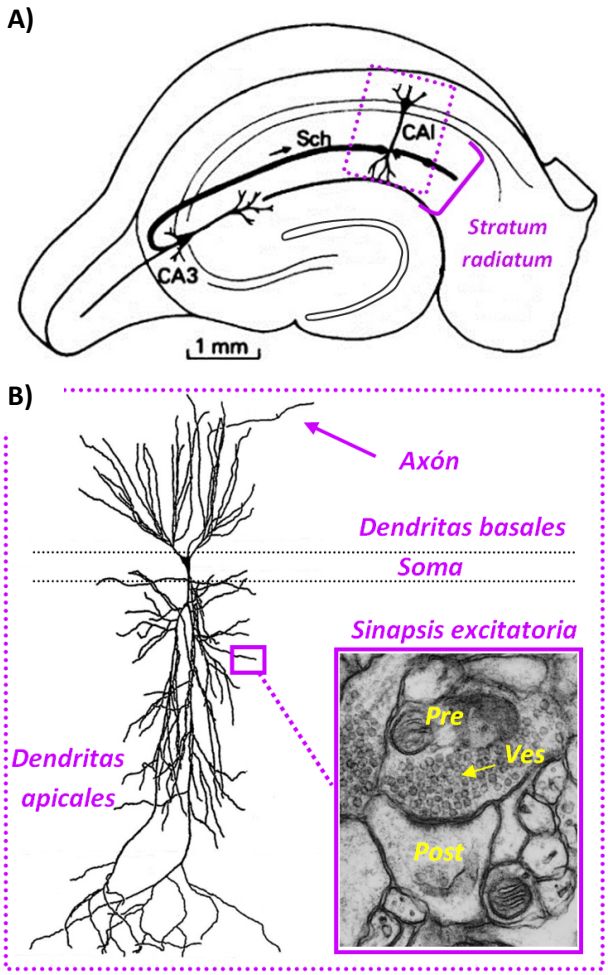


Figura 1. Neuronas piramidales en el hipocampo. A) Red neuronal en una rodaja de hipocampo. Las células piramidales CA3 proyectan hacia las células piramidales CA1 por medio de las colaterales de Schaffer ("Sch"). Las dendritas apicales de las neuronas CA1 forman el *stratum radiatum*. **B)** Detalle de una neurona piramidal del campo CA1 del hipocampo. Las sinapsis CA3-CA1 son sinapsis excitatorias, como la que se muestra en la imagen de microscopía electrónica de la derecha. En una sinapsis química, la comunicación neuronal depende de la liberación de neurotransmisores (glutamato en el caso de las sinapsis excitatorias). El neurotransmisor se almacena en vesículas sinápticas ("Ves") alojadas en el terminal presináptico ("Pre"). Cuando éste se despolariza, el neurotransmisor es liberado al espacio sináptico; la activación de receptores específicos en el terminal postsináptico ("Post") permite la generación de una señal eléctrica que garantiza el flujo de información. Adaptado de Ishizuka et al. 1995; Wedding and Stevens 2009.

3. Transmisión sináptica glutamatérgica.

El principal neurotransmisor excitatorio en el hipocampo, y en general en el sistema nervioso central de los mamíferos, es el glutamato. El glutamato que se libera desde la terminal presináptica puede activar diferentes tipos de receptores a nivel de la membrana postsináptica. Los receptores de glutamato se dividen en dos categorías funcionalmente diferenciadas: los receptores ionotrópicos de glutamato, que son canales iónicos activados por ligando, y los receptores metabotrópicos (mGluRs), cuyos efectos están mediados por sistemas de segundos mensajeros acoplados a proteínas G (Simeone et al. 2004).

Los receptores ionotrópicos de glutamato se nombraron en función de los agonistas específicos capaces de activarlos de forma selectiva: ácido α-amino-3-hidroxi-5-metilisoxazol-propiónico (AMPA), N-metil-D-aspartato (NMDA) y receptores de kainato. Los receptores AMPA y NMDA son los miembros de esta familia directamente implicados en la generación y expresión de la plasticidad sináptica de la transmisión excitatoria.

- **Receptores AMPA (AMPARs):** están compuestos por cuatro subunidades (GluA1-GluA4) que se organizan como dímeros de dímeros. Cada subunidad contiene un extremo N-terminal extracelular, cuatro dominios hidrofóbicos (TM1-4) y un extremo C-terminal intracelular. El extremo N-terminal se expresa en la superficie exterior de la neurona, y contiene el centro de interacción con el ligando; las regiones TM1, TM3 y TM4 atraviesan la membrana plasmática, mientras que la región TM2 forma un bucle en horquilla en el lado intracelular de la membrana (Traynelis et al. 2010). El extremo C-terminal intracelular de los AMPARs es el lugar de interacción para una amplia gama de proteínas, muchas de las cuales están implicadas en la regulación del tráfico del receptor y los fenómenos de plasticidad sináptica (Malinow and Malenka 2002; Henley 2003). Los AMPARs se encuentran en casi todas las sinapsis excitatorias del hipocampo y todos los subtipos son permeables a iones Na^{+}; por el contrario, la entrada de iones de Ca^{2+} depende de su composición de subunidades. El ARN que codifica para la subunidad GluA2 se edita en la posición 607 (Q607R); los AMPARs cuyas subunidades GluA2 contienen arginina tienen una permeabilidad baja a los iones de Ca^{2+} (Burnashev et al. 1992; Swanson et al. 1997), y no presentan rectificación en sentido entrante sino una relación intensidad-voltaje lineal o que rectifica ligeramente en sentido saliente (Verdoorn et al. 1991; Dingledine et al. 1992).

- **Receptores NMDA (NMDARs):** Los NMDARs están compuestos por cuatro subunidades que pertenecen a tres categorías diferentes: GluN1, GluN2 y GluN3. Funcionan como

estructuras heterotetraméricas en las que típicamente las subunidades de tipo GluN1 se asocian con las de tipo GluN2 o una combinación de subunidades GluN2 y GluN3. De forma similar a los AMPARs, las subunidades de los NMDARs presentan cuatro módulos: el extremo N-terminal extracelular contiene el dominio de unión al agonista; el domino transmembrana está compuesto por tres hélices transmembrana más un bucle que se alinea con el filtro de selectividad de iones; y un extremo C-terminal, particularmente largo en el caso de los NMDARs, y que está implicado en el tráfico y anclaje de receptores, y en el acoplamiento de moléculas de señalización. Las siguientes características distinguen a los NMDARs de otros receptores ionotrópicos: 1) el canal iónico está sujeto a un bloqueo dependiente de voltaje por Mg^{2+}, que se anula cuando el terminal postsináptico se despolariza; 2) los canales NMDAR son altamente permeables al Ca^{2+}, cuyo influjo a través de los NMDARs juega un papel muy importante en la plasticidad sináptica a largo plazo; 3) su activación requiere la presencia no sólo de glutamato, sino también de un co-agonista (glicina o D-serina) (Traynelis et al. 2010).

Sin embargo, se ha visto que los fenómenos de plasticidad sináptica no dependen únicamente de los receptores ionotrópicos de glutamato. Algunas formas de plasticidad sináptica a largo plazo requieren de la activación de receptores metabotrópicos de glutamato (mGluRs). A diferencia de los receptores ionotrópicos, los mGluRs contienen siete segmentos transmembrana y están acoplados a proteínas G que unen nucleótidos, las cuales median la mayoría de sus acciones. Al contrario que en el caso de los AMPARs y NMDARs, cuyo papel en transmisión sináptica ha sido bien caracterizado, los papeles fisiológicos de los receptores mGluR todavía no se comprenden en profundidad. La activación del grupo I de receptores postsinápticos (mGluR1 y 5) conduce a una despolarización celular y una tasa incrementada de disparo y por lo tanto, al aumento de la excitabilidad neuronal y la activación de ciertas vías de señalización (Niswender and Conn 2010). Por el contrario, los grupos II (mGluR 2, 3) y III (mGluR 4, 6, 7, 8) de receptores presinápticos inhiben la liberación de neurotransmisor (Niswender and Conn 2010).

4. Transmisión glutamatérgica excitatoria y tráfico de receptores AMPA.

La modulación de la eficacia sináptica, tanto durante el desarrollo neuronal como durante la plasticidad condicionada por la experiencia, depende íntimamente del tráfico regulado de AMPARs (Esteban 2003). Los AMPARs no son entidades estáticas en las sinapsis, sino que presentan un comportamiento eminentemente dinámico (Shepherd and Huganir 2007; Henley et al. 2011). De hecho, la eficacia de la transmisión sináptica depende al menos en parte de la

adición regulada a las sinapsis y la eliminación de las mismas de los AMPARs. Por lo tanto, para asegurar una comunicación neuronal apropiada, el número y la localización sináptica de los AMPARs deben estar sujetos a un estricto control.

Para mantener la distribución subcelular de los AMPARs, específica de función, las neuronas cuentan con una amplia gama de proteínas que median su distribución intracelular, su retención y su eliminación en los lugares de destino. El tipo de interacción proteína-proteína más prevalente cuando se trata de tráfico intracelular de proteínas es la que se establece entre una secuencia corta de aminoácidos típicamente presente en el extremo C-terminal de la proteína que se transporta (AMPARs en este caso) y un dominio PDZ en la proteína con la que interacciona. La abreviatura PDZ procede del nombre de tres proteínas en las que se identificó originalmente este motivo estructural de aproximadamente 90 aminoácidos: PSD-95 (proteína de la densidad postsináptica de 95 kDa de peso molecular), DlgA (proteína de los discos imaginales de la larva de Drosophila) y ZO-1 (proteína de las uniones estrechas epiteliales) (Sheng and Sala 2001). Los AMPARs establecen interacciones PDZ con dominios de los grupos I y II, dependiendo de la subunidad específica involucrada en la interacción (Barry and Ziff 2002; Malinow and Malenka 2002; Song and Huganir 2002; Bredt and Nicoll 2003).

La regulación de la distribución intracelular de AMPARs puede tener lugar en diferentes localizaciones subcelulares y estados de actividad de las neuronas:

- El tráfico de AMPARs desde las dendritas hacia las espinas se modula de forma diferencial durante la transmisión basal y durante la inducción de patrones de actividad que disparan fenómenos de plasticidad sináptica. Además, depende en gran medida de la composición de subunidades de los receptores, fundamentalmente debido a que las interacciones PDZ que establecen los AMPARs con las proteínas a las que se unen son también específicas de subunidad.
- Por otra parte, antes de alcanzar su destino en las dendritas, los AMPARs tienen que ser transportados desde el cuerpo celular, donde se sintetizan mayoritariamente, hasta las proximidades de las espinas a lo largo de las dendritas. Este transporte de larga distancia es dependiente de microtúbulos (MTs) y llevado a cabo por proteínas motoras específicas y sus adaptadores (Hirokawa and Takemura 2005; Kapitein and Hoogenraad 2011).

4.1. Mantenimiento de la transmisión basal y plasticidad sináptica.

Los AMPARs funcionan como hetero-oligómeros compuestos por diferentes combinaciones de cuatro subunidades, de GluA1 a GluA4. GluA4 se expresa fundamentalmente durante el desarrollo postnatal temprano (Zhu et al. 2000). En el hipocampo adulto, se han descrito dos complejos mayoritarios de AMPARs: aquellos que contienen las subunidades GluA1 y GluA2, y los oligómeros GluA2-GluA3 (Wenthold et al. 1996).

Estas dos poblaciones diferenciadas de AMPARs contribuyen a la transmisión sináptica de manera distinta. Los AMPARs de tipo GluA2-GluA3 mantienen la eficacia sináptica ciclando continuamente hacia las sinapsis y fuera de ellas; la llamada "vía constitutiva" es por tanto responsable de la continua adición a las sinapsis y eliminación de las mismas de los AMPARs. Por el contrario, el transporte hacia las sinapsis de la población de tipo GluA1-GluA2 requiere de la inducción de actividad neuronal (Passafaro et al. 2001; Shi et al. 2001). Este tráfico diferencial parece estar controlado en gran medida por las interacciones específicas establecidas entre el dominio carboxilo terminal de la subunidad GluA1 (cola larga, grupo I de dominios PDZ) y de las subunidades GluA2-GluA3 (cola corta, grupo II de dominios PDZ) con las proteínas que contienen los dominios PDZ.

Así, se ha propuesto un modelo en el que la inserción y eliminación local de AMPARs de las sinapsis estarían gobernadas por dos mecanismos regulatorios diferentes (Hayashi et al. 2000; Malinow et al. 2000). La "vía constitutiva" (receptores GluA2-GluA3) permitiría el mantenimiento de la eficacia sináptica ante el reemplazo proteico, actuando de una forma relativamente rápida (vida media de minutos). Por el contrario, la "vía regulada" (receptores de tipo GluA1-GluA2) actuaría de forma transitoria como consecuencia de la inducción de plasticidad conduciendo a la potenciación de la fuerza sináptica de larga duración que conocemos como potenciación a largo plazo o LTP. La vía regulada sería pues responsable de la formación de nuevas memorias, mientras que la vía constitutiva lo sería de su mantenimiento. Este modelo implicaría que la composición de subunidades de los AMPARs es la que dicta la disponibilidad de receptores para su transporte hacia las sinapsis o su eliminación de las mismas.

4.2. Plasticidad sináptica: potenciación a largo plazo (LTP) y depresión a largo plazo (LTD).

Actualmente se piensa que los fenómenos de LTP y LTD, cambios de larga duración en la función sináptica dependientes de actividad, son responsables de la formación de la memoria (Bliss and Collingridge 1993; Bear 1996; Kemp and Manahan-Vaughan 2007). Como

consecuencia, los mecanismos moleculares que conducen a ambos procesos han sido objeto de una intensa investigación durante los últimos 25 años.

La potenciación a largo plazo es un incremento en la eficacia sináptica de larga duración producido por patrones específicos de actividad sináptica en el sistema nervioso central. Una disminución de larga duración en la eficacia sináptica se conoce como depresión a largo plazo.

4.2.1 Potenciación a largo plazo o LTP.

La potenciación a largo plazo o LTP, por sus siglas en inglés, fue descrita por primera vez por Bliss y Lomo en el hipocampo de conejo (Bliss and Lomo 1973). Estos autores encontraron que una estimulación repetitiva de las fibras de la vía perforante resultaba en la potenciación de la respuesta registrada en la células granulares del giro dentado que duraba entre 30 minutos y 10 horas. Desde entonces, la LTP ha sido extensamente estudiada en las sinapsis excitatorias del hipocampo de roedores. Concretamente, gran parte del trabajo sobre la LTP se ha centrado en las conexiones sinápticas establecidas entre las colaterales de Schaffer y las células CA1 piramidales. La estimulación eléctrica de las colaterales de Schaffer genera potenciales postsinápticos excitatorios (EPSPs) en las células CA1. Si las colaterales de Schaffer se estimulan a baja frecuencia, la amplitud de los EPSPs se mantiene constante. Sin embargo, un tren de estímulos breve pero a alta frecuencia provoca LTP, que se manifiesta como un incremento en la amplitud de los EPSPs de larga duración.

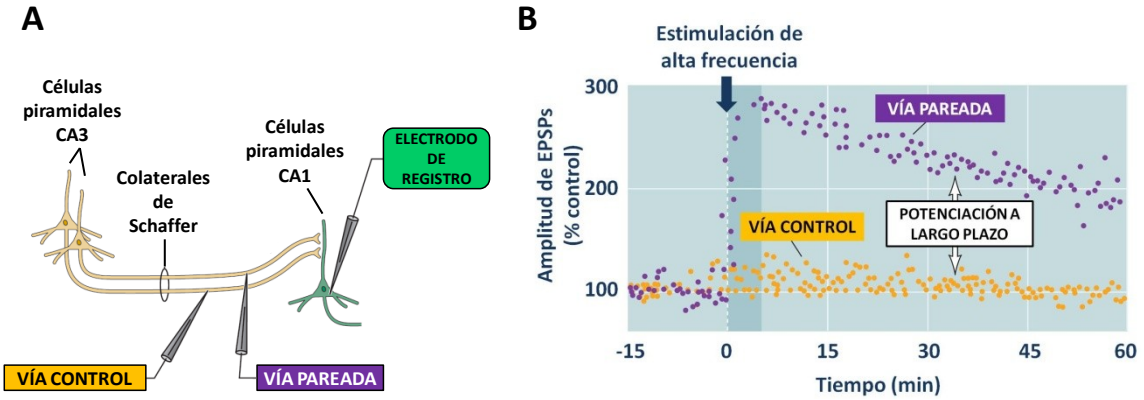


Figura 2. Potenciación a largo plazo de las sinapsis entre las colaterales de Schaffer y las células CA1. **A)** Las colaterales de Schaffer (los axones de las células piramidales CA3 que proyectan hacia el área CA1) se estimulan con un electrodo estimulador a alta frecuencia ("vía pareada"). La respuesta sináptica de la célula piramidal CA1 correspondiente se registra con un electrodo de registro ("registro"). La "vía control" activa una población independiente de colaterales de Schaffer que no se someten a estimulación de alta frecuencia, por lo tanto actuando como control. **B)** Evolución temporal de los cambios en la amplitud de los potenciales postsinápticos excitatorios (EPSP, por sus siglas en inglés) evocados por estimulación de las vías pareadas y control. Una potenciación estable y prolongada resulta de la estimulación a alta frecuencia de la vía pareada. Sin embargo, las respuestas sinápticas que corresponden a la vía control no cambian. Adaptado de Purves 2004.

Con respecto a su base molecular, la potenciación a largo plazo de la eficacia sináptica es consecuencia de aumentos en la función de los AMPARs sinápticos (Kauer et al. 1988; Muller and Lynch 1988; Davies et al. 1989; Isaac et al. 1995; Liao et al. 1995; Durand et al. 1996) que dependen de una activación transitoria de los NMDARs (Bliss and Collingridge 1993). La función de los AMPARs puede verse aumentada por medio de cambios en el número o en la composición de los receptores en las sinapsis y/o cambios en sus propiedades, como un aumento de su conductancia (Benke et al. 1998; Derkach et al. 1999).

Se ha demostrado que la incorporación a las sinapsis de nuevos AMPARs es un mecanismo fundamental que contribuye a la expresión de LTP, y como se ha mencionado previamente, depende de la composición de subunidades de los receptores. Primero se demostró que la subunidad GluA1 (fusionada a GFP como proteína recombinante sobre-expresada) era transportada a las espinas dendríticas sólo tras la inducción de LTP en rodajas de hipocampo, y que esta redistribución era dependiente de la activación de NMDARs (Shi et al. 1999). Un estudio posterior evidenció que los receptores que contenían subunidades de tipo GluA1 eran insertados en las sinapsis tras la inducción de LTP en un proceso dependiente de las interacciones de tipo PDZ establecidas a través del dominio C-terminal de la subunidad GluA1 (Hayashi et al. 2000). La evidencia definitiva del papel desempeñado por la subunidad GluA1 en LTP y ciertas formas de aprendizaje se obtuvo poco después por medio de ratones modificados genéticamente que no expresan dicha subunidad (Zamanillo et al. 1999; Reisel et al. 2002).

4.2.2 Depresión a largo plazo o LTD.

Si la plasticidad dependiente de actividad operase sólo para aumentar la fuerza sináptica, al final tendría lugar una saturación de la eficacia sináptica. Una red neuronal compuesta únicamente por sinapsis cuya fuerza o peso sináptico fueran máximos sería incapaz de adquirir nuevas memorias. Un mecanismo disparado por actividad que permitiera un borrado, o de-potenciación, de la LTP garantizaría la flexibilidad computacional de la red. Además, si un mecanismo adicional, independiente de LTP, permitiera una depresión a largo plazo dependiente de actividad desde los valores basales de fuerza sináptica, entonces la flexibilidad del sistema y su capacidad de almacenamiento se verían potenciados en mayor medida.

La depresión a largo plazo en el hipocampo fue descrita por primera vez en las sinapsis entre las colaterales de Schaffer y las células CA1 (Dunwiddie and Lynch 1978). Este trabajo

evidenció que, además de LTP, las sinapsis hipocampales podían experimentar reducciones en la eficacia sináptica de larga duración y dependientes de actividad.

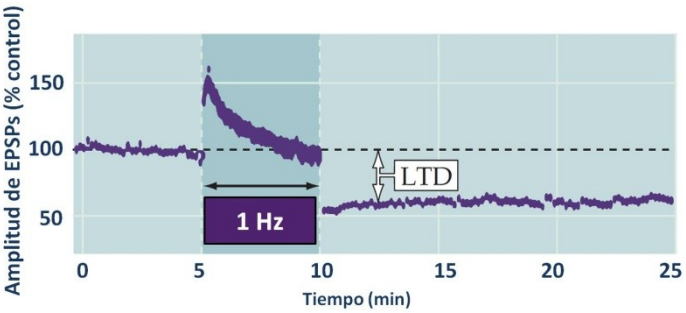


Figura 3. Depresión a largo plazo de las sinapsis hipocampales. La estimulación a baja frecuencia (1 pulso/segundo) de las colaterales de Schaffer garantiza una depresión prolongada y estable de los potenciales postsinápticos excitatorios (EPSPs) que se registran en las células CA1. Adaptado de Purves 2004.

Dudek y Bear (Dudek and Bear 1992) demostraron que se podía inducir LTD eléctricamente por medio de trenes de estimulación de baja frecuencia (figura 3), y que esta inducción era dependiente de la activación de NMDARs. El debate inicial sobre si la inducción de LTD en el área CA1 era dependiente de NMDARs, como habían demostrado Dudek y Bear, o dependiente de mGluRs (Bashir and Collingridge 1994) se zanjó gracias al trabajo de Oliet et al. (Oliet et al. 1997), que demostraba que era posible obtener cualquiera de los dos resultados manipulando el protocolo de inducción y por tanto, confirmando la existencia de formas independientes de LTD. En el área CA1 del hipocampo, la LTD dependiente de mGluRs se induce por exposición a DHPG, un agonista de grupo 1 (Palmer et al. 1997) o por medio de trenes de estimulación a baja frecuencia basados en la aplicación de pares de pulsos a un intervalo inter-pulso apropiado (Kemp et al. 2000).

Los mecanismos moleculares responsables de la expresión de LTD se han estudiado extensamente, particularmente en el caso de la LTD dependiente de NMDARs. La inducción y la expresión de la LTD dependiente de NMDARs son postsinápticas, mientras que la expresión de la LTD dependiente de mGluRs parece implicar componentes tanto presinápticos como postsinápticos. En cualquier caso, el evento que conduce a la depresión de la eficacia sináptica es la endocitosis desde las sinapsis, regulada y dependiente de actividad, de los AMPARs. Al contrario de lo que ocurre durante la LTP, está mucho menos claro qué poblaciones de AMPARs se ven reguladas por las vías que conducen a LTD en las neuronas hipocampales. En ratones dobles *knock-out* para las subunidades GluA2 y GluA3, la transmisión sináptica basal se ve severamente alterada pero la LTD es completamente normal, sugiriendo que los AMPARs que contienen GluA1 son objeto de eliminación regulada de las sinapsis (Meng et al. 2003). Por otra parte, cuando se interfiere con la función de las proteínas que contienen dominios PDZ y que interaccionan específicamente con la subunidad GluA2, como GRIP1/2 y PICK1 (de las que se hablará más adelante), no se produce LTD (Daw et al. 2000), indicando así que la subunidad

GluA2 podría también ser primordial para esta forma de plasticidad sináptica. En conclusión, es posible que, al contrario de lo que ocurre con la LTP, la eliminación regulada de los AMPARs durante la LTD afecte por igual a todas las subpoblaciones (Lee et al. 2002).

4.2.3 Vías de señalización activadas durante plasticidad sináptica.

Actualmente se considera suficientemente probado que la apertura de NMDARs y la entrada concomitante de iones Ca^{2+} en la terminal postsináptica son los eventos que disparan la adición y eliminación regulada de AMPARs en las sinapsis. Se piensa que son muchas las vías de señalización que se activan en respuesta a este aumento de calcio postsináptico, y que son las complejas interacciones establecidas entre ellas las que determinan que se produzca un aumento o una disminución en el número final de AMPARs en las sinapsis.

La fosforilación de los AMPARs juega un papel fundamental en la regulación de la plasticidad sináptica. En el caso de la LTP, la apertura de los NMDARs genera un aumento suficiente en la concentración de calcio en la espina dendrítica como para que se active la calcio calmodulina quinasa II (CamKII), la cual se encuentra en espinas en una concentración muy elevada y cuya activación es necesaria para que se dispare la LTP (Lisman et al. 2002). La CamKII fosforila directamente la subunidad GluA1 en la serina 831 (Mammen et al. 1997; Barria et al. 1997a; Barria et al. 1997b) durante la LTP (Lee et al. 2000), aumentando así la conductancia de los AMPARs (Benke et al. 1998), uno de los mecanismos postsinápticos que contribuye al menos a la fase temprana de la LTP. Además, se ha visto que el incremento en la actividad de CamKII contribuye directamente a la inserción de los AMPARs en la membrana postsináptica (Ehlers 2000).

Aunque se sabe que la activación de la CamKII es uno de los principales requisitos de disparo de la LTP, todavía no se comprenden en su totalidad las cascadas de señalización que se ponen en marcha durante la inducción y mantenimiento de la LTP. Más recientemente se ha podido demostrar que la CamKII activa la trifosfatasa de guanosina (GTPasa) Ras (Chen et al. 1998), lo que conduce a la inserción de AMPARs en la sinapsis vía la activación de efectores como las proteínas quinasas activadas por mitógenos (MAPK) y/o la fosfatidil inositol 3-quinasa (PI3K) (Seger and Krebs 1995; Zhu et al. 2002). De hecho, se ha demostrado que la actividad de la PI3K y la disponibilidad de su producto de fosforilación, el fosfoinosítido-3,4,5-trisfosfato (PIP3), son necesarios para el transporte de nuevos AMPARs hacia las sinapsis en respuesta a la activación de los NMDARs (Man et al. 2003) y para su anclaje a nivel de la membrana postsináptica (Arendt et al. 2010).

La vía de señalización de la proteína quinasa A (PKA) está también implicada en la regulación de la plasticidad sináptica. En particular, se ha visto que la fosforilación de GluA1 por la PKA es necesaria para el transporte hacia las sinapsis de los AMPARs (Lee et al. 2000; Esteban et al. 2003), controlando así mismo el reciclaje de receptores entre la membrana plasmática y los compartimentos endosomales (Ehlers 2000). Sin embargo, parece que la fosforilación de GluA1 por la PKA es necesaria pero no suficiente para disparar el transporte regulado de AMPARs (Esteban et al. 2003), y que se requiere la activación concomitante de la cascada de señalización mencionada anteriormente (CamKII-Ras-MAPK) para el tráfico de AMPARs que tiene lugar durante la LTP. Más recientemente se ha implicado también a las tirosina quinasas que actúan sobre los NMDARs y favorecen su funcionamiento en la inducción de la LTP (Salter and Kalia 2004) (figura 4).

Si la LTP supone la activación de varias quinasas y la LTD representa la situación inversa a la LTP, una hipótesis lógica sería que la LTD requiriera preferentemente la activación de fosfatasas. De hecho, uno de los primeros modelos que se dieron a conocer proponía que la LTD dependiente de NMDARs requería la activación de la proteína fosfatasa calcineurina (PP2B) y de la proteína fosfatasa 1 (PP1) (Lisman 1989). En efecto, excelentes trabajos posteriores han permitido demostrar la implicación de ambas fosfatasas en la LTD, quizá por medio de la modificación del estado de fosforilación de los AMPARs (Mulkey et al. 1993; Mulkey et al. 1994; Carroll et al. 2001).

En cualquier caso, se considera bien establecido que la regulación del estado de fosforilación de los AMPARs es crucial no sólo para la expresión de LTP sino también de LTD. Durante la LTD en el hipocampo, el sitio PKA de la subunidad GluA1, la serina 845, se desfosforila, mientras que la inducción de LTD en sinapsis previamente potenciadas conduce a la desfosforilación del sitio CamKII, la serina 831 (Lee et al. 2000). Los ratones que tienen ambos sitios mutados exhiben grandes déficits en LTD y en la internalización de AMPARs inducida por la activación de NMDARs (Lee et al. 2003). Actualmente se desconoce el mecanismo por el cual el estado de fosforilación de la subunidad GluA1 se traduce en la internalización de AMPARs, pero puede que la regulación diferencial de las proteínas que interaccionan con los AMPARs tenga algo que ver. Además, cabe recordar que no sólo los AMPARs sino también los fosfoinosítidos se desfosforilan durante la LTD. De forma análoga a la conexión entre la formación de PIP3 y la potenciación sináptica, se ha visto que el reemplazo de PIP3 por la fosfatasa de lípidos PTEN está implicado en la depresión sináptica (Wang et al. 2006; Jurado et al. 2010).

No obstante, y contrariamente a la idea original de que la LTD dependía de la activación de fosfatasas, investigaciones más recientes han permitido comprobar que de hecho varias quinasas también juegan un papel en la expresión y el mantenimiento de la LTD. Así por ejemplo, la eliminación de los AMPARs de las sinapsis durante la LTD se correlaciona con la fosforilación de la subunidad GluA2 llevada a cabo por la proteína quinasa C (PKC) (Daw et al. 2000; Kim et al. 2001). Según el modelo más aceptado, la interacción de la subunidad GluA2 con las proteínas GRIP1/ABP (ver más abajo) estaría favoreciendo la estabilización de los receptores en las sinapsis, y su fosforilación en la serina 880 conduciría a la disociación de GluA2 de GRIP1/ABP, con su consiguiente unión a PICK1 (ver más abajo) y eliminación de los AMPARs de las sinapsis. Otras quinasas implicadas en LTD serían la MAPK, por medio de la vía de señalización p38-MAPK (Zhu et al. 2002) y sorprendentemente, y de acuerdo a un trabajo recientemente publicado, la CamKII (Coultrap et al. 2014).

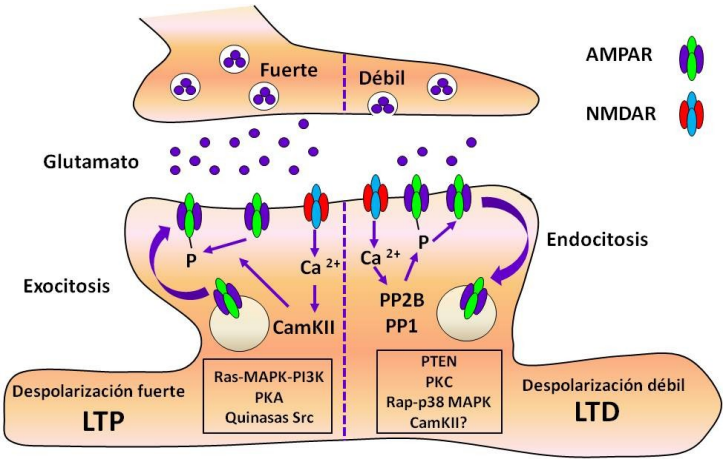


Figura 4. Mecanismos postsinápticos de expresión de LTP y LTD. Izquierda, una potente activación presináptica combinada con una fuerte despolarización postsináptica dispara la LTP, en parte por medio de la vía CamKII, la fosforilación de receptores y su exocitosis. Además de CamKII, otras quinasas (rectángulo) podrían estar implicadas en la expresión de LTP. **Derecha,** la activación débil de la neurona presináptica conduce a una despolarización modesta del terminal postsináptico y al flujo moderado de calcio a través de los NMDARs. Este fenómeno activa preferentemente a fosfatasas que desfosforilan a los AMPARs, favoreciendo así su endocitosis. Se ha propuesto que muchas otras proteínas (rectángulo) podrían estar mediando los mecanismos moleculares que disparan la endocitosis de AMPARs durante la LTD. Adaptado de Luscher and Malenka 2012.

Como se ha descrito, las vías de señalización activadas durante la LTD son considerablemente complicadas (figura 4), y evidentemente se requiere más trabajo para clarificar el modo en que contribuyen a la regulación de la distribución intracelular de los AMPARs una vez que estos han sido endocitados. De hecho, para evitar que los AMPARs ya internalizados vuelvan a la membrana plasmática, de modo que se mantenga la depresión, los AMPARs pueden ser degradados por medio de las vías lisosomal o proteosomal, las cuales están sujetas también a una complicada regulación. Así, se ha demostrado recientemente que el balance entre el

reciclaje de receptores (desde los endosomas de reciclaje al compartimento postsináptico, en un proceso que depende de la activación de Rab11) y su degradación (tráfico dependiente de Rab7 hacia los lisosomas) determina el grado de depresión sináptica después de la inducción de LTD (Fernandez-Monreal et al. 2012).

4.3. Proteínas que interaccionan con los AMPARs.

4.3.1. Proteína de fusión sensible a N-etilmaleimida (NSF). NSF juega un papel fundamental en los eventos de fusión de membranas como la exocitosis de vesículas sinápticas (Rothman 1994). La interacción entre NSF y el extremo C-terminal de la subunidad GluA2 de los AMPARs es un factor crucial en la regulación de la expresión en superficie de éstos. Cuando se interfiere con dicha interacción, la amplitud de las corrientes sinápticas disminuye rápidamente, lo que sugiere que se están perdiendo AMPARs sinápticos (Nishimune et al. 1998; Luscher et al. 1999; Noel et al. 1999).

4.3.2. Proteína de interacción con receptores de glutamato 1 (GRIP1), Proteína de unión a receptores AMPA (ABP, también llamada GRIP2) y Proteína de Interacción con la quinasa C 1 (PICK1). GRIP1 y ABP (o GRIP2) son proteínas con múltiples dominios de tipo PDZ que se unen a las subunidades GluA2 y GluA3 de los AMPARs (Dong et al. 1997; Srivastava et al. 1998; Dong et al. 1999). Mientras que GRIP1 contiene 7 dominios PDZ, ABP/GRIP2 existe en dos isoformas con 6 y 7 dominios PDZ respectivamente. La forma más corta se une a las subunidades GluA2/GluA3 (Srivastava et al. 1998) y es funcionalmente indistinguible de GRIP1.

Parece que GRIP1 y ABP/GRIP2 desarrollan muchas y variadas funciones en la célula, aunque todavía existe mucha controversia al respecto. Algunos estudios proponen que GRIP1/2 puedan estar jugando un papel en el mantenimiento de los AMPARs en las sinapsis (Osten et al. 2000). Sin embargo, también se ha propuesto que la unión de GRIP1/2 a las subunidades GluA2/GluA3 contribuiría a la estabilización intracelular de aquellos AMPARs que han sido previamente endocitados, evitando así su reinserción en la membrana sináptica (Daw et al. 2000). Otros autores defienden que GRIP1/2 podrían estar, por el contrario, favoreciendo la reinserción en la membrana postsináptica de los receptores endocitados (Mao et al. 2010).

PICK1 también une las subunidades GluA2 y GluA3 (Dev et al. 1999; Xia et al. 1999), por medio de sus extremos C-terminales. Como en el caso de GRIP1/2, se han propuesto múltiples funciones para PICK1, incluyendo la regulación de la inserción y de la composición de subunidades de los AMPARs en las sinapsis (Daw et al. 2000; Terashima et al. 2004; Hanley 2008).

4.3.3. Proteína asociada a la sinapsis 97 (SAP97) and Proteína 4.1. SAP97 es otra proteína estructural multidominio que interacciona con los AMPARs, pero a través de la subunidad GluA1 (Leonard et al. 1998). SAP97 podría estar jugando un papel en el transporte de los AMPARs que contienen GluA1 a las espinas dendríticas, puesto que se ha podido comprobar que SAP97 llega a las espinas bajo el control de la fosforilación mediada por CamKII (Mauceri et al. 2004).

La proteína 4.1R es una proteína del citoesqueleto que se identificó por primera vez en eritrocitos. Los homólogos neuronales de la proteína 4.1, 4.1N y 4.1G, interaccionan con la subunidad GluA1 de los AMPARs (Shen et al. 2000); se cree que podrían favorecer la interacción de la subunidad GluA1 con el citoesqueleto de actina, promoviendo así su estabilización en la membrana postsináptica.

4.3.4. Proteínas transmembrana reguladoras de receptores AMPA (TARPs). Cabe mencionar también esta familia de proteínas, más recientemente descrita, en la que se incluye stargazin. Actúan como subunidades auxiliares de los AMPARs que controlan tanto el tráfico de receptores y su anclaje a nivel de la membrana postsináptica, como las propiedades del canal (Ziff 2007; Jackson and Nicoll 2011).

4.4. Transporte de AMPARs dependiente de microtúbulos a lo largo de las dendritas.

Los AMPARs se sintetizan fundamentalmente en el cuerpo celular. Por tanto, necesitan ser transportados largas distancias a lo largo de las dendritas para poder alcanzar las espinas dendríticas y ser insertados en la membrana postsináptica. Además de garantizar el mantenimiento de la forma celular, el citoesqueleto microtubular que se prolonga a lo largo de las dendritas proporciona “raíles” a lo largo de los cuales se transportan vesículas que incluyen proteínas de membrana como los AMPARs. Las proteínas motoras asociadas a microtúbulos de las superfamilias de las kinesinas y las dineínas hacen posible esta forma de transporte activo (Goldstein and Yang 2000; Hirokawa and Takemura 2005).

Los mecanismos que gobiernan la especificidad con respecto al cargo que se transporta y la direccionalidad del movimiento no se conocen todavía demasiado bien. Parece que las proteínas adaptadoras que sirven de puente entre el cargo y los motores moleculares podrían estar mediando dicha especificidad. Así, GRIP1 interacciona directamente con la cadena pesada de la kinesina convencional (Setou et al. 2002). Como se ha mencionado anteriormente, GRIP1 une las subunidades GluA2 y GluA3 de los AMPARs, y por lo tanto, podría funcionar como puente de unión entre los AMPARs y el citoesqueleto microtubular a

través de los motores moleculares. Además, el complejo GRIP1-GluA2 puede asociarse con liprin-α. Esta interacción parece ser también crítica para el tráfico de los AMPARs, puesto que un mutante de liprin-α que no puede unir GRIP1 altera la expresión en superficie de los AMPARs en neuronas hipocampales (Wyszynski et al. 2002). Curiosamente, liprin-α interacciona con un miembro de la familia de las kinesinas, KIF1, y se ha visto que los AMPARs pueden ser co-inmunoprecipitados con KIF1 a partir de lisados de cerebro (Shin et al. 2003).

Por otra parte, cabe recordar que las espinas dendríticas carecen básicamente de microtúbulos y se caracterizan por poseer un rico citoesqueleto de actina, responsable de todos aquellos cambios estructurales que subyacen a la inducción de plasticidad sináptica (Matus 2000). Por lo tanto, los AMPARs que se transportan a lo largo de los "raíles" constituidos por los microtúbulos tienen que ser transferidos en algún momento al citoesqueleto de actina para poder alcanzar las sinapsis. Se desconocen los mecanismos moleculares que median esta transición, pero se ha propuesto a la proteína 4.1 como candidato razonable. No obstante, cabe mencionar así mismo que recientemente se ha demostrado la entrada transitoria de microtúbulos dinámicos en las espinas dendríticas (Jaworski et al. 2009).

5. Los microtúbulos en las neuronas. Proteínas estructurales asociadas a microtúbulos.

Los microtúbulos juegan papeles fundamentales en las neuronas. Son imprescindibles para el establecimiento de la polaridad y morfología neuronales durante el desarrollo, y para mantener la citoarquitectura de las neuronas maduras. Proveen de una base estructural para el transporte intracelular en axones y dendritas, y además, actúan como plataformas para moléculas de señalización (Gundersen and Cook 1999; Davies and Morris 2004; Conde and Caceres 2009).

Una propiedad característica de los microtúbulos es su habilidad para experimentar rápidos ciclos de crecimiento y desensamblaje. Este comportamiento de los microtúbulos, que se observa tanto *in vitro* como *in vivo*, se conoce como "inestabilidad dinámica". La dinámica de los microtúbulos y también sus interacciones con otros componentes celulares están reguladas por proteínas que se unen a ellos. Las "proteínas asociadas a microtúbulos" o MAPs fueron las primeras de estas proteínas en identificarse. Favorecen que la tubulina se ensamble para formar microtúbulos y además, permanecen unidas a la superficie de los microtúbulos, estabilizándolos (Davies and Morris 2004; Conde and Caceres 2009). Una de estas proteínas es la proteína asociada a microtúbulos 1B (MAP1B).

6. Proteína asociada a microtúbulos 1B (MAP1B): fisiología y patología.

6.1 Particularidades moleculares de MAP1B.

MAP1B es una proteína grande con un peso molecular aparente de 320 kDa (Noble et al. 1989). Está compuesta por 2464 aminoácidos. MAP1B se codifica como un precursor poliproteico que después se corta en una cadena pesada (MAP1B-HC) y una ligera (MAP1B-LC) (Hammarback et al. 1991). El sitio de corte se localiza cerca de la posición 2100 de la secuencia de aminoácidos (figura 5). MAP1B-LC se asocia de forma no covalente con la región N-terminal de la cadena pesada para formar un complejo proteico.

MAP1B-HC contiene un sitio de unión a microtúbulos cerca del extremo N-terminal (Noble et al. 1989) y un sitio de unión a actina (Cueille et al. 2007); la cadena ligera también incluye ambos dominios (Zauner et al. 1992; Togel et al. 1998). Como las dos cadenas de MAP1B son capaces de unirse a los microtúbulos, se ha propuesto que MAP1B podría estar funcionando como una proteína capaz de entrecruzar los microtúbulos (Hammarback et al. 1991; Zauner et al. 1992; Pedrotti et al. 1996a).

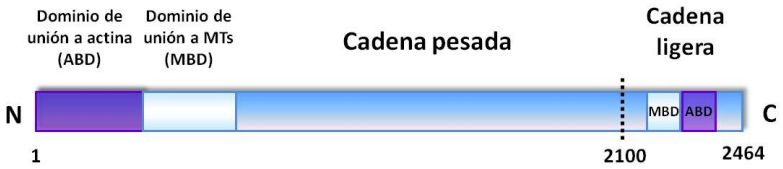


Figure 5. Proteína MAP1B. MAP1B está compuesta por 2464 aminoácidos. Los dominios de unión a actina (ABD) se localizan en el extremo N-terminal de la cadena pesada (1-517) y también en la cadena ligera (2336-2459). El dominio de unión a microtúbulos (MBD) en MAP1B-HC (517-848) se caracteriza por presentar 21 repeticiones de tipo KKEK cerca del extremo N-terminal; MAP1B-LC contiene otro MBD (2210-2336). Adaptado de Riederer 2007.

En homogenados de cerebro de rata, MAP1B-LC existe en una proporción de 6:1 a 8:1 con respecto a MAP1B-HC (Mei et al. 2000). Se ha propuesto que MAP1B-LC tendría una vida media mayor, dado que ambas cadenas se sintetizan en una proporción de 1:1 (Mei et al. 2000). Como MAP1B-LC existe en exceso sobre MAP1B-HC *in vivo*, se ha sugerido que la cadena ligera podría tener funciones adicionales a aquellas que desempeña la fracción acomplejada por la cadena pesada (Mei et al. 2000). En un estudio que pretendía analizar posibles funciones independientes de la cadena ligera cuando no está acomplejada por la pesada, se propuso un modelo en el que la cadena pesada actuaría como subunidad reguladora de la ligera, controlando así su actividad, en el complejo formado por ambas (Togel et al. 1998). De hecho, una de las observaciones más importantes del estudio es que, aunque las dos cadenas contienen dominios de unión a microtúbulos, la actividad estabilizadora de

microtúbulos más potente corresponde sin duda a la cadena ligera. Cuando MAP1B-LC se une a los microtúbulos, les confiere una apariencia ondulada característica y una estabilidad incrementada frente a la acción de agentes despolimerizantes (Togel et al. 1998; Noiges et al. 2002), que es característica de la cadena ligera de MAP1B en comparación con otras MAPs, como MAP2.

6.2 Función de MAP1B.

MAP1B es la primera MAP que se expresa durante el desarrollo neuronal (Tucker et al. 1989). Se encuentra en axones, soma y dendritas (Matus and Riederer 1986; Tucker et al. 1989). Es especialmente abundante en los axones en desarrollo, y su expresión disminuye con el desarrollo (Schoenfeld et al. 1989); aún así, se ha podido demostrar que MAP1B está presente en el compartimento somatodendrítico también en el cerebro adulto (Kawakami et al. 2003; Peng et al. 2004; Collins et al. 2005; Tortosa et al. 2011).

MAP1B se ha estudiado clásicamente como un modulador de la axogénesis por medio de su interacción con el citoesqueleto microtubular (Gonzalez-Billault et al. 2004). De acuerdo con el modelo clásico de polarización neuronal (Kirschner and Mitchison 1986a; Kirschner and Mitchison 1986b), la formación del axón se debe a cambios dramáticos en la organización y la dinámica de los microtúbulos en una región concreta de una neurona; por lo tanto, un factor importante que influencia este proceso es la existencia de una proteína que actúe como un estabilizador de microtúbulos, como MAP1B. La evidencia que apunta al papel de MAP1B en la formación y elongación del axón durante el desarrollo es abundante (Gonzalez-Billault et al. 2004; Montenegro-Venegas et al. 2010); de hecho, diferentes grupos han podido demostrar una alteración severa del desarrollo cerebral como consecuencia de la interrupción del gen de MAP1B (Edelmann et al. 1996; Takei et al. 1997; Gonzalez-Billault et al. 2000; Meixner et al. 2000; Takei et al. 2000).

Además, se ha visto que la interacción entre los citoesqueletos de microtúbulos y actina en los conos de crecimiento es un factor crítico en la elongación axonal durante el desarrollo. MAP1B ha sido propuesta como mediadora de dicha interacción, por su habilidad para unirse tanto a microtúbulos como a filamentos de actina. La observación de que MAP1B modula la actividad de pequeñas GTPasas implicadas en la regulación del citoesqueleto de actina durante la elongación y ramificación del axón, como Rac1, Cdc42 y RhoA, apoya esta hipótesis (Montenegro-Venegas et al. 2010). Algunos autores han propuesto que es la cadena ligera de

MAP1B la que modula la actividad de Rac1 por medio de su unión a Tiam1 (un factor de intercambio de nucleótidos de guanosina para Rac1) (Henriquez et al. 2012).

Más recientemente, se ha propuesto que MAP1B podría estar desarrollando funciones novedosas a nivel del compartimento postsináptico en relación con su habilidad para regular las pequeñas GTPasas, y por tanto, el citoesqueleto de actina. Se ha visto que MAP1B es imprescindible para la correcta morfogénesis de las espinas dendríticas (Tortosa et al. 2011). Además, resultados de nuestro grupo de investigación apuntan a que MAP1B sería necesaria para mediar la eliminación regulada de los AMPARs de las sinapsis que acontece tras la inducción de LTD por medio de la activación de Rac1 (Benoist et al. 2013). De hecho, se ha demostrado que los niveles de expresión de MAP1B se incrementan después del tratamiento de neuronas en cultivo con DHPG, un agonista específico de los receptores mGluR, lo cual también induce endocitosis de AMPARs; esta observación apoyaría la hipótesis de que MAP1B actúa como un regulador esencial en el tráfico de AMPARs que ocurre durante los fenómenos de plasticidad sináptica en el hipocampo (Davidkova and Carroll 2007).

Además se ha propuesto que MAP1B podría actuar como proteína de andamiaje, dado que es capaz de interaccionar con muchas otras proteínas, tanto directamente como por medio de tubulina o actina. Además, estas interacciones podrían ser diferentes para la cadena pesada y la cadena ligera (Riederer 2007).

Cabe destacar que MAP1B ha sido implicada también en la regulación de la distribución subcellular de diferentes tipos de receptores y canales iónicos, mediando su interacción con el citoesqueleto microtubular. Así por ejemplo, MAP1B regula la localización sináptica de receptores GABA ionotrópicos en las sinapsis retinales por medio de su unión a la subunidad GABAc ρ1 (Hanley et al. 1999). MAP1B podría estar también regulando el tráfico de NMDARs, puesto que interacciona con la subunidad NR3A (Eriksson et al. 2010). En el caso concreto de la cadena ligera, se ha visto que puede unirse al canal de sodio voltaje-dependiente Na_v 1.6, facilitando su transporte a la superficie neuronal (O'Brien et al. 2012). Además, se ha demostrado que dos subtipos de receptores de serotonina pueden interaccionar directamente con MAP1B-LC: el receptor 5-HT_6, cuya interacción con MAP1B-LC favorece su expresión en la superficie celular (Kim et al. 2014); y el receptor $5HT_{3A}$, que por el contrario se expresa menos en superficie cuando se une a MAP1B-LC (Sun et al. 2008). De forma equivalente, la sobre-expresión de MAP1B-LC produce una disminución de la expresión en superficie del canal de calcio tipo N Ca_v2.2 (Gandini et al. 2014). Especialmente relevante en relación con el tráfico de AMPARs es la interacción que se ha descrito entre MAP1B-LC y GRIP1, que parece ocurrir por

medio de un dominio no-PDZ de GRIP1 (Seog 2004; Davidkova and Carroll 2007). Actualmente se desconoce cuál es el papel de dicha interacción.

Es importante recordar que la función de MAP1B está modulada por fosforilación (Gonzalez-Billault et al. 2004; Riederer 2007). Se han descrito dos modos de fosforilación para MAP1B que se regulan de forma independiente durante el desarrollo y presentan diferentes distribuciones subcelulares en las neuronas. Quinasas dependientes de prolina (como cdc2, glucógeno sintasa 3, cdk5 y JNK) catalizan el modo I de fosforilación, que está presente en axones en desarrollo (con un gradiente de fosforilación creciente hacia el cono de crecimiento). Un segundo modo de fosforilación, el modo II, es dependiente de la caseín quinasa II (CKII) y está presente tanto en axones como en dendritas, también en la etapa adulta (Diaz-Nido et al. 1988; Ulloa et al. 1993a; Avila et al. 1994; Gonzalez-Billault et al. 2004). Las proteínas fosfatasas que contribuyen a regular los niveles totales de fosforilación de MAP1B son PP2A y PP2B, que actúan sobre el modo I de fosforilación (Ulloa et al. 1993c; Gong et al. 2000) y la fosfatasa PP1, que junto con la PP2A, regula el modo II (Ulloa et al. 1993c; Gong et al. 2000).

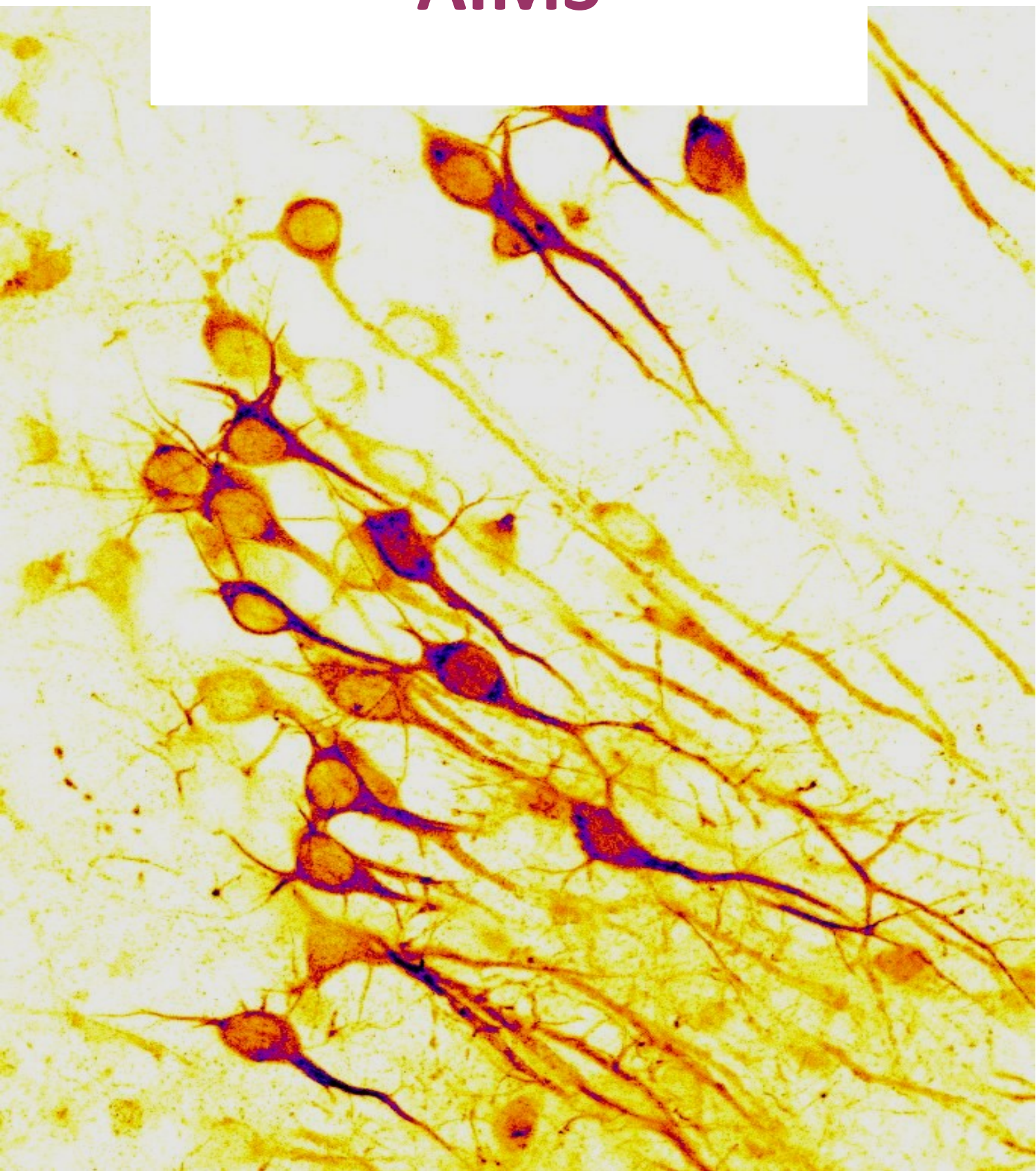
6.3 Patología relacionada con MAP1B.

El Síndrome X-frágil (FXS) y la Neuropatía Axonal Gigante (GAN) son las dos patologías en las que más claramente se ha demostrado la implicación de MAP1B.

El FXS es la causa más frecuente de retraso mental heredado y resulta de la ausencia de la proteína del retraso mental X-frágil (FMRP). FMRP controla la traducción de varios ARNm, incluyendo el de MAP1B. La falta de FMRP conduce a la expresión anormalmente elevada de MAP1B, lo que da lugar a una estabilidad incrementada de los microtúbulos. Se ha propuesto que la dinámica anómala de los microtúbulos que resulta de la falta de FMRP podría ser un factor relevante en la patogénesis del retraso mental asociado al X-frágil (Lu et al. 2004), dado que se ha observado un desarrollo retardado de espinas dendríticas en estos pacientes.

GAN es un desorden autosómico recesivo causado por una mutación en gigaxonina, una proteína que se une a microtúbulos y filamentos intermedios, y que también interacciona con la cadena ligera de MAP1B (Ding et al. 2002). La relevancia clínica de esta interacción se ha podido demostrar en pacientes que presentaban degeneración axonal y muerte neuronal, en los que se han encontrado dos mutaciones específicas en la gigaxonina que impedían la interacción gigaxonina-MAP1B (Ding et al. 2002).

AIMS



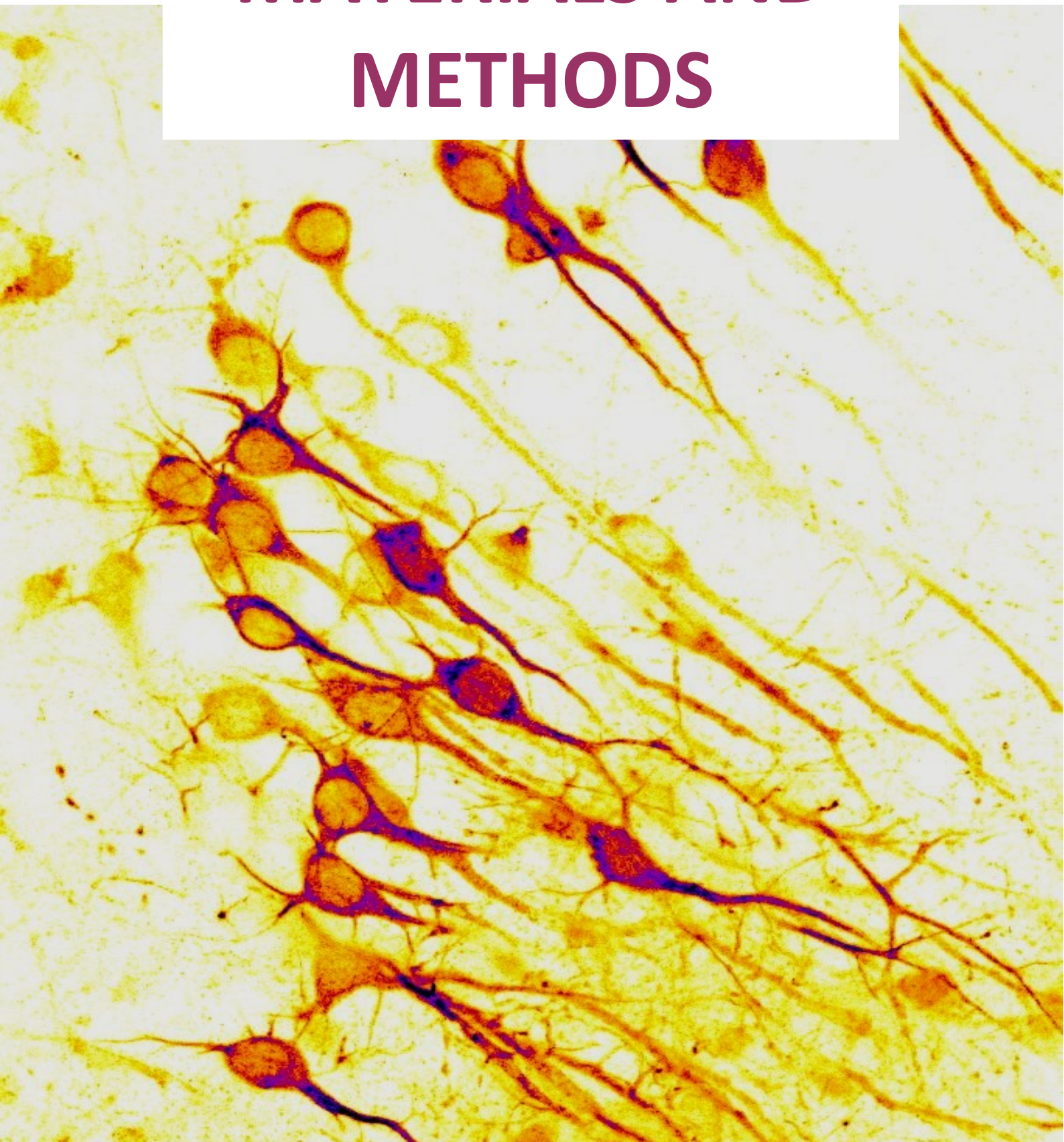
In this work, we aimed to:

1. Develop molecular tools to acutely manipulate MAP1B levels in CA1 hippocampal neurons.

2. Characterize the effects on basal synaptic transmission and synaptic plasticity of MAP1B over-expression and MAP1B acute down-regulation.

3. Explore the potential molecular mechanisms through which MAP1B regulates AMPAR trafficking in CA1 hippocampal neurons.

MATERIALS AND METHODS



1. MATERIALS.

1.1 Reagents and drugs.

Most of the ordinary reagents and media used to perform the experiments that follow were purchased from Sigma-Aldrich, Merck and Bio-Rad. Other providers were: Macherey-Nagel, Panreac, Electron Microscopy Sciences, Conda Pronadisa Laboratories, GIBCO, Roche and Falcon.

The following reagents and drugs were also employed:

PRODUCT	REFERENCE
Syber Safe DNA Gel Stain	Life technologies (Invitrogen)
Complete MINI EDTA-free protease inhibitor cocktail tablets	Roche
Immobilon Western Chemiluminescent HRP Substrate (ECL)	Millipore
Immobilon transfer membranes	Millipore
ProLong Gold Antifade Reagent	Life Technologies (Invitrogen)
Biocytin	Life Technologies (Invitrogen)
Streptavidin-Alexa 555	Life Technologies (Invitrogen)
Lipofectamine 2000 Transfection Reagent	Life Technologies (Invitrogen)
Actin protein purified from rabbit skeletal muscle (95% pure)	Cytoskeleton
Tubulin protein (>99% pure) from bovine brain	Cytoskeleton
Helios Gene Gun System and accessories	Bio-rad
Protein G sepharose 4 fast flow	GE Healthcare
Glutathione sepharose high performance beads	GE Healthcare

DRUG	USE	REFERENCE
Rolipram	Chemical induction of LTP	Sigma-Aldrich
Forskolin	Chemical induction of LTP	Sigma-Aldrich
NMDA	Chemical induction of LTD	Sigma-Aldrich
(R,S)-3,5-Dihydroxyphenylglycine (DHPG)	mGluR-dependent LTD	Biogen (Tocris)
DL-2-Amino-5-phosphonopentanoic acid (DL-APV)	mGluR-dependent LTD	Biogen (Tocris)
Vinblastine	Microtubule depolymerization	Sigma-Aldrich
Taxol (semi-synthetic paclitaxel)	Microtubule polymerization/stabilization	Sigma-Aldrich
Picrotoxin	Electrophysiology	Sigma-Aldrich

2-chloroadenosine	Electrophysiology	Sigma-Aldrich
Spermine	Rectification experiments (electrophysiology)	Sigma-Aldrich
Chloroquine	Lentiviral production	Sigma-Aldrich

1.2 Antibodies.

Listed below are the primary and secondary antibodies used in Western-blot and Immunofluorescence procedures. Dilutions of use are also indicated.

Primary Antibody	Protein	Species	Reference	Dilution WB	Dilution IF
Anti-MAP1B (N-19)	MAP1B (N-terminus)	Goat	Santa-Cruz	1:1000	
Anti-MAP1B (H-130)	MAP1B (C-terminus)	Rabbit	Santa-Cruz	1:1000	1:100
Anti-actin, clone C4	Actin	Mouse	Chemicon (Millipore)	1:2000	
Anti-GFP	GFP	Mouse	Roche	1:1000	1:200
Anti-GFP	GFP	Rabbit	Life technologies (Invitrogen)		1:100
Anti-tyrosine tubulin, clone TUB-1A2	Tyrosinated tubulin	Mouse	Sigma-Aldrich		1:2000
Anti-tubulin, detyrosinated	Detyrosinated tubulin	Rabbit	Chemicon (Millipore)		1:200
Anti-acetylated tubulin, clone 6-11B-1	Acetylated tubulin	Mouse	Sigma-Aldrich		1:1000
Anti-DsRed	RFP (tandem dimer 2, "tdimer2")	Rabbit	Clontech		1:200
Anti-mCherry	mCherry	Rabbit	GeneTex		1:500
Anti-GluA2, extracellular, clone 6C4	GluA2 (N-terminus)	Mouse	Chemicon (Millipore)		1:100
Anti-GluA2 glutamate receptor, clone L21/32	GluA2 (C-terminus)	Mouse	Neuromab		1:100

Anti-GluA1-NT, clone RH95	GluA1 (N-terminus)	Mouse	Chemicon (Millipore)		1:500
Anti-Glutamate Receptor 1 (AMPA subtype) antibody	GluA1 (C-terminus)	Rabbit	Abcam		1:500
Anti-NMDAR2A&B antibody, pan	NR2A and NR2B	Rabbit	Chemicon (Millipore)	1:1000	
Anti-MKLP1 (N-19)	MKLP1 (KIF23) (N-terminus)	Rabbit	Santa Cruz	1:200	
Anti-GRIP1, C-terminus	GRIP1 (C-terminus)	Rabbit	Chemicon (Millipore)		1:100
Anti-Rac1	Rac1	Mouse	BD Biosciences	1:1000	
Anti-RhoA	RhoA	Mouse	BD Biosciences	1:100	

Secondary Antibody	**Protein**	**Species**	**Reference**	**Dilution WB**	**Dilution IF**
Anti-goat IgG (whole molecule), peroxidase	IgG goat	Rabbit	Sigma-Aldrich	1:10.000	
Anti-rabbit IgG (whole molecule), peroxidase	IgG rabbit	Goat	Sigma-Aldrich	1:10.000	
Anti-mouse IgG (whole molecule), peroxidase	IgG mouse	Rabbit	Sigma-Aldrich	1:10.000	
Alexa Fluor 488 goat anti-mouse IgG_1 (γ1)	IgG_1 mouse	Goat	Life technologies (Invitrogen)		1:500
Alexa Fluor 555 donkey anti-mouse IgG (H+L)	IgG heavy chains and light chains mouse	Donkey	Life technologies (Invitrogen)		1:500
Alexa Fluor 488 donkey anti-rabbit IgG (H+L)	IgG heavy chains and light chains rabbit	Donkey	Life technologies (Invitrogen)		1:500
Alexa Fluor 555 donkey anti-rabbit IgG (H+L)	IgG heavy chains and light chains rabbit	Donkey	Life technologies (Invitrogen)		1:500

1.3 Plasmids.

A complete list of the set of plasmids used in this work is presented below. A thorough description of how they were generated is included in the "methods" section.

Plasmid	Expression
pSR5-MAP1B-LC-GFP	MAP1B-LC-GFP
FG12.CMV-MAP1B-HC-GFP	MAP1B-HC-GFP
pSR5-MAP1B-LC-delABD-GFP	MAP1B-LC-delABD-GFP
pSR5-MAP1B-LC-delMBD-GFP	MAP1B-LC-delMBD-GFP
pSR5-MAP1B-LC-Dendra	MAP1B-LC-Dendra
pSR5-Dendra-tubulin	Dendra-tubulin
KH1-LV-mCherry-shRNA 2012	shRNA against MAP1B
KH1-LV-mCherry-scrambled shRNA	Scrambled shRNA
pMAP1B-LC-GFP	MAP1B-LC-GFP
pMAP1B-LC-mCherry	MAP1B-LC-mCherry
pGFP-GluR2 (RQ)	GFP-GluR2 (RQ)
pRFP-GluR2 (RQ)	RFP-GluR2 (RQ)
pSR5-GFP-GluR2 (RQ)	GFP-GluR2 (RQ)
pGFP-GluR1-ires-tCamKII	GFP-GluR1 + tCamKII
pBA-TfR-GFP	TfR-GFP
pJPA5-TfR-mCherry	TfR-mCherry
pGEX-GST-PBD	Rac1 binding domain of PAK1 (fused to GST)
pGEX-GST-RBD	RhoA binding domain of Rhotekin (fused to GST)

2. METHODS.

2.1. Cloning of DNA constructs.

For the cloning of the DNA constructs of interest, several restriction enzymes together with their specific buffers from New England Biolabs were employed, according to manufacturer´s instructions. In general, digestions were carried out at 37ºC over-night. The ligation kit from Takara (Conda Pronadisa laboratories) was the kit of choice. Ligation proceeded at 16ºC over 12-16 hours. To ensure efficient transformation, commercial XL2-blue ultracompetent bacteria (Stratagene) were required; in some cases, SURE R2 super competent bacteria (Stratagene) were needed to avoid recombination of the plasmids being cloned. Heat shock was performed at 42ºC for 45 seconds, and afterwards, bacteria were grown in LB medium at 37ºC for two

hours. Next, bacteria were plated on agarose plates with antibiotics (ampicillin or kanamycin, depending on the specific plasmid, at 100 µg/µL). The appropriate restriction pattern of the newly generated plasmids was checked by running the samples on agarose gels and dying the DNA with Syber Safe (Life Technologies, Invitrogen). Positive clones were grown over-night in liquid LB medium supplemented with antibiotics. DNA was purified with "miniprep" and "maxiprep" kits from Macherey-Nagel ("NucleoSpin plasmid" and "NucleoBond Xtra Maxi" kits, respectively).

A complete description of the cloning strategies designed to generate the plasmids of interest is following. For those plasmids that weren´t cloned, their origin is indicated:

- **pUHD-10-3-MAP1B-LC-GFP and pSR5-MAP1B-LC-GFP.**

The plasmid pMT22Tet (pUHD-10-3-MAP1B-LC-Myc) (Togel et al. 1998) was kindly provided by Dr. Jesús Ávila, from Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid (Spain). The Myc tag of the original plasmid was replaced by EGFP through digestion with NheI and PspOMI restriction enzymes, to generate pUHD-10-3-MAP1B-LC-GFP.

Next, MAP1B-LC-GFP sequence was cloned into pSinRep5 vector (for Sindbis virus production). To do so, pUHD-10-3-MAP1B-LC-GFP was digested with SpeI and PspOMI. After ligation with the fragment resulting from XbaI-PspOMI digestion of pSinRep5, the construct pSR5-MAP1B-LC-GFP was generated.

- **pUHD-10-3-MAP1B-HC-GFP.**

Similarly to MAP1B-LC, the MAP1B-HC recombinant protein was made by in-frame ligation of the EGFP coding sequence with the heavy chain sequence (aminoacids 1-2100) of MAP1B in pMT17Tet plasmid (pUHD-10-3-MAP1B-HC-Myc) (Togel et al. 1998), also provided by Dr. Jesús Ávila.

- **FG12.CMV-MAP1B-HC-GFP.**

The vector FG12.CMV (Verhaegen et al. 2006) was a generous gift from Dr. María S. Soengas, from Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid (Spain). The sequence of MAP1B-HC tagged with GFP was cloned into FG12.CMV vector (previously digested with NheI and BsrGI) via digestion of pUHD-10-3-MAP1B-HC-GFP construct with SpeI and BsrGI enzymes.

- **pSR5-MAP1B-LC-delABD-GFP.**

The plasmid pUHD-10-3-MAP1B-LC-GFP was used to make a mutant of the wild type protein lacking the actin-binding domain. To do so, the sequence of MAP1B-LC corresponding to the actin-binding domain of the wild type protein (aminoacids 2336-2459) was removed by digesting the original plasmid with BstAPI and BmtI restriction enzymes. Blunt ends were generated (by using T4 DNA polymerase, New England Biolabs) to proceed to ligation. This way, the plasmid pUHD-10-3-MAP1B-LC-delABD-GFP was created. The whole sequence corresponding to MAP1B-LC-delABD-GFP (selected via digestion of pUHD-10-3-MAP1B-LC-delABD-GFP with SpeI and PspOMI) was then transferred into pSinRep5 vector (previously digested with XbaI and PspOMI) to finally generate pSR5-MAP1B-LC-delABD-GFP.

- **pSR5-MAP1B-LC-delMBD-GFP.**

The generation of this vector was analogous to the previous one in every step, except for the restriction enzymes initially used to produce the mutant of interest. The sequence of the wild type protein containing only the actin-binding domain (and so, resulting from the deletion of the microtubule-binding domain, aminoacids 2210-2336 of the wild type protein) was generated by digesting pUHD-10-3-MAP1B-LC-GFP plasmid with AvrII and BstAPI.

- **pSR5-MAP1B-LC-Dendra.**

The pUHD-10-3-MAP1B-LC-GFP vector was digested with NheI and BsrGI enzymes to remove the EGFP tag. Then, the EGFP was replaced by Dendra2 tag, previously generated from pDendra2-fibrillarin plasmid (Evrogen) via digestion with the same restriction enzymes. The whole sequence of MAP1B-LC-Dendra was then recloned into pSinRep5 vector following the usual strategy (digestion of pUHD-10-3-MAP1B-LC-Dendra plasmid with SpeI and PspOMI, and digestion of pSinRep5 vector with XbaI and PspOMI).

- **pMT-Dendra-tubulin and pSR5-Dendra-tubulin.**

The pMT-Dendra-tubulin construct (Jolly et al. 2010) was a generous gift from Vladimir I. Gelfand, from Northwestern University Feinberg School of Medicine, Chicago (USA). The sequence corresponding to Dendra-tubulin was recloned afterwards into pSinRep5 plasmid (José A. Esteban´s laboratory at Centro de Biología Molecular “Severo Ochoa”).

- **KH1-LV-mCherry-shRNA 2012.**

Short 21 bp oligos (Sigma-Aldrich), specifically targeting MAP1B sequence (target sequence at position 2012 of the mRNA from mouse: 5´-GCCCAAGAAAGAAGTGGTTAA-3´), were annealed and cloned into SmaI-XbaI sites of the lentiviral vector KH1-LV (Lois et al. 2002), kindly provided by Dr. María S. Soengas. This vector contains an H1 promoter and independent mCherry expression driven by an ubiquitin-C promoter. An additional scrambled shRNA (5´-GACATACAGTGAGCGGATAAA-3´) was designed and cloned following the same strategy to be used as a control.

- **pMAP1B-LC-GFP and pMAP1B-LC-mCherry.**

pMAP1B-LC-GFP was cloned by substitution of EGFP in pC1-EGFP vector (Clontech), digested with NheI and PspOMI enzymes, by MAP1B-LC-GFP sequence, taken from pUHD-10-3-MAP1B-LC-GFP plasmid via digestion with restriction enzymes SpeI and PspOMI. In the resulting plasmid, the expression of MAP1B-LC-GFP is driven by CMV promoter (needed for biolistic method of expression).

To generate pMAP1B-LC-mCherry, the green fluorophore in pMAB1B-LC-GFP was exchanged by mCherry, via digestion of the precursor plasmid with NheI and BsrGI enzymes.

- **pGFP-GluA2 (RQ), pRFP-GluA2 (RQ), pSR5-GFP-GluA2 (RQ) and pGFP-GluA1-ires-tCamKII.**

The GFP-tagged AMPA receptor subunits (GFP-GluA2-R607Q and GFP-GluA1), and the truncated αCaMKII construct (Shi et al. 1999; Hayashi et al. 2000; Shi et al. 2001) were a generous gift from Dr. Roberto Malinow, from the University of California San Diego, California (USA). RFP fusion GluA2 construct is equivalent to its GFP counterpart, but using the red fluorescent protein variant tdimer2.

- **pBA-TfR-GFP and pJPA5-TfR-mCherry.**

The pBA-TfR-GFP (Burack et al. 2000) and pJPA5-TfR-mCherry (Wang et al. 2008) were kindly provided by Dr. Daniel Choquet, from the Interdisciplinary Institute for Neuroscience, Bordeaux (France).

- **pGEX-GST-PBD and pGEX-GST-RBD.**

The pGEX-GST-PBD plasmid (Benard et al. 1999) and the pGEX-GST-RBD plasmid (Ren et al. 1999) were a generous gift from Dr. Fernando Martin-Belmonte, from Centro de Biología Molecular "Severo Ochoa", Madrid (Spain).

2.2. Cell culture and tissue culture.

In this work, two culture systems were used as models: hippocampal primary culture (cell culture) and hippocampal organotypic slice culture (tissue culture). They were prepared following the procedures described below.

2.2.1 Hippocampal primary culture.

Methods for preparing hippocampal primary cultures have been previously described (Banker and Cowan 1977; Dotti et al. 1988). Briefly, hippocampi were dissected from the brains of E18 mouse embryos or E19 rat embryos, treated with 2.5% trypsin for 20 min at 37ºC, thoroughly washed with Hank´s balanced salt solution (supplemented with 10 mM HEPES, pH 7.4 and antibiotics) and dissociated by repeated passage through a constricted Pasteur pipette. Neurons were then plated onto poly-L-lysine-coated coverslips (12 mm diameter, or 24x24 mm square coverslips used in live imaging), and maintained in Minimum Essential Medium (MEM) containing 20% D-glucose and 10% Fetal Bovine Serum (FBS) for 3-4 hours at 37ºC and 5% CO_2. Once the cells had attached to the substrate, the media was replaced with Neurobasal medium (Gigco) supplemented with B27 and glutamine. Cells were cultured at 37ºC and 5% CO_2 for variable periods of time (from 2 to 3 weeks), depending on the specific experiments.

2.2.2 Hippocampal organotypic slice culture.

Organotypic slice cultures were prepared as described (Gahwiler et al. 1997; Fuller and Dailey 2007). Hippocampi were dissected from young rats or mice (postnatal day 5 to 7) in cold dissection medium (10 mM D-glucose, 4 mM KCl, 26 mM $NaHCO_3$, 233.7 mM sucrose, 5 mM $MgCl_2$, and 1 mM $CaCl_2$) and cut transversely at an interval of 400 µm using a tissue chopper. Using thin spatulas, individual slices were then separated in culture medium (MEM supplemented with 20% horse serum, 1 mM glutamine, 1 mM $CaCl_2$, 2 mM MgSO4, 1 mg/mL insulin, 0.0012% ascorbic acid, 30 mM HEPES, 13 mM D-glucose, 5.2 mM $NaHCO_3$) and transferred to a 6-well plate containing culture inserts equipped with semi-porous membranes, and placed on culture medium. The hippocampal slices deposited on culture

inserts were maintained at 35.5ºC and 5% CO_2 for variable periods of time, depending on the requirements of the specific experiments (from 3-4 days to 14 days).

2.3. Expression of recombinant proteins.

2.3.1 Viral vectors.

- **Sindbis Expression System.**

The Sindbis expression system can be used to transiently over-express recombinant proteins in many eukaryotic cell lines as well as in organotypic hippocampal slice cultures (Schlesinger 1993; Schlesinger and Dubensky 1999).

In this work, the Sindbis expression system from Invitrogen was used. This system is composed of two different DNA constructs that have first to be linearized for the in vitro production of RNA transcripts.

The gene(s) of interest were previously cloned into pSinRep5 vector. It contains the Sindbis virus non-structural protein genes 1-4 for replicating RNA transcripts in vivo under the control of the SP6 promoter, and a subgenomic promoter that allows the transcription of the heterologous genes (the gene(s) of interest). A multiple cloning site permits the insertion of the gene(s) of interest into the vector behind the subgenomic promoter.

The second plasmid is DH-BB (that stands for Defective Helper, deleted between BspMII and BamHI). It is a DNA template that contains the genes for the four structural proteins required for packaging of Sindbis viral genome. When RNA from DH-BB is co-transfected by electroporation in BHK (Baby Hamster Kidney) cells with the recombinant RNA from pSinRep5, expression of the structural proteins allow packaging of the recombinant RNA into virus-like particles or pseudovirions. Infection of hippocampal primary neurons or organotypic slices with pseudovirions permits the delivery of recombinant RNA into the cytoplasm, resulting in the prompt production of high levels of the desired protein(s). These virions can undergo just one round of infection as they do not contain the helper RNA (DH-BB plasmid does not contain a packaging signal). Pseudovirions are collected 48 hours after the electroporation of BHK cells by ultracentrifugation of the supernatant at 30.000 r.p.m. for 2 hours. After making aliquots, they are stored at -80 ºC.

- **Lentivirus.**

To produce a stable down-regulation of MAP1B protein in cells, a short 21 bp oligo specifically targeting MAP1B sequence was cloned into KH1-LV lentiviral vector (Lois et al. 2002). Lentiviruses are the most suitable vectors for RNAi knockdown in neuronal cells for two reasons: 1) they can transduce both actively proliferating and non-dividing cells and 2) the genetic material is integrated into the host cell allowing for stable, long-term expression of the shRNA.

In this work, a system composed of three plasmids was used:

- The transfer vector (KH1-LV) containing the shRNA.
- A packaging vector (pCMV-dR8.74) (Dull et al. 1998) including several elements of the viral genome: Gag, which codes for precursors of structural proteins; Pol, encoding for viral enzymes: reverse transcriptase, RNase H, integrase and protease; and Rev, regulator of the expression of virion proteins by facilitating the export of unspliced and incompletely spliced viral RNAs from the nucleus to the cytoplasm.
- And an envelope plasmid (in this case, pMD2.G) (Dull et al. 1998), encoding for VSVG (glycoprotein of vesicular stomatitis virus), which enables viral entry into the infected cell.

pCMV-dR8.74 and pMD2.G were kindly provided by Dr. Jesús Ávila.

The defective lentiviral particles were produced by co-transfection of HEK-293T (Human Embryonic Kidney) cells with the three plasmids mentioned above. The transfection of HEK cells was achieved by calcium phosphate precipitation. Before transfection, normal DMEM (Invitrogen)-10% FBS cell medium was replaced by medium containing 25 µM chloroquine (it increases transfection efficiency). Transfected cells were incubated at 37ºC and 5% CO_2 for 8-12 hours, before replacing medium with chloroquine by fresh, normal medium to remove the drug. After 48 hours, viral particles released to the culture medium were concentrated by ultracentrifugation (30.000 r.p.m., 2 hours), aliquoted and stored at -80ºC.

- **Infection of hippocampal primary cultures and organotypic slices.**

Hippocampal primary neurons were infected with Sindbis virus or lentivirus by directly adding viral particles into the cell culture medium (Malinow et al. 2010). In the case of organotypic slices, a micro-injection of the virus-containing solution with a glass pipette was required

(Malinow et al. 2010). The Picospritzer system (Parker Instruments) permits the delivery of the solution deep into the tissue by supplying repeatable pressure pulses of nitrogen.

2.3.2 Transfection of hippocampal primary neurons.

When transfection or co-transfection of hippocampal primary neurons were required, Lipofectamine 2000 (Invitrogen) was the transfection reagent of choice. One-week old hippocampal primary neurons were used for efficient transfection. 12-mm round coverslips (3 coverslips per well) or 24x24 mm square coverslips (used for live imaging, 1 coverslip per well) were transferred to 6-well plates, and their culture medium was replaced by 1.5 mL of warm Neurobasal medium per well. Per condition, 1 µg of each DNA construct was mixed with 50 µL of Neurobasal medium; at the same time, 3 µL of Lipofectamine 2000 were mixed with 50 µL Neurobasal medium, and incubated for 5 minutes at room temperature. Then DNA and lipofectamine solutions were mixed together, and incubated again for 15 minutes at room temperature. The mixture was immediately added dropwise to hippocampal neurons in culture. An incubation period of 2 hours at 37ºC and 5% CO_2 followed, and afterwards, the culture medium was replaced again. The over-expression of the proteins of interest was analyzed 48 hours after transfection.

2.3.3. Biolistic transfection (gene gun).

In organotypic hippocampal slice cultures, co-expression of several proteins can be achieved using the biolistic method (Lo et al. 1994; Wellmann et al. 1999), with a combination of different plasmids bearing mammalian expression promoters, such as the cytomegalovirus (CMV) promoter. This method of expression may also be appropriate when a DNA construct cannot be packed into lentiviral particles because of its size (in the case of MAP1B-HC-GFP in this work).

To proceed to biolistic transfection, the Bio-Rad hand-held Helios gene gun and accessories were used. In essence, 1.6 µm gold particles that serve as microcarriers were coated with the DNA of interest by precipitation. Then, the DNA-coated gold particles were transferred into a polypropylene tube; the internal surface of the tube had been previously covered with a solution of polyvinylpyrrolidone (PVP) in ethanol to facilitate adhesion of the gold particles. The suspension containing the gold particles was homogenously distributed over the internal surface of the tube and dried using a nitrogen flow; then, the plastic tube was cut into small pieces to make “bullets”. To finish, the bullets were loaded into the cartridge holder of the

hand-held gene gun, and the DNA-coated microcarriers were propelled into cells at high speed using a pulse of helium gas.

2.4. Biochemical procedures.

2.4.1 Protein extracts.

To perform biochemical analysis, protein extracts were prepared from organotypic hippocampal slices in culture. They were collected and homogenized in cold buffer (80-100 µL per insert with 4 to 5 slices) containing 20 mM HEPES, pH 7.4, 100 mM NaCl, 5 mM EDTA and 1% Triton X-100. A cocktail of protease inhibitors (Complete MINI EDTA-free, protease inhibitor cocktail tablets from Roche) was added fresh to the homogenization buffer every time. After centrifugation (13.000 r.p.m., 5 min, 4ºC), the supernatant was collected; the total concentration of proteins was quantified by Bradford colorimetric assay (after addition of the dye, absorbance at 595 nm is measured in a spectrophotometer. The total concentration of proteins in every sample is calculated by comparison with the values of absorbance obtained for different dilutions of BSA (bovine serum albumin) prepared at known concentrations).

2.4.2 Protein electrophoresis and Western-blot.

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) (SDS-PAGE) was used to separate proteins contained in complex mixtures. Protein extracts were boiled for 5 minutes after the addition of a loading buffer containing urea (5x loading buffer: 120 mM Tris-HCl, pH 6.8; 4% SDS, 4M urea, 20% glycerol, 5% β-mercaptoethanol), and then loaded into polyacrylamide gels of different concentrations, according to the specific molecular weight of the proteins of interest. In general, 6% polyacrylamide gels were needed to visualize MAP1B-HC (apparent molecular weight 243 KDa, Hammarback et al. 1991), and 10% polyacrylamide gels were used to analyze MAP1B-LC (apparent molecular weight 27 KDa, Hammarback et al. 1991), for example.

Proteins were then electro-transferred to poly(vinylidene fluoride) (PVDF) membranes (at 0.05 mA over-night at room temperature, or at 0.33 mA at 4ºC for 3 hours). The efficiency of the process was verified by Red Ponceau staining of the membranes. Afterwards, membranes were blocked in blocking solution (5% dried non-fat milk in TBS-0.1% Tween-20(v/v)) for 1 hour at room temperature. The primary antibodies and secondary antibodies (tagged to peroxidase, HRP) used for immunodetection were also diluted in blocking solution. The incubation with the

primary antibody was maintained for 2 hours at room temperature or over-night at 4ºC, and the incubation with the secondary antibody for just 1 hour at room temperature; between both, TBS-0.1% Tween-20 solution was used for washing. The ECL system permitted the visualization of immunoreactive proteins.

2.4.3 Co-immunoprecipitation.

Co-immunoprecipitation is a common technique used to identify physiologically relevant protein-protein interactions. It is based on the use of specific antibodies against a target protein to capture and purify the primary target as well as other macromolecules that are bound to the target by native interactions in the sample solution. These protein complexes can then be analyzed to identify new binding partners, binding affinities, the kinetics of binding and the function of the target protein.

In this work, Protein G Sepharose beads (GE Healthcare) were employed as a matrix to immobilize the primary antibody against the target protein (the primary antibody is bound to the beads through the recognition of its constant fraction). Protein extracts were generated from hippocampal organotypic slices by homogenization in the lysis buffer described above. In this case, only CA1 region was taken for analysis (micro-dissection under the scope was required to cut CA1 region in hippocampal organotypic slices). The aim was to enrich the protein sample in the over-expressed protein (viral infection with Sindbis virus was carried out just in CA1 region to ensure a post-synaptic effect of the manipulation of protein levels).

A fraction of the supernatant was put aside for subsequent SDS-PAGE analysis as “input” (representative of the total amount of the proteins of interest in the initial protein extracts). After equilibration of the beads with lysis buffer, a mixture containing the equilibrated beads, the specific primary antibody against the target protein and the total protein extracts was incubated at 4ºC for 4 hours in a rotating wheel. A mild centrifugation cycle (at 3.000 r.p.m. for 1 minute) followed by several washes with lysis buffer permitted the separation of the fraction of proteins “bound” to beads (ideally composed of the protein complex immobilized together with the beads through interaction of the target protein with the primary antibody) from the “unbound” fraction (containing those proteins unable to interact with the target protein). After addition of loading buffer, all protein samples were analyzed by SDS-PAGE and western blot.

As a specificity control, the primary antibody against the target protein was replaced by an unspecific IgG (produced in the same animal species) in a parallel co-immunoprecipitation. In

the co-immunoprecipitation presented in this work, 3 µg of specific primary antibody or unspecific IgG as a control (1 µg per 100 µg of total protein), were mixed together with 300 µg of total protein and 100 µL of sepharose matrix.

2.4.4 Actin and microtubule co-sedimentation assays.

- **Actin co-sedimentation assay.**

To assess the ability of the deletion mutants of MAP1B-LC to interact with filamentous actin, an actin co-sedimentation assay was performed. A similar procedure has been described elsewhere (Pedrotti and Islam 1996; Noiges et al. 2002). The basic principles of the assay involve an incubation of the protein of interest with filamentous actin, an ultracentrifugation step to pellet filamentous actin and the analysis of the proteins co-sedimenting with it.

Fist, actin protein (purified from rabbit skeletal muscle, from Cytoskeleton) was solubilized in a buffer containing 5 mM Tris pH 8, 0.2 mM $CaCl_2$, 0.2 mM ATP and 0.5 mM DTT, and centrifuged at 20.000 g in a Beckman ultracentrifuge for 10 min at 4ºC to remove any aggregates. Then, actin was polymerized for 1 hour at room temperature by the addition of 50 mM KCl, 1 mM ATP and 2 mM $MgCl_2$. The proteins of interest were extracted from BHK cells previously infected with Sindbis viruses carrying the constructs of interest. After 16 to 24 hours, proteins were extracted in a buffer containing 10 mM Tris-HCl, pH 7.5 and 1 mM DTT by scrapping and sonicating. A first centrifugation to eliminate cellular debris was carried out at 13.000 r.p.m. at 4ºC for 5 minutes, and supernatants were collected. Then, they were subjected to centrifugation at 100.000 g for 20 minutes at 22ºC to pre-clear the mixture of any possible aggregate. After that, protein extracts were incubated with filamentous actin (mass ratio actin: total protein 3:1) for 1 hour at room temperature. The sedimentation of filamentous actin and interacting proteins was driven by ultracentrifugation at 100.000 g for 20 minutes at 22ºC. Finally, equal volumes of supernatants and pellets were analyzed by SDS-PAGE and western blot.

- **Microtubule co-sedimentation assay.**

Very similarly, the ability of the proteins of interest to interact with microtubules was analyzed by a microtubule co-sedimentation assay. This procedure was carried out as described (Campbell and Slep 2011) with modifications. Taxol-stabilized microtubules were prepared by diluting pure tubulin (> 99% pure from bovine brain, from Cytoskeleton) in BRB80 buffer (80 mM K-Pipes, 1 mM $MgCl_2$, 1 mM EGTA, pH to 6.8) supplemented with GTP and DTT (1 mM

each), incubating at 4ºC for 5 minutes, and then warming at 37ºC and introducing taxol stepwise to reach a final concentration of 20 µM.

Also in this case, BHK cells were used to produce large amounts of recombinant proteins. They were infected with Sindbis viruses carrying the constructs of interest, and after 16 to 24 hours, proteins were extracted in BRB80 buffer by scrapping and sonicating. After an initial centrifugation step (13.000 r.p.m., 5 minutes, 4ºC), supernatants were subjected to a new centrifugation at 100.000 g at 25ºC for 30 minutes to clarify. Taxol-stabilized microtubules and clarified total protein extracts were mixed (mass ratio 3:1) and incubated at room temperature for 20 minutes. Samples were then layered onto a glycerol cushion, and centrifuged at 100.000 g for 30 minutes at 25ºC. To finish, supernatant and pellet fractions were collected, mixed with protein loading buffer and analyzed by SDS-PAGE and western blot.

A microtubule co-sedimentation assay was also performed to evaluate the potential ability of MAP1B-LC to influence the interaction of GluA2 with microtubules. In this case, infected hippocampal slices were used as the source of the proteins of interest (a micro-dissection of the CA1 subfield was required to enrich the sample in the over-expressed proteins). In addition, three different concentrations of tubulin were assessed to test a possible dose-dependent effect on GluA2 co-sedimentation with assembled microtubules.

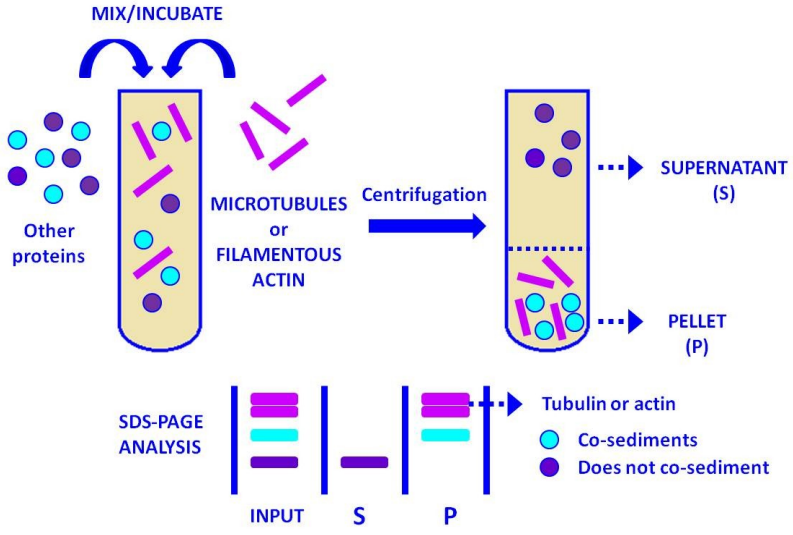


Figure 1. Schematic representation of actin/microtubule co-sedimentation assays. The proteins of interest are mixed with stabilized microtubules or filamentous actin. After incubation, the mixture is centrifuged. Supernatant and pellet fractions are subsequently recovered, and analyzed by SDS-PAGE. Proteins that appear in the supernatant do not sediment with microtubules or filamentous actin; proteins recovered in the pellet sediment thanks to their interaction with microtubules or filamentous actin. This type of assay allows the functional characterization of proteins alleged to bind cellular components that pellet under the application of a centrifugal force.

2.4.5 Pull-down experiments: assessment of Rac1 and RhoA activities.

PAK1 (p21 activated kinase) is the effector protein of Rac1, a small GTPase. The region known as PBD (p21 binding domain) is able to interact with the active form of Rac1 (Rac1-GTP), but its affinity for the GDP-bound form of Rac1 is negligible. That is the reason why PBD is widely used in assays evaluating the activity of Rac1 GTPase. In parallel, Rhotekin is a protein able to interact with the active form of RhoA GTPase, GTP-RhoA, through its RhoA-Binding Domain (RBD).

The Rac1-binding domain of PAK1 (PBD) and the RhoA-binding domain of Rhotekin (RBD) were expressed in Escherichia coli as fusion proteins with glutathione S-transferase (GST). The bacteria culture was grown upon IPTG induction. The purification of GST-fused proteins was then carried out by generating a bacterial lysate (in "buffer A": 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1% triton and protease inhibitors from Roche) with a French press, prior to incubation with GST sepharose beads for 1 hour at 4ºC in a rotating wheel and subsequent washing (wash buffer identical to buffer A, except for the concentration of triton: 0.5% triton).

Protein extracts were generated from hippocampal organotypic slices over-expressing the proteins of interest via Sindbis virus infection. Only CA1 region was taken for analysis to enrich the protein sample in the over-expressed proteins. They were homogenized in a specific lysis buffer (0.1% SDS, 1% triton, 10 mM $MgCl_2$, 0.5 mM DTT and protease inhibitors in TBS) and centrifuged at 13.000 r.p.m., at 4ºC, for 2 minutes. Supernatants were then incubated with PBD or RBD-coated GST sepharose beads for 25 minutes at 4ºC in a rotating wheel. A fraction of supernatants was used to generate the "inputs" (representative of the total amount of the proteins of interest in the initial lysates). After the incubation period, "bounds" (in this case, active Rac1 or RhoA) and "unbounds" (inactive Rac1 or RhoA) fractions were separated. Loading buffer was added to inputs, bounds and unbounds before proceeding to SDS-PAGE in 12% polyacrylamide gels. Immunodetection on PVDF membranes was performed using specific antibodies against either Rac1 or RhoA GTPases.

2.7. Pharmacological treatments.

When needed, the following pharmacological treatments were applied:

2.7.1. Chemical induction of long-term potentiation (LTP) in hippocampal organotypic slices.

To biochemically analyze the effects of LTP induction on protein expression, the inserts containing the hippocampal organotypic slices in culture (6-8 days in vitro, DIV) were first placed on submersion-type holding chambers containing artificial cerebro-spinal fluid, ACSF (119 mM NaCl, 2.5 mM KCl, 1 mM NaH_2PO_4, 11 mM D-glucose, 26 mM $NaHCO_3$, 4 mM $MgCl_2$, 4 mM $CaCl_2$, pH 7.4) gassed with carbogen (95% O_2, 5% CO_2), for 5 minutes. Then, inserts were transferred to the LTP-inducing solution, consisting of ACSF without $MgCl_2$, and 0.1 µM rolipram, 50 µM forskolin and 100 µM picrotoxin, for 15 minutes at room temperature. Rolipram is a phosphodiesterase inhibitor and forskolin acts as a powerful activator of adenylate cyclase. Together, they contribute to increase the concentration of cyclic adenosine monophosphate inside the cell, which has been proved to induce long-term potentiation in hippocampal slices (Slack and Pockett 1991; Barad et al. 1998; Otmakhov et al. 2004). Picrotoxin is an antagonist of $GABA_A$ receptors; its addition to the mixture of forskolin and rolipram seems to facilitate the induction of LTP.

After the 15-minute incubation, some slices were immediately collected and homogenized in cold lysis buffer; others inserts were placed back on holding chambers containing normal ACSF for recovery. After variable periods of time, those slices were homogenized, too. To finish, total extracts were used for protein analysis via SDS-PAGE and western blot.

The chemical induction of LTP (using the same cocktail of drugs) was also employed when monitoring changes in the mobility of MAP1B-LC-Dendra by live imaging techniques. Hippocampal slices were placed in a live imaging chamber and perfused with ACSF gassed with carbogen (see below). For LTP induction, the ACSF containing rolipram, forskolin and picrotoxin was let flow throughout the perfusion system for 15 minutes. Afterwards, it was replaced by normal ACSF.

2.7.2. Vinblastine.

Vinblastine is a depolymerizing agent acting on microtubules. It was used to analyze possible changes in the sub-cellular distribution of over-expressed MAP1B-LC-GFP in CA1 neurons as a consequence of microtubule depolymerization.

First, hippocampal slices previously infected with Sindbis virus to over-express MAP1B-LC-GFP were treated for 3 hours with vinblastine at 10 µM. The drug was added to the culture

medium; during treatment, hippocampal slices were maintained at normal culture conditions (in an incubator at 35.5ºC, 5% CO_2). Afterwards, the treated hippocampal slices were fixed in 4% PFA, 4% sucrose in PBS over-night at 4ºC, and a typical immunofluorescence experiment was performed (see below).

2.7.3. Induction of mGluR-dependent LTD in organotypic hippocampal slices.

mGluR-dependent LTD in hippocampal slices was assessed by electrophysiology. To induce depression, (R,S)-3,5-dihydroxyphenylglycine (DHPG) was added at 50 µM (Huber et al. 2001) to the ACSF filling in the perfusion system. A 5-minute washout of the drug with normal ACSF followed.

2.7.4. Chemical induction of NMDAR-dependent LTD in hippocampal organotypic slices.

The brief application of NMDA at 20 µM has been described to induce NMDAR-dependent LTD in hippocampal slices (Lee et al. 1998). The chemical induction of LTD was used in the present work when analyzing the mobility of recombinant MAP1B-LC in CA1 neurons by live imaging.

The hippocampal slices in the live imaging chamber were perfused with ACSF gassed with carbogen. To induce LTD, NMDA was added at 20 µM to the perfusion solution in contact with the slices, and let fill in the system for 5 minutes. Then, the ACSF containing NMDA was replaced by normal ACSF (washout of the drug).

2.6. Electrophysiology.

Electrophysiology is the technique that allows the on-line recording of electrical activity in neurons. In this work, the properties of the CA3-CA1 hippocampal synapse were studied. Schaffer collaterals (axon collaterals given off by CA3 pyramidal cells) were stimulated, and subsequent electrical responses were recorded in CA1 neurons.

All the electrophysiological recordings compiled in this work were performed in whole-cell, patch clamp configuration, and most of them, in voltage clamp configuration. The patch clamp configuration allows the establishment of a direct contact between the recording microelectrode placed inside a glass pipette, and the cytoplasm of the cell whose electrical activity is being registered. The glass pipette is filled in with “internal solution”, a solution matching the ionic composition of the cytoplasm (115 mM $CsMeSO_3$, 20 mM CsCl, 10 mM

HEPES, 2.5 mM $MgCl_2$, 4 mM Na_2-ATP, 0.4 mM Na-GTP, 10 mM Na-phosphocreatine, 0.6 mM EGTA). The voltage clamp technique is based on the measurement of an electrical current while maintaining the membrane potential of the cell at a constant value (the voltage is "clamped").

A typical electrophysiological setup is composed of the following elements: a plastic chamber that holds the biological sample and is connected to a perfusion system to maintain physiological conditions. A microscope, usually equipped with a mercury lamp so that the fluorescence from the cells under study can be visualized. Micromanipulators, which are used to precisely locate two types of electrodes, stimulating electrodes and recording electrodes, on the tissue. A recording electrode consists of a silver filament introduced inside a glass pipette whose resistance varies between 4 and 6 MΩ. All the components are localized on an air table that absorbs mechanical vibrations, and inside a Faraday cage to block the electrical noise. A Multiclamp 700 A/B amplifier (Molecular Devices) and a digitizer are needed to accurately measure the electrical currents recorded from neurons in the range of picoamps (processing done with pClamp software).

In the experiments listed below, organotypic hippocampal slices placed on a plastic chamber were maintained alive by filling in the perfusion system with oxygenated ACSF kept at 29ºC. 2-chloroadenosine (adenosine receptor agonist, 200 µM) and picrotoxin (antagonist of $GABA_A$ receptors, 100 µM) were added to the ACSF before starting recordings. Both drugs contribute to decrease the excitability and spontaneous activity of neurons in culture.

2.6.1. Recording of basal transmission.

In these experiments, whole-cell recordings were simultaneously obtained from nearby pairs of CA1 pyramidal neurons, one over-expressing the protein (or shRNA) of interest, and the other one as a control neuron ("pair recordings"). Because only CA1 cells (and not CA3 cells) were infected or transfected, this configuration ensured that recombinant proteins were always expressed exclusively in the post-synaptic cell. Synaptic responses were elicited with bipolar electrodes using single-voltage pulses (200 ms, up to 25 V).

Synaptic AMPAR-mediated responses were measured by setting the membrane potential of the cell at -60 mV, and NMDAR-mediated responses at +40 mV, at a latency when AMPAR responses had fully decayed (100 ms).

2.6.2. Peptide pep2m.

Peptide 2m (pep2m) is a decapeptide corresponding to the NSF-binding domain of the GluA2 subunit of AMPARs. It has been described to produce a marked, progressive decrease of AMPAR-mediated synaptic transmission when loaded into CA1 hippocampal neurons (Nishimune et al. 1998; Song et al. 1998; Luscher et al. 1999; Noel et al. 1999).

Peptide 2m was dissolved at 1 mM in internal solution together with the protease inhibitors bestatin (100 µM) and leupeptin (10 µM). The addition of the peptide to the internal solution permitted its constant infusion into the cell once the seal established between the cellular membrane and the glass pipette was opened. AMPAR-dependent synaptic currents were recorded as described, considering the first two minutes of recording as the baseline value for analysis.

2.6.3. Rectification experiments.

For rectification studies, AMPAR responses were recorded at −60 mV and +40 mV in the presence of 100 µM D,L-2-amino-5-phosphonovaleric acid (APV, to block NMDA receptors) in the perfusion solution, and 100 µM spermine (a voltage-dependent channel blocker of GluA2-lacking AMPA receptors) in the internal solution. The rectification index was calculated as the ratio between the AMPAR synaptic responses at -60 mV and at +40 mV.

2.6.4. Synaptic plasticity.

To assess the effects on synaptic plasticity of the manipulation of MAP1B levels in CA1 pyramidal neurons, different stimulating protocols were applied:

- **NMDAR-dependent LTD** was induced by pairing low-frequency pre-synaptic stimulation (300 or 500 pulses, as indicated, at 1 Hz) with moderate post-synaptic depolarization (at -40 mV).
- **mGluR-dependent LTD:** the induction of mGluR-dependent LTD was carried out by adding (R,S)-3,5-DHPG at 50 µM to circulating ACSF. The treatment with the drug was maintained for 5 minutes; then, a washout period of 5 minutes was required. To evaluate the ability of the drug to induce the generation of action potentials in the post-synaptic cell, voltage clamp mode was switched to current clamp configuration during the application of the drug and the 5-minute washout. The internal solution in the patch pipette was, hence, specific for current clamp configuration (115 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, 4 mM Na_2-ATP, 0.3 mM Na_3-GTP, pH 7.25).

- **LTP** was induced with a pairing protocol, by stimulating Schaffer collateral fibers at 3 Hz (300 pulses) while depolarizing the post-synaptic cell at 0 mV.

2.7. Fluorescence microscopy.

2.7.1. Immunofluorescence: hippocampal organotypic slices.

Hippocampal slices were infected with Sindbis virus to over-express the protein of interest. 16 to 24 hours later, they were fixed over-night at 4ºC in fixation solution for slices (4% PFA-, 4% sucrose in PBS, pH 7.4). After extensive washing with PBS, they were blocked in blocking solution (3% BSA, 2% horse serum, 0.3% triton in PBS) for 1 hour at room temperature. The incubation with the primary antibody (diluted in blocking solution) was performed over-night at 4ºC. After washing repeatedly with PBS, the slices were incubated with the secondary antibody (also diluted in blocking solution) for 1 hour at room temperature. To mount the samples after washing off the secondary antibody, Prolong Gold antifade reagent was employed.

2.7.2. Immunofluorescence: hippocampal primary cultures.

To analyze MAP1B-LC-GFP co-localization with post-translationally modified tubulin, and to perform the evaluation of GRIP1 distribution along dendrites in primary neurons, fixation with methanol was required. Briefly, hippocampal primary neurons plated on coverslips were infected with Sindbis virus to over-express the protein of interest. After 16 to 24 hours, they were washed with TBS and fixed carefully in methanol supplemented with 1 mM EGTA for 20 minutes at -20ºC. The subsequent washes were performed with TBS-0.1% triton; an additional step of permeabilization with TBS-0.5% triton was required. A solution composed of 3% BSA, 0.1% triton and 0.1% sodium azide in TBS was used as blocking solution. The blocking step lasted just 10 minutes at room temperature. The primary antibody (diluted in blocking solution) was incubated with the sample for 1 hour at room temperature; the incubation with the secondary antibody (after washing) also lasted 1 hour. Samples were then washed with milli Q water, dried and mounted on microscope slides with Prolong Gold.

To perform GluA2/GluA1 surface versus total staining experiments and evaluation of MAP1B down-regulation with a specific shRNA, fixation with PFA was considered the method of choice. As with the hippocampal slices, the fixation step was carried out with a solution consisting of 4% PFA, 4% sucrose in PBS. Only 20 minutes of fixation at room temperature were required in the case of primary neurons. To label only surface receptors, the blocking

step was performed primarily with a solution (named PGT) lacking triton (0.22% fish gelatin in PBS). After blocking, primary and secondary antibodies (diluted in PGT) were incubated with the sample for 1 hour at room temperature each (both incubations were separated by extensive washing with PBS). The primary antibodies selected for this step of the procedure were the ones directed against the N-terminal region of GluA2/GluA1 subunits. Then, a step of permeabilization with triton followed (PGT supplemented with 0.1% triton, for at least 30 minutes at room temperature). The primary antibodies directed against the C-terminal domain of GluA2/GluA1 subunits (to label the whole population of receptors, not just those located on the surface) were incubated with the sample for 1 hour at room temperature, and a new incubation with the corresponding secondary antibodies was performed afterwards. Once rinsed with milli Q water and dried, the coverslips were mounted on microscope slides with Prolong Gold.

2.7.3. Confocal fluorescence imaging on fixed tissue/cultures.

- **Dendritic spine quantification.**

To perform a complete analysis of dendritic spine morphology and density in MAP1B-LC-GFP over-expressing CA1 neurons compared to uninfected, control neurons, organotypic hippocampal slices were first infected with Sindbis virus. After 16 to 24 hours, uninfected and infected neurons were filled in, in parallel, with biocytin (an intracellular marker that allows the visualization of the complete morphology of the cell). To do so, the electrophysiology setup described above was required. The patch clamp technique was employed to target the cell of interest, and establish a direct contact between its cytoplasm and the internal solution supplemented with biocytin contained in the patch pipette. Once the seal membrane-patch pipette was opened, biocytin could flow into the cell; this flow was maintained for a period of 10 minutes, approximately. Immediately afterwards, slices were fixed (4% PFA, 4% sucrose in PBS) over-night at 4ºC, and a typical immunofluorescence experiment as the one previously described was performed. Streptavidin (that recognizes biocytin) fused to a fluorescent tag was added to the solution containing the anti-GFP primary antibody.

A confocal microscope LSM510 coupled to an inverted microscope Axiovert200 M (Zeiss) was used for image acquisition. Images were acquired using a 63x oil immersion objective (zoom 3x), at a resolution of 1024 x 1024 pixels and setting the pinhole value at 0.8 µm. To image the whole apical dendrite, several stacks (at 0.14 µm) were acquired. For quantification, the maximum intensity projection of stacks was used.

Images were processed for deconvolution with Huygens software. Volume Integration and Alignment System (Vias) software was used to reconstruct a whole primary apical dendrite per neuron. Quantification of head diameter and length of dendritic spines, as well as number of dendritic spines per micron along the selected apical dendrite, was carried out with NeuronStudio software.

- **Spine/dendrite ratio for GluA2 and GluA1.**

RFP-GluA2 was co-expressed with MAP1B-LC-GFP (or GFP as a control) in CA1 neurons by biolistic transfection. The same technique was employed to co-express GFP-GluA1-ires-tCamKII with either MAP1B-LC-mCherry or mCherry alone. A typical immunofluorescence procedure on hippocampal organotypic slices was then carried out.

A multiphoton microscope was used for image acquisition (confocal and multiphoton microscopes LSM710 and LSM510 coupled to an inverted microscope AxioObserver and a vertical microscope AxioImager M1, respectively, from Zeiss). Images were acquired using a 63x oil immersion objective. Maximum intensity projections were used for analysis. The quantification was performed by measuring fluorescence intensity in dendritic spines, fluorescence intensity in the adjacent dendritic branch, and calculating a ratio.

The same procedure was applied to evaluate GFP-GluA2 presence in spines of CA1 neurons in hippocampal organotypic slices prepared from wild type mice (MAP1B +/+) or heterozygous animals for MAP1B (MAP1B +/-). To over-express GFP-GluA2, organotypic slices (DIV 5-7) were infected with Sindbis virus.

2.7.4. Epifluorescence imaging on fixed primary cultures.

- **GluA2/GluA1 surface staining versus GluA2/GluA1 total staining. Quantification of GRIP1 staining.**

For both experiments, images were acquired with an inverted microscope Axiovert200 coupled to a CCD camera (Zeiss), using the 63x oil immersion objective.

For GluA2/GluA1 surface staining versus GluA2/GluA1 total staining, the analysis was carried out as follows. Using the tool “segmented line” from Image J, dendrites were delineated in the green channel (corresponding to GFP staining). Having transformed lines into areas, the mean fluorescence intensity of each specified area was calculated, in the channel corresponding to surface staining and in the channel corresponding to total staining. Three dendrites per neuron

were quantified. Mean intensity values for surface and total staining, and ratio between them, were compared among cells.

A similar processing was applied for the analysis of GRIP1 distribution in primary neurons over-expressing MAP1B-LC-GFP (or GFP as a control). Dendrites were delineated in the green channel using the "segmented line" tool of Image J. In this case, only the main prominent dendrite coming out from the soma of the cell was considered for analysis. Mean fluorescence intensity in the area occupied by the delineated dendrite (50 µm) was then measured in the channel corresponding to GRIP1 staining. The mean intensity in the soma of the cell was also calculated, and total values of fluorescence in soma and dendrite, as well as the ratio dendrite/soma, were compared among different neurons. For cluster analysis, the "Log3D" plugin from Image J (Sage et al. 2005) was applied to image selections corresponding to the delineated dendrites. This plugin facilitates the definition of clusters. Then, the same intensity threshold was applied for every cell, and the thresholded selection was imported into the original 32-bit image of the dendrite to calculate the number of clusters per 50 µm of dendrite, the total fluorescence intensity and the area of each cluster (using the "analyze particles" command of Image J).

2.7.4. Fluorescence imaging on live tissue: multiphoton.

- **FRAP experiments on dendrites and spines.**

FRAP (Fluorescence Recovery After Photobleaching) experiments were performed in a multiphoton microscope. Live hippocampal slices were placed in an imaging chamber connected to a perfusion system filled in with ACSF gassed with carbogen. The temperature of the circulating ACSF was kept constant (37ºC) thanks to an external heating system.

During FRAP experiments, a specific region of a CA1 neuron over-expressing the protein of interest is "bleached". This means that the laser used to excite the fluorophore (IR laser, 910 nm) is flashed at this specific region at a high intensity so that the dye is rendered unable to fluoresce again. The idea behind this method is to use FRAP to measure the mobility of the protein of interest inside the neuron. If the protein fluorescently tagged is indeed mobile, the fluorescence in the bleached area will be recovered over time as a consequence of non-bleached molecules moving around.

In the FRAP experiments presented in this work, the bleaching flash of the laser was applied on dendrites and/or dendritic spines of CA1 neurons over-expressing the proteins of interest. To

compare rates of recovery (for example, to analyze the mobility in dendrites of recombinant GluA2/GluA1 in the presence or absence of MAP1B-LC-GFP), the bleached area on the dendrite was always of the same size. The quantification consisted of the measurement of fluorescence intensity in the bleached area, before (normalized to 1) and at different time points after the bleaching event (value normalized to zero immediately after the bleaching). Normalization to an adjacent stretch of non-bleached dendrite was required to compensate for ongoing photobleaching during image acquisition.

- **Photoactivation experiments on dendrites.**

Photoactivation experiments represent a complementary approach to FRAP experiments to study the mobility of a given protein inside the cell. In this case, the protein of interest is tagged to a photoactivatable fluorophore (dendra2). The photoactivatable fluorophore emits normally in the green channel; when it is irradiated with a high intensity light, green fluorescence is converted into red fluorescence. From that moment on, the movement of a discreet subcellular protein pool can be monitored in the red channel.

In the set of experiments presented in this thesis, both MAP1B-LC and tubulin were tagged to dendra2. The photoactivation of the protein of interest was carried out by illuminating a specific region of a dendrite of a CA1 neuron with 405 nm laser, at 10% intensity for MAP1B-LC-dendra and 50% intensity for Dendra-tubulin. IR laser (910 nm) was used for image acquisition before and after photoactivation. Red fluorescence in the illuminated spot was quantified before photoactivation (zero fluorescence) and at different time points afterwards (the fluorescence intensity just after photoactivation was normalized to 1). Photoactivation experiments were also performed in a multiphoton microscope, using the same setup described for FRAP experiments.

2.7.5. Fluorescence imaging on live tissue: epifluorescence.

The inverted microscope Axiovert200 coupled to a CCD camera was the one chosen to image the movement of transferrin receptor (TfR) clusters along dendrites in live hippocampal neurons.

Primary neurons were either co-transfected with MAP1B-LC-GFP (or GFP as a control) and TfR-mCherry with Lipofectamine 2000, or first infected with a lentivirus producing a shRNA against MAP1B (or scrambled shRNA as a control), and 8 days later, transfected with Lipofectamine 2000 to over-express TfR-GFP. Live imaging was performed 48 hours after transfection with

Lipofectamine 2000 in both cases. At the microscope, primary neurons were maintained alive thanks to the incubation system of the microscope (temperature set at 35.5ºC) and the specific buffer filling in the chamber used for imaging (146 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgSO_4$, 1.6 mM $NaHCO_3$, 0.15 mM NaH_2PO_4, 8 mM D-glucose, 20 mM HEPES, pH 7.4). 63x oil immersion objective was employed for acquisition. Images of TfR clusters were acquired every 2-3 seconds for a period of 1 minute (the exposure time of the camera was adjusted according to the intensity of fluorescence of TfR clusters in every cell under study).

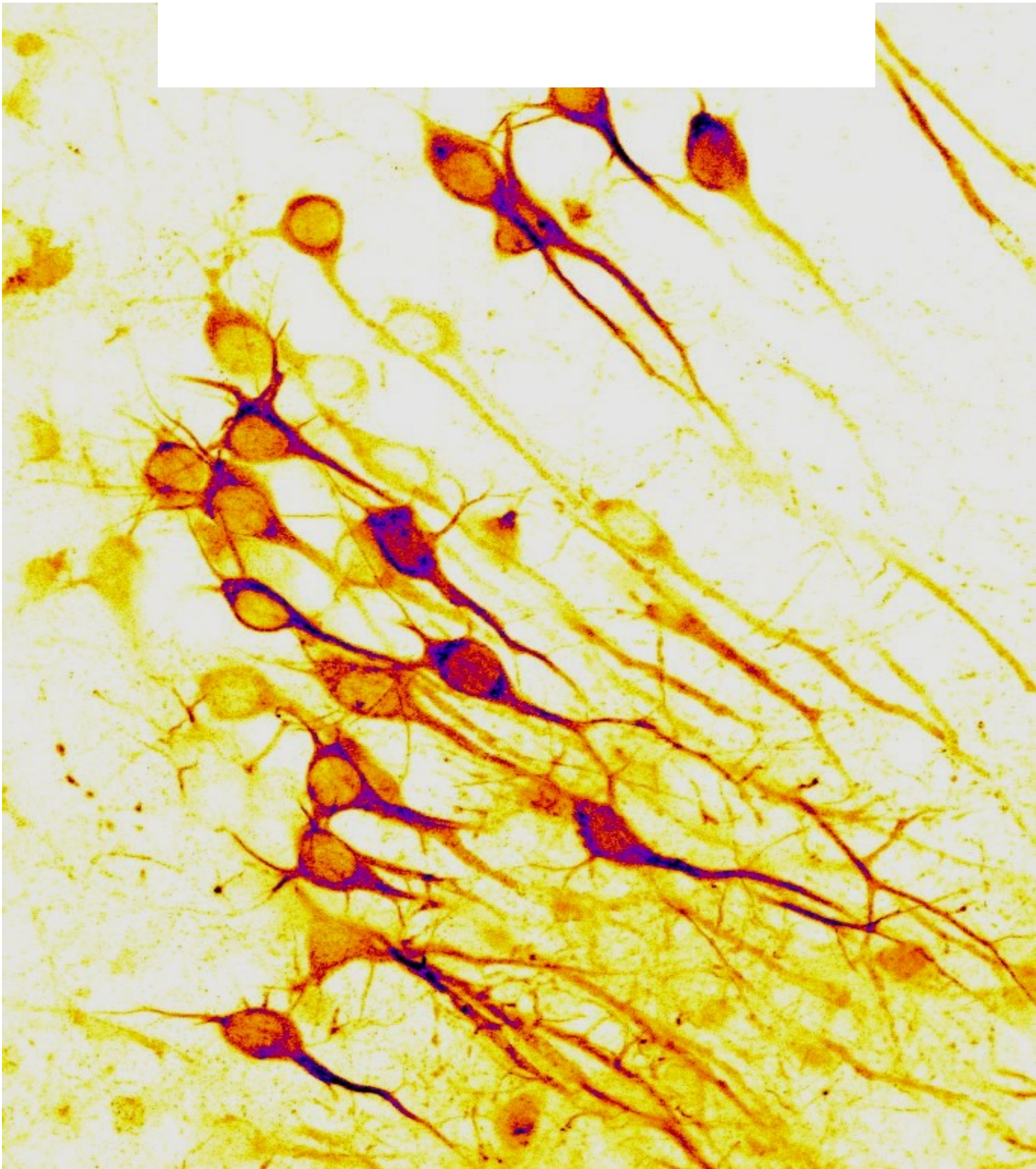
For analysis, kymographs were obtained from time-lapse images with Image J software ("MultipleKymograph" plugin, submitted by J. Rietdorf and A. Seitz, European Molecular Biology Laboratory, Heidelberg, Germany). Kymographs are a way to represent a dynamic process in a single image. They result from the graphical representation of spatial position (x axis) over time (y axis). This way, each cluster in the original set of images is converted into a line in the kymograph, representing how its position in the dendrite changed over time. A moving cluster results in a diagonal line in the kymograph; if a cluster stays in the same position during image acquisition, then it will give rise to a vertical, straight line.

On kymographs, the tracks generated by every moving particle were manually outlined with Image J software. A single track is composed of multiple individual trajectories that we defined as "events of transport". Using the tracks on kymographs, we calculated the instantaneous speed of each event of transport (depending on the space travelled and the time of movement), with the "read velocities from tsp" macro (also available on-line).

2.8. Statistical analysis.

All graphs represent average values ± s.e.m. Statistical differences were calculated according to non-parametric tests. When significant differences were observed, p-values for pairwise comparisons were calculated according to two-tailed Mann-Whitney test (for unpaired data) or Wilcoxon test (for paired data). Comparisons between cumulative distributions were calculated with the two-sample Kolmogorov-Smirnov test.

RESULTS



PART I: modulation of MAP1B levels of expression and its effects on synaptic transmission and plasticity.

The main objective of the present study was to analyze the possible involvement of MAP1B in the processes regulating synaptic strength of glutamatergic neurotransmission in hippocampus. To this aim, we adopted two strategies based on the manipulation of MAP1B protein levels and the study of its consequences in hippocampal neurons:

- After verifying the increase in MAP1B expression during synaptic plasticity, we decided to assess the consequences of MAP1B *gain of function* through over-expression in CA1 hippocampal neurons.

- To complement the previous approach, we also studied MAP1B loss of function via lentiviral-mediated acute down-regulation.

A) MAP1B over-expression.

1. MAP1B expression is up-regulated during the induction of LTP.

A change in the levels of expression of a given protein during the induction or the progression of a specific biological process is a good indication of the potential involvement of the protein in the process under study. Thus, to assess the potential role of MAP1B in regulating the intracellular sorting of AMPARs in hippocampal neurons, we first analyzed changes in the expression of MAP1B light chain and heavy chain during synaptic plasticity, specifically during the chemical induction of LTP in organotypic hippocampal slices. It is worth noting that a translation-dependent increase in the dendritic levels of MAP1B after treatment of cultured neurons with DHPG and NMDA was previously reported (Davidkova and Carroll 2007).

LTP was induced chemically as described (Otmakhov et al. 2004). As shown in figure 1, the expression of both MAP1B-LC and MAP1B-HC increases after the induction of LTP. Therefore, it seems that the activation of the signaling cascades associated with the insertion of AMPARs at synapses during LTP controls also MAP1B expression. This result would suggest a possible involvement of MAP1B in the processes orchestrating the trafficking of AMPARs in CA1 neurons.

The fact that MAP1B expression was enhanced upon the induction of LTP prompted us to study possible changes in the strength of synaptic transmission both in basal conditions and during synaptic plasticity as a result of MAP1B over-expression.

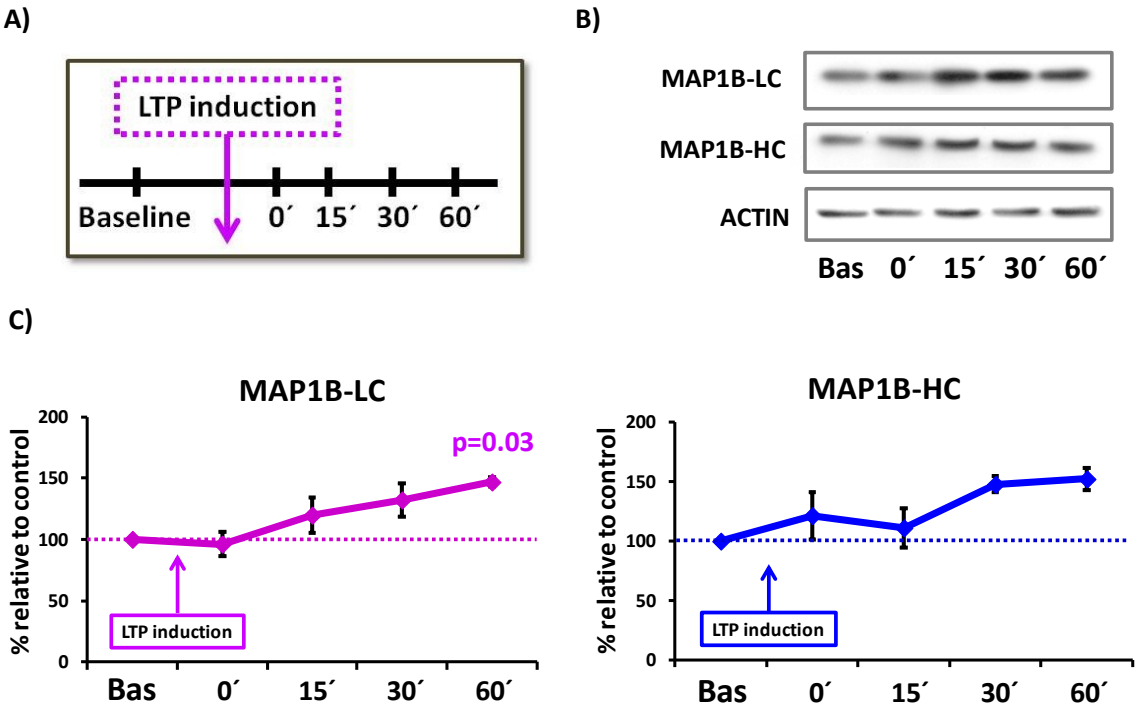


Figure 1. MAP1B expression is regulated by synaptic plasticity. A) Protein levels were assessed in control conditions ("baseline"), just after the chemical induction of LTP ("0 minutes") with 0.1 µM rolipram, 50 µM forskolin and 100 µM picrotoxin, and at different time points during the recovery period in ACSF ("15", "30" and "60 minutes"). **B)** Representative western blots of MAP1B-LC, MAP1B-HC and actin total levels of expression before and after LTP induction. **C)** Quantification of MAP1B-LC (left) and MAP1B-HC (right) levels normalized to the control condition, from experiments as the one shown above (MAP1B-LC, n=6 independent experiments; MAP1B-HC, n=5 independent experiments). "p" corresponds to the statistical significance value comparing "60 minutes" to the control condition calculated with Wilcoxon test. Error bars represent s.e.m.

2. MAP1B-GFP over-expression decreases basal transmission.

To analyze the potential role of the light chain and the heavy chain of MAP1B in regulating synaptic transmission, we decided to over-express both chains of the protein separately in CA1 hippocampal neurons and measure basal transmission using electrophysiological recordings. Thus, we over-expressed either MAP1B-LC-GFP or MAP1B-HC-GFP in CA1 pyramidal neurons and measured AMPAR- and NMDAR-dependent synaptic currents (figure 2).

As shown in figure 2A, MAP1B-LC-GFP over-expression was accompanied by a significant decrease in AMPA currents. On the contrary, NMDAR-dependent transmission was unaltered in infected cells comparing to uninfected, control neurons. When MAP1B-HC-GFP was over-

expressed (figure 2B), both AMPAR- and NMDAR-mediated currents were reduced. Therefore, the over-expression of MAP1B-HC-GFP was generally affecting synaptic transmission mediated by AMPA and NMDA receptors, whereas MAP1B-LC-GFP was specifically acting on AMPA receptors, which encouraged us to further study its mechanism of action.

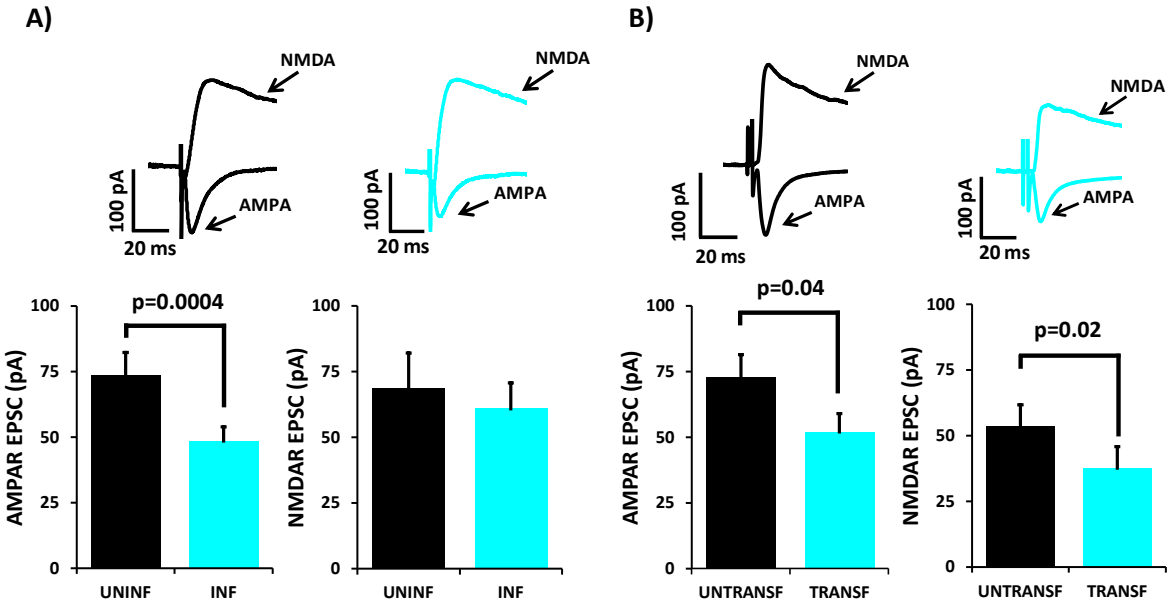


Figure 2. MAP1B-GFP over-expression affects basal transmission. A) Synaptic responses were recorded from pairs of neighboring CA1 neurons over-expressing MAP1B-LC-GFP (infected, INF) and control neurons (uninfected, UNINF), in the presence of picrotoxin at -60 mV for AMPARs (n=42 pairs), and at +40 mV for NMDARs (NMDA responses were collected at a latency of 100 ms; n=29 pairs). **B)** CA1 neurons were biolistically transfected with MAP1B-HC-GFP. Synaptic responses were measured from pairs of neighboring transfected (TRANSF) and untransfected (UNTRANSF) neurons (AMPARs, n=23 pairs; NMDARs, n=14 pairs). "p" corresponds to the statistical significance value calculated by Wilcoxon test. Error bars represent s.e.m.

3. Over-expressed MAP1B-LC-GFP displays a filamentous pattern of distribution probably due to its binding to microtubules.

3.1 Immunofluorescence on fixed hippocampal cultures.

First, we decided to characterize MAP1B-LC-GFP distribution and behavior when over-expressed in CA1 pyramidal neurons. To evaluate the ability of MAP1B-LC-GFP to interact with endogenous MAP1B-HC, we performed a co-immunoprecipitation with a specific antibody raised against MAP1B-HC (figure 3). Endogenous MAP1B-LC was co-immunoprecipitated almost completely with MAP1B-HC. On the contrary, recombinant MAP1B-LC was found mainly in the unbound fraction. This result demonstrated that MAP1B-LC-GFP was not

primarily forming a complex with the heavy chain in CA1 pyramidal neurons when over-expressed.

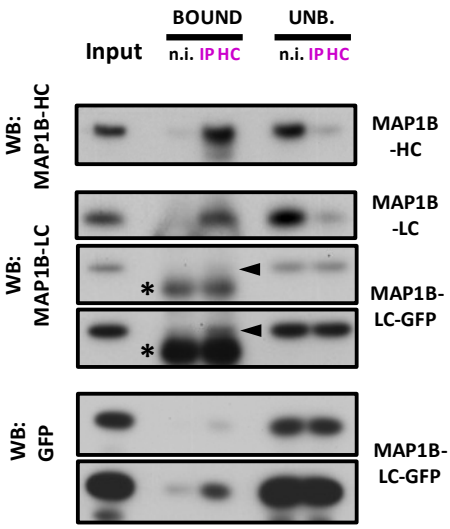


Figure 3. MAP1B-HC immunoprecipitation. Total protein extracts from hippocampal slices were immunoprecipitated with anti-MAP1B-HC ("IP HC") or with a non-immune ("n.i.") antibody. Input, bound fraction and unbound fraction ("UNB.") for each protein of interest are shown. Western blots of immunoprecipitated proteins were performed with antibodies against MAP1B-HC (upper panel); MAP1B-LC (middle panel), showing endogenous and recombinant MAP1B-LC (two different exposures); and GFP (lower panel) (two different exposures). The arrowheads mark the position of the band corresponding to recombinant MAP1B-LC, only visible when the exposure of the film is long enough. The asterisks point to IgG bands unspecifically recognized by the secondary antibody.

Next, we wondered about the ability of MAP1B-LC-GFP to interact with microtubules, as described (Zauner et al. 1992). When over-expressed, MAP1B-LC-GFP appeared clearly filamentous in the cell body and dendrites of CA1 hippocampal neurons, resembling a microtubule-like pattern of distribution (figure 4). A 3-hour treatment with vinblastine (a drug used to depolymerize microtubules) produced a complete loss of the characteristic filamentous pattern of distribution of MAP1B-LC-GFP (figure 5), resulting in a diffuse cytoplasmic localization of the recombinant protein. This result indicates that, being mainly free from MAP1B-HC, over-expressed MAP1B-LC-GFP is probably interacting with microtubules inside CA1 pyramidal neurons.

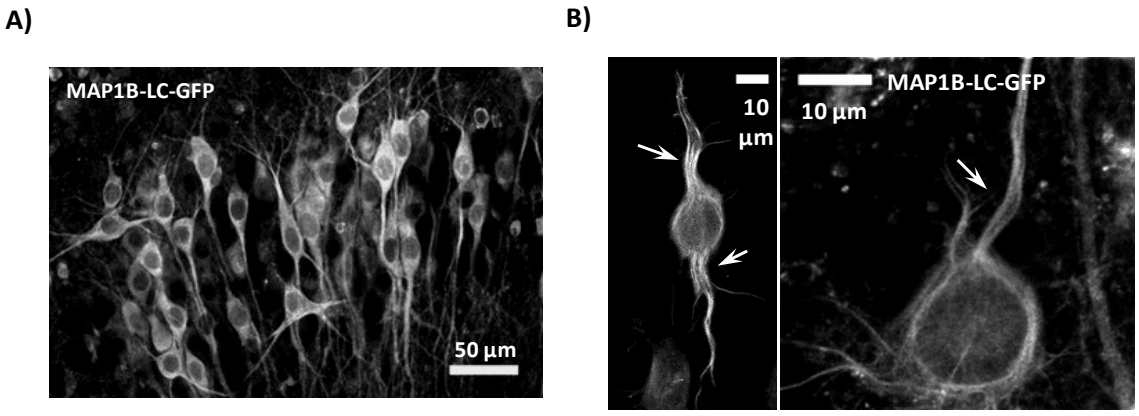


Figure 4. MAP1B-LC-GFP displays a filamentous pattern of distribution. A) Representative confocal image of hippocampal CA1 neurons over-expressing MAP1B-LC-GFP after 24 hours from infection with Sindbis virus. **B)** MAP1B-LC-GFP displays a filamentous pattern of distribution in soma and dendrites of infected CA1 neurons. Arrows indicate marked filaments in dendrites.

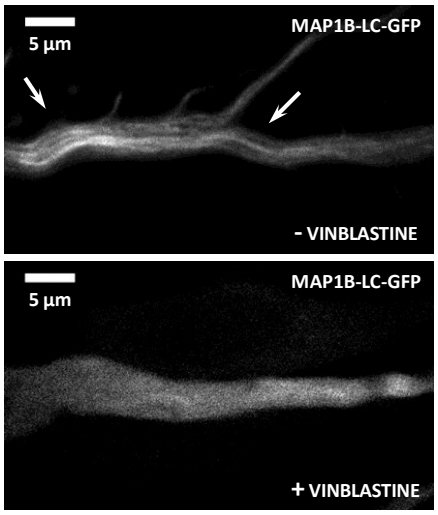


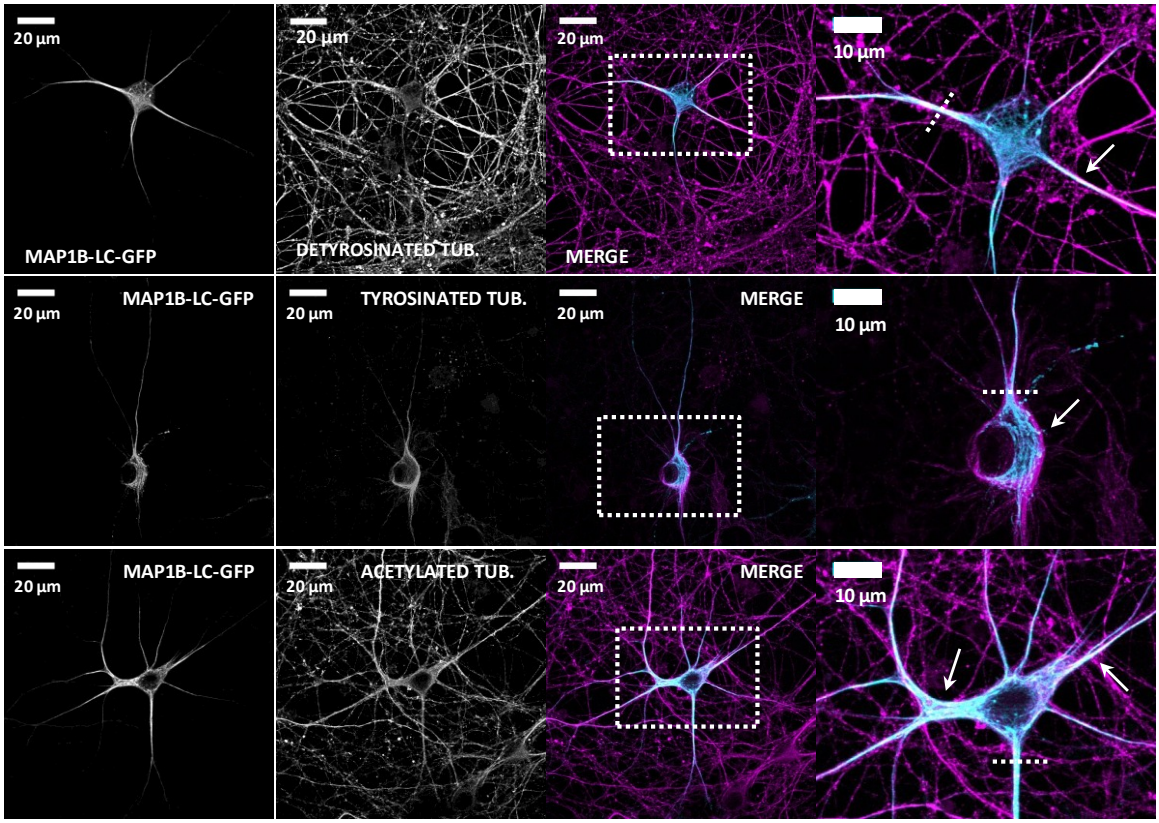
Figure 5. MAP1B-LC-GFP filamentous pattern of distribution is presumably due to its binding to microtubules. A 3-hour treatment with vinblastine (10 µM, microtubule depolymerizing agent) results in a complete loss of typical filamentous appearance (arrows) of MAP1B-LC-GFP in dendrites of CA1 hippocampal neurons.

To note, recombinant MAP1B-LC was hardly visualized in dendritic spines, despite its reported interaction *in vitro* and *in vivo* with filamentous actin (Togel et al. 1998). A similar behavior was previously described for the endogenous MAP1B in cultured hippocampal neurons (Tortosa et al. 2011). MAP1B-LC predominant distribution along dendritic shafts and absence from spines could reflect an increased affinity for microtubules comparing to actin filaments, as suggested elsewhere (Noiges et al. 2002).

To gather further evidence of MAP1B-LC-GFP binding to microtubules, we performed immunofluorescence experiments on primary hippocampal neurons over-expressing the recombinant protein. The immunostaining was performed against three post-translational modifications (PTMs) of tubulin: detyrosinated and acetylated tubulin, associated with stable microtubules (Kreis 1987; Piperno et al. 1987; Schulze et al. 1987), and tyrosinated tubulin, which is considered to be a hallmark of dynamic microtubules (Webster et al. 1987). As expected because of its ability to stabilize microtubules (Pedrotti and Islam 1995; Togel et al. 1998; Noiges et al. 2002), MAP1B-LC-GFP was found to co-localize with stable microtubules (figure 6).

For semi-quantitative analysis, a line was drawn across the dendrites of neurons over-expressing MAP1B-LC-GFP (cyan) and stained against the corresponding PTM of tubulin (magenta). Fluorescence along the dotted line was quantified in both channels to assess the degree of coincidence in the pattern of distribution of the proteins under study. Indeed, line plots showed spatial coincidence of MAP1B-LC-GFP with detyrosinated and acetylated tubulin, and an exclusive pattern with tyrosinated tubulin (figure 6).

A)



B)

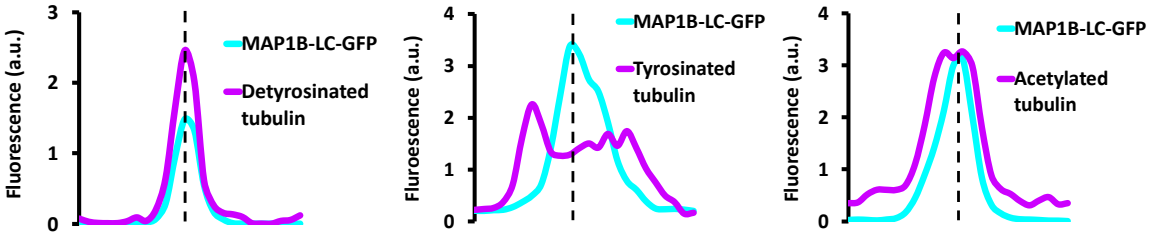


Figure 6. MAP1B-LC-GFP co-localizes with stable microtubules. A) Immunostaining against different post-translational modifications (PTMs) of tubulin (detyrosinated tubulin, tyrosinated tubulin and acetylated tubulin) in MAP1B-LC-GFP over-expressing primary neurons (DIV 19-21). Overlay and higher magnification of insets are shown in the right panels. Note co-localization or exclusion of MAP1B-LC-GFP with each PTM of tubulin (arrows). **B)** Line plots showing quantification of fluorescence intensity across dendrites of neurons over-expressing MAP1B-LC-GFP and stained against detyrosinated, tyrosinated and acetylated tubulin, like the ones shown in (A). A. u., arbitrary units.

3.2 Live imaging of recombinant MAP1B-LC in CA1 hippocampal neurons.

3.2.1 Mobility of recombinant MAP1B-LC in dendrites of CA1 neurons.

To further explore MAP1B-LC-GFP interaction with microtubules, we characterized its mobility inside CA1 pyramidal neurons. Performing FRAP (Fluorescence Recovery After Photobleaching) experiments on dendrites of neurons over-expressing the recombinant protein, we were able

to show (figure 7) that only a 20% fraction of the initial GFP fluorescence was slowly recovered after more than 1 hour from the photobleaching event. This slow recovery profile was coherent with the idea of MAP1B-LC-GFP being mostly anchored inside the cell.

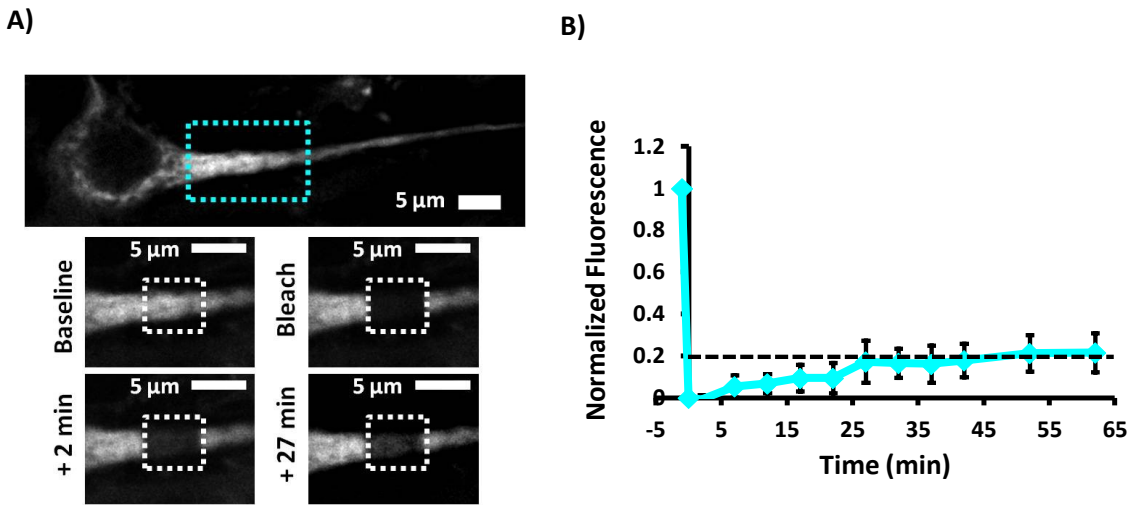


Figure 7. MAP1B-LC-GFP is mainly immobile in dendrites of CA1 neurons. A) Representative images of a FRAP experiment performed on the dendrite of a hippocampal CA1 neuron over-expressing MAP1B-LC-GFP. The inset in the upper picture represents the area amplified in the images below. The dashed rectangle in the amplified pictures corresponds to the area that was bleached during the experiment. "Baseline", before photobleaching; "Bleach", just after the photobleaching event. **B)** Quantification of 3 independent experiments. Error bars represent s.e.m.

To test if microtubules behave as MAP1B-LC-GFP, we cloned a photoactivatable form of MAP1B-LC and of tubulin, and carried out photoactivation experiments in CA1 neurons over-expressing either MAP1B-LC-Dendra or Dendra-tubulin, as shown in figure 8. To note, photoactivation of Dendra-tubulin was performed after 24 hours from the infection with Sindbis virus and only in those dendrites in which Dendra-tubulin appeared as parallel arrays (figure 8A), probably indicating that the recombinant tubulin had already been incorporated into assembled microtubules. MAP1B-LC and tubulin displayed the same dynamic behavior in this set of experiments, with almost over-lapping curves of fluorescence decay over time, which was possibly due to a slow turnover of both structural proteins in dendrites of CA1 neurons. Thus, live imaging experiments allowed us to confirm that MAP1B-LC-GFP/Dendra slow mobility was analogous to that of stable microtubules themselves, strengthening the possibility that MAP1B-LC-GFP/Dendra could be forming a protein complex with microtubules in vivo.

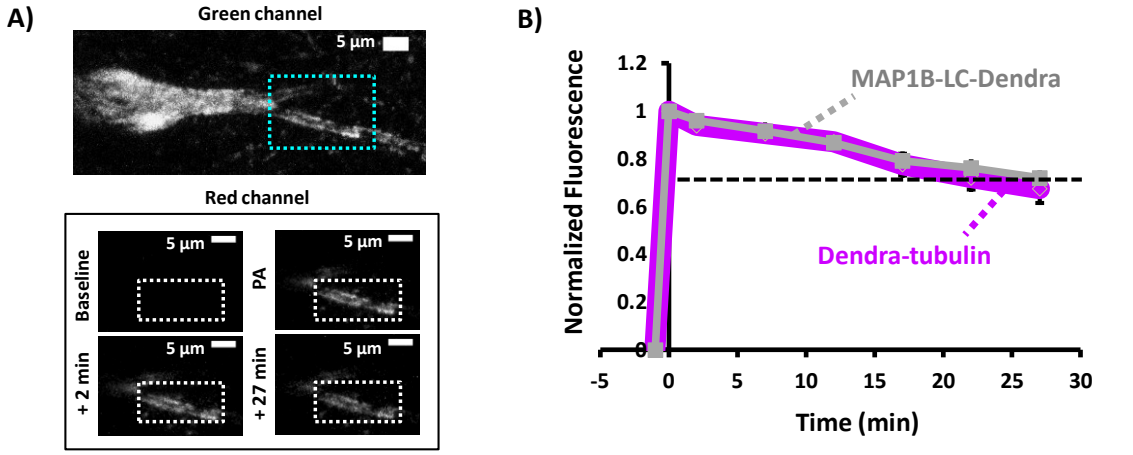


Figure 8. The mobility of Dendra-tubulin is equivalent to that of MAP1B-LC-Dendra in CA1 neurons. A) Representative images of a photoactivation (PA) experiment performed on the dendrite of a hippocampal CA1 neuron over-expressing Dendra-tubulin. The inset in the upper picture represents the area amplified in the images below. The dashed rectangle in the amplified pictures represents the photoactivated region. "Baseline", before PA. "PA", just after performing PA. **B)** Quantification of 4 PA experiments performed on dendrites of CA1 neurons over-expressing Dendra-Tubulin (magenta line). The gray line corresponds to the quantification of PA experiments for MAP1B-LC-Dendra (n=8

Nevertheless, this scenario changed dramatically when synaptic plasticity was induced in hippocampal slices over-expressing MAP1B-LC-GFP. First, we analyzed MAP1B-LC-GFP mobility by FRAP experiments that were performed before, immediately after or 30 minutes after chemically inducing LTD in hippocampal slices. As shown in figure 9A and 9B, the mobility of the recombinant protein increased notably just after the induction of LTD; however, if the FRAP experiment was carried out half an hour after applying the pharmacological treatment, the mobility of MAP1B-LC-GFP was comparable to that measured under basal conditions (figure 9B). This result would point to a transient detachment of MAP1B-LC-GFP from microtubules just after the induction of synaptic plasticity in hippocampal slices.

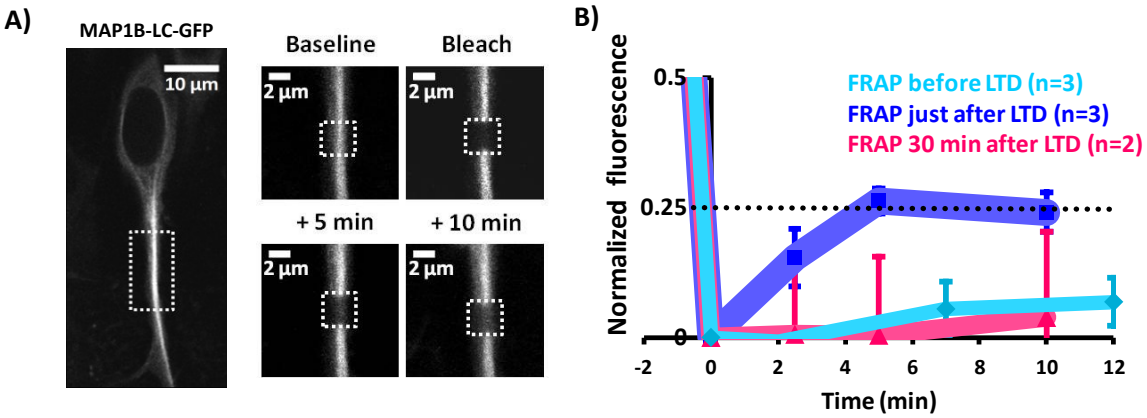


Figure 9. MAP1B-LC-GFP mobilizes transiently during the induction of long-term depression (LTD) (FRAP experiments). A) Left, representative picture of a CA1 neuron over-expressing MAP1B-LC-GFP. The inset in the left picture represents the area amplified in the images on the right. The dashed rectangle in the amplified pictures represents the bleached region (just after LTD, NMDA 20 µM for 5 min). **Right,** representative pictures of the same dendrite before photobleaching ("baseline"), immediately after the photobleaching event ("bleach"), and at several time points during fluorescence recovery. **B)** Quantification of FRAP experiments before (cyan) and just after LTD (blue) or 30 minutes after LTD (magenta). Error bars, s.e.m.

To confirm this hypothesis, we assessed the mobility of recombinant MAP1B-LC after the induction of either LTD or LTP via PA experiments, which provide a complementary approach to FRAP experiments (figure 10.1). As figure 10.2 demonstrates, both LTD and LTP triggered an enhancement in MAP1B-LC-Dendra mobility, manifested as a notorious increase in the slope of the line representing the fluorescence decay over time; however, this enhancement was transient, as the rate of fluorescence decay previous to the induction of synaptic plasticity was quickly recovered after the induction period was over. These data would suggest that the signaling cascades activated upon the induction of synaptic plasticity have the potential to impact on MAP1B-LC behavior and distribution inside the cell.

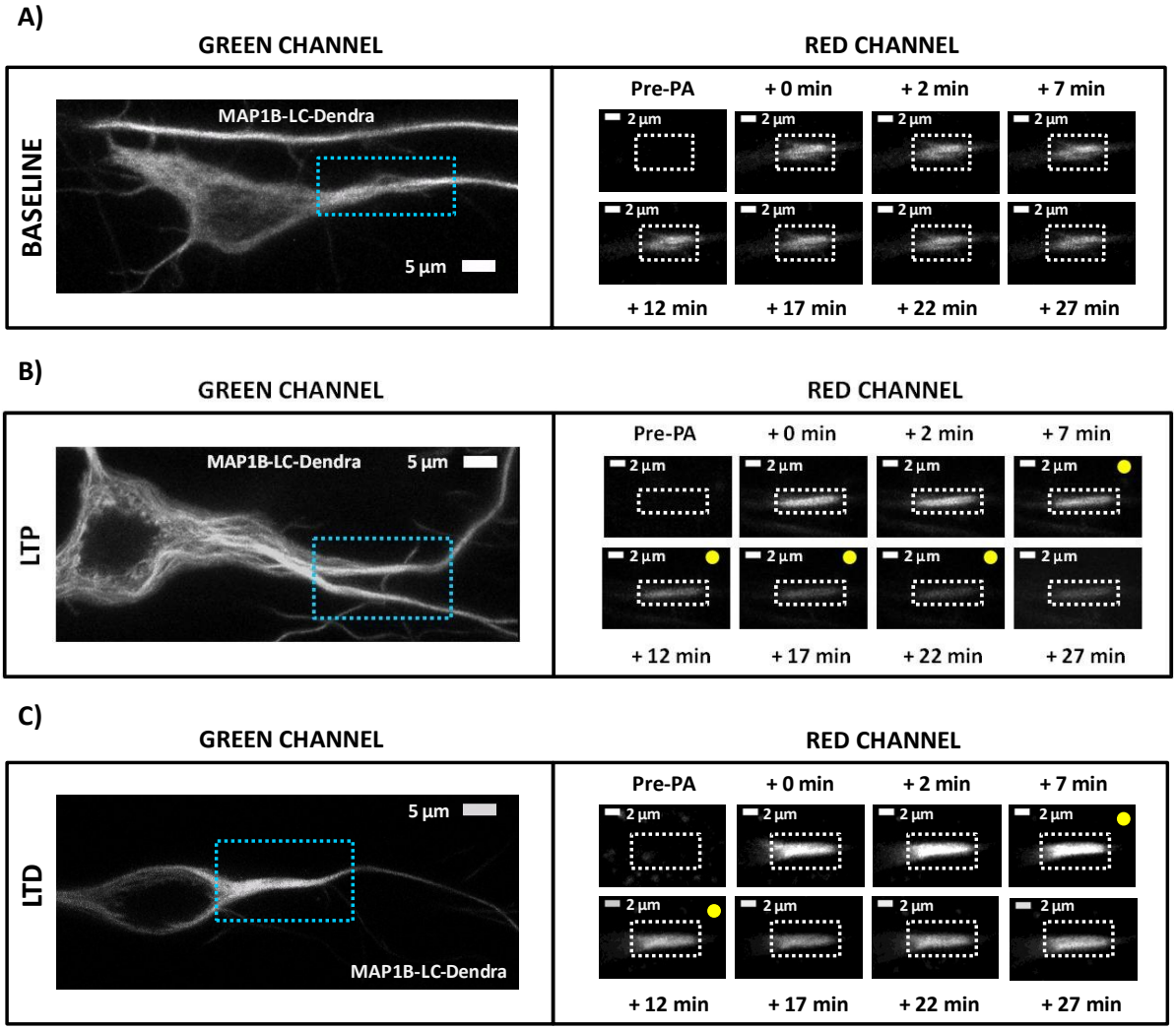


Figure 10.1. MAP1B-LC-Dendra mobilizes transiently during the induction of synaptic plasticity (PA experiments). A) Left, representative picture of a CA1 neuron over-expressing MAP1B-LC-Dendra before undergoing a PA experiment. The inset in the left picture represents the area amplified in the images on the right. The dashed rectangle in the amplified pictures represents the area of interest on which the 405 nm laser was irradiated to proceed to PA. **Right,** representative images of the dendrite of the same neuron before PA ("Pre-PA"), just after undergoing PA ("+0 min"), and at different time points during the experiment. **B)** Same as in A, but for a PA experiment during which LTP was chemically induced (yellow dots). **C)** Same as in A, but for a PA experiment during which LTD was chemically induced (yellow dots).

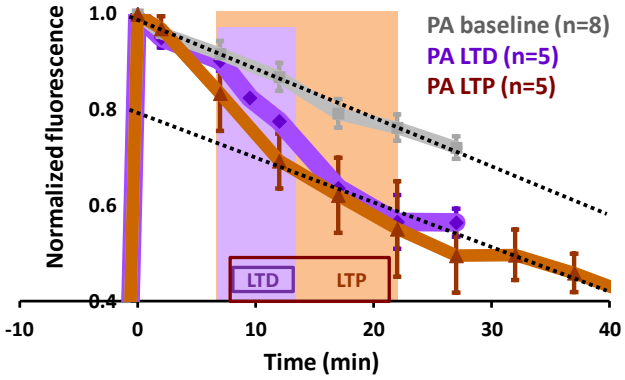


Figure 10.2. MAP1B-LC-Dendra mobilizes transiently during the induction of synaptic plasticity (PA experiments). Quantification of fluorescence intensity after PA on dendrites of CA1 neurons over-expressing MAP1B-LC-Dendra, for experiments like those shown in figure 10.1.

A transient entry of dynamic microtubules from the dendritic shaft into spines was reported in primary hippocampal cultures (Jaworski et al. 2009). This event was shown to be promoted by the stimulation of synaptic NMDARs (Merriam et al. 2011) and synaptic calcium influx (Merriam et al. 2013). As previously mentioned, LTD and LTP have been demonstrated to occur upon a more or less sustained local calcium rise inside the spine, too. Thus, we wanted to test if the transient mobilization of recombinant MAP1B-LC during the induction of synaptic plasticity was an intrinsic property of the protein or could be attributed to some kind of modulation of microtubule dynamics. To do so, we performed PA experiments in dendrites of CA1 pyramidal neurons over-expressing Dendra-tubulin and compared its fluorescence decay profile over time with that of MAP1B-LC-Dendra, previously quantified. Opposite to MAP1B-LC-Dendra, the mobility of Dendra-tubulin was unaffected by the induction of LTD, as evidenced in figure 11. We concluded thereby that the short-term mobilization of recombinant MAP1B-LC during the induction of synaptic plasticity was not due to a hypothetical local reorganization of microtubules but to the potential of synaptic plasticity paradigms to impact on MAP1B-LC functionality.

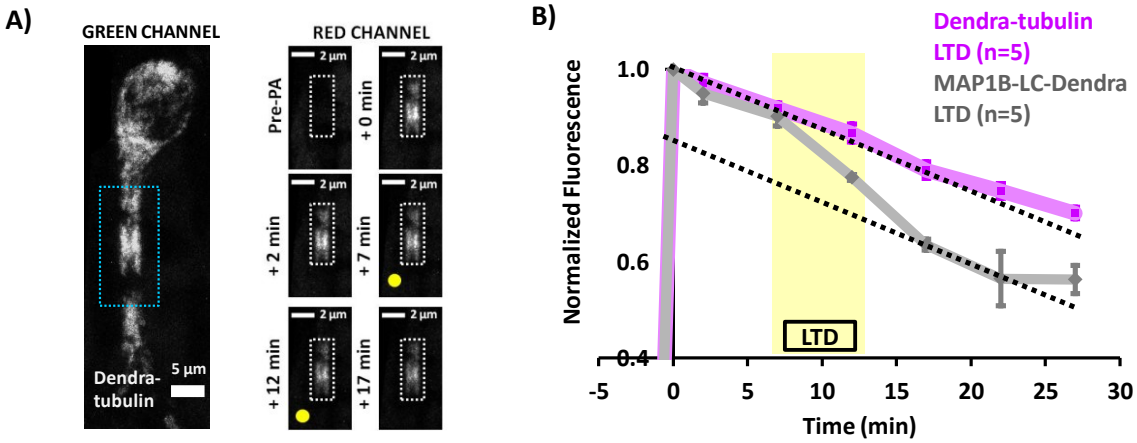


Figure 11. The mobility of Dendra-tubulin does not change upon the induction of LTD. A) Left, representative picture of a neuron over-expressing Dendra-tubulin. The inset corresponds to the area amplified in the pictures on the right**. Right,** representative images of a PA experiment performed on the dendrite of the neuron on the left. LTD (yellow dots) was induced after PA. Dashed rectangles represent the photoactivated area. **B)** Quantification of PA experiments for Dendra-tubulin as the one shown in A, and comparison with PA experiments for MAP1B-LC-Dendra. Error bars, s.e.m.

3.2.2 Mobility of recombinant MAP1B-LC in spines of CA1 neurons.

Next, we wanted to evaluate if the infrequent detection of MAP1B-LC-GFP in dendritic spines in fixed samples could be due to a transient, short-term entry of the recombinant protein into the spine compartment as described for dynamic microtubules. To test this possibility, we imaged MAP1B-LC-GFP in dendritic spines in live CA1 hippocampal neurons for a period of thirty minutes. As shown in figure 12, once in dendritic spines, MAP1B-LC-GFP stayed stably at this location and did not seem to become mobile. Therefore, we concluded that MAP1B-LC-GFP localized rarely at dendritic spines in the absence of stimulation, but if found in any spine, it seemed to be accumulated enduringly in this subcellular compartment, maybe anchored to the actin cytoskeleton.

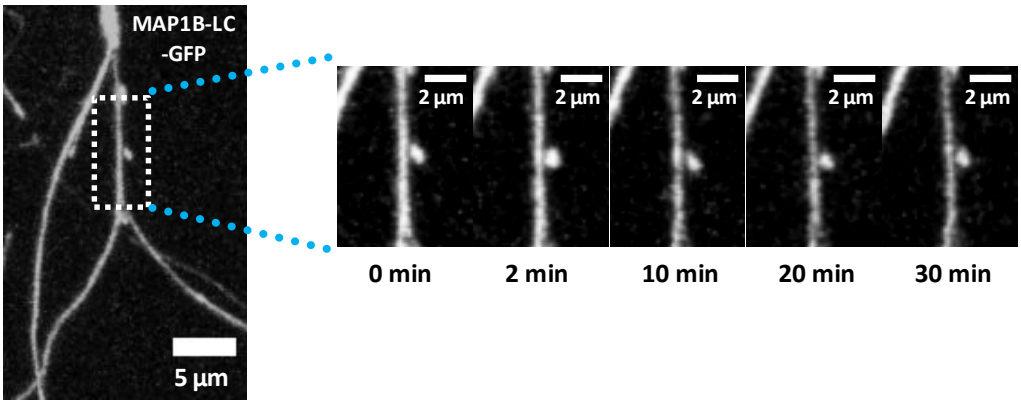


Figure 12. MAP1B-LC-GFP stays stably in spines in the absence of stimulation. Left, representative picture of a CA1 neuron over-expressing MAP1B-LC-GFP in which the recombinant protein appears accumulated in a dendritic spine. The inset shows the area amplified in the images on the right. **Right,** the same spine was imaged along 30 minutes in the absence of stimulation. No movement of MAP1B-LC-GFP could be detected.

In parallel to the assessment of MAP1B-LC-GFP/Dendra mobility in dendrites upon the induction of synaptic plasticity, we decided to evaluate if the recombinant protein found in dendritic spines could also mobilize during LTD or LTP. To do so, we chemically induced LTD or LTP after photoactivation in neurons over-expressing MAP1B-LC-Dendra. Indeed, although only a few examples were collected, we could image MAP1B-LC-Dendra slowly leaving dendritic spines after the induction of both LTD and LTP (figure 13). This result would strengthen the notion that MAP1B-LC mobilization triggered upon the induction of synaptic plasticity is an intrinsic property of the protein independent of its specific subcellular location.

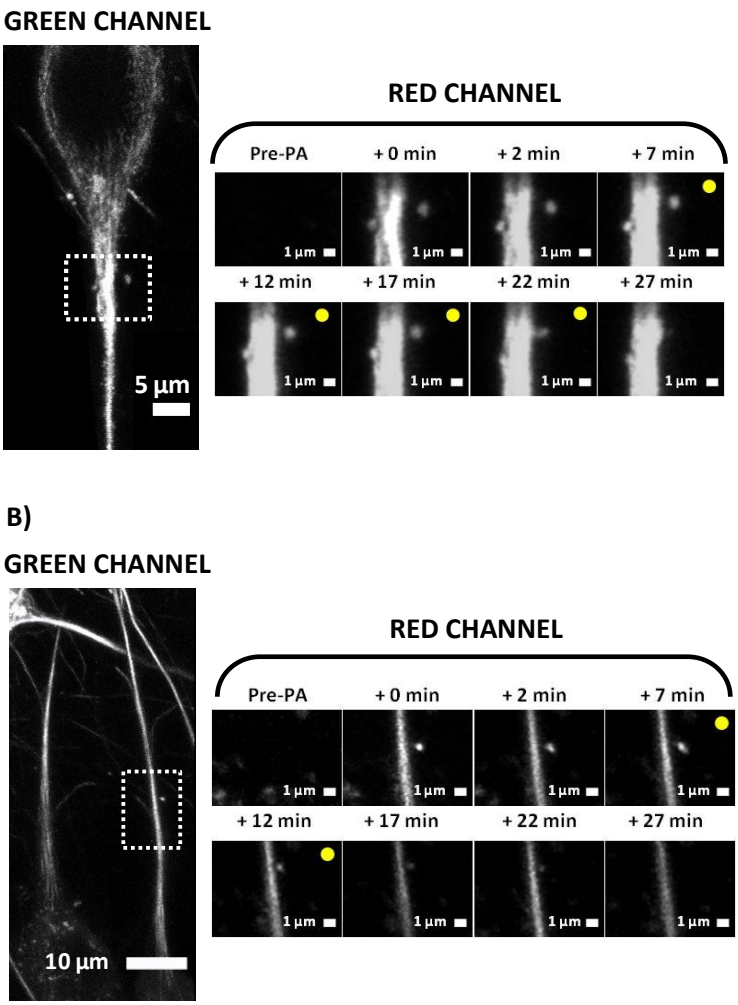


Figure 13. MAP1B-LC-Dendra leaves dendritic spines when synaptic plasticity is induced. A) Left, representative picture of a CA1 neuron over-expressing MAP1B-LC-Dendra; the recombinant protein is accumulated in a dendritic spine. The inset shows the area amplified in the images on the right. **Right,** representative pictures of a dendritic spine containing MAP1B-LC-Dendra. After PA, LTP was chemically induced (yellow dots). **B) Left,** representative picture of a CA1 neuron over-expressing MAP1B-LC-Dendra in which a dendritic spine containing the recombinant protein was detected. The inset shows the area amplified in the images on the right. **Right,** representative pictures of a dendritic spine containing MAP1B-LC-Dendra. After PA, LTD was chemically induced (yellow dots).

4. LTP is enhanced in MAP1B-LC-GFP over-expressing neurons.

To fulfill MAP1B-LC characterization, we wondered if the over-expression of MAP1B-LC-GFP could have any impact on the induction or expression of synaptic plasticity in CA1 pyramidal neurons. Importantly, MAP1B has been demonstrated to be required for the regulated endocytosis of AMPA receptors after the induction of LTD (Benoist et al. 2013). Therefore, we first assessed NMDAR-dependent LTD in CA1 neurons over-expressing MAP1B-LC-GFP and in non-infected, control neurons (figure 14A). After stimulation (300 pulses at 1 Hz) at mild depolarization (-40 mV), AMPAR-mediated synaptic responses were equally depressed in both conditions.

A selective role of MAP1B in DHPG-mediated endocytosis of AMPA receptors has been proposed (Davidkova and Carroll 2007), so we next tested mGluR-dependent LTD expression in infected and non-infected cells. As shown in figure 14B, no difference in the level of depression in infected cells versus non-infected cells could be observed. We concluded, then, that the

over-expression of the light chain of MAP1B does not have any effect either on NMDAR-dependent or mGluR-dependent LTD in CA1 hippocampal neurons.

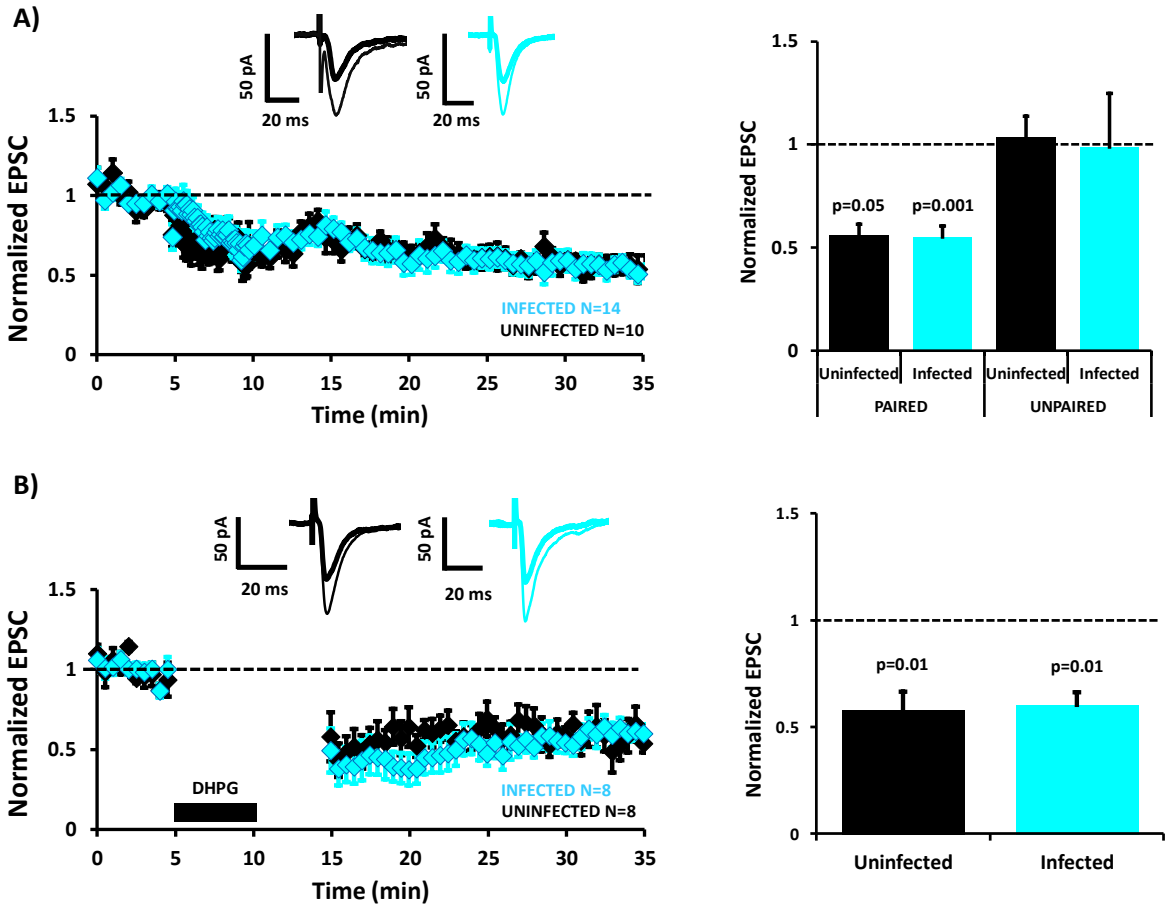


Figure 14. MAP1B-LC-GFP over-expression does not affect LTD. AMPAR-mediated synaptic responses were recorded from CA1 neurons and normalized to the average baseline value before the induction of plasticity. **A) Left,** time course of NMDAR-dependent LTD (300 pulses at 1 Hz, depolarization at -40 mV) in control neurons (black) and neurons over-expressing MAP1B-LC-GFP (cyan), with representative traces above. **Right,** average responses collected from the last 5 minutes of the recording and normalized to the baseline. Left columns (paired) correspond to the induced pathway; both uninfected and infected cells were significantly depressed with respect to their baseline. "p" values correspond to the statistical significance values calculated according to the Wilcoxon test. Right columns (unpaired) correspond to the pathway that was not stimulated during the induction. **B) Left,** mGluR-dependent LTD was induced with 50 µM of (RS)-3,5-DHPG for 5 minutes, as indicated with the black bar. Representative traces are shown above. Right, average responses collected from the last 5 minutes of the recording, and normalized to the baseline; uninfected and infected cells were significantly depressed with respect to their baseline. "p" corresponds to the statistical significance value calculated with the Wilcoxon test.

We then induced LTP in neurons over-expressing MAP1B-LC-GFP and control neurons with a pairing protocol following a baseline period of transmission. A 2-fold potentiation was observed in uninfected neurons (figure 15). Nevertheless, the potentiation was significantly higher in infected neurons, of approximately 4 fold. Two different models could fit into this scenario: either the increased potentiation is a consequence of the decrease in AMPAR-

dependent basal transmission due to the over-expression of MAP1B-LC-GFP or MAP1B-LC is somehow promoting the induction or expression of LTP in CA1 hippocampal neurons. To answer this question, we proceed to characterize the effects on basal transmission and synaptic plasticity of MAP1B down-regulation.

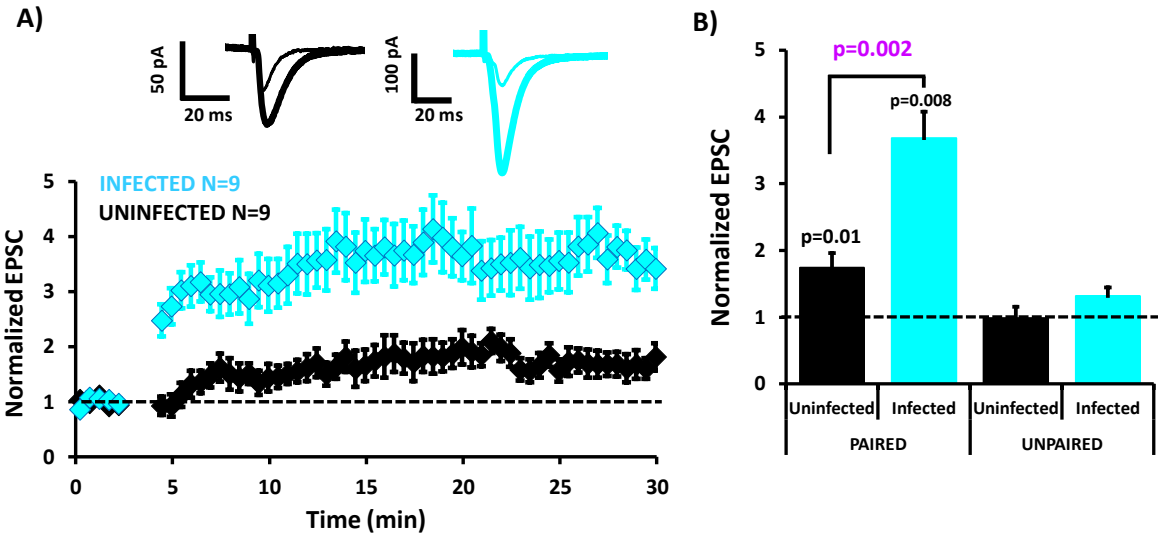


Figure 15. LTP is enhanced upon MAP1B-LC-GFP over-expression. AMPAR-mediated synaptic responses were recorded from CA1 neurons and normalized to the average baseline value before the induction of LTP. **A)** Time course of LTP (300 pulses at 3 Hz, depolarization at 0 mV) for control neurons and neurons over-expressing MAP1B-LC-GFP, with representative traces shown above. **B)** Average responses collected from the last 5 minutes of the recording and normalized to the baseline. Left columns (paired) correspond to the induced pathway; uninfected and infected cells were significantly potentiated with respect to their baseline ("p" values shown just above each column correspond to the statistical significance values calculated according to the Wilcoxon test). The "p" value on top of the graph (in magenta) corresponds to the statistical significance value comparing the extent of potentiation in uninfected versus infected neurons (Mann-Whitney test). Error bars represent s.e.m. Right columns (unpaired) correspond to the pathway that was not stimulated during the induction.

B) MAP1B down-regulation.

1. MAP1B is effectively down-regulated with a lentiviral-mediated strategy.

As described in Materials and Methods, a short hairpin RNA (shRNA) targeting mouse MAP1B gene was cloned into a lentiviral vector. To test its efficiency in down-regulating MAP1B *in vivo*, two different strategies were followed. First, the lentiviral vector carrying the shRNA against MAP1B was used to infect mouse hippocampal primary neurons. MAP1B depletion was corroborated with an immunofluorescence performed against endogenous MAP1B ten days after the infection (figure 16A).

On the other hand, CA1 neurons in hippocampal slices were infected with the lentiviral vector and a SDS-PAGE analysis of protein expression was carried out ten days after. As shown in

figure 16B, MAP1B level of expression in protein extracts was comparable to that of MAP1B heterozygous mice (MAP1B +/-). To perform a semi-quantitative analysis of MAP1B down-regulation, CA1 subfields in theory containing a majority of infected cells were microdissected before proceeding to protein extraction. However, the rate of viral infection never reaches 100% of the cells in a specific area, so it is very likely that the region selected for analysis contained also non-infected cells expressing normal levels of MAP1B. Still, a considerable degree of protein down-regulation could be observed, supporting the idea that MAP1B depletion upon shRNA expression was almost complete in the infected cells.

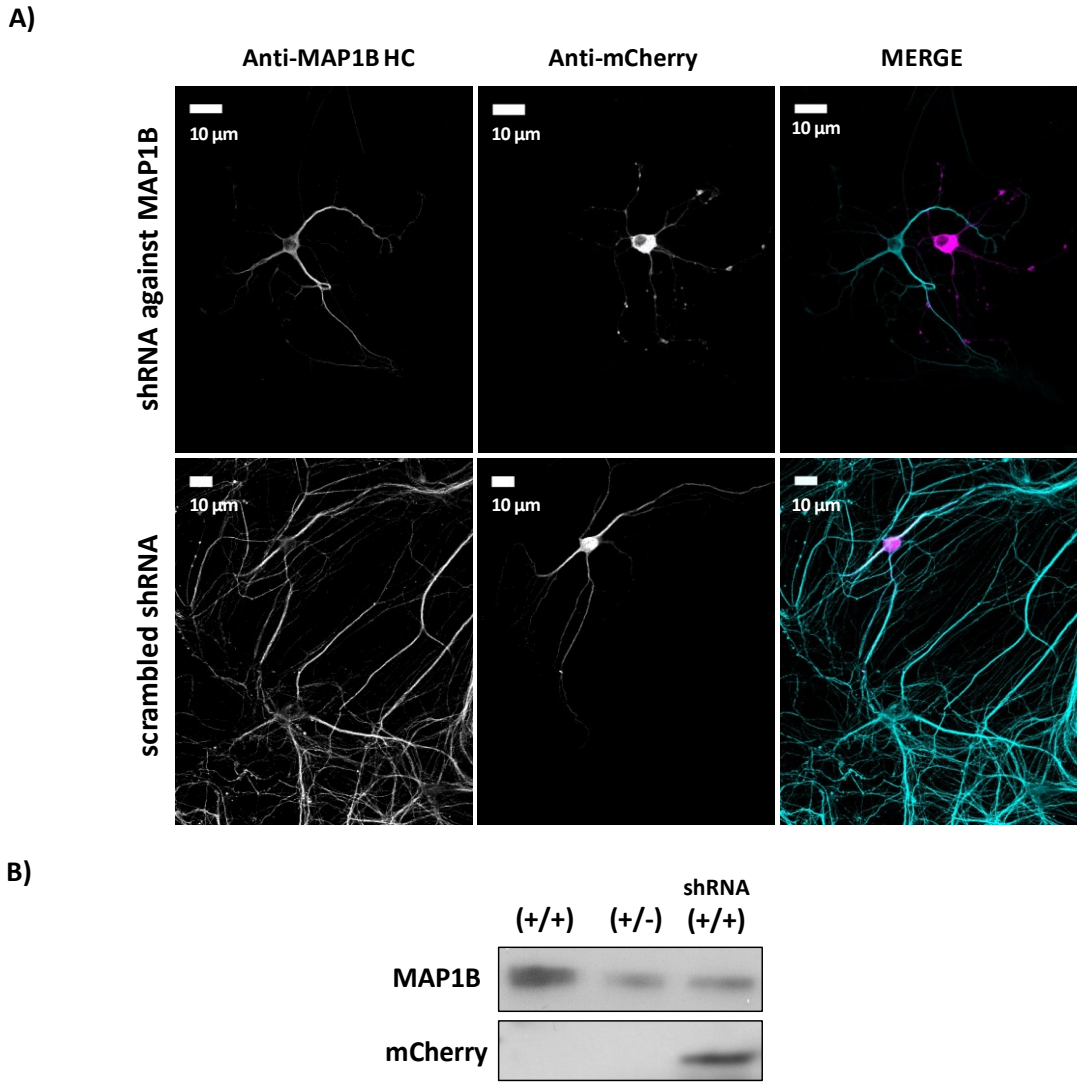


Figure 16. Acute down-regulation of MAP1B. A) Representative confocal pictures of hippocampal primary neurons (DIV 20) infected with a lentiviral vector expressing either a shRNA against MAP1B protein (upper panel) or a scrambled shRNA (lower panel). The immunostaining was performed against MAP1B HC (left) and mCherry (middle). The image on the right depicts the merge of the two previous ones. **B) Up,** western blot showing the expression levels of MAP1B in organotypic slices from wild-type (MAP1B +/+) and heterozygous (MAP1B +/-) mice, and from wild-type animals after the expression of the shRNA against MAP1B. **Down,** western blot showing mCherry expression in lentiviral-infected wild-type slices.

2. MAP1B down-regulation does not alter basal synaptic transmission.

Notably, the effects of MAP1B deficiency on basal synaptic transmission and synaptic plasticity were previously explored in our group (Benoist et al. 2013). In this work, adult hypomorphus mice for MAP1B were used to obtain acute brain slices in which AMPA/NMDA ratios and LTD/LTP expression were measured, with specific stimulation protocols for field recordings. Thereby, both the system and the protocols used were different from the ones presented below. In addition, in such a study, it is difficult to discern which effects might be due to the continuous lack of MAP1B during the development of the nervous system and to a hypothetical compensational mechanism of such a deficiency. Therefore, to complete MAP1B characterization in synaptic plasticity, we decided to complement the previous study with a different approach: the acute down-regulation of the full-length MAP1B protein in CA1 pyramidal neurons in cultured hippocampal slices.

We first tested basal synaptic transmission in CA1 neurons expressing the shRNA against MAP1B. As shown in figure 17, no difference in either AMPAR- or NMDAR-mediated transmission was observed between infected and non-infected, control cells. This result was coherent with that reported in the study by Benoist et al. showing no change in AMPA/NMDA ratios in MAP1B +/- comparing to MAP1B +/+ mice.

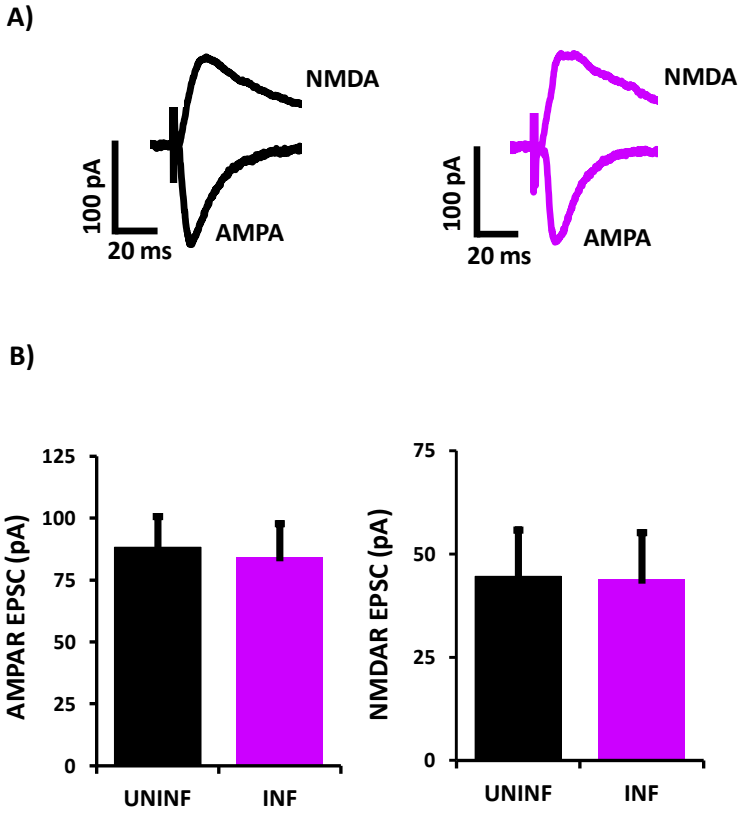


Figure 17. The acute down-regulation of MAP1B does not affect basal synaptic transmission. A) Representative traces for AMPAR and NMDAR responses recorded from control, uninfected CA1 neurons (black), or neurons expressing the shRNA against MAP1B (magenta). **B)** Synaptic responses were recorded from pairs of neighboring control CA1 neurons (uninfected, UNINF) and infected neurons expressing the shRNA against MAP1B (INF), in the presence of picrotoxin at -60 mV for AMPARs (n=21 pairs), and at +40 mV for NMDARs (n=13 pairs). Error bars, s.e.m.

3. MAP1B down-regulation impairs NMDAR-dependent LTD but does not affect LTP.

Next, we decided to evaluate NMDAR-dependent LTD in MAP1B-depleted CA1 neurons comparing to control neurons. A low-frequency train of stimuli was delivered to induce depression (500 pulses at 1 Hz). As indicated in figure 18, uninfected neurons showed a 50% degree of depression; however, this was not paralleled by shRNA-expressing neurons, which only experienced a mild (20%) long-lasting depression. A scrambled shRNA sequence was included in the experiment to rule out a blockade of depression due to lentiviral-infection. This result was consistent with the lack of depression in MAP1B +/- mice reported by Benoist et al. and strengthened the idea that MAP1B is required for AMPAR-endocytosis during LTD.

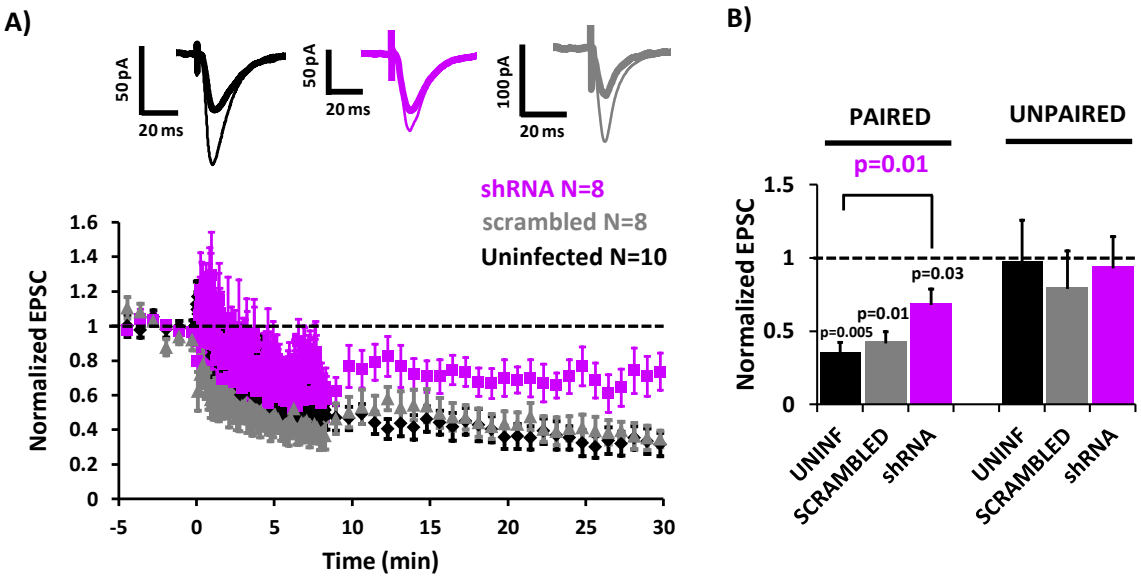


Figure 18. Effect of MAP1B down-regulation on LTD. AMPAR-mediated synaptic responses were recorded from CA1 neurons and normalized to the average baseline value before the induction of plasticity. **A)** Time course of NMDAR-dependent LTD (500 pulses at 1 Hz) in control neurons, and neurons expressing either the shRNA against MAP1B, or a scrambled control. Representative traces are shown above the graph. **B)** Average responses collected from the last 5 min of the recording and normalized to the baseline. Left columns (paired) correspond to the induced pathway. Uninfected and infected cells (expressing either the shRNA against MAP1B, or the scrambled shRNA) were significantly depressed with respect to their baseline ("p" values shown just above each column correspond to the statistical significance values calculated according to the Wilcoxon test). "p" corresponds to the statistical significance value comparing the extent of depression in uninfected versus infected neurons expressing the shRNA against MAP1B (Mann-Whitney test) (in magenta). Right columns (unpaired) correspond to the pathway that was not stimulated during the induction. Error bars, s.e.m.

Benoist et al. reported no change in LTP in MAP1B +/- mice comparing to MAP1B +/+ animals when potentiation was induced with high frequency stimulation. Nevertheless, when a milder

protocol of stimulation was applied, an enhanced potentiation could be observed in slices from heterozygous animals comparing to slices from control animals.

In CA1 neurons expressing the shRNA against MAP1B, the degree of potentiation after LTP induction (300 pulses, 3 Hz) was comparable to that expressed by control neurons (figure 19). It is likely that a ceiling effect of the stimulation protocol explains the unaltered LTP we observe upon MAP1B depletion, similarly to the phenotype described by Benoist and colleagues with high frequency stimulation. However, we did not explore the potential involvement of MAP1B in LTP any further.

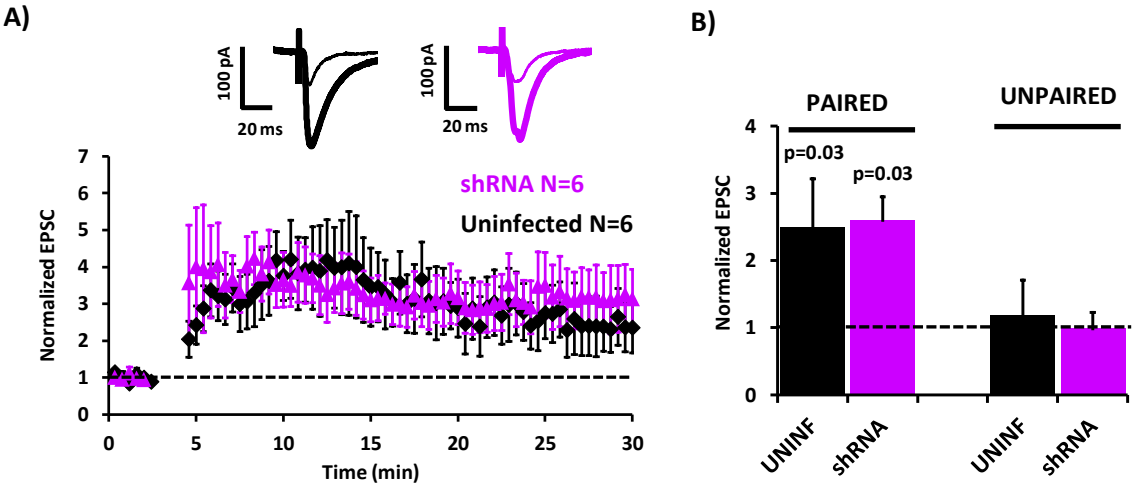


Figure 19. The acute down-regulation of MAP1B does not alter LTP. AMPAR-mediated synaptic responses were recorded from CA1 neurons and normalized to the average baseline value before the induction of LTP. **A)** Time course of LTP (300 pulses at 3 Hz) for control neurons and neurons expressing the shRNA against MAP1B. Representative traces for uninfected and infected neurons are shown above. **B)** Average responses collected from the last 5 min of the recording and normalized to the baseline. Left columns (paired) correspond to the induced pathway; uninfected (UNINF) and infected cells (shRNA) were significantly potentiated with respect to their baseline ("p" values shown just above each column correspond to the statistical significance values calculated according to the Wilcoxon test). Right columns (unpaired) correspond to the pathway that was not stimulated during the induction. Error bars, s.e.m.

PART II: dissecting the molecular mechanism of action of MAP1B-LC.

The over-expression of the light chain of MAP1B gave us information about its potential to impact on AMPAR-mediated basal transmission, and therefore, to modify some forms of long-term synaptic plasticity in CA1 hippocampal neurons. We next aimed to uncover its molecular mechanism of action:

- First, we tried to ascertain the possible role of each functional domain of MAP1B-LC (actin-binding domain and microtubule-binding domain) in the AMPAR-dependent depression subsequent to the over-expression of the wild type protein.
- Then, we wondered whether the depression in basal transmission observed in the presence of enhanced levels of MAP1B-LC could be due to an alteration in the number or morphology of dendritic spines and/or to defects in AMPAR trafficking.
- Further research allowed us to finally identify GRIP1 as the molecular link between MAP1B-LC and AMPAR trafficking in CA1 neurons.

A) MAP1B-LC mutants: MAP1B-LC-delABD and MAP1B-LC-delMBD.

1. Testing the functionality of MAP1B-LC mutants.

As described, MAP1B-LC harbors two distinct domains: a microtubule-binding domain (MBD) and an actin-binding domain (ABD). We initially hypothesized that the reduced AMPAR-dependent transmission observed upon the over-expression of the wild type MAP1B-LC could be due to its potential to modulate the microtubule cytoskeleton, the actin cytoskeleton or both. To evaluate this possibility, we decided to generate two deletion mutants of MAP1B-LC, one lacking the ABD and the other one lacking the MBD, and test the consequences of their over-expression in CA1 neurons.

Once the mutants were generated, we first analyzed their distribution in CA1 pyramidal neurons. The mutant lacking the ABD (the so called MAP1B-LC-delABD-GFP) was expected to be absent from dendritic spines and to display a filamentous pattern of distribution (similar to the wild type protein) as it was the one supposedly maintaining the ability to interact with

microtubules. As shown in figure 20A, this pattern was not as evident as with the whole MAP1B-LC, but a certain degree of filamentous arrangement could be observed in the periphery of the soma and the initial stretch of apical dendrites in CA1 neurons.

In the case of the mutant lacking the MBD (MAP1B-LC-delMBD-GFP), as it was the mutant supposedly able to interact with the actin cytoskeleton, our original expectation was to find it concentrated in dendritic spines, a compartment where filamentous actin is largely accumulated in neurons. To our surprise, we could not clearly identify spines containing this mutant of MAP1B-LC when it was over-expressed. As shown in figure 20B, MAP1B-LC-delMBD-GFP displayed a patchy distribution in the cell body and dendrites of CA1 neurons.

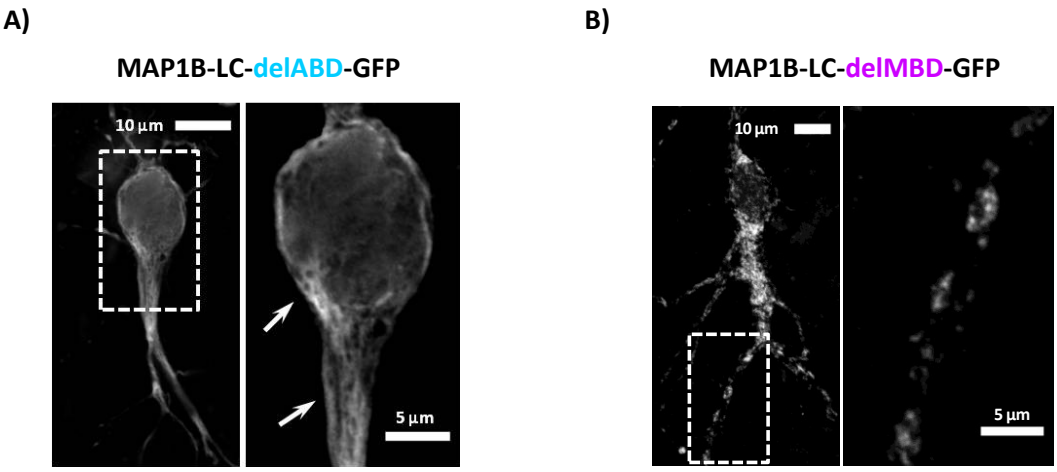


Figure 20. Over-expression of MAP1B-LC mutants in CA1 neurons. A) Confocal pictures showing the pattern of distribution in CA1 neurons of a deletion mutant of MAP1B-LC-GFP obtained when the actin-binding domain of the wt protein is removed (MAP1B-LC-delABD-GFP). The rectangle in the left picture shows the area amplified in the right picture. Arrows point to filamentous appearance in the soma and apical dendrites. **B)** Same as in A, but corresponding to the over-expression of a mutant of MAP1B-LC-GFP generated by deleting the microtubule-binding domain of the wt protein (MAP1B-LC-delMBD-GFP).

The analysis of the distribution of MAP1B-LC mutants in CA1 neurons was not really informative about their binding to the microtubule or actin cytoskeletons. To clearly test their functionality, we performed *in vitro* co-sedimentation experiments, which are based on the ability of a protein that interacts with microtubules (or microfilaments) to co-sediment with them. In these experiments, a protein extract containing the protein of interest is mixed with purified microtubules (or microfilaments) and after centrifugation, its presence in supernatant and pellet fractions is analyzed.

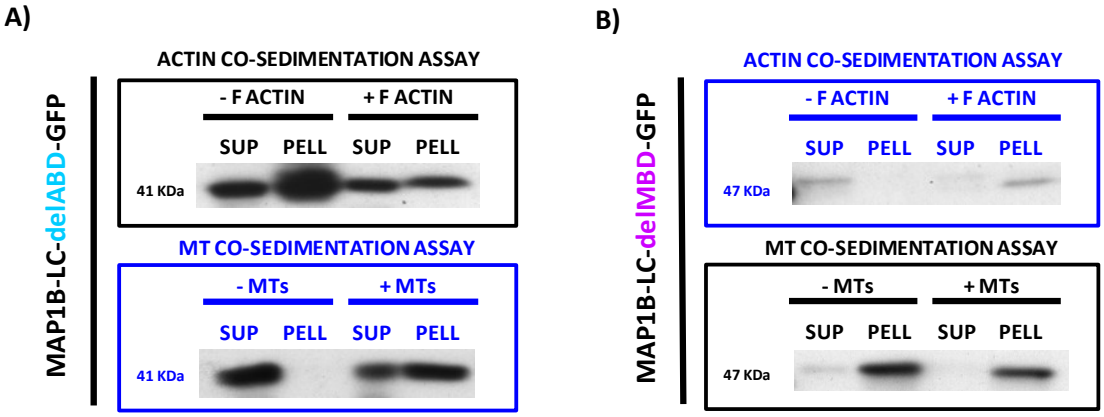


Figure 21. MAP1B-LC-delABD-GFP co-sediments with microtubules and MAP1B-LC-delMBD-GFP co-sediments with actin. A) Western-blots of actin and microtubule co-sedimentation assays for MAP1B-LC-delABD-GFP. F ACTIN= filamentous actin; SUP=supernatant; PELL=pellet. **B)** Same as in C, but for MAP1B-LC-delMBD-GFP.

In the case of the mutant lacking the ABD, its distribution in supernatant and pellet fractions was equivalent in the absence and in the presence of filamentous actin (figure 21A, upper panel), meaning that filamentous actin does not change the ability of the mutant to sediment because both proteins do not interact. On the contrary, if assembled microtubules were added to the protein extract containing MAP1B-LC-delABD-GFP, the mutant shifted its distribution from being exclusively in the supernatant to being concentrated mainly in the pellet (figure 21A, lower panel). The interaction with microtubules, which sediment under the centrifugal force applied during the procedure, drags the mutant to the pellet, too.

The opposite scenario is observed with the mutant lacking the MBD. MAP1B-LC-delMBD-GFP maintains the ability of the wild type protein to interact with actin, and so, it is present mainly in the pellet when filamentous actin is added to the protein extract (figure 21B, upper panel). The fact that it is recovered in the supernatant in the absence of exogenously added actin indicates that the mutant does not sediment on its own in these conditions. In contrast, its distribution between supernatant and pellet does not change if microtubules are incubated with the protein extract prior to the centrifugation step. In any case, the protein tends to be present in the pellet (figure 21B, lower panel).

In sum, these experiments confirmed that, although they do not display the expected subcellular distribution, the generated mutants of MAP1B-LC were effectively able to interact with either the microtubule cytoskeleton or the actin cytoskeleton, and so, they were valid to be tested as potential modulators of AMPAR-dependent basal transmission in CA1 pyramidal neurons.

2. The over-expression of MAP1B-LC mutants does not affect basal synaptic transmission in CA1 neurons.

To analyze the potential role of the interaction with microtubules and/or actin in the decrease in basal transmission mediated by the wt protein, we over-expressed MAP1B-LC-delABD-GFP and MAP1B-LC-delMBD-GFP in CA1 neurons and recorded AMPAR and NMDAR currents. As shown in figure 22, no change in either AMPAR- or NMDAR-mediated transmission was observed upon the over-expression of the mutant lacking the ABD or the mutant lacking the MBD. Having previously tested their functionality, this result pointed to the fact that both domains of MAP1B-LC were required to mediate the effect of the wild type protein on AMPAR-dependent transmission in CA1 neurons.

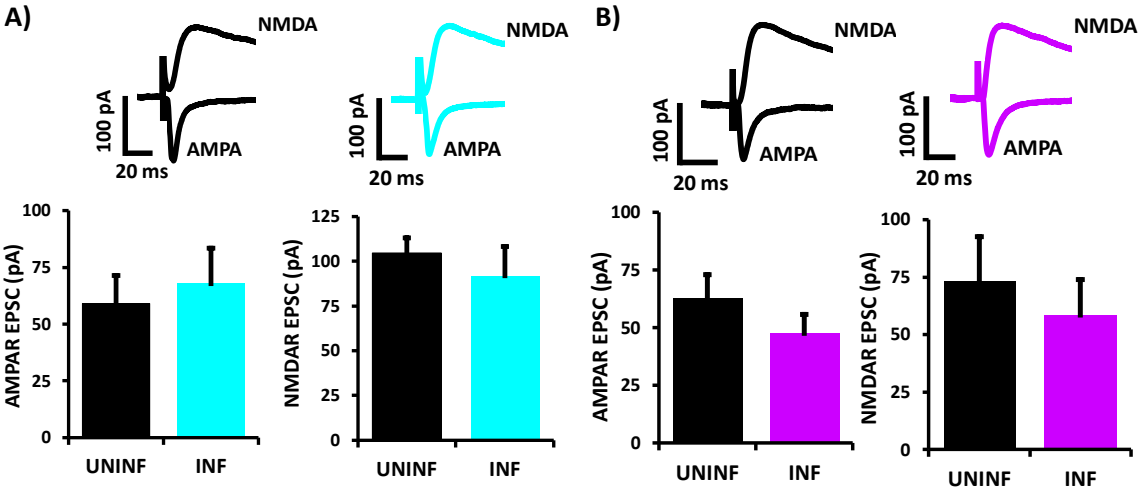


Figure 22. The over-expression of deletion mutants of MAP1B-LC does not affect basal synaptic transmission in CA1 neurons. A) Synaptic responses were recorded from pairs of neighboring CA1 neurons over-expressing MAP1B-LC-delABD-GFP (infected, INF) and control neurons (uninfected, UNINF), in the presence of picrotoxin at -60 mV for AMPARs (n=12 pairs), and at +40 mV for NMDARs (NMDAR responses were collected at a latency of 100 ms; n=11 pairs). Representative traces are shown above. **B)** Same experiment as in A, but corresponding to the over-expression of MAP1B-LC-delMBD-GFP (AMPAR responses, n=22 pairs; NMDAR responses, n=17 pairs). Error bars, s.e.m.

B) Dendritic spine remodeling or AMPAR trafficking?

1. MAP1B-LC over-expression does not alter the size or number of dendritic spines.

MAP1B has been previously reported to be necessary for dendritic spine development and maturation (Tortosa et al. 2011). In cultured neurons from MAP1B knock-out mice (which die perinatally), dendritic spines are less abundant and more immature (most of them are

filopodia, long thin protrusions without a distinguishable head). This phenotype correlates with a decreased postsynaptic function evidenced by the recording of AMPAR-mediated miniature synaptic currents in cultured neurons from MAP1B knock-out animals (Tortosa et al. 2011). Therefore, we first tested the possibility that the decrease in AMPAR-dependent transmission observed upon MAP1B-LC over-expression could result from an alteration in dendritic spine morphology or number. Similar to the data reported by Tortosa et al., one could expect a decrease in dendritic spine number or size/maturity in the face of a reduced basal synaptic transmission.

To perform a morphometric analysis of dendritic spines in the presence of MAP1B-LC-GFP, infected and uninfected, control neurons were intracellularly labeled with biocytin (figure 23A). After a 3D-reconstruction and quantification, we did not find any difference in head diameter, spine length or density between neurons over-expressing MAP1B-LC-GFP and control neurons (figure23B).

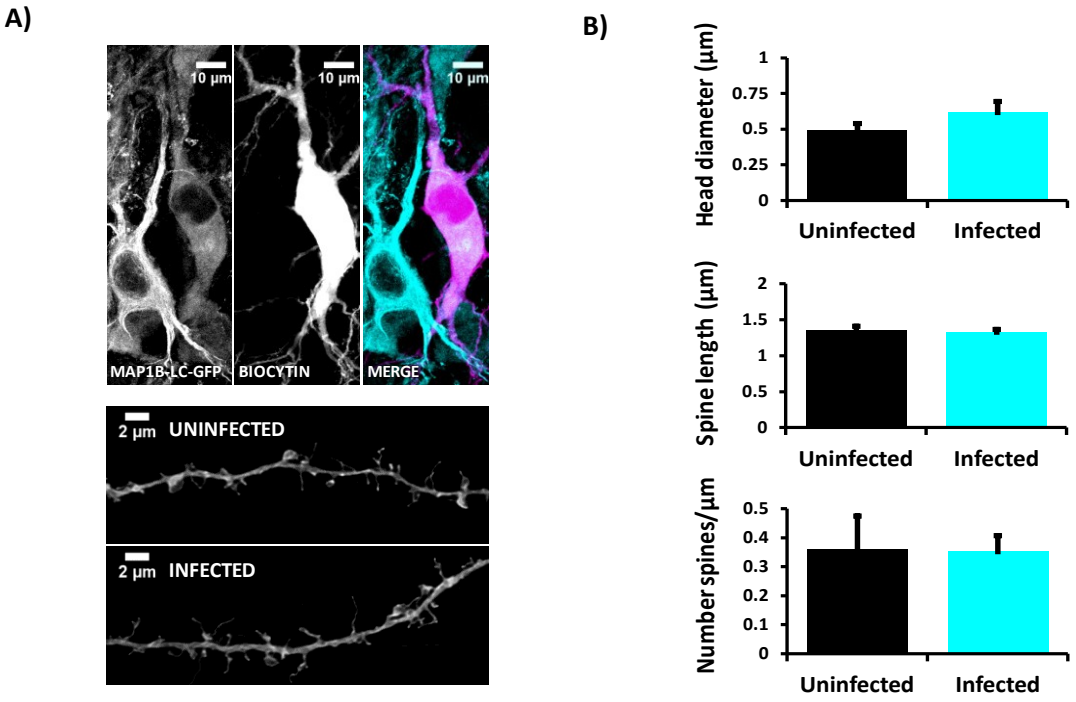


Figure 23. Neither morphology nor number of dendritic spines are affected upon MAP1B-LC-GFP over-expression. A) Upper panel, representative image of a CA1 neuron over-expressing MAP1B-LC-GFP that was filled in with biocytin to reveal its complete morphology. **Lower panel,** representative confocal deconvoluted images of dendritic spines in apical dendrites of uninfected, control CA1 neurons and infected neurons over-expressing MAP1B-LC-GFP. **B)** Quantification of average head diameter, spine length and spine density in uninfected (n=5) and infected (n=4) neurons. Error bars represent s.e.m.

2. Rac1/RhoA activities are not altered upon MAP1B-LC-GFP over-expression.

The ability of MAP1B to regulate axonal development (Montenegro-Venegas et al. 2010), dendritic morphogenesis (Tortosa et al. 2011) and AMPAR endocytosis after LTD induction (Benoist et al. 2013) has been reported to rely on its ability to modulate the activity of small GTPases, and therefore, the actin cytoskeleton. It has been shown that MAP1B deficiency is associated with decreased Rac1 activity and increased RhoA activity (Montenegro-Venegas et al. 2010; Tortosa et al. 2011). Furthermore, it has been proposed that it is the light chain of MAP1B the one that binds Tiam1 (Rac1 GEF), and thus, directly mediates changes in Rac1 activation (Henriquez et al. 2012). Therefore, we wondered if the over-expression of MAP1B-LC-GFP could have any impact on Rac1 or RhoA activity that could explain the observed phenotype.

In organotypic hippocampal slices over-expressing MAP1B-LC-GFP versus GFP-expressing, control slices, no difference in the activity of small GTPases Rac1 or RhoA could be assessed (figure 24A and B). Importantly, this result fits well with the above described normal dendritic spine morphology and number in MAP1B-LC-GFP over-expressing neurons.

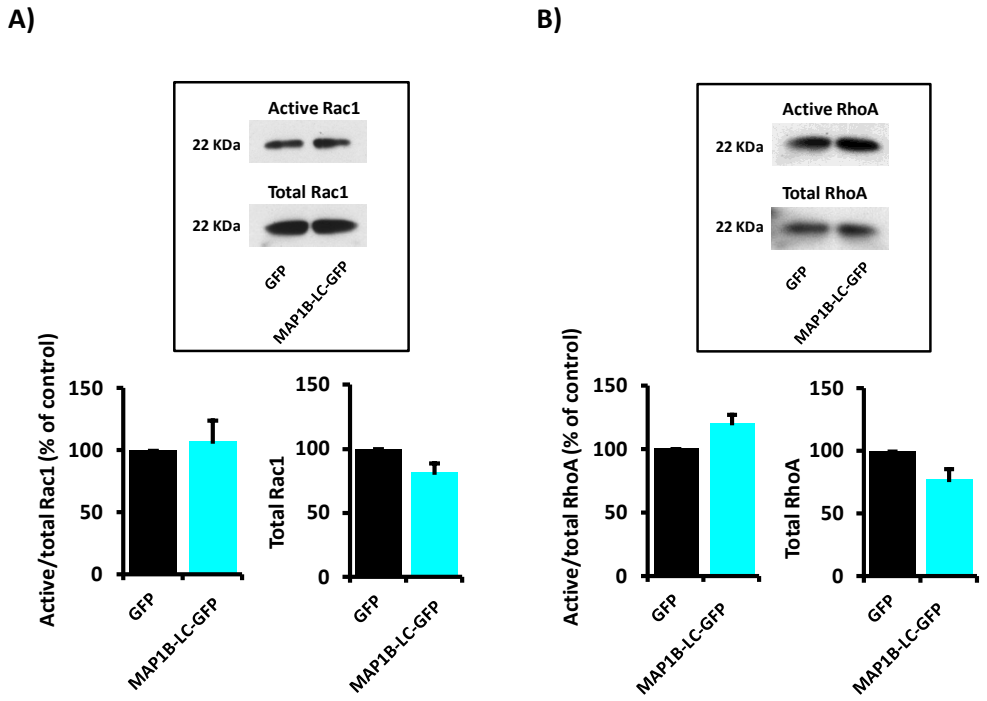


Figure 24. The over-expression of the light chain of MAP1B does not seem to imbalance neither Rac1 nor RhoA activity in CA1 neurons. A) Upper panel, representative western blot of a pull-down experiment to measure Rac1 activity using protein extracts from hippocampal slices previously infected with Sindbis virus to over-express either GFP (as a control) or MAP1B-LC-GFP. **Lower panel,** quantification of active Rac1 versus total Rac1 (left), and total levels of Rac1 (right), in MAP1B-LC-GFP-expressing hippocampal slices relative to control (n=7 experiments). **B) Upper panel,** same as in A, but showing a pull-down experiment to measure active RhoA. **Lower panel,** same as in A, but referred to active RhoA versus total RhoA (left), and total levels of RhoA (right) (n=6 experiments). Error bars, s.e.m.

3. The constitutive cycling of GluA2-GluA3 AMPA receptors is impaired upon the over-expression of recombinant MAP1B-LC.

3.1 Imaging of fluorescently-tagged GluA2 in hippocampal slices.

3.1.1 Co-expression of recombinant MAP1B-LC.

As the over-expression of MAP1B-LC-GFP in CA1 neurons did not seem to affect dendritic spine morphology or small GTPases activity, we next tested if recombinant MAP1B-LC was exerting any effect on the trafficking of AMPARs from dendritic compartments to spines.

As previously mentioned, the GluA2-GluA3 population of AMPARs is the one thought to be responsible for the maintenance of basal synaptic transmission in the face of protein turnover, as this pathway replaces continuously existing synaptic receptors in a manner not requiring neuronal activity (Passafaro et al. 2001; Shi et al. 2001). Hence, this population would be the one expected to be specifically targeted by MAP1B-LC-GFP.

To corroborate this hypothesis, we first analyzed GluA2 accumulation in dendritic spines in the presence or absence of MAP1B-LC-GFP. To this end, we co-expressed RFP-GluA2 together with MAP1B-LC-GFP, or GFP as control, in organotypic hippocampal slices via biolistic transfection (figure 25A). Homomers of recombinant GluA2-GluA2 subunits have been proved to constitutively traffic to synapses like the endogenous GluA2-GluA3 population (Shi et al. 2001).

The spine/dendrite ratio of RFP fluorescence is a measurement of RFP-GluA2 accumulation in spines comparing to dendritic shafts. As shown in figure 25C, the cumulative distribution of spine/dendrite ratios in neurons over-expressing MAP1B-LC-GFP was left-shifted when compared to the GFP condition. Therefore, the whole population of quantified spines in MAP1B-LC-GFP over-expressing neurons contained less RFP-GluA2 in spines than GFP over-expressing neurons, as the average value of spine/dendrite ratios for both conditions (figure 25D) demonstrates as well.

To rule out a general effect on AMPAR trafficking independent of subunit composition, we repeated the same experiment but co-expressing the GluA1 subunit of AMPARs instead of GluA2 together with MAP1B-LC-mCherry or mCherry as a control (figure 25B). The co-expression of the catalytic domain of CamKII (tCamKII) is required to guarantee GluA1 homomers gaining access into dendritic spines (Hayashi et al. 2000). As shown in figure 25C, cumulative distributions of spine/dendrite ratios for GluA1 in the presence of MAP1B-LC-

mCherry or mCherry alone were basically overlapping. There was no difference in the average value of spine/dendrite ratios for both conditions either (figure 25D). These results would point to a specific impairment in the trafficking of GluA2-GluA3 AMPARs in the presence of enhanced levels of MAP1B-LC.

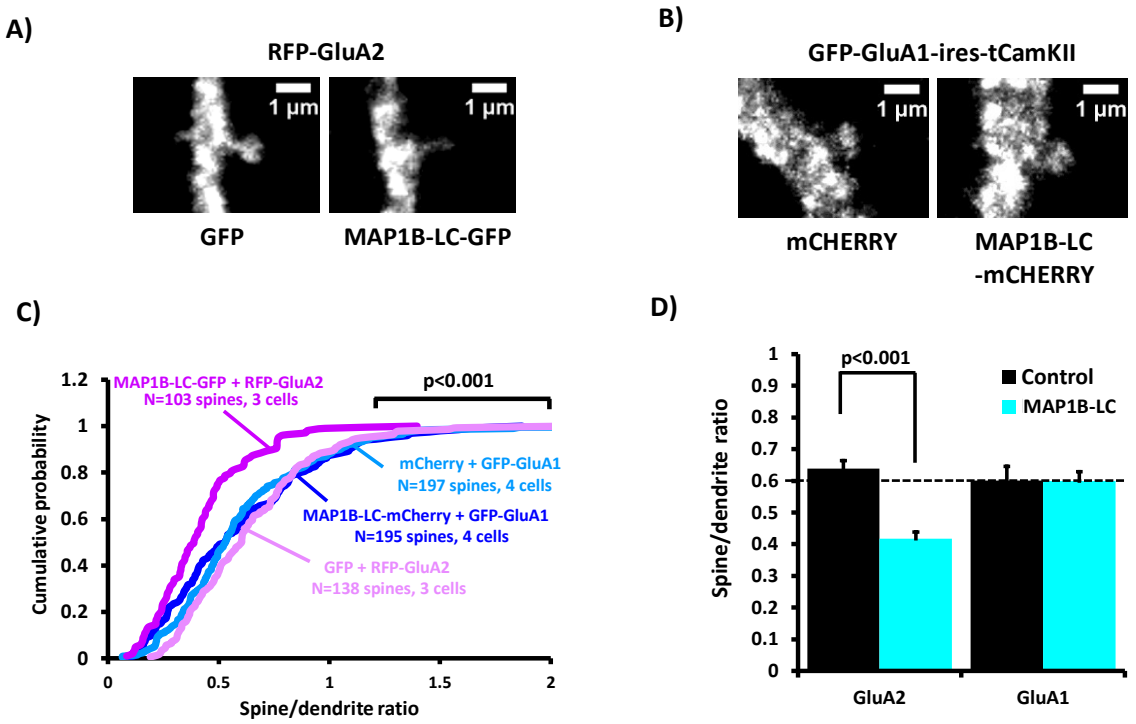


Figure 25. Over-expression of MAP1B-LC-GFP decreases GluA2, but not GluA1, accumulation in dendritic spines. A) Representative confocal images of dendritic spines from neurons co-expressing RFP-GluA2 and MAP1B-LC-GFP, or GFP as a control. **B)** Representative confocal images of GFP-GluA1 in dendritic spines of neurons co-expressing MAP1B-LC-mCherry, or mCherry as a control. **C)** Quantification of fluorescence intensity in spines versus adjacent dendrite from neurons like those in (A and B). Data are presented as cumulative distributions of spine/dendrite ratios. Significance calculated by Kolmogorov-Smirnov test. **D)** Average spine/dendrite ratio for recombinant GluA2 and GluA1 in the presence of over-expressed MAP1B-LC, or in control conditions. "p" corresponds to the statistical significance value calculated by Mann-Whitney test, considering the total number of quantified dendritic spines per condition. Error bars represent s.e.m.

3.1.2 Down-regulation of MAP1B.

If the over-expression of MAP1B-LC results in a reduced presence of GluA2-GluA3 AMPARs in dendritic spines, MAP1B deficiency may be translated into an accumulation of this population of AMPARs in the spine compartment. To test this possibility, we prepared hippocampal slices out of MAP1B +/+ and MAP1B +/- animals and quantified GFP-GluA2 presence in spines of CA1 neurons after infection. As shown in figure 26, the cumulative distributions and average values of spine/dendrite ratios are similar for both genotypes. Therefore, the excess of MAP1B-LC

impacts negatively on GluA2-GluA3 accumulation in spines whereas the deficiency of the full length protein does not seem to alter this process. This result is consistent with the unaffected basal transmission recorded in CA1 neurons expressing the shRNA against MAP1B and in slices from MAP1B heterozygous mice (Benoist et al. 2013).

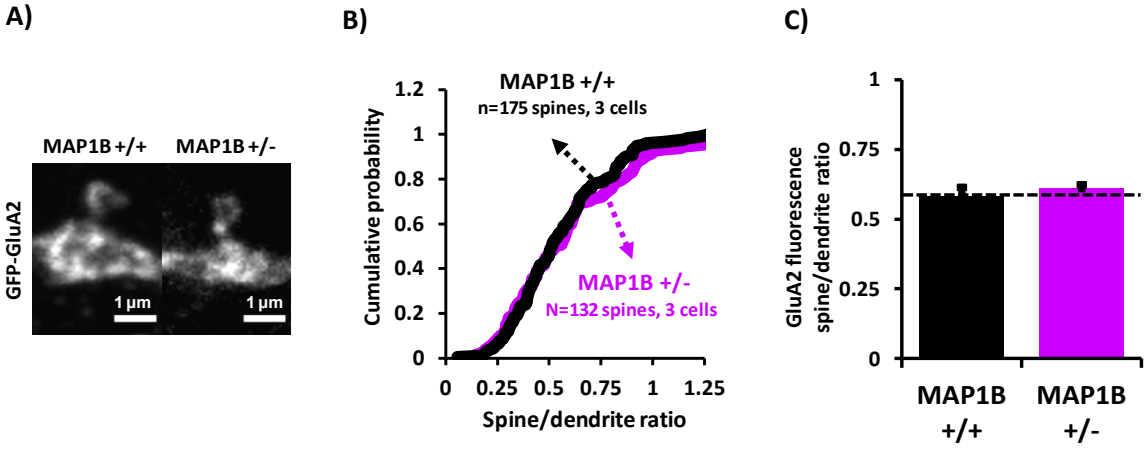


Figure 26. GluA2 accumulation in dendritic spines is unchanged upon MAP1B depletion. A) Representative confocal pictures of GFP-GluA2 in spines of CA1 neurons from MAP1B +/+ and MAP1B +/- mouse hippocampal slices. **B)** Quantification of fluorescence intensity in spines versus the adjacent dendritic shaft from neurons like those in (A). Data are presented as cumulative distributions of spine/dendrite ratios. **C)** Average spine/dendrite ratio for GFP-GluA2 in CA1 neurons from MAP1B +/+ and MAP1B +/- slices. Error bars correspond to s.e.m.

3.2 Electrophysiological recordings in the presence of pep2m peptide.

The constitutive cycling of GluA2-GluA3 AMPARs in and out of synapses has been demonstrated to depend on the interaction between NSF and the C-terminus of the GluA2 subunit. A small peptide termed “pep2m” mimicking the NSF-binding site of GluA2 can be used to interfere with this interaction (Nishimune et al. 1998; Song et al. 1998; Luscher et al. 1999; Noel et al. 1999). When pep2m is infused intracellularly, a continuous reduction of AMPAR-mediated synaptic transmission can be observed as a consequence of AMPARs being removed from synapses, but failing to be inserted (or stabilized at synapses) again.

To corroborate that the impaired ability of GluA2 homomers to accumulate in spines was responsible for the decrease in basal transmission observed upon MAP1B-LC-GFP over-expression, we recorded AMPAR-dependent basal transmission in CA1 neurons over-expressing MAP1B-LC-GFP and in uninfected, control neurons adding pep2m to the internal solution contained in the patch pipette. As shown in figure 27, a rundown of AMPAR-mediated synaptic transmission was observed in uninfected neurons, as expected. Nevertheless, synaptic

transmission was stable over time in MAP1B-LC-GFP over-expressing CA1 neurons. This result clearly illustrates that, in the presence of MAP1B-LC-GFP, less GluA2-GluA3 AMPARs reach the synapse. As a consequence, infusing pep2m in CA1 hippocampal neurons over-expressing MAP1B-LC-GFP does not decrease basal synaptic transmission as it does in control neurons.

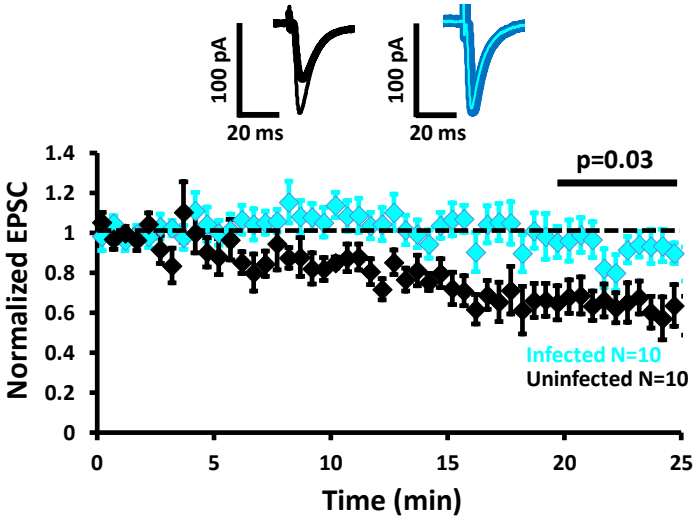


Figure 27. MAP1B-LC-GFP over-expression impairs the constitutive cycling of GluA2-GluA3 AMPA receptors. Time course of AMPAR EPSCs recorded in patch clamp configuration at -60 mV in the presence of picrotoxin, during the infusion of pep2m (included in patch pipette) in neurons over-expressing MAP1B-LC-GFP (cyan) and control neurons (black). Uninfected neurons undergo a significant depression of AMPAR response (p=0.007, between average response during 2-minute baseline, and average response between 20 and 25 minutes of recording, significance calculated with Wilcoxon test), whereas infected neurons do not (p=0.46, significance calculated with Wilcoxon test). The level of depression of AMPAR-dependent responses between 20 and 25 minutes of recording in uninfected cells is significantly different from that measured in infected cells. “p” corresponds to the statistical significance value calculated by Mann-Whitney test. Error bars represent s.e.m. Sample traces for uninfected and infected cells are shown above the plot.

3.3 Rectification index of endogenous AMPARs in the presence of MAP1B-LC-GFP.

To confirm that the only population of AMPARs targeted by the over-expressed MAP1B-LC was the GluA2-GluA3 population, we measured the rectification properties of AMPARs in CA1 neurons over-expressing MAP1B-LC-GFP versus uninfected neurons (figure 28).

Opposite to the AMPARs that contain the GluA2 subunit, GluA2-lacking AMPARs (mainly GluA1-GluA1 homomers) are inwardly rectifying (Boulter et al. 1990; Hollmann et al. 1991; Verdoorn et al. 1991). Their presence at synapses can be estimated by measuring their rectification index, defined as the ratio of AMPAR-mediated responses recorded at -60 mV divided by the amplitude of AMPAR currents at +40 mV. As shown in figure 28, no difference in

rectification index could be observed in CA1 neurons over-expressing MAP1B-LC-GFP versus uninfected neurons. Thus, the synaptic delivery of GluA2-lacking receptors is not affected by the over-expression of MAP1B-LC-GFP.

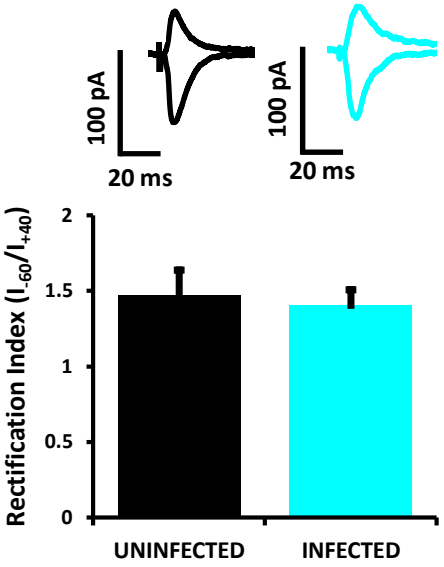


Figure 28. Rectification index in CA1 neurons over-expressing MAP1B-LC-GFP (cyan) and control neurons (black). AMPAR-mediated responses were recorded at -60 mV and +40 mV in the presence of spermine in the patch pipette. The rectification index was calculated as the ratio of responses at both holding potentials. Error bars represent s.e.m. Sample traces are shown above the corresponding columns of the plot.

Taken together, these data demonstrate that MAP1B-LC has the potential to specifically modulate the trafficking of the GluA2-GluA3 population of AMPARs. The over-expression of MAP1B-LC-GFP affects the ability of GluA2-GluA3 AMPARs to reach dendritic spines, and as a consequence, it has an impact on basal synaptic transmission.

4. MAP1B-LC over-expression reduces the mobile fraction of recombinant GluA2 AMPA receptors in dendrites.

4.1 Analysis of GFP-GluA2 mobility in spines.

To better understand how MAP1B-LC-GFP impairs the mobility of GluA2-GluA3 receptors, we co-expressed GFP-GluA2 with either MAP1B-LC-mCherry, or mCherry as a control, in organotypic hippocampal slices via biolistic transfection and analyzed GFP-GluA2 mobility in different compartments of the cell.

First, we assessed the mobility of recombinant GluA2 homomers in dendritic spines (figure 29A). A FRAP experiment was performed on spines expressing GFP-GluA2 in the presence or absence of recombinant MAP1B-LC; after the photobleaching event, the extent of fluorescence recovery was measured over 30 minutes (figure 29B). To our surprise, the same recovery profile was obtained for GFP-GluA2 in spines of cells expressing either MAP1B-LC-mCherry or

mCherry alone. The fraction of recovery and the dynamics of recovery were almost identical in both conditions. This was a striking result given the reduced accumulation of recombinant GluA2 in dendritic spines in the presence of augmented levels of the light chain of MAP1B.

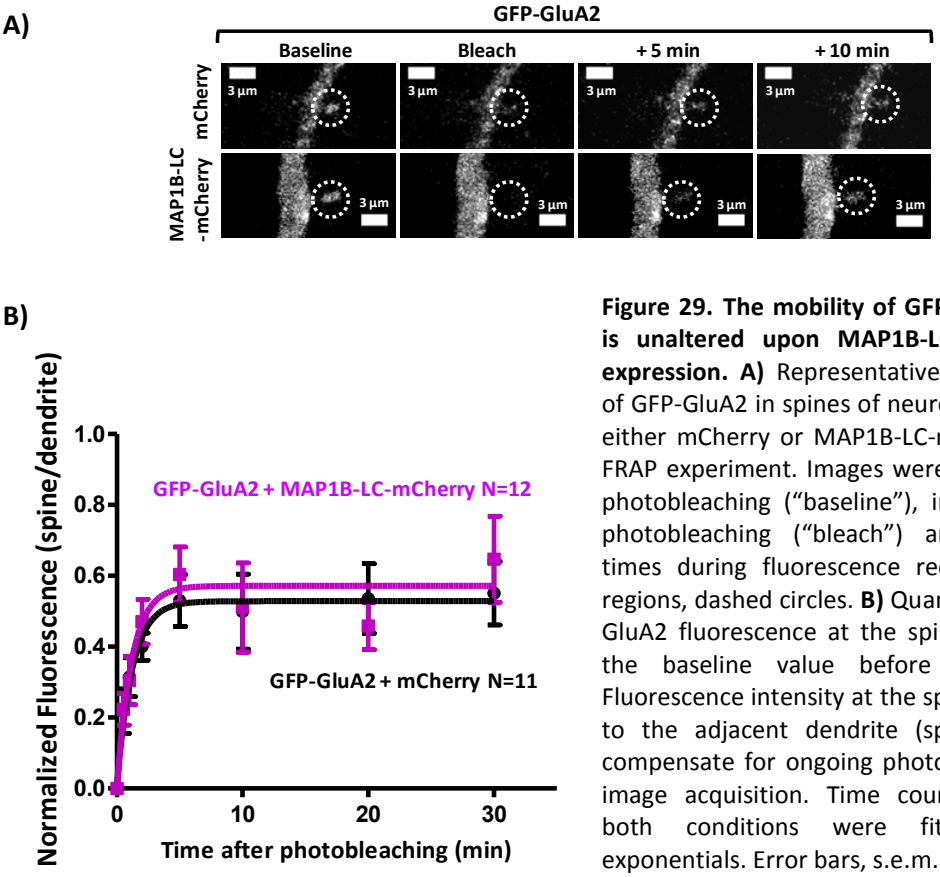


Figure 29. The mobility of GFP-GluA2 in spines is unaltered upon MAP1B-LC-mCherry over-expression. A) Representative confocal images of GFP-GluA2 in spines of neurons co-expressing either mCherry or MAP1B-LC-mCherry during a FRAP experiment. Images were acquired before photobleaching ("baseline"), immediately after photobleaching ("bleach") and at indicated times during fluorescence recovery. Bleached regions, dashed circles. **B)** Quantification of GFP-GluA2 fluorescence at the spine normalized to the baseline value before photobleaching. Fluorescence intensity at the spine is normalized to the adjacent dendrite (spine/dendrite) to compensate for ongoing photobleaching during image acquisition. Time courses obtained in both conditions were fitted to single exponentials. Error bars, s.e.m.

4.2 Analysis of GFP-GluA2 mobility in dendrites.

Next, we tested the mobility of recombinant GluA2 homomers in dendrites. Photobleaching of GFP-GluA2 was performed on the dendritic shafts of CA1 neurons co-expressing either MAP1B-LC-mCherry or mCherry alone, and the recovery of green fluorescence was followed for 20 minutes. As shown in figures 30A and 30B, a fast and almost complete fluorescence recovery could be measured in dendrites of mCherry-expressing cells. Already at 5 minutes, an 80% of the initial fluorescence was restored in the area of the dendrite that had undergone photobleaching. On the contrary, a much smaller fluorescence recovery was measured in the dendrites of cells over-expressing MAP1B-LC-mCherry. At 20 minutes after the bleaching, only a 50% fraction of the initial fluorescence was restored. Nevertheless, the dynamics of fluorescence recovery were comparable in both situations, as revealed by the fitting of both time courses to single exponentials. This result allowed us to hypothesize that the over-

expression of MAP1B-LC was yielding a smaller fraction of GluA2-GluA3 AMPARs able to traffic along dendrites, and as a consequence, it was reducing the pool of AMPARs available to be continuously delivered into dendritic spines. Yet, those that reached dendritic spines did it with the same dynamics as in control conditions.

In accordance with previous experiments, we then evaluated if the observed effect on AMPARs mobility along dendrites was specific for the homomeric GluA2 population. We repeated the FRAP experiments on dendrites described above, but co-expressing GFP-GluA1 instead of GFP-GluA2 with either MAP1B-LC-mCherry or mCherry alone. As shown in figures 30C and 30D, the recovery profile for both conditions was basically overlapping. This result again supported our hypothesis that MAP1B-LC exerts its effects on a specific population of AMPARs.

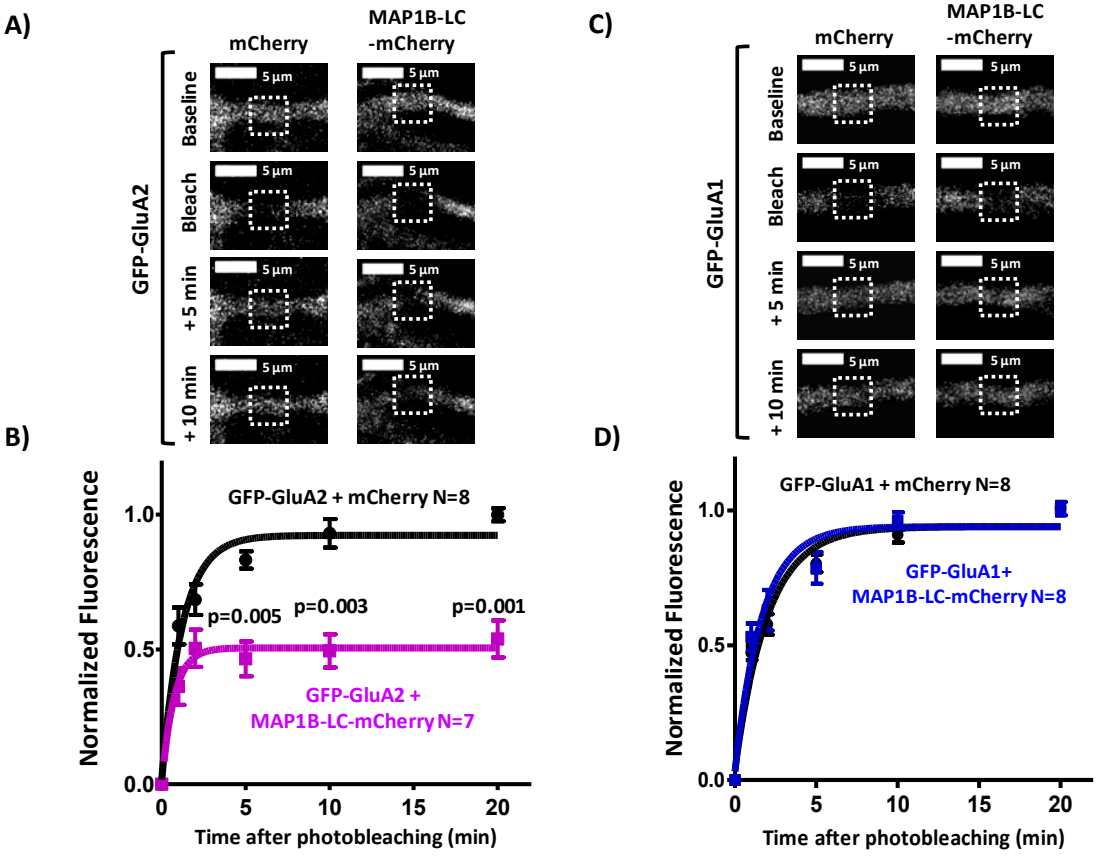


Figure 30. MAP1B-LC-mCherry over-expression impairs GluA2 trafficking along dendrites. A) Representative confocal images of GFP-GluA2 in dendritic branches of neurons expressing either mCherry or MAP1B-LC-mCherry during a FRAP experiment. Images were acquired before photobleaching ("baseline"), immediately after photobleaching ("bleach") and at indicated times during fluorescence recovery. Bleached regions, dashed squares. **B)** Quantification of GFP-GluA2 fluorescence at the dendrite normalized to the baseline value before photobleaching. Fluorescence intensity at the bleached area is normalized to a reference, "non-bleached" region of the dendrite to compensate for ongoing photobleaching during image acquisition. Time courses in both conditions were fitted to single exponentials. "p" values correspond to statistical significance values calculated according to Mann-Whitney test. Error bars represent s.e.m. **C)** and **D)** Same experiment as in (A) and (B), but co-expressing GFP-GluA1 with either mCherry or MAP1B-LC-mCherry, instead of GFP-GluA2.

5. The surface expression of the endogenous GluA2 subunit of AMPARs is diminished upon MAP1B-LC-GFP over-expression.

To verify if the immobilization of AMPARs we had observed upon MAP1B-LC over-expression could result in intracellular trapping, we aimed to characterize the surface expression of the GluA2 subunit of AMPARs in hippocampal primary neurons in culture. Primary neurons were infected with Sindbis virus to over-express either MAP1B-LC-GFP or GFP, as a control, and immunostained against either the surface population or the total population of GluA2-containing AMPARs.

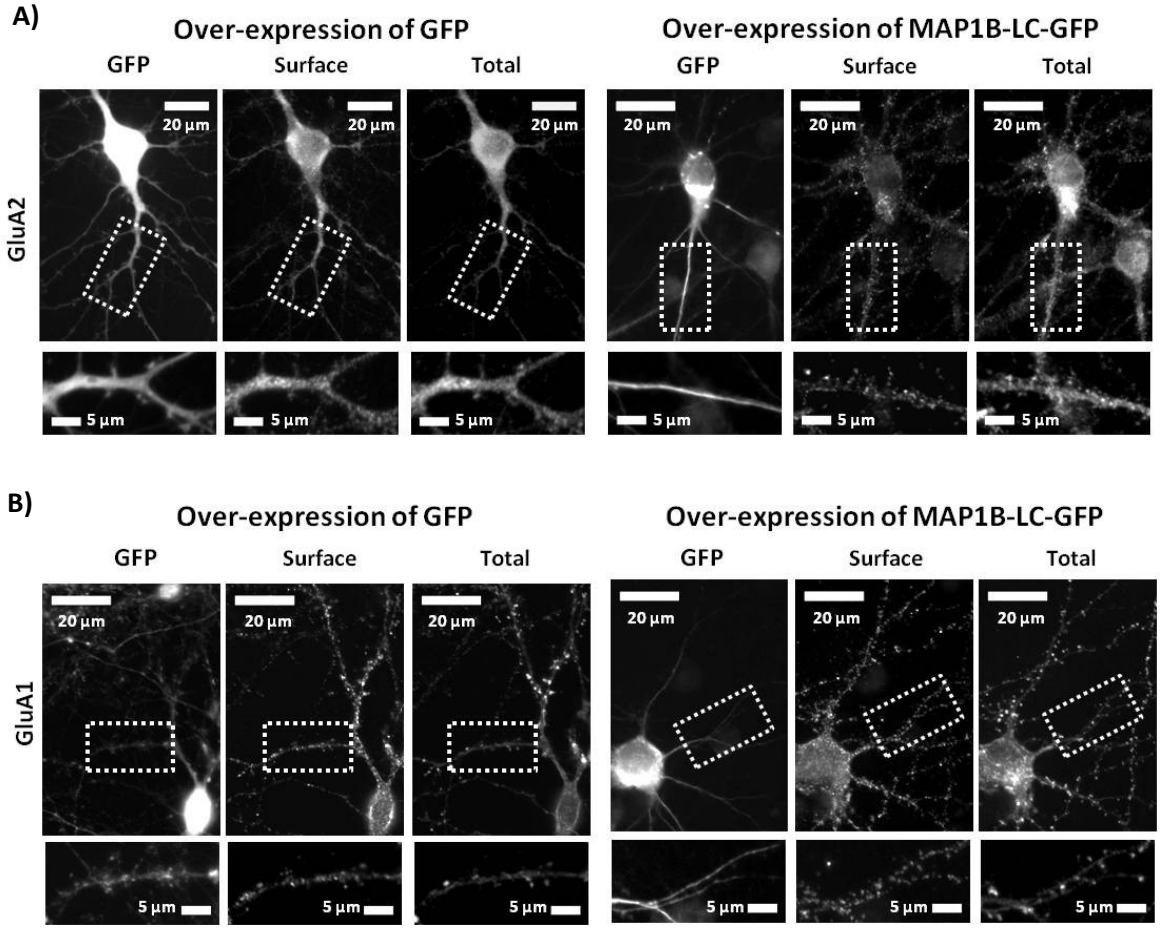


Figure 31. MAP1B-LC-GFP over-expression impairs GluA2 surface delivery in hippocampal primary neurons (DIV 16-21). A) Left, representative images of hippocampal primary neurons over-expressing GFP and stained against surface and total GluA2. **Right,** hippocampal neurons over-expressing MAP1B-LC-GFP and stained against surface and total GluA2. White rectangles indicate insets of representative dendritic branches shown in lower panels. **B)** Same experiment as in A, but staining against surface and total GluA1 instead of GluA2.

As shown in figures 31A and 32A, a significant decrease in the surface expression of the endogenous GluA2 subunit was observed in neurons over-expressing MAP1B-LC-GFP. This

reduction was not due to a down-regulation of total expression levels of the protein (figures 32A and 32B).

To demonstrate the subunit specificity of this effect, we performed the same experiment but looking at the GluA1 subunit. As expected, the surface levels of expression of this subunit were unaffected in the presence of over-expressed MAP1B-LC-GFP (figures 31B and 32A); total levels of expression were not changed either (figures 32A and 32B). This result supported the idea that MAP1B-LC is able to modulate the surface delivery of endogenous GluA2-containing AMPARs without affecting the GluA1 population.

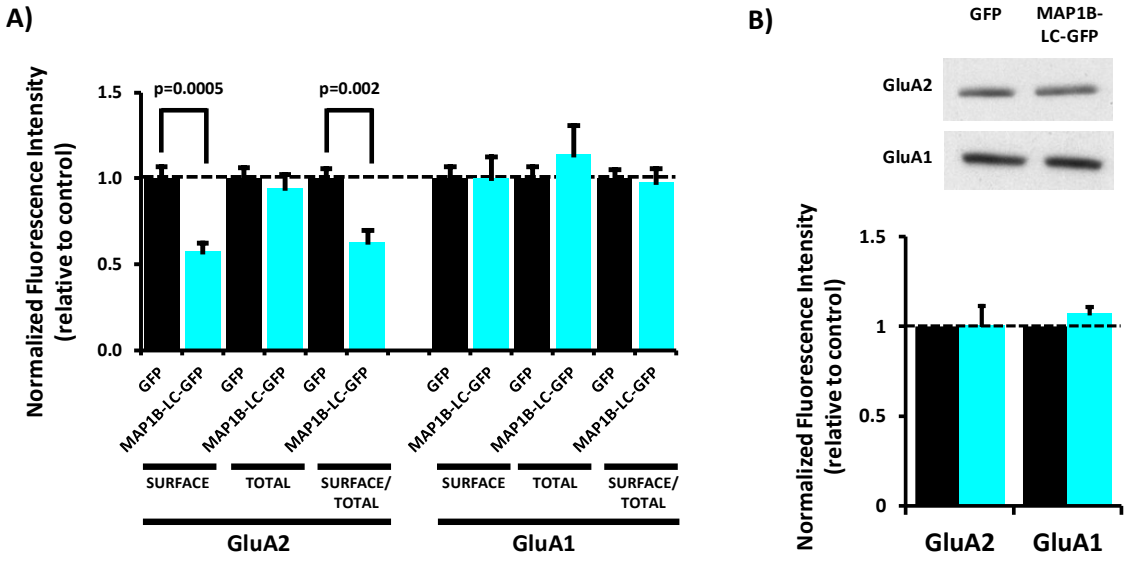


Figure 32. Quantification of the impairment in GluA2 surface delivery observed upon MAP1B-LC-GFP over-expression. A) Quantification of fluorescence intensity corresponding to surface, total and surface over total GluA2 and GluA1 in dendritic branches of neurons over-expressing either GFP or MAP1B-LC-GFP, like those shown in figures 30A and 30B. Statistical significance calculated according to Wilcoxon test (n=20 cells per condition, 2 independent experiments). **B) Upper panel,** western blot of total GluA2 and GluA1 levels in hippocampal primary neurons over-expressing either GFP or MAP1B-LC-GFP. **Lower panel,** quantification of total GluA2 and GluA1 levels from 3 independent experiments. Error bars, s.e.m.

6. MAP1B regulates microtubule-dependent transport of transferring receptor.

6.1 Over-expression of MAP1B-LC.

We had demonstrated that MAP1B-LC is able to regulate the surface delivery of a specific population of AMPARs. As a consequence, it modulates the strength of synaptic transmission in neurons. Still, the molecular mechanism underlying these effects on AMPARs needed to be unraveled.

MAP1B-LC has been shown to associate with microtubules and stabilize them against the action of depolymerizing drugs (Pedrotti and Islam 1995; Togel et al. 1998). Actually, we have been able to show in this work that, when over-expressed, MAP1B-LC-GFP co-localizes mainly with stable microtubules (figure 6). In neurons, vesicles containing AMPARs as well as many other membrane-associated proteins are transported along dendrites in a microtubule-dependent manner, thanks to microtubule-associated motor proteins as kinesins and dyneins (Hirokawa and Takemura 2005; Kapitein and Hoogenraad 2011). Some authors have suggested a certain degree of interference between microtubule-associated proteins, which decorate microtubules, and motor proteins that traffic and carry their cargo along them (Seitz et al. 2002; Tokuraku et al. 2007; Dixit et al. 2008).

To test the possibility that MAP1B-LC might be modulating the trafficking of AMPARs along dendrites through the regulation of microtubule-dependent transport, we analyzed the speed of transport of transferring receptor (TfR) along dendrites of hippocampal primary neurons over-expressing either MAP1B-LC-GFP or GFP as a control (figure 33). TfR has been characterized as a transmembrane protein specifically sorted into dendrites by means of microtubule-dependent transport (West et al. 1997; Burack et al. 2000), and so, it has been largely used as a reporter of such a process in neurons.

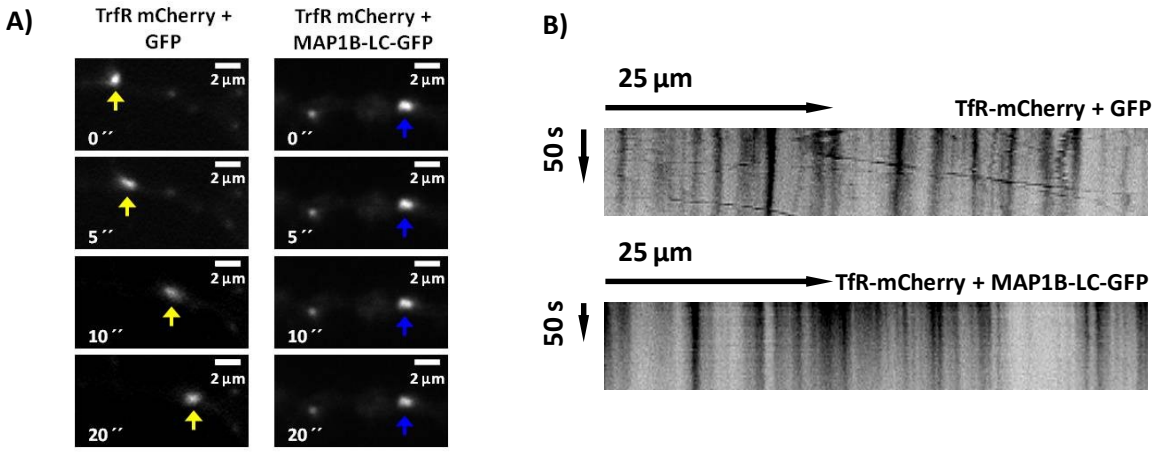


Figure 33. Imaging of TfR-mCherry clusters moving along dendrites of primary hippocampal neurons (DIV 9-10) co-expressing either GFP or MAP1B-LC-GFP. A) Representative sequential pictures of TfR clusters imaged over time in dendrites of hippocampal primary neurons co-transfected with TfR-mCherry + GFP, or TfR-mCherry + MAP1B-LC-GFP. **B)** Representative examples of kymographs generated from time-lapse images like those shown in (A).

After co-transfection, a series of time-lapse images were acquired in neurons co-expressing TfR-mCherry and MAP1B-LC-GFP or GFP; the movement of TfR-mCherry clusters along dendrites was followed and measured using kymographs (figure 33). As shown in figure 34, the cumulative distribution corresponding to the instantaneous speed of events of transport (see

"Materials and Methods") for TfR-mCherry clusters in neurons co-expressing MAP1B-LC-GFP was clearly left-shifted. That is, TfR-mCherry clusters moved significantly slower in the presence of over-expressed MAP1B-LC-GFP. This result suggested that indeed enhanced levels of MAP1B-LC have the potential to slow down microtubule-dependent transport of TfR.

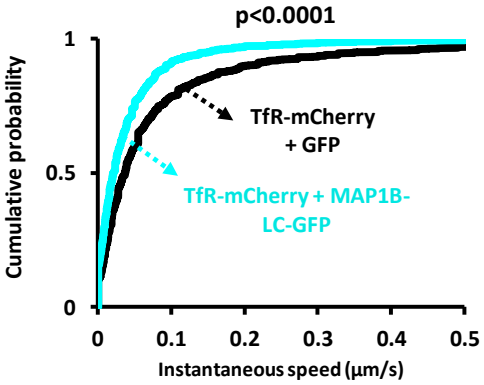


Figure 34. MAP1B-LC-GFP over-expression delays microtubule-dependent transport of TfR. Cumulative distribution of instantaneous speed of events of transport for TfR in primary neurons (DIV 9-10) co-expressing GFP (black) (N=2663 events of transport, 3 cells from 2 independent experiments) or MAP1B-LC-GFP (cyan) (N=2943 events, 3 cells, 2 independent experiments).

6.2 Down-regulation of MAP1B.

Nevertheless, the ability of MAP1B-LC to modulate microtubule-based transport was observed upon over-expression. We wanted to test if such a capacity was only due to over-expression or could be attributed to the endogenous MAP1B protein, too. To this end, we chose to down-regulate MAP1B expression in hippocampal primary neurons, using a scrambled shRNA sequence as a control. We then transfected these neurons with TfR-GFP and followed the movement of TfR-GFP clusters along dendrites. As shown in figure 35, the cumulative distribution of instantaneous speed for TfR-GFP clusters was right-shifted if MAP1B protein was down-regulated, meaning that microtubule-dependent transport seemed to be accelerated in the absence of MAP1B. This result was supported by a previous report linking MAP1B to retrograde transport of mitochondria in axons (Jimenez-Mateos et al. 2006).

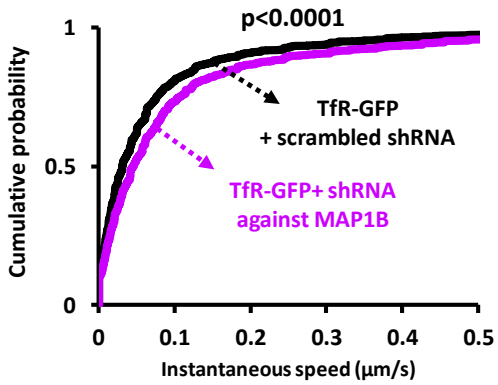


Figure 35. MAP1B acute down-regulation slightly accelerates microtubule-dependent transport of TfR. Hippocampal primary neurons were infected at DIV 0 with lentiviral vectors expressing a shRNA against MAP1B or a scrambled shRNA, and transfected with TfR-GFP at DIV 8. The graph shows the cumulative distribution for instantaneous speed of events of transport for TfR in neurons (DIV 10) expressing a shRNA against MAP1B (N=2819 events of transport, 4 cells, 4 independent experiments) or a scrambled shRNA (N=2125 events, 4 cells, 4 independent experiments). Statistical significance calculated according to Kolmogorov-Smirnov test.

The quantification of the percentage of immobile events of transport (velocity <0.1 µm/s) and the mean speed of mobile events of transport (velocity >0.1 µm/s) upon MAP1B-LC over-expression or MAP1B down-regulation is shown in figure 36. In a coherent manner, the over-expression of MAP1B-LC-GFP yields a higher proportion of immobile events of transport and a lower velocity of those that are mobile, whereas the down-regulation of the protein is associated with a lower proportion of immobile events, and a higher mean velocity of those that move at the expected velocities for microtubule-dependent transport. Hence, these results would imply a bi-directional modulation of microtubule-dependent transport by MAP1B, so that transport will get delayed if MAP1B levels are increased, and accelerated upon MAP1B depletion.

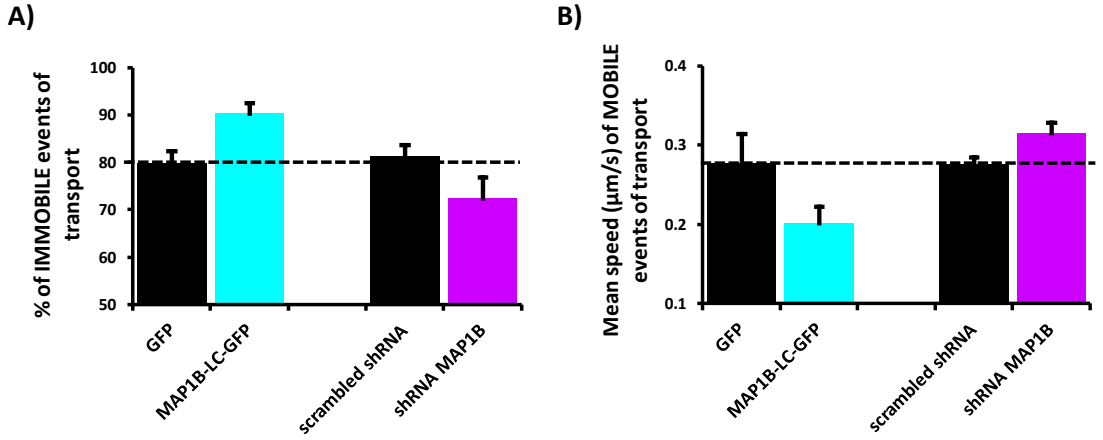


Figure 36. MAP1B modulates microtubule-dependent transport of Transferrin Receptor (TfR) in hippocampal primary neurons (DIV 9-10). A) Quantification of the frequency of immobile events of transport for TfR in neurons co-expressing either GFP or MAP1B-LC-GFP, or a shRNA against MAP1B or a scrambled shRNA. **B)** Mean speed of mobile events of transport. Error bars, s.e.m.

7. MAP1B-LC might enhance the interaction of GluA2 with microtubules.

The quantification of TfR transport along dendrites is informative about the global process of microtubule-dependent transport in neurons, but does not provide a direct evidence of how specific populations of AMPARs are transported along dendrites. Our premise is that MAP1B-LC targets specifically the GluA2-GluA3 population of AMPARs without affecting the GluA1-GluA2 population. The fact that direct imaging of AMPARs being transported along microtubules in dendrites has been proven technically unfeasible so far has made it impossible to demonstrate our hypothesis of a subunit-specific effect of MAP1B-LC also in the microtubule-dependent transport of AMPARs.

Although still lacking a molecular link between AMPARs and MAP1B-LC, we reasoned that if MAP1B-LC was able to decrease the mobile population of GluA2-GluA3 AMPARs in dendrites, it

could maybe be due to an increased interaction of AMPARs with microtubules upon the over-expression of MAP1B-LC.

As an attempt to demonstrate an increased interaction of AMPARs with microtubules in the presence of MAP1B-LC-GFP, we designed a microtubule co-sedimentation experiment in which three different concentrations of assembled microtubules were used to potentially co-sediment GluA2. We were expecting to observe a dose-dependent effect in GluA2-microtubule interaction with the increasing concentration of tubulin polymers. After centrifugation, three fractions were distinguished and analyzed separately by SDS-PAGE: supernatant, interphase and pellet. Hippocampal slices over-expressing GFP instead of MAP1B-LC-GFP were used as control.

We first analyzed the amount of tubulin present in each fraction in MAP1B-LC-GFP and GFP conditions. As shown in figure 37A, tubulin was equally distributed in the three fractions in both conditions. The quantification of tubulin in the soluble fraction (supernatant plus interphase) (figure 37C) shows no difference between MAP1B-LC-GFP- and GFP-expressing slices. This uniformity prevented the possible bias of more sedimented GluA2 in the condition with more sedimented tubulin, if that had been the case.

Next, we analyzed GluA2 presence in supernatant, interphase and pellet. In protein samples from GFP-expressing slices, GluA2 was mainly present in the pellet fraction but could also be detected in the soluble fraction, more clearly in the case of the two extreme concentrations of tubulin (1.3 mg/mL and 0.14 mg/mL). Interestingly, the over-expression of MAP1B-LC-GFP seemed to facilitate GluA2 accumulation in the pellet, as supernatant and interphase fractions appeared devoid of the protein at least for the concentrations of tubulin previously mentioned (figure 37C). As a dose-dependent effect of microtubule concentration on GluA2 ability to co-sediment was not obvious, we quantified GluA2 presence in soluble and insoluble fractions for the three concentrations of tubulin used in the assay and pooled these data together. A clear difference in the amount of GluA2 in the soluble fraction could be ascertained in GFP- versus MAP1B-LC-GFP-expressing samples (figure 37C).

To validate this result, negative controls were considered next. GluA1 was not probably a good control, as its possible presence in the insoluble fraction could just reflect its ability to interact with the GluA2 subunit. We had previously shown that MAP1B-LC decreases AMPAR-dependent transmission but does not affect NMDAR-dependent transmission, so it is reasonable to think that the trafficking of NMDARs is not altered in the presence of MAP1B-LC-

GFP. Indeed, the distribution of the NMDARs subunits 2A and 2B in soluble and insoluble fractions did not change in the MAP1B-LC-GFP condition comparing to the GFP condition (figure 37B).

KIF23 (a member of the kinesin family playing a role in the organization of the mitotic spindle during mitosis) was also used as a negative control. KIF23 interacts with microtubules, but being involved in a biological process independent of microtubule-based transport of AMPARs in neurons, such interaction was not expected to be modulated by MAP1B-LC. As shown in figure 37B, KIF23 equally co-sediments with microtubules in samples from GFP-expressing slices and MAP1B-LC-GFP-expressing slices.

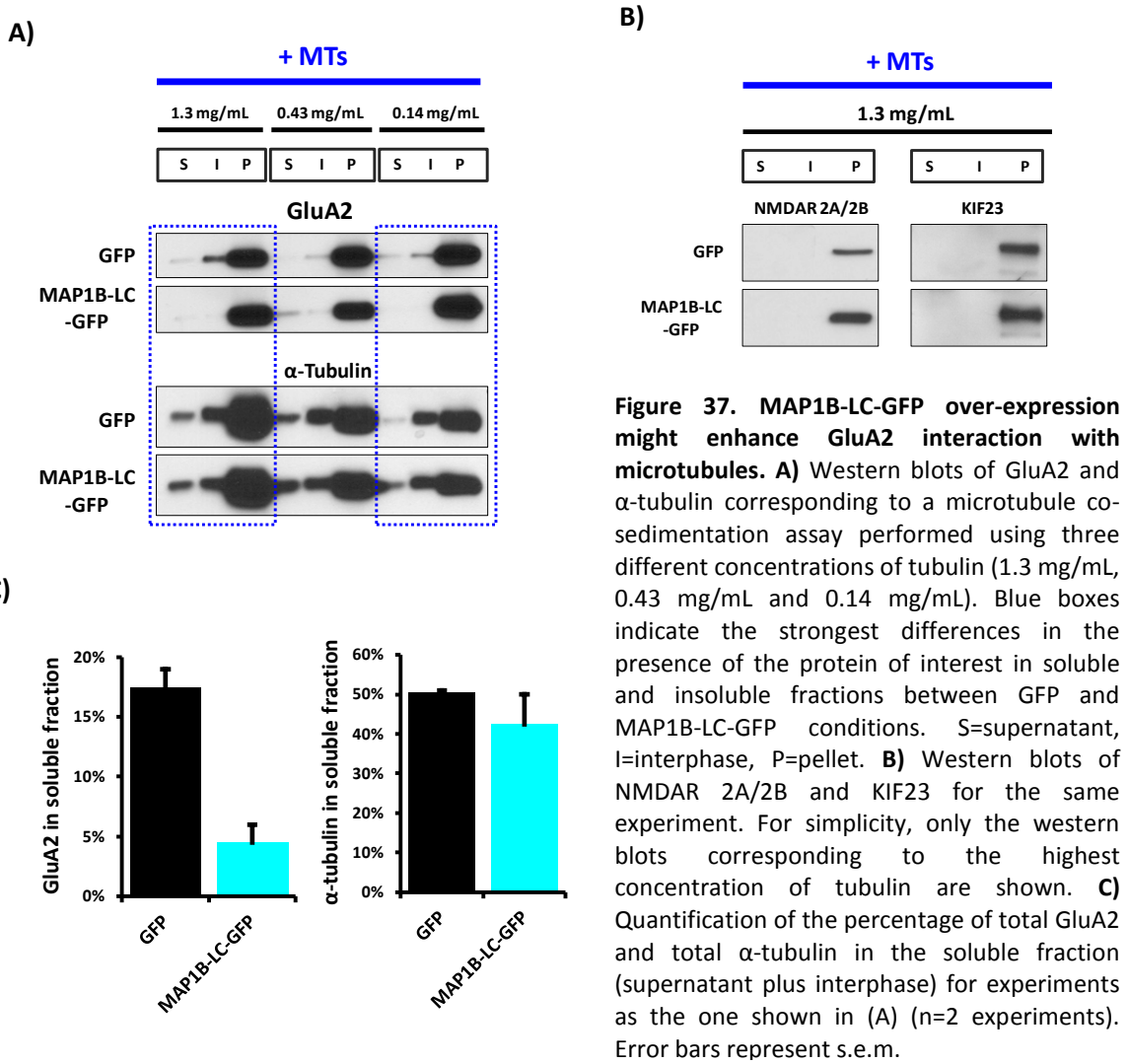


Figure 37. MAP1B-LC-GFP over-expression might enhance GluA2 interaction with microtubules. A) Western blots of GluA2 and α-tubulin corresponding to a microtubule co-sedimentation assay performed using three different concentrations of tubulin (1.3 mg/mL, 0.43 mg/mL and 0.14 mg/mL). Blue boxes indicate the strongest differences in the presence of the protein of interest in soluble and insoluble fractions between GFP and MAP1B-LC-GFP conditions. S=supernatant, I=interphase, P=pellet. **B)** Western blots of NMDAR 2A/2B and KIF23 for the same experiment. For simplicity, only the western blots corresponding to the highest concentration of tubulin are shown. **C)** Quantification of the percentage of total GluA2 and total α-tubulin in the soluble fraction (supernatant plus interphase) for experiments as the one shown in (A) (n=2 experiments). Error bars represent s.e.m.

It is worth noting that the results presented above correspond to two independent experiments. In further repetitions, we frequently found that the distribution of tubulin among supernatant, interphase and pellet was variable; this rendered the direct comparison of GluA2

amount in soluble and insoluble fractions between the GFP and the MAP1B-LC-GFP conditions invalid. Independently, we performed microtubule co-sedimentation assays with protein extracts from MAP1B +/+ and MAP1B +/- animals, in an attempt to confirm a bi-directional modulation of the interaction between GluA2 and microtubules by MAP1B (data not shown). Again, we obtained very variable results. In sum, we decided that this approach might not be reproducible among experiments and that in any case, it was not solving the question about the molecular link between MAP1B-LC and AMPARs.

C) GRIP1 as the molecular link between MAP1B-LC and AMPAR trafficking.

1. MAP1B-LC-GFP impairs GRIP1 dendritic targeting in hippocampal primary neurons.

Regarding the trafficking of AMPARs along dendrites, we demonstrated that the over-expression of MAP1B-LC-GFP exerts a specific effect on the GluA2-GluA3 population of AMPARs but not on the GluA1-GluA2 population. In this scenario, a new intervening partner was required to justify such specificity.

MAP1B-LC has been shown to interact with GRIP1 (Seog 2004; Davidkova and Carroll 2007). On the other hand, GRIP1 has been proposed to act as the adaptor protein for GluA2-containing AMPARs in kinesin-dependent transport along microtubules in dendrites (Setou et al. 2002). To test if the observed effect of MAP1B-LC on the dendritic trafficking of AMPARs could be related to MAP1B-LC interaction with GRIP1, we measured GRIP1 distribution in hippocampal primary neurons over-expressing MAP1B-LC-GFP or GFP as a control.

As shown in figure 38A, dendritic regions of 50 µm length were defined in neurons over-expressing either MAP1B-LC-GFP or GFP, and the mean fluorescence intensity due to GRIP1 was measured. The average fluorescence intensity in the cell body was also quantified. In neurons over-expressing MAP1B-LC-GFP, the fluorescence intensity corresponding to GRIP1 was increased in the cell body and decreased in dendrites, yielding a reduced dendrite/soma ratio of intensities comparing to neurons over-expressing GFP (figure 38B). These data would point to an altered delivery of GRIP1 from the cell body towards the dendrites in the presence of MAP1B-LC-GFP.

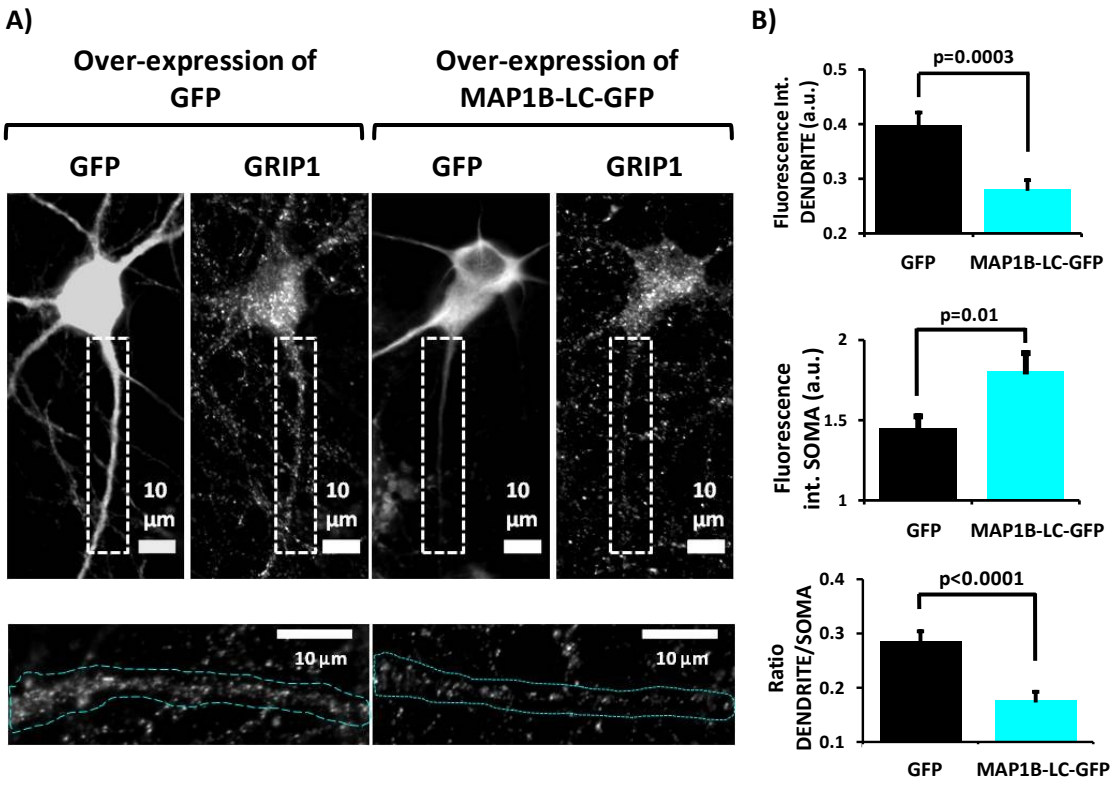


Figure 38. The over expression of MAP1B-LC-GFP impairs the dendritic targeting of GRIP1 in hippocampal primary neurons (DIV 19-22). A) Representative examples of hippocampal primary neurons over-expressing either GFP (control) or MAP1B-LC-GFP, and stained against GRIP1. Insets correspond to dendrites shown below in higher magnification. A precise delineation of dendrites (cyan lines) was carried out before proceeding to quantification of mean fluorescence intensity for GRIP1. **B)** Quantification of fluorescence intensity for GRIP1 in dendrites (top) and soma (middle) of cells expressing either GFP (n=36 cells) or MAP1B-LC-GFP (n=30 cells) from 3 independent experiments. A.u., arbitrary units. The ratio dendrite/soma for both types of neurons is shown below. Statistical significance calculated according to Mann-Whitney test. Error bars, s.e.m.

Then, we measured total number, size and total fluorescence intensity of GRIP1 clusters in 50-µm dendritic regions, as described in figure 39A. As shown in figure 39B, the number of GRIP1 clusters was significantly reduced in neurons over-expressing MAP1B-LC-GFP comparing to those over-expressing GFP, although the size of these clusters and the quantity of GRIP1 in them (related to the total fluorescence intensity of each cluster) seemed to be equivalent in both conditions. Taken together, these data would support the idea that GRIP1 dendritic targeting is impaired upon the over-expression of MAP1B-LC-GFP; as a consequence, the dendritic transport of GluA2-containing AMPARs would be delayed. Under these circumstances, the constitutive access of the GluA2-GluA3 population of AMPARs to dendritic spines and synapses might be impeded resulting in a net reduction of basal synaptic transmission.

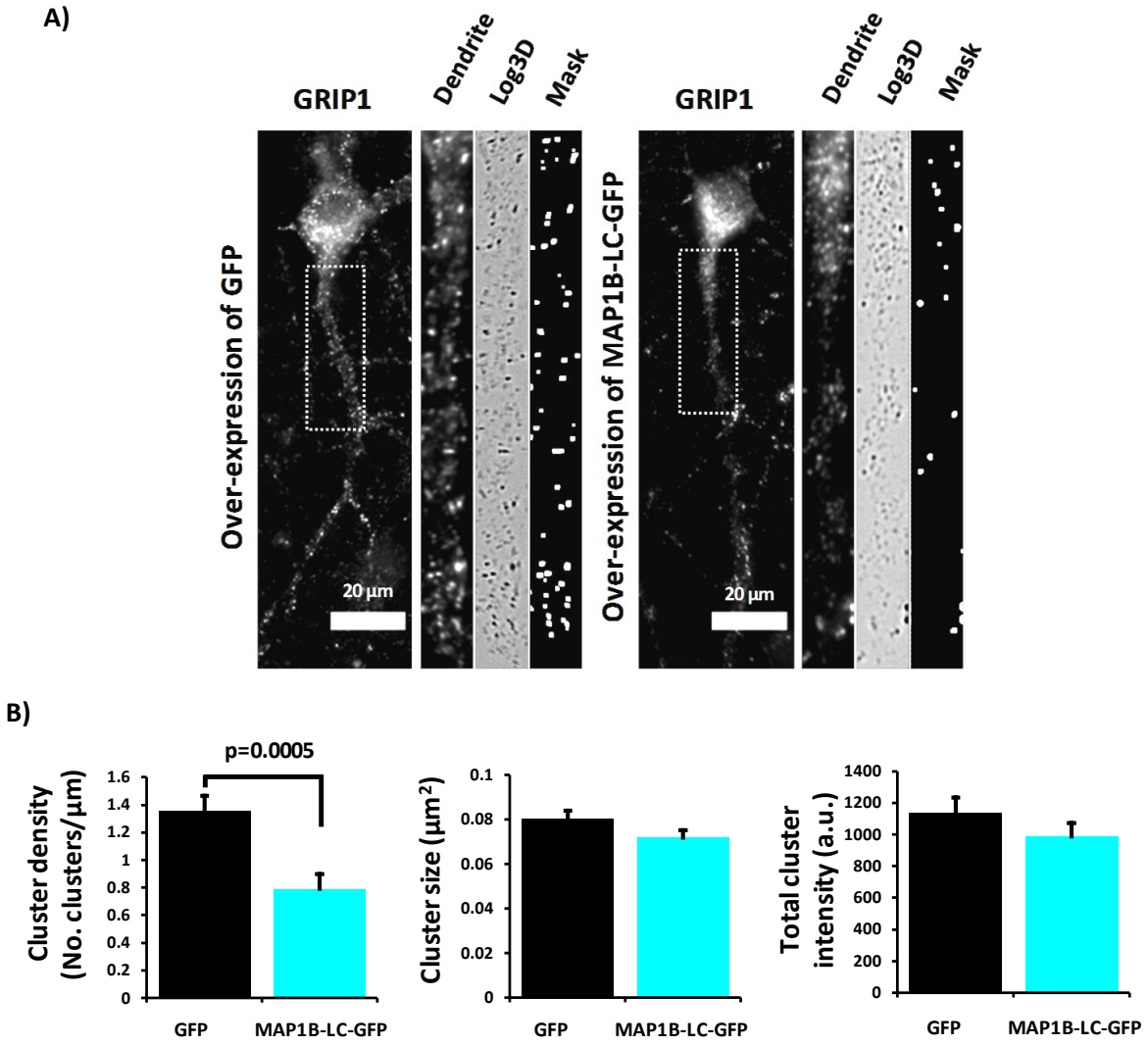
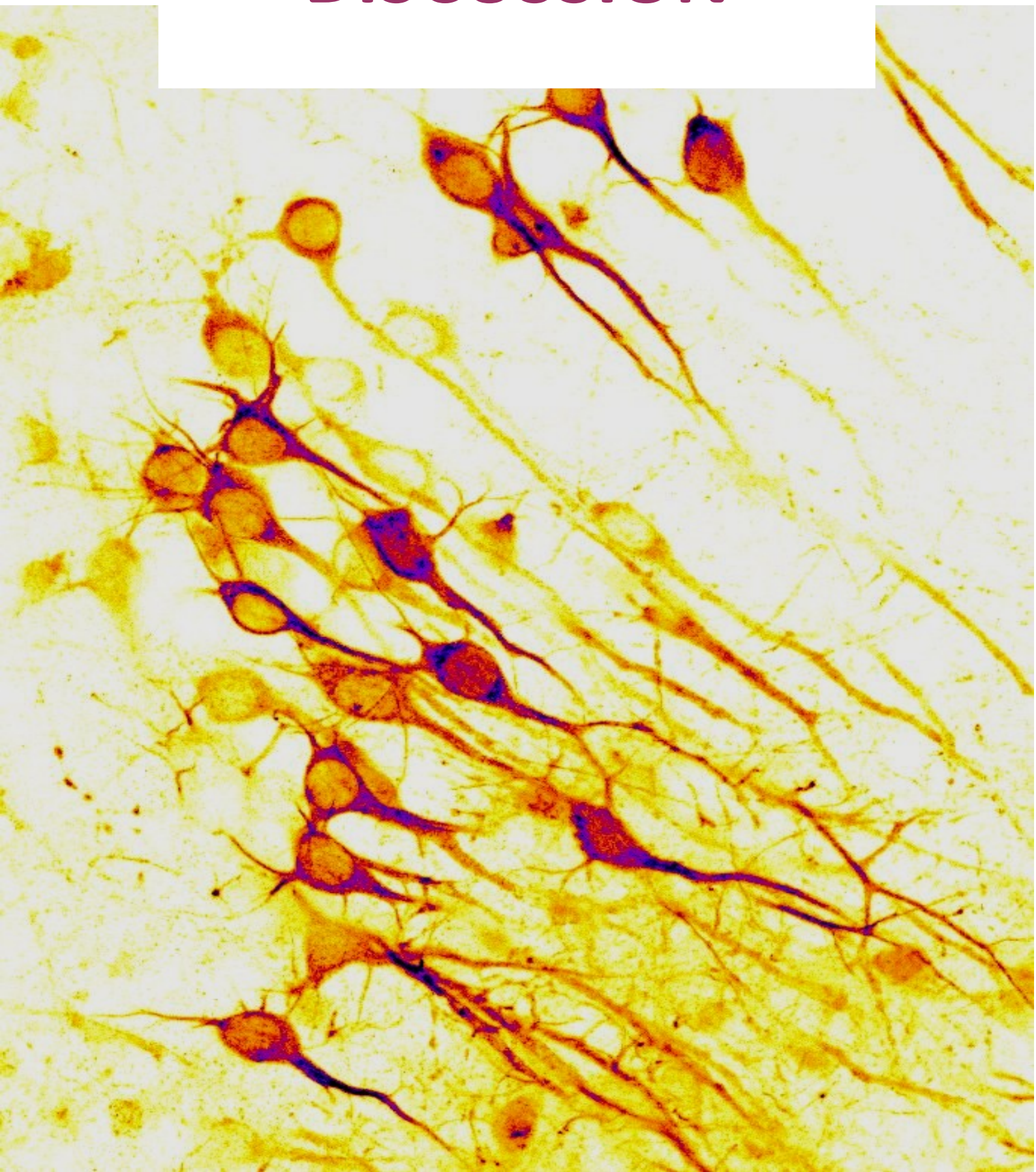


Figure 39. The over-expression of MAP1B-LC-GFP decreases the number of clusters of GRIP1 along dendrites of hippocampal primary neurons (DIV 19-22). A) Left, the number of clusters of GRIP1 along 50 µm of dendrite was analyzed in GFP-expressing hippocampal neurons, like the one shown in the picture. The delineated segment of dendrite ("dendrite") was used as template for the Log3D-plugin (Image J) transformation ("Log3D"), and the generation of a mask that illustrates the result of the application of an intensity threshold ("mask") on the Log3D image. **Right,** the same kind of analysis was performed in neurons over-expressing MAP1B-LC-GFP. **B)** Quantification of density, size and total fluorescence intensity of GRIP1 clusters for hippocampal primary neurons over-expressing GFP (n=26 cells) or MAP1B-LC-GFP (n=23 cells) from 3 independent experiments. Statistical significance according to Mann-Whitney test. Error bars represent s.e.m. A.u., arbitrary units.

DISCUSSION



The main goal of the work compiled in this thesis has been to describe the role played by the light chain of MAP1B in the trafficking of AMPARs in hippocampal CA1 neurons. As a result, MAP1B-LC has been characterized as a crucial factor mediating the dendritic trafficking and surface expression of the GluA2-GluA3 population of AMPARs, very likely through its interaction with GRIP1.

A) Dynamics of MAP1B-LC in CA1 hippocampal neurons.

1. Anchoring to microtubules vs. transient mobilization during the induction of synaptic plasticity.

1.1 MAP1B-LC is mainly bound to microtubules.

Regarding MAP1B-LC distribution inside CA1 neurons, the data previously presented indicate that MAP1B-LC is likely anchored to microtubules. Immunofluorescence experiments on hippocampal slices demonstrate that over-expressed MAP1B-LC displays a filamentous pattern of distribution that is reminiscent of the microtubular network; in addition, this appearance is lost when neurons are pre-treated with a microtubule-depolymerizing drug (vinblastine). In primary hippocampal neurons, MAP1B-LC staining is coincident with that of stable microtubules in dendrites (detyrosinated and acetylated tubulin), and live imaging experiments evidence that its basal mobility is comparable to that of microtubules themselves.

A co-immunoprecipitation experiment using an antibody against MAP1B-HC revealed that MAP1B-LC-GFP hardly interacts with the heavy chain of the endogenous protein *in vitro*. This circumstance would be in favor of MAP1B-LC-GFP binding to microtubules. It has been described that the light chain of MAP1B exerts distinct effects on the morphological appearance and stability of the microtubule network as long as it is free from the heavy chain. Indeed, as the interaction of the light chain of MAP1B with microtubules has been shown to be diminished in the presence of the heavy chain, MAP1B-HC has been proposed to act as the inhibitory subunit of the full length protein (Noiges et al. 2002). Therefore, the observations reported in the present work would result from MAP1B-LC acting on its own on the microtubule network, without the inhibitory influence of the heavy chain.

At this point, it is important to recall that MAP1B-LC exists naturally in a considerable excess over the heavy chain (Mei et al. 2000); thereby, it is reasonable to think that the light chain

might have additional functions *in vivo* outside of the complex with the heavy chain. The over-expression of MAP1B-LC would represent a suitable model for this situation.

1.2 Dynamics in basal conditions vs. dynamics during the induction of synaptic plasticity.

In basal conditions, the mobility of recombinant MAP1B-LC is negligible, as shown by live imaging experiments. In FRAP experiments, only a 20% of the initial fluorescence is recovered after more than 60 minutes from the bleaching event. Complementarily, a very slow decay of fluorescence can be observed after photoactivation of MAP1B-LC-Dendra, which could be due, at least in part, to photobleaching. It is interesting that a similar behavior can be observed for Dendra-tubulin in photoactivation experiments.

In neurons, different organelles and membrane-associated proteins are rapidly transported along axons in a motor-based process known as “fast axonal transport”; dendritic transport of this kind has been demonstrated to occur as well. In contrast, the term “slow axonal transport” refers to the movement of the proteins that comprise the cytoskeleton itself, including tubulin and microtubule-associated proteins, which occurs at a much lower rate (Baas and Buster 2004). It is likely that the slow mobility of recombinant MAP1B-LC and tubulin observed in live imaging experiments corresponds to this modality of transport taking place in dendrites, too; it could also reflect a slow turnover of both structural proteins. In any case, it strengthens the idea that recombinant MAP1B-LC is forming a complex with microtubules in dendrites of CA1 pyramidal neurons.

Surprisingly, a transient increase in MAP1B-LC mobility could be assessed upon the induction of both LTD and LTP. Such an increase could not be observed for Dendra-tubulin in photoactivation experiments, indicating that it is probably an intrinsic property of MAP1B-LC.

The binding of MAP1B to microtubules and microfilaments seems to be modulated by phosphorylation, although the underlying mechanism is not well understood yet. Concerning MAP1B interaction with microtubules, current evidence is conflicting. On the one hand, it has been described that the inhibition of PP2A and PP2B, two of the protein phosphatases that act on MAP1B (Ulloa et al. 1993c), is correlated with a decreased binding of MAP1B to microtubules (Gong et al. 2000). This would imply that the lack of dephosphorylation, and so an increased phosphorylation, detaches MAP1B from microtubules. On the other hand, the depletion of the catalytic subunits of CKII, one of the kinases known to phosphorylate MAP1B, has been reported to reduce MAP1B binding to the microtubule cytoskeleton (Ulloa et al.

1993b; Ulloa et al. 1993c), suggesting that it is phosphorylation and not dephosphorylation that promotes MAP1B attachment to microtubules.

Both PP2B and CKII have been involved in hippocampal synaptic plasticity (Charriaut-Marlangue et al. 1991; Mulkey et al. 1993). It is tempting to speculate that the signaling cascades activated during synaptic plasticity in hippocampus, involving the activation of numerous mediators such as kinases and phosphatases whose activity is interconnected, might lead to temporal changes in the phosphorylation state of MAP1B that result in a transient detachment from the microtubule cytoskeleton. However, the phosphorylation sites identified in MAP1B sequence so far are all located at the heavy chain (Riederer 2007). We have demonstrated that over-expressed MAP1B-LC is not primarily forming a complex with MAP1B-HC, so it does not seem very reasonable that the binding of recombinant MAP1B-LC to microtubules is modulated by putative changes in MAP1B-HC phosphorylation. One possibility would be that the light chain harbors also phosphorylation sites that have not been described yet, or that its binding to microtubules is regulated through an alternative post-translational modification.

Intriguingly, the mobility of MAP1B-LC is enhanced only during the induction of synaptic plasticity in CA1 neurons, but not shortly afterwards. This observation would suggest that the transient activation of signaling cascades subsequent to the induction of synaptic plasticity in hippocampus might have the potential to impact on MAP1B functionality. Furthermore, the precise temporal regulation of MA1PB-LC dynamics would imply a possible participation of the protein in processes occurring only during the induction but not during the expression or maintenance of LTD or LTP.

MAP1B-LC transient mobilization could also suggest that it needs to change its subcellular location to play a specific role during the induction of LTD or LTP. Our observations point to the fact that MAP1B-LC detaches from microtubules but stays in the dendritic shaft during synaptic plasticity induction, as we did not observe the recombinant protein moving into other subcellular compartments such as dendritic spines. Two possible mechanisms might explain this observation. On the one hand, we have demonstrated that recombinant MAP1B-LC is largely decorating microtubules in our preparations; it is possible that its detachment from microtubules responds to the fact that the microtubule lattice needs to be unobstructed for the movement of organelles to occur during the induction of synaptic plasticity. On the other hand, MAP1B-LC might function to locate other components in the proximity of the microtubule cytoskeleton in basal conditions; the transient mobilization of MAP1B-LC during

LTD or LTP induction may reflect the subcellular reorganization or relocation of such components as a result to stimulation.

2. MAP1B-LC presence in dendritic spines.

The study of MAP1B-LC subcellular distribution in CA1 neurons revealed that the protein is occasionally present in dendritic spines. Such an observation has been made before for the endogenous MAP1B (Tortosa et al. 2011). In this work, the authors indicated that approximately 1% of dendritic spines contained MAP1B, and it was suggested that MAP1B presence at this compartment might be a consequence of the reported transient entry of dynamic microtubules into dendritic spines (Jaworski et al. 2009). However, live imaging experiments of MAP1B-LC-GFP presented in this thesis support the notion that the recombinant protein is stably anchored inside dendritic spines. MAP1B-LC could be acting as a stabilizing factor for dynamic microtubules that would have transiently penetrated dendritic spines; alternatively, it may reside in dendritic spines as a consequence of its binding to the actin cytoskeleton.

Remarkably, MAP1B-LC-GFP seems to leave dendritic spines after the induction of synaptic plasticity. This phenomenon was observed both after LTD and LTP induction. Opposite to MAP1B-LC behavior in dendrites, the process of mobilization seems to be initiated slowly after the induction phase, lasting for several minutes and resulting in an irreversible disappearance of MAP1B-LC-GFP from dendritic spines. This interesting observation was not explored any further in the present work, but it may well parallel dynamic changes of the actin cytoskeleton inside dendritic spines resulting from synaptic plasticity occurrence.

B) Regulation of basal synaptic transmission and plasticity by MAP1B-LC.

1. Over-expression of MAP1B-LC.

The over-expression of MAP1B-LC-GFP in CA1 neurons was shown to produce a decrease in basal transmission unrelated to dendritic spine morphology or Rac1/RhoA activation. Other authors have reported increased Rac1 activity subsequent to MAP1B-LC over-expression (Henriquez et al. 2012). However, these experiments were performed in a heterologous system because, as the authors declare, the presence of endogenous MAP1B masks the effect

of the light chain on Rac1 activity, which would perfectly fit with the results presented in this thesis.

Interestingly, the decrease in basal synaptic transmission observed upon MAP1B-LC-GFP over-expression was shown to be specific for AMPAR-mediated transmission, as NMDA currents resulted unaffected. In contrast, the over-expression of MAP1B-HC-GFP yielded a general depression of transmission, involving both AMPA and NMDA receptors. The mechanisms by which the heavy chain of MAP1B unspecifically depresses transmission were not explored any further; however, as it also contains a microtubule-binding domain (Noble et al. 1989; Zauner et al. 1992), it is tempting to speculate that enhanced levels of MAP1B-HC might drive a change in the organization of the microtubule cytoskeleton that may affect general processes of trafficking of AMPA and NMDA receptors towards synapses.

On the basis of several imaging experiments, it can be argued that AMPARs are retained in dendrites upon the over-expression of MAP1B-LC. This effect is, importantly, subunit-dependent. The decreased surface expression of endogenous GluA2 versus GluA1 in primary hippocampal neurons points to a subunit-specific intracellular retention of AMPARs in the presence of enhanced levels of MAP1B-LC. In addition, FRAP experiments show a reduction in the mobile population of recombinant GluA2 receptors, while the GluA1 population results unaffected. Indeed, the quantification of fluorescence intensity of recombinant GluA2 receptors in spines versus dendrites evidences that such retention at the level of dendrites impairs the accumulation of receptors in spines, again in a subunit-specific manner.

As a consequence of MAP1B-LC retention of the GluA2-GluA3 population of AMPARs in an intracellular compartment, most probably at the level of dendrites, these receptors cannot continuously cycle and contribute to basal transmission; therefore, basal synaptic transmission is reduced. As previously mentioned, this population is the one thought to maintain basal synaptic transmission in neurons in the face of protein turnover (Shi et al. 2001).

Concerning the impact of MAP1B-LC over-expression on synaptic plasticity, a model can be proposed in which the effects seen on LTP and LTD can be explained on account of the initial decrease in basal transmission. On the one hand, it has been shown that LTP induction drives the insertion of the GluA1-GluA2 population of AMPARs into synapses (Hayashi et al. 2000; Passafaro et al. 2001; Shi et al. 2001). In the presence of MAP1B-LC-GFP, a fraction of the GluA2-GluA3 population would be retained in dendrites whereas the GluA1-GluA2 population would be able to traffic freely towards synapses upon the appropriate stimuli. Under these

circumstances, a normal amount of LTP combined with a decreased basal transmission would yield an increased potentiation (figure 1). Actually, a similar phenotype has been described in a knock-out mouse for the GluA2 subunit of AMPARs (Jia et al. 1996).

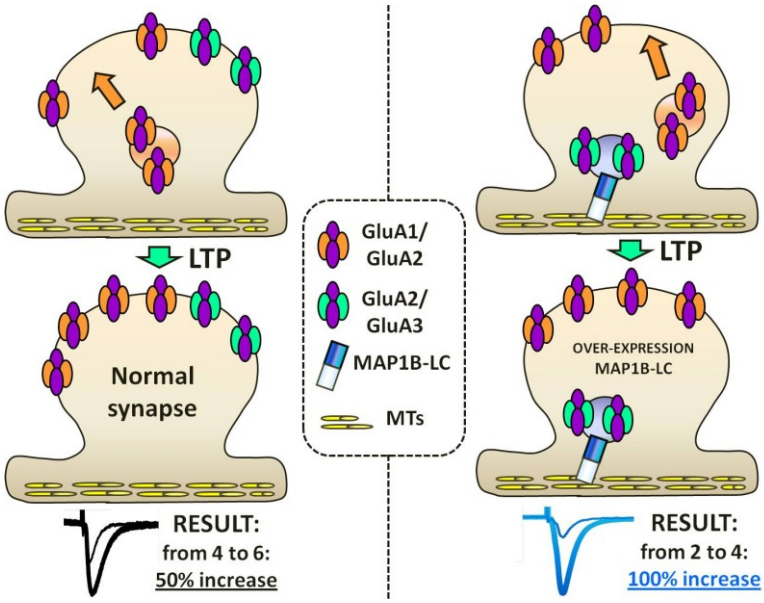


Figure 1. Mechanism of LTP enhancement upon MAP1B-LC over-expression. Left, in a normal synapse, GluA1-GluA2 and GluA2-GluA3 AMPARs are present at the synapse. Upon LTP induction, only GluA1-GluA2 AMPARs are driven to the synapse. The final enhancement of transmission depends on the net increase in the total number of AMPARs at the synapse. **Right,** when MAP1B-LC is over-expressed, GluA2-GluA3 AMPARS are retained in dendrites and do not contribute to basal transmission. As a result, the net increase in transmission is larger when GluA1-GluA2 AMPARs are driven to the synapse upon LTP induction.

On the other hand, the regulated endocytosis of AMPARs after LTD induction has been proposed to be subunit-independent (Chung et al. 2000; Kim et al. 2001; Perez et al. 2001; Lee et al. 2002; Meng et al. 2003). Therefore, GluA1-GluA2 and GluA2-GluA3 populations of AMPARs would be equally removed from synapses so that transmission is reduced to a certain extent, independently of the initial level of transmission prior to LTD induction (figure 2).

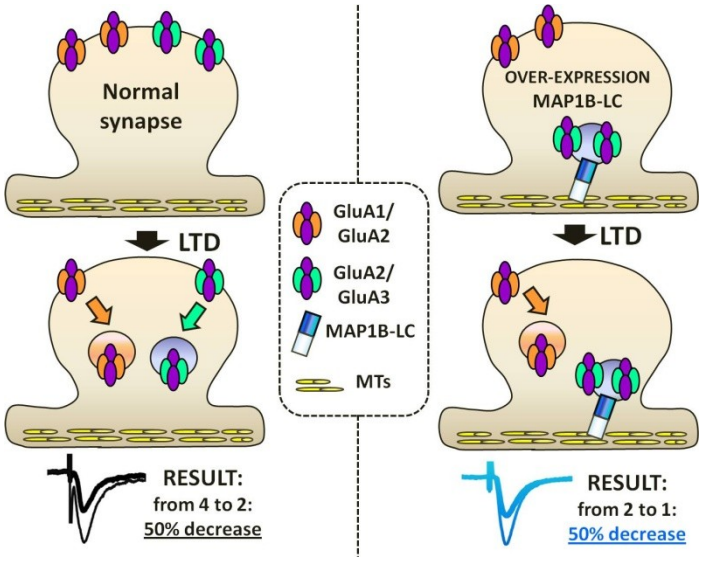


Figure 2. LTD is unaltered upon MAP1B-LC over-expression. LTD has been proposed to involve the endocytosis of both populations of AMPARs (GluA1-GluA2 and GluA2-GluA3). Independently of the initial level of transmission, AMPARs are endocytosed to a certain extent to generate depression. The degree of depression is equivalent in a normal synapse (left) and in a synapse over-expressing MAP1B-LC (right).

2. MAP1B acute depletion.

MAP1B acute depletion in CA1 neurons does have an impact on synaptic plasticity as well. With the protocol of stimulation we have used for cultured slices, LTP seems unaffected. However, we have previously reported an increased LTP in heterozygous animals for MAP1B. In this case, a milder protocol was required to avoid a possible ceiling effect (Benoist et al. 2013). It is likely that the unaffected LTP reported in this thesis is also due to a ceiling effect of the stimulation protocol. We did not characterize MAP1B role in LTP any further.

On the contrary, LTD is partially impaired. We have shown that MAP1B deficiency results in decreased Rac1 activation, which is required to drive AMPAR endocytosis after LTD (Benoist et al. 2013). Nevertheless, we do not know if the light chain of MAP1B is specifically playing any role during this process, although it has been shown that it is the light chain the one that binds Tiam1 (a GEF of Rac1) (Henriquez et al. 2012). At this point, it is important to note that the depletion of the complete MAP1B protein does not represent the opposite situation to the over-expression of its light chain. The lack of the heavy chain of MAP1B may influence the phenotype observed upon acute depletion of the protein and thereby, embroil the interpretation of the results.

C) MAP1B regulates the dendritic transport of AMPARs.

According to live imaging experiments following the movement of TfR clusters along dendrites in primary hippocampal neurons, MAP1B would have the potential to modulate microtubule-dependent transport bidirectionally. MAP1B-LC over-expression reduces the fraction of mobile clusters and the mean speed of those that move, whereas MAP1B down-regulation accelerates transport by increasing the proportion of clusters that move and the speed at which they travel along dendrites. Indeed, MAP1B deficiency was previously associated to an increased velocity of microtubule-based transport of mitochondria in axons (Jimenez-Mateos et al. 2006).

AMPARs are transported along dendrites in an active process powered by molecular motors (Hirokawa and Takemura 2005; Kapitein and Hoogenraad 2011). GluA2-containing AMPARs are linked to different proteins of the kinesin superfamily through GRIP1 (Setou et al. 2002; Shin et al. 2003). However, it is not clear how the transport of GluA1-containing AMPARs proceeds. SAP97, a PDZ domain-containing protein that interacts with the C-terminus of GluA1, has been proposed to play a role in regulating AMPAR addition to hippocampal

pyramidal cell synapses (Leonard et al. 1998; Sans et al. 2001). As a binding partner of KIF1Bα (Mok et al. 2002), SAP97 would seem a reasonable candidate to mediate GluA1 transport along dendrites; nevertheless, further investigations would be required to clarify this aspect.

As previously mentioned, FRAP experiments in the presence of enhanced levels of MAP1B-LC revealed a subunit-specific effect on AMPAR transport along dendrites; this would suggest that GluA1 and GluA2 are transported in different vesicles along microtubules. In studies addressing the subcellular location and distribution of vesicles containing AMPARs in neurons, distinct profiles were obtained for GluA1 and GluA2 subunits in biochemical fractionation experiments (Lee et al. 2001). Indeed, GluA2 seems to be present in many of the fractions containing also TfR. Furthermore, in a study aiming at characterizing the dendritic transport of GluA1 and GluA2 in hippocampal primary neurons, different dynamics of movement along dendrites were found for both varieties of recombinant receptors; this observation would be suggestive of distinct mechanisms of dendritic transport for different populations of AMPARs (Perestenko and Henley 2003).

Does the delay in microtubule-dependent transport upon MAP1B-LC over-expression explain the specific retention in dendrites of the GluA2-GluA3 population of AMPARs? Or is it the consequence of AMPARs being immobilized on microtubular tracks? TfR is a valid tool to study microtubule-dependent transport in neurons because it has been widely characterized as a transmembrane protein specifically sorted into dendrites by means of this type of transport (West et al. 1997; Burack et al. 2000); however, it is not useful to solve the question about the subunit-specific effect of MAP1B-LC over-expression on AMPAR trafficking along dendrites.

Some authors have suggested a certain degree of interference between microtubule-associated proteins, which decorate microtubules, and motor proteins that traffic and carry their cargo along them (Seitz et al. 2002; Tokuraku et al. 2007; Dixit et al. 2008). However, as GluA1 trafficking along dendrites is not affected in the presence of enhanced levels of MAP1B-LC, it seems unlikely that the observed delay in TfR transport results from a general impairment of microtubule-dependent transport in CA1 neurons. A possible explanation might be that GluA2 and TfR travel together in the same vesicles whereas GluA1-containing AMPARs are transported in independent vesicles or by alternative mechanisms. This could respond to the fact that GluA2 and TfR trafficking and sorting are constitutive in neurons, whereas the processes regulating the trafficking of GluA1-containing AMPARs seem to be tightly controlled by activity.

D) MAP1B-LC and GRIP1: an integrated model.

The series of experiments presented in this thesis support a novel role for MAP1B-LC as a crucial mediator of AMPAR trafficking in CA1 hippocampal neurons. According to our data, MAP1B-LC has the potential to regulate the subcellular distribution of AMPARs and consequently, their specific contribution to basal transmission and synaptic plasticity. On the basis of imaging experiments revealing an altered dendritic distribution of GRIP1 in the presence of over-expressed MAP1B-LC, we speculate that MAP1B-LC mediates AMPAR trafficking in CA1 neurons via its specific interaction with GRIP1.

1. Proposed functions of GRIP, PICK1 and NSF in AMPAR trafficking.

GRIP1/GRIP2 (collectively referred to as GRIP hereafter) and PICK1 are PDZ domain-containing proteins well characterized as interacting partners of the GluA2/GluA3 subunits of AMPARs (Dong et al. 1997; Srivastava et al. 1998; Xia et al. 1999). Despite decades of investigation, their specific contribution to the trafficking of AMPARs is still a matter of intense debate. This situation is favored by the existence of a complex network of proteins that cooperate to regulate the subcellular sorting of AMPARs, both during the constitutive and regulated processes that orchestrate AMPAR delivery from dendrites to spines and backwards.

A classical model proposes a dual role for GRIP in neurons. On the one hand, GRIP would be favoring the clustering and stabilization of GluA2-containing AMPARs at synapses, by limiting their endocytic rate (Osten et al. 2000). To enable endocytosis, the phosphorylation of GluA2 at Ser 880 would be required. Phosphorylation by PKC at this site has been described to disrupt GluA2 interaction with GRIP without affecting the binding to PICK1 (Matsuda et al. 1999; Chung et al. 2000); additionally, the induction of LTD in hippocampal neurons was shown to increase phosphorylation of GluA2 at Ser 880 (Daw et al. 2000; Kim et al. 2001). Thereby, it has been proposed that PICK1 would be substituting GRIP in GluA2-binding during LTD to promote the endocytosis of AMPARs.

On the other hand, for AMPARs to stably incorporate into an intracellular pool of receptors after endocytosis, interaction with GRIP has also been proposed to play a part (Daw et al. 2000). Using a short peptide that disrupts GluA2 interactions with PDZ proteins, the authors propose that GRIP would be acting as a retention factor for AMPARs in an undefined intracellular compartment whereas PICK1 would favor AMPARs recycling back to the plasma membrane. This way, GRIP would function to prevent unregulated reinsertion whereas PICK1

would associate with the “mobile” pool of receptors. The hypothesis of GRIP acting as a mechanism to retain AMPARs intracellularly after stimulation would also be supported by the work of Braithwaite and colleagues (Braithwaite et al. 2002).

Nevertheless, the models discussed above are not devoid of controversy. More recently, the work by Mao and colleagues (Mao et al. 2010) has completely challenged the previous conception of the role played by GRIP in AMPAR trafficking. Via an acute deletion of GRIP1 in hippocampal primary cultures obtained from GRIP2 knock-out animals, they show that neither the surface expression nor the endocytosis rate of AMPARs (after NMDA application) is altered upon GRIP depletion. In contrast, the recycling of the endocytosed AMPARs is retarded. Rather strikingly, these data would imply that GRIP is not needed to cluster or stabilize AMPARs neither at the postsynaptic membrane nor at intracellular compartments; on the contrary, it would be required to promote AMPARs recycling back to the plasma membrane after their regulated endocytosis. This result would be supported by a subsequent work from the same group showing that the palmitoylation of GRIP1b (an isoform of GRIP1), which favors GRIP1b association to membranes, accelerates GluA2 recycling after regulated endocytosis (Thomas et al. 2012). Still, this last result would contradict the data reported by Hanley and Henley (Hanley and Henley 2010) that suggest that GRIP1a (the non-palmitoylatable form of GRIP1) would be involved specifically in restricting NMDAR-induced AMPAR endocytosis or, alternatively, promoting recycling of GluA2-containing AMPARs whereas GRIP1b (the palmitoylatable isoform) would either contribute to endocytosis or reduce receptor recycling.

The analysis of GRIP subcellular and ultrastructural distribution in neurons did not help to settle this controversy. Via biochemical studies, GRIP was originally described to be membrane associated and concentrated at the postsynaptic density (Wyszynski et al. 1998; Dong et al. 1999), although to a lesser extent than PSD95. In a subsequent study based on electron microscopy, GRIP was found to be associated with asymmetric synapses, mainly concentrated over the postsynaptic membrane and the postsynaptic density. In addition, GRIP was described to be present not only within spines but also along dendrites, typically associated with microtubules (Wyszynski et al. 1999). When analyzing its subcellular distribution in hippocampal neurons in culture with immunocytochemical techniques, GRIP appeared to be concentrated in sparse puncta of large diameter along the soma and dendrites, a distribution highly similar to the one reported in this thesis. More recently, GRIP1 has been described to appear also at recycling endosomes, co-localizing with Rab11 and TfR but not with EEA-1 (a marker of early endosomes) (Mao et al. 2010). On the contrary, Hanley and Henley (Hanley

and Henley 2010) have shown that GRIP1 co-localizes with EEA-1, and so with early endosomes, after NMDA-mediated stimulation of hippocampal primary cultures.

Apart from GRIP and PICK1, NSF protein interacts with the GluA2 subunit of AMPARs as well. The pep2m-mediated disruption of the NSF-GluA2 interaction was originally described to result in a reduction of AMPAR surface expression and a rundown of AMPAR-dependent basal transmission in CA1 neurons (Luscher et al. 1999; Noel et al. 1999). In the complex scenario of AMPAR trafficking routes seemingly dominated by GRIP and PICK1, how would NSF fit? Although its involvement in AMPAR trafficking was reported decades ago, it is not clear yet whether NSF is responsible for the insertion of AMPARs at the postsynaptic membrane or for their later stabilization at this location. NSF being involved in the direct insertion of GluA2-containing AMPARs at synapses would be compatible with studies showing no synaptic incorporation of GluA2 mutated at the NSF-interacting site (Shi et al. 2001). Other studies, however, have suggested that NSF would be required for the stabilization of AMPARs at synapses, preventing their regulated endocytosis (Braithwaite et al. 2002).

2. A model for MAP1B-LC/GRIP1 interaction in AMPAR trafficking.

As previously mentioned, GRIP1 dendritic targeting is impaired upon MAP1B-LC over-expression in hippocampal primary neurons. MAP1B-LC has been reported to interact with GRIP1, specifically via its actin-binding domain (Seog 2004; Davidkova and Carroll 2007). In addition, GRIP1 has been shown to link the GluA2 subunit of AMPARs to the heavy chain of conventional kinesin (Setou et al. 2002), thereby acting as an adaptor protein in the microtubule-dependent transport of AMPARs along dendrites. Thus, it is possible that MAP1B-LC interaction with GRIP1 is retaining the GRIP1/GluA2-3 complex in the proximity of the microtubule lattice. As a consequence, the progression of the GRIP1/GluA2-3 tandem along the microtubule tracks towards the distal part of dendrites would be retarded or impeded, resulting in the abnormal dendritic distribution of GRIP1 we have observed.

What would be then the function of the interaction between MAP1B-LC and GRIP1 in CA1 hippocampal neurons? Considering the evidence presented in this thesis, we propose a model in which MAP1B-LC plays a role in the regulation of the subcellular distribution of the GRIP1/GluA2-3 complex via its direct interaction with GRIP1. The fine tuning of the subcellular allocation of the GRIP1/GluA2-3 complex would have a direct impact on its availability to interact with other key players such as NSF and PICK1, and consequently, it would result in the

modulation of AMPAR trafficking both during basal conditions and synaptic plasticity (figure 3.1).

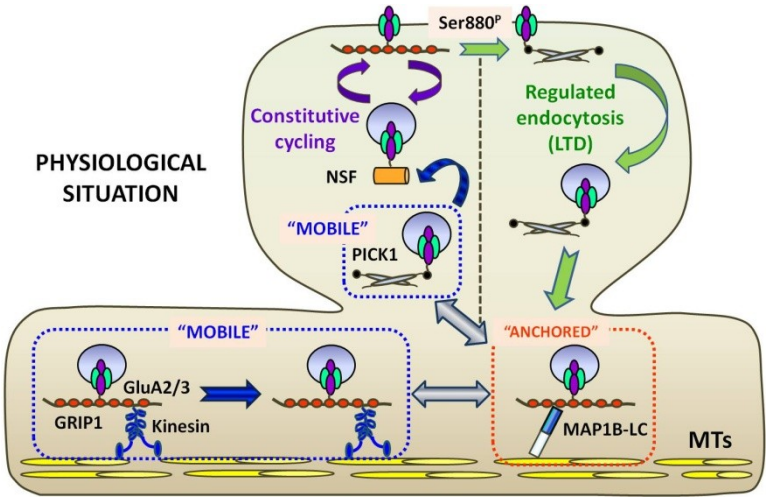


Figure 3.1. GRIP1 subcellular distribution and functions in a physiological situation. GRIP1 has been proposed to play a role in AMPARs stabilization at synapses and intracellular retention after regulated endocytosis (LTD). In addition, GRIP1 functions as an adaptor protein in the microtubule-dependent transport of AMPARs along dendrites. We propose GRIP1/GluA2-3 intracellular retention is mediated by MAP1B-LC through the anchoring to microtubules.

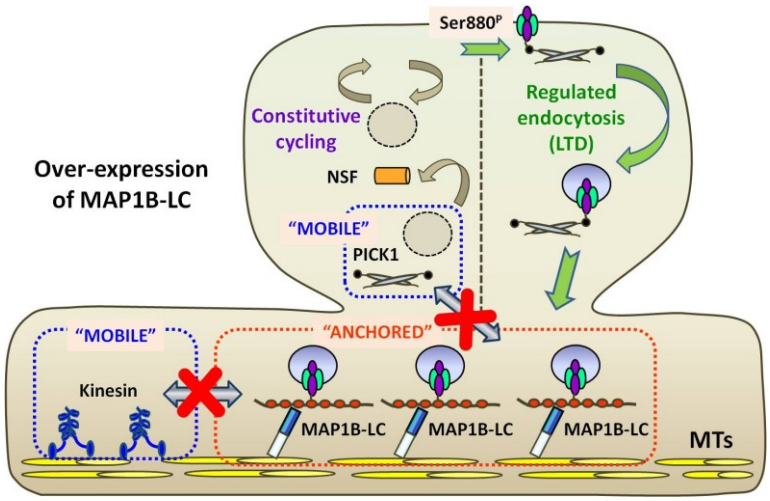


Figure 3.2. The GRIP1/GluA2-3 complex is retained in the proximity of microtubules upon MAP1B-LC over-expression. The intracellular retention of the GRIP1/GluA2-3 complex prevents it to interact with NSF/PICK1, which mediate the constitutive cycling of the receptor. The dendritic trafficking of GluA2-GluA3 AMPARs is also impaired.

As over-expressed MAP1B-LC is very likely anchored to microtubules, it is reasonable to think that it could be retaining the GRIP1/GluA2-3 complex at the level of the microtubule cytoskeleton. This idea sounds reasonable considering that electron microscopy studies have evidenced that GRIP1 appears frequently associated with microtubules in dendrites (Wyszynski et al. 1999). In addition, we have shown via FRAP experiments that a fraction of GluA2-containing AMPARs are indeed immobilized at dendrites in the presence of over-expressed MAP1B-LC. According to the different models previously discussed, this anchoring might be preventing the interaction between NSF/PICK1 and the GluA2 subunit of AMPARs that controls the surface expression of the constitutive pool (figure 3.2).

One can think of two consequences derived from the intracellular retention of the GRIP1/GluA2-3 complex in the proximity of the microtubule cytoskeleton. First, the decreased availability of mobile receptors would be somewhat mimicking the effect of peptide pep2m,

designed to interfere with the NSF-GluA2 interaction. This would be the reason why infusing pep2m in CA1 neurons over-expressing MAP1B-LC does not have any impact on basal synaptic transmission. Second, the immobilization of a fraction of GluA2-containing AMPARs at dendrites would impair its surface expression and consequently, AMPAR-dependent transmission would be decreased in basal conditions, as demonstrated.

At this point, it is worth recalling that MAP1B-LC modulates specifically the trafficking of the GluA2- but not the GluA1-containing population of AMPARs. According to our model, what would be the reason for this specificity? The possibility that MAP1B-LC regulates the intracellular sorting of AMPARs via its interaction with GRIP1 would explain also this observation, because GRIP1 has been demonstrated to interact with the GluA2 and GluA3 subunits of AMPARs (the ones that integrate the constitutive pool of receptors (Shi et al. 2001)) but not with the GluA1 or GluA4 subunits (Dong et al. 1997).

Interestingly, the behavior of both mutants of MAP1B-LC, MAP1B-LC-delABD-GFP and MAP1B-LC-delMBD-GFP, would support this theory as well. MAP1B-LC mutants were generated to examine the possible contribution of each domain to the function of the wild type protein. We concluded actin-binding and microtubule-binding were both required to mediate the decrease in AMPAR-dependent basal transmission observed upon MAP1B-LC over-expression.

In fact, the actin-binding domain of MAP1B-LC is the one described to interact with GRIP1 as well. Thereby, it would make sense that the interaction with GRIP1 via the actin-binding domain together with the binding to microtubules via the microtubule-binding domain were both essential to mediate the anchoring of the GRIP1/GluA2-3 tandem to the microtubule cytoskeleton. Therefore, this interpretation would justify why both domains of the wild type protein need to function cooperatively. Furthermore, it would suggest that it is not only MAP1B-LC binding to GRIP1 but the concomitant anchoring to the microtubule cytoskeleton that modulates AMPAR trafficking.

According to the work by Daw and colleagues, GRIP1 would be playing a role in the intracellular retention of AMPARs after its regulated endocytosis (Daw et al. 2000). The model we propose would be compatible with this possibility as well. As MAP1B-LC over-expression does not affect LTD, the interpretation would be that GRIP1 binding to MAP1B-LC does not prevent GRIP1 from executing its function in the intracellular retention of the endocytosed receptors.

On the other hand, it is worth noting that MAP1B-LC mobilizes transiently during the induction of synaptic plasticity. Hanley and Henley (Hanley and Henley 2010) have shown that both isoforms of GRIP1, GRIP1a and GRIP1b, co-localize with endosomal markers only after NMDA application, suggesting a reorganization of the endosomal trafficking machinery driven by GRIP1 upon LTD induction. It is possible, then, that MAP1B-LC mobilization is required to release GRIP1 from microtubules so that GRIP1 is free to orchestrate the trafficking of endosomes after the induction of LTD. Furthermore, this assumption would also make our model compatible with the role for GRIP1 in AMPAR recycling after LTD proposed by Mao and colleagues (Mao et al. 2010). These authors have shown that AMPAR recycling after LTD is retarded in GRIP1/GRIP2 knock out neurons. Our model would imply that upon MAP1B-LC mobilization, GRIP1 release from its anchoring to microtubules would allow it to favor AMPAR recycling to the plasma membrane to maintain a given degree of depression after LTD induction.

Regarding the effects of MAP1B-LC over-expression on synaptic plasticity in CA1 neurons, our model would also be consistent with an increased potentiation after LTP induction resulting from a decreased basal transmission. To date, GRIP1 has not been directly involved in the induction, expression or maintenance of LTP. As explained in a previous section, the phenotype observed upon MAP1B-LC over-expression would be a consequence of the intracellular retention of the GluA2-containing population of AMPARs via GRIP1 in basal conditions and would not necessarily respond to a specific effect of MAP1B-LC on LTP.

On the other hand, how would the proposed model explain the phenotype observed upon MAP1B depletion? MAP1B acute depletion through a specific shRNA does not affect basal synaptic transmission. In the absence of MAP1B, the GluA2-GluA3 population of AMPARs would not be retained intracellularly; it would normally interact with NSF/PICK1, which adjust the surface expression of the constitutive cycling pool of receptors to maintain basal transmission. Or alternatively, GRIP1 would be free from intracellular anchoring to locate at the synaptic plasma membrane and play a role in the stabilization of AMPARs at this location.

On the contrary, MAP1B knock-down has been demonstrated to impair LTD expression in CA1 neurons, both in heterozygous animals and after its acute depletion through a specific shRNA. According to our model, the lack of anchoring to the microtubule cytoskeleton or the absence of regulation of the intracellular population of GRIP1 would prevent it from retaining AMPARs after LTD (figure 3.3). This idea would not be incompatible with the impaired activation of Rac1 GTPase that has been demonstrated to underlie the insufficient endocytosis of AMPARs after

LTD induction in MAP1B +/- neurons. It could be indicating a dual role for MAP1B in regulating the subcellular distribution of the GRIP1/GluA2-3 complex and providing enough Rac1 activity during LTD.

The idea of misallocation of GRIP1 in the absence of MAP1B would also be consistent with the proposed role for GRIP1 in favoring the recycling of AMPARs after LTD (Mao et al. 2010; Thomas et al. 2012). The lack of intracellular retention of GRIP1 could be promoting its association with trafficking vesicles, and therefore, the increased recycling of AMPARs after LTD that would decrease the degree of depression. As previously mentioned, palmitoylation of GRIP1b has been shown to favor its association with recycling endosomes and has been linked to an accelerated recycling of receptors after NMDA application in hippocampal primary neurons (Thomas et al. 2012). Although we looked at the total population of GRIP1 including GRIP1b and GRIP1a, it is tempting to speculate that the anchoring of GRIP1 to the microtubule cytoskeleton by MAP1B-LC might be restricting the access of palmitoylating enzymes to GRIP1, and therefore, might be regulating its association with the endosomal compartment to promote AMPAR recycling.

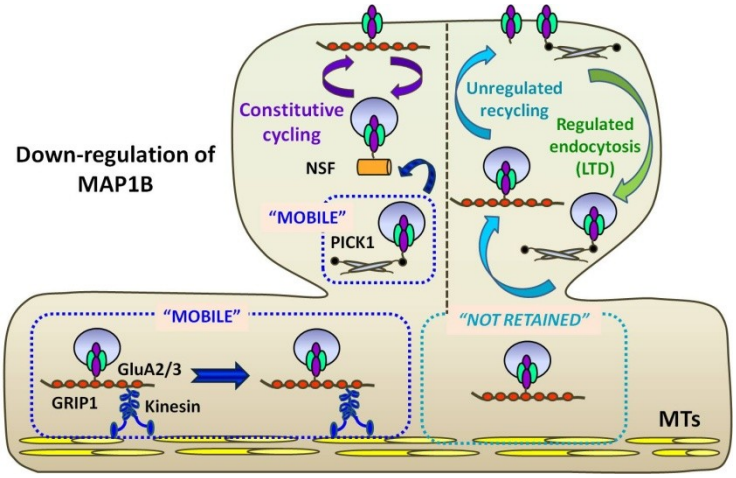
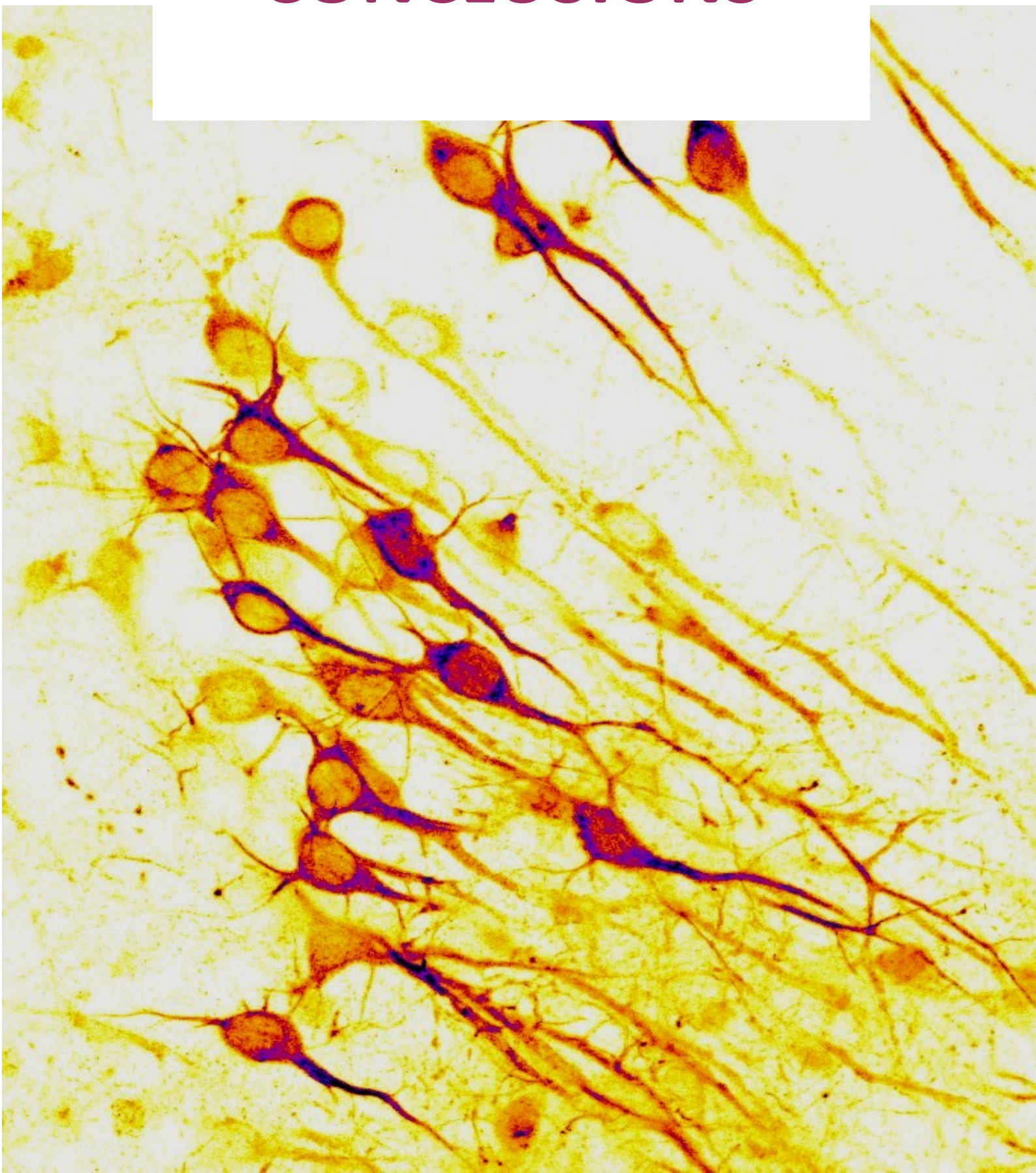


Figure 3.3. MAP1B depletion eliminates GRIP1/GluA2-3 intracellular retention. The lack of intracellular retention of the GRIP1/GluA2-3 complex does not affect its availability to interact with NSF/PICK1 but accelerates its recycling after LTD, resulting in a reduced depression.

In sum, the evidence compiled in this thesis points to a novel role of the light chain of MAP1B as a key player in the subcellular sorting of a specific population of AMPARs. Its binding to GRIP1 together with its ability to interact with microtubules would be essential to determine the surface expression of the GluA2-GluA3 population of AMPARs, and consequently, the degree to which they contribute to basal transmission in neurons. Furthermore, this model would assign a functional meaning to the interaction between MAP1B-LC and GRIP1 for the first time and would strengthen the increasing evidence on the role of microtubules and microtubule-associated proteins in the regulation of AMPAR dynamics in and out of dendritic spines and therefore, of synaptic transmission.

CONCLUSIONS



The following **conclusions** can be drawn from the experimental evidence presented in this thesis:

1. The light chain of MAP1B decreases specifically AMPAR-dependent synaptic transmission in CA1 hippocampal neurons, whereas the heavy chain depresses globally AMPAR-dependent and NMDAR-dependent basal transmission upon over-expression.

2. When over-expressed, the light chain of MAP1B co-localizes mainly with stable microtubules in dendrites. It hardly interacts with the endogenous heavy chain, which possibly facilitates MAP1B-LC binding to microtubules. **Our experimental model could be reproducing the behavior of the fraction of MAP1B-LC that exists naturally in excess over the heavy chain and that is thought to play an independent role.** Upon the induction of synaptic plasticity, MAP1B-LC is transiently dynamic. This mobilization is not a consequence of the depolymerization of microtubules in dendrites.

3. MAP1B-LC acts on AMPAR-dependent synaptic transmission through the modulation of the trafficking of the GluA2-GluA3 subtype of AMPARs. In the time scale of our experiments, neither the activity of small GTPases (Rac1, RhoA) nor the morphology or number of dendritic spines are altered upon MAP1B-LC over-expression.

4. The over-expression of the light chain of MAP1B results in a partial retention of GluA2-GluA3 AMPARs at the level of dendrites. As a consequence, these receptors cannot accumulate in dendritic spines, reach synapses and contribute to basal transmission through their constitutive cycling.

5. MAP1B-LC targets exclusively the GluA2-GluA3 population of AMPARs without affecting the normal trafficking of the GluA1-GluA2 population. Recombinant GluA1 AMPARs are ordinarily transported along dendrites and accumulate in spines to a normal extent in the presence of over-expressed MAP1B-LC. The decreased synaptic incorporation of GluA2-containing receptors does not result in an enhanced incorporation of GluA2-lacking receptors.

6. The retention of a fraction of GluA2-GluA3 AMPARs at dendrites upon MAP1B-LC over-expression does not prevent **a normal induction and expression of LTD in CA1 hippocampal neurons. On the contrary, LTP is enhanced,** very likely as a consequence of the initial depression in basal transmission.

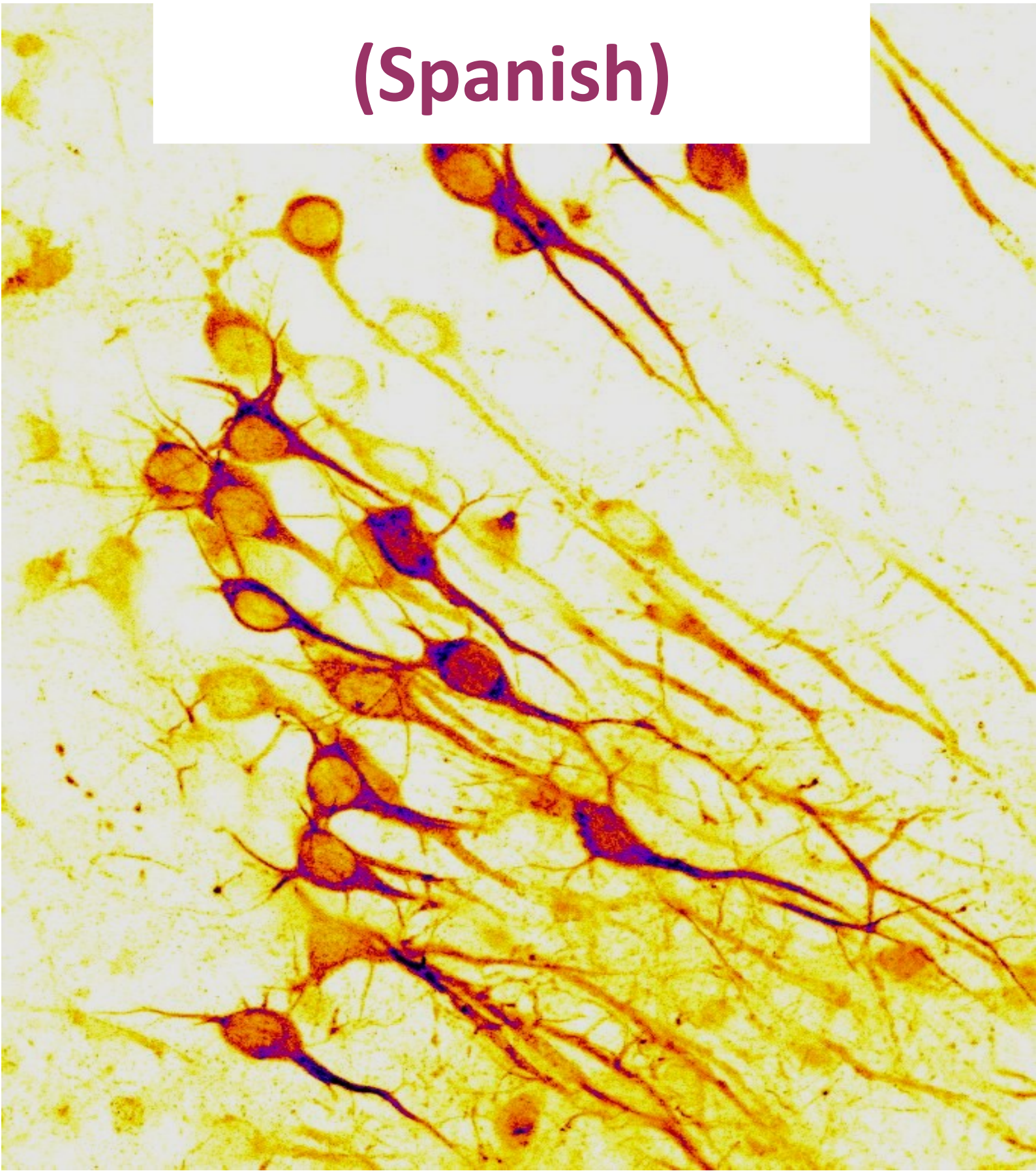
7. Probably, MAP1B-LC regulates the trafficking of the GluA2-GluA3 population of AMPARs through its direct interaction with GRIP1.

8. Both domains of MAP1B-LC, the actin-binding domain and the microtubule-binding domain, need to function cooperatively to regulate AMPAR trafficking. The actin-binding domain is the one that interacts with GRIP1 as well. The fact that microtubule-binding is also required suggests that MAP1B-LC might be anchoring the GRIP1/GluA2-3 complex to the microtubule cytoskeleton.

9. Preliminary data point to the fact that MAP1B-LC over-expression might be potentiating the interaction of the GluA2 subunit of AMPARs with microtubules. If MAP1B-LC is regulating the anchoring of the GRIP1/GluA2-3 complex to the microtubule cytoskeleton, **it is likely that an excessive retention of the complex upon MAP1B-LC over-expression results in an impairment of microtubule-dependent transport.** We used TfR as a reporter of microtubule-dependent transport, although it does not provide information about the specificity of the effect.

10. MAP1B depletion has distinct effects on synaptic plasticity and microtubule-dependent transport. **The fact that both chains of the protein are depleted makes it difficult to directly compare the effects due to full-length MAP1B down-regulation with the phenotype observed upon MAP1B-LC over-expression.** It is possible that the light chain and the heavy chain of MAP1B have distinct functions related to the regulation of AMPAR trafficking in basal conditions and synaptic plasticity; however, more experiments would be required to clarify this aspect.

CONCLUSIONS (Spanish)



A partir de los datos experimentales recopilados en esta tesis, podemos extraer las siguientes **conclusiones:**

1. La cadena ligera de MAP1B reduce de forma específica la transmisión sináptica dependiente de receptores AMPA en neuronas hipocampales CA1, mientras que la cadena pesada deprime globalmente tanto la transmisión basal dependiente de receptores AMPA como la dependiente de receptores NMDA cuando se sobre-expresa.

2. Cuando se sobre-expresa, la cadena ligera de MAP1B co-localiza fundamentalmente con microtúbulos estables en dendritas. Apenas interacciona con la cadena pesada endógena, lo que probablemente facilita su unión a los microtúbulos. **Nuestro modelo experimental podría estar reproduciendo el comportamiento de la fracción de MAP1B-LC que existe naturalmente en exceso con respecto a la cadena pesada y que podría estar jugando un papel autónomo o independiente.** Cuando se induce plasticidad sináptica, la cadena ligera de MAP1B se moviliza de forma transitoria. Dicha movilización no es consecuencia de la despolimerización de microtúbulos en dendritas.

3. MAP1B-LC actúa sobre la transmisión sináptica dependiente de receptores AMPA por medio de la modulación del tráfico del subtipo de receptores GluA2-GluA3. En la escala temporal de nuestros experimentos, ni la actividad de las pequeñas GTPasas (Rac1, RhoA) ni la morfología o número de espinas dendríticas se vieron alteradas como consecuencia de la sobre-expresión de MAP1B-LC.

4. La sobre-expresión de la cadena ligera de MAP1B resulta en la retención parcial de los receptores AMPA GluA2-GluA3 a nivel de las dendritas. Como consecuencia, estos receptores no se acumulan en espinas dendríticas, no alcanzan las sinapsis y no contribuyen al mantenimiento de la transmisión sináptica basal por medio de su reciclaje constitutivo.

5. MAP1B-LC actúa exclusivamente sobre la población GluA2-GluA3 de receptores AMPA, sin afectar el tráfico normal de la población GluA1-GluA2. Cuando MAP1B-LC se sobre-expresa, los receptores AMPA GluA1 recombinantes se transportan de manera ordinaria a lo largo de las dendritas y se acumulan normalmente en las espinas. La incorporación disminuida a las sinapsis de los receptores que contienen GluA2 no se traduce en una incorporación incrementada de aquellos que carecen de la subunidad GluA2.

6. La retención de una fracción de receptores GluA2-GluA3 en las dendritas cuando MAP1B-LC se sobre-expresa no impide la **normal inducción y expresión de LTD en neuronas**

hipocampales CA1. Por el contrario, la LTP se ve incrementada, probablemente como consecuencia de una reducción inicial en la transmisión basal.

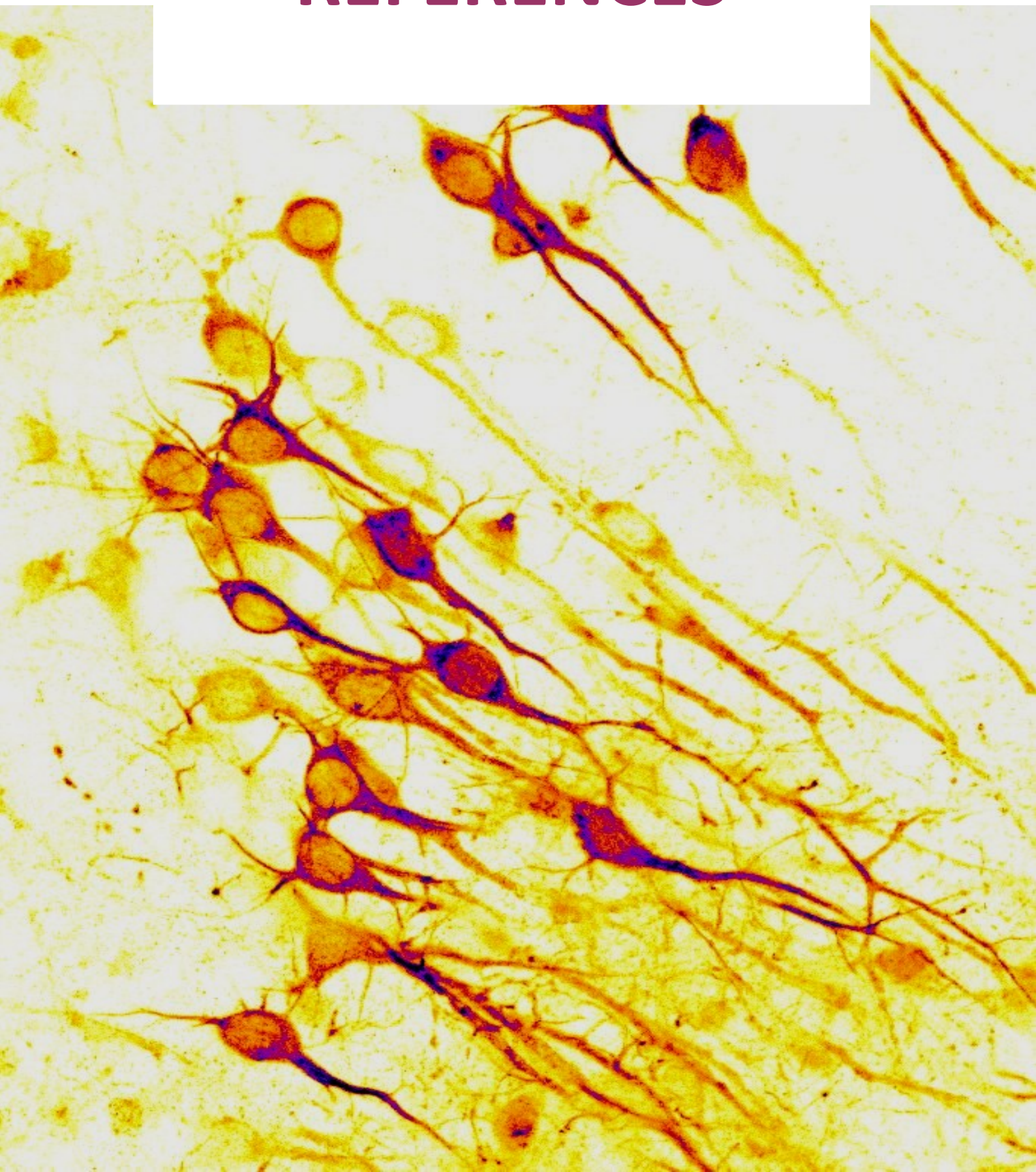
7. Probablemente, MAP1B-LC regula el tráfico intracelular de los receptores AMPA de tipo GluA2-GluA3 por medio de su interacción directa con GRIP1.

8. Los dos dominios de MAP1B-LC, el dominio de unión a actina y el dominio de unión a microtúbulos, necesitan funcionar de forma conjunta para regular el tráfico de receptores AMPA. El dominio de unión a actina es también el que interacciona con GRIP1. El hecho de que se requiera la unión a microtúbulos de forma concomitante sugiere que MAP1B-LC podría estar actuando por medio del anclaje del complejo GRIP1/GluA2-3 al citoesqueleto microtubular.

9. La sobre-expresión de MAP1B-LC podría estar potenciando la interacción de la subunidad GluA2 de receptores AMPA con los microtúbulos, de acuerdo con los datos preliminares presentados en esta memoria. Si, en efecto, MAP1B-LC es capaz de regular el anclaje del complejo GRIP1/GluA2-3 al citoesqueleto microtubular, **es posible que una retención excesiva de dicho complejo debida a la sobre-expresión de MAP1B-LC resulte en un enlentecimiento del transporte mediado por microtúbulos.** En este trabajo, hemos empleado el receptor de transferrina (TfR) como reportero del transporte mediado por microtúbulos, aunque no nos proporciona información sobre la especificidad del efecto observado.

10. La depleción de MAP1B tiene efectos característicos sobre plasticidad sináptica y sobre el transporte dependiente de microtúbulos. **El hecho de que ambas cadenas de la proteína resulten eliminadas hace que la comparación directa de los efectos debidos a la regulación a la baja de la proteína MAP1B completa y el fenotipo observado como consecuencia de la sobre-expresión de su cadena ligera sea muy complicada.** Es posible que la cadena ligera y la cadena pesada ejerzan diferentes funciones en relación a la regulación del tráfico de receptores AMPA tanto en condiciones basales como durante los fenómenos de plasticidad sináptica; sin embargo, se requieren más experimentos para clarificar este punto.

REFERENCES



Andersen, P. (2007). The hippocampus book. Oxford ; New York, Oxford University Press.

Arendt, K. L., Royo, M., Fernandez-Monreal, M., Knafo, S., Petrok, C. N., Martens, J. R. and Esteban, J. A. (2010). "PIP3 controls synaptic function by maintaining AMPA receptor clustering at the postsynaptic membrane." Nat Neurosci **13**(1): 36-44.

Avila, J., Ulloa, L., Gonzalez, J., Moreno, F. and Diaz-Nido, J. (1994). "Phosphorylation of microtubule-associated proteins by protein kinase CK2 in neuritogenesis." Cell Mol Biol Res **40**(5-6): 573-9.

Baas, P. W. and Buster, D. W. (2004). "Slow axonal transport and the genesis of neuronal morphology." J Neurobiol **58**(1): 3-17.

Banker, G. A. and Cowan, W. M. (1977). "Rat hippocampal neurons in dispersed cell culture." Brain Res **126**(3): 397-42.

Barad, M., Bourtchouladze, R., Winder, D. G., Golan, H. and Kandel, E. (1998). "Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory." Proc Natl Acad Sci U S A **95**(25): 15020-5.

Barria, A., Derkach, V. and Soderling, T. (1997a). "Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor." J Biol Chem **272**(52): 32727-30.

Barria, A., Muller, D., Derkach, V., Griffith, L. C. and Soderling, T. R. (1997b). "Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation." Science **276**(5321): 2042-5.

Barry, M. F. and Ziff, E. B. (2002). "Receptor trafficking and the plasticity of excitatory synapses." Curr Opin Neurobiol **12**(3): 279-86.

Bashir, Z. I. and Collingridge, G. L. (1994). "An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus." Exp Brain Res **100**(3): 437-43.

Bear, M. F. (1996). "A synaptic basis for memory storage in the cerebral cortex." Proc Natl Acad Sci U S A **93**(24): 13453-9.

Benard, V., Bohl, B. P. and Bokoch, G. M. (1999). "Characterization of rac and cdc42 activation in chemoattractant-stimulated human neutrophils using a novel assay for active GTPases." J Biol Chem **274**(19): 13198-204.

Benke, T. A., Luthi, A., Isaac, J. T. and Collingridge, G. L. (1998). "Modulation of AMPA receptor unitary conductance by synaptic activity." Nature **393**(6687): 793-7.

Benoist, M., Palenzuela, R., Rozas, C., Rojas, P., Tortosa, E., Morales, B., Gonzalez-Billault, C., Avila, J. and Esteban, J. A. (2013). "MAP1B-dependent Rac activation is required for AMPA receptor endocytosis during long-term depression." EMBO J **32**(16): 2287-99.

Bliss, T. V. and Collingridge, G. L. (1993). "A synaptic model of memory: long-term potentiation in the hippocampus." Nature **361**(6407): 31-9.

Bliss, T. V. and Lomo, T. (1973). "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path." J Physiol **232**(2): 331-56.

Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. and Heinemann, S. (1990). "Molecular cloning and functional expression of glutamate receptor subunit genes." Science **249**(4972): 1033-7.

Braithwaite, S. P., Xia, H. and Malenka, R. C. (2002). "Differential roles for NSF and GRIP/ABP in AMPA receptor cycling." Proc Natl Acad Sci U S A **99**(10): 7096-101.

Bredt, D. S. and Nicoll, R. A. (2003). "AMPA receptor trafficking at excitatory synapses." Neuron **40**(2): 361-79.

Burack, M. A., Silverman, M. A. and Banker, G. (2000). "The role of selective transport in neuronal protein sorting." Neuron **26**(2): 465-72.

Burgess, N., Maguire, E. A. and O'Keefe, J. (2002). "The human hippocampus and spatial and episodic memory." Neuron **35**(4): 625-41.

Burnashev, N., Monyer, H., Seeburg, P. H. and Sakmann, B. (1992). "Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit." Neuron **8**(1): 189-98.

Campbell, J. N. and Slep, K. C. (2011). "alphabeta-Tubulin and microtubule-binding assays." Methods Mol Biol **777**(2011): 87-97.

Carroll, R. C., Beattie, E. C., von Zastrow, M. and Malenka, R. C. (2001). "Role of AMPA receptor endocytosis in synaptic plasticity." Nat Rev Neurosci **2**(5): 315-24.

Charriaut-Marlangue, C., Otani, S., Creuzet, C., Ben-Ari, Y. and Loeb, J. (1991). "Rapid activation of hippocampal casein kinase II during long-term potentiation." Proc Natl Acad Sci U S A **88**(22): 10232-6.

Chen, H. J., Rojas-Soto, M., Oguni, A. and Kennedy, M. B. (1998). "A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II." Neuron **20**(5): 895-904.

Chung, H. J., Xia, J., Scannevin, R. H., Zhang, X. and Huganir, R. L. (2000). "Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins." J Neurosci **20**(19): 7258-67.

Collins, M. O., Yu, L., Coba, M. P., Husi, H., Campuzano, I., Blackstock, W. P., Choudhary, J. S. and Grant, S. G. (2005). "Proteomic analysis of in vivo phosphorylated synaptic proteins." J Biol Chem **280**(7): 5972-82.

Conde, C. and Caceres, A. (2009). "Microtubule assembly, organization and dynamics in axons and dendrites." Nat Rev Neurosci **10**(5): 319-32.

Coultrap, S. J., Freund, R. K., O'Leary, H., Sanderson, J. L., Roche, K. W., Dell'Acqua, M. L. and Bayer, K. U. (2014). "Autonomous CaMKII mediates both LTP and LTD using a mechanism for differential substrate site selection." Cell Rep **6**(3): 431-7.

Cueille, N., Blanc, C. T., Popa-Nita, S., Kasas, S., Catsicas, S., Dietler, G. and Riederer, B. M. (2007). "Characterization of MAP1B heavy chain interaction with actin." Brain Res Bull **71**(6): 610-8.

Davidkova, G. and Carroll, R. C. (2007). "Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors in hippocampus." J Neurosci **27**(48): 13273-8.

Davies, R. W. and Morris, B. J. (2004). Molecular biology of the neuron. Oxford, Oxford University Press.

Davies, S. N., Lester, R. A., Reymann, K. G. and Collingridge, G. L. (1989). "Temporally distinct pre- and post-synaptic mechanisms maintain long-term potentiation." Nature **338**(6215): 500-3.

Daw, M. I., Chittajallu, R., Bortolotto, Z. A., Dev, K. K., Duprat, F., Henley, J. M., Collingridge, G. L. and Isaac, J. T. (2000). "PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses." Neuron **28**(3): 873-86.

Derkach, V., Barria, A. and Soderling, T. R. (1999). "Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors." Proc Natl Acad Sci U S A **96**(6): 3269-74.

Dev, K. K., Nishimune, A., Henley, J. M. and Nakanishi, S. (1999). "The protein kinase C alpha binding protein PICK1 interacts with short but not long form alternative splice variants of AMPA receptor subunits." Neuropharmacology **38**(5): 635-44.

Diaz-Nido, J., Serrano, L., Mendez, E. and Avila, J. (1988). "A casein kinase II-related activity is involved in phosphorylation of microtubule-associated protein MAP-1B during neuroblastoma cell differentiation." J Cell Biol **106**(6): 2057-65.

Ding, J., Liu, J. J., Kowal, A. S., Nardine, T., Bhattacharya, P., Lee, A. and Yang, Y. (2002). "Microtubule-associated protein 1B: a neuronal binding partner for gigaxonin." J Cell Biol **158**(3): 427-33.

Dingledine, R., Hume, R. I. and Heinemann, S. F. (1992). "Structural determinants of barium permeation and rectification in non-NMDA glutamate receptor channels." J Neurosci **12**(10): 4080-7.

Dixit, R., Ross, J. L., Goldman, Y. E. and Holzbaur, E. L. (2008). "Differential regulation of dynein and kinesin motor proteins by tau." Science **319**(5866): 1086-9.

Dong, H., O'Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F. and Huganir, R. L. (1997). "GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors." Nature **386**(6622): 279-84.

Dong, H., Zhang, P., Song, I., Petralia, R. S., Liao, D. and Huganir, R. L. (1999). "Characterization of the glutamate receptor-interacting proteins GRIP1 and GRIP2." J Neurosci **19**(16): 6930-41.

Dotti, C. G., Sullivan, C. A. and Banker, G. A. (1988). "The establishment of polarity by hippocampal neurons in culture." J Neurosci **8**(4): 1454-68.

Dudek, S. M. and Bear, M. F. (1992). "Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade." Proc Natl Acad Sci U S A **89**(10): 4363-7.

Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D. and Naldini, L. (1998). "A third-generation lentivirus vector with a conditional packaging system." J Virol **72**(11): 8463-71.

Dunwiddie, T. and Lynch, G. (1978). "Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency." J Physiol **276**: 353-67.

Durand, G. M., Kovalchuk, Y. and Konnerth, A. (1996). "Long-term potentiation and functional synapse induction in developing hippocampus." Nature **381**(6577): 71-5.

Edelmann, W., Zervas, M., Costello, P., Roback, L., Fischer, I., Hammarback, J. A., Cowan, N., Davies, P., Wainer, B. and Kucherlapati, R. (1996). "Neuronal abnormalities in microtubule-associated protein 1B mutant mice." Proc Natl Acad Sci U S A **93**(3): 1270-5.

Ehlers, M. D. (2000). "Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting." Neuron **28**(2): 511-25.

Eriksson, M., Samuelsson, H., Bjorklund, S., Tortosa, E., Avila, J., Samuelsson, E. B., Benedikz, E. and Sundstrom, E. (2010). "MAP1B binds to the NMDA receptor subunit NR3A and affects NR3A protein concentrations." Neurosci Lett **475**(1): 33-7.

Esteban, J. A. (2003). "AMPA receptor trafficking: a road map for synaptic plasticity." Mol Interv **3**(7): 375-85.

Esteban, J. A., Shi, S. H., Wilson, C., Nuriya, M., Huganir, R. L. and Malinow, R. (2003). "PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity." Nat Neurosci **6**(2): 136-43.

Fernandez-Monreal, M., Brown, T. C., Royo, M. and Esteban, J. A. (2012). "The balance between receptor recycling and trafficking toward lysosomes determines synaptic strength during long-term depression." J Neurosci **32**(38): 13200-5.

Fuller, L. and Dailey, M. E. (2007). "Preparation of rodent hippocampal slice cultures." CSH Protoc **2007**: pdb prot4848.

Gahwiler, B. H., Capogna, M., Debanne, D., McKinney, R. A. and Thompson, S. M. (1997). "Organotypic slice cultures: a technique has come of age." Trends Neurosci **20**(10): 471-7.

Gandini, M. A., Henriquez, D. R., Grimaldo, L., Sandoval, A., Altier, C., Zamponi, G. W., Felix, R. and Gonzalez-Billault, C. (2014). "Ca2.2 channel cell surface expression is regulated by

the light chain 1 (LC1) of the microtubule-associated protein B (MAP1B) via UBE2L3-mediated ubiquitination and degradation." Pflugers Arch.

Goldstein, L. S. and Yang, Z. (2000). "Microtubule-based transport systems in neurons: the roles of kinesins and dyneins." Annu Rev Neurosci **23**: 39-71.

Gong, C. X., Wegiel, J., Lidsky, T., Zuck, L., Avila, J., Wisniewski, H. M., Grundke-Iqbal, I. and Iqbal, K. (2000). "Regulation of phosphorylation of neuronal microtubule-associated proteins MAP1b and MAP2 by protein phosphatase-2A and -2B in rat brain." Brain Res **853**(2): 299-309.

Gonzalez-Billault, C., Demandt, E., Wandosell, F., Torres, M., Bonaldo, P., Stoykova, A., Chowdhury, K., Gruss, P., Avila, J. and Sanchez, M. P. (2000). "Perinatal lethality of microtubule-associated protein 1B-deficient mice expressing alternative isoforms of the protein at low levels." Mol Cell Neurosci **16**(4): 408-21.

Gonzalez-Billault, C., Jimenez-Mateos, E. M., Caceres, A., Diaz-Nido, J., Wandosell, F. and Avila, J. (2004). "Microtubule-associated protein 1B function during normal development, regeneration, and pathological conditions in the nervous system." J Neurobiol **58**(1): 48-59.

Good, M. A. (2002). "Spatial memory and hippocampal formation: Where are we now?" Psicologica **23**(1): 109-138.

Gundersen, G. G. and Cook, T. A. (1999). "Microtubules and signal transduction." Curr Opin Cell Biol **11**(1): 81-94.

Hammarback, J. A., Obar, R. A., Hughes, S. M. and Vallee, R. B. (1991). "MAP1B is encoded as a polyprotein that is processed to form a complex N-terminal microtubule-binding domain." Neuron **7**(1): 129-39.

Hanley, J. G. (2008). "PICK1: a multi-talented modulator of AMPA receptor trafficking." Pharmacol Ther **118**(1): 152-60.

Hanley, J. G., Koulen, P., Bedford, F., Gordon-Weeks, P. R. and Moss, S. J. (1999). "The protein MAP-1B links GABA(C) receptors to the cytoskeleton at retinal synapses." Nature **397**(6714): 66-9.

Hanley, L. J. and Henley, J. M. (2010). "Differential roles of GRIP1a and GRIP1b in AMPA receptor trafficking." Neurosci Lett **485**(3): 167-72.

Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C. and Malinow, R. (2000). "Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction." Science **287**(5461): 2262-7.

Henley, J. M. (2003). "Proteins interactions implicated in AMPA receptor trafficking: a clear destination and an improving route map." Neurosci Res **45**(3): 243-54.

Henley, J. M., Barker, E. A. and Glebov, O. O. (2011). "Routes, destinations and delays: recent advances in AMPA receptor trafficking." Trends Neurosci **34**(5): 258-68.

Henriquez, D. R., Bodaleo, F. J., Montenegro-Venegas, C. and Gonzalez-Billault, C. (2012). "The light chain 1 subunit of the microtubule-associated protein 1B (MAP1B) is responsible for Tiam1 binding and Rac1 activation in neuronal cells." PLoS One **7**(12): e53123.

Hirokawa, N. and Takemura, R. (2005). "Molecular motors and mechanisms of directional transport in neurons." Nat Rev Neurosci **6**(3): 201-14.

Hollmann, M., Hartley, M. and Heinemann, S. (1991). "Ca2+ permeability of KA-AMPA--gated glutamate receptor channels depends on subunit composition." Science **252**(5007): 851-3.

Huber, K. M., Roder, J. C. and Bear, M. F. (2001). "Chemical induction of mGluR5- and protein synthesis--dependent long-term depression in hippocampal area CA1." J Neurophysiol **86**(1): 321-5.

Isaac, J. T., Nicoll, R. A. and Malenka, R. C. (1995). "Evidence for silent synapses: implications for the expression of LTP." Neuron **15**(2): 427-34.

Ishizuka, N., Cowan, W. M. and Amaral, D. G. (1995). "A quantitative analysis of the dendritic organization of pyramidal cells in the rat hippocampus." J Comp Neurol **362**(1): 17-45.

Jackson, A. C. and Nicoll, R. A. (2011). "The expanding social network of ionotropic glutamate receptors: TARPs and other transmembrane auxiliary subunits." Neuron **70**(2): 178-99.

Jaworski, J., Kapitein, L. C., Gouveia, S. M., Dortland, B. R., Wulf, P. S., Grigoriev, I., Camera, P., Spangler, S. A., Di Stefano, P., Demmers, J., Krugers, H., Defilippi, P., Akhmanova, A. and Hoogenraad, C. C. (2009). "Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity." Neuron **61**(1): 85-100.

Jia, Z., Agopyan, N., Miu, P., Xiong, Z., Henderson, J., Gerlai, R., Taverna, F. A., Velumian, A., MacDonald, J., Carlen, P., Abramow-Newerly, W. and Roder, J. (1996). "Enhanced LTP in mice deficient in the AMPA receptor GluR2." Neuron **17**(5): 945-56.

Jimenez-Mateos, E. M., Gonzalez-Billault, C., Dawson, H. N., Vitek, M. P. and Avila, J. (2006). "Role of MAP1B in axonal retrograde transport of mitochondria." Biochem J **397**(1): 53-9.

Jolly, A. L., Kim, H., Srinivasan, D., Lakonishok, M., Larson, A. G. and Gelfand, V. I. (2010). "Kinesin-1 heavy chain mediates microtubule sliding to drive changes in cell shape." Proc Natl Acad Sci U S A **107**(27): 12151-6.

Jurado, S., Benoist, M., Lario, A., Knafo, S., Petrok, C. N. and Esteban, J. A. (2010). "PTEN is recruited to the postsynaptic terminal for NMDA receptor-dependent long-term depression." EMBO J **29**(16): 2827-40.

Kapitein, L. C. and Hoogenraad, C. C. (2011). "Which way to go? Cytoskeletal organization and polarized transport in neurons." Mol Cell Neurosci **46**(1): 9-20.

Kauer, J. A., Malenka, R. C. and Nicoll, R. A. (1988). "A persistent postsynaptic modification mediates long-term potentiation in the hippocampus." Neuron **1**(10): 911-7.

Kawakami, S., Muramoto, K., Ichikawa, M. and Kuroda, Y. (2003). "Localization of microtubule-associated protein (MAP) 1B in the postsynaptic densities of the rat cerebral cortex." Cell Mol Neurobiol **23**(6): 887-94.

Kemp, A. and Manahan-Vaughan, D. (2007). "Hippocampal long-term depression: master or minion in declarative memory processes?" Trends Neurosci **30**(3): 111-8.

Kemp, N., McQueen, J., Faulkes, S. and Bashir, Z. I. (2000). "Different forms of LTD in the CA1 region of the hippocampus: role of age and stimulus protocol." Eur J Neurosci **12**(1): 360-6.

Kim, C. H., Chung, H. J., Lee, H. K. and Huganir, R. L. (2001). "Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression." Proc Natl Acad Sci U S A **98**(20): 11725-30.

Kim, S. H., Kim, D. H., Lee, K. H., Im, S. K., Hur, E. M., Chung, K. C. and Rhim, H. (2014). "Direct Interaction and Functional Coupling between Human 5-HT6 Receptor and the Light Chain 1 Subunit of the Microtubule-Associated Protein 1B (MAP1B-LC1)." PLoS One **9**(3): e91402.

Kirschner, M. and Mitchison, T. (1986a). "Beyond self-assembly: from microtubules to morphogenesis." Cell **45**(3): 329-42.

Kirschner, M. W. and Mitchison, T. (1986b). "Microtubule dynamics." Nature **324**(6098): 621.

Kreis, T. E. (1987). "Microtubules containing detyrosinated tubulin are less dynamic." EMBO J **6**(9): 2597-606.

Lee, H. K., Barbarosie, M., Kameyama, K., Bear, M. F. and Huganir, R. L. (2000). "Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity." Nature **405**(6789): 955-9.

Lee, H. K., Kameyama, K., Huganir, R. L. and Bear, M. F. (1998). "NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus." Neuron **21**(5): 1151-62.

Lee, H. K., Takamiya, K., Han, J. S., Man, H., Kim, C. H., Rumbaugh, G., Yu, S., Ding, L., He, C., Petralia, R. S., Wenthold, R. J., Gallagher, M. and Huganir, R. L. (2003). "Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory." Cell **112**(5): 631-43.

Lee, S. H., Liu, L., Wang, Y. T. and Sheng, M. (2002). "Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD." Neuron **36**(4): 661-74.

Lee, S. H., Valtschanoff, J. G., Kharazia, V. N., Weinberg, R. and Sheng, M. (2001). "Biochemical and morphological characterization of an intracellular membrane compartment containing AMPA receptors." Neuropharmacology **41**(6): 680-92.

Leonard, A. S., Davare, M. A., Horne, M. C., Garner, C. C. and Hell, J. W. (1998). "SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit." J Biol Chem **273**(31): 19518-24.

Liao, D., Hessler, N. A. and Malinow, R. (1995). "Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice." Nature **375**(6530): 400-4.

Lisman, J. (1989). "A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory." Proc Natl Acad Sci U S A **86**(23): 9574-8.

Lisman, J., Schulman, H. and Cline, H. (2002). "The molecular basis of CaMKII function in synaptic and behavioural memory." Nat Rev Neurosci **3**(3): 175-90.

Lo, D. C., McAllister, A. K. and Katz, L. C. (1994). "Neuronal transfection in brain slices using particle-mediated gene transfer." Neuron **13**(6): 1263-8.

Lois, C., Hong, E. J., Pease, S., Brown, E. J. and Baltimore, D. (2002). "Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors." Science **295**(5556): 868-72.

Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell W, T., Li, W., Warren, S. T. and Feng, Y. (2004). "The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development." Proc Natl Acad Sci U S A **101**(42): 15201-6.

Luscher, C. and Malenka, R. C. (2012). "NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD)." Cold Spring Harb Perspect Biol **4**(6).

Luscher, C., Xia, H., Beattie, E. C., Carroll, R. C., von Zastrow, M., Malenka, R. C. and Nicoll, R. A. (1999). "Role of AMPA receptor cycling in synaptic transmission and plasticity." Neuron **24**(3): 649-58.

Maguire, E. A., Burgess, N., Donnett, J. G., Frackowiak, R. S., Frith, C. D. and O'Keefe, J. (1998). "Knowing where and getting there: a human navigation network." Science **280**(5365): 921-4.

Maguire, E. A., Burgess, N. and O'Keefe, J. (1999). "Human spatial navigation: cognitive maps, sexual dimorphism, and neural substrates." Curr Opin Neurobiol **9**(2): 171-7.

Malinow, R., Hayashi, Y., Maletic-Savatic, M., Zaman, S. H., Poncer, J. C., Shi, S. H., Esteban, J. A., Osten, P. and Seidenman, K. (2010). "Introduction of green fluorescent protein (GFP) into hippocampal neurons through viral infection." Cold Spring Harb Protoc **2010**(4): pdb prot5406.

Malinow, R., Mainen, Z. F. and Hayashi, Y. (2000). "LTP mechanisms: from silence to four-lane traffic." Curr Opin Neurobiol **10**(3): 352-7.

Malinow, R. and Malenka, R. C. (2002). "AMPA receptor trafficking and synaptic plasticity." Annu Rev Neurosci **25**: 103-26.

Mammen, A. L., Kameyama, K., Roche, K. W. and Huganir, R. L. (1997). "Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II." J Biol Chem **272**(51): 32528-33.

Man, H. Y., Wang, Q., Lu, W. Y., Ju, W., Ahmadian, G., Liu, L., D'Souza, S., Wong, T. P., Taghibiglou, C., Lu, J., Becker, L. E., Pei, L., Liu, F., Wymann, M. P., MacDonald, J. F. and Wang, Y. T. (2003). "Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons." Neuron **38**(4): 611-24.

Mao, L., Takamiya, K., Thomas, G., Lin, D. T. and Huganir, R. L. (2010). "GRIP1 and 2 regulate activity-dependent AMPA receptor recycling via exocyst complex interactions." Proc Natl Acad Sci U S A **107**(44): 19038-43.

Matsuda, S., Mikawa, S. and Hirai, H. (1999). "Phosphorylation of serine-880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor-interacting protein." J Neurochem **73**(4): 1765-8.

Matus, A. (2000). "Actin-based plasticity in dendritic spines." Science **290**(5492): 754-8.

Matus, A. and Riederer, B. (1986). "Microtubule-associated proteins in the developing brain." Ann N Y Acad Sci **466**: 167-79.

Mauceri, D., Cattabeni, F., Di Luca, M. and Gardoni, F. (2004). "Calcium/calmodulin-dependent protein kinase II phosphorylation drives synapse-associated protein 97 into spines." J Biol Chem **279**(22): 23813-21.

Mei, X., Sweatt, A. J. and Hammarback, J. A. (2000). "Regulation of microtubule-associated protein 1B (MAP1B) subunit composition." J Neurosci Res **62**(1): 56-64.

Meixner, A., Haverkamp, S., Wassle, H., Fuhrer, S., Thalhammer, J., Kropf, N., Bittner, R. E., Lassmann, H., Wiche, G. and Propst, F. (2000). "MAP1B is required for axon guidance and Is involved in the development of the central and peripheral nervous system." J Cell Biol **151**(6): 1169-78.

Meng, Y., Zhang, Y. and Jia, Z. (2003). "Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3." Neuron **39**(1): 163-76.

Merriam, E. B., Lumbard, D. C., Viesselmann, C., Ballweg, J., Stevenson, M., Pietila, L., Hu, X. and Dent, E. W. (2011). "Dynamic microtubules promote synaptic NMDA receptor-dependent spine enlargement." PLoS One **6**(11): e27688.

Merriam, E. B., Millette, M., Lumbard, D. C., Saengsawang, W., Fothergill, T., Hu, X., Ferhat, L. and Dent, E. W. (2013). "Synaptic regulation of microtubule dynamics in dendritic spines by calcium, F-actin, and drebrin." J Neurosci **33**(42): 16471-82.

Milner, B. (1972). "Disorders of learning and memory after temporal lobe lesions in man." Clin Neurosurg **19**: 421-46.

Mok, H., Shin, H., Kim, S., Lee, J. R., Yoon, J. and Kim, E. (2002). "Association of the kinesin superfamily motor protein KIF1Balpha with postsynaptic density-95 (PSD-95), synapse-associated protein-97, and synaptic scaffolding molecule PSD-95/discs large/zona occludens-1 proteins." J Neurosci **22**(13): 5253-8.

Montenegro-Venegas, C., Tortosa, E., Rosso, S., Peretti, D., Bollati, F., Bisbal, M., Jausoro, I., Avila, J., Caceres, A. and Gonzalez-Billault, C. (2010). "MAP1B regulates axonal development by modulating Rho-GTPase Rac1 activity." Mol Biol Cell **21**(20): 3518-28.

Morris, R. G., Garrud, P., Rawlins, J. N. and O'Keefe, J. (1982). "Place navigation impaired in rats with hippocampal lesions." Nature **297**(5868): 681-3.

Mulkey, R. M., Endo, S., Shenolikar, S. and Malenka, R. C. (1994). "Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression." Nature **369**(6480): 486-8.

Mulkey, R. M., Herron, C. E. and Malenka, R. C. (1993). "An essential role for protein phosphatases in hippocampal long-term depression." Science **261**(5124): 1051-5.

Muller, D. and Lynch, G. (1988). "Long-term potentiation differentially affects two components of synaptic responses in hippocampus." Proc Natl Acad Sci U S A **85**(23): 9346-50.

Nishimune, A., Isaac, J. T., Molnar, E., Noel, J., Nash, S. R., Tagaya, M., Collingridge, G. L., Nakanishi, S. and Henley, J. M. (1998). "NSF binding to GluR2 regulates synaptic transmission." Neuron **21**(1): 87-97.

Niswender, C. M. and Conn, P. J. (2010). "Metabotropic glutamate receptors: physiology, pharmacology, and disease." Annu Rev Pharmacol Toxicol **50**: 295-322.

Noble, M., Lewis, S. A. and Cowan, N. J. (1989). "The microtubule binding domain of microtubule-associated protein MAP1B contains a repeated sequence motif unrelated to that of MAP2 and tau." J Cell Biol **109**(6 Pt 2): 3367-76.

Noel, J., Ralph, G. S., Pickard, L., Williams, J., Molnar, E., Uney, J. B., Collingridge, G. L. and Henley, J. M. (1999). "Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism." Neuron **23**(2): 365-76.

Noiges, R., Eichinger, R., Kutschera, W., Fischer, I., Nemeth, Z., Wiche, G. and Propst, F. (2002). "Microtubule-associated protein 1A (MAP1A) and MAP1B: light chains determine distinct functional properties." J Neurosci **22**(6): 2106-14.

O'Brien, J. E., Sharkey, L. M., Vallianatos, C. N., Han, C., Blossom, J. C., Yu, T., Waxman, S. G., Dib-Hajj, S. D. and Meisler, M. H. (2012). "Interaction of voltage-gated sodium channel Nav1.6 (SCN8A) with microtubule-associated protein Map1b." J Biol Chem **287**(22): 18459-66.

O'Keefe, J. and Dostrovsky, J. (1971). "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat." Brain Res **34**(1): 171-5.

O'Keefe, J. and Nadel, L. (1978). The hippocampus as a cognitive map. Oxford
New York, Clarendon Press ;
Oxford University Press.

Oliet, S. H., Malenka, R. C. and Nicoll, R. A. (1997). "Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells." Neuron **18**(6): 969-82.

Osten, P., Khatri, L., Perez, J. L., Kohr, G., Giese, G., Daly, C., Schulz, T. W., Wensky, A., Lee, L. M. and Ziff, E. B. (2000). "Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor." Neuron **27**(2): 313-25.

Otmakhov, N., Khibnik, L., Otmakhova, N., Carpenter, S., Riahi, S., Asrican, B. and Lisman, J. (2004). "Forskolin-induced LTP in the CA1 hippocampal region is NMDA receptor dependent." J Neurophysiol **91**(5): 1955-62.

Palmer, M. J., Irving, A. J., Seabrook, G. R., Jane, D. E. and Collingridge, G. L. (1997). "The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus." Neuropharmacology **36**(11-12): 1517-32.

Passafaro, M., Piech, V. and Sheng, M. (2001). "Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons." Nat Neurosci **4**(9): 917-26.

Pedrotti, B., Francolini, M., Cotelli, F. and Islam, K. (1996a). "Modulation of microtubule shape in vitro by high molecular weight microtubule associated proteins MAP1A, MAP1B, and MAP2." FEBS Lett **384**(2): 147-50.

Pedrotti, B. and Islam, K. (1995). "Microtubule associated protein 1B (MAP1B) promotes efficient tubulin polymerisation in vitro." FEBS Lett **371**(1): 29-31.

Pedrotti, B. and Islam, K. (1996). "Dephosphorylated but not phosphorylated microtubule associated protein MAP1B binds to microfilaments." FEBS Lett **388**(2-3): 131-3.

Peng, J., Kim, M. J., Cheng, D., Duong, D. M., Gygi, S. P. and Sheng, M. (2004). "Semiquantitative proteomic analysis of rat forebrain postsynaptic density fractions by mass spectrometry." J Biol Chem **279**(20): 21003-11.

Perestenko, P. V. and Henley, J. M. (2003). "Characterization of the intracellular transport of GluR1 and GluR2 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons." J Biol Chem **278**(44): 43525-32.

Perez, J. L., Khatri, L., Chang, C., Srivastava, S., Osten, P. and Ziff, E. B. (2001). "PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2." J Neurosci **21**(15): 5417-28.

Piperno, G., LeDizet, M. and Chang, X. J. (1987). "Microtubules containing acetylated alpha-tubulin in mammalian cells in culture." J Cell Biol **104**(2): 289-302.

Purves, D. (2004). Neuroscience. Sunderland, Mass., Sinauer Associates, Publishers.

Reisel, D., Bannerman, D. M., Schmitt, W. B., Deacon, R. M., Flint, J., Borchardt, T., Seeburg, P. H. and Rawlins, J. N. (2002). "Spatial memory dissociations in mice lacking GluR1." Nat Neurosci **5**(9): 868-73.

Ren, X. D., Kiosses, W. B. and Schwartz, M. A. (1999). "Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton." EMBO J **18**(3): 578-85.

Riederer, B. M. (2007). "Microtubule-associated protein 1B, a growth-associated and phosphorylated scaffold protein." Brain Res Bull **71**(6): 541-58.

Rothman, J. E. (1994). "Mechanisms of intracellular protein transport." Nature **372**(6501): 55-63.

Sage, D., Neumann, F. R., Hediger, F., Gasser, S. M. and Unser, M. (2005). "Automatic tracking of individual fluorescence particles: application to the study of chromosome dynamics." IEEE Trans Image Process **14**(9): 1372-83.

Salter, M. W. and Kalia, L. V. (2004). "Src kinases: a hub for NMDA receptor regulation." Nat Rev Neurosci **5**(4): 317-28.

Sans, N., Racca, C., Petralia, R. S., Wang, Y. X., McCallum, J. and Wenthold, R. J. (2001). "Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway." J Neurosci **21**(19): 7506-16.

Schlesinger, S. (1993). "Alphaviruses--vectors for the expression of heterologous genes." Trends Biotechnol **11**(1): 18-22.

Schlesinger, S. and Dubensky, T. W. (1999). "Alphavirus vectors for gene expression and vaccines." Curr Opin Biotechnol **10**(5): 434-9.

Schoenfeld, T. A., McKerracher, L., Obar, R. and Vallee, R. B. (1989). "MAP 1A and MAP 1B are structurally related microtubule associated proteins with distinct developmental patterns in the CNS." J Neurosci **9**(5): 1712-30.

Schulze, E., Asai, D. J., Bulinski, J. C. and Kirschner, M. (1987). "Posttranslational modification and microtubule stability." J Cell Biol **105**(5): 2167-77.

Seger, R. and Krebs, E. G. (1995). "The MAPK signaling cascade." FASEB J **9**(9): 726-35.

Seitz, A., Kojima, H., Oiwa, K., Mandelkow, E. M., Song, Y. H. and Mandelkow, E. (2002). "Single-molecule investigation of the interference between kinesin, tau and MAP2c." EMBO J **21**(18): 4896-905.

Seog, D. H. (2004). "Glutamate receptor-interacting protein 1 protein binds to the microtubule-associated protein." Biosci Biotechnol Biochem **68**(8): 1808-10.

Setou, M., Seog, D. H., Tanaka, Y., Kanai, Y., Takei, Y., Kawagishi, M. and Hirokawa, N. (2002). "Glutamate-receptor-interacting protein GRIP1 directly steers kinesin to dendrites." Nature **417**(6884): 83-7.

Shen, L., Liang, F., Walensky, L. D. and Huganir, R. L. (2000). "Regulation of AMPA receptor GluR1 subunit surface expression by a 4. 1N-linked actin cytoskeletal association." J Neurosci **20**(21): 7932-40.

Sheng, M. and Sala, C. (2001). "PDZ domains and the organization of supramolecular complexes." Annu Rev Neurosci **24**: 1-29.

Shepherd, J. D. and Huganir, R. L. (2007). "The cell biology of synaptic plasticity: AMPA receptor trafficking." Annu Rev Cell Dev Biol **23**: 613-43.

Shi, S., Hayashi, Y., Esteban, J. A. and Malinow, R. (2001). "Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons." Cell **105**(3): 331-43.

Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold, R. J., Svoboda, K. and Malinow, R. (1999). "Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation." Science **284**(5421): 1811-6.

Shin, H., Wyszynski, M., Huh, K. H., Valtschanoff, J. G., Lee, J. R., Ko, J., Streuli, M., Weinberg, R. J., Sheng, M. and Kim, E. (2003). "Association of the kinesin motor KIF1A with the multimodular protein liprin-alpha." J Biol Chem **278**(13): 11393-401.

Simeone, T. A., Sanchez, R. M. and Rho, J. M. (2004). "Molecular biology and ontogeny of glutamate receptors in the mammalian central nervous system." J Child Neurol **19**(5): 343-60; discussion 361.

Slack, J. R. and Pockett, S. (1991). "Cyclic AMP induces long-term increase in synaptic efficacy in CA1 region of rat hippocampus." Neurosci Lett **130**(1): 69-72.

Song, I. and Huganir, R. L. (2002). "Regulation of AMPA receptors during synaptic plasticity." Trends Neurosci **25**(11): 578-88.

Song, I., Kamboj, S., Xia, J., Dong, H., Liao, D. and Huganir, R. L. (1998). "Interaction of the N-ethylmaleimide-sensitive factor with AMPA receptors." Neuron **21**(2): 393-400.

Spruston, N. (2008). "Pyramidal neurons: dendritic structure and synaptic integration." Nat Rev Neurosci **9**(3): 206-21.

Srivastava, S., Osten, P., Vilim, F. S., Khatri, L., Inman, G., States, B., Daly, C., DeSouza, S., Abagyan, R., Valtschanoff, J. G., Weinberg, R. J. and Ziff, E. B. (1998). "Novel anchorage of GluR2/3 to the postsynaptic density by the AMPA receptor-binding protein ABP." Neuron **21**(3): 581-91.

Sun, H., Hu, X. Q., Emerit, M. B., Schoenebeck, J. C., Kimmel, C. E., Peoples, R. W., Miko, A. and Zhang, L. (2008). "Modulation of 5-HT3 receptor desensitization by the light chain of microtubule-associated protein 1B expressed in HEK 293 cells." J Physiol **586**(3): 751-62.

Swanson, G. T., Kamboj, S. K. and Cull-Candy, S. G. (1997). "Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition." J Neurosci **17**(1): 58-69.

Takei, Y., Kondo, S., Harada, A., Inomata, S., Noda, T. and Hirokawa, N. (1997). "Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1B (MAP1B) gene." J Cell Biol **137**(7): 1615-26.

Takei, Y., Teng, J., Harada, A. and Hirokawa, N. (2000). "Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes." J Cell Biol **150**(5): 989-1000.

Terashima, A., Cotton, L., Dev, K. K., Meyer, G., Zaman, S., Duprat, F., Henley, J. M., Collingridge, G. L. and Isaac, J. T. (2004). "Regulation of synaptic strength and AMPA receptor subunit composition by PICK1." J Neurosci **24**(23): 5381-90.

Thomas, G. M., Hayashi, T., Chiu, S. L., Chen, C. M. and Huganir, R. L. (2012). "Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking." Neuron **73**(3): 482-96.

Togel, M., Wiche, G. and Propst, F. (1998). "Novel features of the light chain of microtubule-associated protein MAP1B: microtubule stabilization, self interaction, actin filament binding, and regulation by the heavy chain." J Cell Biol **143**(3): 695-707.

Tokuraku, K., Noguchi, T. Q., Nishie, M., Matsushima, K. and Kotani, S. (2007). "An isoform of microtubule-associated protein 4 inhibits kinesin-driven microtubule gliding." J Biochem **141**(4): 585-91.

Tortosa, E., Montenegro-Venegas, C., Benoist, M., Hartel, S., Gonzalez-Billault, C., Esteban, J. A. and Avila, J. (2011). "Microtubule-associated protein 1B (MAP1B) is required for dendritic spine development and synaptic maturation." J Biol Chem **286**(47): 40638-48.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J. and Dingledine, R. (2010). "Glutamate receptor ion channels: structure, regulation, and function." Pharmacol Rev **62**(3): 405-96.

Tucker, R. P., Garner, C. C. and Matus, A. (1989). "In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain." Neuron **2**(3): 1245-56.

Ulloa, L., Avila, J. and Diaz-Nido, J. (1993a). "Heterogeneity in the phosphorylation of microtubule-associated protein MAP1B during rat brain development." J Neurochem **61**(3): 961-72.

Ulloa, L., Diaz-Nido, J. and Avila, J. (1993b). "Depletion of casein kinase II by antisense oligonucleotide prevents neuritogenesis in neuroblastoma cells." EMBO J **12**(4): 1633-40.

Ulloa, L., Dombradi, V., Diaz-Nido, J., Szucs, K., Gergely, P., Friedrich, P. and Avila, J. (1993c). "Dephosphorylation of distinct sites on microtubule-associated protein MAP1B by protein phosphatases 1, 2A and 2B." FEBS Lett **330**(1): 85-9.

Verdoorn, T. A., Burnashev, N., Monyer, H., Seeburg, P. H. and Sakmann, B. (1991). "Structural determinants of ion flow through recombinant glutamate receptor channels." Science **252**(5013): 1715-8.

Verhaegen, M., Bauer, J. A., Martin de la Vega, C., Wang, G., Wolter, K. G., Brenner, J. C., Nikolovska-Coleska, Z., Bengtson, A., Nair, R., Elder, J. T., Van Brocklin, M., Carey, T. E., Bradford, C. R., Wang, S. and Soengas, M. S. (2006). "A novel BH3 mimetic reveals a mitogen-activated protein kinase-dependent mechanism of melanoma cell death controlled by p53 and reactive oxygen species." Cancer Res **66**(23): 11348-59.

Wang, Y., Cheng, A. and Mattson, M. P. (2006). "The PTEN phosphatase is essential for long-term depression of hippocampal synapses." Neuromolecular Med **8**(3): 329-36.

Wang, Z., Edwards, J. G., Riley, N., Provance, D. W., Jr., Karcher, R., Li, X. D., Davison, I. G., Ikebe, M., Mercer, J. A., Kauer, J. A. and Ehlers, M. D. (2008). "Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity." Cell **135**(3): 535-48.

Webster, D. R., Gundersen, G. G., Bulinski, J. C. and Borisy, G. G. (1987). "Differential turnover of tyrosinated and detyrosinated microtubules." Proc Natl Acad Sci U S A **84**(24): 9040-4.

Wedding, D. and Stevens, M. J. (2009). "Psychology: IUPsyS Global Resource (Edition 2009) [CD-ROM]." International Journal of Psychology, 44(Suppl. 1).

Wellmann, H., Kaltschmidt, B. and Kaltschmidt, C. (1999). "Optimized protocol for biolistic transfection of brain slices and dissociated cultured neurons with a hand-held gene gun." J Neurosci Methods **92**(1-2): 55-64.

Wenthold, R. J., Petralia, R. S., Blahos, J., II and Niedzielski, A. S. (1996). "Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons." J Neurosci **16**(6): 1982-9.

West, A. E., Neve, R. L. and Buckley, K. M. (1997). "Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor." J Neurosci **17**(16): 6038-47.

Wyszynski, M., Kim, E., Dunah, A. W., Passafaro, M., Valtschanoff, J. G., Serra-Pages, C., Streuli, M., Weinberg, R. J. and Sheng, M. (2002). "Interaction between GRIP and liprin-alpha/SYD2 is required for AMPA receptor targeting." Neuron **34**(1): 39-52.

Wyszynski, M., Kim, E., Yang, F. C. and Sheng, M. (1998). "Biochemical and immunocytochemical characterization of GRIP, a putative AMPA receptor anchoring protein, in rat brain." Neuropharmacology **37**(10-11): 1335-44.

Wyszynski, M., Valtschanoff, J. G., Naisbitt, S., Dunah, A. W., Kim, E., Standaert, D. G., Weinberg, R. and Sheng, M. (1999). "Association of AMPA receptors with a subset of glutamate receptor-interacting protein in vivo." J Neurosci **19**(15): 6528-37.

Xia, J., Zhang, X., Staudinger, J. and Huganir, R. L. (1999). "Clustering of AMPA receptors by the synaptic PDZ domain-containing protein PICK1." Neuron **22**(1): 179-87.

Zamanillo, D., Sprengel, R., Hvalby, O., Jensen, V., Burnashev, N., Rozov, A., Kaiser, K. M., Koster, H. J., Borchardt, T., Worley, P., Lubke, J., Frotscher, M., Kelly, P. H., Sommer, B., Andersen, P., Seeburg, P. H. and Sakmann, B. (1999). "Importance of AMPA receptors

for hippocampal synaptic plasticity but not for spatial learning." Science **284**(5421): 1805-11.

Zauner, W., Kratz, J., Staunton, J., Feick, P. and Wiche, G. (1992). "Identification of two distinct microtubule binding domains on recombinant rat MAP 1B." Eur J Cell Biol **57**(1): 66-74.

Zhu, J. J., Esteban, J. A., Hayashi, Y. and Malinow, R. (2000). "Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity." Nat Neurosci **3**(11): 1098-106.

Zhu, J. J., Qin, Y., Zhao, M., Van Aelst, L. and Malinow, R. (2002). "Ras and Rap control AMPA receptor trafficking during synaptic plasticity." Cell **110**(4): 443-55.

Ziff, E. B. (2007). "TARPs and the AMPA receptor trafficking paradox." Neuron **53**(5): 627-33.

ANNEX:
PUBLICATIONS