Activity-dependent changes in synaptic efficacy at glutamatergic and GABAergic connections in the immature hippocampus

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DECLARATION

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CONTENTS

ABBREVIATIONS

[ ]	concentration
ACSF	Artificial CerebroSpinal Fluid
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ATP	Adenosine 5'-triphosphate
BAPTA	1,2-bis(o-Aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid
BDNF	Brain-derived neurotrophic factor
CA1/4	*Cornu Ammonis* regions 1/4
cAMP	3'-5'-cyclic Adenosine MonoPhospate
CCK	Cholecystokinin
CNQX	6-Cyano-7-NitroQuinoXaline-2,3-dione
CNS	Central Nervous System
D-AP5	D-2-amino-5-phosphonovalerate
DCG-IV	(2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl)glycine
DG	Dentate Gyrus
DMSO	DiMethylSulphOxide
DNQX	6,7-DiNitroQuinoXaline-2,3-dione
EGTA	Ethylene Glycol-bis(2-aminoethylether)-*N-N-N'-N'*-tetraacetic acid
EPSC	Excitatory PostSynaptic Current
EPSP	Excitatory PostSynaptic Potential
ERK	Extracellular signal-Regulated Kinases
GABA	γ-aminobutyric acid
GAD	Glutamic Acid Decarboxylase

GAT	γ-aminobutyric acid transporter
GDP	Giant Depolarizing Potential
HEPES	4-(2-hydroxyethyl)-1-piperazine-1-ethanesulfonic acid
KAR	Kainate Receptor
KCC2	K^+/Cl^- co-transporter isoform 2
L-AP4	L(+)-2-amino-4-phosphonobutyric acid
LTD	Long-Term Depression
LTP	Long-Term Potentiation
MF	Mossy Fiber
mGluR	metabotropic glutamate receptor
nAChR	nicotinic acetylcholine receptor
NKCC1	Na-K-Cl cotransporter isoform 1
NMDA	N-methyl-D-aspartate
O-LM	Oriens–Lacunosum Moleculare
O-OR	Oriens–Oriens and Radiatum
P-LM	Pyramidale–Lacunosum Moleculare
PCR	Polymerase Chain Reaction
PKA	Protein Kinase A
PPR	Paired-Pulse Ratio
PTX	Picrotoxin
R–LM	Radiatum–Lacunosum Moleculare
SNARE	SNAP (Soluble NSF Attachment Protein) REceptors

STD-LTD	spike-timing-dependent-long-term depression
STD-LTP	spike-timing-dependent-long-term potentiation
TASK	TWIK-related acid-sensitive K^+ channel
TrkB	Tropomyosin-related kinase receptor B
TTX	Tetrodotoxin
VGAT	Vesicular GABA Transporter

ABSTRACT

During development, correlated network activity plays a crucial role in establishing functional synaptic contacts, thus contributing to the development of adult neuronal circuits. In the hippocampus, a region of the brain involved in the formation of declarative memories, network-driven giant depolarizing potentials or GDPs are generated by the synergistic action of glutamate and GABA, which at this developmental stage is depolarizing and excitatory. The depolarizing action of GABA during GDPs results in calcium flux via NMDA receptors and voltage-dependent calcium channels. Calcium, in turn activates different signaling pathways necessary for several developmental processes including synaptogenesis. In previous work from our laboratory it was demonstrated that GDPs and associated calcium transients act as coincident detectors for enhancing synaptic efficacy at poorly developed mossy fibre-CA3 synapses in a Hebbian type of way.

In the first paper presented in my thesis we asked whether GDPs can enhance synaptic efficacy at emerging glutamatergic Schaffer collateral-CA1 synapses. To this aim, the rising phase of GDPs was used to trigger Schaffer collateral stimulation in such a way that synchronized network activity was coincident with presynaptic activation of afferent input. This procedure resulted in a persistent increase in spontaneous and evoked AMPA-mediated glutamatergic currents. No potentiation was observed when a delay of 3 s was introduced between GDPs and afferent stimulation. The induction of pairing-induced synaptic potentiation required calcium influx into the postsynaptic cell through L-type of calcium channels, since it was prevented by nifedipine, a broad voltage-dependent calcium channel blocker. However, the expression of this form of synaptic plasticity was presynaptic as suggested by the decrease in PPR and the increase in frequency, but not in the amplitude, of spontaneous EPSCs. Pairing-induced potentiation of synaptic currents was mimicked by exogenous application of BDNF and was prevented by a scavenger of endogenous BDNF or TrkB receptors antagonists. Blocking TrkB receptors in the postsynaptic cell did not prevent the effects of pairing, suggesting that BDNF, possibly secreted from the postsynaptic cell during the depolarizing action of GDPs, acts on TrkB receptors localized on the presynaptic neurons. In addition, pairing-induced synaptic potentiation was blocked by ERK inhibitors,

indicating that BDNF activates the MAPK/ERK cascade, which may lead to transcriptional regulation and new protein synthesis in the postsynaptic neuron. Therefore, BDNF can act presynaptically to alter the probability of glutamate release but also postsynaptically to produce the morphological modifications necessary for the formation of new synapses and the refinement of the adult neuronal circuit. In conclusion, these results support the hypothesis that during a critical period of postnatal development, GDPs are instrumental in tuning excitatory glutamatergic connections and provide new insights into the molecular mechanisms involved in this process.

In the second part of my thesis, we investigated whether another associative form of synaptic plasticity, the spike-timing dependent plasticity or STDP, can modify synaptic strength at immature mossy fibres (MF)-CA3 synapses, which early in postnatal life are GABAergic. Spike-timing dependent plasticity is essential for information processing in the brain, and this process depends on the temporal order of pre and postsynaptic spiking. We first demonstrated that GABA, released from MF terminals depolarizes tagged cells. Then we found that when back propagating action potential followed the presynaptic stimulation of MF, a persistent increase in amplitude of GABAA-mediated postsynaptic current (GPSCs) was observed, an effect that was associated with a decrease in the number of failures and a reduction in the paired-pulse ratio. Positive pairing (pre versus post) performed in current clamp conditions, produced a stable potentiation of the slope of GPSPs which often triggered action potentials. Therefore, after pairing, the probability of firing increased significantly. In contrast, when the back propagating action potential preceded the presynaptic stimulation, a significant reduction in amplitude of GPSCs was detected. The temporal window of the potentiation or depression lasted for ~50 ms in both directions. The potentiating effect required a rise of calcium in the postsynaptic cell via voltage-dependent calcium channels since this effect was prevented by intracellular BAPTA or by nifedipine. While the induction of this form of synaptic plasticity was postsynaptic, the expression was presynaptic as suggested by the changes in failure rate and paired-pulse ratio, which accompanied modifications in amplitude of GPSCs (in both directions). This suggests the existence of a cross talk between the post and the presynaptic neuron and the involvement of a transcellular retrograde signal that was identified in the BDNF. BDNF, released by the postsynaptic neuron during spike-induced membrane depolarization may act on presynaptic

Trk B receptor to enhance transmitter release. Thus, Trk B receptor antagonists prevented spike-time dependent potentiation and exogenous application of BDNF mimicked the effects of pairing, further strengthening the initial observation. We found also that the effects of BDNF on synaptic potentiation required the activity of protein kinase A. These results show that, during postnatal development, pairing back propagating action potentials with MF-GPSPs persistently enhances synaptic efficacy and brings CA3 principal cells to fire, thus providing a reliable way to convey information from granule cells to the CA3 associative network at a time when glutamatergic synapses are still poorly developed. It should be stressed that in this study, to prevent possible contamination with glutamatergic transmission, we routinely used D-APV and DNQX to block NMDA and AMPA/kainate receptors, respectively. We noticed however, that when GYKI was used to block only AMPA receptors, addition of DNQX caused an increase in MF-GPSCs, suggesting that presynaptic kainate receptors (KARs) down regulate the release of GABA from MF terminals. Therefore, in the third paper the functional role of KARs in regulating GABA release from MF terminals was carefully investigated.

Presynaptic kainate receptors regulate synaptic transmission in several brain areas but are not known to have this action at immature mossy fibers (MF) terminals which release mainly GABA. We found that, during the first week of postnatal life, application of DNQX or UBP 302, a selective antagonist for GluK1 receptors, increased the amplitude of MF-GPSCs, an effect associated with a decrease in failure rate and in PPR, indicating the involvement of GluK1 receptors in the regulation of GABA release. These receptors were tonically activated by “ambient” glutamate present in the extracellular space, since decreasing the extracellular concentration of glutamate with a glutamate scavenger, prevented the activation of GluK1 receptors and mimicked the effects of KAR antagonists. In addition, the depressant effect of GluK1 on GABA release was dependent on a metabotropic type of mechanism since the potentiating effects of both UBP 302 and the glutamate scavenger on GPSCs were fully blocked by Pertussis toxin. In the presence of U73122, a selective inhibitor of phospholipase C, along the transduction pathway downstream to G protein, GluK1 activation enhanced the probability of GABA release. This was probably mediated by the ionotropic action of GluK1 and was in line which the enhanced MF excitability which was probably dependent on the direct depolarization of MF terminals via calcium-permeable cation channels. In addition, we

found that GluK1 dynamically regulated the direction of STDP being able to shift spike-time dependent potentiation into depression. The GluK1-induced depression of MF-GPSCs would prevent the excessive activation of the CA3 associative network by the excitatory action of GABA and the onset of seizures.

INTRODUCTION

The Hippocampus

"Memories, light the corners of the mind" - So goes the popular song from the 1970s, but actually it is believed that memories are formed deep within the brain rather than in the corners. One brain structure known to be involved in the complex processes of forming, sorting, and storing memories is the hippocampus. The hippocampus is a well characterized brain structure, named by the sixteenth century anatomist *Julius Caesar Aranzi (1529 – 1589)*, in his book *"De Humano Foetu liber"* (Rome, 1564), from the Greek word for sea horse, *hippos* and *kampos,* meaning '*horse*' and '*sea monster*', respectively. This reference follows the resemblance of its curved shape to that of a sea horse. The resemblance of the hippocampus to the horns of a ram, prompted another name: *"cornu arietis"*, later changed by Rene Croissant de Garengeot (1688 – 1759) to *"cornu ammonis"* because of the ancient Egyptian God of Theba Amun, who was presented as a ram headed man, or a ram headed sphinx. The shape of the hippocampus is conserved across a wide range of mammalian species. Ramon y Cajal (1852 – 1934) contributed in a major way in understanding the microscopic structure of the nervous system using a modification of the Golgi staining method. This method stains a relatively small proportion of cells. With this technique, the discoveries of the cellular architecture of the hippocampal formation and the associated subdivisions still remain a key stone for understanding the functional structure of the hippocampus.

A paired structure with mirror-image halves in either hemisphere of the brain, the hippocampus is located inside the medial temporal lobe. Along with other structures in the temporal lobe, the hippocampus is involved in learning and memory (Kandel, 2001), and plays an important role in storage of long-term traces in humans and other mammalian species (Milner et al., 1998; Kim and Baxter, 2001; Burgess et al., 2002). In the rodents, the hippocampus plays a crucial role in spatial information processing, where specific cells called "place cells" take part with a high rate of firing, when the animal moves through a specific location in the environment (O'Keefe, 1983).

Anatomy

Anatomically, located on the medial wall of the later ventricle, the hippocampus is an elongated structure with its longitudinal axis forming a semicircle around the thalamus. The hippocampus proper and the surrounding regions, the dentate gyrus (DG), subiculum and entorhinal cortex, together comprise the "hippocampal formation" (Figure 1). Cajal classified the regions into areas CA1 – CA4, based on the size and structure of neurons. CA1 and CA3 form the larger zones, with the CA4 (or the hilus) corresponding to the initial part of CA3.

Although there is a lack of consensus relating to terms describing the hippocampus and the adjacent cortex, the term hippocampal formation generally applies to the dentate gyrus, fields CA1-CA3 and the subiculum (see also *Cornu ammonis*). The CA1 and CA3 fields make up the hippocampus proper. Information flowing through the hippocampus proceeds from dentate gyrus to CA3, CA1 and the subiculum. CA2 represents only a very small portion of the hippocampus and its presence is often neglected. The CA3 region is critical for the initiation of hippocampal interictal-like activity. This depends on the presence of abundant recurrent excitatory connections between CA3 pyramidal cells (Miles and Wong, 1983). In area CA3, Lorente de No (1934) has identified 3 subareas by anatomical position – CA3a, CA3b and CA3c, corresponding to the bend of CA3, the ventral portion between the bend and the lateral end of the DG and the portion encapsulated by the blades of the DG, respectively. A recent study has suggested that the CA3a region is involved in initiation of ensemble activity (Wittner and Miles, 2007). This may be due to the fact that CA3a pyramidal cells are more excitable and fire in bursts more frequently than CA3b cells. Hence, at least in guinea pigs, CA3a cells have more complex dendritic arbors than CA3b cells especially in zones targeted by recurrent synapses (Wittner and Miles, 2007).

Pyramidal cell soma are arranged in the stratum pyramidale in all the regions. Other regions identified include the stratum oriens where the basal dendrites of the pyramidal cells could be found, the stratum radiatum and stratum lacunosum moleculare, where the apical dendrites are radially oriented.

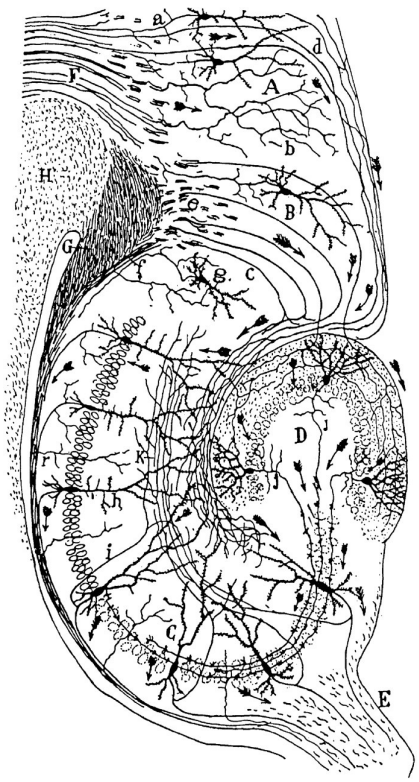

Figure 1: Neuronal elements of the hippocampal formation. Labelled areas include the subiculum, part of the entorhinal cortex, the fornix, the dentate gyrus and the regions CA1 to CA4. The hippocampus *proper* is divided into *alveus, stratus oriens, stratum pyramidale, stratus lucidum, stratum radiatum* and *stratum lacunosum-moleculare.* (Ramon y Cajal, 1911).

The Hippocampal Circuit

From the entorhinal cortex, a region which receives and integrates multisensory inputs from the touch, auditory, olfactory and visual pathways as well inputs from the cingulated gyrus, output fibers cross (perforate) the surrounding cortex and terminate into the dentate gyrus. The so-called "perforant path" is generally considered the main input to the hippocampus. The dentate granule cells have relatively short axons, called the "mossy fibers" which connect the nearest part of the pyramidal cell population. These axons have been called 'mossy' by Ramon y Cajal (Ramon y Cajal, 1911) because of their particular appearance at the light microscopic level that reminds, as the mossy fibers in the cerebellum, the shape of the moss on trees. Mossy fibers (MF) give rise to large *en passant* swellings and terminal expansions on CA3 principal neurons or mossy cells seen as giant boutons at the electron microscopic level. These presynaptic swellings adapt very well to specialized postsynaptic elements present on proximal dendrites of CA3 principal cells, called *thorny excrescences*.

The MF synaptic complex contains multiple active zones (up to 50) associated with postsynaptic densities. In addition MF make synaptic contacts with GABAergic interneurons present in the *hilus* and in the CA3 area and these represent the majority of all MF connections (Frotscher et al., 2006). In a seminal paper, Acsády et al., (1998) demonstrated that MF connections with interneurons have either the shape of small boutons or filopodial extensions. Differences in morphology between MF terminals at principal cells and interneurons may account for the distinct functional properties of these synapses which appear to be regulated in a target specific way (Nicoll and Schmitz, 2005).

The axons of CA3 principal cells project to the septum trough the fornix. In addition, they send collaterals (Schaffer collateral) that make synaptic connections with the apical dendrites of CA1 pyramidal neurons. The axons of CA3 pyramidal cells form also recurrent excitatory synapses with neighboring principal cells giving rise to an auto-associative network crucial for learning and memory functions. As already mentioned, the auto associative CA3 network plays an important role in initiating ensemble network activity during seizures.

The CA1 pyramidal cells project to a nearby cortical area, the subiculum which in turn projects to the septum through the fornix.

For its relatively simple organization, the hippocampus is particularly well suited for studies on slices, because it is constructed as a series of lamellae, each of which contain the whole three synaptic circuit.

MAJOR NEURONAL TYPES IN THE HIPPOCAMPUS

Granule cells in the dentate gyrus

The principal neurons of the DG are the granule cells. These have a small, ovoid cell body with a single dendritic tree which extends into the molecular layer and terminate near the hippocampal fissure. Dendrites are heavily studded with spines. They receive a main excitatory input from the enthorhinal cortex *via* the perforant path (Amaral and Witter, 1995). Granule cells receive also a number of inhibitory inputs from many different types of interneurons, mostly from those present in the hilus. One peculiar characteristic of granule cells in the dentate gyrus is that they are still post-mitotic at birth and continue to develop until the second week of postnatal life. In addition, they continuously undergo turnover throughout the life (Henze et al., 2000) since they are constantly generated from stem cells in the hilus.

CA1 and CA3 pyramidal neurons

Pyramidal cells, which account for about 90% of hippocampal neurons, constitute a rather uniform cell population with homogenous morphology. They are named for their somatic shape and possess apical and basal dendrites. The basal dendrites occupy *Stratum Oriens* while apical dendrites occupy *Stratum Radiatum* and *Stratum-lacunosum Moleculare*. The dendrites are studded with spines, which are the sites of most excitatory connections (Ramon y Cajal, 1904; Lorente de No, 1934; Bannister and Larkman, 1995; Megias et al., 2001). Therefore, spine density can be used as a reasonable measure of excitatory synapses density (Gray, 1959; Andersen et al., 1966a, 1966b; Megias et al., 2001). As already mentioned, CA3 pyramidal cells posses a particular class of spines, the "*thorny excrescences*" which are present mainly on the apical dendrites (~40 on each neuron; Blackstad and Kjaerheim, 1961; Amaral and Witter, 1989; Chicurel and Harris, 1992), where they form the postsynaptic targets of mossy fibers. Spines contain several organelles including mitochondria which contribute to calcium handling (in addition to serving as a primary source of energy) and sarco-endoplasmic reticulum calcium ATPase which play a crucial role in calcium buffering and release.

Pyramidal cells are excitatory and liberate the neurotransmitter glutamate (Storm-Mathisen, 1977; Roberts and Sharif, 1981). Pyramidal cells receive inhibitory inputs from local

GABAergic interneurons. These control pyramidal cell excitability *via* feed-back or feed-forward inhibition. Feed-forward inhibition is mediated by GABAergic interneurons activated by extra- or intra hippocampal inputs (Buzsáki, 1984; Lacaille et al., 1987). Feed-back inhibition is mediated by local interneurons excited by pyramidal cells *via* recurrent collaterals (Andersen et al., 1964). These inhibitory mechanisms play an important role in synchronizing the activity of widely distributed neurons (Cobb et al., 1995) and have been proposed to control the generation of oscillatory activity in neural networks (Buzsáki and Chrobak, 1995).

Mossy cells

Mossy cells are part of numerous cell types present in the polymorphic layer (hilus) between the granule cells in the DG and the CA3 pyramidal neurons (Amaral, 1978). These cells were carefully characterized primarily because they were the first to degenerate during epilepsy and ischemia. They consist of ovoid cell bodies with several primary dendrites. Mossy cells receive excitatory input from MF and additional excitatory inputs from a subset of CA3 pyramidal neurons that send axon collaterals back into the hilus. They also receive substantial inhibitory inputs, probably from basket cells of the dentate gyrus, from aspiny hilar interneurons and GABAergic input from the medial septum. Their axons make excitatory glutamatergic synapses with granule cells in the DG and with GABAergic interneurons in the hilus (Buckmaster et al., 1996).

GABAergic interneurons

Inhibitory interneurons, which form about 10% of the total hippocampal neuronal population constitute a heterogeneous group of cells which liberate the transmitter γ-aminobutyric acid (GABA, Freund and Buzsáki, 1996). They play a critical role in controlling communication between pyramidal neurons and rythmogenesis. Interneurons are distributed across all layers of the hippocampus and have a highly variable morphology. They differ from pyramidal cells in their active and passive membrane properties. For instance, pyramidal cells accommodate (during a steady current pulse the firing is not maintained but declines with time) while interneurons usually do not accommodate and can fire at frequencies up to 400 Hz with little reduction in frequency (Lacaille, 1991). This may be due to major differences in transcript expressions for distinct subunits of voltage-gated K^+ channels, which shape the action potential phenotypes of principal neurons and

interneurons in the cortex (Martina et al., 1998). The resting membrane potential of pyramidal cells and interneurons also differs, with interneurons being more depolarized than pyramidal cells. As shown for subicular interneurons (Richards et al., 2007), this difference could be due to the expression of distinct α-subunits of the Na^+/K^+-ATPase in interneurons and pyramidal cells. The Na^+/K^+-ATPase is known to contribute to the regulation of neuronal membrane potential, since transmembrane Na^+ and K^+ exchange is electrogenic. Moreover, in comparison with principal cells, interneurons exhibit a higher input resistance (Morin et al., 1996; Cauli et al., 1997; Savic et al., 2001). These differences seem to depend on the differential expression of pH-sensitive leak potassium channel TASK, a subtype of KCNK channel that regulates resting membrane potential and input resistance. TASK channel subunits 1 and 3 seem to be more highly expressed in pyramidal cells than in interneurons (Taverna et al., 2005).

Pyramidal cells and interneurons also differ in peptide composition, neurotransmitter receptor subunit expression and neurotransmitter release mechanisms (Buzsáki, 2001). Further studies and novel techniques including gene expression profile may uncover additional differences between these two classes of neurons. Microarray mRNA expression analysis has been used to compare the cellular heterogeneity of excitatory and inhibitory neurons in different brain areas (Sugino et al., 2006) including the CA1 region of the hippocampus (Kamme et al., 2003). Immunocytochemical (Macdonald and Olsen 1994; Pickard et al., 2000), combined patch and single cell PCR (Martina et al., 1998; Taverna et al., 2005) and mRNA expression profile experiments (Telfeian et al., 2003) have revealed differences in Glutamate and GABA receptor subunits composition between principal cells and interneurons, which may contribute to the different behavior of these cells.

The term interneuron was originally used to describe invertebrate cells that were neither input nor output neurons. As the concept of synaptic inhibition developed (Eccles, 1964), it came to be used as a synonym for inhibitory cells with short axons. These cells were seen to play an essential role in the regulation of local circuit excitability, while principal cells, with longer axons, were thought to project information to distant brain regions. The continued emergence of functional, biochemical and anatomical data on principal and inhibitory cells has revealed exceptions to this definition such that the original view on interneurons is clearly an oversimplification. Glutamatergic cells make synapses locally as well as at a distance, and GABAergic cells can make synapses at a distance as well as locally. Since most,

if not all, non-principal cells use GABA as a transmitter (Freund and Buzsáki, 1996), the definition “GABAergic non-principal cells” appears to be the most adequate for hippocampal interneurons.

Based on Golgi staining, Ramon y Cajal (1893) and Lorenté de No (1934) distinguished ~20 different types of hippocampal interneurons (Klausberger and Somogyi, 2008; Figure 2). Distinct cell types were first described according to features of their axonal or dendritic processes (e.g. basket cell, horizontal cell and stellate cell). Labeling techniques can now reveal the entire dendritic and axonal processes of recorded cells (Buhl et al., 1994). The large number of interneuron types was matched by an equally rich terminology including both classical descriptions such as basket cells and new terms emphasizing different aspects of interneuron anatomy (Gulyas et al., 1993). The postsynaptic target domain has been highlighted for the ‘axo–axonic cells’, which innervate the axon initial segment of pyramidal cells (Buhl et al.,1994). The site of layers containing the soma and axonal processes is noted in the names of O-LM cells (*Oriens–Lacunosum Moleculare*, Freund and Buzsáki, 1996), P-LM cells (*Pyramidale–Lacunosum Moleculare*, Oliva et al., 2000) and O-OR bistratified cells (*Oriens–Oriens* and *Radiatum*, Maccaferri et al., 2000). Other names have emphasized the orientation of interneuron dendrites, as in stellate cells, and the vertical or horizontal cells of stratum oriens, as well as the nearby excitatory synapses on post-synaptic sites such as the Schaffer collateral-associated interneurons.

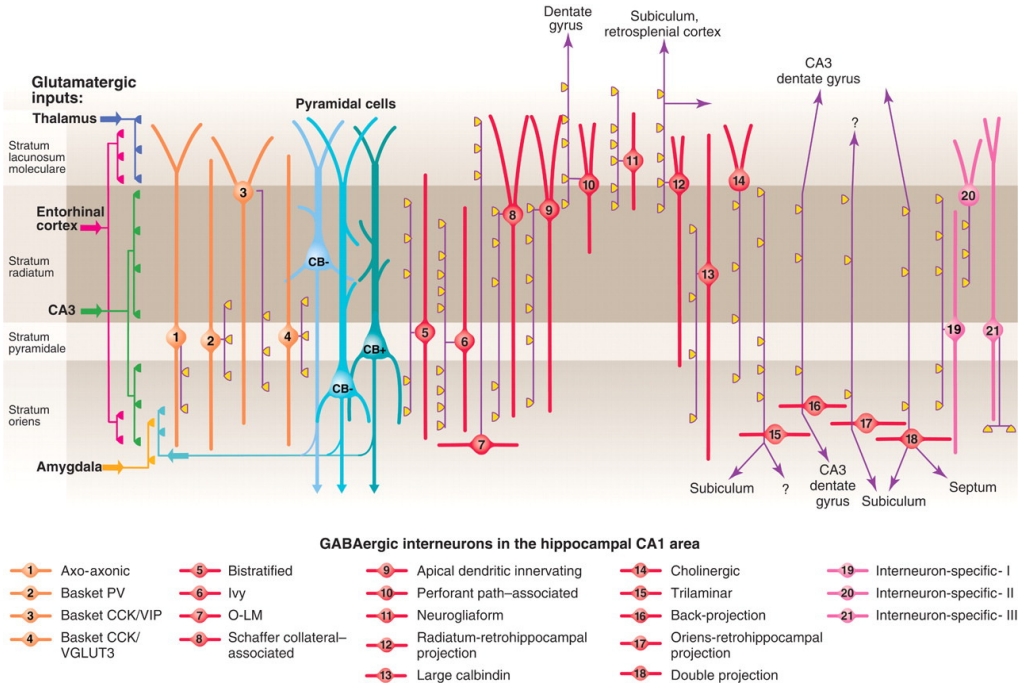

Figure 2: Three types of pyramidal cell are accompanied by at least 21 classes of interneurons in the hippocampal CA1 area. The main termination of five glutamatergic inputs are indicated on the left. The somata and dendrites of interneurons innervating pyramidal cells (blue) are orange, and those innervating mainly other interneurons are pink. Axons are purple; the main synaptic terminations are yellow. Note the association of the output synapses of different interneuron types with the perisomatic region (left) and either the Schaffer collateral/commissural or the entorhinal pathway termination zones (right), respectively. VIP, vasoactive intestinal polypeptide; VGLUT, vesicular glutamate transporter; O-LM, oriens lacunosum moleculare (Klausberger and Somogyi, 2008).

Interneurons (except mossy cells) all contain GABA as a neurotransmitter (Storm-Mathisen et al., 1983) and the GABA synthesizing enzymes GAD65 and GAD67 (Ribak, 1978). Specific interneuron populations contain different peptides, including somatostatin, cholecystokinin (CCK) and substance P, or Ca^{2+}-binding proteins, such as calbindin, parvalbumin and calretinin, (Somogyi et al., 1984). These markers have been used to produce a neurochemical classification which may also imply functional differences (Freund and Buzsáki, 1996; Baraban and Tallent, 2004). Parvalbumin, calbindin or calretinin expression seems to discriminate between interneurons with different dendritic geometry, postsynaptic target selection and synaptic input density (Gulyas et al., 1999). However, morphologically defined interneurons may co-exist in a single neurochemically identified subgroup. For example, O–LM, O–bistratified, P–LM and R–LM interneurons of the CA1 hippocampal subfield are all immunoreactive for somatostatin (Katona et al., 1999; Maccaferri et al., 2000; Oliva et al., 2000; Losonczy et al., 2002). Similarly, parvalbumin is expressed by basket and axo–axonic cells (Kosaka et al., 1987; Klausberger et al., 2003) as well as at lower levels in the soma and dendrites but not terminals of O–LM cells (Maccaferri et al., 2000; Losonczy et al., 2002). Furthermore, distinct markers may be expressed in morphologically similar interneurons with different functional properties. Basket cells for instance express parvalbumin or CCK (Freund, 2003). Therefore, a combined neurochemical and anatomical classification will be more useful than a scheme based on either one of these parameters.

Principal cells and GABAergic interneurons receive neuromodulatory inputs from a number of subcortical areas. These include the cholinergic projection from basal forebrain cholinergic neurons in the medial septum, diagonal band, substantia innominata and nucleus basalis of Meynert (reviewed by McKinney, 2005), the noradrenergic, serotonergic and dopaminergic inputs from the locus coeruleus, the raphe and the ventral tegmental area, respectively. In particular, the cholinergic innervation, mediated *via* metabotropic (G-

protein-coupled) muscarinic and ionotropic nicotinic acetylcholine receptors contributes to enhance activity-dependent synaptic plasticity processes. Deficits in the cholinergic system produce impairment of cognitive functions that are particularly relevant during senescence and in age-related neurodegenerative pathologies. In particular, Alzheimer's disease is characterized by a selective loss of cholinergic neurons in the basal forebrain (Bartus et al., 1982) and nAChRs in particular regions controlling memory processes such as the cortex and the hippocampus (Paterson and Nordberg, 2000).

Glial cells

These include micro and macro glia. Microglial cells, which are distributed throughout the CNS, form about 20% of all glial cells contained in the brain (Lawson et al., 1990). They have a myeloid origin, deriving from bone marrow precursor cells (Ling and Wong, 1993) and form a resident, stable population of innate immune cells whose phenotype seems to represent an adaptation of the monocyte and macrophage cells of the blood to a neural environment. In normal brain, microglial phenotype is down regulated and characterized by a low expression of the CD45 leukocyte antigen. Microglias normally have no phagocytotic or endocytotic activity and proteins that induce or mediate typical macrophage functions are poorly expressed or absent (Kreutzberg, 1996). Normal levels of neuronal activity apparently act to maintain this inactive microglial cell phenotype via neurotrophin signaling (Neumann et al., 1998; Wei and Jonakait, 1999) as well as neurotransmitter and peptide levels (Hetier et al., 1991; Delgado et al., 1998). It has recently been suggested that neurons signal directly to microglia, in cell-to-cell fashion, with an inhibitory signal involving the membrane glycoprotein OX2 (Hoek et al., 2000; Wright et al., 2000).

Macroglia are a large cell class including oligodendrocytes and astrocytes. As neurones they originate from the differentiation of multipotent neural stem cells. Oligodendrocytes, located in the white matter, act primarily to form myelin (Bunge, 1968), an insulation of modified plasma membrane that surrounds axons. They govern the speed and efficacy of axonal impulse conduction and so are essential for CNS function. Astrocytes were thought to support neuronal function by supplying essential substrates and removing toxic substrates, but recent data suggests they are intimately involved in the neurogenesis occurring in restricted brain regions of adult mammals including the hippocampus (Doetsch and Scharff, 2001; Song et al., 2002). Astrocytes can retain stem cell like properties (Doetsch, 2003) and

induce differentiation in adult neural stem cells (Song et al., 2002) or direct differentiation in neuronal cell types (Berninger et al., 2007). Another interesting role of astrocytes is as a third partner, with pre- and post-synaptic elements in the structural and functional organization of the synapse. Porter and colleagues have shown that astrocytes express receptors and respond to several neurotransmitters including glutamate (Porter and McCarthy, 1996; Porter and McCarthy, 1997) and GABA (Kang et al., 1998; Araque et al., 2002). Astrocytes modulate also neurotransmission by controlling ambient transmitter levels with glutamate transporters and instruct the development, maintenance and recovery of synapses. Synaptic stimuli from neurones can evoke astrocytic responses including increases in intracellular Ca^{2+} and release of "gliotransmitter". Self propagating waves of Ca^{2+} signals are reported to spread long distances to other glial cells via gap junctions or ATP signals (Newman, 2001). Reports also suggest that astrocytes can release glutamate (Bezzi et al., 1998; Araque et al., 2000; Pasti et al., 2001; D'Ascenzo et al., 2007; Fellin et al., 2007) or D-Serine, an endogenous ligand for NMDA receptors (Mothet et al., 2005) and depolarize neurons in several regions including hippocampus (Hassinger et al., 1995). Further, it has been shown that interneuronal firing elicits a GABABR-mediated elevation of calcium in neighboring astrocytes, which in turn potentiates inhibitory synaptic transmission, thereby suggesting an involvement of astrocytes as an intermediate player in activity-dependent modulation of inhibitory synapses in the hippocampus (Kang et al., 1998).

DEVELOPMENT OF THE HIPPOCAMPUS

Cell Proliferation

Along with the neocortex, the hippocampus derives from the telencephalic pallium. During early development, the undifferentiated germinal cells (neuroepithelium) are localized in the ventricular zone. From the neuroepithelial cells, pluripotent progenitors are born which in turn lead to the generation of neuronal and glial precursors. Migration of the first postmitotic cells happens in a radial manner, out of the neuroepithelium to form the first recognizable cortical layer, called the preplate (or early marginal zone). In the rat, the preplate is present at day 10–15 after conception.

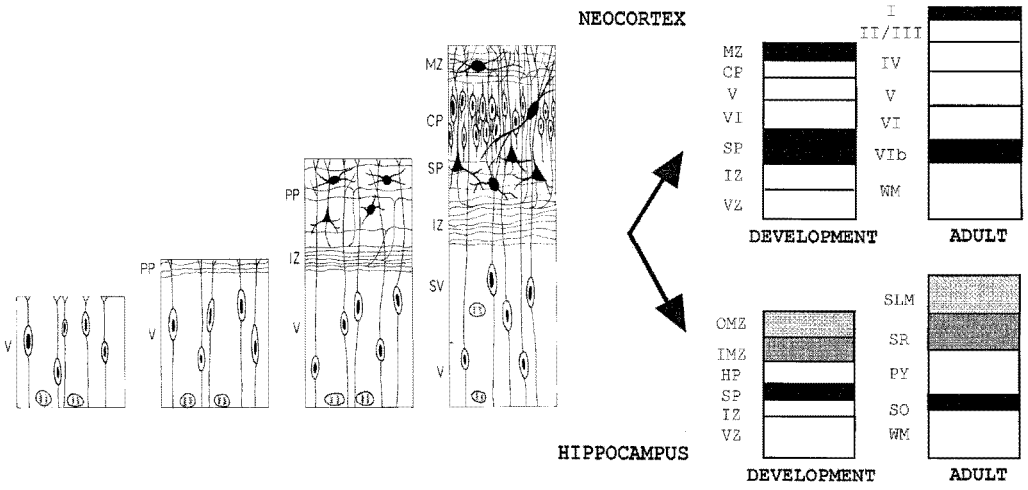

Figure 3: Left panel - Schematic representation of early developmental stages of cortical lamination in the telencephalic wall. In the pseudostratified cerebral wall a preplate forms that contains the pioneer neurons. Subsequently, post-mitotic neurons, migrating along radial glial cells, form a cortical plate within the preplate. Abbreviations: V, ventricular zone; PP, preplate; IZ, intermediate zone; SV, subventricular zone; SP, subplate; CP, cortical plate; MZ, marginal zone (Uylings *et al.* 1990). **Right Panel -** A schematic illustration showing the different developmental organizations of hippocampal and neocortical lamination. Initially, all cortices have a similar lamination pattern (a ventricular zone and a preplate) and formation of this pattern is followed by the formation of a dense cellular layer, the cortical plate. Hereafter, cortical lamination shows a different development. In the reptilian cortex, the cell-dense cortical plate remains, likewise in the hippocampus. However, in the neocortex the cell-dense cortical plate differentiates into several sublayers (layers II/III–VIa). The marginal zone develops into the molecular layer in reptiles and into the stratum lacunosum-moleculare and radiatum in the hippocampus, and into layer I in the neocortex. The existence of a separate subplate layer in reptiles is less clear, but it is tempting to speculate that the subcellular layer is derived from

this preplate layer. In the hippocampus it forms the stratum oriens, in the neocortex layer VIb, and in primates it disappears. Abbreviations: VZ, ventricular zone; PP, preplate; CP, cortical plate; MZ, marginal zone; SP, subplate; SL, subcellular layer; IZ, intermediate zone; OMZ, outer marginal zone; IMZ, inner marginal zone; HP, hippocampal plate; ML, molecular layer; CL, cellular layer; EL, ependymal layer; SLM, stratum lacunosum-moleculare; SR, stratum radiatum; PY, pyramidal cell layer; SO, stratum oriens; WM, white matter; I–VIb, neocortical layers.

The adult rat neocortex, where the cortical plate expands and forms cortical layers, consists of six layers. Layers I and VIb are from the preplate layers (Figure 3). In the developing hippocampus, the marginal zone is subdivided into two segregated sublayers: the inner marginal zone (IMZ) and the outer marginal zone (OMZ). The developing hippocampal cortical plate (HP) does not become a multi-laminar cortex but remains a cell dense pyramidal cell layer (PY)**.** In the adult hippocampus, the OMZ becomes the stratum lacunosum moleculare (SLM), the IMZ the stratum radiatum (SR), and the subplate the stratum oriens (SO).

Neurogenesis and neuronal migration

The hippocampal pyramidal neurons are generated between the embryonic day 16 (E16) and 20 (E20), in the ventricular zone of the pallium (Bayer, 1980a; Bayer, 1980b). From here, they migrate towards the hippocampal plate (Rakic, 1995), using the radial glial fibres. The migration features an inside-out sequence with the newly arrived neurons settling in the upper level of the already migrated cells. The pyramidal cell layer becomes recognizable in areas CA1 and CA3 around E20. Once the migration is completed, the radial glial cells retract their end feet, undergo changes in their morphology and become astrocytes. This migration process is supposed to be calcium dependent and regulated by NMDA, GABA receptors and voltage-dependent calcium channels (Spitzer, 2002; Komuro and Rakic, 1998). At about the same time, the granule cells in the dentate gyrus are also generated, although most of the DG neurogenesis occurs later. Approximately 85% of the granule cells are generated during the first postnatal month (Bayer, 1980a; Bayer, 1980b). These are generated mainly in the ventricular zone and in the hilus, with only a small proportion being generated within the DG itself. Granule cells migrate along glia fibres. GABAergic cells in the hippocampus are generated before pyramidal neurons between E14 and E15. They migrate tangentially from the ventricular zone of the subpallial telencephalon to their final destination mainly prenatally between E16 and E17. GABA depolarizes neuronal progenitor cells thus favouring

calcium entry *via* voltage-dependent calcium channels. Calcium signalling in turn activates several metabolic pathways necessary for cell survival and differentiation including DNA synthesis (LoTurco et al., 1995). At this early developmental stage, neurotransmitters can influence neurogenesis by acting at distance from their release sites (growth cones) in a paracrine way (Demarque et al., 2002). Thus, "ambient" GABA and glutamate, present in the extracellular space, may influence neuronal signalling by binding mainly to extrasynaptic receptors. The release of neurotransmitters may occur in a non-vesicular fashion and does not require vesicular release proteins. Later in development, with the occurrence of conventional synapses, this system is replaced by a more focal form of neuronal communication. Synaptic signalling is controlled by proteins (transporters), predominantly expressed on axons and surrounding glial cells which facilitate the clearance of the neurotransmitters from the synaptic cleft. Besides neurotransmitters, other factors such as extracellular matrix proteins, neurotrophins and adhesion molecules contribute to regulate neurogenesis (Super et al., 1998).

It is worth noting that in granule cells neurogenesis persists in adulthood (van Praag et al., 2002). Also in this case, before being innervated by the pre-existing functional circuitry (Ge et al., 2006), newborn granule cells in the adult dentate gyrus are tonically activated by ambient GABA.

Studies on rats (Tyzio et al., 1999; Hennou et al., 2002) and non-human primates (Khazipov et al., 2001) have shown that in both interneurons and pyramidal cells, functional GABAergic synapses are formed before glutamatergic ones. In acute rat hippocampal slices at P0, only few GABAergic interneurons (5%) were found to be non-innervated, 17% exhibited only GABAergic synapses and 78% both GABAergic and glutamatergic synapses (Tyzio et al., 1999; Khasipov et al., 2001; Hennou et al., 2002). In contrast, 80% of pyramidal cells in the CA1 area were found to lack apical dendrites and were electrophysiologically "silent"; 10% expressed small apical dendrite restricted to the stratum radiatum and exhibited only GABAergic synapses while the remaining 10% apical dendrites reaching stratum lacunosum moleculare and exhibited both GABAergic and glutamatergic synapses. The sequential expression of GABA and glutamate synapses was not related to the length of axons that was found relatively well developed in all neurons. Interestingly, the GABA–glutamate sequence is retained in newborn granule cells during adult neurogenesis (Ge et al., 2006).

GABAERGIC SIGNALLING EARLY IN POSTNATAL DEVELOPMENT

Early in postnatal life, GABA depolarizes and excites target cells.

GABA is the main inhibitory neurotransmitter in the adult CNS. It inhibits neuronal firing by activating two different classes of receptors: $GABA_A$ and $GABA_B$. The $GABA_A$ receptors are ligand-gated ion channels, permeable mainly to Cl^- and to HCO_3^-which are competitively antagonized by bicuculline (Cherubini and Conti, 2001). The opening of the channels causes a net flux of anions inside the cells with consequent membrane hyperpolarization and a reduction of cell firing. The $GABA_B$ receptors do not contain an integral ion channel but are coupled with cationic channels (usually inwardly rectifying potassium channels) *via* G_i and G_o proteins (reviewed in Bettler et al., 2004; Couve et al., 2000). Activation of $GABA_B$ receptors causes a membrane hyperpolarization and a reduction of cell firing (Lüscher et al., 1997). They are selectively activated by baclofen and antagonized by saclofen or phaclofen. Activation of $GABA_B$ receptors also modulates cAMP production (Hashimoto and Kuriyama, 1997) leading to a wide range of actions on ion channels and proteins that are targets of the cAMP-dependent kinase (protein kinase A, PKA), and thus modulate neuronal and synaptic functions (Gerber and Gähwiler, 1994; Sakaba and Neher, 2003).

$GABA_A$ and $GABA_B$ receptors are localized also on presynaptic membranes. Activation of $GABA_A$ receptors localized on GABAergic or glutamatergic terminals may contribute to modulate spontaneous GABAergic and glutamatergic transmission. Using mechanically dissociated rat hippocampal CA3 neurons (which still have intact presynaptic nerve endings) Jang et al. (2006) found that exogenous application of muscimol increased the frequency of spontaneous EPSCs, an effect that was blocked $GABA_A$ receptor antagonists. This was probably due to GABA-induced depolarization of glutamatergic terminals. At mossy fiber-CA3 synapses, presynaptic $GABA_A$ receptor activation resulted in a reduction or increase of mossy fiber excitability depending on the $[Cl^-]_i$.

Activation of presynaptic $GABA_B$ receptors (localized on both GABAergic and glutamatergic terminals), decreases neurotransmitter release by inhibiting voltage-activated calcium channels of the N or P/Q types (Amico C et al., 1995; Mintz and Bean, 1993; Poncer JC, 1997).

Interestingly, in the immediate postnatal period, when glutamatergic synapses are still poorly developed (Hosokawa et al.,1994; Tyzio et al., 1999), GABA *via* $GABA_A$ receptors depolarizes and excites target cells through an outwardly-directed flux of chloride. Towards the end of the second postnatal week, GABA action shifts from depolarizing to the hyperpolarizing direction as in adulthood (Ben-Ari et al., 1989). The first observations of a developmentally regulated shift of GABA function was made by Obata et al., (1978), who found that application of GABA or glycine depolarized 6-days and hyperpolarized 10-days old chick spinal cord neurons in culture.

In the developing hippocampus, the depolarizing action of GABA firstly investigated with intracellular recordings from CA3 pyramidal cells (Ben-Ari et al., 1989) was shown to be dependent on the elevated intracellular chloride concentration. Subsequent work has demonstrated that in pyramidal neurons of the rat hippocampus, the ontogenetic change in $GABA_A$-mediated responses from depolarizing to hyperpolarizing was coupled to a developmental induction of the expression of the neuronal (Cl^-)-extruding K^+/Cl^- co-transporter, KCC2 (Kaila et al., 1999). The intracellular chloride homeostasis is under control of two main Cl^- co-transporters, the NKCC1 and KCC2 that enhance and lower $[Cl^-]_i$, respectively (Blaesse et al., 2009; Figure 4).

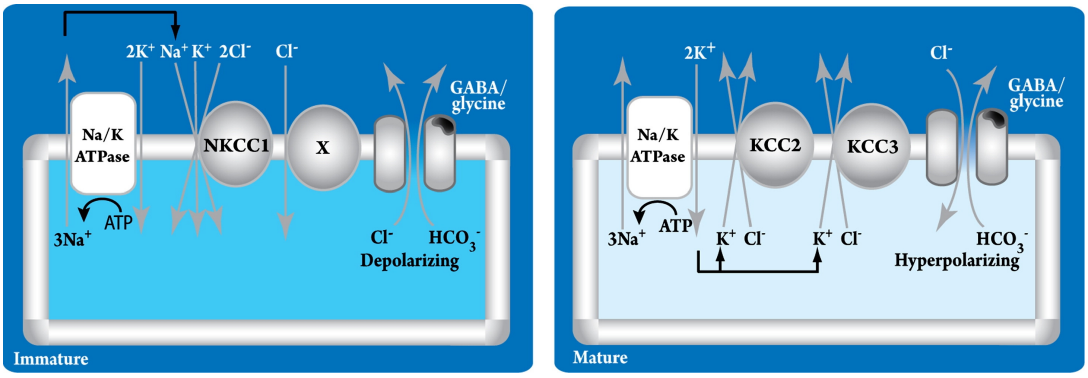

Figure 4: Ion-Transport Mechanisms Underlying $GABA_A$ and Glycine Receptor-Mediated Responses in Immature and Mature Neurons. (Left) NKCC1 mediates Cl^- uptake in immature hippocampal and cortical neurons, while an as yet unidentified Cl^- transporter denoted by X operates in some neurons either in parallel with NKCC1 or in its absence. (Right) In mature CNS neurons, KCC2 is the principal K-Cl cotransporter, and it is often coexpressed with KCC3. Cl^- transport by all CCCs is fuelled by the Na^+ and K^+ gradients generated by the Na-K ATPase. Because the intracellular concentration of Cl^- is high in immature neurons (indicated by the shade of blue), E_{GABA} and E_{Gly} are not directly affected by the

intracellular HCO_3^- concentration, while in mature neurons, the channel-mediated net efflux of HCO_3^- can have a major effect on the reversal potentials (Blaesse et al., 2009).

Due to the low expression of the KCC2 extruder at birth, chloride accumulates inside the neuron *via* NKCC1. The developmentally up regulated expression of KCC2 towards the end of the first postnatal week is responsible for the shift of GABA from the depolarizing to the hyperpolarizing direction (Rivera et al., 1999). KCC2 extrudes K^+ and Cl^- using the electrochemical gradient for K^+. Cl^- extrusion is weak in immature neurons and increases with neuronal maturation (Khirug et al., 2005; Luhmann & Prince, 1991). Transfection of the KCC2 gene into immature hippocampal neurons converts the action of GABA from excitatory to inhibitory (Chudotvorova et al., 2005). Using *in-utero* electroporation to inject a bicistronic construct encoding KCC2 and EGFP in a subpopulation of immature neurons, Cancedda et al., (2007) were able to eliminated the excitatory action of GABA in a subpopulation of rat ventricular progenitors and cortical neurons derived from these progenitors, as assessed by changes in the reversal potential of GABA-induced currents and the resting membrane potential after $GABA_A$ receptor blockade. Suppression of the excitatory action of GABA resulted in a severe impairment of the morphological maturation of cortical neurons *in vivo*, without significant effect on their radial migration. Furthermore, reducing neuronal excitability of cortical neurons *in vivo* by overexpressing an inward-rectifying K^+ channel, which lowered the resting membrane potential, they were able to mimic the effect of premature KCC2 expression. These data strongly suggest that the depolarizing action of GABA is essential for the morphological maturation of neonatal cortical neurons in vivo (Cancedda et al., 2007; Figure 5).

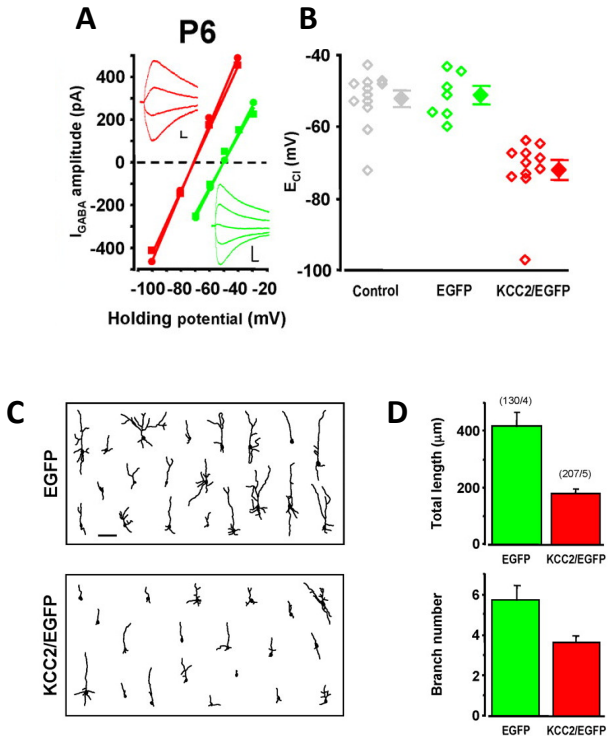

Figure 5: GABA excitation was essential for morphological maturation. **A.** Examples of I–V relationships for membrane current responses elicited by puffing GABA at the soma of layer II/III neurons in acute slices of somatosensory cortex from P4–P6 rats, which were transfected with KCC2/EGFP (red) or EGFP (green). Insets represent sample traces of GABA-induced currents at different holding potentials. Calibration: 100 ms, 100 pA. **B.** E_{Cl} values determined by GABA puffing for P4–P6 neurons untransfected (gray) or transfected with EGFP alone (green) or with KCC2/EGFP (red). Average values (± SEM) are indicated on the right. **C.** 2-D projection tracing of the dendritic arbor of 20 randomly sampled neurons in layer II/III of P6 cortices transfected with EGFP or KCC2/EGFP. Scale bar, 100 µm. **D.** Total dendritic length and branch number of cortical neurons at P6 transfected with EGFP or KCC2/EGFP. Data represent mean ± SEM. For both parameters, the values of KCC2/EGFP-transfected cells were significantly different from those of EGFP-transfected cells (p<0.001). Numbers in parentheses refer to the total number of cells/rats analyzed. (Adapted from Cancedda et al., 2007).

Similarly, using RNA interference to knock down *in vivo* the expression of the Na-K-2Cl co-transporter NKCC1, it was found that changes in the direction of GABA action led to a disruption of dendritic development associated with an alteration in the formation of excitatory synapses (Wang and Kriegstein, 2008). Blocking NKCC1 with the diuretic

bumetanide (Dzhala et al., 2005; Sipilä et al., 2006) caused similar effects. Interestingly, these effects could be rescued by over expressing voltage-independent NMDA receptors, indicating that GABA depolarization cooperates with NMDA receptor activation (the depolarizing action of GABA would help relieving the magnesium block from the NMDA receptors) to regulate the formation of excitatory synapses (Wang and Kriegstein, 2008; Figure 6).

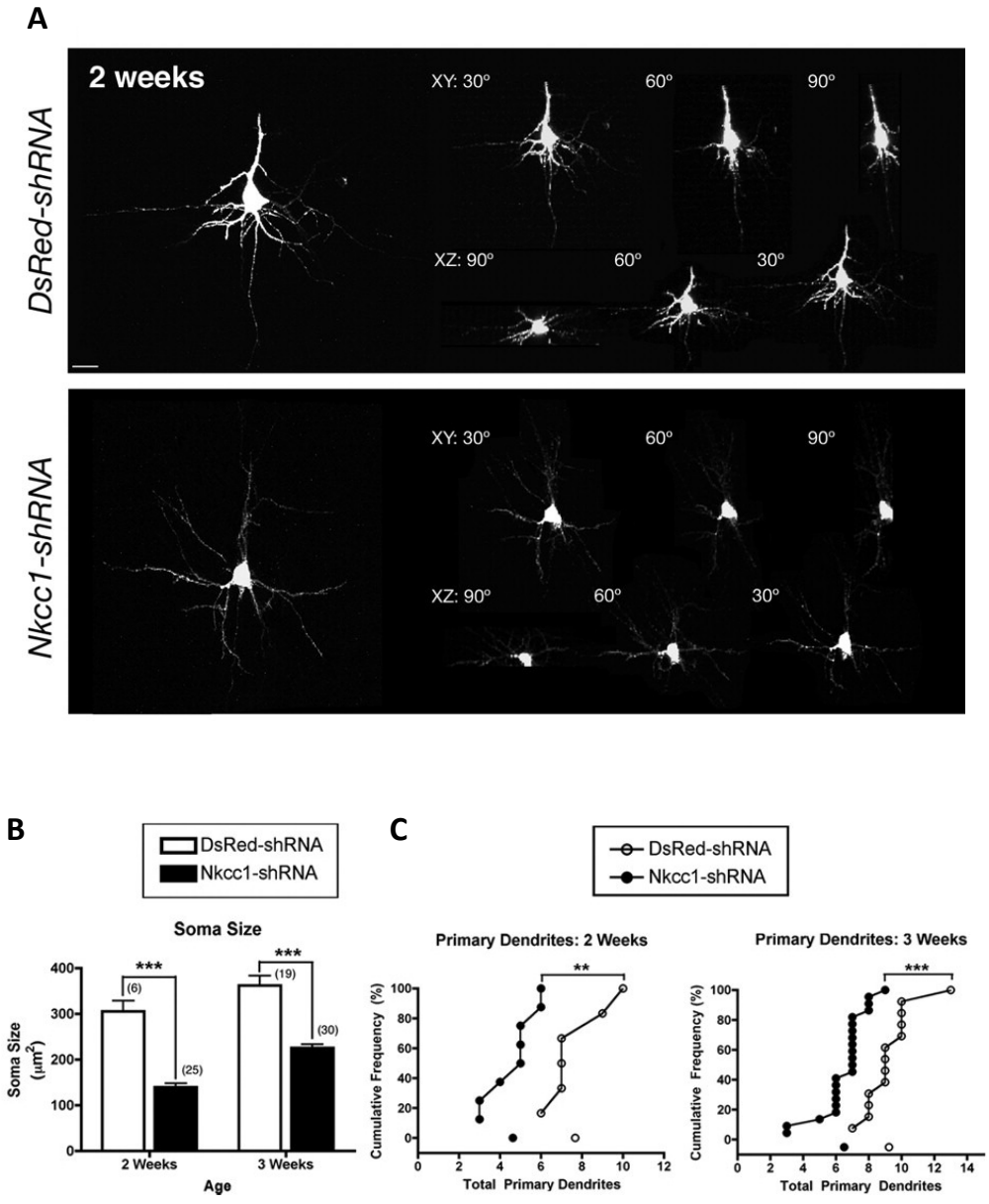

Figure 6: Nkcc1-shRNA expression disrupts morphology of cortical neurons. **A.** Confocal 3-D reconstruction of dendrites of control or Nkcc1-shRNA-expressing cortical neurons at 2 or 3

weeks postnatal. The left panels show the flattened stack of a neuron, and the right panels show multiple rotational views as indicated by the plane and angle of rotation. Scale bar, 20 μm. **B, C.** Quantification of average soma size **(B)** and number of primary dendrites **(C)** of GFP+ cortical neurons demonstrate that, compared with control pyramidal neurons, Nkcc1-shRNA-expressing neurons display smaller soma size and fewer primary dendrites at both 2 and 3 weeks. Bar graphs indicate mean ± SEM (**p < 0.005; ***p < 0.0001; t test). The numbers of neurons are indicated in parentheses. (Adapted from Wang and Kriegstein, 2008).

Cooperation with NMDA receptor activation represents an early form of coincidence detection between GABAergic and glutamatergic inputs. More recently, it was found that in NKCC1 knock out mice, the diminished GABAergic excitation resulted in reduced and less correlated spontaneous network activity and in a delayed maturation of glutamatergic and GABAergic synapses in the absence of any detectable morphological modification (Pfeffer et al., 2009). In a closed related study, based on another strain of animals (Sipila et al., 2009), it was found that the loss of GABAergic excitation in NKCC1 knock out mice resulted in unperturbed KCC2 expression and in enhanced AMPA receptor dependent network activity. Although according to Sipila et al., (2009) the lack of depolarizing response to GABA would promote a homeostatic compensatory increase in intrinsic excitability of glutamatergic neurons, discrepancy between these two reports is unclear.

According to Ganguly et al., (2001), GABA itself may control the shift from excitation to inhibition because the shift would not occur in cultured neurons exposed to $GABA_A$ receptor antagonists. However, these data have been questioned in subsequent studies which have shown that the developmental up regulation of KCC2 can take place in the complete absence of $GABA_A$ receptor signaling (Ludwig et al., 2003; Titz et al., 2003). Furthermore, during embryonic development in VIAAT knockout mice, KCC2 mRNA and protein levels are unaffected, despite the absence of GABAergic synaptic transmission (Wojcik et al., 2006).

The developmental shift of GABA from the depolarizing to the hyperpolarizing direction is likely to involve neurotrophins (Aguado et al., 2003; Rivera et al., 2004) although the exact underlying molecular mechanisms are not known. Opposite results concerning the role of neurotrophins on the regulation of the KCC2 transporter have been reported in different preparations. Thus, while the *in vivo* overexpression of BDNF results in higher rates of synaptogenesis, GABA production and expression of KCC2 (Aguado et al., 2003), exposure of

hippocampal slices to BDNF resulted in a reduction of KCC2 mRNA and protein (Rivera et al., 2002).

As already mentioned, $GABA_A$ receptors are permeable to two physiologically relevant anions, Cl^- and $HCO3^-$ (Farrant, 2007; Kaila, 1994). Passive re-distribution of H^+ and $HCO3^-$ ions would lead to a more acidic intraneuronal pH (Roos and Boron, 1981; Lee et al., 1996). Hence, the electrochemical equilibrium potential for $HCO3^-$ would be much less negative (typically ~ -15 mV) than the resting membrane potential. Taking into account the relatively low $HCO3^-$ *vs* Cl^- permeability ratio (0.2 - 0.4) of $GABA_A$ receptors, simple considerations based on the Goldman-Hodgkin-Katz equation show that, when the intracellular chloride concentration is low as in adulthood, the reversal potential of $GABA_A$ receptor-mediated responses deviates significantly from the equilibrium potential of Cl^- (Kaila et al., 1993). However, the depolarizing $GABA_A$ receptor-mediated responses in immature neurons are generally based on a high intracellular Cl^- concentration (Payne et al., 2003), which is often higher than expected on the basis of passive distribution. Hence, the equilibrium potential of Cl^- is less negative than the resting membrane potential and this creates a driving force that accounts for the depolarizing action of GABA in immature cells (Cherubini et al., 1991; Rivera et al., 2005).

The development of fluorescent chloride indicators has allowed the direct measurement of $[Cl^-]_i$ (Mukhtarov et al., 2008, Markova et al., 2008). Using this tool it was clearly demonstrated that $[Cl^-]_i$ decreases during development of hippocampal neurons in culture (Kuner and Augustine, 2000).

It is worth mentioning that GABA-induced depolarization may still result in inhibition of cell firing if the positive shift in the membrane potential is associated with a strong “shunting inhibition” (Lamsa et al., 2000). In organotypic hippocampal cultures, GABA released from GABAergic interneurons depolarizes principal cells (Mohajerani and Cherubini, 2005). However, while in the majority of cells (11/14), the depolarizing action was found to be associated with inhibition of cell firing, in a few neurons (3/14) it was accompanied by a strong increase in cell firing, an effect that was prevented by the $GABA_A$ receptor antagonist bicuculline (Mohajerani and Cherubini, 2005). This indeed indicates that GABA-induced membrane depolarization can still cause a “shunting inhibition” *via* an increase in Cl^- conductance.

In the immature hippocampus, the depolarizing action of GABA enables the induction of synchronized activity, the so called giant depolarizing potentials or GDPs, which consist in recurrent membrane depolarization with superimposed fast action potentials, separated by quiescent intervals (Ben-Ari et al., 1989). GDPs which have been proposed to be the *in vitro* counterpart of "sharp waves" recorded in rat pups during immobility periods, sleep and feeding (Leinekugel et al., 2002) can be considered a primordial form of synchrony between neurons, which precedes more organized forms of activity such as theta and gamma rhythms (Buzsáki and Draguhn, 2004). Correlated network activity constitutes a hallmark of developmental networks, well preserved during evolution that has been observed not only in the hippocampus but in almost every brain structure examined, including the retina (Feller et al., 1997), the neocortex (Owens et al., 1996; Dammerman et al., 2000; Maric et al., 2001), the hypothalamus (Chen et al., 1996), the cerebellum (Yuste and Katz, 1991; Eilers et al., 2001) and the spinal cord (Wang et al., 1994; O'Donovan, 1999).

The depolarizing action of GABA during GDPs results in calcium influx through the activation of voltage-dependent calcium channels and N-methyl-D-aspartate (NMDA) receptors (Leinekugel et al., 1997; Garaschuk et al., 1998; Figure 7). GDPs and associated calcium transients lead to the activation of intracellular signaling pathways known to contribute to several developmental processes including morphological maturation and synaptogenesis (Owens and Kriegstein, 2002). More recently, GDPs and associated calcium transients have been shown to act as coincident detectors for enhancing synaptic efficacy at poorly developed synapses (Kasyanov et al., 2004; Mohajerani et al., 2007).

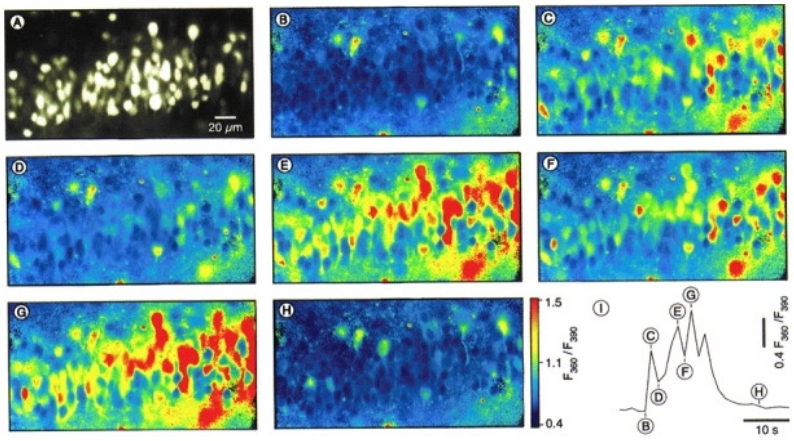

Figure 7: Spatially resolved changes in $[Ca^{2+}]i$ in individual CA1 pyramidal neurones during a spontaneous Ca^{2+} burst. **A**. An epifluorescence image (taken at 390 nm excitation light) of

the cell body layer in the CA1 region of a hippocampal slice from a 3-day-old rat taken with a × 40 objective lens. **B-H**. Pseudocolour ratio images were taken at various time points, as indicated in **I**, during a Ca^{2+} burst consisting of four Ca^{2+} transients. **I**. time course of the Ca^{2+} burst calculated as a mean of all individual changes in $[Ca^{2+}]i$ (n= 107 cells). (Adapted from Garaschuk et al., 1998).

How GDPs are generated is still a matter of debate. In the disinhibited hippocampus, population synchrony has been proposed to depend on an active process consisting in a build up period during which synaptic traffic and cell firing exceeds a certain threshold (Menendez de la Prida et al., 2006). Functionally excitatory synaptic interactions would facilitate neuronal synchronization and the initiation of population bursts (Traub and Wong, 1982; Miles and Wong, 1987; Traub and Miles, 1991). A similar process may be involved in the generation of GDPs early in postnatal life (Menendez de la Prida and Sanchez-Andres, 1999; 2000) when synaptic interactions are facilitated by the excitatory action of GABA (Cherubini et al., 1991; Ben-Ari, 2002) and glutamate (Bolea et al., 1999). The presence of intrinsically bursting neurons, which by virtue of their spontaneous discharge and large spike output, can drive other neurons to fire in synchrony could contribute to GDPs generation (Menendez de la Prida and Sanchez-Andres, 2000; Sanabria et al., 2001; Sipila et al., 2005). A tonic $GABA_A$-mediated conductance, well developed at birth (Marchionni et al., 2007) would bring the membrane to the voltage window where voltage-dependent bursts are generated (Sipila et al., 2005).

Early in postnatal development, the main neurotransmitter released from mossy fiber terminals is GABA

As already mentioned, in adults, the MF input to CA3 is glutamatergic and comprises the second synapse of the classical tri-synaptic hippocampal circuit. In particular conditions however, MF can release in addition to glutamate also GABA. Thus, in the hippocampus of epileptic animals (Gutiérrez and Heinemann, 2001; Romo-Parra et al., 2003) monosynaptic GABAergic inhibitory postsynaptic potentials (IPSPs) occur in principal cells in response to *dentate gyrus* stimulation. Seizures are associated with a transient up regulation of the GABAergic markers GAD65 and GAD67 (Schwarzer and Sperk, 1995; Sloviter et al., 1996) as well as the mRNA for the vesicular GABA transporter, VGAT (Lamas et al., 2001). Interestingly, both GAD67 and its product GABA appear to be constitutively expressed in MF.

Further evidence suggests that MF can release glutamate and GABA also in physiological conditions. Hence, Walker et al., (2001) and Gutiérrez et al., (2003) have demonstrated the presence of both monosynaptic GABAergic and glutamatergic responses following activation of granule cells in the *dentate gyrus* in juvenile guinea pigs and rats. In our laboratory, Safiulina et al., (2006) have demonstrated that during the first week of postnatal life the main neurotransmitter released by MF terminals into principal cells and interneurons is GABA (Figure 8).

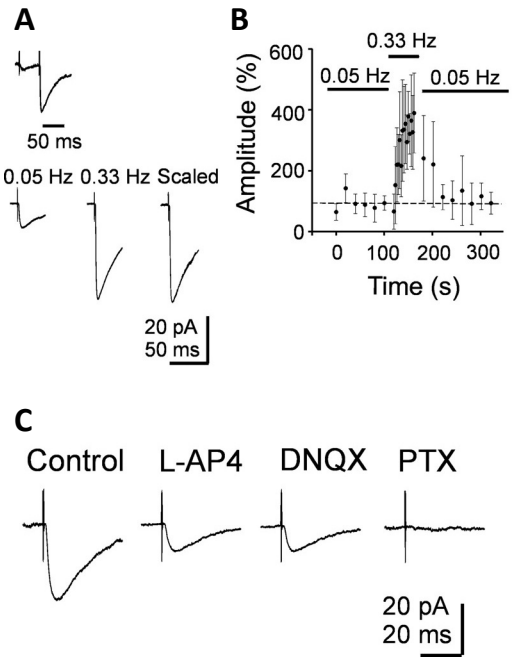

Figure 8: Minimal stimulation of granule cells in the dentate gyrus evokes $GABA_A$-mediated monosynaptic responses in CA3 pyramidal cells. **A.** Paired stimuli delivered at 50 ms interval to granule cells in the dentate gyrus evoked at -70 mV synaptic responses exhibiting strong paired-pulse facilitation (average of 50 individual responses). Bottom traces represent mean amplitude of synaptic responses evoked by stimulation of granule cells in the dentate gyrus at 0.05 and 0.33 Hz. On the right, the two responses are normalized and superimposed. **B**. The mean amplitude of synaptic currents evoked at 0.05 and 0.33 Hz (bars) is plotted against time. Note the slow buildup of facilitation of synaptic responses at 0.33 Hz that completely reversed to control values after returning to 0.05 Hz stimulation. Error bars represent SEM. **C**. Synaptic responses obtained before and during application of L-AP-4 (10 μM) and L-AP-4 plus DNQX (20 μM). Addition of picrotoxin (PTX; 100 μM) completely abolished synaptic currents. Each response is the average of 20 individual traces (including failures). Note that

GABAA-mediated synaptic currents were depressed by L-AP-4 but were unaffected by DNQX. (Adapted from Safiulina et al., 2006).

Single fiber MF inputs to principal cells or interneurons were identified on the basis of their sensitivity to group III mGluR agonist L-AP4. In this regard, neonatal rats behave differently from adult animals which are sensitive to DCG-IV but insensitive to L-AP4 (Lanthorn et al., 1984). It should be stressed however, that both group II and III mGluRs have been found on rat MF terminals: while group III mGluRs are located predominantly in presynaptic active zones, group II in preterminal rather than terminal portions of axons (Shigemoto et al., 1997). The inability of DCG-IV in modulating MF responses in immature neurons can be attributed to the different expression and/or location of group II/III mGluRs early in postnatal development. One intriguing aspect to be considered is how mGluRs are activated early in postnatal days, when MFs seem to release only GABA. One possibility is that they are constitutively activated by ambient glutamate present in the extracellular medium.

Interestingly, monosynaptic inhibitory responses obtained in principal cells by stimulating GABAergic interneurons were found to be insensitive to mGluR agonists (Walker et al., 2001; Doherty et al., 2004; Kasyanov et al., 2004; Gutiérrez, 2005).

MF inputs were also identified on the basis of their strong paired pulse facilitation and short term frequency-dependent facilitation (Salin et al., 1996). Strong paired pulse facilitation was observed particularly at MF-CA3 synapses, which were often "silent" in response to the first stimulus. At MF-interneuron synapses both paired pulse facilitation and depression were observed while at interneuron-CA3 or interneuron-interneuron synapses the most common feature was paired pulse depression. The degree of frequency-dependent facilitation is another peculiar aspect of MF responses which probably depends on the enhanced probability of neurotransmitter release following the large rise of intraterminal calcium concentration and activation of calcium/calmodulin-dependent kinase II (Salin et al., 1996). Alternatively, synaptic facilitation may occur as the consequence of the progressive and local saturation of calcium by the endogenous fast calcium buffer calbindin, which is highly expressed in MF terminals. This would produce a gradual increase in calcium concentration at releasing sites (Blatow et al., 2003). In our case, frequency facilitation occurred already when the stimulation frequency was shifted from 0.05 to 0.3 Hz. However, the increment in size of synaptic responses was larger at MF-CA3 principal cell connections than at MF-interneuron synapses (see also Toth et al., 2000).

As expected for $GABA_A$-mediated synaptic events, synaptic currents evoked in CA3 principal cells or GABAergic interneurons by minimal stimulation of granule cells in the *dentate gyrus* were completely blocked by picrotoxin or bicuculline. These responses which were highly sensitive to L-AP4, exhibited a strong paired pulse facilitation and short term frequency-dependent facilitation. Moreover, they were potentiated by NO-711, a blocker of the GABA transporter GAT-1 and by flurazepam, an allosteric modulator of $GABA_A$ receptors.

Additional fibers releasing both glutamate and GABA into CA3 principal cells and interneurons could be recruited by increasing the strength of stimulation (Safiulina et al., 2006). In comparison with MF-induced GABAergic currents, glutamatergic responses occurred with a shorter latency. These responses were mediated by MF, since they were reversibly depressed by L-AP4 and were abolished when DNQX (10 μM) was added to picrotoxin. Using a low chloride intracellular solution (E_{Cl} -90 mV) to simultaneously record AMPA- and $GABA_A$-mediated events it was found that synaptic currents (intermingled with response failures) fluctuated between outward, biphasic and inward, suggesting that GABA and glutamate can be released independently from the same fiber.

Altogether these experiments are in line with previous reports showing the sequential expression of GABAergic and glutamatergic synapses early in postnatal development (Hosokawa et al., 1994; Tyzio et al., 1999) and clearly demonstrate that GABA is the main neurotransmitter released from MFs during postnatal development. Further evidence in favor of GABA as a transmitter at MF synapses is the observation that the vesicular GABA transporter VGAT was found in MF terminals (Safiulina et al., 2006; but see Chaudhry et al., 1998). However, in order to generate synaptic responses, GABA should not only be present in synaptic vesicles and released in an activity-dependent manner but should bind to post synaptic $GABA_A$ receptors. Although evidence for the presence of $GABA_A$ receptors facing immature MF terminals early in development is still lacking, a previous study on adult rats has demonstrated the presence of AMPA and $GABA_A$ receptors co-localized in front of the respective active zones (Bergersen et al., 2003). GABA may also spills out to activate neighboring extrasynaptic $GABA_A$ receptors localized away from the release sites as suggested for juvenile guinea pigs and rats (Walker et al., 2001). However, the relatively fast rise time of GABAergic responses found in the present experiments, similar to that of glutamatergic synaptic currents makes this hypothesis unlikely.

Overall, the data of Safiulina et al. (2006) support the hypothesis that early in development, MF contain two different set of low and high threshold fibers releasing GABA and GABA plus glutamate respectively. While the first would disappear with maturation the second would persist longer or would reappear in pathological conditions such as in epilepsy (Walker et al., 2001; Gutiérrez et al., 2003). Comparable to our results it has been recently shown that minimal stimulation of the granule cell layer in hippocampal slices obtained from P4-P6 mice evoked GABAergic currents that were insensitive to 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D-AP5 but were blocked by picrotoxin (Uchigashima et al., 2007). According to these authors, these currents were due to GABA released from low threshold GABAergic interneurons. It should be stressed however, that unlike putative MF responses described here, which occurred with very low probability (responses were often silent to first pulse) and exhibited strong short-term frequency-dependent facilitation, those reported by Uchigashima et al., (2007) displayed only minimal facilitation.

Paired recordings from granule cells and postsynaptic neurons would probably allow better understanding how MF establish their contact at early developmental stages. However, we can not exclude the possibility that, during the first week of postnatal life, synaptic currents evoked by minimal stimulation of granule cell in the *dentate gyrus* are generated by GAD or GAD mRNA positive interneurons which early in development migrate to the upper and middle portions of the granule layer cells (Dupuy and Houser, 1997) while maintaining all the functional properties of later appearing glutamatergic MF responses.

SYNAPTIC PLASTICITY IN THE DEVELOPING HIPPOCAMPUS

During the construction of neuronal circuits, both activity-dependent and activity-independent mechanisms interact, shaping several aspects of neuronal development including migration and differentiation, axonal path finding and the establishment of functional synaptic connections. Synaptic plasticity relies in the ability of changing the strength of synaptic connections. This constitutes the cellular basis for learning and memory processes. On the basis of theoretical studies in 1949 Hebb proposed a coincidence-detection rule, in which a synapse linking two cells is strengthened if the cells are active in close temporal contiguity (Hebb, 1949). This prediction was verified subsequently at excitatory connections between the perforant pathway and granule cells in the dentate gyrus. Brief trains of high frequency stimulation to the perforant pathway induced an abrupt and sustained increase in synaptic efficacy into granule cells that persisted for days or week (Bliss and Lomo, 1973). This phenomenon, called long-term potentiation or LTP, has been found not only at excitatory but also at inhibitory synapses and in several brain structures. In addition, much progress has been made in elucidating the mechanisms regulating its induction and expression. Neuronal circuits possess a high degree of flexibility and evidence has been provided that they can modify synaptic strength in both directions. A persistent decrease in synaptic strength caused by low-frequency stimulation of presynaptic fibers or in an associative manner by asynchronous pairing of presynaptic and postsynaptic activity constitutes the long-term depression or LTD (Bear and Abraham, 1996). Thus, a synapse that is originally potentiated can be depotentiated. This resetting prevents saturation of LTP and makes the synapses more responsive and temporally flexible that passive decay. In addition, LTD of previously potentiated synapses could serve as a forgetting mechanism for information storage, Furthermore, in a network of synaptic contacts, LTD may accentuate the signals from neighbouring potentiated synapses in a manner analogous to the way lateral inhibition function to promote edge detection in the visual system.

The importance of the temporal order of pre- and postsynaptic activity in long-term changes in synaptic efficacy has been explored in a variety of glutamatergic and GABAergic synapses in different brain regions (Bell et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Egger et al., 1999; Sivakumaran et al., 2009; Woodin et al., 2003; for review, see Dan and Poo, 2006). It was observed that when presynaptic spiking preceded the postsynaptic spiking (pre *versus*

post), LTP was induced while when the order of stimulation was reversed (post *versus* pre), LTD was observed. The temporal window in either direction was several tens of milliseconds, with the width of the window varying between different synapses or brain regions. This form of activity dependent change in plasticity is now referred to as spike timing dependent plasticity (STDP, Figure 9). One important characteristic of STDP is that, unlike conventional protocols where high-frequency stimulation induces LTP and low-frequency stimulation induces LTD, the same stimulation frequency can be used to induce both LTP and LTD.

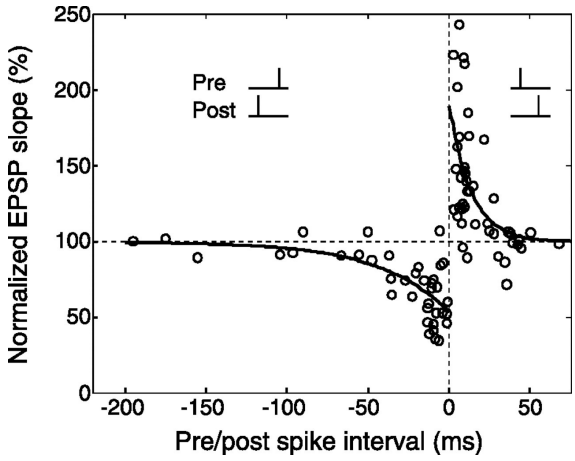

Figure 9: Synaptic modification (% change in EPSP slope) induced by repetitively pairing pre- and postsynaptic spikes in layer 2/3 of visual cortical slices from the rat. Each symbol represents result from one experiment. Curves are single exponential, least-squares fits of the data. *Insets* depict the sequence of spiking in the pre- and postsynaptic neurons. (From Dan and Poo, 2006).

Persistent modifications in synaptic strength may involve changes in the expression of postsynaptic receptors or modifications in the probability of transmitter release, including convertion of “silent” synapses into active ones or *vice versa*. Synapses can be silent because of either post or presynaptic mechanisms. “Presynaptically” silent synapses produce no responses because no neurotransmitter is released whereas “postsynaptically” silent synapses are unable to detect release due to the lack of postsynaptic receptors (Voronin and Cherubini, 2004). Synapse unsilencing, due to the insertion of new AMPARs on the postsynaptic membrane has been suggested to be particularly relevant during postnatal development, when a significant fraction of excitatory synapses has been shown to express only NMDA but not AMPAR. The number of cells expressing also AMPARs increases during development (Durand et al., 1996). Immunogold studies indicate that maturation requires incorporation of new AMPARs at pure NMDA synapses (Isaac, 2003). However this view has

been recently challenged by the following observations which strongly support presynaptic modifications in the release machinery. Thus, Groc et al., (2002) found that spontaneous AMPAR- and NMDAR-mediated synaptic events were present from birth and their relative amplitude and frequency remained constant during the first postnatal week. Experiments on evoked responses in which AMPAR-mediated currents to a second of two paired stimuli could be detected in apparently silent synapses allow similar conclusions to be drawn (Gasparini et al., 2000; Maggi et al., 2003). Maturation of presynaptic machinery may also account for the increased number of AMPA responses observed during development of cultured neurones (Renger et al., 2001). Interestingly, interfering with presynaptic vesicle fusion by exposing mature cultures to tetanus toxin (which cleaves SNARE proteins) was able to reconvert functional synapses into silent ones (Renger et al., 2001).

Since the pioneering work of Hubel and Wiesel (1959, 1962, 1963, 1968) on the effects of visual deprivation on the development of the visual system, it has become increasingly clear that electrical activity is essential for establishing the appropriate network connections in many parts of the brain (Katz and Shatz, 1996; Zhang and Poo, 2001). In particular, neurotransmitters, such as glutamate and GABA released during spontaneous or experience-driven electrical activity have been shown to regulate many aspects of neuronal network formation from migration to synaptogenesis (Manent and Represa, 2007). In this respect it is worth noting that: I. neurotransmitters and receptors are expressed early in brain development; II. in the immature brain neurotransmitters may act as paracrine signaling molecules acting at distance from the release site; III. Neurotransmitters regulate intracellular calcium and transduction signaling pathway required for many cellular functions, including dynamic changes of the cytoskeleton. As already mentioned, in recent years evidence has been provided that in the immature hippocampus GABA acts as a developmental signal regulating many developmental related aspects including cell proliferation, migration, growth and synapse formation (Ben-Ari et al., 2007).

Plasticity processes at glutamatergic CA3-CA1 synapses

Unlike adulthood, before postnatal day 9 (P9), high frequency stimulation of the Schaffer collateral induces in CA1 pyramidal neurons a form of LTP which requires the activation of cyclic AMP-dependent protein kinase A (PKA) but not calcium/calmodulin-dependent protein kinase II (CaMKII; Yasuda et al., 2003). During the first week of postnatal life, the

levels of CaMKII are extremely low and most synapses present on CA1 principal cells occur on the dendritic shafts. Interestingly, the number of synapses occurring on spines increases dramatically between P1 and P12 in concomitance with the time when LTP become dependent on CaMKII activation. Therefore, cAMP dependent PKA would provide the signaling pathway necessary for spine formation.

It should be stressed that, early in development, LTP would be facilitated in a associative way by the depolarizing action of GABA which would activate voltage-dependent calcium channels and NMDA receptors (GABA would be instrumental in relieving the magnesium block from NMDA receptors).

A sustained low frequency stimulation of the Schaffer collateral (at 5 Hz for 3-4 min) induced a form of LTD which, unlike adults, is independent of NMDA receptor activation but requires the combined activation of voltage-dependent calcium channels and metabotropic glutamate receptors (Bolshakov and Siegelbaum, 1994). Although in this case the induction was postsynaptic, LTD expression was related to a reduced transmitter release from Schaffer collaterals implying the involvement of a retrograde messenger (Bolshakov and Siegelbaum, 1994).

Plasticity processes at MF-CA3 synapses

As already mentioned, during the first week of postnatal life, MF-CA3 synapses are mainly GABAergic. These synapses are characterized by a low probability of transmitter release and are often “presynaptically” silent. It was found in our laboratory that, by pairing MF stimulation with network-driven GDPs, produced a persistent increase in synaptic efficacy that was restricted to the active synapse. In addition, the pairing protocol was able to convert silent synapses into actives ones (Kasyanov et al., 2004; Spitzer, 2004; Figure 10). The potentiation depended on calcium signals generated by GDPs *via* activation of voltage-dependent calcium channels as demonstrated by the fact that synaptic potentiation was blocked by nifedipine, a broad spectrum calcium channel blocker but not by the NMDA receptor antagonist D-AP5. This Hebbian type of synaptic plasticity may be instrumental for promoting synapse development and the refinement of synaptic circuits.

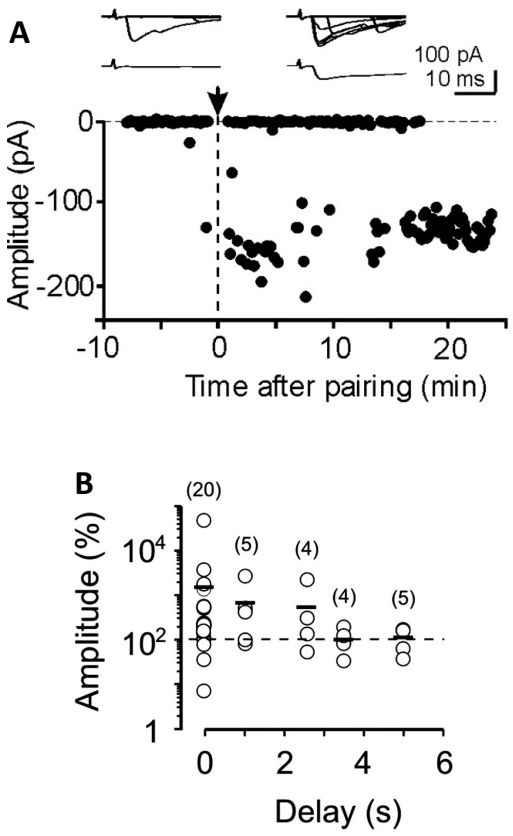

Figure 10: Pairing GDPs with MF stimulation persistently enhances synaptic efficacy at MF-CA3 synapses. **A.** Amplitudes of synaptic responses (dots) evoked by stimulation of MF before and after pairing (arrow at time 0) are plotted against time. The traces above the graph represent 7 individual responses evoked before (Left) and 15 min after pairing (Right); lower traces represent the average of 48 individual responses (successes and failures) obtained before (Left) and 15 min after pairing (Right). **B.** Temporal specificity of the correlated activity required for LTP induction. During the pairing protocol, a delay of 0-5 s was introduced between GDP rise time and synaptic stimulation. Each symbol represents the mean percentage increase of EPSCs amplitude as a function of the delay for the number of cells indicated (into brackets). Small bars represent the average of all open circles in a column. Note that the maximum potentiating effect was achieved when pre- and postsynaptic signals were coincident. (Adapted from Kasyanov et al., 2004).

In addition, a study on the intact hippocampal formation has revealed that spontaneous ongoing synaptic activity is necessary for the normal development of GABAergic synapses since blocking this activity for one day with TTX prevented the increase in frequency of mini $GABA_A$-mediated synaptic events which is an index of the formation of new functional

GABAergic synapses (Colin-Le Brun et al., 2004). Similar results were obtained when spontaneous activity was blocked with CNQX and D-AP5, indicating that a network-driven activity requiring glutamatergic and GABAergic receptors is involved in the formation of functional GABAergic synapses (Colin-Le Brun et al., 2004; see also Kuczewski et al., 2008a).

During the second postnatal week, with the appearance of glutamatergic synapses, the coexistence of two distinct forms of LTD, associative and non-associative, was identified at MF-CA3 synapses. Thus, while a high frequency stimulation train delivered to the MF in stratum lucidum induced a form of LTD which was independent of NMDA or mGlu receptors activation, low frequency stimulation of afferent inputs induced a form of LTD, which, like in adults, was dependent on presynaptic mGluR. While the first form required postsynaptic calcium rise, the second one was independent of calcium rise in the postsynaptic cell (Domenici et al., 1998).

A novel form of LTD has been recently described at juvenile MF-CA3 synapses but not at MF-interneuron connections. Here, a transient tonic depolarization of CA3 principal cells induced a rise in postsynaptic calcium leading to a persistent depression of glutamate release from presynaptic terminals associated with a switch of calcium permeable AMPA receptors into calcium impermeable ones (Ho et al., 2007). Similar results were obtained with bursts firing instead of a steady membrane depolarization (Ho et al., 2009). It should be stressed that the early participation of calcium-permeable AMPA receptors to nascent glutamatergic MF-pyramidal cell synapses may provide a postsynaptic calcium signal important for synapse maturation.

Plasticity processes at GABAergic synapses

Both LTP and LTD have been reported to occur at GABAergic synapses.

Repetitive depolarizing pulses delivered to CA3 principal cells in the presence of the NMDA and AMPA receptor antagonists D-AP5 and CNQX, respectively, induced long lasting changes in amplitude and frequency of spontaneous and evoked $GABA_A$-mediated postsynaptic events in hippocampal slice obtained from P0 and P8 (Caillard et al., 1999; Gubellini et al., 2001; Gubellini et al., 2005). The induction mechanism of this form of LTP relied on postsynaptic calcium rise via voltage-gated calcium channels since LTP was prevented by chelating intracellular calcium with BAPTA or blocking voltage-dependent calcium channels with nimodipine (Caillard et al., 2000). In few cases this conditioning protocol was able to

switch on previous silent synapses. In contrast with the induction that was clearly postsynaptic, the expression of this form of LTP was presynaptic as suggested by the increase in frequency of $GABA_A$-mediated events. This required a retrograde signal which was identified in the BDNF and TrkB receptors (Gubellini et al., 2005; Figure 11). Thus, LTP did not develop in the presence of TrkB immunoglobulin, a "scavenger" on endogenous BDNF, or in the presence of k252a, an inhibitor of TrkB receptors coupled-tyrosine kinase. BDNF was probably released by the postsynaptic neuron by back propagating action potentials (Kuczewski et al., 2008b).

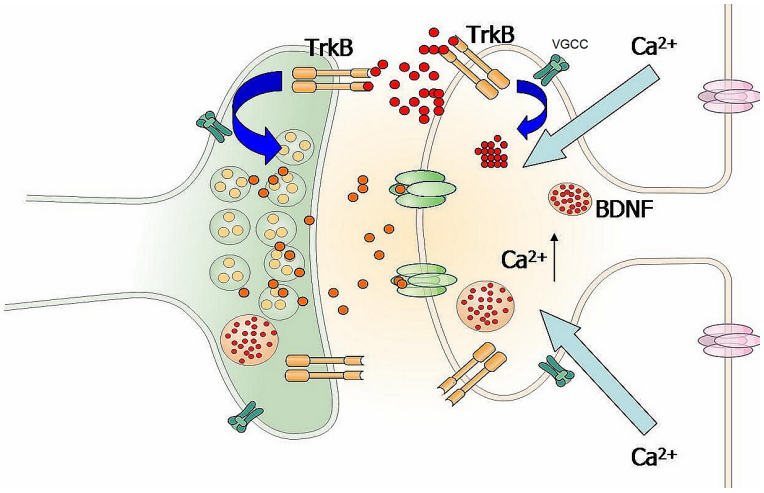

Figure 11: Retrograde signaling by BDNF. Calcium influx in the postsynaptic cell *via* voltage-gated calcium channels (VGCC) leads to rise of intracellular calcium (Ca^{2+}), triggering release of BDNF (red circles) into the synaptic cleft. BDNF can act on both pre- and postsynaptic TrkB receptors (yellow). At the presynaptic level, activation of TrkB by BDNF results in enhanced neurotransmitter release.

In conditions in which NMDA receptors were not blocked, the same conditioning protocol caused LTD of $GABA_A$-mediated synaptic currents (instead of LTP) indicating that this NMDA-dependent form of synaptic plasticity required the combined activation of NMDA and $GABA_A$ receptors (Caillard et al., 1999). As for LTP, also LTD induction was postsynaptic while its expression was presynaptic. Both LTP and LTD were only induced at the time when GABA was depolarizing and excitatory (McLean et al., 1996).

Interestingly, $LTD_{GABA\text{-}A}$ required calcium entry through NMDA receptors and release of calcium from intracellular calcium stores (Caillard et al., 2000).

Changes in the strength of GABAergic inputs could also result from modifications in the reversal of GABAergic synaptic currents in the depolarizing or hyperpolarizing directions. Hence a reduction in synaptic strength was observed following prolonged postsynaptic

spiking which induced a Ca^{2+}-dependent downregulation of KCC2. This led to a positive shift in E_{Cl}^- (without affecting $GABA_A$ receptor conductance) and to a reduced GABAergic inhibition. In contrast, an increase in synaptic efficacy (in this case, synaptic inhibition) was observed in concomitance with a hyperpolarizing shift of E_{Cl}^-, following repetitive postsynaptic spiking within ± 5 ms of GABAergic stimulation of terminals and this effect was prevented when the inward transport of Cl^- through NKCC1 was blocked with bumetanide (Balena and Woodin, 2008; Woodin et al., 2003; Fiumelli et al., 2005).

AIM OF THE STUDY

Correlated neuronal activity exerts a critical control over the functional refinement of synaptic connections. In the developing hippocampus, giant depolarizing potentials or GDPs might play a major role in such processe. In previous work from our laboratory it was demonstrated that GDPs may act as coincidence detector signals for enhancing synaptic efficacy at mossy fibre-CA3 synapses (Kasyanov et al., 2004) which at this early stage of development are mainly GABAergic. Pairing GDPs with mossy fibers stimulation induced a persistent increase in synaptic efficacy, further supporting Hebb' postulate on activity-dependent synaptic strengthening. GDPs occur synchronously over the entire hippocampus and we hypothesized that activation of GDPs in coincidence with Schaffer collateral inputs might play a major role in enhancing synaptic strength at emerging glutamatergic synapses.

Therefore, at the beginning of my PhD course, I joined Majid H. Mohajerani (final year PhD student in our lab) in a project aimed at understanding whether during a critical period of postnatal development (between P0 and P6), $GABA_A$-mediated GDPs control synaptic plasticity at glutamatergic synapses. We used combined electrophysiological and immunocytochemical approaches. In electrophysiological experiments (whole-cell patch clamp recordings in current and voltage clamp mode) we recorded spontaneous glutamatergic events from CA1 pyramidal cells in acute hippocampal slices prepared from neonatal rats. Immature CA3-CA1 synapses have the advantage to bear only a single functional release site and therefore the amplitude distribution of sEPSCs matches very closely that of miniature events recorded in the presence of tetrodotoxin. In some experiments we recorded also excitatory postsynaptic currents evoked in CA1 principal cells by stimulation of the Schaffer collateral. The rising phase of $GABA_A$-mediated GDPs was used to trigger Schaffer collateral stimulation in such a way that the synchronized network activity was coincident with the activation of presynaptic afferent inputs. We found that this procedure produced a persistent increase in amplitude of evoked EPSCs and in frequency of spontaneous glutamatergic events. This effect required the influx of calcium into the postsynaptic neuron *via* L-type calcium channels, and was independent of NMDA receptor activation. Upon introduction of a temporal delay between GDPs and afferent stimulation, no potentiation was observed, thereby indicating that coincident pre- and postsynaptic activity was essential for synaptic potentiation. The presynaptic expression of this form of LTP required the activation of a retrograde signaling molecule that was identified in the

BDNF. This neurotrophin, released from the postsynaptic neuron may act on both pre and postsynaptic Trk B receptors. Activation of presynaptic receptors would lead to the increased transmitter release while activation of postsynaptic receptors would trigger the activation of the ERK pathway leading to transcriptional regulation and new protein synthesis in the postsynaptic neuron.

This part of the work is published in paper N. 1.

Subsequently, I focused on another Hebbian form of synaptic plasticity, called spike timing dependent plasticity or STDP, which has been extensively studied to investigate changes in synaptic strength occurring at glutamatergic synapses. I studied whether STDP may occur also at MF-CA3 synapses which early in development release mainly GABA. In a first set of experiments I found that during the first week of postnatal life, GABA released from MF terminals depolarize and excite tagged cells. Then, I paired MF stimulation with postsynaptic spikes. This procedure caused a persistent change in synaptic efficacy (either an increase or a decrease) depending on the temporal order of pre and postsynaptic stimulation. Then, I identified the transduction signaling pathway responsible for pairing-induced synaptic potentiation. As for GDPs-induced increase in synaptic efficacy at MF-CA3 and CA3-CA1 synapses, the persistent increase observed here was independent of NMDA receptor activation but required a rise of Ca^{2+} in the postsynaptic neuron *via* voltage-dependent calcium channels. As for emerging glutamatergic synapses, also at MF-CA3 synapses, pairing-induced persistent changes in synaptic efficacy required BDNF and the activation of cyclic AMP dependent-PKA which probably exerted a permissive role.

This part of the work is published in paper N. 2.

To avoid the involvement of ionotropic glutamate receptors activated by glutamate released during synaptic stimulation, STDP was routinely induced in the presence of the ionotropic AMPA and NMDA glutamate receptors antagonists. However, I noticed that a high concentration of DNQX, which blocks AMPA and kainate receptors, caused an increase in amplitude of MF $GABA_A$-mdiated postsynaptic currents suggesting the involvement of presynaptic kainate receptors.

Therefore, in collaboration with, Maddalena Delma Caiati, first year PhD student, we investigated in detail this issue. We found that endogenous activation of presynaptic kainate

receptors (localized on MF) by ambient glutamate reversibly suppress GABA release from MF terminals. We identified the type of kainate receptor involved, the GluK1, which has the peculiarity to be an ionotropic and metabotropic receptor, since it is coupled with a G-protein (Lerma et al., 2001). The depressant effect of GluK1 on GABA release was mediated by a metabotropic type of action since it was prevented by treating the slices with pertussis toxin. Interestingly, in the absence of kainate receptor antagonists, pairing presynatic MF stimulation with postsynaptic spiking induced a long-term depression instead of potentiation, indicating that GluK1 receptors control this form of synaptic plasticity.

This work which has been submitted for publication is described in paper N. 3.

Paper **N. 1.**

Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3–CA1 connections in the hippocampus.

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Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3–CA1 connections in the hippocampus

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At early developmental stages, correlated neuronal activity is thought to exert a critical control on functional and structural refinement of synaptic connections. In the hippocampus, between postnatal day 2 (P2) and P6, network-driven giant depolarizing potentials (GDPs) are generated by the synergistic action of glutamate and GABA, which is depolarizing and excitatory. Here the rising phase of GDPs was used to trigger Schaffer collateral stimulation in such a way that synchronized network activity was coincident with presynaptic activation of afferent input. This procedure produced a persistent increase in spontaneous and evoked α-amino-3-hydroxy-5-methyl-4-isoxadepropionic acid-mediated glutamatergic currents, an effect that required calcium influx through postsynaptic L-type calcium channels. No potentiation was observed when a delay of 3 sec was introduced between GDPs and afferent stimulation. Pairing-induced potentiation was prevented by scavengers of endogenous BDNF or tropomyosin-related kinase receptor B (TrkB) receptor antagonists. Blocking TrkB receptors in the postsynaptic cell did not prevent the effects of pairing, suggesting that BDNF, possibly secreted from the postsynaptic cell during GDPs, acts on TrkB receptors localized on presynaptic neurons. Application of exogenous BDNF mimicked the effects of pairing on synaptic transmission. In addition, pairing-induced synaptic potentiation was blocked by ERK inhibitors, suggesting that BDNF activates the MAPK/ERK cascade, which may lead to transcriptional regulation and new protein synthesis in the postsynaptic neuron. These results support the hypothesis that, during a critical period of postnatal development, $GABA_A$-mediated GDPs are instrumental in tuning excitatory synaptic connections and provide insights into the molecular mechanisms involved in this process.

development | giant depolarizing potential | excitatory postsynaptic current | synaptic pairing | TrkB receptors

Spontaneously occurring neuronal oscillations constitute a hallmark of developmental networks (1). In the immature hippocampus, giant depolarizing potentials (GDPs) represent a primordial form of synchrony between neurons, which precedes more organized forms of activity, such as the theta and gamma rhythms (2). These events, which are characterized by recurrent membrane depolarization with superimposed fast-action potentials separated by long and variable intervals of several seconds, are generated when the synaptic traffic and cell firing within the network increase to a threshold level (3). GDPs are synaptic in origin and involve the action of both glutamate and GABA, which, during a restricted period of postnatal development, is depolarizing and excitatory (4–6). The depolarizing action of GABA during GDPs results in the activation of voltage-dependent calcium channels and *N*-methyl-D-aspartate receptors (7). GDPs also can be recorded *in vivo* in rat pups, in which they occur during immobility periods, sleep, and feeding (8). GDP-associated calcium waves are thought to be crucial for the structural refinement of the neuronal connectivity and the establishment of the adult neuronal circuit during a critical period of synapse formation (9, 10). Rewiring would involve electrical activity and the cooperative and competitive interactions between converging inputs.

As suggested by the Hebb postulate for associative learning (11), correlated spiking of pre- and postsynaptic neurons can result in strengthening or weakening of synapses depending on the temporal order of spiking. GDPs are therefore ideal for allowing associative modifications of coincident signals. We previously demonstrated that at mossy fiber–CA3 connections, which during the first postnatal week are mainly GABAergic (12), pairing GDPs with afferent stimulation induced a persistent increase in synaptic efficacy, which is usually restricted to the activated synapse (13).

In the present work, we examined whether excitatory glutamatergic synapses can be modified in an associative way by pairing $GABA_A$-mediated GDPs with Schaffer collateral stimulation. Our findings suggest that "pairing" persistently enhances synaptic efficacy at these synapses, an effect that depends on the rise of calcium in the postsynaptic cell. In addition, compared with our previous work (13), in the present study, we identified the intracellular signaling pathway mediating pairing-induced potentiation, which involves the activation of BDNF and the ERK pathway.

Results

Pairing GDPs with Schaffer Collateral Stimulation Enhances Synaptic Efficacy at CA3–CA1 Synapses. Whole-cell recordings in current-clamp mode from 170 CA1 pyramidal neurons in hippocampal slices from P2- to P6-old rats revealed the presence of GDPs (Fig. 1*B*). In accord with a previous work from CA3 pyramidal cells, we used a pairing procedure to correlate GDPs with Schaffer collateral activation (Fig. 1*A*). For this purpose, the rising phase of GDPs (which occurred at the frequency of 0.069 ± 0.012 Hz; $n = 40$) was used to trigger Schaffer collateral stimulation in stratum radiatum in such a way that synchronized network activity was coincident with presynaptic activation of the afferent input (see Fig. 1*B*). Before pairing, minimal stimulation of afferent fibers (at 0.05 Hz) evoked in CA1 principal cells held at −53 mV (corresponding to E_{GABA}) synaptic currents intermingled with response failures. Synaptic currents were mediated by α-amino-3-hydroxy-5-methyl-4-isoxadepropionic

Author contributions: E.C. designed research; M.H.M., S.S., P.Z., and P.A. performed research; M.H.M., S.S., and P.A. analyzed data; and E.C. wrote the paper.

The authors declare no conflict of interest.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxadepropionic acid; BAPTA, 1,2-bis (2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; D-AP5, *D*-(−)-2-amino-5-phosphonopentaoic acid; GDP, giant depolarizing potential; LTP, long-term potentiation; P*n*, postnatal day *n*; PPR, paired-pulse ratio; sEPSC, spontaneous excitatory postsynaptic current; TrkB, tropomyosin-related kinase receptor B.

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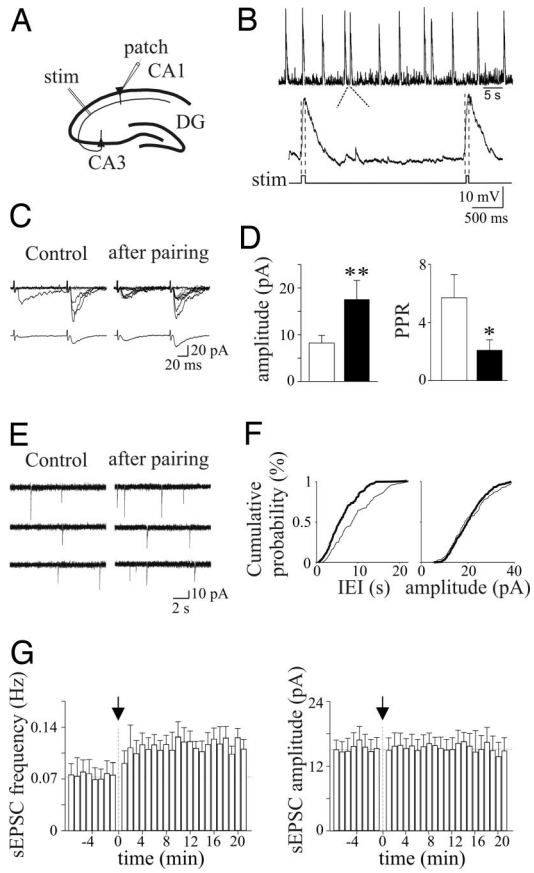

Fig. 1. Pairing GDPs with Schaffer collateral stimulation persistently enhances synaptic efficacy at CA3–CA1 connections. (*A*) Diagram of the hippocampus showing a CA1 pyramidal neuron receiving a synaptic input from a CA3 principal cell. The stimulating electrode (stim) was positioned in stratum radiatum. (*B*) GDPs recorded from a CA1 cell in current-clamp mode. Below the trace, two GDPs are shown on an expanded time scale. Note the absence of spikes riding on the top of GDPs because of a block of the sodium channel with intracellular QX-314. The rising phase of GDPs (between the dashed lines) was used to trigger synaptic stimulation (stim). (*C*) (*Upper*) Nine superimposed individual responses (successes and failures) evoked by Schaffer collateral stimulation and obtained in control and 20 min after pairing. (*Lower*) Average of 19 trials (successes and failures) obtained in the same experimental conditions. (*D*) Each bar represents the mean peak amplitude of synaptic responses (including failures; $n = 8$) and PPR ($n = 8$) obtained before (open bars) and 20 min after (filled bars) pairing. *, $P < 0.05$; **, $P < 0.01$. (*E*) Consecutive traces from the same neuron described in *C* showing sEPSCs obtained in control and 20 min after pairing. (*F*) Cumulative distribution of interevent interval (IEI) (*Left*) and amplitude (*Right*) for the cell shown in *E* before (thin line) and after (thick line) pairing. (*G*) Summary plots showing the mean frequency (*Left*) and amplitude (*Right*) of sEPSCs obtained before and after pairing (arrows indicate time 0; $n = 14$).

acid (AMPA) receptors because they were readily blocked by 20 μM 6,7-dinitroquinoxaline-2,3-dione ($n = 6$; data not shown).

After a control period of 7–10 min, the patch was switched from voltage- to current-clamp mode, and Schaffer collateral responses were paired for 7 min with GDPs. In this period, the mean number of GDPs was 26.1 ± 3.4. As illustrated in Fig. 1 *C* and *D*, the pairing procedure produced a strong and persistent potentiation of Schaffer collateral-mediated synaptic currents (from 8.2 ± 1.6 to 17.6 ± 4.1 pA, 20 min after pairing; $P < 0.01$; $n = 8$) that was associated with a significant increase in the number of successes (from 15.3 ± 4.1% to 37.8 ± 5.5%; $P < 0.01$). In double-pulse experiments ($n = 8$), pairing-induced long-term potentiation (LTP) was accompanied with a significant reduction of the paired-pulse ratio (PPR) (from 5.7 ± 1.6 to 2.1 ± 0.7 pA; $P < 0.05$) (Fig. 1*D*) and a significant increase in the inverse squared value of the coefficient of variation of response amplitude (from 0.08 ± 0.03 to 0.94 ± 0.2 pA; $P < 0.05$; $n = 8$), suggesting that an increased probability of glutamate release accounted for LTP expression.

One interesting question is whether the pairing procedure may also affect AMPA-mediated spontaneous excitatory postsynaptic currents (sEPSCs). Therefore, sEPSCs were analyzed in the same cells used for the evoked responses. At immature CA3–CA1 synapses, the amplitude distribution of sEPSCs matches closely that of miniature events recorded in the presence of tetrodotoxin because these synapses bear only a single functional release site (14, 15). sEPSCs occurred at the frequency of 0.11 ± 0.03 Hz and had a mean amplitude of 14.3 ± 2.4 pA ($n = 21$). They were reversibly blocked by 20 μM 6,7-dinitroquinoxaline-2,3-dione, indicating that they were mediated by glutamate acting on AMPA receptors ($n = 10$; data not shown). Pairing GDPs with afferent stimulation (same cell shown in Fig. 1*C*) significantly and persistently (for at least 20 min) enhanced the frequency but not the amplitude of individual events (Fig. 1 *E* and *F*). On average, 20 min after pairing, the frequency of sEPSCs was 71% higher than in controls (it changed from 0.07 ± 0.02 to 0.12 ± 0.02 Hz; $n = 14$; $P < 0.01$) (Fig. 1*G*). This effect was not associated with significant changes in amplitude of individual events (15.2 ± 2.1 and 14.4 ± 3.2 pA before and after pairing, respectively; $P > 0.5$) (Fig. 1*G*). The increase in frequency, but not in amplitude, of sEPSCs suggests a presynaptic site of action.

In the absence of pairing, no significant changes in synaptic efficacy could be detected. "No pairing" consisted of switching the patch for 7 min from voltage- to current-clamp mode. In this period, GDPs occurred randomly in the absence of afferent stimulation. In these cells, the mean peak amplitude currents were 7.9 ± 2.1 and 7.1 ± 1.9 pA in control and 20 min after switching from current- to voltage-clamp conditions, respectively. Similarly, the success rate varied from 21.1 ± 5.8% to 18.4 ± 4.8% ($n = 5$; $P > 0.5$; data not shown), and the frequency of sEPSCs varied from 0.12 ± 0.02 to 0.11 ± 0.03 Hz; $P > 0.5$; $n = 7$).

To test the temporal specificity for LTP induction in 10 cells, a 3-sec delay between GDPs and synaptic stimulation was introduced. In agreement with a previous study (13), we found that with this delay the pairing procedure failed to induce synaptic potentiation. Before and after pairing, the amplitude of the evoked responses was not significantly different (6.4 ± 1.1 and 4.4 ± 0.9 pA, respectively; $P > 0.5$). In addition, no significant changes in the frequency (0.14 ± 0.06 and 0.13 ± 0.07 Hz; $P > 0.5$) or amplitude (9.9 ± 0.4 and 8.5 ± 0.2 pA, respectively) of sEPSCs were detected [supporting information (SI) Fig. 6].

Overall, these experiments clearly show that pairing with Schaffer collateral stimulation GDPs causes a persistent potentiation of the amplitude of evoked sEPSCs and the frequency of sEPSCs. Although otherwise stated in the following experiments, we focused only on pairing-induced long-term changes on sEPSCs on CA1 pyramidal cells, in which minimal stimulation of Schaffer collaterals evoked sEPSCs to the first or second stimulus.

Pairing-Induced Synaptic Potentiation Requires a Calcium Rise in the Postsynaptic Cell. A common trigger for LTP is a postsynaptic rise in intracellular calcium concentration (16). To test whether a rise of postsynaptic calcium during GDPs is responsible for a pairing-induced increase in synaptic efficacy, cells were loaded with a 20 mM concentration of the calcium chelator 1,2-bis (2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA). In these cases, the pairing procedure failed to cause any persistent change in synaptic strength ($n = 6$; $P > 0.1$) (Fig. 2). A tendency

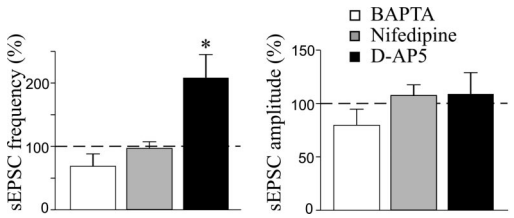

Fig. 2. Pairing-induced potentiation requires a rise of intracellular calcium concentration in the postsynaptic cell via voltage-dependent calcium channels. Mean frequency (*Left*) and amplitude (*Right*) of sEPSCs expressed as a percentage of controls (dashed lines) obtained 20 min after pairing in the presence of 20 mM intracellular BAPTA (white bars; $n = 6$), 10 μM extracellular nifedipine (gray bars; $n = 7$), or 50 μM D-AP5 (black bars; $n = 6$). *, $P < 0.05$.

toward depression was observed, although it did not reach a significant level (in the presence of BAPTA, the frequency of sEPSCs measured in control and 20 min after pairing was 0.08 ± 0.03 and 0.06 ± 0.03 Hz, whereas the amplitude was 13.1 ± 1.9 and 10.1 ± 3.9 pA before and after pairing, respectively). These experiments suggest that, at CA3–CA1 synapses, a pairing-induced persistent increase in the frequency of sEPSCs depends on a calcium rise in the postsynaptic cell.

A rise of intracellular calcium may occur via voltage-dependent calcium channels or NMDA receptors (7, 17). To identify the source of calcium responsible for GDP-induced potentiation in the frequency of sEPSCs, additional experiments were performed in the presence of a 50 μM concentration of the NMDA receptor antagonist *D*-(−)-2-amino-5-phosphonopentaoic acid (D-AP5) ($n = 6$) or a 10 μM concentration of the voltage-dependent calcium channel blocker nifedipine ($n = 7$). D-AP5 failed to prevent pairing-induced persistent changes in the frequency of sEPSCs (208.1 ± 34.8% of control; $P < 0.05$), whereas nifedipine blocked the changes (96.9 ± 10.5% of control; $P > 0.5$) (Fig. 2). It is worth noting that nifedipine and D-AP5 did not modify the shape or amplitude of GDPs (data not shown). These results indicate that, early in postnatal life, a calcium rise through the voltage-dependent calcium channel is the trigger for activity-dependent changes in synaptic strength.

To mimic the effects of GDPs on synaptic potentiation, we repeatedly depolarized postsynaptic neurons with bursts of action or plateau potentials evoked in the presence of 20 μM D-AP5. However, as illustrated in SI Fig. 7, a postsynaptic calcium rise through theta bursts or plateau potentials failed to enhance synaptic efficacy.

Pairing-Induced Synaptic Potentiation Requires the Activation of BDNF and the ERK Pathway. The present data clearly show that, although the induction of LTP is postsynaptic (dependent on the postsynaptic rise of calcium through GDPs), its expression is presynaptic, as suggested by the decrease in PPR and increase in frequency, but not in the amplitude, of sEPSCs. Therefore, the postsynaptic cell provides a transcellular retrograde signal to the presynaptic neuron. One attractive candidate is BDNF, which can be released in a calcium-dependent way by depolarization of the postsynaptic cell (18–20) and plays a crucial role in synaptic plasticity (21). BDNF acts on tropomyosin-related kinase receptor B (TrkB), and this interaction activates different signaling pathways. First, we proved that, at immature CA3–CA1 connections, BDNF is able to mimic the effects of pairing on synaptic strength. As illustrated in Fig. 3, bath application of 40 μg/ml BDNF produced a strong potentiation of Schaffer collateral-mediated synaptic currents (from 6.4 ± 1.8 in control to 24.8 ± 9.4 pA 10 min after BDNF; $P < 0.05$; $n = 6$) that was associated with a significant increase in the number of successes (from 23.6 ± 8.4% to 68.0 ± 10.1%; $P < 0.05$) and a decrease in PPR (from 2.9 ± 0.5 to 0.9 ± 0.1 pA; $P < 0.05$). In addition,

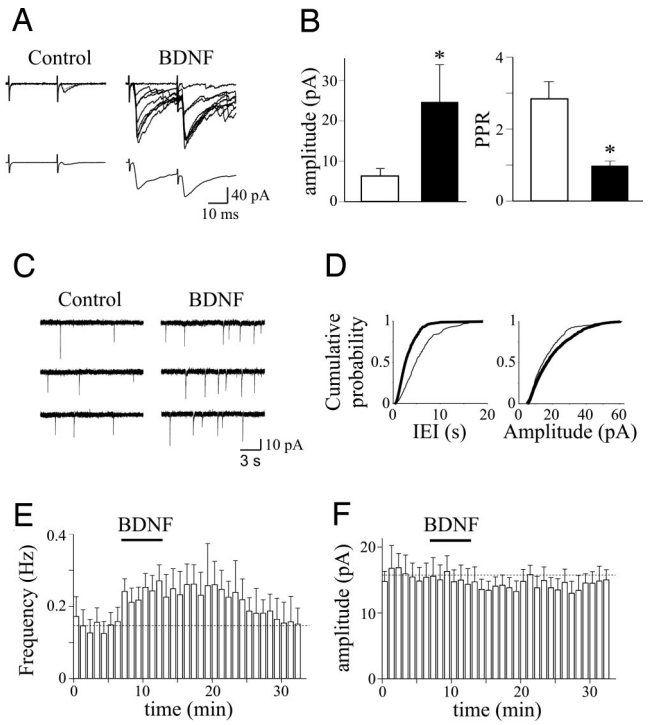

Fig. 3. BDNF enhances synaptic efficacy at CA3–CA1 connections. (*A*) (*Upper*) Nine superimposed individual responses (successes and failures) evoked by Schaffer collateral stimulation and obtained in control and during bath application of 40 μg/ml BDNF. (*Lower*) Average of 19 trials (successes and failures) obtained in the same experimental conditions. (*B*) Each bar represents the mean peak amplitude of synaptic responses (including failures; $n = 6$) and PPR ($n = 6$) obtained in control (open bars) and during superfusion of BDNF (filled bars). *, $P < 0.05$. (*C*) Consecutive traces from the same cell shown in *A* illustrating sEPSCs obtained in control and during application of BDNF. (*D*) Cumulative distribution of interevent interval (IEI) (*Left*) and amplitude (*Right*) for the cell shown in *C* before (thin line) and after (thick line) pairing. (*E*) Summary plot showing the mean frequency (*Left*) and amplitude (*Right*) of sEPSCs obtained before and after pairing ($n = 8$).

BDNF enhanced the frequency of sEPSCs (from 0.14 ± 0.02 to 0.23 ± 0.02 pA; $P < 0.01$; $n = 8$) without altering their amplitude (from 15.9 ± 1.5 to 15.2 ± 2.1 pA; $P > 0.1$). These effects were prevented by bath application of 150 nM k-252a, a specific inhibitor of protein kinase coupled to Trk receptors ($n = 3$) (SI Fig. 8) (22). However, when k-252a was included in the patch pipette to block postsynaptic TrkB receptors (20), it failed to prevent BDNF-induced potentiation of sEPSCs ($n = 6$) (SI Fig. 8), indicating that BDNF enhances synaptic efficacy acting on presynaptic TrkB receptors. In a second set of experiments, the involvement of endogenous BDNF in pairing-induced synaptic potentiation was assessed with k-252a. Bath application of 150 nM k-252a fully prevented pairing-induced enhancement of the frequency of sEPSCs. Mean frequency values were 0.14 ± 0.03 and 0.16 ± 0.04 Hz before and after pairing, respectively ($P > 0.5$; $n = 9$) (Fig. 4), whereas mean amplitude values were 14.9 ± 1.1 and 15.7 ± 2.3 pA before and after pairing, respectively ($P > 0.5$) (Fig. 4). In the absence of pairing, k-252a alone did not modify the frequency and amplitude of sEPSCs (frequency values were 0.11 ± 0.03 vs. 0.12 ± 0.02 Hz and amplitude values were 14.2 ± 4.2 vs. 12.7 ± 3.4 pA in control and in the presence of k-252a, respectively). Similar results were obtained with 150 nM k-252b, which is a weaker inhibitor of protein kinase coupled to Trk receptors (23). On average, in six cells before and after pairing, the frequency values of sEPSCs were 0.08 ± 0.02 and 0.07 ± 0.02 Hz, respectively ($P > 0.5$), whereas the amplitude

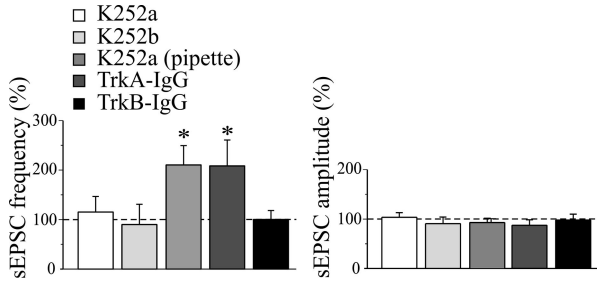

Fig. 4. Pairing-induced increase in frequency of sEPSCs requires the activation of TrkB receptors by BDNF. Mean frequency (*Left*) and amplitude (*Right*) of sEPSCs (normalized to prepairing control values; dashed lines) recorded before and 20 min after pairing in the presence of 150 nM K252a in the bath ($n = 9$), 150 nM K252b in the bath ($n = 6$), 150 nM K252a into the patch pipette ($n = 6$), 1 μg/ml TrkA–IgG ($n = 5$), and 1 μg/ml TrkB–IgG ($n = 6$). Note the increase in sEPSC frequency in the presence of TrkA–IgG.

values were 15.9 ± 2.5 and 14.8 ± 1.4 pA, respectively ($P > 0.5$) (Fig. 4). To better assess the contribution of endogenous TrkB ligands to GDP-induced potentiation, we incubated the slices for at least 3 h with a soluble form of TrkB receptor (TrkB–IgG) engineered as an immunoadhesin to prevent TrkB activation (24, 25). In slices preincubated with 1 μg/ml TrkB–IgGs, the pairing procedure did not affect the frequency or amplitude of sEPSCs (mean frequency values were 0.08 ± 0.03 Hz in control and 0.08 ± 0.02 Hz after pairing; $n = 6$; $P > 0.5$; mean amplitude values were 18.9 ± 2.7 pA in control and 17.7 ± 2.2 pA after pairing; $P > 0.5$) (Fig. 4). TrkB–IgGs did not alter the frequency or amplitude of synaptic events (frequency values were 0.09 ± 0.02 vs. 0.08 ± 0.03 Hz and amplitude values were 15.8 ± 2.7 vs. 12.1 ± 1.6 pA in the absence or presence of TrkB–IgG, respectively). In contrast, when slices were incubated with TrkA–IgGs, which sequester endogenous nerve growth factor, but not endogenous BDNF (26), GDP pairing produced a persistent increase in the frequency of sEPSCs (Fig. 4). On average, 20 min after pairing, the frequency of sEPSCs was 107% higher than in control (mean frequency values were 0.07 ± 0.02 and 0.14 ± 0.03 Hz before and after pairing, respectively; $n = 7$; $P < 0.05$), whereas the amplitude was slightly reduced to 87.1 ± 9% of control (mean amplitude values were 16.3 ± 1.9 and 14.2 ± 1.6 pA before and after pairing, respectively; $P > 0.5$). Additionally, in the case of TrkA–IgGs, no changes in the frequency or amplitude of spontaneous synaptic activity were detected in the absence of pairing. Although BDNF is probably released from the postsynaptic cell during GDP-induced membrane depolarization (20), it probably acts on TrkB receptors present on presynaptic terminals because k-252a in the patch pipette was unable to block pairing-induced potentiation ($n = 6$) (Fig. 4). Altogether, these data show that endogenous TrkB, but not TrkA ligands, is required for pairing-induced persistent potentiation in the frequency of sEPSCs.

One of the most common signaling pathways activated by BDNF after binding to TrkB receptor is the ERK, a subfamily of MAPK (27). Upon phosphorylation, ERK is translocated into the nucleus, where it activates the transcription factor cAMP response element-binding protein, thus contributing to the persistent modifications in synaptic efficacy (28–31). To assess the role of ERK in synaptic potentiation, the pairing procedure was performed in the presence of the ERK inhibitors U0126 and PD98059. Bath application of 20 μM U0126 for 10 min did not modify the frequency and amplitude of sEPSCs, which remained at predrug levels (before and after U0126 application, the mean frequency values were 0.12 ± 0.02 and 0.11 ± 0.01 Hz, whereas the mean amplitude values were 14.9 ± 2.2 and 15.6 ± 1.5 pA; $P > 0.5$ for both frequency and amplitude; $n = 6$). However, after

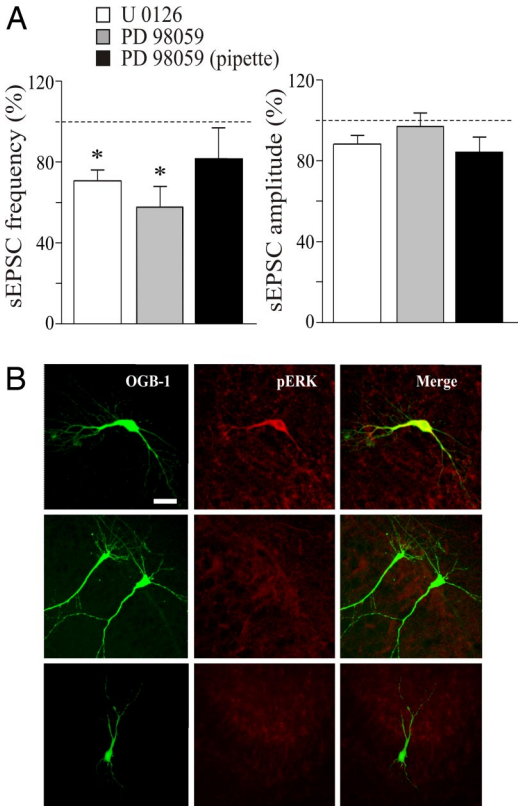

Fig. 5. Pairing-induced increase in synaptic efficacy requires the activation of the ERK pathway. (*A*) Mean frequency (*Left*) and amplitude (*Right*) of sEPSCs (normalized to prepairing control values; dashed lines) recorded before and 20 min after pairing in the presence of the following ERK inhibitors: 20 μM U0126 ($n = 5$), 50 μM PD98059 added to the extracellular medium ($n = 6$), and 50 μM PD98059 added to the intrapipette solution ($n = 5$). *, $P < 0.05$. *(B)* (*Left* and *Center*) Immunofluorescence images showing different CA1 pyramidal cells stained with Oregon green BAPTA 488–1 (OGB-1) (*Left*), with polyclonal antibodies recognizing the doubly phosphorylated form of ERK1/2 (pERK) (*Center*). (*Right*) Merged images. Pairing GDPs with Schaffer collateral stimulation induced ERK phosphorylation in the recorded neuron (*Top*), whereas no ERK phosphorylation was present in the absence of pairing (*Middle*) or when pairing was performed in the presence of a 20 μM concentration of the ERK inhibitor U0126 (*Bottom*). (Scale bar: 20 μm.)

pairing, U0126 induced a persistent and significant reduction in the frequency of sEPSCs. On average, 20 min after pairing the frequency of sEPSCs was 71% of controls (frequency varied from 0.21 ± 0.04 to 0.15 ± 0.04 Hz before and after pairing, respectively; $P < 0.05$), whereas the amplitude was not significantly modified (16.6 ± 2.2 and 14.9 ± 0.9 pA before and after pairing, respectively; $P > 0.5$) (Fig. 5*A*). Similar effects were obtained with PD98059. Slices were preincubated for 10 min with 50 μM PD98059. Although PD98059 did not modify the frequency or amplitude of synaptic events (frequency values were 0.11 ± 0.02 and 0.09 ± 0.02 Hz and amplitude values were 14.9 ± 2.7 and 12.8 ± 1.8 pA before and after drug application, respectively; $P > 0.5$ for both frequency and amplitude), it prevented the potentiating effect of pairing and caused a significant reduction of the frequency of sEPSCs (mean frequency values were 0.15 ± 0.04 and 0.08 ± 0.02 Hz before and after pairing, respectively; 57% of controls; $P < 0.05$), but not in the amplitude (mean amplitude values were 15.2 ± 2.7 and 14.3 ± 3.1 pA before and after pairing, respectively; $P > 0.5$) of sEPSCs ($n = 6$) (Fig. 5*A*).

To test whether ERK activation occurred into the postsynaptic cell, PD98059 was applied directly into the patch pipette (32).

Additionally, in this condition, 50 μM PD98059 prevented the effects of ERK upon paired-induced potentiation of the frequency of sEPSCs (mean frequency values were 0.09 ± 0.03 and 0.07 ± 0.02 Hz before and after pairing, respectively; $P > 0.1$; mean amplitude values were 16.1 ± 1.3 and 13.8 ± 1.9 pA before and after pairing, respectively; $P > 0.1$; $n = 5$) (Fig. 5*A*).

The involvement of ERK in GDP-induced changes in synaptic efficacy was further validated by immunocytochemical experiments, which demonstrated that the pairing procedure was able to induce ERK phosphorylation ($n = 5$), an effect that was prevented by 20 μM U0126 ($n = 4$) (Fig. 5*B*). In the absence of pairing, no ERK phosphorylation was observed (Fig. 5*B*). These data suggest that the persistent changes in frequency of sEPSCs after GDP pairing require the activation of the ERK pathway. ERK phosphorylation also was prevented by the TrkB receptor inhibitor k-252a in the patch pipette ($n = 4$) or in the bath ($n = 3$), suggesting that, in the present experiments, ERK phosphorylation was triggered by BDNF (SI Fig. 9).

Discussion

The present data clearly show that, during the first week of postnatal life, correlated presynaptic (Schaffer collateral) and postsynaptic (GDPs) activity induced a persistent enhancement of synaptic efficacy at glutamatergic CA3–CA1 connections. This outcome required a transient rise of calcium in the postsynaptic cell and the involvement of BDNF and the ERK pathway.

The pairing procedure not only produced a strong potentiation of the sEPSCs evoked by the Schaffer collateral stimulation but also persistently enhanced the frequency of spontaneous glutamatergic events.

In CA3 pyramidal neurons, evidence has been provided that, during a restricted period of postnatal development, long-term changes in GABAergic synaptic transmission can be induced by repetitive depolarizing pulses (25, 33, 34). Although in these studies the conditioning protocol used for LTP induction could have been of physiological relevance, in the present case, the persistent enhancement of glutamatergic transmission at CA3–CA1 synapses depended on a calcium rise through a voltage-dependent calcium channel activated by the depolarizing action of GABA during GDPs. Interestingly, when the afferent stimulation was paired with theta bursts or plateau potentials in the postsynaptic cell to mimic GDPs, it failed to persistently enhance the frequency of sEPSCs. When paired with afferent stimulation, plateau potentials only briefly enhanced the frequency of sEPSCs, which declined to a control level a few minutes after pairing. In the adult hippocampus, repetitive voltage pulses to the postsynaptic cell have been shown to induce a short-lasting potentiation of excitatory synaptic transmission (35). However, unlike the present case, transient changes in synaptic strength observed in the adult primarily depended on postsynaptic mechanisms because membrane depolarization not only enhanced the frequency of miniature events but also the amplitude of responses to exogenously applied AMPA. Whatever the mechanisms, our results indicate that the way by which calcium enters into the cell is crucial for pairing-induced potentiation. We cannot exclude, however, that in the present study GDPs may concomitantly stimulate presynaptic terminals to further strengthen synaptic currents. This result may, at least in part, explain why depolarizing the postsynaptic cell with theta bursts or plateau potentials failed to persistently enhance synaptic strength.

In the present experiments, the involvement of BDNF in GDP-induced synaptic potentiation was demonstrated by the observation that scavengers of endogenous BDNF or blockers of protein kinase coupled to Trk receptors were able to prevent pairing-induced persistent enhancement of the frequency of sEPSCs. The present results do not indicate whether endogenous BDNF was released from the pre- or postsynaptic cell. However, evidence was recently provided that a moderate postsynaptic depolarization can elicit the release of BDNF from the postsynaptic cell in a calcium-dependent way (20). Once released, BDNF would affect TrkB receptors localized on both pre- and postsynaptic membranes. Although at the presynaptic level BDNF-induced activation of TrkB receptors would enhance transmitter release (20, 36, 37), at the postsynaptic level it would activate fast dendritic calcium transients (38) and different intracellular signaling pathways. One of the most common signaling pathways activated by BDNF is the MAPK/ERK cascade (28, 39). Activation of ERK would lead to transcriptional regulation and new protein synthesis (27) required for the enduring forms of synaptic plasticity (31). As in the late phase of LTP (31), in the present case the activation of ERK was targeted to the postsynaptic neuron as demonstrated by electrophysiological and immunocytochemical experiments. However, we cannot exclude a concomitant activation of ERK in the presynaptic neuron. The postsynaptic action of ERK may further support structural changes (triggered by BDNF activation of TrkB receptors), such as dendritic growth and increase in spine density (30, 40). The ERK pathway also can be activated by calcium entry by NMDA receptors (41). However, this event is unlikely because pairing-induced potentiation was independent of NMDA receptor activation. Therefore, BDNF can act presynaptically to alter the probability of glutamate release but also postsynaptically to produce the morphological modifications necessary for the formation of new synapses and the refinement of the adult neuronal hippocampal circuit.

In conclusion, our data demonstrate that network-driven GABAergic oscillations such as GDPs are essential for the functional maturation of glutamatergic synapses.

Materials and Methods

Slice Preparation. Experiments were performed on hippocampal slices from P2–P6 Wistar rats as previously described (13). Briefly, animals were decapitated after being anesthetized with an i.p. injection of 2 g/kg urethane. The brain was quickly removed from the skull and placed in ice-cold artificial cerebrospinal fluid containing 130 mM NaCl, 3.5 mM KCl, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1.3 mM $MgCl_2$, 2 mM $CaCl_2$, and 25 mM glucose saturated with 95% O_2 and 5% CO_2 (pH 7.3–7.4). Transverse hippocampal slices (400 μm thick) were cut with a vibratome and stored at room temperature in a holding bath containing the same solution. After a recovery period of at least 1 h, an individual slice was transferred to the recording chamber, where it was continuously superfused with oxygenated artificial cerebrospinal fluid at a rate of 2 to 3 ml/min at 33–34°C.

Electrophysiological Recordings. Electrophysiological experiments were performed with CA1 pyramidal cells by using the whole-cell configuration of the patch-clamp technique in current- or voltage-clamp mode. Synaptic responses were evoked at 0.05 Hz by minimal stimulation of the Schaffer collateral. They were recorded in voltage-clamp conditions from a holding potential of −53 mV (E_{GABA}). In most cases, paired stimuli were applied at 50-msec intervals. Patch electrodes were pulled from Borosilicate glass capillaries (Hingelberg, Malsfeld, Germany). They had a resistance of 4 to 6 MΩ when filled with an intracellular solution containing 135 mM K-gluconate, 20 mM KCl, 10 mM Hepes, 4 mM MgATP, 0.3 mM GTP, 0.5 mM EGTA, and 5 mM QX-314. In some experiments, recordings were performed with patch pipettes containing 20 mM BAPTA (Sigma–Aldrich, Milan, Italy). When 20 mM BAPTA was added to the pipette solution, K-gluconate was reduced from 135 to 115 mM. For plateau potential experiments, K-gluconate was substituted with $CsMeSO_4$. In some experiments, cells were visualized with 100 μM Oregon green 488 BAPTA-1 (Molecular Probes, Eugene, OR).

Recordings were made with a patch-clamp amplifier (Axo-

patch 200A; Axon Instruments, Foster City, CA). Series resistance compensation was used only for current-clamp recordings. The stability of the patch was checked by repetitively monitoring the input and series resistance during the experiment. Cells exhibiting >20% changes in series resistance were excluded from the analysis. The following drugs were used: D-AP5 and 6,7-dinitroquinoxaline-2,3-dione (Tocris Cookson Ltd., Bristol, U.K.); nifedipine and BDNF (Sigma–Aldrich); and k-252a, k-252b, U0126, and PD 98059 (Calbiochem, La Jolla, CA). Immunoadhesins TrkA–IgG and TrkB–IgG were gifts from A. Cattaneo (International School for Advanced Studies).

All drugs except D-AP5 and BDNF were dissolved in DMSO. The final concentration of DMSO in the bathing solution was 0.1%. At this concentration, DMSO alone did not modify the shape or kinetics of synaptic currents. Drugs were applied in the bath by a three-way tap system by changing the superfusion solution to one differing only in its content of drugs. The ratio of flow rate to bath volume ensured complete exchange within 2 min.

Immunofluorescence Staining. Hippocampal slices with paired or unpaired neurons were fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer. After 2 h of preincubation in basic buffer, primary antibodies to doubly phosphorylated ERK1/2 (Cell Signaling Technology, Danvers, MA) were applied at a 1:200 ratio overnight at 4°C. After washing, the resulting immune complexes were visualized with Alexa Fluor 594-labeled goat anti-rabbit antibody (Invitrogen, Carlsbad, CA). Slices were imaged with the DM-IRE2 (Leica, Wetzlar, Germany) confocal system by using sequential dual-channel recording of the Oregon green-injected cells.

Data Acquisition and Analysis. Data were stored on a disk after digitization with an A/D converter (Digidata 1322A; Axon Instruments, Union City, CA). Data were sampled at 20 kHz and filtered with a cutoff frequency of 2 kHz. Data acquisition was done by using pClamp 9 (Axon Instruments). sEPSCs and evoked EPSCs were analyzed offline with the Clampfit 9 program. sEPSCs were first collected by using the template function of Clampfit and then reviewed by visual inspection. The mean amplitude of sEPSCs was obtained by averaging successes and failures. PPR was calculated as the ratio between the mean amplitude of sEPSC2 and sEPSC1. The coefficient of variation of response amplitude was determined as the ratio between the standard deviation and the mean. Values are given as mean ± SEM. Significance of differences was assessed by Student's *t* test and Wilcoxon signed rank test ($P < 0.05$).

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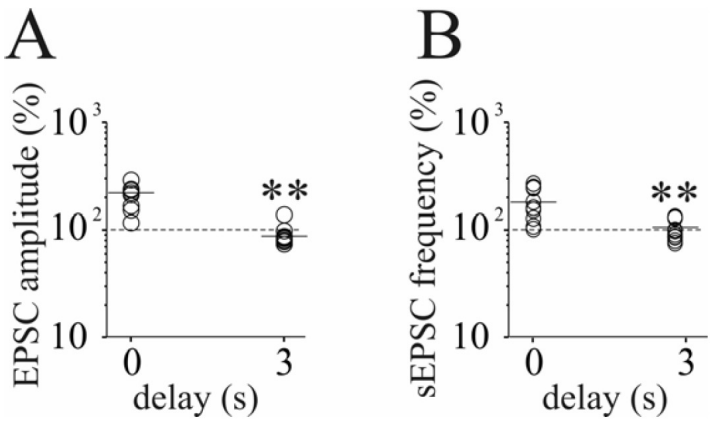
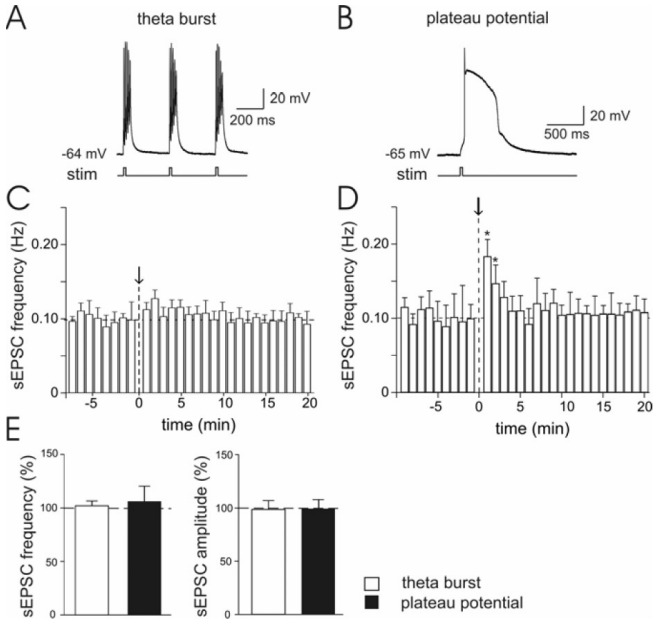
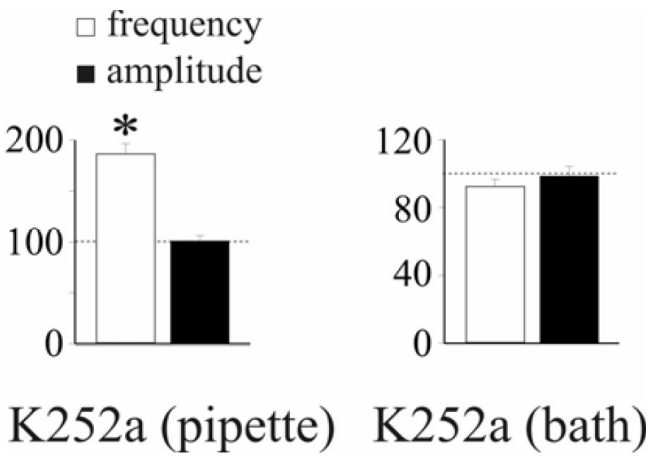
1. Ben-Ari Y (2001) *Trends Neurosci* 24:353–360.
2. Buzsaki G, Draguhn A (2004) *Science* 304:1926–1929.
3. Menendez de la Prida LM, Huberfeld G, Cohen I, Miles R (2006) *Neuron* 49:131–142.
4. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) *J Physiol* 416:303–325.
5. Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) *Trends Neurosci* 14:515–519.
6. Ben-Ari Y (2002) *Nat Rev Neurosci* 3:728–739.
7. Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R (1997) *Neuron* 18:243–255.
8. Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsaki G (2002) *Science* 296:2049–2052.
9. Garaschuk O, Linn J, Eilers J, Konnerth A (2000) *Nat Neurosci* 3:452–459.
10. Voigt T, Opitz T, de Lima AD (2005) *J Neurosci* 25:4605–4615.
11. Hebb D (1949) *The Organization of Behavior* (Wiley, New York).
12. Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) *J Neurosci* 26:597–608.
13. Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) *Proc Natl Acad Sci USA* 101:3967–3972.
14. Hsia AY, Malenka RC, Nicoll RA (1998) *J Neurophysiol* 79:2013–2024.
15. Hanse E, Gustafsson B (2001) *J Physiol* 531:467–480.
16. Malenka RC, Nicoll RA (1999) *Science* 285:1870–1874.
17. Garaschuk O, Hanse E, Konnerth A (1998) *J Physiol* 507:219–236.
18. Goodman LJ, Valverde J, Lim F, Geschwind MD, Federoff HJ, Geller AI, Hefti F (1996) *Mol Cell Neurosci* 7:222–238.
19. Lessmann V, Gottmann K, Malcangio M (2003) *Prog Neurobiol* 69:341–374.
20. Magby JP, Bi C, Chen ZY, Lee FS, Plummer MR (2006) *J Neurosci* 26:13531–13536.
21. Poo MM (2001) *Nat Rev Neurosci* 2:24–32.
22. Knusel B, Hefti F (1992) *J Neurochem* 59:1987–1996.
23. Tanaka T, Saito H, Matsuki N (1997) *J Neurosci* 17:2959–2966.
24. Sermasi E, Margotti E, Cattaneo A, Domenici L (2000) *Eur J Neurosci* 12:1411–1419.
25. Gubellini P, Ben-Ari Y, Gaiarsa JL (2005) *J Neurosci* 25:5796–5802.
26. Pesavento E, Margotti E, Righi M, Cattaneo A, Domenici L (2000) *Neuron* 25:165–175.
27. Sweatt JD (2004) *Curr Opin Neurobiol* 14:311–317.
28. Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) *J Neurosci* 22:1532–1540.
29. Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002) *Learn Mem* 9:224–237.
30. Alonso M, Medina JH, Pozzo-Miller L (2004) *Learn Mem* 11:172–178.
31. Kelleher RJ, III, Govindarajan A, Tonegawa S (2004) *Neuron* 44:59–73.
32. Shen MR, Chou CY, Browning JA, Wilkins RJ, Ellory JC (2001) *J Physiol* 537:347–362.
33. Caillard O, Ben-Ari Y, Gaiarsa JL (1999) *J Physiol* 518:109–119.
34. Gubellini P, Ben-Ari Y, Gaiarsa JL (2001) *Eur J Neurosci* 14:1937–1946.
35. Wyllie DJ, Manabe T, Nicoll RA (1994) *Neuron* 12:127–138.
36. Lohof AM, Ip NY, Poo MM (1993) *Nature* 363:350–353.
37. Tao HW, Poo M (2001) *Proc Natl Acad Sci USA* 98:11009–11015.
38. Lang SB, Stein V, Bonhoeffer T, Lohmann C (2007) *J Neurosci* 27:1097–1105.
39. Huang EJ, Reichardt LF (2003) *Annu Rev Biochem* 72:609–642.
40. Segal RA, Greenberg ME (1996) *Ann Rev Neurosci* 19:463–489.
41. Hardingham GE, Arnold FJ, Bading H (2001) *Nat Neurosci* 4:565–566.



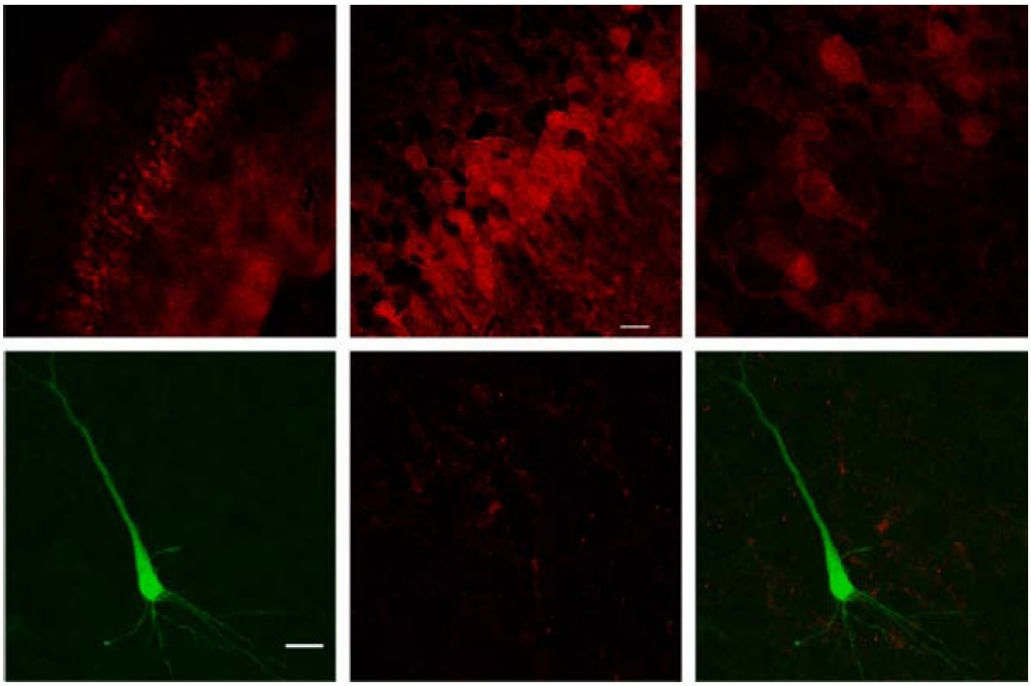
Supp. Info: Fig. 6. Temporal specificity of the correlated activity required for GDPs-induced synaptic potentiation. During the pairing protocol, a delay of 0 or 3 sec was introduced between GDP rise time and synaptic stimulation. (*A*) Each symbol represents the mean percentage increase in amplitude of EPSCs evoked by Schaffer collateral stimulation (successes and failures) as a function of the delay (0-sec delay, $n = 9$; 3-sec delay, $n = 10$). Horizontal bars represent the average of all open circles in a column. (*B*) As in *A*, but for the frequency of sEPSCs. No potentiating effect was found when pre- and postsynaptic signals were delayed by 3 sec. Evoked and spontaneous activity was recorded from the same cells. **, $P < 0.01$.



Supp. Info: Fig. 7. Pairing theta bursts or plateau potentials with Schaffer collateral stimulation fails to persistently enhance synaptic strength. (*A* and *B*) The rising phase of theta bursts (five action potentials at 100 Hz evoked in the postsynaptic cell by short depolarizing current pulses, repeated 10 times at the theta frequency every 10 sec) (*A*) or plateau potentials (20 plateau potentials at 0.1 Hz evoked in the postsynaptic cells loaded with calcium to block most of the potassium conductances) (*B*) were used to trigger Schaffer collateral stimulation (stim). (*C* and *D*) Summary plots showing the mean frequency of sEPSCs recorded in control conditions and after pairing (arrows at 0) with theta bursts (*C*) (n = 8) or plateau potentials (*D*) (n = 10). Note the transient increase in frequency of sEPSCs after pairing (*, $P < 0.05$). (*E*) Mean frequency (*Left*) and amplitude (*Right*) of sEPSCs expressed as percentage of controls (dashed lines) obtained 20 min after pairing with theta bursts (empty bars) or plateau potentials (filled bars). The frequency of sEPSCs was 0.1 ±0.02 and 0.09 ± 0.02 Hz before and 20 min after pairing with theta bursts, respectively (n = 8; $P > 0.5$); and the amplitude was 16.6 ±1.7 and 16.2 ± 2.3 pA (n = 8; $P > 0.5$) before and 20 min after pairing, respectively. The frequency of sEPSCs was 0.1 ±0.04 and 0.11 ± 0.02 Hz before and 20 min after pairing with plateau potentials, respectively; and the amplitude was 13.6 ±1.9 and 13.4 ± 2.3 pA before and 20 min after pairing, respectively ($P > 0.5$ for both frequency and amplitude; n = 8). These experiments demonstrate that a calcium rise in the postsynaptic cell through voltage-dependent calcium channel is not sufficient to persistently enhance glutamate release.



Supp. Info: Fig. 8. The increase in frequency of sEPSCs induced by BDNF was dependent on the activation of TrkB receptors localized on presynaptic neurons. Each bar represents the mean frequency (white) and amplitude (black) of sEPSCs expressed as percentage of controls (dashed lines) obtained by applying 40 mg/ml BDNF in the presence of 150 nM K252a into the patch pipette (*Left*) (n = 6) or in the bath (*Right*) (n = 3). *, $P < 0.05$. Note that BDNF significantly enhanced sEPSCs only when K252a was present into the patch pipette.



Supp. Info: Fig. 9. Pairing-induced ERK phosphorylation is triggered by BDNF. (*Upper*) Hippocampal slice (obtained from a P4 rat) incubated with 40 mg/ml BDNF for 1 h induced ERK phosphorylation in pyramidal neurons (stained with polyclonal antibodies recognizing the doubly phosphorylated form of ERK1/2). (Magnification: *Left*, ×20; *Center*, ×40; *Right*, ×60.) (*Lower*) CA1 pyramidal cell (from a P5 rat) stained with Oregon green BAPTA 488-1 (*Left*) and polyclonal antibodies recognizing the doubly phosphorylated form of ERK1/2. (*Right*) Merged picture. The cell was paired in the presence of a 150 nM concentration of the TrkB receptor antagonist K252a. Notice that K252a prevented ERK phosphorylation. (Scale bars: *Upper*, 80 mm; *Lower*, 20 mm.)

Paper **N. 2.**

At immature mossy fiber-CA3 synapses, correlated pre and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA.

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Development/Plasticity/Repair

At Immature Mossy-Fiber–CA3 Synapses, Correlated Presynaptic and Postsynaptic Activity Persistently Enhances GABA Release and Network Excitability via BDNF and cAMP-Dependent PKA

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In the adult rat hippocampus, the axons of granule cells in the dentate gyrus, the mossy fibers (MF), form excitatory glutamatergic synapses with CA3 principal cells. In neonates, MF release into their targets mainly GABA, which at this developmental stage is depolarizing. Here we tested the hypothesis that, at immature MF–CA3 synapses, correlated presynaptic [single fiber-evoked $GABA_A$-mediated postsynaptic potentials (GPSPs)] and postsynaptic activity (back propagating action potentials) may exert a critical control on synaptic efficacy. This form of plasticity, called spike-timing-dependent plasticity (STDP), is a Hebbian type form of learning extensively studied at the level of glutamatergic synapses. Depending on the relative timing, pairing postsynaptic spiking and single MF-GPSPs induced bidirectional changes in synaptic efficacy. In case of positive pairing, spike-timing-dependent-long-term potentiation (STD-LTP) was associated with a persistent increase in GPSP slope and in the probability of cell firing. The transduction pathway involved a rise of calcium in the postsynaptic cell and the combined activity of cAMP-dependent PKA (protein kinase A) and brain-derived neurotrophic factor (BDNF). Retrograde signaling via BDNF and presynaptic TrkB receptors led to a persistent increase in GABA release. In "presynaptically" silent neurons, the enhanced probability of GABA release induced by the pairing protocol, unsilenced these synapses. Shifting E_{GABA} from the depolarizing to the hyperpolarizing direction with bumetanide failed to modify synaptic strength. Thus, STD-LTP of GPSPs provides a reliable way to convey information from granule cells to the CA3 associative network at a time when glutamatergic synapses are still poorly developed.

Key words: **hippocampal mossy fibres; excitatory action of GABA; spike-timing-dependent plasticity; increased synaptic efficacy; BDNF; cAMP-dependent PKA**

Introduction

Spike-timing-dependent plasticity (STDP) is a particular form of associative Hebbian type of learning, crucial for information coding. STDP consists in a bidirectional modification of synaptic strength which relies on the temporal order of presynaptic and postsynaptic spiking (Dan and Poo, 2006). Thus, positively correlated presynaptic and postsynaptic spiking (pre before post) within a critical window leads to long-term potentiation (LTP), whereas a negative correlation (post before pre) induces long-term depression (LTD) (Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Feldman, 2000) (for review, see Dan and Poo, 2006). While at glutamatergic synapses STDP has been extensively studied (Dan and Poo, 2004, 2006; Caporale and Dan, 2008), at GABAergic connections the information available is limited (Caporale and Dan, 2008). In the hippocampus of juvenile animals, correlated spiking activity has been shown to induce a downregulation of the K^+-Cl^- cotransporter KCC2 with consequent increase in $[Cl^-]_i$, change in GABA equilibrium potential (E_{GABA}), and weakening of synaptic inhibition (Woodin et al., 2003; Fiumelli et al., 2005; Fiumelli and Woodin, 2007). Whether STDP may occur also at immature GABAergic synapses when the action of GABA is depolarizing (Cherubini et al., 1991; Ben-Ari, 2002) is still unclear. The depolarizing action of GABA may facilitate synaptic plasticity processes via a rise of $[Ca^{2+}]_i$ through voltage-dependent calcium channels and NMDA receptors (Cherubini et al., 1991; Ben-Ari et al., 2007). However, these effects may be overwhelmed by the concomitant $GABA_A$-mediated shunting inhibition (Staley and Mody, 1992; Lamsa et al., 2000; Mohajerani and Cherubini, 2005; Banke and McBain, 2006).

We examined this issue at mossy fiber (MF)–CA3 synapses, which in the immediate postnatal period release mainly GABA (Safiulina et al., 2006). Pairing single postsynaptic spikes with unitary MF $GABA_A$-mediated postsynaptic potentials (GPSPs) consistently upregulated or downregulated synaptic strength ac-

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cording to the temporal order of stimulation. Positive pairing induced persistent changes in GPSPs slope, which often reached the threshold for action potential generation. STD-LTP required a rise of calcium in the postsynaptic cell via voltage-dependent calcium channel and the activation of brain-derived neurotrophic factor (BDNF) and cAMP-dependent protein kinase A. STD-LTP was prevented when GPSPs shifted from the depolarizing to the hyperpolarizing direction with bumetanide, a selective inhibitor of neuronal Cl^- uptake mediated by the Na^+-K^+-$2Cl^-$ cotransporter isoform 1 NKCC1 (Sipilä et al., 2006).

These results indicate that in the immediate postnatal period, at mossy fiber synapses, the precise temporal correlation between GPSPs and postsynaptic spiking powerfully control synaptic strengthening or weakening thus contributing to the functional refinement of developing neuronal circuits.

Materials and Methods

Slice preparation. Experiments were performed on hippocampal slices from P2–P5 Wistar rats as described previously (Gasparini et al., 2000). All experiments were performed in accordance with the European Community Council Directive of 24 November 1986 (86/609EEC) and were approved by local authority veterinary service. Briefly, animals were decapitated after being anesthetized with an intraperitoneal injection of urethane (2 g/kg). The brain was quickly removed from the skull and placed in ice-cold ACSF containing the following (in mM): 130 NaCl, 3.5 KCl, 1.2 NaH_2PO_4, 27 $NaHCO_3$, 1.3 $MgCl_2$, 2 $CaCl_2$, 25 glucose, saturated with 95% O_2 and 5% CO_2 (pH 7.3–7.4). Transverse hippocampal slices (400 μm thick) were cut with a vibratome and stored at room temperature (20−24°C) in a holding bath containing the same solution as above. After a recovery period of at least 1 h, an individual slice was transferred to the recording chamber where it was continuously superfused with oxygenated ACSF at a rate of 2–3 ml/min at 33−35°C.

Electrophysiological recordings. Electrophysiological experiments were performed from CA3 pyramidal cells using the whole-cell configuration of the patch-clamp technique in current or voltage-clamp mode. Neurons were visualized using an upright microscope (Olympus BX51WI) equipped with differential interference contrast (DIC) optics and infrared video camera. Patch electrodes were pulled from borosilicate glass capillaries (Hingelberg). They had a resistance of 4–6 MΩ when filled with an intracellular solution containing the following (in mM): 140 KCl, 1 $MgCl_2$, 10 HEPES, 4 MgATP, 0.5 EGTA, pH 7.3. In some experiments, recordings were performed with patch pipettes containing the calcium chelator 1,2-bis (2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA 20 mM, Sigma). In these cases, to maintain the same osmolarity (~290 mOsm), the intrapipette concentration of KCl was reduced to 120 mM. Recordings were made with a patch-clamp amplifier (Axopatch 200A; Axon Instruments). Series resistance was assessed repetitively every 5 min and in current-clamp recordings compensated at 75% throughout the experiment. Cells exhibiting >15–20% changes in series resistance were excluded from the analysis.

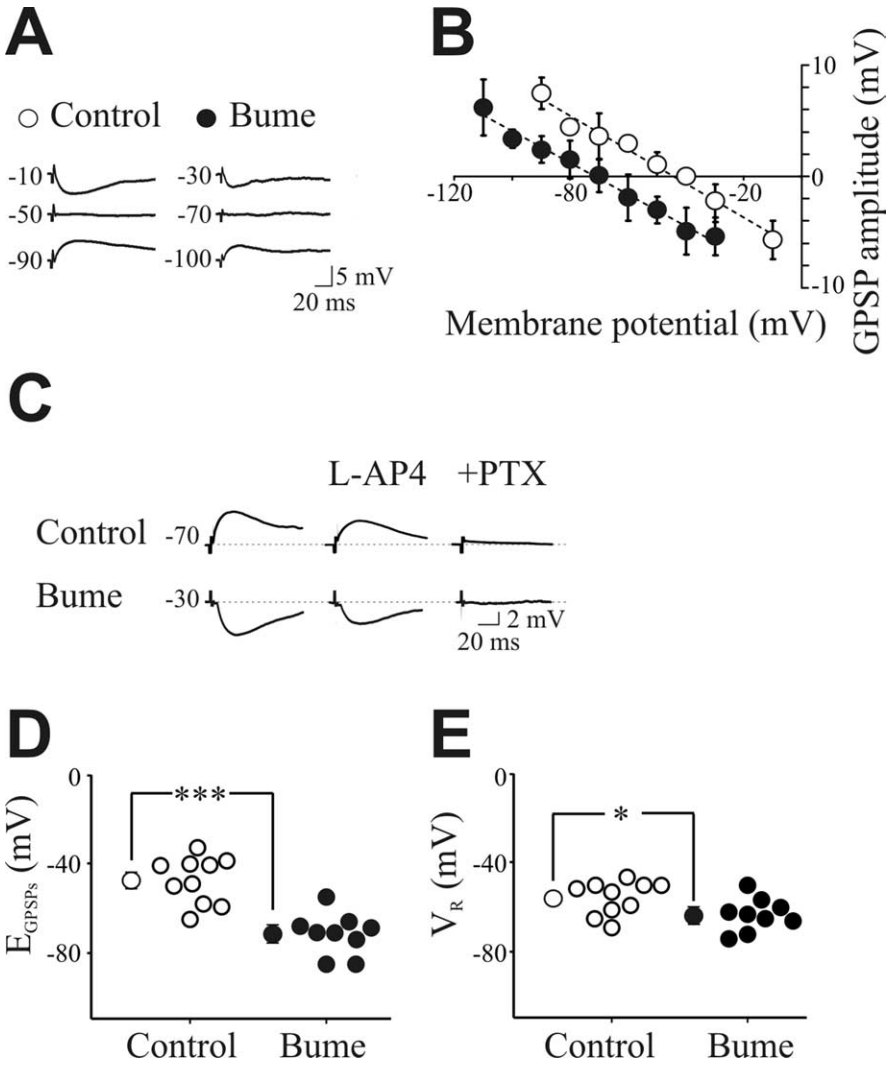

Figure 1. GABA released from MF terminals depolarizes principal cells. ***A***, Synaptic responses (successes plus failures) evoked at three different holding potentials (to the left of the traces) by stimulation of granule cells in the dentate gyrus in control (○) and in the presence of bumetanide (●, 10 μM). ***B***, Plot of GPSP amplitudes as a function of membrane potentials in control (○, $n = 10$) and in the presence of bumetanide (●, $n = 9$). Note that in bumetanide, E_{GPSP} shifted toward more negative values (−71.5 mV vs −47.6 mV). ***C***, GPSPs obtained in control and in the presence of bumetanide were sensitive to L-AP4 (10 μM) and to picrotoxin (100 μM). In bumetanide, GPSPs were recorded at −30 mV to increase the driving force for Cl^-. ***D***, ***E***, Each symbol represents E_{GPSPs} (***D***) and V_R (***E***) of individual cells obtained in control (○) and in the presence of bumetanide (●). The average values are shown on the left of each group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

$GABA_A$-mediated synaptic potentials or currents (GPSPs or GPSCs) were evoked at 0.05 Hz from a holding potential of –70 mV in the presence of DNQX (20 μM) and D-AP5 (50 μM) to block AMPA- and NMDA-mediated synaptic responses, respectively. We used minimal stimulation of the granule cells in the dentate gyrus to activate only one or few presynaptic fibers. According to the technique described by Jonas et al. (1993) and Allen and Stevens (1994), the stimulation intensity was decreased until only a single axon was activated. This was achieved when the mean amplitude of the postsynaptic currents and failure probability remained constant over a range of stimulus intensities near threshold for detecting a response (Safiulina et al., 2006). An abrupt increase in the mean peak amplitude of synaptic currents was observed when the stimulus intensity was further increased. This all or none behavior let us to assume that only a single fiber was stimulated. In addition, the latency and the shape of individual synaptic responses remained constant for repeated stimuli. The monosynaptic nature of synaptic currents was supported by the unimodal and narrow latencies and rise time distributions

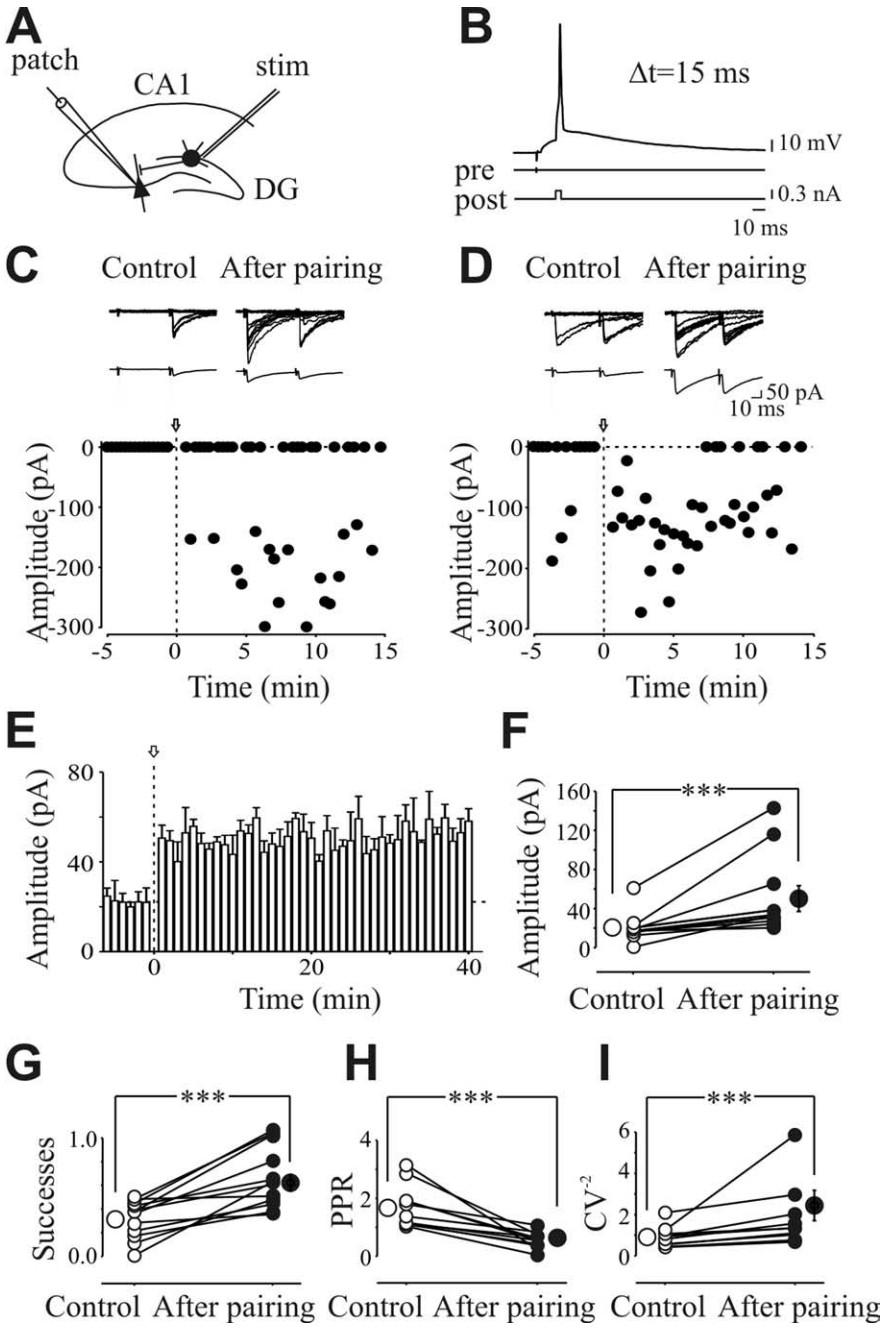

Figure 2. Spike-timing dependent LTP induced by pairing MF GPSPs with postsynaptic spiking. ***A***, Schematic representation of the experimental design. ***B***, Spike-timing protocol. GPSP preceded the postsynaptic spike by 15 ms (Δt). ***C***, ***D***, Peak amplitude of MF GPSCs in presynaptically silent (***C***) and low probability neurons (***D***) evoked before and after pairing (arrows at time 0) as a function of time. Insets above the graphs represent individual (top traces) and averaged GPSCs (bottom traces) evoked before and after pairing. ***E***, Summary plot of mean GPSCs amplitude recorded before and after pairing versus time ($n = 12$). ***F–I***, Amplitude (***F***), successes (***G***), paired-pulse ratio (***H***), and inverse squared of CV (***I***) measured in individual cells before (○) and 40 min after pairing (●). Bigger symbols represent averaged values. $^{***}p < 0.001$.

which remained constant when the extracellular Ca^{2+}/Mg^{2+} concentration ratio was reduced from 2:1.3 to 1:3 (Safiulina et al. 2006).

When the probability of synaptic failures in response to a first stimulus was near 1 (failures were estimated by visual discrimination), we applied a second pulse at 50 ms interval. If a response to a second stimulus appeared in 15–30 consecutive trials (silent to the first stimulus), we considered this synapse presynaptically silent.

MF inputs were identified on the basis of their sensitivity to group III mGluR agonist 2-amino-4-phosphonobutyric acid (L-AP4 10 μM) (Gutiérrez et al., 2003; Kasyanov et al., 2004; Safiulina et al., 2006), their strong paired pulse facilitation and short term frequency-dependent facilitation (Safiulina et al., 2006). They were blocked by bicuculline or picrotoxin, confirming their GABAergic nature. In contrast to MF inputs, GABAergic inputs from interneurons were insensitive to L-AP4 (Walker et al., 2001; Safiulina et al., 2006).

Experiments were also performed using the gramicidin-perforated patch (Kyrozis and Reichling, 1995) to preserve the anionic conditions of the recorded cells. In this case, the pipette solution contained the same intracellular solution plus 80 μg/ml gramicidin D (Sigma). A 20 mg/ml stock of gramicidin in dimethylsulphoxide (DMSO) was prepared freshly (<2 h before recording) and sonicated. This was diluted with gramicidin-free solution, sonicated again for 20–30 s, and centrifuged. Patch pipettes were back filled with a gramicidin containing solution and then the tip of the pipette was dipped into and filled with a gramicidin–free solution by applying a negative pressure for 20–30 s to facilitate cell-attached formation (seal resistance >3 GΩ). After ~40 min, series resistance decreased and stabilized around 30 MΩ. The perforated patch condition was constantly monitored and in the case of membrane rupture recording was discontinued. In fact, due to the high intracellular $[Cl^-]$, the break of the membrane was associated with a clear change in membrane capacitance and a shift of E_{GPSPs} near 0 mV (Tyzio et al. 2007).

Usually, synaptic currents were recorded in voltage-clamp mode for 5–10 min. Then, changes in synaptic efficacy, induced by pairing (in current-clamp conditions) presynaptic activity with postsynaptic spiking (10 spikes evoked at 0.1 Hz) at various time intervals, were recorded for additional 15–60 min. Some experiments were performed in current-clamp mode to correlate pairing-induced changes in GPSPs slope with the probability of cell firing.

Drugs used were as follows: D-(-)-2-amino-5-phosphonopentaoic acid (D-AP5), 6,7-dinitroquinoxaline-2,3-dione (DNQX), L-AP4, PTX, bumetanide, Rp-cAMPS (all from Tocris Bioscience); nifedipine, forskolin, BDNF (all from Sigma); K252a, protein kinase A inhibitor 6–22 amide (PKI 6–22), protein kinase A inhibitor 14–22 amide, cell-permeable (PKI 14–22) (all from Calbiochem). All drugs were dissolved in either distilled water or ethanol, as required, except DNQX, forskolin, and bumetanide, which were dissolved in DMSO. The final concentration of DMSO in the bathing solution was 0.1%. At this concentration, DMSO alone did not modify the shape or the kinetics of synaptic currents. Drugs were applied in the bath via a three-way tap system, by changing the superfusion solution to one differing only in its content of drug(s). The ratio of flow rate to bath volume ensured complete exchange within 1–2 min.

Data acquisition and analysis. Data were acquired and digitized with an A/D converter (Digidata 1200, Molecular Devices) and stored on a computer hard disk. Acquisition and analysis of evoked responses were performed with Clampfit 9 (Molecular Devices). Data were sampled at 20 kHz and filtered with a cutoff frequency of 1 kHz. Mean GPSCs amplitude was obtained by averaging successes and failures. The paired pulse

ratio (PPR) was calculated as the mean amplitude of the synaptic response evoked by the second stimulus over that evoked by the first one. The coefficient of variation was calculated as the ratio between the SD of synaptic currents amplitude and the mean. Unless otherwise stated, data are presented as mean ± SEM. Quantitative comparisons were based on students paired or unpaired *t* test, as required and *p* values <0.05 were considered as significant.

Results

MF inputs to principal cells were identified on the basis of their strong paired pulse facilitation, their sensitivity to group III mGluR agonist L-AP4 and their short term frequency-dependent facilitation (Kasyanov et al., 2004; Safiulina et al., 2006). MF inputs were mainly GABAergic since they were readily and reversibly blocked by bicuculline methiodide (20 μM) or picrotoxin (100 μM) (Walker et al., 2001; Safiulina et al., 2006). In the present experiments, GPSPs or GPSCs elicited by minimal stimulation of granule cells in the dentate gyrus were routinely recorded at −60 mV from CA3 pyramidal cells (at P2–P5) in the presence of DNQX (20 μM) and D-AP5 (50 μM) to block AMPA and NMDA receptors, respectively. These synapses were characterized by the low probability of GABA release. In several cases ($n = 22$), stimulation of granule cells in the dentate gyrus failed to produce any synaptic response over 15–30 consecutive trials. However, occasional responses to a second stimulus could occur, suggesting that these synapses were presynaptically silent (Gasparini et al., 2000; Kasyanov et al., 2004) (see Fig. 2*C*).

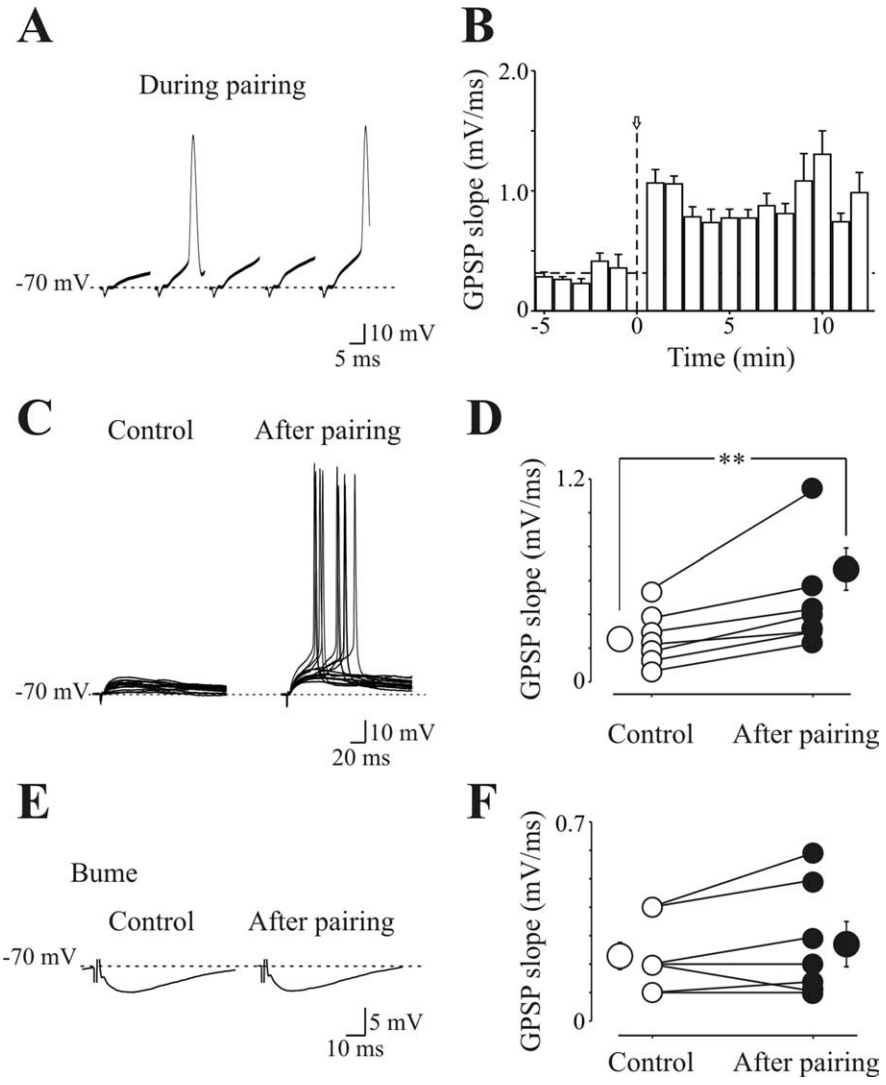

Figure 3. Pairing-induced increase in GPSPs slope was associated with enhanced firing probability. ***A***, Representative traces from one neuron showing the rising phases of GPSPs (evoked at 0.1 Hz) during pairing. Note the progressive increase of GPSPs slope which occasionally gave rise to action potentials (with <15 ms delay). ***B***, The GPSPs slope obtained before and after pairing (arrows at time 0) is plotted as a function of time ($n = 7$). ***C***, Superimposed traces from a single neuron before and after pairing. ***D***, Each symbol represents the mean value of GPSPs slope obtained from individual cells before (○) and 15 min after pairing (●). Larger symbols represent averaged values ($n = 7$). ***E***, Representative examples of GPSPs (average of 15 traces) evoked in the presence bumetanide before and after pairing. ***F***, Each symbol represents the mean value of GPSPs slope obtained from individual cells before (○) and 15 min after pairing (●) in the presence of bumetanide. Bigger symbols represent averaged values ($n = 7$). $^{**}p < 0.01$.

GABA released from MF terminals depolarizes principal cells

A first set of experiments, using gramicidin-perforated patch to preserve the intracellular chloride concentration (Kyrozis and Reichling, 1995) was aimed at evaluating whether GABA released from MF terminals exerts on principal cells a depolarizing or a hyperpolarizing action. The representative example of Figure 1*A* (Control) shows averaged responses (successes plus failures) evoked by granule cell stimulation at three different holding potentials. The reversal of GPSPs (E_{GPSP}) was −50 mV. Since the resting membrane potential (V_R) of this cell was −60 mV, the driving force for GABA, defined as $E_{GPSP} - V_R$, was 10 mV. This indicates that GABA was depolarizing (Sipilä et al., 2006; Tyzio et al., 2007). Overall, in 10 cells, E_{GPSP} was −47.6 ± 3.3 mV (Fig. 1*B*,*D*, open circles) while V_R was −56 ± 2.2 mV (Fig. 1*E*, open circles). E_{GPSP} value was very close to the threshold for action potential generation (−49.3 ± 2.4 mV; $n = 21$).

In accord with their MF origin (Gutiérrez et al., 2003, 2005; Kasyanov et al., 2004; Safiulina et al., 2006), bath application of L-AP4 (10 μM) significantly reduced the amplitude of GPSPs (34.7 ± 8.2% of control; $n = 8$; $p < 0.01$), which were completely blocked by picrotoxin (100 μM) (Fig. 1*C*). The reduction of GPSCs amplitude induced by L-AP4 was very similar to that found in a previous study for MF-evoked synaptic currents (66 ± 8%; Safiulina et al., 2006).

To see whether accumulation of chloride inside the cell via the cation-chloride cotransporter NKCC1 was responsible for the depolarizing action of GABA, slices were incubated for 30–50 min with bumetanide (10 μM), a selective inhibitor of NKCC1 (Dzhala et al., 2005; Sipilä et al., 2006). In all cells tested ($n = 9$), bumetanide caused a slight membrane hyperpolarization (on average, V_R was −63.6 ± 2.3 mV; $p < 0.05$ vs controls) (Fig. 1*E*, closed circles) and a shift of E_{GPSP} toward more negative values (−71.5 ± 3.09 mV; $p < 0.001$ respect to controls) (Fig. 1*A*,*B*,*D*, closed circles), indicating that GABA was hyperpolarizing. Also in this case, bath application of L-AP4 induced a significant reduction in amplitude of GPSPs (34.9 ± 8.6% of control) thus confirming their MF origin ($n = 5$; $p < 0.01$) (Fig. 1*C*).

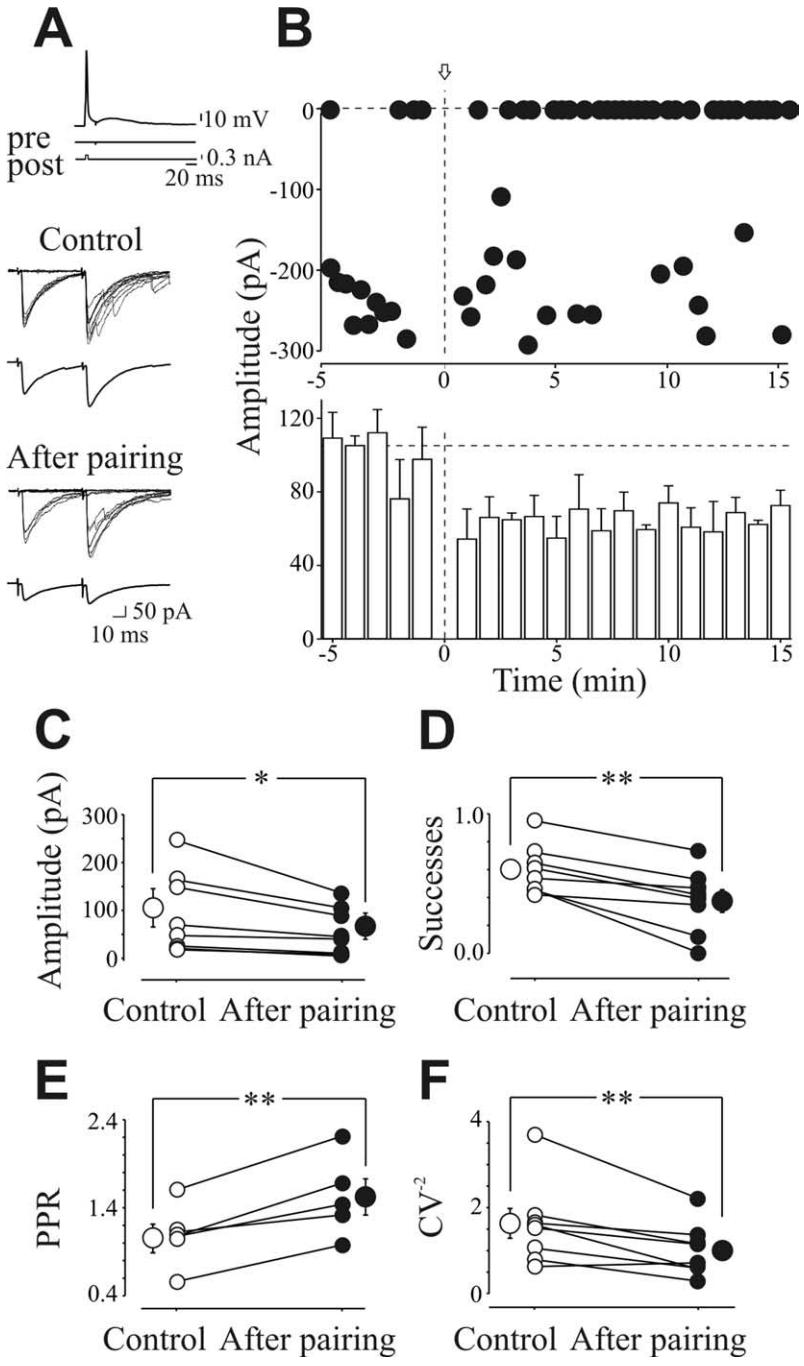

Figure 4. Spike-timing dependent LTD induced by pairing MF GPSPs with postsynaptic spiking. ***A***, The inset represents the spike-timing protocol. The postsynaptic spike (post) preceded the GPSP (pre) by 15 ms. Below, Individual (top) and averaged (bottom) traces of GPSCs evoked before (Control) and after pairing. ***B***, In the top graph, the peak amplitudes of GPSCs (shown in ***A***) obtained before and after pairing (arrows at time 0) are plotted against time. In the bottom graph, summary plot of GPSCs amplitude versus time ($n = 8$). ***C–F***, Amplitude (***C***), successes (***D***), paired-pulse ratio (***E***) and inverse squared of CV (***F***) measured in individual cells before (○) and after pairing (●). Bigger circles represent average values. ($n = 8$ for each group except for PPR in which $n = 5$). $^{*}p < 0.05$; $^{**}p < 0.01$.

These data demonstrate that at MF–CA3 synapses, due to the accumulation of Cl^- inside the cell by NKCC1, GABA exerts a depolarizing action.

Bidirectional modifications of synaptic strength associated with STDP

In the next series of experiments, we used a pairing procedure to verify whether STDP could modify synaptic efficacy. STDP was induced in current-clamp mode by pairing 10 postsynaptic spikes (at 0.1 Hz) with afferent stimulation, varying the relative timing of presynaptic and postsynaptic activity (Fig. 2*A*,*B*). Postsynaptic firing without presynaptic activation caused no changes in synaptic efficacy (the amplitude of GPSCs was -28.6 ± 3.2 pA and -29.3 ± 2.8 pA, before and after postsynaptic firing, respectively, $n = 6$). In addition, no changes in synaptic efficacy were detected changing stimulation frequency from 0.05 to 0.1 Hz in the absence of postsynaptic spikes ($n = 4$; data not shown). Coincident presynaptic and postsynaptic activity (0 ms delay) did not modify synaptic strength. However, a delay of 15 ms (pre before post) (Fig. 2*B*) caused a strong potentiation of GPSCs. Examples of presynaptically silent or low probability synapses are shown in Figure 2, *C* and *D*, respectively. In both cases, correlated activity (arrows) induced an increase in GPSCs amplitude and successes rate which persisted without decrement for periods of time variable from 40 to 60 min indicating that changes in synaptic efficacy were persistent as in LTP. The time course of pairing-induced modifications of synaptic strength observed in 12 cells (including one presynaptically silent) recorded for at least 40 min after pairing is illustrated in Figure 2*E*. On average, the peak amplitude of GPSCs (successes plus failures) was 21.5 ± 4.1 pA and 51.9 ± 13.1 pA, before and 15 min after pairing ($p < 0.001$) (Fig. 2*F*), respectively, while the success rate changed from 0.31 ± 0.04 to 0.62 ± 0.07 ($p < 0.001$) (Fig. 2*G*). These effects were associated with a significant reduction of the PPR (from 1.66 ± 0.22 to 0.64 ± 0.09; $p < 0.001$) (Fig. 2*H*) and a significant increase in the inverse squared value of the coefficient of variation (CV^{-2}) of responses amplitude (from 0.91 ± 0.14 to 2.43 ± 0.73; $p < 0.001$; $n = 19$, Fig. 2*I*). The PPR was measured only in nonsilent cells. After pairing the potency of synaptic responses (mean amplitude of successes without failures) increased significantly (from 53.9 ± 12.1 pA to 90.1 ± 13.9 pA; $p < 0.01$).

Positive pairing (with 15 ms delay) was also performed in current-clamp conditions (using gramicidin-perforated patch) to measure changes in GPSPs slope and in the probability of firing. As shown in Figure 3, *B* and *D*, pairing always (7/7) induced a stable potentiation of GPSP slope (from 0.25 ± 0.05 to 0.67 ± 0.12; $n = 7$; $p < 0.01$) which sometimes initiated during the pairing protocol (Fig. 3*A*; note that in this case changes in GPSPs slope triggered action potentials). Due to the larger size of synaptic events, after pairing it was easier to reach the threshold for action potential generation (after pairing, the probability of cell firing persistently increased from 0.04 to 0.5) (Fig. 3*C*).

STD-LTP did not depend on changes in the equilibrium po-

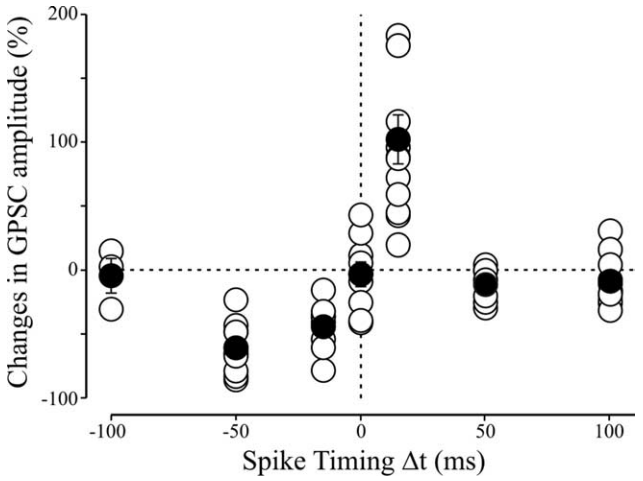

Figure 5. Temporal window for spike-timing dependent enhancement and reduction in the efficacy of MF GPSCs. Plot of GPSC amplitude (as percentage of controls) from individual cells (○) as a function of Δt. Closed symbols represent the averaged values for each Δt. No changes in amplitude were detected when the postsynaptic action potential was coincident with presynaptic GPSPs ($\Delta t = 0$ ms). Note that the magnitude of synaptic potentiation reached the maximum at $\Delta t = 15$ ms which corresponds to the peak of GPSP.

tential for GABA since similar E_{GPSP} values were found before and after pairing (-48.3 ± 2.5 mV and -43.8 ± 4.1 mV, respectively; $n = 7$) (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). These data indicate that correlating presynaptic and postsynaptic activity strongly enhances the amplitude of GABA-mediated synaptic events leading to excitation of principal cells. In cells treated with bumetanide (10 μM), the pairing protocol did not modify the slope of hyperpolarizing GPSPs (Fig. 3*E*,*F*) ($n = 7$).

STD-LTP probably did not depend on general changes in intrinsic membrane properties of principal cells or changes in presynaptic cell excitability, because the pairing procedure did modify neither the threshold for action potential generation (-49.3 ± 1.5 mV and -47.9 ± 2.1 mV before and after pairing, respectively; $n = 7$) nor the firing frequency in response to depolarizing current pulses (supplemental Fig. S2, available at www.jneurosci.org as supplemental material).

Negative pairing (post before pre) (Fig. 4*A*) with a 15 ms delay was assessed in synapses with relatively high probability of release. This procedure induced a consistent depression of MF-mediated GPSCs. After pairing, the mean peak amplitude of GPSCs (successes plus failures) shifted from 105.3 ± 40.0 pA to 66.9 ± 27.4 pA ($n = 8$; $p < 0.05$) (Fig. 4*A*,*B*). This was mainly caused by a decrease in the number of successes (from 0.61 ± 0.06 to 0.38 ± 0.08, before and after pairing, respectively; $n = 8$; $p < 0.01$) (Fig. 4*B*,*D*). The potency of the responses (mean amplitude of successes without failures) was almost unchanged (174 ± 49.7 pA and 150.5 ± 36.9 pA before and after pairing, respectively; $p > 0.1$; data not shown). As expected for a reduction in the probability of GABA release, the increased number of failures was associated with a significant increase in PPR (from 1.04 ± 0.16 to 1.52 ± 0.20; $n = 5$; $p < 0.01$) (Fig. 4*E*) and a significant decrease of CV^{-2} (from 1.63 ± 0.35 to 1.0 ± 0.2; $n = 8$; $p < 0.01$) (Fig. 4*F*).

The time window for bidirectional activity-dependent changes in synaptic efficacy is summarized in Figure 5. When the action potential was coincident with the GPSP, no changes in synaptic strength were detected. However, when the postsynaptic spike followed the GPSP onset with 15 ms delay (which corresponds to the peak of the synaptic event), the magnitude of synaptic potentiation reached its maximum. GPSP potentiation declined toward control level with delays of >50 ms. On the contrary, when the postsynaptic spike preceded the GPSP we observed a depression of the synaptic responses that regained the control level with delays of >50 ms. Overall, these data indicate that activity-dependent changes in synaptic efficacy strongly rely on the temporal relationship between presynaptic and postsynaptic activation being the critical window for STDP similar to that described for most glutamatergic and GABAergic synapses (Dan and Poo, 2006).

Pairing-induced synaptic potentiation requires a rise of calcium in the postsynaptic cell via voltage-dependent calcium channels

A common trigger for LTP is a postsynaptic rise in intracellular calcium concentration (Malenka and Nicoll, 1999). To test whether a rise of postsynaptic calcium during STDP is responsible for the increase in synaptic efficacy, cells were loaded with the calcium chelator BAPTA (20 mM). In these cases, the pairing procedure failed to cause any persistent increase of synaptic strength. Instead, a persistent depression of synaptic currents was observed (Fig. 6*A*,*B*) (Kasyanov et al. 2004). In the presence of BAPTA, the mean amplitude of GPSCs measured before and after pairing was -36.9 ± 5.3 pA and -22.1 ± 2.6 pA, respectively ($n = 6$; $p = 0.01$). These experiments suggest that at MF–CA3 synapses, pairing-induced LTP depends on calcium rise in the postsynaptic cell. This may occur via voltage-dependent calcium channels or via NMDA receptors (Leinekugel et al., 1997; Garaschuk et al., 1998; Kasyanov et al., 2004). NMDA receptors were not involved, because as already reported, all experiments were routinely performed in the presence of D-AP5 (50 μM) (Fig. 6*A*,*B*). However, pairing-induced synaptic potentiation was prevented by nifedipine (10 μM), indicating the involvement of voltage-dependent L-type of calcium channels. The mean amplitude of GPSCs, measured before and after pairing was -46.3 ± 9.2 pA and -22.2 ± 6.8 pA, respectively; $n = 5$; $p > 0.1$ (Fig. 6*A*,*B*). These results indicate that, early in postnatal life, calcium rise through VDCC triggers STD-LTP. In the following experiments, we focused only on the signaling pathways involved in STD-LTP.

Pairing-induced synaptic potentiation requires the activation of TrkB receptors by BDNF

The present data clearly show that the induction of STD-LTP is postsynaptic while its expression is presynaptic as suggested by the decrease in PPR and by the increase in CV^{-2} of MF-mediated synaptic responses. Therefore, the postsynaptic cell shall provide a transcellular retrograde signal to the presynaptic neuron. One attractive candidate is BDNF, which can be released in a calcium-dependent way by depolarization of the postsynaptic cell (Goodman et al., 1996; Lessmann et al., 2003; Magby et al., 2006; Kuczewski et al., 2008b). Previous studies from the immature hippocampus have shown that BDNF is the retrograde messenger required for increasing the probability of GABA and glutamate release at GABAergic and glutamatergic synapses, respectively (Gubellini et al., 2005; Mohajerani et al., 2007). Therefore, we tested whether BDNF was able to mimic the effects of STD-LTP on MF-evoked GPSCs. As illustrated in Figure 7*A*, in the absence of BDNF, no changes in synaptic efficacy were detected ($n = 5$). Bath application of BDNF (40 ng/ml for 5 min), however, produced a significant increase in amplitude of GPSCs (from 20.3 ± 4.2 pA to 44.7 ± 7.5 pA; $p < 0.01$; $n = 7$) which persisted for at

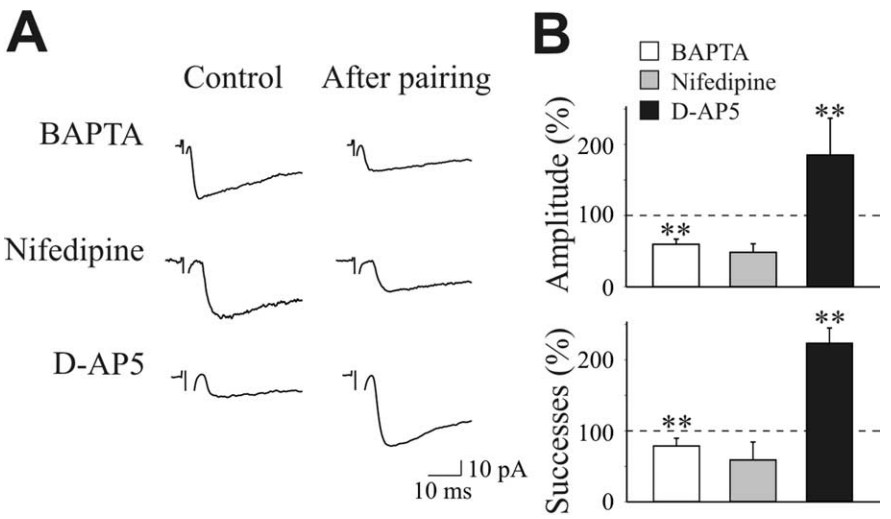

Figure 6. Pairing-induced potentiation requires a postsynaptic rise of intracellular calcium via voltage-dependent calcium channels. ***A***, Averaged traces of GPSC evoked before and 15 min after pairing in neurons loaded with intracellular BAPTA (20 mM) or exposed to extracellular solutions containing nifedipine (10 μM) or D-AP5 (50 μM). ***B***, Pairing-induced changes in the mean amplitude and mean number of successes expressed as percentage of controls from all cells tested (BAPTA, $n = 6$), nifedipine ($n = 5$) and D-AP5 ($n = 5$). $^{**}p < 0.01$.

least 10–15 min after BDNF was washed out (Fig. 7*B*,*C*). This effect was associated with a significant increase in the number of successes (from 0.24 ± 0.05 to 0.59 ± 0.07; $p < 0.01$) (Fig. 7*D*) and a significant decrease in PPR (from 2.63 ± 0.4 to 0.52 ± 0.22; $p < 0.01$) (Fig. 7*E*). The effects of BDNF were prevented by bath application of K252a (150 nM), a specific inhibitor of protein tyrosine kinase coupled to TrkB receptors ($n = 5$) (supplemental Fig. S3, available at www.jneurosci.org as supplemental material) (Knüsel and Hefti, 1992), indicating that this neurotrophin was acting on TrkB receptors. The involvement of endogenous BDNF in pairing-induced synaptic potentiation was assessed using K252a. Bath application of K252a (150 nM) fully prevented STD-LTP. Surprisingly, in the presence of K252a, a persistent depression of synaptic currents was observed (mean GPSCs amplitude was 45.2 ± 6.7 pA and 32.1 ± 4.4 pA, before and after pairing, respectively; $n = 7$; $p < 0.05$) (Fig. 7*F–H*). This effect was associated with a reduced number of successes and an increase in PPR (0.75 ± 0.18 and 0.99 ± 0.21, before and after pairing, respectively, $p < 0.01$) (Fig. 7*I*,*J*) suggesting a presynaptic site of action. The mechanisms underlying K252a-induced synaptic depression are still unclear and further experiments need to elucidate this issue. In the absence of pairing, K252a alone did not modify the amplitude or kinetics of GPSCs (amplitude: 38.2 ± 4.6 pA and 37.9 ± 5.8 pA, in control and in the presence of K252a, respectively; $n = 5$).

Altogether, these data show that at immature MF–CA3 synapse endogenous BDNF is crucial for STD-LTP induction

Pairing-induced synaptic potentiation requires the activation of cAMP-dependent PKA

In the neonatal rodent hippocampus, LTP requires the activation of cAMP-dependent protein kinase A (PKA) (Yasuda et al., 2003). Therefore, in the following experiments we tested whether at MF–CA3 synapses STD-LTP requires the activation of cAMP-dependent PKA. First, we tested the effects of forskolin, an activator of adenylyl cyclase. Bath application of forskolin (50 μM for 5 min) mimicked the effects of BDNF and induced a significant increase in amplitude of GPSCs (from 45.4 ± 11.1 pA in control to 72.6 ± 12.9 pA, 10 min after forskolin; $p < 0.01$; $n = 6$) (Fig. 8*A*,*B*) which persisted for at least 15 min after forskolin was washed out. This effect was associated with a significant increase in the number of successes (from 0.37 ± 0.08 to 0.63 ± 0.10; $p < 0.01$) (Fig. 8*C*), in the CV^{-2} (from 0.69 ± 0.18 to 2.79 ± 1.04; $p < 0.05$) (Fig. 8*E*) and a decrease in PPR (from 1.12 ± 0.29 to 0.38 ± 0.13; $p < 0.05$) (Fig. 8*D*).

In subsequent experiments, the involvement of endogenous cAMP-dependent PKA in STD-LTP was assessed using the selective cell permeant inhibitor Rp-cAMPS. As illustrated in Figure 9, in the presence of Rp-cAMPS (20 μM), positive pairing failed to potentiate MF-mediated synaptic currents suggesting that changes in synaptic efficacy requires the activation of both BDNF and cAMP-dependent PKA (peak amplitudes were 20.1 ± 5.4 pA and 17.1 ± 4.3 pA, successes rate 0.34 ± 0.1 and 0.31 ± 0.1, the PPR 1.09 ± 0.2 and 1.03 ± 0.2 and CV^{-2} 0.51 ± 0.08 and 0.6 ± 0.06, before and after pairing, respectively).

Since cAMP has been shown to regulate the recruitment of TrkB receptors to the plasma membrane (Meyer-Franke et al., 1998) and to gate the effects of BDNF on hippocampal neurons (Ji et al., 2005), we tested whether inhibitors of cAMP or PKA were able to prevent the potentiating effects of BDNF on GPSCs. In two separate set of experiments, bath application of Rp-cAMPS (20 μM; $n = 8$) or PKI 14–22 (1 μM; $n = 6$), a cell-permeable inhibitor of PKA, fully prevented the effects of BDNF on GPSCs (supplemental Fig. S4, available at www.jneurosci.org as supplemental material). Finally, to identify the presynaptic or postsynaptic site of PKA action, positive pairing was performed in cells loaded with the membrane impermeable form of PKI (PKI 6–22, 1 μM; $n = 7$). In these conditions, correlated presynaptic and postsynaptic activity failed to produce any persistent change in synaptic strength (Fig. 10*A*,*B*). In the presence of PKI 6–22, peak amplitudes of GPSCs were 26.0 ± 3.6 pA and 24.9 ± 7.3 pA, successes rate 0.34 ± 0.06 and 0.33 ± 0.07, PPR 1.47 ± 0.12 and 1.33 ± 0.28 and CV^{-2} 0.76 ± 0.11 and 0.75 ± 0.17, before and after pairing, respectively.

This indicates that PKA activity in the postsynaptic neuron plays a crucial role in STDP.

Discussion

It is known that, in particular conditions, MF can release, in addition to glutamate, GABA (Walker et al., 2001; Gutiérrez et al., 2003, 2005), and a recent study from our laboratory has demonstrated that, early in development, the main neurotransmitter released from MF is GABA (Safiulina et al., 2006). During postnatal development, granule cells transiently express both mRNA coding for GAD 67 and GAD 67 protein (Dupuy and Houser, 1997; Frahm and Draguhn, 2001). This GABAergic phenotype has been attributed either to principal cells synthesizing GABA, which would be downregulated in adulthood (Frahm and Draguhn, 2001) or to GAD-positive interneurons which would transiently migrate to the upper and middle portions of the granule cell layer (Dupuy and Houser, 1997; Uchigashima et al., 2007). Whatever their origin, MF-mediated GPSCs exhibited all characteristics of MF responses such as strong paired pulse facilitation,

short term frequency-dependent facilitation and sensitivity to the group III mGluR agonist L-AP4 (Salin et al., 1996; Safiulina et al., 2006). In addition, hyperpolarizing GPSPs evoked by dentate gyrus stimulation in the presence of bumetanide were also sensitive to L-AP4 further indicating their MF origin (monosynaptic responses obtained in principal cells by stimulating GABAergic interneurons were unaffected by L-AP4) (Walker et al., 2001; Doherty et al., 2004; Kasyanov et al., 2004; Gutiérrez, 2005; Safiulina et al., 2006).

GABA released from MF exerted a depolarizing action on CA3 pyramidal neurons as assessed by measuring the driving force for GABA (E_{GPSP}-V_R)which was positive to V_R. GABA not only exerted a depolarizing action but after positive pairing triggered action potential firing. This effect was not due to changes in intrinsic membrane properties of tagged cells or modifications in E_{GPSP}, since similar firing properties and E_{GPSP} values were observed before and after pairing. Unlike the present experiments, in cultured hippocampal neurons bearing depolarizing responses to GABA, repetitive postsynaptic spiking within ± 5 ms of $GABA_A$-mediated synaptic transmission was able to downregulate the expression of the cation-chloride cotransporter NKCC1 leading to a reduction in $[Cl^-]_i$ and a shift of E_{Cl} below V_R (Balena and Woodin, 2008). It should be stressed, however, that while in our experiments the STDP protocol consisted in 10 pairs of single postsynaptic spikes with unitary GPSPs, cultured neurons were stimulated with 150 pairs of presynaptic and postsynaptic spikes at 5 Hz. In addition, in our case the temporal correlation between weak inputs (single MF-mediated synaptic events) and strong inputs (back propagating action potential) dictated the direction of synaptic changes toward potentiation or depression. The temporal window responsible for LTP and LTD was very similar to that reported in previous studies for glutamatergic synapses (Magee and Johnston, 1997; Markram et al., 1997; Bi and Poo, 1998; Debanne et al. 1998; Zhang et al., 1998), suggesting the similar learning rules regulate STDP at GABAergic connections.

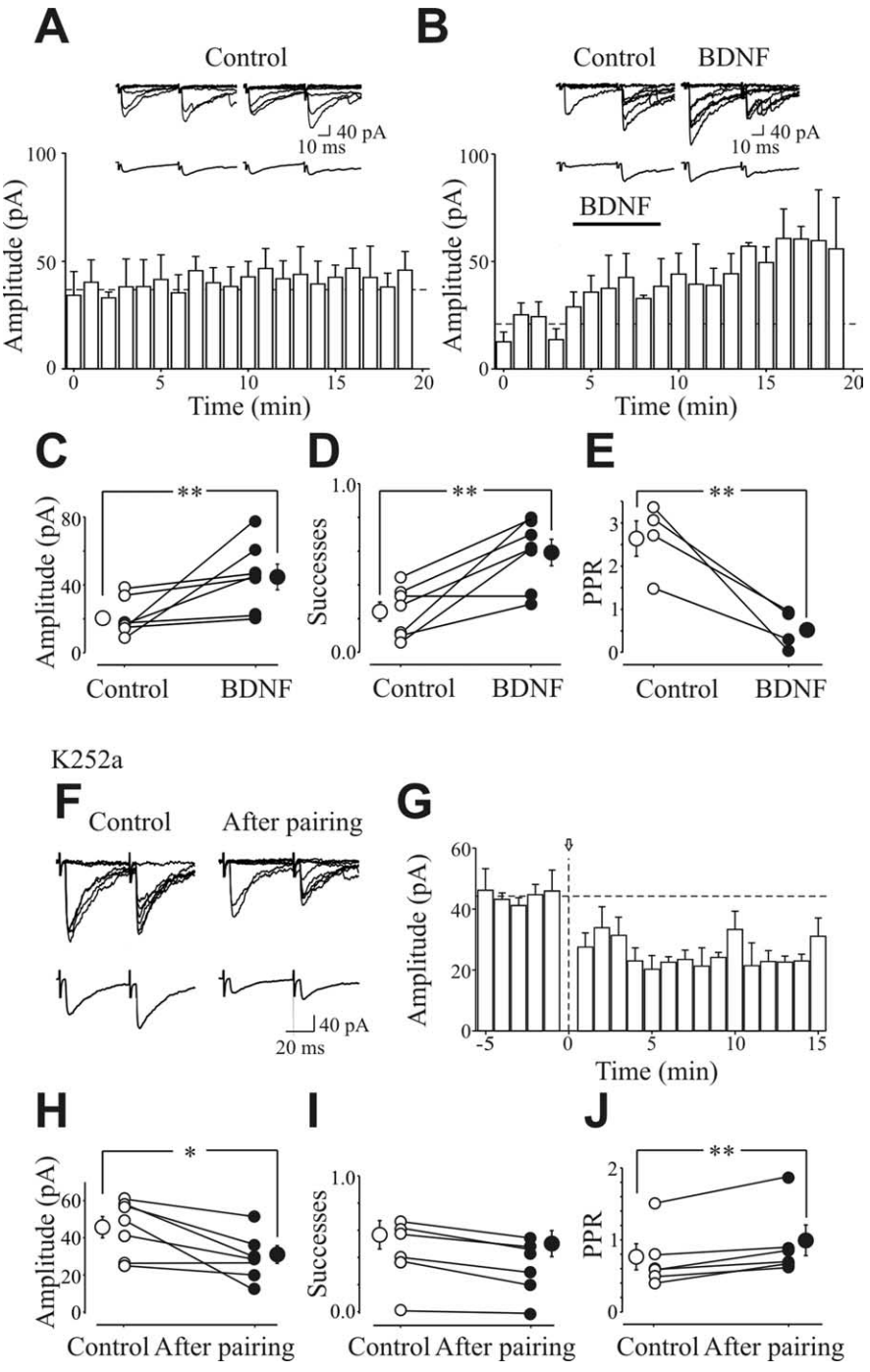

Figure 7. Pairing-induced synaptic potentiation requires the activation of TrkB receptors by BDNF. ***A***, Summary plot showing the mean amplitude of GPSCs versus time in the absence of BDNF (Control; $n = 5$). Insets above the graph refer to GPSCs (individual traces above and averaged traces below) recorded during the first (left) and the last (right) 5 min. ***B***, Summary plot showing the mean amplitude of GPSC ($n = 7$) evoked before, during and after bath application of BDNF (bar). The horizontal dashed line represents the mean amplitude of GPSCs recorded in control before BDNF application. Insets above the graph represent superimposed individual (top) and averaged (bottom) traces of GPSCs evoked before (Control) and 15 min after the application of BDNF (40 ng/ml). ***C–E***, Amplitude (***C***), successes (***D***) and paired-pulse ratio (***E***) measured in individual cells before (○) and 15 min after BDNF (●). Larger symbols represent averaged values. ***F–J***, As in Figure 2 but in the presence of K252a ($n = 7$). $^*p < 0.05$; $^{**}p < 0.01$.

As in other forms of developmental plasticity (Gaiarsa et al., 2002; Kasyanov et al., 2004; Mohajerani et al., 2007), calcium entering into the cell through VDCCs was responsible for positive changes in synaptic efficacy as demonstrated by the lack of STD-LTP when BAPTA was present in the recording pipette or when nifedipine was added to the extracellular solution. In accord to the calcium hypothesis, the direction of synaptic changes would be determined by distinct calcium signals which would activate different molecular pathways (Caporale and Dan, 2008). However, in the present case, positive pairing in the presence of BAPTA not only prevented LTP but induced LTD, suggesting that synaptic depression is tightly regulated by the amount of calcium entering into the postsynaptic

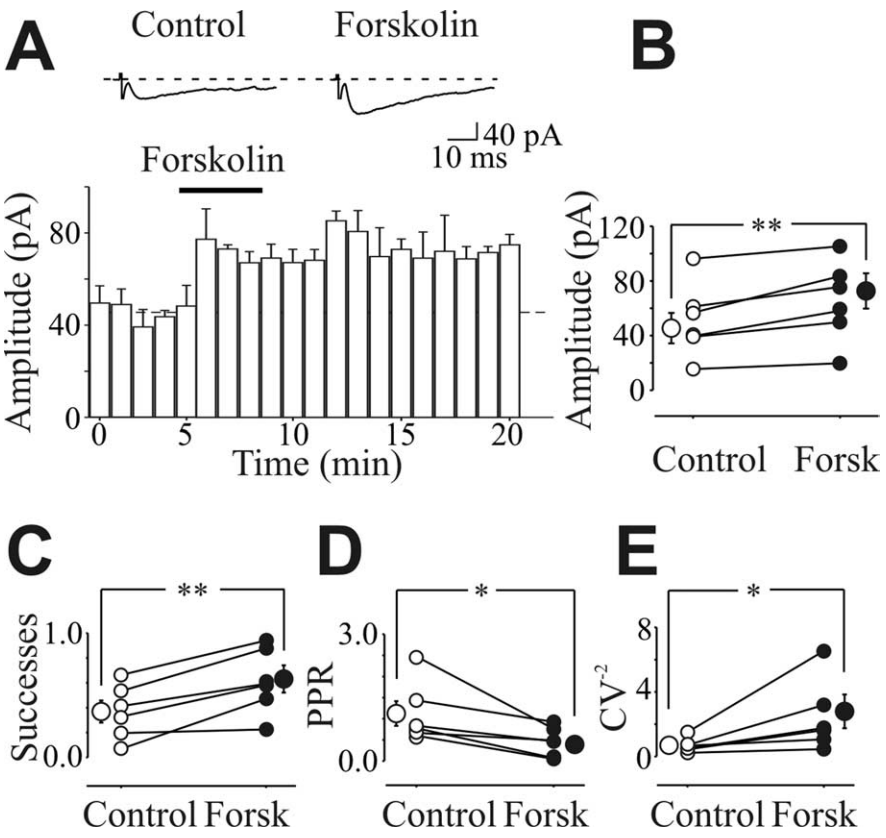

Figure 8. Forskolin mimics the effects of BDNF. ***A***, Summary plot showing the mean amplitude of GPSC ($n = 6$) evoked before, during and after bath application of forskolin (50 μM, bar). Insets above the graph show averaged traces (15 GPSCs from a representative neuron) evoked before and during bath application of forskolin. ***B–E***, Amplitude (***B***), successes (***C***), paired-pulse ratio (***D***) and inverse squared of CV (***E***) measured before (○) and during bath application of forskolin (●). Larger symbols represent averaged values. *$p < 0.05$; **$p < 0.01$.

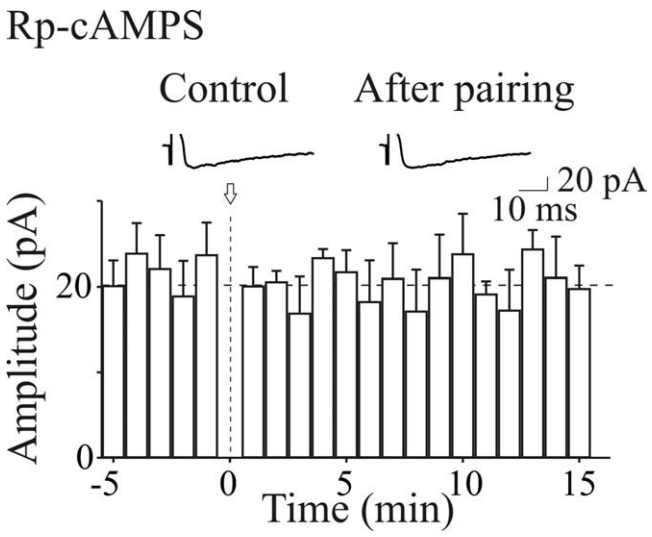

Figure 9. Pairing-induced synaptic potentiation requires the activation of cAMP-dependent PKA. Summary plot showing the mean amplitude of GPSC ($n = 5$; average of 12 traces) evoked in the presence of Rp-cAMPS before and after pairing (arrows at time 0) as a function of time. Insets above the graph represent individual traces of GPSCs evoked before and after pairing.

cell. While the induction of STD-LTP was clearly postsynaptic, its expression was presynaptic, as indicated by the pairing-induced decrease in failures rate, in paired-pulse facilitation, and increase in CV^{-2}, all traditional indices of changes in presynaptic release probability (Zucker, 1989). Changes in failure rate and CV^{-2} could result from the insertion of new receptors on the subsynaptic membrane in silent synapses (Kullmann, 2003). Moreover, pairing-induced appearance of GPSCs in apparently "presynaptically silent" neurons is consistent with an increase in release probability (Gasparini et al., 2000). Similar to our findings, even in mature GABAergic synapses, activity-dependent modifications required a postsynaptic calcium rise through VDCCs (Woodin et al., 2003; Haas et al., 2006). However, in these cases, a presynaptic expression mechanism was excluded because no changes in paired pulse ratio were detected.

The postsynaptic induction of STD-LTP and its presynaptic expression implies the existence of cross talk between the post and presynaptic neuron and the involvement of a transcellular retrograde signal. One attractive candidate is BDNF, which plays a crucial role in synaptic plasticity (Poo, 2001), in $GABA_A$-mediated LTP (Gubellini et al., 2005), and is known to be a potent regulator of spontaneous correlated network activity at early developmental stages (Aguado et al., 2003; Carmona et al., 2006). Although several lines of evidence indicate that BDNF can be released in a calcium-dependent way by depolarization of the postsynaptic cell (Goodman et al., 1996; Lessmann et al., 2003; Magby et al., 2006; Kuczewski et al., 2008a,b), the present data do not allow identifying the presynaptic or postsynaptic site of BDNF release. However, the recent observation of a calcium-dependent dendritic release of BDNF following spontaneous or evoked back propagating action potentials in hippocampal neurons in culture, strongly support a postsynaptic locus (Kuczewski et al., 2008b). Interestingly, as in the present experiments, only a few spikes (eight at 4 Hz) were sufficient to trigger BDNF secretion (Kuczewski et al., 2008b). Once released, BDNF would interact with high affinity TrkB receptors localized on both presynaptic and postsynaptic membranes. Activation of presynaptic TrkB receptors would lead to an increase in transmitter release (Li et al., 1998) (for review, see Poo, 2001; Du and Poo, 2004). In the present experiments, the involvement of BDNF in STD-LTP was demonstrated by the capability of this neurotrophin to mimic the effects of pairing on amplitude, successes rate and PPR of GPSCs and by the observation that SDT-LTP was prevented by K252a, a tyrosine kinase inhibitor. It is worth noting that in the presence of K252a the pairing procedure led to synaptic depression. Although this effect was clearly presynaptic, as indicated by the increase in PPR, the underlying mechanism is unclear. A tonic activation of TrkB receptor by endogenous BDNF can be excluded since K252a alone did not affect synaptic transmission.

At excitatory synapses, the long-term effects of BDNF on activity-dependent synaptic plasticity, has been found to be regulated by cAMP-dependent PKA. This molecule would exert a permissive role by enhancing the sensitivity to BDNF (Boulanger and Poo, 1999), by increasing TrkB phosphorylation (Ji et al.,

2005) and by recruiting TrkB receptors to dendritic spines (Meyer-Franke et al., 1998) (for review, see Nagappan and Lu, 2005). Consistent with these studies, STD-LTP of MF-GPSCs was blocked by Rp-cAMPS and PKI 14–22, indicating that endogenous BDNF required the activity of cAMP-dependent PKA. In the present experiments, however, forskolin alone was able to mimic the effects of BDNF, and STD-LTP was prevented when the postsynaptic cell was loaded with the membrane impermeable PKA inhibitor PKI 6–22. This suggests that cAMP-dependent PKA may regulate intracellular calcium rise (Obrietan and van den Pol, 1997; Dunn et al., 2006), which in turn would affect BDNF release from the postsynaptic cell. The precise molecular mechanisms regulating this cascade remain to be elucidated.

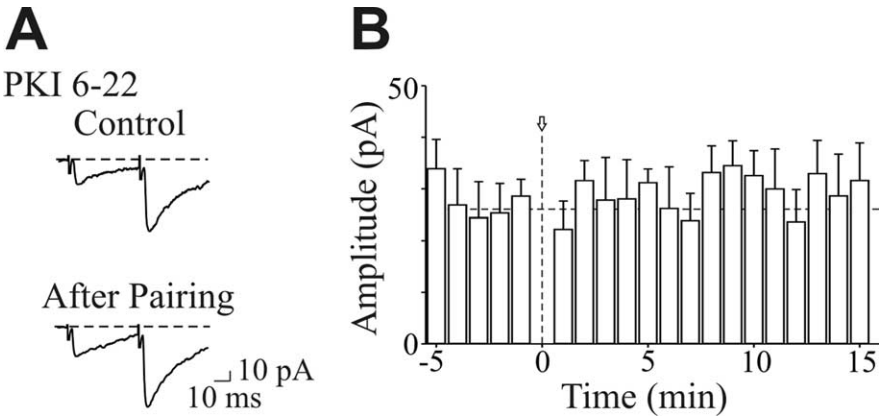

Figure 10. Pairing-induced potentiation of GPSCs was prevented by inhibiting PKA in the postsynaptic neuron. ***A***, Individual traces (each is the average of 10 responses) obtained from a CA3 pyramidal neuron recorded with a pipette containing PKI 6–22 (1 μM) before (Control) and 15 min after pairing. ***B***, Summary plot showing the mean amplitude of GPSCs (recorded with a patch pipette containing PKI 6–22) evoked before and after pairing (arrows at time 0) as a function of time. In the presence of PKI 6–22 into the patch pipette, pairing was unable to modify MF-mediated synaptic responses.

In conclusion, our results show that, during postnatal development, pairing back propagating action potentials with MF-GPSPs persistently enhances synaptic efficacy and brings CA3 principal cells to fire, thus providing a reliable way to convey information from granule cells to the CA3 associative network at a time when glutamatergic synapses are still poorly developed.

References

Aguado F, Carmona MA, Pozas E, Aguiló A, Martínez-Guijarro FJ, Alcantara S, Borrell V, Yuste R, Ibañez CF, Soriano E (2003) BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- cotransporter KCC2. Development 130:1267–1280.

Allen C, Stevens CF (1994) An evaluation of causes for unreliability of synaptic transmission. Proc Natl Acad Sci U S A 91:10380–10383.

Balena T, Woodin MA (2008) Coincident pre- and postsynaptic activity downregulates NKCC1 to hyperpolarize E(Cl) during development. Eur J Neurosci 27:2402–2412.

Banke TG, McBain CJ (2006) GABAergic input onto CA3 hippocampal interneurons remains shunting throughout development. J Neurosci 26:11720–11725.

Ben-Ari Y (2002) Excitatory action of GABA during development: the nature of the nurture. Nat Rev Neurosci 3:728–739.

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215–1284.

Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J Neurosci 18:10464–10472.

Boulanger L, Poo MM (1999) Gating of BDNF-induced synaptic potentiation by cAMP. Science 284:1982–1984.

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a hebbian learning rule. Annu Rev Neurosci 31:25–46.

Carmona MA, Pozas E, Martínez A, Espinosa-Parrilla JF, Soriano E, Aguado F (2006) Age-dependent spontaneous hyperexcitability and impairment of GABAergic function in the hippocampus of mice lacking trkB. Cereb Cortex 16:47–63.

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515–519.

Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23–30.

Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. Physiol Rev 86:1033–1048.

Debanne D, Gähwiler BH, Thompson SM (1998) Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. J Physiol 507:237–247.

Doherty JJ, Alagarsamy S, Bough KJ, Conn PJ, Dingledine R, Mott DD (2004) Metabotropic glutamate receptors modulate feedback inhibition in a developmentally regulated manner in rat dentate gyrus. J Physiol 561:395–401.

Du JL, Poo MM (2004) Rapid BDNF-induced retrograde synaptic modification in a developing retinotectal system. Nature 429:878–883.

Dunn TA, Wang CT, Colicos MA, Zaccolo M, DiPilato LM, Zhang J, Tsien RY, Feller MB (2006) Imaging of cAMP levels and protein kinase A activity reveals that retinal waves drive oscillations in second-messenger cascades. J Neurosci 26:12807–12815.

Dupuy ST, Houser CR (1997) Developmental changes in GABA neurons of the rat dentate gyrus: an in situ hybridization and birthdating study. J Comp Neurol 389:402–418.

Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ (2005) NKCC1 transporter facilitates seizures in the developing brain. Nat Med 11:1205–1213.

Feldman DE (2000) Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron 27:45–56.

Fiumelli H, Woodin MA (2007) Role of activity-dependent regulation of neuronal chloride homeostasis in development. Curr Opin Neurobiol 17:81–86.

Fiumelli H, Cancedda L, Poo MM (2005) Modulation of GABAergic transmission by activity via postsynaptic Ca2+-dependent regulation of KCC2 function. Neuron 48:773–786.

Frahm C, Draguhn A (2001) GAD and GABA transporter (GAT-1) mRNA expression in the developing rat hippocampus. Brain Res Dev Brain Res 132:1–13.

Gaiarsa JL, Caillard O, Ben-Ari Y (2002) Long-term plasticity at GABAergic and glycinergic synapses: mechanisms and functional significance. Trends Neurosci 25:564–570.

Garaschuk O, Hanse E, Konnerth A (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. J Physiol 507:219–236.

Gasparini S, Saviane C, Voronin LL, Cherubini E (2000) Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc Natl Acad Sci U S A 97:9741–9746.

Goodman LJ, Valverde J, Lim F, Geschwind MD, Federoff HJ, Geller AI, Hefti F (1996) Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol Cell Neurosci 7:222–238.

Gubellini P, Ben-Ari Y, Gaïarsa JL (2005) Endogenous neurotrophins are required for the induction of GABAergic long-term potentiation in the neonatal rat hippocampus. J Neurosci 25:5796–5802.

Gutiérrez R (2005) The dual glutamatergic-GABAergic phenotype of hippocampal granule cells. Trends Neurosci 28:297–303.

Gutiérrez R, Romo-Parra H, Maqueda J, Vivar C, Ramìrez M, Morales MA, Lamas M (2003) Plasticity of the GABAergic phenotype of the "glutamatergic" granule cells of the rat dentate gyrus. J Neurosci 23:5594–5598.

Haas JS, Nowotny T, Abarbanel HD (2006) Spike-timing-dependent plas-

ticity of inhibitory synapses in the entorhinal cortex. J Neurophysiol 96:3305–3313.
Ji Y, Pang PT, Feng L, Lu B (2005) Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. Nat Neurosci 8:164–172.
Jonas P, Major G, Sakmann B (1993) Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. J Physiol 472:615–663.
Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci U S A 101:3967–3972.
Knüsel B, Hefti F (1992) K-252 compounds: modulators of neurotrophin signal transduction. J Neurochem 59:1987–1996.
Kuczewski N, Langlois A, Fiorentino H, Bonnet S, Marissal T, Diabira D, Ferrand N, Porcher C, Gaiarsa JL (2008a) Spontaneous glutamatergic activity induces a BDNF-dependent potentiation of GABAergic synapses in the newborn rat hippocampus. J Physiol 586:5119–5128.
Kuczewski N, Porcher C, Ferrand N, Fiorentino H, Pellegrino C, Kolarow R, Lessmann V, Medina I, Gaiarsa JL (2008b) Backpropagating action potentials trigger dendritic release of BDNF during spontaneous network activity. J Neurosci 28:7013–7023.
Kullmann DM (1993) Quantal variability of excitatory transmission in the hippocampus: implications for the opening probability of fast glutamate-gated channels. Proc Biol Sci 253:107–116.
Kyrozis A, Reichling DB (1995) Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. J Neurosci Methods 57:27–35.
Lamsa K, Palva JM, Ruusuvuori E, Kaila K, Taira T (2000) Synaptic GABA(A) activation inhibits AMPA-kainate receptor-mediated bursting in the newborn (P0–P2) rat hippocampus. J Neurophysiol 83:359–366.
Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R (1997) Ca2+ oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA receptors in the neonatal hippocampus. Neuron 18:243–255.
Lessmann V, Gottmann K, Malcangio M (2003) Neurotrophin secretion: current facts and future prospects. Prog Neurobiol 69:341–374.
Li YX, Zhang Y, Lester HA, Schuman EM, Davidson N (1998) Enhancement of neurotransmitter release induced by brain-derived neurotrophic factor in cultured hippocampal neurons. J Neurosci 18:10231–10240.
Magby JP, Bi C, Chen ZY, Lee FS, Plummer MR (2006) Single-cell characterization of retrograde signaling by brain-derived neurotrophic factor. J Neurosci 26:13531–13536.
Magee JC, Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. Science 275:209–213.
Malenka RC, Nicoll RA (1999) Long-term potentiation–a decade of progress? Science 285:1870–1874.
Markram H, Lübke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science 275:213–215.
Meyer-Franke A, Wilkinson GA, Kruttgen A, Hu M, Munro E, Hanson MG Jr, Reichardt LF, Barres BA (1998) Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. Neuron 21:681–693.
Mohajerani MH, Cherubini E (2005) Spontaneous recurrent network activity in organotypic rat hippocampal slices. Eur J Neurosci 22:107–118.
Mohajerani MH, Sivakumaran S, Zacchi P, Aguilera P, Cherubini E (2007) Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3 CA1 connections in the hippocampus. Proc Natl Acad Sci U S A 104:13176–13181.
Nagappan G, Lu B (2005) Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. Trends Neurosci 28:464–471.
Obrietan K, van den Pol AN (1997) GABA activity mediating cytosolic Ca2+ rises in developing neurons is modulated by cAMP-dependent signal transduction. J Neurosci 17:4785–4799.
Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24–32.
Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597–608.
Salin PA, Scanziani M, Malenka RC, Nicoll RA (1996) Distinct short-term plasticity at two excitatory synapses in the hippocampus. Proc Natl Acad Sci U S A 93:13304–13309.
Sipilä ST, Schuchmann S, Voipio J, Yamada J, Kaila K (2006) The cation-chloride cotransporter NKCC1 promotes sharp waves in the neonatal rat hippocampus. J Physiol 573:765–773.
Staley KJ, Mody I (1992) Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABAA receptor-mediated postsynaptic conductance. J Neurophysiol 68:197–212.
Tyzio R, Holmes GL, Ben-Ari Y, Khazipov R (2007) Timing of the developmental switch in GABA(A) mediated signaling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings. Epilepsia 48:96–105.
Uchigashima M, Fukaya M, Watanabe M, Kamiya H (2007) Evidence against GABA release from glutamatergic mossy fiber terminals in the developing hippocampus. J Neurosci 27:8088–8100.
Walker MC, Ruiz A, Kullmann DM (2001) Monosynaptic GABAergic signaling from dentate to CA3 with a pharmacological and physiological profile typical of mossy fiber synapses. Neuron 29:703–715.
Woodin MA, Ganguly K, Poo MM (2003) Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl- transporter activity. Neuron 39:807–820.
Yasuda H, Barth AL, Stellwagen D, Malenka RC (2003) A developmental switch in the signaling cascades for LTP induction. Nat Neurosci 6:15–16.
Zhang LI, Tao HW, Holt CE, Harris WA, Poo MM (1998) A critical window for cooperation and competition among developing retinotectal synapses. Nature 395:37–44.
Zucker RS (1989) Short-term synaptic plasticity. Annu Rev Neurosci 12:13–31.

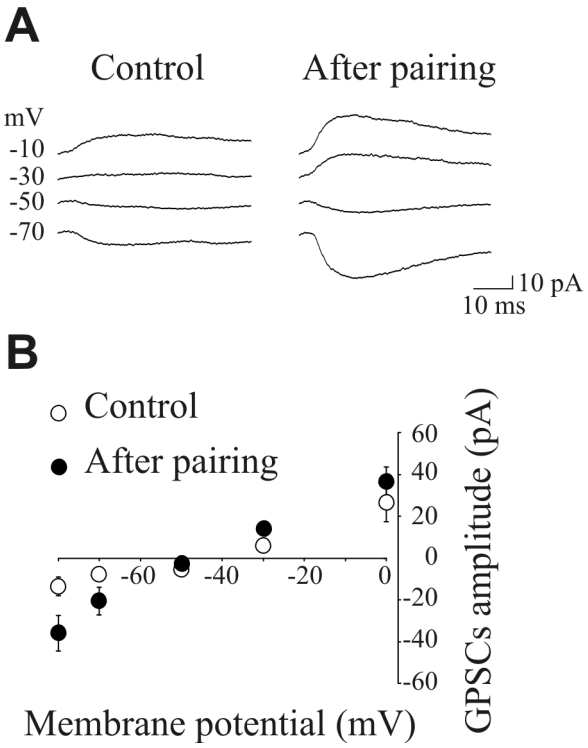

Figure S1. STD-LTP does not modify EGPSP

A, Synaptic responses (successes plus failures) evoked at four different holding potentials (on the left of the traces) by stimulation of granule cells in the dentate gyrus before (Control), and 10 min after positive pairing. B, Plot of GPSPs amplitudes as a function of membrane potentials before (○) and after pairing (●). EGPSP was -48.3233 ± 2.51 mV and -43.8688 ± 4.103, (n=7), before and after pairing respectively. Note the increase in amplitude of GPSPs after pairing. Recordings were performed with perforated patch.

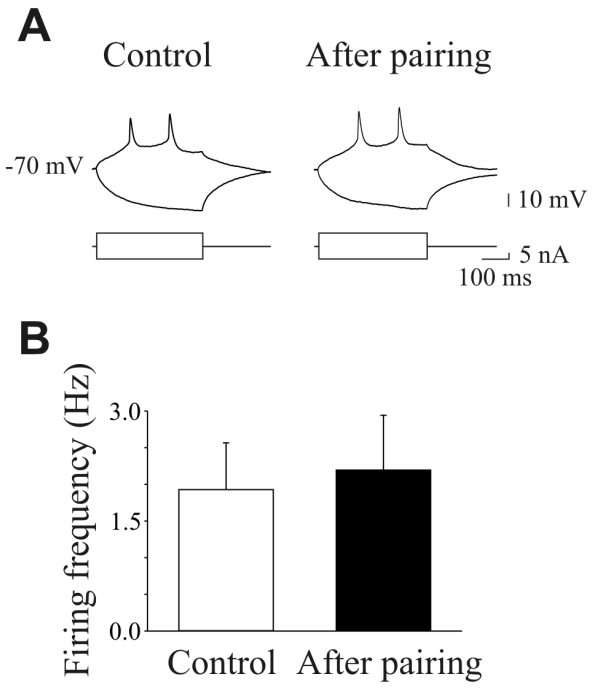

Figure S2. STD-LTP does not change the firing frequency of principal cells.

A, Firing patterns evoked in one CA3 pyramidal cell (at P3) by depolarizing current pulses (below the traces) before (Control) and 10 min after positive pairing. Note that pairing did not modify cell excitability or input resistance of the recorded cell. B, Each column represents the mean firing frequency of principal cells before (white) and 10 min after pairing (n=6; black). Bars represent the SEM. Recordings were performed with perforated patch.

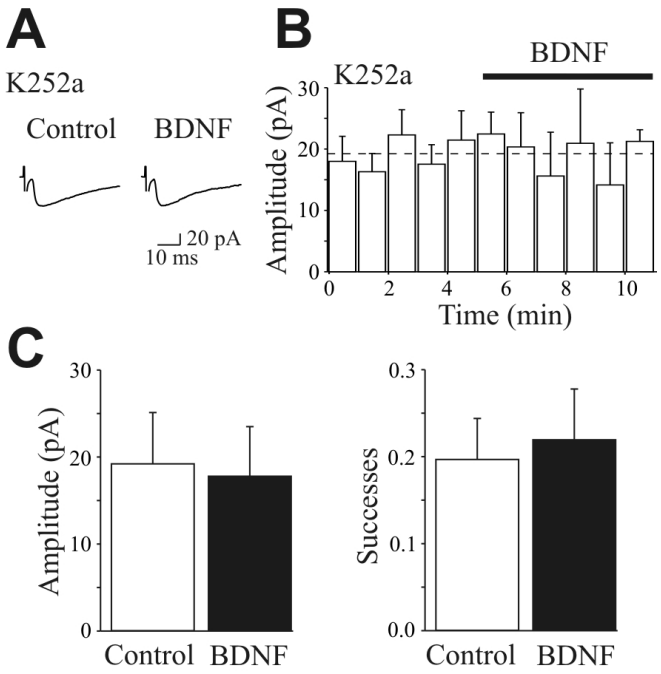

Figure S3. K252a prevents the effect of BDNF on synaptic efficacy

A, Averaged traces (from a representative neuron) evoked in the presence of K252a before and during bath application of BDNF. B, Summary plot showing the mean amplitude of GPSC (n=4) evoked in the presence of K252a before and during bath application of BDNF (bar). C, Each column represents the mean amplitude (n=4) and mean number of successes (n=4) of GPSCs evoked in the presence of K252a before and during bath application of BDNF.

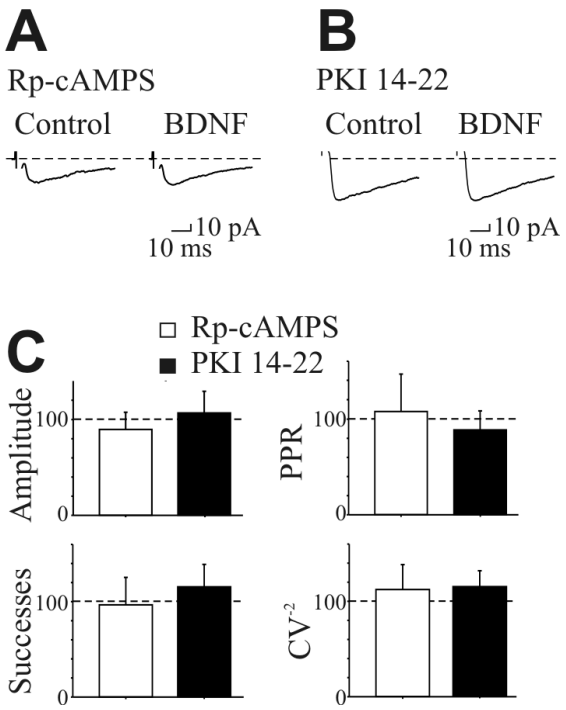

Figure S4. The potentiating effect BDNF on synaptic efficacy is prevented by bath application of Rp-cAMPS and PKI 14-22

A-B, Averaged traces (from two representative neurons; each trace is the average of 10 responses) evoked in the presence of Rp-cAMPS (20 µM; A) and PKI 14-22 (1 µM; B) before (Control) and during application of BDNF (40 ng/ml). Note the lack of BDNFinduced potentiation in both cases. C, Each column represents the mean GPSCs amplitude, mean number of successes, mean PPR and inverse squared of CV, obtained during bath application of BDNF and expressed as percentage of Controls (dashed lines) in slices superfused with of Rp-cAMPS (white columns, n=8) or PKI 14-22 (black columns, n=6). Note the lack of BDNF-induced potentiation in the presence of RpcAMPS and PKI 14-22.

Paper **N. 3.**

In the developing rat hippocampus, endogenous activation of presynaptic kainate receptors reduces GABA release from mossy fiber terminals

Delma Caiati*, **Sudhir Sivakumaran*** and Enrico Cherubini.

Submitted. (* Equal Contribution)

ABSTRACT

Presynaptic kainate receptors regulate synaptic transmission in several brain areas but are not known to have this action at immature mossy fibers (MF) terminals which release mainly GABA. Here, we report that during the first week of postnatal life, endogenous activation of GluK1 receptors by ambient glutamate severely depresses MF-mediated GABAergic currents (GPSCs). This effect was mediated by a metabotropic process sensitive to Pertussis toxin. In the presence of U73122, a selective inhibitor of phospholipase C, along the transduction pathway downstream to G protein, GluK1 activation increased the probability of GABA release thus unveiling the ionotropic action of this receptor. In addition, we found that GluK1 enhanced MF excitability by directly depolarizing MF terminals via calcium-permeable cation channels. Furthermore, GluK1 dynamically regulated the direction of STDP occurring by pairing MF stimulation with postsynaptic spiking and shifted spike time-dependent potentiation into depression. The GluK1-induced depression of MF-GPSCs would prevent excessive activation of the CA3 associative network by the excitatory action of GABA and the emergence of seizures in the immature brain.

INTRODUCTION

Kainate receptors (KARs) are highly expressed in the CNS where they are involved in different physiological functions (for a review see Jane et al. 2009). Five different receptor subtypes have been so far cloned, named GluK1-5, which co-assemble in various combinations to form functional receptors with distinct pharmacological and physiological properties (Collingridge et al. 2009). Their role depends on the precise subcellular localization at presynaptic, postsynaptic and extrasynaptic sites. While at some excitatory connections KARs have been shown to carry at least in part, current charges of synaptic responses (Castillo et al 1997; Vignes and Collingridge, 1997; Mulle et al. 1998; Cossart et al. 2002), at presynaptic sites they exert a powerful control of transmitter release at both excitatory and inhibitory synapses (Lerma, 2003). In addition, evidence has been provided that, in several CNS regions, KARs control cell excitability through a metabotropic type of action, which may involve the activation of G protein and intracellular signal cascades (Rodriguez-Moreno and Lerma, 1998; Rozas et al. 2003; Melyan et al. 2004; Lerma, 2006; Rodriguez-Moreno and Sihra, 2007). The hippocampus is endowed with KARs with preponderant presynaptic localization (Represa et al. 1987; Ben Ari and Cossart, 2000). In particular, activation of KA autoreceptors localized on glutamatergic mossy fibres (MFs), the axons of dentate gyrus granule cells, has been shown to enhance glutamate release particularly during frequency-dependent facilitation, a form of short-term plasticity characteristic of MF-CA3 synapses (Contractor et al. 2001; Lauri et al. 2001b; Schmitz et al. 2001; but see Kwon and Castillo, 2008). This effect has been attributed to kainate-induced depolarization of presynaptic boutons or axon terminals (Lauri et al. 2001a; Kamiya et al. 2002; Nicoll and Schmitz, 2005) and the release of calcium from local stores (Scott et al. 2008). Interestingly, KARs are present also on presynaptic GABAergic terminals where they control GABA release. Activation of these receptors relies on the source of glutamate, on the spatio-temporal feature of glutamate release in the extracellular space, on the localization of the receptors and their affinity for glutamate. According to the targeted cells (interneurons or pyramidal cells), stimulation of KARs localized on GABAergic interneurons has been shown to exert either a facilitatory (Mulle et al. 2000; Cossart et al. 2001) or a depressant (Rodriguez-Moreno et al. 1997; Clarke et al. 1997; Rodriguez-Moreno and Lerma, 1998; Maingret et al. 2005) action on GABA release.

Several lines of evidence suggest a role of KARs in neuronal development. In the hippocampus, the expression of mRNA encoding for different KAR subunits including GluK1 is high early in development and declines later to lower adult levels (Bettler et al 1990; Bahn et al. 1994). Signalling *via* presynaptic GluK1 containing KARs has been shown to be critical for regulating the number of functional glutamatergic synapses (Vesikansa et al. 2007) and the balance between GABAergic and glutamatergic transmission which control correlated network activity (Lauri et al. 2005). Moreover, KARs localized on MF terminals have been shown to regulate filopodia motility known to facilitate the establishment of initial synaptic contacts and the stabilization of newly formed ones (Tashiro et al. 2003). Interestingly, during the first week of postnatal life, the main neurotransmitter released from MF terminals is GABA (Safiulina et al. 2006) and glutamatergic MF-evoked excitatory postsynaptioc currents comprising kainate components start appearing after P6 (Marchal and Mulle, 2004).

Here, we addressed whether, immediately after birth, MF-induced $GABA_A$-mediated postsynaptic currents (GPSCs) in CA3 principal cells are controlled by presynaptic KARs. We report that, at this developmental stage, MF-GPSCs are down regulated by G protein-coupled GluK1 receptors, which are endogenously activated by glutamate present in the extracellular medium. We further demonstrated that, at these synapses, GluK1 receptors dynamically regulate the direction of spike-time dependent plasticity.

Slice preparation

Experiments were performed on hippocampal slices from P2-P5 Wistar rats as previously described (Gasparini et al., 2000). All experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609EEC) and were approved by local authority veterinary service. Briefly, animals were decapitated after being anaesthetized with an i.p. injection of urethane (2g/kg). The brain was quickly removed from the skull and placed in ice-cold ACSF containing (in mM): NaCl 130, KCl 3.5, NaH_2PO_4 1.2, $NaHCO_3$ 27, $MgCl_2$ 1.3, $CaCl_2$ 2, glucose 25, saturated with 95% O_2 and 5% CO_2 (pH 7.3-7.4). Transverse hippocampal slices (400 µm thick) were cut with a vibratome and stored at room temperature (20-24 ^{o}C) in a holding bath containing the same solution as above. After a recovery period of at least one-hour, an individual slice was transferred to the recording chamber where it was continuously superfused with oxygenated ACSF at a rate of 2-3 ml/min at 33-35^{o}C.

Electrophysiological recordings

Electrophysiological experiments were performed from CA3 pyramidal cells using the whole-cell configuration of the patch-clamp technique in current or voltage-clamp mode. Neurons were visualized using an upright microscope (Olympus BX51WI) equipped with differential interference contrast (DIC) optics and infrared video camera. Patch electrodes were pulled from borosilicate glass capillaries (Hingelberg, Malsfeld, D). They had a resistance of 4-6 MΩ when filled with an intracellular solution containing (in mM): KCl 140, $MgCl_2$ 1, HEPES 10, MgATP 4, EGTA 0.5 (pH 7.3). In some cases Guanosine 5'-[□-thio]di-phosphate (GDP□S, from Sigma) at the concentration of 0.3 mM was included into the patch pipette to block postsynaptic G-protein coupled receptors. Recordings were made with a patch clamp amplifier (Axopatch 200A; Axon Instruments, Foster City, CA). The access resistance was repetitively monitored every five min with a voltage step and was < 20 MΩ and the results were discarded if it changed more than 15-20%.

Mossy fibers $GABA_A$-mediated postsynaptic currents (GPSCs) were evoked at 0.05 Hz from a holding potential of –70 mV. We used minimal stimulation of the granule cells in the dentate gyrus in order to activate only one or few presynaptic fibers. According to the technique described by Jonas et al., (1993) and Allen and Stevens (1994) the stimulation intensity was decreased until only a single axon was activated. This was achieved when the mean amplitude of the postsynaptic currents and failure probability remained constant over a range of stimulus intensities near threshold for detecting a response (see Safiulina et al., 2006; Fig. 1S). An abrupt increase in the mean peak amplitude of synaptic currents was observed when the stimulus intensity was further increased. This all or none behavior let us to assume that only a single fiber was stimulated. In addition, the latency and the shape of individual synaptic responses remained constant for repeated stimuli. The monosynaptic nature of synaptic currents was supported by the unimodal and narrow latencies and rise time distributions which remained constant when the extracellular Ca^{2+}/Mg^{2+} concentration ratio was reduced from 2:1.3 to 1:3 (Safiulina et al. 2006).

When the probability of synaptic failures in response to a first stimulus was near 1 (failures were estimated by visual discrimination), we applied a second pulse at 50 ms interval. If a response to a second stimulus appeared in 15-30 consecutive trials (silent to the first stimulus) we considered this synapse “presynaptically” silent.

MF inputs were identified on the basis of their sensitivity to group III mGluR agonist 2-amino-4-phosphonobutyric acid (L-AP4 10 µM; Gutierrez et al., 2003; Kasyanov et al., 2004; Safiulina et al., 2006), their strong paired pulse facilitation and short term frequency-dependent facilitation (Safiulina et al., 2006). GPSCs were blocked by bicuculline or picrotoxin. In contrast to MF inputs, GABAergic inputs from interneurons were insensitive to L-AP4 (Walker et al., 2001; Safiulina et al., 2006). To block G protein mediated signals, slices were incubated overnight in a medium containing pertussis toxin (PTx, 5 µg/ml).

In some experiments, antidromic action potentials were recorded from visually identified granule cells in dentate gyrus. In this case the intracellular solution contained K-gluconate (150 mM) instead of KCl. Extracellular stimuli (at 0.3 Hz, duration 150 s) were delivered *via* a stimulation electrode positioned in stratum lucidum ~ 200 µm away from granule cell layer.

Spike-time dependent plasticity (STDP) was induced in current clamp mode by pairing MF stimulation with postsynaptic spikes. MF-GPSCs were recorded first in voltage clamp mode

for 5-10 min to obtain a stable baseline. Then, STDP was induced by pairing presynaptic stimulation of granule cells in the dentate gyrus with postsynaptic spiking (10 spikes at 0.1 Hz, 15 ms delay pre *versus* post). Changes in synaptic efficacy were monitored by recording synaptic currents for additional 20-30 min after pairing.

Drugs used were: 6,7-dinitroquinoxaline-2,3-dione (DNQX), L(+)-2-amino-4-phosphonobutyric acid (L-AP4), Picrotoxin (PTX), 4-(8-methyl-9H-1,3-dioxolo[4,5-h][2,3]benzodiazepin-5-yl)-benzenamine hydrochloride (GYKI), (S)-1-(2-Amino-2-carboxyethyl)-3-(2-carboxybenzyl)pyrimidine-2,4-dione (UBP 302), (RS)-2-Amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propanoic acid (ATPA), D-(-)-2-amino-5-phosphonopentaoic acid (D-AP5), LY341495, Philanthotoxin (PhTx), Pertussis toxin (PTx), U73122 (all purchased from Tocris Cookson Ltd, Bristol, UK); glutamate scavenger system (glutamic-pyruvic transaminase [GPT] + pyruvate), 4-[[4-Formyl-5-hydroxy-6-methyl-3-[(phosphonooxy)methyl]-2-pyridinyl]azo]-1,3-benzenedisulfonic acid tetrasodium salt (PPADS), dihydro-beta erythroidine (DHβE), atropine, Guanosine 5'-[β-thio]diphosphate (GDPβS), baclofen and CGP 52432 were purchased from Sigma (Milan, Italy).

All drugs were dissolved in water, ethanol or dimethylsulphoxide (DMSO), as required. UBP 302 was dissolved in 1 eq. NaOH. DNQX, GYKI, LY341495 were dissolved in DMSO. The final concentration of DMSO in the bathing solution was 0.1%. At this concentration, DMSO alone did not modify the shape or the kinetics of synaptic currents. Drugs were applied in the bath *via* a three-way tap system, by changing the superfusion solution to one differing only in its content of drug(s). The ratio of flow rate to bath volume ensured complete exchange within 1-2 min.

Data acquisition and analysis

Data were acquired and digitized with an A/D converter (Digidata 1200, Molecular Devices) and stored on a computer hard disk. Acquisition and analysis of evoked responses were performed with Clampfit 9 (Axon Instruments, Foster City, CA). Data were sampled at 20 kHz and filtered with a cut off frequency of 1 kHz. Mean GPSCs amplitude was obtained by averaging successes and failures. The paired pulse ratio (PPR) was calculated as the mean amplitude of the synaptic response evoked by the second stimulus over that evoked by the first one. The coefficient of variation was calculated as the ratio between the standard deviation of synaptic currents amplitude and the mean. Unless otherwise stated, data are

presented as mean ± SEM. Quantitative comparisons were based on students paired or unpaired t-test, as required and *p* values < 0.05 were considered as significant.

RESULTS

Tonic activation of presynaptic GluK1 receptors down regulates GABA release from MF terminals

Whole cell patch clamp recordings were performed from CA3 principal cells in hippocampal slices obtained from P2-P5 old rats. In agreement with previous studies (Gutierrez et al., 2003; Kasyanov et al., 2004; Safiulina et al. 2006; Safiulina and Cherubini, 2009; Sivakumaran et al., 2009), during the first week of postnatal life, minimal stimulation of granule cells in the dentate gyrus elicited low probability GABA-mediated postsynaptic currents (GPSCs), which were completely blocked by bicuculline (10 µM) or picrotoxin (100 µM). Depolarizing the cell to positive potentials (+40 mV) produced outward currents with latency, onset and deactivation kinetics similar to those obtained at -70 mV and sensitive to bicuculline, further indicating that they were mediated by $GABA_A$ receptors (data not shown; see also Safiulina et al., 2006). Consistent with previous observations (Safiulina et al., 2006; Safiulina and Cherubini, 2009), bath application of GYKI (30 µM) which at this concentration selectively blocks AMPA receptors, did not modify the amplitude, the shape, the latency and rise time of individual responses, indicating that AMPA receptors do not contribute to synaptic currents (Fig. 1A-D). On average, on five cells, the amplitude of MF-evoked GPSCs was 40 ± 2 pA and 39 ± 1 pA (*p*=0.8), the rise time 1.8 ± 0.2 ms and 1. 9 ± 0.3 ms (*p*=0.4) and latency 2.8 ± 0.2 and 2.8 ± 0.3 ms (*p*=0.8), before and during GYKI application, respectively.

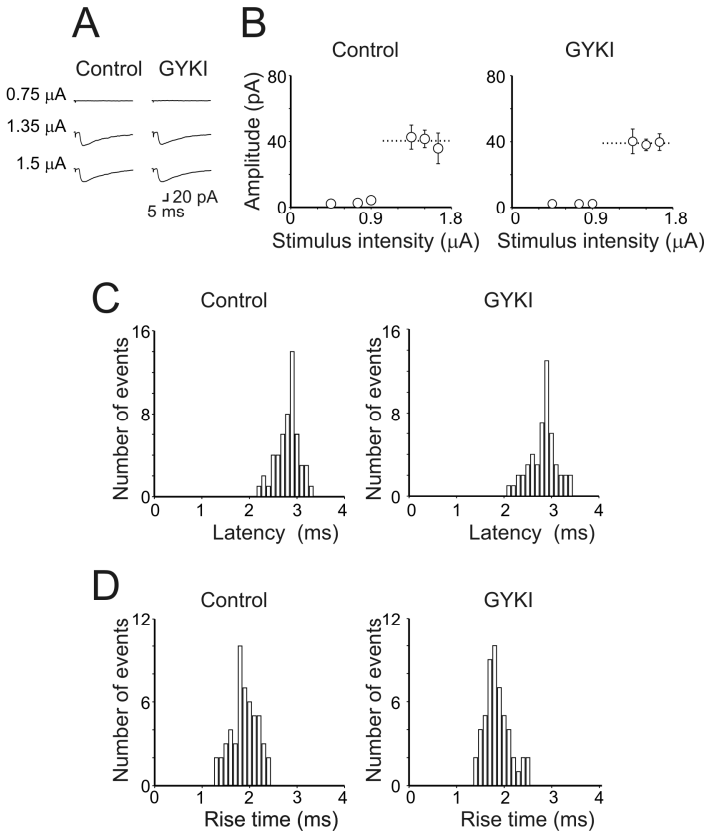

Figure 1. AMPA receptors do not contribute to unitary postsynaptic currents evoked in CA3 principal cells by minimal stimulation of granule cells in the dentate gyrus.

A. Unitary synaptic currents evoked in a CA3 pyramidal cell at P4 with different stimulation intensities before (Control) and during application of GYKI (30 μM). Each trace is the average of 15-20 responses (including failures). B. Peak amplitudes of synaptic currents represented in A are plotted as a function of stimulus intensities. Bars are SEM, dashed lines the averaged amplitude of GPSCs. Latency (C) and rise time (D) distributions of individual currents evoked in a CA3 pyramidal cell by minimal stimulation of granule cells in the dentate gyrus in the absence (Control) and in the presence of GYKI. Note that in the presence of GYKI the unimodal distribution of latencies and rise times of individual responses did not change.

Interestingly, the addition of DNQX (50 μM, which blocks both AMPA and kainate receptors) to GYKI, significantly enhanced the amplitude of single fibre-evoked GPSCs, an effect that was associated with an increase in the number of successes (Fig. 2A). On average, in 8 cells, the peak amplitude of GPSCs (successes plus failures) was 30 ± 4 pA and 53 ± 10 pA, before and after DNQX, respectively; (p=0.01; Fig. 2 B and C) while the success rate changed from 0.34 ± 0.05 to 0.57 ± 0.08 (p=0.006; Fig. 2D). These effects were associated with a significant reduction of the PPR from 1.33 ± 0.23 to 0.67 ± 0.11 (p=0.001; Fig. 2E) and a significant increase in the inverse squared value of the coefficient of variation (CV^{-2}) of responses

amplitude from 0.73 ± 0.15 to 2.28 ± 0.44 (p=0.03; Fig. 2F). While changes in successes rate may be indicative of pre or postsynaptic changes, PPR and CV^{-2} are largely used to evaluate changes in probability of transmitter release (Zucker and Regehr, 2002). These effects occurred in the absence of any change in input resistance or in the holding current of the recorded neurons. As expected for MF-mediated responses, GPSCs were significantly reduced in amplitude by group III mGluR agonist L-AP4 (10 µM) and completely blocked by picrotoxin (100 µM; Fig. 2B; see also Gutierrez, 2003; Kasyanov et al. 2004; Safiulina et al., 2006). Synaptic currents originated from GABAergic interneurons could be readily distinguished from MF-GPSCs since unlike the latter which exhibited paired-pulse facilitation, they exhibited paired pulse depression and were insensitive to L-AP4 (the peak amplitude of synaptic currents was 39 ± 8 pA and 36 ± 9 pA before and during L-AP4, respectively; p=0.1; n=7; see also Walker et al. 2001 and Safiulina et al. 2006; data not shown).

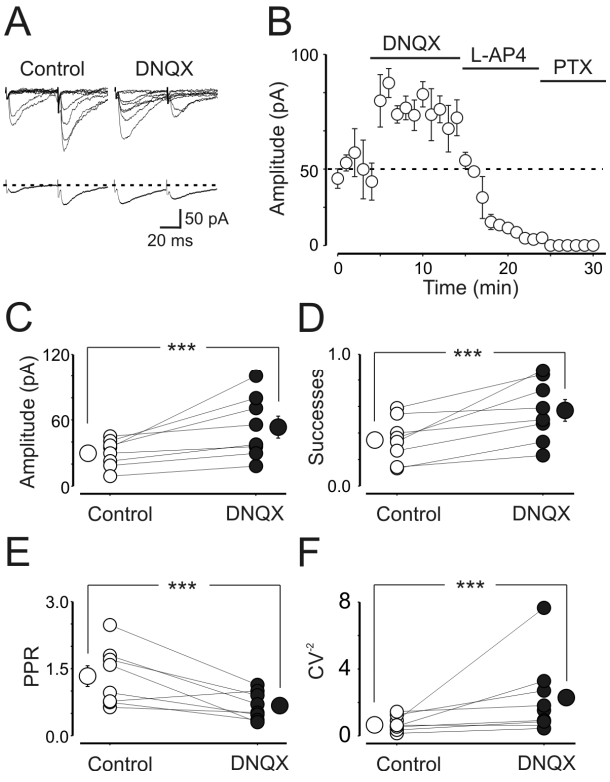

Figure 2. Endogenous activation of presynaptic kainate receptors down regulate MF-GPSCs.

A. Superimposed individual traces of MF-GPSCs evoked in the presence of GYKI (30 µM) and GYKI plus DNQX (50 µM). Note that in this and in the following experiments, GYKI was always present in the bathing solution in control conditions. Below: averaged traces (successes plus failures). Note that DNQX enhanced the amplitude of the first response and reduced the number of synaptic failures. B.

Summary plot showing the mean amplitude of GPSCs obtained in 8 cells in the presence of DNQX, L-AP4 and PTX. Vertical bars are SEM. The horizontal dashed line refers to the mean amplitude value measured before DNQX. C-F. Amplitude (C), Successes (D), PPR (E) and inversed square of CV (F) measured in individual cells before and after application of DNQX. Bigger symbols represent averaged values. ***p<0.001

To exclude the possibility of an indirect effect *via* kainate-induced modulation of other receptors known to depress transmitter release such as $GABA_B$ receptors (Safiulina and Cherubini, 2009), nicotine and muscarinic acetylcholine receptors (Maggi et al., 2004), purinergic P2Y receptors (Zhang et al. 2003; Safiulina et al. 2005) and metabotropic glutamate receptors (mGluRs, Scanziani et al., 1997), in a set of experiments (n=18) DNQX was applied in the presence of CGP 52432 (1 µM), DHβE (50 µM), atropine (1 µM), PPADS (50 µM), LY341495 (100 µM), selective antagonists for these receptors. Also in this case, DNQX induced a significant increase in GPSCs amplitude similar to that obtained in the absence of the blockers, suggesting a direct effect of DNQX on kainate receptors localized on MF terminals (Fig. 1S).

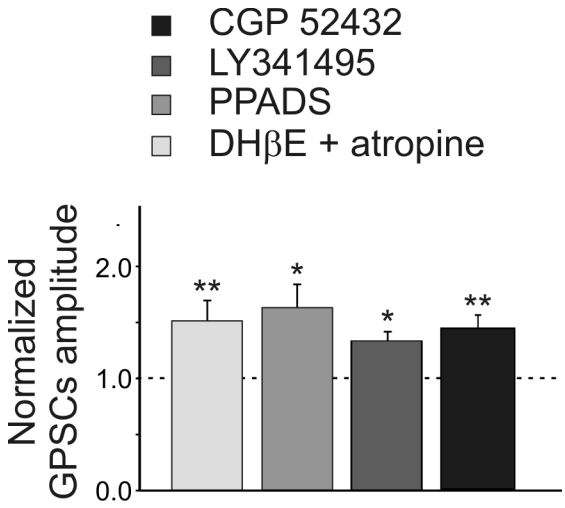

Figure 1S. At immature MF-CA3 synapses, the effects of kainate receptors on GABA release are not indirectly mediated *via* $GABA_B$, muscarinic, nicotinic, purinergic or mGlu- receptors.

Each column represents the mean amplitude of GPSCs obtained in the presence of GYKI (30 µM) and DNQX (50 µM) plus DHβE (50 µM; n=3), atropine (1 µM; n=3), PPADS (50 µM; n=3), LY341495 (100 µM; n=5) and CGP 52432 (3 µM; n=6). The amplitude of the GPSCs was normalized to that obtained in the absence of the drugs (dashed line). The groups are not significantly different from each other, but are significantly different from controls. * p<0.05; ** p<0.01.

In order to identify which kainate receptor subtype was involved in the observed effects, we used UBP 302 which is a selective GluK1 kainate receptor antagonist (More et al., 2004; Jane

et al., 2009). The rationale behind was that, during postnatal development, GluK1 receptors are highly expressed in the hippocampus (Bahn et al., 1994; Ritter et al. 2002) and previous work from immature CA3 pyramidal cells demonstrated the involvement of GluK1 in modulation of spontaneous, network-driven giant depolarizing potentials (GDPs; Lauri et al. 2005) which are highly expressed in the CA3 area (Ben-Ari et al., 2007). Like DNQX, UBP 302 (10 µM) caused a significant increase in amplitude of GPSCs (from 48 ± 8 pA to 84 ± 15 pA; n=19; *p*=0.001; Fig. 3A-C) and in successes rate (from 0.4 ± 0.04 to 0.7 ± 0.03; *p*=0.001; Fig. 3D). These effects were associated with a significant decrease in PPR from 1.4 ± 0.2 to 0.6 ± 0.1 (n=16; *p*=0.001; Fig. 3E) and a significant increase in CV^{-2} from 0.97 ± 0.2 to 3.3 ± 0.9 (*p*=0.001; n= 19, Fig. 3F).

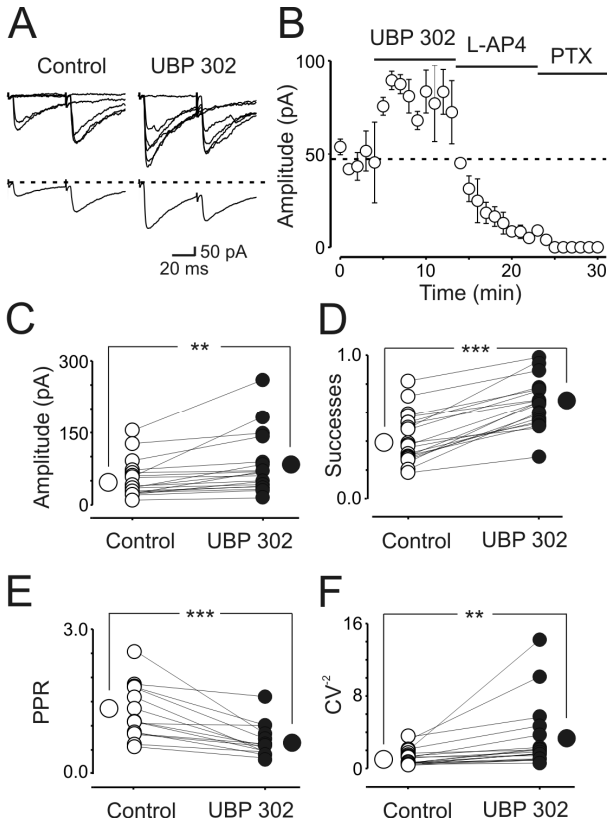

Figure 3. Endogenous activation GluK1 kainate receptors depress GABA release from MF terminals.

A. Superimposed individual MF-GPSCs evoked in the presence of GYKI (30 µM, Control) and GYKI plus UBP 302 (10 µM). Below: average traces (successes plus failures). Note that UBP 302 enhanced the amplitude of the first response and reduced the number of synaptic failures. B. Summary plot showing the mean amplitude of GPSCs obtained in 19 cells in the presence of UBP 302, L-AP4 and PTX. C-F. Amplitude (C), Successes (D), PPR (E) and inversed square of CV (F) measured in individual cells before and after application of UBP 302. Bigger symbols represent averaged values. ** *p*<0.01; ***p*<0.001.

In six cases, stimulation of granule cells in the dentate gyrus, failed to produce any synaptic response to the first stimulus (over at least 30 consecutive trials). However, occasional responses to the second stimulus suggested that these synapses were "presynaptically" silent (Gasparini et al. 2000; Kasyanov et al. 2004; Sivakumaran et al. 2009; Safiulina and Cherubini, 2009). Application of UBP 302 to “presynaptically” silent cells induced the appearance of synaptic responses to the first stimulus (Fig. 2S). In these cases, the PPR could not be measured because calculated ratios are infinitely large when the mean amplitude of the first response is close to zero. Compatible with a lack of quantal release, in these cases also the CV^{-2} was close to zero.

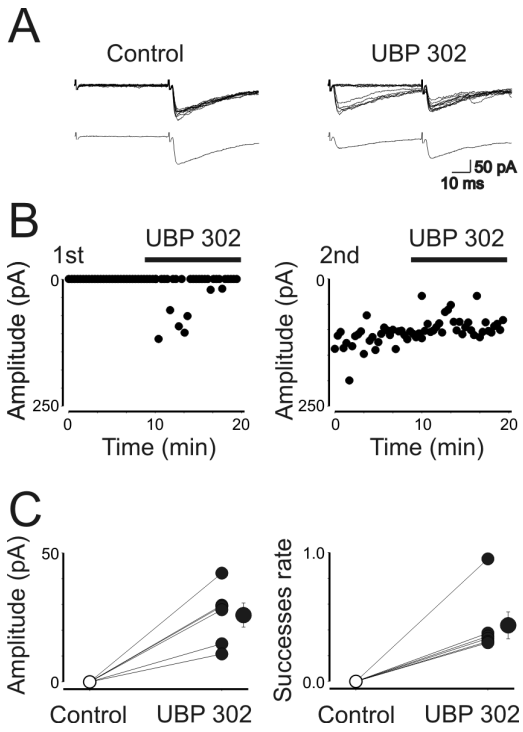

Figure 2S. Blocking GluK1 receptors with UBP 302 enhances the probability of GABA release and converts "presynaptically" silent synapses into active ones.

A. Superimposed individual (up) and averaged traces (down) of a “presynaptically” silent MF-CA3 connection before (Control) and during application of UBP 302. Note the appearance of successes in response to the first stimulus in UBP 302. B. Time course of the peak amplitude of the first (left) and second (right) response shown in A, in control and during bath application of UBP (bars). C. Summary data (amplitude and successes rate) for 6 “presynaptically” silent cells.

In contrast to UBP 302, pharmacological activation of GluK1 receptors with ATPA, a selective GluK1 agonist (Clarke et al., 1997), reversibly decreased the amplitude of MF-GPSCs (from 54 ± 9 pA to 22 ± 4 pA; n=11; p=0.001; Fig. 4A-C), an effect that was accompanied with a decrease in the number of successes (0.6 ± 0.1 to 0.35 ± 0.1; *p*=0.001; Fig. 4D), an increase in PPR (0.8 ± 0.1 to 1.3 ± 0.2; n=7; *p*=0.008; Fig. 4E) and a decrease in the CV^{-2} (1.5 ± 1.3 to 1.1 ± 0.2; n=11; *p*=0.01; Fig. 4F). Taken together, these data strongly indicate that presynaptic GluK1 receptors depress GABA release from immature MF terminals.

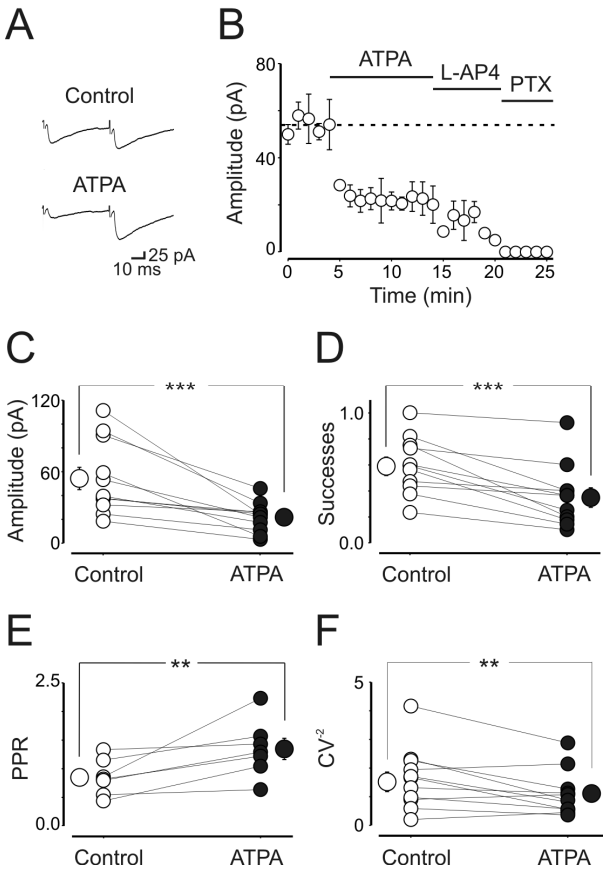

Figure 4. Bath application of the selective GluK1 agonist ATPA mimic the effects of endogenous glutamate on kainate receptors.

A. Averaged traces of GPSCs evoked in a CA3 pyramidal cell by stimulation of granule cells in the dentate gyrus, in control condition and after addiction of ATPA (1µM). B. Summary plot for 11 cells. C-F. Amplitude (C), Successes (D), PPR (E) and inversed square of CV (F) measured in individual cells before and after application of ATPA. ** *p*<0.01; ***p*<0.001.

Presynaptic GluK1 receptors are endogenously activated by glutamate present in the extracellular space

How could presynaptic GluK1 receptors be activated if, early in postnatal development, MF release mainly GABA? One possibility is that these receptors are tonically activated by endogenous glutamate present in the extracellular space. To test this hypothesis, in another set of experiments, we used an enzymatic glutamate scavenger system (glutamic-pyruvic transaminase [GPT] + pyruvate) (Overstreet et al., 1997; Min et al., 1998) on the assumption that enhancing the clearance of glutamate from the extracellular space prevents the activation of presynaptic kainate receptors. GPT catalyzes the conversion of glutamate and pyruvate to □-ketoglutarate and alanine. To exclude any effect *via* metabotropic glutamate receptors (mGluRs, Scanziani et al. 1997), we routinely added in the bathing solution the broad spectrum $mGluR_{1-8}$ antagonist LY341495 (100 µM, Fitzjohn at al., 1998). The scavenger mimicked in all respects the facilitating effects of UBP 302 on GPSCs (the peak amplitude of GPSCs was 44 $\pm$ 8 pA and 73 $\pm$ 10 pA, before and after the scavenger, respectively; n=7; *p*=0.003; Fig. 5A and B). The scavenger caused also a significant increase in successes rate (from 0.5 $\pm$ 0.1 to 0.8 $\pm$ 0.1; *p*=0.01; Fig. 5C), a significant decrease in PPR (from 1.5 $\pm$ 0.2 to 0.7 $\pm$ 0.1; n=7; *p*=0.008; Fig. 5D), and a significant increase in CV^{-2} (from 1.02 $\pm$ 0.2 to 3.4 $\pm$ 0.94; n=7; *p*=0.01; Fig. 5E), further suggesting a presynaptic site of action. In addition, the facilitating effect of the scavenger on GPSCs was fully occluded by subsequent application of UBP (the peak amplitude of GPSCs was 73 $\pm$ 10 pA and 70 $\pm$ 12 pA, in the presence of the scavenger and the scavenger plus UBP 302, respectively; n=7; Fig. 5B). Application of the scavenger after UBP 302 was also ineffective (n=5; p=0.3; data not shown). These results indicate that early in postnatal life, depression of GABA release from MF terminals occurs *via* activation of GluK1 receptors by endogenous glutamate present in the extracellular space.

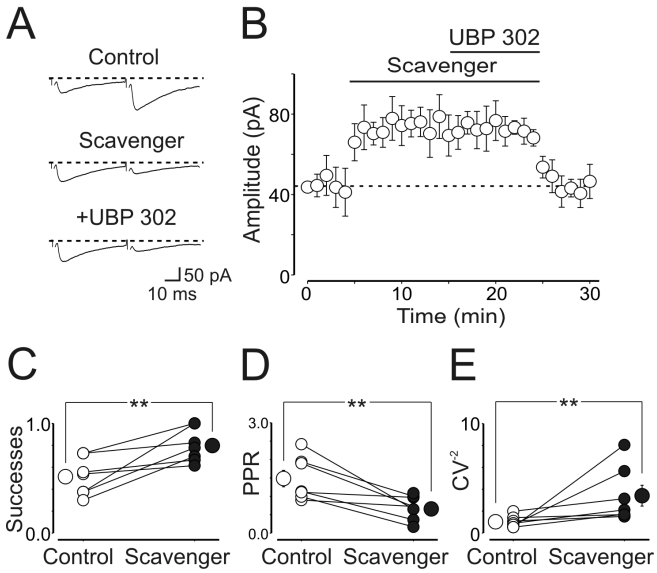

Figure 5. Ambient glutamate activates presynaptic GluK1 kainate receptors on mossy fibres terminals.

A. Averaged traces of GPSCs evoked by stimulation of granule cells in the dentate gyrus, before and after addiction of the glutamate scavenger (glutamic-pyruvic transaminase [GPT] + pyruvate) and the scavenger plus UBP 302. The broad spectrum $mGluR_{1-8}$ antagonist LY341495 (100 µM) was present throughout the experiments. B. Pooled data from 7 cells to show that the glutamate scavenger increased the mean peak amplitude of GPSCs and fully occluded the effects of UBP 302. C-E. Successes (C), PPR (D) and inversed square of CV (E) measured in individual cells before and after application of the glutamate scavenger. ** p<0.01.

The depressant effect of kainate on MF GPSCs is mediated by G-coupled receptors

At inhibitory synapses, kainate-induced depression of GABA release has been shown to involve the presynaptic activation of a pertussis toxin (PTx)-sensitive $G_{i/o}$ protein coupled kainate receptor (Lerma, 2006; Rodriguez-Moreno and Sihra, 2007). G-protein in turn would activate phospholipase C (PLC) leading to the release of calcium from intracellular stores. The concomitant activation of protein kinase C (PKC) would inhibit voltage-dependent N-type calcium channels known to control transmitter release (Castillo et al., 1994; Dunlap et al. 1995; Li et al. 2007). To elucidate whether the effects of kainate on GPSCs involved a G protein, hippocampal slices were incubated overnight with a solution containing PTx (5

μg/ml). In these conditions, bath application of the $GABA_B$ receptor agonist baclofen (from a holding potential of -60 mV), failed to evoked any outward current (data not shown; see also Lauri et al. 2005). In the presence of PTx, UBP 302 did not modify GPSCs amplitude (the peak amplitude of GPSCs was 71 ± 14 pA and 61 ± 13 pA, in the absence or in the presence of UBP 302, respectively; n= 10; *p*=0.2; Fig. 6A, closed circles). Similarly, the amplitude of GPSC was unaltered when the scavenger was applied to slices incubated in PTx (on average, in 6 cells the peak amplitude of GPSCs was 61 ± 14 pA and 59 ± 16 pA, in the absence and in the presence of the scavenger, respectively; *p*=0.6; Fig. 6B, closed circles). Incubation with PTx affects G protein-coupled receptors present on both pre and postsynaptic membranes. To see whether the depression of MF-GPSCs involved G-coupled receptors localized on the presynaptic site, additional experiments (n=5) were performed using GDPβS (0.3 mM) into the patch pipette. This treatment completely blocked postsynaptic $GABA_B$ receptors. Thus, in the presence of GDPβS, the $GABA_B$ receptor agonist baclofen (20 μM) failed to induce outward currents (baclofen evoked-currents were 53 ± 5 pA and 5 ± 2 pA in the absence or in the presence of GDPβS, respectively, n=6; *p*=0.001; Fig. 6C and D). However, as shown in the example of Fig. 6C, baclofen, *via* presynaptic $GABA_B$ receptors, depressed spontaneous ongoing synaptic currents. Bath application of UBP 302 to neurons recorded with GDP□S, was still able to enhance GPSCs amplitude (the peak amplitude of GPSCs varied from 29 ± 4 pA to 43 ± 5 pA; n=5; *p*=0.003; Fig. 6E and F), indicating that the depression of MF-GPSCs is mediated by G protein-coupled kainate receptors, present on MF terminals.

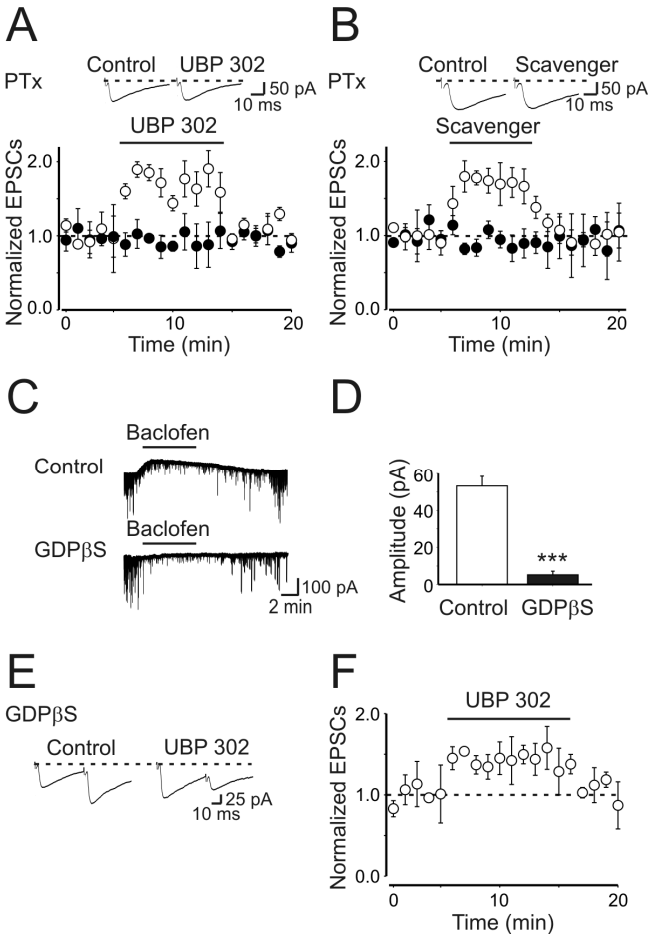

Figure 6. The depressant effect of kainate on GPSCs involves a G protein-mediated mechanism.

A. Summary plot showing the effects of UBP 302 on the peak amplitude of GPSCs in controls (open symbols) and after overnight treatment with pertussis toxin (PTx, closed symbols). Note that in cells treated with PTx, UBP 302 was ineffective (see also inset above the graph representing average traces obtained in the presence of GYKI and GYKI plus UBP 302). B. as in A but in the presence of the glutamate scavenger showing that also the effect of the scavenger is dependent on G protein. C. Application of baclofen (20 μM, bar) induced an outward currents associated with a reduction in spontaneous synaptic events (upper trace). Application of baclofen to a cell recorded with a patch pipette containing GDPβS, failed to produce an outward current, but was still able to depress the ongoing synaptic activity (bottom trace). D. Each column represents the mean amplitude of baclofen-induced currents recorded with a normal intrapipette solution (Control) and with one containing GDPβS (n=6). E-F. GDPβS into the intrapipette solution did not affect UBP 302-induced GPSCs facilitation. E. representative traces. F. summary plot from 5 cells. ***p<0.001.

As already mentioned, the most common transduction signaling pathway stimulated by G protein involves PLC activation (Rodriguez-Moreno and Lerma, 1998; Rozas et al., 2003). Therefore, in the following experiments, we tried to disrupt the intracellular cascade

downstream to G-protein activation with U73122 (10 µM), a selective PLC blocker. Surprisingly, we found that, unlike PTx, application of UBP 302 in the presence of U73122 caused a significant depression of MF-GPSCs, probably unmasking the ionotropic action of GluK1. The amplitude of MF-GPSCs was 50 ± 9 pA and 29 ± 11 pA, in the absence or in the presence of UBP 302, respectively (n=6; *p*=0.01; Fig. 7A and B). Differences between PTx and U73122 could be explained by the fact that, incubating the slices with PTx for several hours, may inhibit G proteins not only coupled with KARs, leading to a different effect.

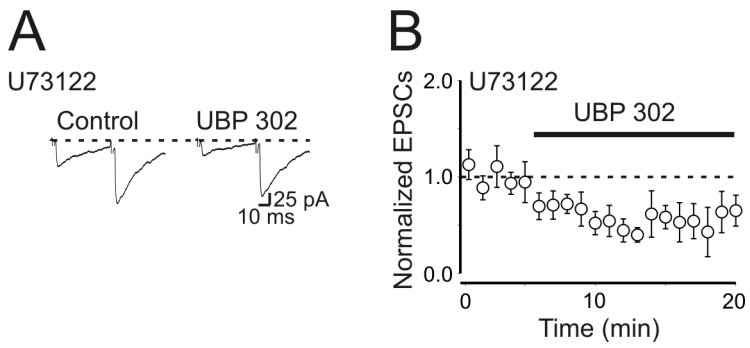

Figure 7. Blocking PLC with U73122 reveals the ionotropic action of GluK1 on MF-GPSCs.

A. Averaged traces of MF-GPSCs evoked in a CA3 principal cell in the presence of U73122 (10 µM, Control) and U73122 plus UBP 302 (10 µM). B. Summary plot showing the mean amplitude of GPSCs obtained in 6 cells in the presence of U73122 before and during application of UBP 302 (bar). The amplitude of synaptic responses are normalized to those obtained before UBP 302 (dashed line).

Activation of presynaptic kainate receptors enhances MF excitability

In juvenile animals, activation of presynaptic kainate receptors has been found to directly depolarize *via* cation channels glutamatergic MF (Kamiya and Ozawa, 2000) or GABAergic terminals (Semyanov and Kullmann, 2001) thus lowering the threshold for antidromic action potential generation. To test whether activation of GluK1 receptors localized on immature MF terminals exhibits similar characteristics, we recorded antidromic spikes from single granule cells (held at -70 mV), in response to stimulation of MFs *via* a stimulation electrode positioned in stratum lucidum. When we used stimuli able to evoke antidromic spikes in > 50 % of trials, application of UBP 302 (10 µM), reversibly decreased the probability of successes from 0.6 ± 0.05 to 0.15 ± 0.02 (n=7; *p*=0.001; Fig. 8A and B). In contrast, ATPA reversibly increased the probability of successes when antidromic spikes were evoked by weak stimuli (< 50 % of successes). The successes rate was 0.38 ± 0.04 and 0.72 ± 0.04, in the absence or in the presence of ATPA, respectively (n=7; *p*=0.001; Fig. 8C and D). Since GluK1 receptors

are known to be highly permeable to calcium (Lauri et al. 2003; Scott et al. 2008), in another set of experiments we applied philanthotoxin (PhTx, 3 µM) which blocks calcium permeable AMPA/kainate receptor (Fletcher and Lodge, 1996). As UBP 302, PhTx significantly reduced the number of successes from 0.59 ± 0.04 to 0.15 ± 0.05 (n=7; *p*=0.001; Fig. 8E and F). In the presence of PhTx, we increased the stimulation intensity to obtain again > 50% of successes and then we applied UBP 302. In this case, the drug failed to produce any effect. The successes rate was 0.58 ± 0.09 and 0.61 ± 0.09, before and during UBP 302, respectively (n=7; *p*=0.08; Fig. 8G and H). These data indicate GluK1 increases MF excitability through the activation of calcium-permeable cationic channels and depolarization of MF terminals.

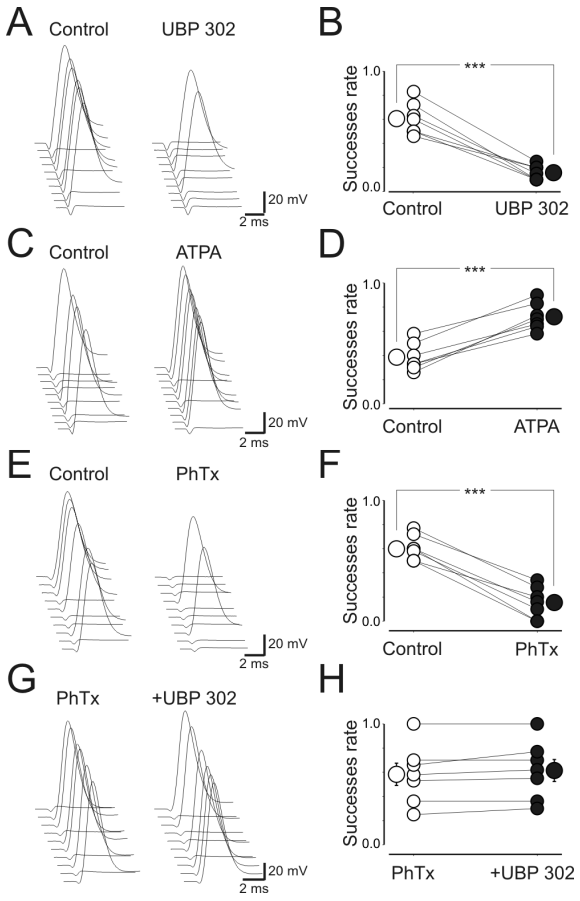

Figure 8. GluK1 receptors sensitive to philantotoxin control MF excitability.

A. Consecutive traces showing antidromic spikes recorded in granule cells upon stimulation of MF in stratum lucidum before and during application of UBP 302 (note that the stimulus strength was set to obtain > than 50 % of successes. B. Summary plot of UBP 302 effects on successes rate (n=7). C-D. In cells with < than 50% of successes, ATPA enhanced MF excitability and the successes rate (n=7). E-F. Philantotoxin (PhTx, 3 µM) mimicked the effects of UBP 302 (n=7). G-H. In the presence of philantotoxin, UBP was not effective (n=7). *** *p*<0.001.

GluK1Rs control the direction of spike-time dependent plasticity (STDP)

In a previous study we demonstrated that, depending on the relative timing, pairing afferent stimulation with postsynaptic spiking induced bidirectional changes in synaptic efficacy (Sivakumaran et al. 2009). In particular, a persistent increase in synaptic strength was observed when MF stimulation preceded postsynaptic spiking. To avoid possible contamination with glutamatergic events, experiments were routinely performed in the presence of D-AP5 and DNQX to block NMDA and AMPA/kainate receptors, respectively. Therefore, to see whether GluK1 receptors control spike-time dependent LTP, in another set of experiments, the pairing procedure was repeated only in the presence of GYKI, to prevent the activation of AMPA receptors. Pairing consisted in correlating (in current clamp mode) MF stimulation with 10 postsynaptic spikes (at 0.1 Hz) using, as in previous work, a delay of 15 ms (corresponding to the peak of the synaptic responses, Fig. 9A and B). As shown in the representative example of Fig. 9C, pairing (arrow) induced a reduction in amplitude of GPSCs which persisted for at least 20 min. On average, the peak amplitude of GPSCs (successes plus failures) was 42 ± 6 pA and 19 ± 2 pA, before and 20 min after pairing, respectively (p= 0.003; n=11; Fig. 9C and E). This effect was associated with a significant decrease in successes rate (from 0.46 ± 0.06 to 0.25 ± 0.06; p=0.005; Fig. 9E), in the inversed square of the coefficient of variation of responses amplitude (from 1.67 ± 0.49 to 0.96 ± 0.29; p=0.02; Fig. 9E) and a significant increase in PPR (from 0.86 ± 0.27 to 1.97 ± 0.47; n=8; p=0.01; Fig. 9E). When UBP 302 was added to GYKI, the pairing procedure produced a persistent potentiation of synaptic responses that was in all respects similar to that already described (Sivakumaran et al. 2009). In summary the amplitude of GPSCs varied from 84 ± 15 pA to 124 ± 18 pA (p=0.001; Fig. 9D and E). This effects was associated with an increase in successes rate (from 0.5 ± 0.05 to 0.8 ± 0.06; p=0.001), in CV^{-2} (from 1.19 ± 0.2 to 4.1 ± 1; p=0.01) and a decrease in PPR (from 1.5 ± 0.2 to 0.8 ± 0.2; n=9; p=0.004; Fig. 9 E). These results indicate that presynaptic GluK1 control the direction of STDP at immature MF-CA3 synapses.

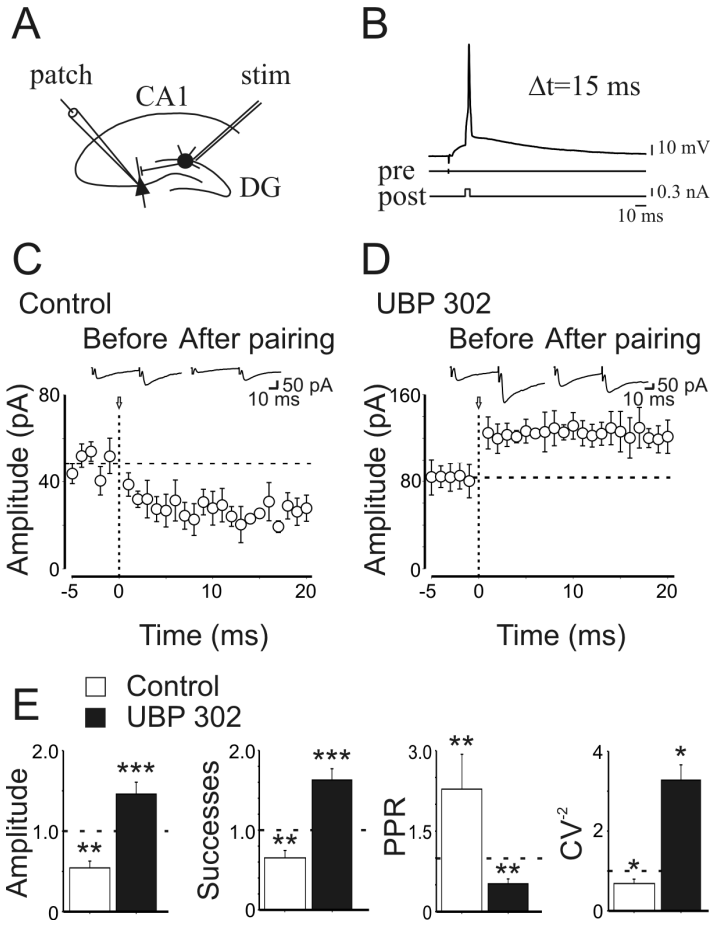

Figure 9. At immature MF-CA3 synapses, presynaptic kainate receptors control the direction of spike-time dependent plasticity (STDP).

A. Schematic representation of the experimental design. B. The stimulation of granule cells in the dentate gyrus (pre) preceded the postsynaptic spike (post) by 15 ms (Δt). C. Summary plot of the mean peak amplitude of GPSCs recorded in the presence of GYKI before and after pairing (arrow at time 0; n=11). The dashed line represents the mean amplitude of GPSCs before pairing. Insets represent averaged GPSCs obtained from a single neuron before and after pairing. Note that pairing induced synaptic depression. D. As in C but in the presence of UBP 302 (n=9). In this case, pairing induced synaptic potentiation E. Each column represents the mean amplitude, successes rate, PPR and inverse squared of CV of GPSCs obtained after pairing and normalized to controls (before paring, dashed lines) in neurons exposed only to GYKI (white) or GYKI plus UBP 302 (black). * p<0.05; ** p<0.01; ***p<0.001.

DISCUSSION

The present experiments from the immature hippocampus have revealed the presence of functional presynaptic kainate receptors on GABAergic MF terminals. Endogenous activation of these receptors by glutamate present in the extracellular medium reduces the probability of GABA release contributing in some cases to synapses silencing. Moreover, GluK1 receptors present on MF terminals dynamically regulate the direction of STDP being able to switch spike time-dependent potentiation into depression.

In previous studies, we provided evidence that, during the first week of postnatal life, the main neurotransmitter released from MF terminals is GABA (Kasyanov et al., 2004; Safiulina et al., 2006; Safiulina and Cherubini, 2009; Sivakumaran et al., 2009). MF-mediated glutamatergic responses start appearing during the second week of postnatal life (Amaral and Dent, 1981). In particular, kainate-mediated EPSCs have been detected only after P6, in coincidence with the appearance of large amplitude AMPA responses and with the onset of low frequency facilitation (Marchal and Mulle, 2004). This period tightly correlates with that in which $GABA_A$-mediated synaptic responses shift from the depolarizing to the hyperpolarizing direction (Cherubini et al. 1991; Ben-Ari et al. 2007). In the present experiments, GPSCs originated from MF since they exhibited strong paired pulse facilitation, and were sensitive to group III mGluR agonist L-AP4, (Gutierrez et al. 2005; Safiulina et al., 2006). In this respect they could be easily distinguished from those originating from GABAergic interneurons which were insensitive to L-AP4 and exhibited paired pulse depression (Walker et al. 2001; Safiulina et al. 2006). MF-mediated synaptic responses did not carry AMPA-mediated components since GYKI, at the concentration used to block AMPA receptors, failed to modify the amplitude, rise time or latency of synaptic currents. However, in the presence of GYKI, synaptic currents were reversibly enhanced by DNQX or UBP 302, indicating that presynaptic KARs down regulate GABA release from MF terminals. In this respect, our results are similar to those obtained by Maingret et al. (2005) on $GABA_A$-mediated postsynaptic currents evoked in neonatal CA1 pyramidal neurons by electrical stimulation of GABAergic axons. In the immature hippocampus, presynaptic GluK1 receptors have been well documented (Bahn et al., 1994; Ritter et al. 2002) and a recent study has provide evidence that at CA3-CA1 synapses functional GluK1 receptors, down regulate

glutamate release (Lauri et al. 2006). Moreover, in the CA3 hippocampal region, activation of GluK1 receptors reversibly blocks spontaneous network-driven bursts such as GDPs (Lauri et al. 2005). In our case, the depression of MF-GPSCs by KARs was not mediated indirectly *via* other signalling molecules known to inhibit GABA release, since DNQX was still able to enhance the amplitude of GPSCs when applied in the presence of various receptor antagonists including those for $GABA_B$, nicotinic, muscarinic, P2Y and mGlu.

Several line of evidence suggest that GluK1 receptors localized on MF terminals reduces the probability of GABA release: i. the increase in amplitude of MF-GPSCs by DNQX or UBP 302 was associated with an enhanced successes rate and paired-pulse ratio, considered an index of presynaptic release probability (Zucker and Regehr, 2002). ii. The GluK1 agonist ATPA induced a powerful depression of MF-evoked synaptic responses, an effect associated with a significant increase in transmitter failures and in the paired-pulse ratio. iii. Decreasing the concentration of "ambient" glutamate with the scavenger, prevented the activation of GluK1 receptors and mimicked the effects of KAR antagonists. Previous studies from different brain structures have demonstrated that physiological modifications in glutamate concentration may cause a switch in KAR function from facilitation to inhibition (Jiang et al. 2001; Delaney and Jahr, 2002; Braga et al. 2003; Youn and Randic, 2004; Lerma, 2006). It is therefore likely that early in postnatal development, a high level of glutamate in the extracellular space, maintained by a less efficient glutamate transport mechanism (Danbolt, 2001; Diamond, 2005) and a poorly developed diffusional barrier (Jansson et al., 2000) facilitates the activation of high affinity KARs with consequent reduction in the probability of transmitter release.

KARs have been shown to exert both a ionotropic and a metabotropic type of action (for a review see Lerma, 2006). In particular, the depression of transmitter release seems to occur *via* G-protein-coupled KARs (Rodriguez-Moreno and Lerma, 1998; Frerking et al., 2001; Cunha et al. 2000; Lauri et al. 2005; 2006). Also in our case, the depressant effect of kainate on GABA release was likely dependent on a metabotropic type of mechanism since the potentiating effects of both UBP 302 and the glutamate scavenger on GPSCs were fully blocked by PTx. The signaling pathway likely involved the release of calcium from intracellular stores, the activation of phospholipase C and PKC with consequent inhibition of voltage-dependent calcium channels (Rodriguez-Moreno and Lerma, 1998; Rozas et al. 2003). Interestingly, after blocking PLC with U73122, downstream to G protein activation, a

facilitatory effect of GluK1 on MF GPSCs was unveiled. This effect was probably dependent on a ionotropic type of action. Similarly, GluK1-induced increase in MF excitability was dependent on calcium flux through calcium-permeable cationic channels, which by depolarizing presynaptic terminals, lowered the threshold for antidromic spikes (see also Kamiya and Ozawa, 2000; Schmitz et al. 2001; Semyanov and Kullmann, 2001; Maingret et al., 2005). How can an increased MF excitability be reconciled with a depression of GABA release? According to Kamiya and Ozawa (2000) this may occur *via* inactivation of Na^{+}/Ca^{2+} channels or electrical shunting but this seems unlikely in view of the recent finding of KA-induced facilitation of action potential evoked calcium entry in MF boutons *via* a calcium store-dependent mechanism (Scott et al., 2008). However, it should be stressed that early in postnatal life MF axons terminate in very small, spherical expansions (Amaral and Dent, 1981) and do not exhibit yet use-dependent facilitation of transmission (Marchal and Mulle, 2004).

It is unclear whether the dual signaling pathways (ionotropic and metabotropic) which depend on the common ionotropic GluK1 subunit are independent or functionally coupled. In a previous study from dorsal root ganglion cells, it has been demonstrated that KA through GluK1 receptors induces a G protein-dependent rise in $[Ca^{2+}]i$, favoring its release from the internal stores (Rozas et al., 2003). As a matter of speculation we favor the hypothesis that calcium entering through calcium-permeable cationic channels (which by enhancing MF excitability should facilitate the probability of GABA release) may directly or indirectly interfere with G protein-mediated signaling leading to a dominant inhibitory action on MF-GPSCs. The interplay between these two different pathways has been recently shown to account for the PKC-dependent autoregulation of membrane KARs (Rivera et al. 2007). However, much work is needed to elucidate this issue.

Early in postnatal development, G protein dependent mechanisms linked to KA activation have been well established particularly at glutamatergic synapses (Lauri et al., 2005; 2006). These receptors have a very high affinity for glutamate a condition required for being endogenously activated by glutamate present in the extracellular medium. With maturation, presynaptic KARs in an activity-dependent manner would be gradually lost and replaced by low affinity ones, not longer able to be activated by ambient glutamate (Lauri et al. 2006). In the present experiments, tonic activation of presynaptic KARs by endogenous glutamate accounted for the persistent depression of MF-GPSCs observed after pairing presynaptic MF

stimulation with postsynaptic spiking as demonstrated by the possibility to switch spike-time dependent depression into potentiation with UBP 302. Spike time dependent potentiation of MF-GPSCs, observed in the presence of KAR antagonists was dependent on intracellular calcium rise and release of BDNF (Sivakumaran et al. 2009). Although the precise mechanisms underlying these phenomenona are still unclear, we can not exclude the possibility that KA-induced synaptic depression may rely on a distinct calcium signal which in turn may activate a different molecular pathway, as suggested by the calcium hypothesis (Caporale and Dan, 2008).

Whatever is the mechanism, it is conceivable that, at immature MF-CA3 synapses, KA-induced depression of GABAergic transmission by ambient glutamate limits the excessive activation of the auto-associative CA3 network by the excitatory action of GABA, thus preventing the onset of seizures. These properties are likely to be critical for information processing at immature MF-CA3 synapses and for the proper development of the proper adult hippocampal circuitry.

REFERENCES

Allen C, Stevens CF (1994) An evaluation of causes for unreliability of synaptic transmission. Proc Natl Acad Sci U S A 91:10380-10383.

Amaral DG, Dent JA (1981) Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. J Comp Neurol 195:51-86.

Bahn S, Volk B, Wisden W (1994) Kainate receptor gene expression in the developing rat brain. J Neurosci 14:5525-5547.

Ben-Ari Y, Cossart R (2000) Kainate, a double agent that generates seizures: two decades of progress. Trends Neurosci 23:580-587.

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215-1284.

Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M, Heinemann S (1990) Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. Neuron 5:583-595.

Braga MF, Aroniadou-Anderjaska V, Xie J, Li H (2003) Bidirectional modulation of GABA release by presynaptic glutamate receptor 5 kainate receptors in the basolateral amygdala. J Neurosci 23:442-452.

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a hebbian learning rule. Annu Rev Neurosci 31:25-46.

Castillo PE, Weisskopf MG, Nicoll RA (1994) The role of Ca2+ channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. Neuron 12:261-269.

Castillo PE, Malenka RC, Nicoll RA (1997) Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature 388:182-186.

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515-519.

Clarke VR, Ballyk BA, Hoo KH, Mandelzys A, Pellizzari A, Bath CP, Thomas J, Sharpe EF, Davies CH, Ornstein PL, Schoepp DD, Kamboj RK, Collingridge GL, Lodge D, Bleakman D (1997) A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. Nature 389:599-603.

Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56:2-5.

Contractor A, Swanson G, Heinemann SF (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron 29:209-216.

Cossart R, Tyzio R, Dinocourt C, Esclapez M, Hirsch JC, Ben-Ari Y, Bernard C (2001) Presynaptic kainate receptors that enhance the release of GABA on CA1 hippocampal interneurons. Neuron 29:497-508.

Cossart R, Epsztein J, Tyzio R, Becq H, Hirsch J, Ben-Ari Y, Crépel V (2002) Quantal release of glutamate generates pure kainate and mixed AMPA/kainate EPSCs in hippocampal neurons. Neuron 35:147-159.

Cunha RA, Malva JO, Ribeiro JA (2000) Pertussis toxin prevents presynaptic inhibition by kainate receptors of rat hippocampal [(3)H]GABA release. FEBS Lett 469:159-62.

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1-105.

Delaney AJ, Jahr CE (2002) Kainate receptors differentially regulate release at two parallel fiber synapses. Neuron 36:475-482.

Diamond JS (2005) Deriving the glutamate clearance time course from transporter currents in CA1 hippocampal astrocytes: transmitter uptake gets faster during development. J Neurosci 25:2906-2916.

Dunlap K, Luebke JI, Turner TJ (1995) Exocytotic Ca2+ channels in mammalian central neurons. Trends Neurosci 18:89-98.

Fitzjohn SM, Bortolotto ZA, Palmer MJ, Doherty AJ, Ornstein PL, Schoepp DD, Kingston AE, Lodge D, Collingridge GL (1998) The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. Neuropharmacology 37:1445-1458.

Fletcher EJ, Lodge D (1996) New developments in the molecular pharmacology of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate and kainate receptors. Pharmacol Ther 70:65-89.

Frerking M, Schmitz D, Zhou Q, Johansen J, Nicoll RA (2001) Kainate receptors depress excitatory synaptic transmission at CA3-->CA1 synapses in the hippocampus via a direct presynaptic action. J Neurosci 21:2958-2966.

Gasparini S, Saviane C, Voronin LL, Cherubini E (2000) Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc Natl Acad Sci U S A 97:9741-9746.

Gutierrez R, Romo-Parra H, Maqueda J, Vivar C, Ramirez M, Morales MA, Lamas M (2003) Plasticity of the GABAergic phenotype of the "glutamatergic" granule cells of the rat dentate gyrus. J Neurosci 23:5594-5598.

Gutierrez R (2005) The dual glutamatergic-GABAergic phenotype of hippocampal granule cells. Trends Neurosci 28:297-303.

Jane DE, Lodge D, Collingridge GL (2009) Kainate receptors: pharmacology, function and therapeutic potential. Neuropharmacology 56:90-113.

Jansson A, Lippoldt A, Mazel T, Bartfai T, Ogren SO, Syková E, Agnati LF, Fuxe K (2000) Long distance signalling in volume transmission. Focus on clearance mechanisms. Prog Brain Res 125:399-413.

Jiang L, Xu J, Nedergaard M, Kang J (2001)A kainate receptor increases the efficacy of GABAergic synapses. Neuron 30:503-513.

Jonas P, Major G, Sakmann B (1993) Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. J Physiol 472:615-663.

Kamiya H, Ozawa S (2000) Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. J Physiol 523:653-665

Kamiya H, Ozawa S, Manabe T (2002) Kainate receptor-dependent short-term plasticity of presynaptic Ca2+ influx at the hippocampal mossy fiber synapses. J Neurosci 22:9237-9243.

Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci U S A 101:3967-3972.

Kwon HB, Castillo PE (2008) Role of glutamate autoreceptors at hippocampal mossy fiber synapses. Neuron 60:1082-1094.

Lauri SE, Delany C, J Clarke VR, Bortolotto ZA, Ornstein PL, T R Isaac J, Collingridge GL (2001a) Synaptic activation of a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fibre synapses. Neuropharmacology 41:907-915.

Lauri SE, Bortolotto ZA, Bleakman D, Ornstein PL, Lodge D, Isaac JT, Collingridge GL (2001b) A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. Neuron 32:697-709.

Lauri SE, Bortolotto ZA, Nistico R, Bleakman D, Ornstein PL, Lodge D, Isaac JT, Collingridge GL (2003) A role for Ca2+ stores in kainate receptor-dependent synaptic facilitation and LTP at mossy fiber synapses in the hippocampus. Neuron 39:327-341.

Lauri SE, Segerstråle M, Vesikansa A, Maingret F, Mulle C, Collingridge GL, Isaac JT, Taira T (2005) Endogenous activation of kainate receptors regulates glutamate release and network activity in the developing hippocampus. J Neurosci 25:4473-4484.

Lauri SE, Vesikansa A, Segerstråle M, Collingridge GL, Isaac JT, Taira T (2006) Functional maturation of CA1 synapses involves activity-dependent loss of tonic kainate receptor-mediated inhibition of glutamate release. Neuron 50:415-429.

Lerma J (2003) Roles and rules of kainate receptors in synaptic transmission. Nat Rev Neurosci 4:481-495.

Lerma J (2006) Kainate receptor physiology. Curr Opin Pharmacol 6:89-97.

Li L, Bischofberger J, Jonas P (2007) Differential gating and recruitment of P/Q-, N-, and R-type Ca2+ channels in hippocampal mossy fiber boutons. J Neurosci 7:13420-13429.

Maggi L, Sola E, Minneci F, Le Magueresse C, Changeux JP, Cherubini E (2004) Persistent decrease in synaptic efficacy induced by nicotine at Schaffer collateral-CA1 synapses in the immature rat hippocampus. J Physiol 559:863-874.

Maingret F, Lauri SE, Taira T, Isaac JT (2005) Profound regulation of neonatal CA1 rat hippocampal GABAergic transmission by functionally distinct kainate receptor populations. J Physiol 567:131-142.

Marchal C, Mulle C (2004) Postnatal maturation of mossy fibre excitatory transmission in mouse CA3 pyramidal cells: a potential role for kainate receptors. J Physiol 561:27-37.

Melyan Z, Lancaster B, Wheal HV (2004) Metabotropic regulation of intrinsic excitability by synaptic activation of kainate receptors. J Neurosci 24:4530-4534.

Min MY, Rusakov DA, Kullmann DM (1998) Activation of AMPA, kainate, and metabotropic receptors at hippocampal mossy fiber synapses: role of glutamate diffusion. Neuron 21:561-570.

More JC, Nistico R, Dolman NP, Clarke VR, Alt AJ, Ogden AM, Buelens FP, Troop HM, Kelland EE, Pilato F, Bleakman D, Bortolotto ZA, Collingridge GL, Jane DE (2004) Characterisation of UBP296: a novel, potent and selective kainate receptor antagonist. Neuropharmacology 47:46-64.

Mulle C, Sailer A, Pérez-Otaño I, Dickinson-Anson H, Castillo PE, Bureau I, Maron C, Gage FH, Mann JR, Bettler B, Heinemann SF (1998) Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. Nature 392:601-615.

Mulle C, Sailer A, Swanson GT, Brana C, O'Gorman S, Bettler B, Heinemann SF (2000) Subunit composition of kainate receptors in hippocampal interneurons. Neuron 28:475-484.

Nicoll RA, Schmitz D (2005) Synaptic plasticity at hippocampal mossy fibre synapses. Nat Rev Neurosci 6:863-876.

Overstreet LS, Pasternak JF, Colley PA, Slater NT, Trommer BL (1997) Metabotropic glutamate receptor mediated long-term depression in developing hippocampus.Neuropharmacology 36:831-844.

Represa A, Tremblay E, Ben-Ari Y (1987) Kainate binding sites in the hippocampal mossy fibers: localization and plasticity. Neuroscience 20:739-748.

Ritter LM, Vazquez DM, Meador-Woodruff JH (2002) Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. Brain Res Dev Brain Res 139:227-236.

Rivera R, Rozas JL, Lerma J (2007) PKC-dependent autoregulation of membrane kainate receptors. EMBO J 26:4359-4367.

Rodríguez-Moreno A, Herreras O, Lerma J (1997) Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. Neuron 19:893-901.

Rodríguez-Moreno A, Lerma J (1998) Kainate receptor modulation of GABA release involves a metabotropic function. Neuron 20:1211-1218.

Rodríguez-Moreno A, Sihra TS (2007) Kainate receptors with a metabotropic modus operandi. Trends Neurosci 30:630-637.

Rozas JL, Paternain AV, Lerma J (2003) Noncanonical signaling by ionotropic kainate receptors. Neuron 39:543-553.

Safiulina VF, Kasyanov AM, Sokolova E, Cherubini E, Giniatullin R (2005) ATP contributes to the generation of network-driven giant depolarizing potentials in the neonatal rat hippocampus. J Physiol 565:981-992.

Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597-608.

Safiulina VF, Cherubini E (2009) At Immature Mossy Fibers-CA3 Connections, Activation of Presynaptic GABA(B) Receptors by Endogenously Released GABA Contributes to Synapses Silencing. Front Cell Neurosci 3:1.

Scanziani M, Salin PA, Vogt KE, Malenka RC, Nicoll RA (1997) Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors. Nature 385:630-634.

Schmitz D, Mellor J, Frerking M, Nicoll RA (2001) Presynaptic kainate receptors at hippocampal mossy fiber synapses. Proc Natl Acad Sci U S A 98:11003-11008.

Scott R, Lalic T, Kullmann DM, Capogna M, Rusakov DA (2008) Target-cell specificity of kainate autoreceptor and Ca2+-store-dependent short-term plasticity at hippocampal mossy fiber synapses. J Neurosci 28:13139-13149.

Semyanov A, Kullmann DM (2001) Kainate receptor-dependent axonal depolarization and action potential initiation in interneurons. Nat Neurosci 4:718-723.

Sivakumaran S, Mohajerani MH, Cherubini E (2009) At immature mossy-fiber-CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA. J Neurosci 29:2637-2647.

Tashiro A, Dunaevsky A, Blazeski R, Mason CA, Yuste R (2003) Bidirectional regulation of hippocampal mossy fiber filopodial motility by kainate receptors: a two-step model of synaptogenesis. Neuron 38:773-84.

Vesikansa A, Sallert M, Taira T, Lauri SE (2007) Activation of kainate receptors controls the number of functional glutamatergic synapses in the area CA1 of rat hippocampus. J Physiol 583:145-157.

Vignes M, Collingridge GL (1997) The synaptic activation of kainate receptors. Nature 388:179-182.

Walker MC, Ruiz A, Kullmann DM (2001) Monosynaptic GABAergic signaling from dentate to CA3 with a pharmacological and physiological profile typical of mossy fiber synapses. Neuron 29:703-715.

Youn DH, Randic M (2004) Modulation of excitatory synaptic transmission in the spinal substantia gelatinosa of mice deficient in the kainate receptor GluR5 and/or GluR6 subunit. J Physiol 555:683-698.

Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S (2003) ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. Neuron 40:971-982

Zucker RS, Regehr WG. (2002) Short-term synaptic plasticity. Annu Rev Physiol 64, 355-405.

FUTURE PERSPECTIVES

The last paper included in my thesis has revealed the presence of presynaptic kainate receptors on GABAergic MF terminals. Endogenous activation of GluK1 receptors by glutamate present in the extracellular medium led to a depression of GABA release and to the shift of spike-time-dependent potentiation into depression. These effects likely involved a G-protein coupled GluK1 receptor since they were blocked by pertussis toxin. The signaling pathway downstream of G-protein activation entails the stimulation of PLC. A first interesting question to ask is whether, blocking PLC downstream of G-protein activation, reveals the ionotropic type of action of GluK1. We have seen that the GluK1-induced enhancement of cell excitability at MF terminals is mediated by the ionotropic receptors since this effect was blocked by philantotoxin and in the presence of philantotoxin the selective GluK1 reecptor antagonist UBP 302 was not anymore effective. Therefore, repeating the pairing protocol for STDP in the presence of a selective PLC blocker would probably allow revealing the ionotropic action of GluK1 receptor in this form of synaptic plasticity. In addition, taking advantage of the two photon microscopy associated with a patch clamp set up present in our lab, it will be of interest to measure the intracellular calcium concentration (in cells loaded with the calcium-sensitive dye Oregon green) before, during and after the STDP protocol. The induction of spike-time dependent LTP recorded in the presence of AMPA/kainate receptor antagonists was dependent on intracellular calcium flux *via* voltage-dependent calcium channels. It would be interesting to see whether spike-time dependent LTD observed in the absence of AMPA/kainate receptor blockers is also associated with an intracellular calcium rise. If this is the case, the next question to be solved is why a rise of $[Ca^{2+}]_i$ leads to the activation of different transduction pathways. May be different intracellular calcium levels may account for different signaling in the case of LTP and LTD, respectively as suggested by the calcium hypothesis, according to which the direction of synaptic changes is determined by distinct calcium signals which would activate different molecular pathways (Caporale and Dan, 2008). In the case of LTD, the possibility that intracellular calcium rise may occur *via* calcium-permeable kainate receptors and/or *via* calcium release from the internal stores following a signaling pathway downstream of G-protein activation should be also considered.

Another point not taken into account in the present work is what happens in the case of negative pairing (postsynaptic spiking before presynaptic stimulation of MF) which in the presence of AMPA/kainate receptor antagonists leads to LTD. Similar experiments should be repeated in the absence of ionotropic glutamatergic blockers to see whether presynaptic kainate receptors modulate also this form of synaptic plasticity.

Another interesting form of synaptic plasticity whih has been described at MF-CA3 synapses of juvenile animals consists in a persistent depression of AMPA-mediated synaptic responses following a transient tonic depolarization of CA3 principal cells (Ho et al., 2007) or bursts firing induced in pyramidal cells by brief trains of suprathreshold current pulses (Ho et al., 2009). Both protocols lead to a rise in postsynaptic calcium and a switch of calcium permeable AMPA receptors into calcium impermeable ones. Early in postnatal development however, the main neurotransmitter released from MF terminals is GABA (Safiulina et al., 2006) and consistent with this study, during my thesis work I have found that GYKI, a selective antagonist of AMPA receptors does not modify the amplitude, latency, rise time or kinetics of individual MF-evoked responses indicating that AMPA receptors do not contribute to synaptic currents. Safiulina et al. (2006) have found that, in some cases, increasing the strength of MF stimulation can lead to the release of both GABA and glutamate probably from the same fiber. This is in line with the work of Marchal and Mulle (2004), performed in the presence of bicuculline which has documented the presence of MF-mediated AMPA responses only after P3. It would be interesting to see whether a persistent depression of AMPA-mediated synaptic currents could be induced by pairing (after P3) GDPs with MF stimulation (in the presence of $GABA_A$ receptor antagonists). In comparison with the transient membrane depolarization or with bursts firing, GDPs would certainly represent a more “physiological” signal. This would allow ascertaining whether GDPs contribute to the developmental shift of calcium-permeable AMPA receptors into calcium impermeable. In previous work from the group of McBain, it was found that the expression of calcium-permeable AMPA receptors on CA3 pyramidal cells is limited to the first three weeks of postnatal life and is regulated by the PDZ domain containing protein interacting with C kinase (PICK1). It would be also of interest to see whether the pairing procedure interferes with the PDZ domain and/or with PICK1 function. If this is the case, by disrupting the interaction between PICK1 and calcium permeable AMPA receptors (using a small interfering

peptide, the EVKI; Ho et al., 2007) should abolish synaptic depression as well as the shift of AMPA receptors from calcium-permeable to calcium impermeable.

In the adult hippocampus, calcium-permeable AMPA receptors have been shown to be expressed mainly on GABAergic interneurons. Parallel experiments may allow investigating whether a similar procedure can shift the expression of calcium permeable AMPA receptors into calcium impermeable on GABAergic interneurons. To this aim we can take advantage of GIN mice, present in our colony, which express EGFP in a subpopulation of GABAergic interneurons containing somatostatin. These interneurons which are highly expressed in stratum orines and project to stratum lacunosum moleculare (O-LM) are actively involved in rhythmogenesis. In preliminary experiments from our lab, the presence of calcium permeable AMPA receptors on O-LM interneurons has been well documented already at P10-P15 when GDPs are still present.

In adult animals, markers of the GABAergic phenotype can be transiently expressed in granule cells of the dentate gyrus during epileptiform activity induced by convulsants, by electrical stimulation of the perforant pathway or kindling (Gutiérrez, 2005). In these animals, the upregulation of GAD 67 and its mRNA in granule cells has been found to be associated with the release of GABA from MF terminals. This gives rise to monosynaptic $GABA_A$-mediated responses in CA3 principal cells which express $GABA_A$ receptors just in apposition to presynaptic MF terminals (Bergersen et al. 2003). It would be of interest to see whether GABA release from MF terminals of seizured animals is controlled by presynaptic GluK1 receptors in the same way as in immature MF terminals. Usually, activation of kainate autoreceptors localized on adult glutamatergic mossy fibres has been show to enhance glutamate release particularly during frequency-dependent facilitation, a form of short-term plasticity characteristic of these synapses (Contractor et al. 2001; Lauri et al. 2001; Schmitz et al. 2001; but see Kwon and Castillo, 2008). Although in animal models of epilepsy GABA released from MF has been shown to exert a strong inhibitory control on CA3 principal cells and interneurons (with subsequent disinhibition) it is unclear whether this mechanism may protect or facilitate seizures. In addition, presynaptic auto ($GABA_A$ and $GABA_B$) and heteroreceptors (kainate) may control the release of this neurotransmitter and activity-dependent plasticity processes such as STDP. Pairing stimulation of MF with postsynaptic spiking and vice versa in animals subjected to seizures, would allow elucidating how information from granule cells to the CA3 associative network is conveyed and processed.

BIBLIOGRAPHY

Acsády L, Kamondi A, Sík A, Freund T, Buzsáki G (1998) GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J Neurosci 18:3386-403.

Aguado F, Carmona MA, Pozas E, Aguiló A, Martínez-Guijarro FJ, Alcantara S, Borrell V, Yuste R, Ibañez CF, Soriano E (2003) BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2. Development 130:1267-80.

Amaral DG (1978) A Golgi study of cell types in the hilar region of the hippocampus in the rat. J Comp Neurol 182:851-914.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31:571-91.

Amico C, Marchetti C, Nobile M, Usai C (1995) Pharmacological types of calcium channels and their modulation by baclofen in cerebellar granules. J Neurosci 15:2839-48.

Andersen P, Eccles JC, Loyning Y (1964) Pathway of postsynaptic inhibition in the hippocampus. J Neurophysiol 27:608-19.

Andersen P, Blackstad TW, Lömo T (1966a) Location and identification of excitatory synapses on hippocampal pyramidal cells. Exp Brain Res 1:236-48.

Andersen P, Holmqvist B, Voorhoeve PE (1966b) Excitatory synapses on hippocampal apical dendrites activated by entorhinal stimulation. Acta Physiol Scand 66:461-72.

Araque A, Li N, Doyle RT, Haydon PG (2000) SNARE protein-dependent glutamate release from astrocytes. J Neurosci 20:666-73.

Araque A, Martín ED, Perea G, Arellano JI, Buño E (2002) Synaptically released acetylcholine evokes Ca^{2+} elevations in astrocytes in hippocampal slices. J Neurosci 22:2443-50.

Balena T, Woodin MA (2008) Coincident pre- and postsynaptic activity downregulates NKCC1 to hyperpolarize E(Cl) during development. Eur J Neurosci 27:2402-12.

Bannister NJ, Larkman AU (1995) Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: II. Spine distributions. J Comp Neurol 360:161-71.

Baraban SC, Tallent MK (2004) Interneuron Diversity series: Interneuronal neuropeptides--endogenous regulators of neuronal excitability. Trends Neurosci 27:135-42.

Bartus RT, Dear RL 3rd, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408-14.

Bayer SA (1980a) Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. J Comp Neurol 190:87-114.

Bayer SA (1980b) Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. J Comp Neurol 190:115-34.

Bear MF, Abraham WC (1996) Long-term depression in hippocampus. Annu Rev Neurosci 19:437-62.

Bell CC, Han VZ, Sugawara Y, Grant K (1997) Synaptic plasticity in a cerebellum-like structure depends on temporal order. Nature 387:278-81.

Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol 416:303-25.

Ben-Ari Y (2002) Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728-39.

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215-84.

Bergersen L, Ruiz A, Bjaalie JG, Kullmann DM, Gundersen V (2003) GABA and GABAA receptors at hippocampal mossy fibre synapses. Eur J Neurosci 18:931-41.

Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, Götz M (2007) Functional properties of neurons derived form in vitro reprogrammed postnatal astroglia. J Neurosci 27:8654-64.

Bettler B, Kaupmann K, Mosbacher J, Gassmann M (2004) Molecular structure and physiological functions of GABA(B) receptors. Physiol Rev 84:835-67.

Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, Pozzan T, Volterra A (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391:281-5.

Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J Neurosci 18:10464-72.

Blackstad TW, Kjaerheim A (1961) Special axo-dendritic synapses in the hippocampal cortex: electron and light microscopic studies on the layer of mossy fibers. J Comp Neurol 117:133-59.

Blaesse P, Airaksinen MS, Rivera C, Kaila K (2009) Cation-chloride cotransporters and neuronal function. Neuron 61:820-38.

Blatow M, Caputi A, Burnashev N, Monyer H, Rozov A (2003) Ca2+ buffer saturation underlies paired pulse facilitation in calbindin-D28k-containing terminals. Neuron 38:79-88.

Bliss TV, 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 232:331–356.

Bolea S, Avignone E, Berretta N, Sanchez-Andres JV, Cherubini E (1999) Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. J Neurophysiol 81:2095-102.

Bolshakov VY, Siegelbaum SA (1994) Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264:1148-52.

Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA (1996) Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. J Comp Neurol 366:271-92

Buhl EH, Halasy K, Somogyi P (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. Nature 368:823-8.

Bunge RP (1968) Glial cells and the central myelin sheath. Physiol Rev 48:197-251.

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

Buzsáki G (1984) Feed-forward inhibition in the hippocampal formation. Prog Neurobiol 22:131-53.

Buzsáki G, Chrobak JJ (1995) Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. Curr Opin Neurobiol 5:504-10.

Buzsáki G, Draguhn A (2004) Neuronal oscillations in cortical networks. Science 304:1926-9.

Caillard O, Ben-Ari Y, Gaiarsa JL (1999) Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. J Physiol 518:109-19.

Caillard O, Ben-Ari Y, Gaïarsa JL (2000) Activation of presynaptic and postsynaptic ryanodine-sensitive calcium stores is required for the induction of long-term depression at GABAergic synapses in the neonatal rat hippocampus. J Neurosci 20:RC94.

Cancedda L, Fiumelli H, Chen K, Poo MM (2007) Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. J Neurosci 27:5224-35.

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a Hebbian learning rule. Annu Rev Neurosci 31:25-46

Cauli B, Audinat E, Lambolez B, Angulo MC, Ropert N, Tsuzuki K, Hestrin S, Rossier J (1997) Molecular and physiological diversity of cortical nonpyramidal cells. J Neurosci 17:3894-906.

Chaudhry FA, Reimer RJ, Bellocchio EE, Danbolt NC, Osen KK, Edwards RH, Storm-Mathisen J (1998) The vesicular GABA transporter, VGAT, localizes to synaptic vesicles in sets of glycinergic as well as GABAergic neurons. J Neurosci 18:9733-50.

Chen G, Trombley PQ, van den Pol AN (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. J Physiol 494:451–464.

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515-9.

Cherubini E, Conti F (2001) Genserating diversity at GABAergic synapses. Trends Neurosci 24:155-62.

Chicurel ME, Harris KM (1992) Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. J Comp Neurol. 325:169-82.

Chudotvorova I, Ivanov A, Rama S, Hubner CA, Pellegrino C, Ben Ari Y, Medina I (2005) Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. J Physiol 566:671–679.

Cobb SR, Buhl EH, Halasy K, Paulsen O, Somogyi P (1995) Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. Nature 378:75-8.

Colin-Le Brun I, Ferrand N, Caillard O, Tosetti P, Ben-Ari Y, Gaïarsa JL (2004) Spontaneous synaptic activity is required for the formation of functional GABAergic synapses in the developing rat hippocampus. J Physiol 559:129-39.

Contractor A, Swanson G, Heinemann SF (2001) Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. Neuron 29(1):209-16.

Couve A, Moss SJ, Pangalos MN (2000) GABAB receptors: a new paradigm in G protein signaling. Mol Cell Neurosci 16:296-312.

Dammerman RS, Flint AC, Noctor S, Kriegstein AR (2000) An excitatory GABAergic plexus in developing neocortical layer 1. J Neurophysiol 84:428-34.

Dan Y, Poo MM (2006) Spike timing-dependent plasticity: from synapse to perception. Physiol Rev 86:1033-48.

D'Ascenzo M, Fellin T, Terunuma M, Revilla-Sanchez R, Meaney DF, Auberson YP, Moss SJ, Haydon PG (2007) mGluR5 stimulates gliotransmission in the nucleus accumbens. Proc Natl Acad Sci U S A. 104:1995-2000.

de la Prida LM, Huberfeld G, Cohen I, Miles R (2006) Threshold behavior in the initiation of hippocampal population bursts. Neuron 49:131-42.

Debanne D, Shulz DE, Fregnac Y (1998) Activity-dependent regulation of 'on' and 'off' responses in cat visual cortical receptive fields. J Physiol 508:523-48.

Delgado R, Carlin A, Airaghi L, Demitri MT, Meda L, Galimberti D, Baron P, Lipton JM, Catania A (1998) Melanocortin peptides inhibit production of proinflammatory cytokines and nitric oxide by activated microglia. J Leukoc Biol 63:740-5.

Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L (2002) Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 36:1051-61.

Doetsch F, Scharff C (2001) Challenges for brain repair: insights from adult neurogenesis in birds and mammals. Brain Behav Evol 58:306-22.

Doetsch F (2003) The glial identity of neural stem cells. Nat Neurosci 6:1127-34.

Doherty JJ, Alagarsamy S, Bough KJ, Conn PJ, Dingledine R, Mott DD (2004) Metabotropic glutamate receptors modulate feedback inhibition in a developmentally regulated manner in rat dentate gyrus. J Physiol 561:395-401.

Domenici MR, Berretta N, Cherubini E (1998) Two distinct forms of long-term depression coexist at the mossy fiber-CA3 synapse in the hippocampus during development. Proc Natl Acad Sci U S A. 95:8310-5.

Dupuy ST, Houser CR (1997) Developmental changes in GABA neurons of the rat dentate gyrus: an in situ hybridization and birthdating study. J Comp Neurol 389:402-18.

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

Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ (2005) NKCC1 transporter facilitates seizures in the developing brain. Nat Med 11:1205-13.

Eccles JC (1964) The Physiology of Synapse.

Egger V, Feldmeyer D, Sakmann B(1999) Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. Nat Neurosci 2:1098-105.

Eilers J, Plant TD, Marandi N, Konnerth A (2001) GABA-mediated Ca2+ signalling in developing rat cerebellar Purkinje neurones. J Physiol 536:429-37.

Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA(A) receptor signalling. Prog Brain Res 160:59-87.

Feller MB, Butts DA, Aaron HL, Rokhsar DS, Shatz CJ (1997) Dynamic processes shape spatiotemporal properties of retinal waves. Neuron 19:293-306.

Fellin T, D'Ascenzo M, Haydon PG (2007) Astrocytes control neuronal excitability in the nucleus accumbens. ScientificWorldJournal 7:89-97.

Fiumelli H, Cancedda L, Poo MM (2005) Modulation of GABAergic transmission by activity via postsynaptic Ca^{2+}-dependent regulation of KCC2 function. Neuron 48:773-86.

Freund TF and Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6: 347-470.

Freund TF (2003) Interneuron diversity series: Rhythm and mood in perisomatic inhibition. Trend Neurosci 26:489-95.

Frotscher M, Jonas P, Sloviter RS (2006) Synapses formed by normal and abnormal hippocampal mossy fibers. Cell Tissue Res 326:361-7.

Ganguly K, Schinder AF, Wong ST, Poo M (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell 105:521-32.

Garaschuk O, Hanse E, Konnerth A (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. J Physiol 507:219-36.

Gasparini S, Saviane C, Voronin LL, Cherubini E (2000) Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc Natl Acad Sci U S A. 97:9741-6.

Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439:589-93.

Gerber U, Gähwiler BH (1994) GABAB and adenosine receptors mediate enhancement of the K+ current, IAHP, by reducing adenylyl cyclase activity in rat CA3 hippocampal neurons. J Neurophysiol 72:2360-7.

Gray EG (1959) Electron microscopy of synaptic contacts on dendritic spines of the cerebral cortex. Nature 183:1592-3.

Groc L, Gustafsson B, Hanse E (2002) Spontaneous unitary synaptic activity in CA1 pyramidal neurons during early postnatal development: constant contribution of AMPA and NMDA receptors. J Neurosci 22:5552-62.

Gubellini P, Ben-Ari Y, Gaïarsa JL (2001) Activity- and age-dependent GABAergic synaptic plasticity in the developing rat hippocampus. Eur J Neurosci 14:1937-46.

Gubellini P, Ben-Ari Y, Gaïarsa JL (2005) Endogenous neurotrophins are required for the induction of GABAergic long-term potentiation in the neonatal rat hippocampus. J Neurosci 25:5796-802.

Gulyás AI, Miles R, Hájos N, Freund TF (1993) Precision and variability in postsynaptic target selection of inhibitory cells in the hippocampal CA3 region. Eur J Neuroci 5:1729-51.

Gulyás AI, Megías M, Emrci Z, Freund TF (1999) Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci 19:10082-97.

Gustafsson B, Wigström H, Abraham WC, Huang YY (1987) Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. J Neurosci 7:774–780.

Gutiérrez R, Heinemann U (2001) Kindling induces transient fast inhibition in the dentate gyrus--CA3 projection. Eur J Neurosci 13:1371-9.

Gutiérrez R, Romo-Parra H, Maqueda J, Vivar C, Ramìrez M, Morales MA, Lamas M (2003) Plasticity of the GABAergic phenotype of the "glutamatergic" granule cells of the rat dentate gyrus. J Neurosci 23:5594-8.

Gutiérrez R (2005) The dual glutamatergic-GABAergic phenotype of hippocampal granule cells. Trends Neurosci 28:297-303.

Hashimoto T, Kuriyama K (1997) In vivo evidence that GABA(B) receptors are negatively coupled to adenylate cyclase in rat striatum. J Neurochem 69:365-70.

Hassinger TD, Atkinson PB, Strecker GJ, Whalen LR, Dudek FE, Kossel AH, Kater SB (1995) Evidence for glutamate-mediated activation of hippocampal neurons by glial calcium waves. J Neurobiol 28:159-70.

Hebb DO (1949) The organization of behavior, New York: Wiley.

Hennou S, Khalilov I, Diabira D, Ben-Ari Y, Gozlan H (2002) Early sequential formation of functional GABA(A) and glutamatergic synapses on CA1 interneurons of the rat foetal hippocampus. Eur J Neurosci 16:197-208.

Henze DA, Urban NN, Barrionuevo G (2000) The multifarious hippocampal mossy fiber pathway: a review. Neuroscience 98:407-27.

Hetier E, Ayala J, Bousseau A, Prochiantz A (1991) Modulation of interleukin-1 and tumor necrosis factor expression by beta-adrenergic agonists in mouse ameboid microglial cells. Exp Brain Res 86:407-13.

Ho MT, Pelkey KA, Topolnik L, Petralia RS, Takamiya K, Xia J, Huganir RL, Lacaille JC, McBain CJ (2007) Developmental expression of Ca2+-permeable AMPA receptors underlies

depolarization-induced long-term depression at mossy fiber CA3 pyramid synapses. J Neurosci 27:11651-62.

Ho MT, Pelkey KA, Pelletier JG, Huganir RL, Lacaille JC, McBain CJ (2009) Burst firing induces postsynaptic LTD at developing mossy fiber-CA3 pyramid synapses. J Physiol. 2009 Jul 27. [Epub ahead of print]

Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD (2000) Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290:1768-71.

Hosokawa Y, Sciancalepore M, Stratta F, Martina M, Cherubini E (1994) Developmental changes in spontaneous GABAA-mediated synaptic events in rat hippocampal CA3 neurons. Eur J Neurosci 6:805-13.

Hubel DH, Wiesel TN (1959) Receptive fields of single neurones in the cat's striate cortex. J Physiol 148:574–591.

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160:106–154.

Hubel DH, Wiesel TN (1963) Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. J Neurophysiol 26:994–1002.

Hubel DH & Wiesel TN (1968) Receptive fields and functional architecture of monkey striate cortex. J Physiol 195:215–243.

Isaac JT (2003) Postsynaptic silent synapses: evidence and mechanisms. Neuropharmacology 45:450-60.

Jang IS, Nakamura M, Ito Y, Akaike N (2006) Presynaptic GABAA receptors facilitate spontaneous glutamate release from presynaptic terminals on mechanically dissociated rat CA3 pyramidal neurons. Neuroscience 138:25-35.

Kaila K, Voipio J, Paalasmaa P, Pasternack M, Deisz RA (1993) The role of bicarbonate in GABAA receptor-mediated IPSPs of rat neocortical neurones. J Physiol 464:273-89.

Kaila K (1994) Ionic basis of GABA(A) receptor channel function in the nervous system. Prog Neurobiol 42:489–537.

Kamme F, Salunga R, Yu J, Tran DT, Zhu J, Luo L, Bittner A, Guo HQ, Miller N, Wan J, Erlander M (2003) Single-cell microarray analysis in hippocampus CA1: demonstration and validation of cellular heterogeneity. J Neurosci 23:3607-15

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030-8.

Kang J, Jiang L, Goldman SA, Nedergaard M (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat Neurosci 1:683-92.

Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci U S A. 101:3967-72.

Katona I, Acsády L, Freund TF (1999) Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus. Neuroscience 88:37-55.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133-8.

Khazipov R, Esclapez M, Caillard O, Bernard C, Khalilov I, Tyzio R, Hirsch J, Dzhala V, Berger B, Ben-Ari Y (2001) Early development of neuronal activity in the primate hippocampus in utero. J Neurosci 21:9770-81.

Khirug S, Huttu K, Ludwig A, Smirnov S, Voipio J, Rivera C, Kaila K, Khiroug L (2005) Distinct properties of functional KCC2 expression in immature mouse hippocampal neurons in culture and in acute slices. Eur J Neurosci 21:899-904.

Kim JJ, Baxter MG (2001) Multiple brain-memory systems: the whole does not equal the sum of its parts. Trends Neurosci 24:324-30.

Klausberger T, Somogyi P (2008) Neuronal diversity and Temporal Dynamics: The Unity of Hippocampal Circuit Operations. Science 321:53-57.

Komuro H, Rakic P (1998) Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca^{2+} fluctuations. J Neurobiol 37:110-30.

Kosaka T, Katsumaru H, Hama K, Wu JY, Heizmann CW (1987) GABAergic neurons containing the Ca^{2+}-binding protein parvalbumin in the rat hippocampus and dentate gyrus. Brain Res 419:119-30.

Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19:312-8.

Kuczewski N, Langlois A, Fiorentino H, Bonnet S, Marissal T, Diabira D, Ferrand N, Porcher C, Gaiarsa JL (2008a) Spontaneous glutamatergic activity induces a BDNF-dependent potentiation of GABAergic synapses in the newborn rat hippocampus. J Physiol 586:5119-28.

Kuczewski N, Porcher C, Ferrand N, Fiorentino H, Pellegrino C, Kolarow R, Lessmann V, Medina I, Gaiarsa JL (2008b) Backpropagating action potentials trigger dendritic release of BDNF during spontaneous network activity. J Neurosci 28:7013-23.

Kuner T, Augustine GJ (2000) A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. Neuron 27:447-59.

Kwon HB, Castillo PE (2008) Role of glutamate autoreceptors at hippocampal mossy fiber synapses. Neuron 60:1082-1094.

Lacaille JC (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampal slices in viro. J Neurophysiol 66:1441-54.

Lacaille JC, Mueller AL, Kunkel DD, Schwartzkroin PA (1987) Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. J Neurosci 7:1979-93.

Lamas M, Gómez-Lira G, Gutiérrez R (2001) Vesicular GABA transporter mRNA expression in the dentate gyrus and in mossy fiber synaptosomes. Brain Res Mol Brain Res 93:209-14.

Lamsa K, Palva JM, Ruusuvuori E, Kaila K, Taira T (2000) Synaptic GABA(A) activation inhibits AMPA-kainate receptor-mediated bursting in the newborn (P0-P2) rat hippocampus. J Neurophysiol 83:359-66.

Lanthorn TH, Ganong AH, Cotman CW (1984) 2-Amino-4-phosphonobutyrate selectively blocks mossy fiber-CA3 responses in guinea pig but not rat hippocampus. Brain Res 290:174-8.

Lauri SE, Bortolotto ZA, Bleakman D, Ornstein PL, Lodge D, Isaac JT, Collingridge GL (2001) A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. Neuron 32:697-709.

Lawson LJ, Perry VH, Dri P, Gordon S (1990) Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 9:151-70.

Lee J, Taira T, Pihlaja P, Ransom BR, Kaila K (1996) Effects of CO2 on excitatory transmission apparently caused by changes in intracellular pH in the rat hippocampal slice. Brain Res 706:210-6.

Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R (1997) Ca2+ oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA receptors in the neonatal hippocampus. Neuron 18:243-55.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsáki G (2002) Correlated bursts of activity in the neonatal hippocampus in vivo. Science 296:2049-52.

Lerma J, Paternain AV, Rodríguez-Moreno A, López-García JC (2001) Molecular physiology of kainate receptors. Physiol Rev 81:971-98.

Ling EA, Wong WC (1993) The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. Glia 7:9-18.

Lorente de Nó R (1934) Studies on the structure of the cerebral cortex – II. Continuation of the study of the ammonic system. J Psychol 46:113-177.

LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15:1287-98.

Losonczy A, Zhang L, Shigemoto R, Somogyi P, Nusser Z (2002) Cell type dependence and variability in the short-term plasticity of EPSCs in identified mouse hippocampal interneurons. J Physiol 542:193-210.

Ludwig A, Li H, Saarma M, Kaila K, Rivera C (2003) Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. Eur J Neurosci 18:3199-206.

Luhmann HJ, Prince DA (1991) Postnatal maturation of the GABAergic system in rat neocortex. J Neurophysiol 65:247-63.

Lüscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA (1997) G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19:687-95.

Maccaferri G, Roberts JD, Szucs P, Cottingham CA, Somogyi P (2000) Cell surface domain specific postsynaptic currents evoked by identified GABAergic neurons in rat hippocampus in vitro. J Physiol 524:91-116.

Macdonald RL and Olsen RW (1994) GABAA receptor channels. Annu Rev Neurosci 17:569-602.

Maggi L, Le Magueresse C, Changeux JP, Cherubini E (2003) Nicotine activates immature "silent" connections in the developing hippocampus. Proc Natl Acad Sci U S A. 100:2059-64.

Manent JB, Represa A (2007) Neurotransmitters and brain maturation: early paracrine actions of GABA and glutamate modulate neuronal migration. Neuroscientist 13:268-79.

Marchal C, Mulle C (2004) Postnatal maturation of mossy fibre excitatory transmission in mouse CA3 pyramidal cells: a potential role for kainate receptors. J Physiol 561:27-37.

Marchionni I, Omrani A, Cherubini E (2007) In the developing rat hippocampus a tonic GABAA-mediated conductance selectively enhances the glutamatergic drive of principal cells. J Physiol 581:515-28

Maric D, Liu QY, Maric I, Chaudry S, Chang YH, Smith SV, Sieghart W, Fritschy JM, Barker JL (2001) GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl- channels. J Neurosci 21:2343-60.

Markova O, Mukhtarov M, Real E, Jacob Y, Bregestovski P (2008) Genetically encoded chloride indicator with improved sensitivity. J Neurosci Methods 170:67-76.

Martina M, Schultz JH, Ehmke H, Monyer H, Jonas P (1998) Functional and molecular differences between voltage-gated K+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. J Neurosci 18:8111-25.

McKinney M, Jacksonville MC (2005) Brain cholinergic vulnerability: relevance to behavior and disease. Biochem Pharmacol 70:1115-24.

McLean HA, Caillard O, Ben-Ari Y, Gaiarsa JL (1996) Bidirectional plasticity expressed by GABAergic synapses in the neonatal rat hippocampus. J Physiol 496:471-7.

Megías M, Emri Z, Freund TF, Gulyás AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102:527-40.

Menendez de la Prida L, Sanchez-Andres JV (1999) Nonlinear transfer function encodes synchronization in a neural network from the mammalian brain. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics 60:3239-43.

Menendez de la Prida L, Sanchez-Andres JV (2000) Heterogeneous populations of cells mediate spontaneous synchronous bursting in the developing hippocampus through a frequency-dependent mechanism. Neuroscience 97:227-41.

Miles R, Wong RK (1987) Latent synaptic pathways revealed after tetanic stimulation in the hippocampus. Nature 329:724-6.

Milner B, Squire LR, Kandel ER (1998) Cognitive neuroscience and the study of memory. Neuron 20:445-68.

Mintz IM, Bean BP (1993) GABAB receptor inhibition of P-type Ca2+ channels in central neurons. Neuron 10:889-98.

Mohajerani MH, Cherubini E (2005) Spontaneous recurrent network activity in organotypic rat hippocampal slices. Eur J Neurosci 22:107-18.

Mohajerani MH, Sivakumaran S, Zacchi P, Aguilera P, Cherubini E (2007) Correlated network activity enhances synaptic efficacy via BDNF and the ERK pathway at immature CA3 CA1 connections in the hippocampus. Proc Natl Acad Sci U S A. 104:13176-81.

Morin F, Beaulieu C, Lacaille JC (1996) Membrane properties and synaptic currents evoked in CA1 interneuron subtypes in rat hippocampal slices. J Neurophysiol 76:1-16.

Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G (2005) Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. Proc Natl Acad Sci U S A. 102:5606-11.

Mukhtarov M, Markova O, Real E, Jacob Y, Buldakova S, Bregestovski P (2008) Monitoring of chloride and activity of glycine receptor channels using genetically encoded fluorescent sensors. Philos Transact A Math Phys Eng Sci 366:3445-62.

Neumann H, Misgeld T, Matsumuro K, Wekerle H (1998) Neurotrophins inhibit major histocompatibility class II inducibility of microglia: involvement of the p75 neurotrophin receptor. Proc Natl Acad Sci U S A 95:5779-84.

Newman EA (2001) Propagation of intercellular calcium waves in retinal astrocytes and Muller cells. J Neurosci 21:2215-23.

Nicoll RA, Schmitz D (2005) Synaptic plasticity at hippocampal mossy fibre synapses. Nat Rev Neurosci 6:863-76.

Obata K, Oide M, Tanaka H (1978) Excitatory and inhibitory actions of GABA and glycine on embryonic chick spinal neurons in culture. Brain Res 144:179-84.

O'Donovan MJ (1999) The origin of spontaneous activity in developing networks of the vertebrate nervous system. Curr Opin Neurobiol 9:94-104.

O'Keefe J (1983) Two spatial systems in the rat brain-implications for the neural basis of learning and memory. Prog Brain Res 58:453-64.

Oliva AA Jr, Jiang M, Lam T, Smith KL, Swann JW (2000) Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J Neurosci 20:3354-68.

Owens DF, Kriegstein AR (2002) Is there more to GABA than synaptic inhibition? Nat Rev Neurosci 3:715-27.

Owens DF, Boyce LH, Davis MB, Kriegstein AR (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414-23.

Pasti L, Zonta M, Pozzan T, Vicini S, Carmignoto G (2001) Cytosolic calcium oscillations in astrocytes may regulate exocytotic release of glutamate. J Neurosci 21:477-84.

Paterson D, Nordberg A (2000) Neuronal nicotinic receptors in the human brain. Prog Neurobiol 61:75-111.

Payne JA, Rivera C, Voipio J, Kaila K (2003) Cation-chloride co-transporters in neuronal communication, development and trauma. Trends Neurosci 26:199-206.

Pfeffer CK, Stein V, Keating DJ, Maier H, Rinke I, Rudhard Y, Hentschke M, Rune GM, Jentsch TJ, Hübner CA (2009) NKCC1-dependent GABAergic excitation drives synaptic network maturation during early hippocampal development. J Neurosci 29:3419-30.

Pickard L, Noël J, Henley JM, Collingridge GL, Molnar E (2000) Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. J Neurosci 20:7922-31.

Poncer JC, McKinney RA, Gähwiler BH, Thompson SM (1997) Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. Neuron 18:463-72.

Porter JT, McCarthy KD (1996) Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J Neurosci 16:5073-81.

Porter JT, McCarthy KD (1997) Astrocytic neurotransmitter receptors in situ and in vivo. Prog Neurobiol 51:439-55.

Rakic P (1995) Radial versus tangential migration of neuronal clones in the developing cerebral cortex. Proc Natl Acad Sci U S A. 92:11323-7.

Ramón y Cajal S (1893) Estructura del asta de Ammon y fascia dentate. *Anat. de la Socied. Espan. de Historia natural* t. XXII (Ann Soc Esp Hist Nat 22).

Ramón y Cajal S (1904) Sobre las variaciones normales y patológicas de las neurofibrillas. *Trab. d. Labor. d. Invest. biol.* t. III, f. 1.

Ramón y Cajal S (1911) *Histologie du Systeme Nerveux de l'Homme et des Vertebretes.* Paris: A. Maloine.

Renger JJ, Egles C, Liu G (2001) A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation. Neuron 29:469-84.

Ribak CE (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. J Neurocytol 7:461-78.

Richards KS, Bommert K, Szabo G, Miles R (2007) Differential expression of Na+/K+-ATPase alpha-subunits in mouse hippocampal interneurones and pyramidal cells. J Physiol 585:491-505.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251-5.

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M (2002) BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. J Cell Biol 159:747-52.

Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipilä S, Payne JA, Minichiello L, Saarma M, Kaila K (2004) Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci 24:4683-91.

Rivera C, Voipio J, Kaila K (2005) Two developmental switches in GABAergic signalling: the K^+-Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. J Physiol 562:27-36.

Roberts PJ and Sharif NA (1981) Radioreceptor binding studies with glutamate and aspartate. Adv Biochem Psychopharmacol 27:295-305.

Romo-Parra H, Vivar C, Maqueda J, Morales MA, Gutiérrez R (2003) Activity-dependent induction of multitransmitter signaling onto pyramidal cells and interneurons of hippocampal area CA3. J Neurophysiol 89:3155-67.

Roos A, Boron WF (1981) Regulation of intracellular pH in barnacle muscle. Kroc Found Ser 15:205-19.

Ruiz A, Fabian-Fine R, Scott R, Walker MC, Rusakov DA, Kullmann DM (2003) GABAA receptors at hippocampal mossy fibers. Neuron 39:961-73.

Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597-608.

Sakaba T, Neher E (2003) Direct modulation of synaptic vesicle priming by GABA(B) receptor activation at a glutamatergic synapse. Nature 424:775-8.

Salin PA, Scanziani M, Malenka RC, Nicoll RA (1996) Distinct short-term plasticity at two excitatory synapses in the hippocampus. Proc Natl Acad Sci U S A. 93:13304-9.

Sanabria ER, Su H, Yaari Y (2001) Initiation of network bursts by Ca2+-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. J Physiol 532:205-16.

Savic N, Pedarzani P, Sciancalepore M (2001) Medium afterhyperpolarization and firing pattern modulation in interneurons of stratum radiatum in the CA3 hippocampal region. J Neurophysiol 85:1986-97.

Schmitz D, Mellor J, Frerking M, Nicoll RA (2001) Presynaptic kainate receptors at hippocampal mossy fiber synapses. Proc Natl Acad Sci U S A 98:11003-11008.

Schwarzer C, Sperk G (1995) Hippocampal granule cells express glutamic acid decarboxylase-67 after limbic seizures in the rat. Neuroscience 69:705-9.

Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S, Mizuno N (1997) Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci 17:7503-22.

Shulga A, Thomas-Crusells J, Sigl T, Blaesse A, Mestres P, Meyer M, Yan Q, Kaila K, Saarma M, Rivera C, Giehl KM (2008) Posttraumatic GABA(A)-mediated [Ca2+]i increase is essential for the induction of brain-derived neurotrophic factor-dependent survival of mature central neurons. J Neurosci 28:6996-7005.

Sipilä ST, Huttu K, Soltesz I, Voipio J, Kaila K (2005) Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. J Neurosci 25:5280-9.

Sipilä ST, Schuchmann S, Voipio J, Yamada J, Kaila K (2006) The cation-chloride cotransporter NKCC1 promotes sharp waves in the neonatal rat hippocampus. J Physiol 573:765-73.

Sipilä ST, Huttu K, Yamada J, Afzalov R, Voipio J, Blaesse P, Kaila K (2009) Compensatory enhancement of intrinsic spiking upon NKCC1 disruption in neonatal hippocampus. J Neurosci 29:6982-8.

Sivakumaran S, Mohajerani MH, Cherubini E (2009) At immature mossy-fiber-CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA. J Neurosci 29:2637-47.

Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL (1996) Basal expression and induction of glutamate decarboxylase and GABA in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. J Comp Neurol 373:593-618.

Somogyi P, Hodgson Aj, Smith AD, Nunzi MG, Gorio A, Wu JY (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. J Neurosci 4: 2590-603.

Song H, Stevens CF, Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417:39-44.

Spitzer NC (2002) Activity-dependent neuronal differentiation prior to synapse formation: the functions of calcium transients. J Physiol Paris 96:73-80.

Spitzer NC (2004) Coincidence detection enhances appropriate wiring of the nervous system. Proc Natl Acad Sci U S A. 101:5311-2.

Storm-Mathisen J (1977) Localization of transmitter candidates in the brain: the hippocampal formation as a model. Prog Neurobio 8:119-81.

Storm-Mathisen J, Leknes AK, Bore AT, Vaaland JL, Edminson P, Haug FM, Ottersen OP (1983) First visualization of glutamate and GABA in neurones by immunocytochemistry. Nature 301:517-20.

Sugino K, Hempel CM, Miller MN, Hattox AM, Shapiro P, Wu C, Huang ZJ, Nelson SB (2006) Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nat Neurosci 9:99-107.

Supèr H, Soriano E, Uylings HB (1998) The functions of the preplate in development and evolution of the neocortex and hippocampus. Brain Res Brain Res Rev 27:40-64.

Taverna S, Tkatch T, Metz AE, Martina M (2005) Differential expression of TASK channels between horizontal interneurons and pyramidal cells of rat hippocampus. J Neurosci 25:9162-70.

Telfeian AE, Tseng HC, Baybis M, Crino PB, Dichter MA (2003) Differential expression of GABA and glutamate-receptor subunits and enzymes involved in GABA metabolism between electrophysiologically identified hippocampal CA1 pyramidal cells and interneurons. Epilepsia 44:143-9.

Titz S, Hans M, Kelsch W, Lewen A, Swandulla D, Misgeld U (2003) Hyperpolarizing inhibition develops without trophic support by GABA in cultured rat midbrain neurons. J Physiol 550:719–730.

Toth K, Suares G, Lawrence JJ, Philips-Tansey E, McBain CJ (2000) Differential mechanisms of transmission at three types of mossy fiber synapse. J Neurosci 20:8279-89.

Traub RD, Miles R (1991) Multiple modes of neuronal population activity emerge after modifying specific synapses in a model of the CA3 region of the hippocampus. Ann N Y Acad Sci 627:277-90.

Traub RD, Wong RK (1982) Cellular mechanism of neuronal synchronization in epilepsy. Science 216:745-7.

Tyzio R, Represa A, Jorquera I, Ben-Ari Y, Gozlan H, Aniksztejn L (1999) The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. J Neurosci 19:10372-82.

Uchigashima M, Fukaya M, Watanabe M, Kamiya H (2007) Evidence against GABA release from glutamatergic mossy fiber terminals in the developing hippocampus. J Neurosci 27:8088-100.

van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH (2002) Functional neurogenesis in the adult hippocampus. Nature 415:1030-4.

Voronin LL, Cherubini E (2004) 'Deaf, mute and whispering' silent synapses: their role in synaptic plasticity. J Physiol 557:3-12.

Walker MC, Ruiz A, Kullmann DM (2001) Monosynaptic GABAergic signaling from dentate to CA3 with a pharmacological and physiological profile typical of mossy fiber synapses. Neuron 29:703-15.

Wang DD, Kriegstein AR (2008) GABA regulates excitatory synapse formation in the neocortex via NMDA receptor activation. J Neurosci 28:5547-58.

Wang J, Reichling DB, Kyrozis A, MacDermott AB (1994) Developmental loss of GABA- and glycine-induced depolarization and Ca^{2+} transients in embryonic rat dorsal horn neurons in culture. Eur J Neurosci 6:1275-80.

Wei R, Jonakait GM (1999) Neurotrophins and the anti-inflammatory agents interleukin-4 (IL-4), IL-10, IL-11 and transforming growth factor-beta1 (TGF-beta1) down-regulate T cell costimulatory molecules B7 and CD40 on cultured rat microglia. J Neuroimmunol 95:8-18.

Witter MP, Van Hoesen GW, Amaral DG (1989) Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. J Neurosci 9:216-28.

Wittner L, Miles R (2007) Factors defining a pacemaker region for synchrony in the hippocampus. J Physiol 584:867-83.

Wojcik SM, Katsurabayashi S, Guillemin I, Friauf E, Rosenmund C, Brose N, Rhee JS (2006) A shared vesicular carrier allows synaptic corelease of GABA and glycine. Neuron 50:575-87.

Woodin MA, Ganguly K, Poo MM (2003) Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl- transporter activity. Neuron 39:807-20.

Wright GJ, Puklavec MJ, Willis AC, Hoek RM, Sedgwick JD, Brown MH, Barclay AN (2000) Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. Immunity 13:233-42.

Yasuda H, Barth AL, Stellwagen D, Malenka RC (2003) A developmental switch in the signaling cascades for LTP induction. Nat Neurosci 6:15-6.

Yuste R, Katz LC (1991) Control of postsynaptic Ca^{2+} influx in developing neocortex by excitatory and inhibitory neurotransmitters. Neuron 6:333-44.

Zhang LI, Poo MM (2001) Electrical activity and development of neural circuits. Nat Neurosci 4 Suppl:1207-14.

ANNEX

Paper N.2., “At immature mossy fiber-CA3 synapses, correlated pre and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA.”, was the subject of a Journal Club review in the Journal of Neuroscience, by Lanore F, Rebola N and Carta M.

Spike-timing-dependent plasticity induces presynaptic changes at immature hippocampal mossy fiber synapses.

Lanore F, Rebola N, Carta M.

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Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Spike-Timing-Dependent Plasticity Induces Presynaptic Changes at Immature Hippocampal Mossy Fiber Synapses

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Review of Sivakumaran et al. (http://www.jneurosci.org/cgi/content/full/29/8/2637)

Activity-dependent modulations of synaptic strength play a crucial role in the structural and functional refinement of neuronal connections (Goda and Davis, 2003), which are thought to be the cellular basis of information storage and memory formation. Long-lasting modifications in the efficacy of synaptic transmission can be induced by coincident presynaptic and postsynaptic activity in a form of plasticity known as spike-timing-dependent plasticity (STDP) (Caporale and Dan, 2008). In this form of plasticity, the precise timing and the order of presynaptic and postsynaptic action potentials determine the magnitude and the direction of the change in synaptic strength.

STDP can occur at both glutamatergic and GABAergic synapses, with slightly different induction rules. At glutamatergic synapses, when a postsynaptic action potential follows a presynaptic spike within a window of tens of milliseconds, long-term potentiation (LTP) usually occurs, whereas the reverse order results in depression (LTD). The induction rules for STDP at GABAergic synapses appear to be more variable (Caporale and Dan, 2008). The current hypothesis suggests that the direction of plasticity depends on the peak amplitude of the postsynaptic intracellular calcium concentrations: high calcium levels lead to LTP, whereas low to moderate levels lead to LTD (Caporale and Dan, 2008).

STDP has been already described in many areas of the brain, but so far it was not known whether it occurs at hippocampal mossy fiber synapses made between granule cells and CA3 pyramidal cells (Mf–CA3 synapses). In adult animals, Mf–CA3 synapses are glutamatergic synapses, but during the first week of development, GABA is the main neurotransmitter released (Safiulina et al., 2006). These synapses display several unique features, including low basal release probability, pronounced paired-pulse facilitation, and frequency facilitation. Moreover, Mf–CA3 synapses also display a particular form of LTP that is expressed presynaptically and is independent of NMDA receptor activation. Conversely, Mf–CA3 synapses are commonly assumed not to express the classical NMDA-dependent LTP associated with postsynaptic AMPA-EPSCs.

At Mf–CA3 synapses, whether presynaptic LTP depends on postsynaptic induction mechanisms has been a matter of debate. Williams and Johnston (1989) and Yeckel et al. (1999) argued that presynaptic LTP requires postsynaptic increases in Ca^{2+} and metabotropic glutamate receptor (mGluR) activation. The dependence of mossy fiber presynaptic LTP on postsynaptic mechanisms was further supported by the observation that pairing presynaptic stimulation of mossy fibers with CA3 pyramidal cell depolarization induces LTP at Mf–CA3 synapses (Urban and Barrionuevo, 1996). However, Mellor and Nicoll (2001) showed that mossy fiber LTP is independent of postsynaptic Ca^{2+} and mGluRs receptor activation, arguing that the postsynaptic cell does not participate in inducing synaptic plasticity at Mf–CA3 synapses. Regardless of this controversy, the question that remains unanswered is whether mossy fibers present a Hebbian-like form of LTP.

In a recent study published in *The Journal of Neuroscience*, Sivakumaran et al. (2009) elegantly explored the presence of STDP at hippocampal Mf–CA3 pyramidal cell synapses. They examined this issue during an early postnatal period (postnatal day 2–5) when these synapses are thought to operate mainly through depolarizing $GABA_A$ synaptic currents (Safiulina et al., 2006). Mossy fiber $GABA_A$-mediated synaptic potentials or currents (Mf-GPSPs or Mf-GPSCs) were evoked at 0.05 Hz from a holding potential of −70 mV in the presence of DNQX (20 μM) and D-AP5 (50 μM) to block AMPA- and NMDA-mediated synaptic responses, respectively. Using a paired-pulse stimulation recording paradigm (interstimulus interval 50 ms), the authors recorded from what they classified as "presynaptic silent synapses." These correspond to synapses at which a synaptic response was detected following the

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second stimulus of a pair, but not after the first stimulus. Semantically, the term "silent synapses" should not be used to refer to synapses with a very low probability of release, which are not very active, but also not silent. Although we agree that the difference between the two is subtle and that the term "silent synapses" is commonly used to refer to these synapses with very low probability of release, it is important to distinguish these from postsynaptically silent synapses whose definition is not dependent on the rate of stimulation. Maybe it is more appropriate to use the definition "whispering" synapses, a term already adopted by Voronin and Cherubini (2004).

By applying a pairing paradigm (10 postsynaptic spikes evoked at 0.1 Hz with afferent stimulation) between presynaptic and postsynaptic activity, the authors observed STDP in immature Mf–CA3 synapses. The observed activity-dependent changes in synaptic efficacy strongly relied on the temporal relationship between presynaptic and postsynaptic activation, with a critical window for STDP similar to that described for most glutamatergic and GABAergic synapses. If a positive pairing of postsynaptic spikes 15 ms after single Mf-GPSCs was used, LTP was observed; however, if postsynaptic spikes preceded Mf-GPSCs by 15 ms, LTD was induced.

Coincident presynaptic and postsynaptic activity had been shown previously to modulate synaptic strength at GABAergic synapses when GABA is still depolarizing (Woodin et al., 2003). However, in the previous study, the modulation of synaptic strength was related to a calcium-dependent decrease of the K^+–Cl^- cotransport activity. This seems not to be the mechanism involved in the activity-dependent modulation of GABAergic synaptic responses at immature mossy fiber synapses, since no change in GABA equilibrium potential was observed after pairing.

Interestingly, both LTP and LTD appeared to be induced postsynaptically and expressed presynaptically. In support of the presynaptic expression, the authors observed that LTP was followed by a decrease in paired-pulse ratio, decrease in the number of failures, and increase in the CV^{-2} (inverse squared value of coefficient of variation), whereas LTD was accompanied by opposite changes in these parameters. However, as mentioned by the authors, these parameters should be analyzed with caution, because insertion or removal of postsynaptic receptors could in the same circumstances produce the same effects.

The authors observed that LTP induction by the pairing protocol led to synapse "fully speaking," further supporting the presynaptic locus of expression of the observed LTP. Moreover, in a recent paper the authors showed that the "whispering" state of mossy fiber synapses at this age seems to be related to presynaptic tonic $GABA_B$ receptor activation inhibiting synaptic release (Safiulina and Cherubini, 2009). In future work, it would be interesting to investigate whether "fully speaking" synapse induced by the pairing protocol operates by modulating tonic $GABA_B$ receptor activation.

Mechanistically, Sivakumaran et al. (2009) found that STDP-LTP of GPSCs depended on postsynaptic calcium rise, postsynaptic protein kinase A (PKA) activity, and TrkB [brain-derived neurotrophic factor (BDNF) receptor] receptor activation. The authors hypothesize that the pairing protocol induced an activity-dependent release of BDNF from CA3 pyramidal cells, which in turn acted presynaptically at mossy fiber terminals to increase probability of release. Consequently, exogenous BDNF application did indeed increase synaptic efficacy at immature Mf–CA3 pyramidal cell synapses through a presynaptic mechanism required on PKA activity. To further support BDNF action through presynaptic TrkB receptors it would have been interesting to test the effect of exogenous BDNF application while blocking postsynaptic PKA activity. This experiment would rule out the possible modulation of synaptic transmission by BDNF acting on postsynaptic TrkB receptors as it has been suggested by Huang et al. (2008). While there are substantial evidence that BDNF can act as a retrograde messenger in several other synapses (Poo, 2001), this is the first time that such a phenomenon is reported to occur at Mf–CA3 synapses.

Surprisingly, when the authors blocked LTP induction by using a TrkB receptor antagonist or by reducing postsynaptic calcium rise using an antagonist of voltage-dependent L-type calcium channels (nifedipine) or with patch pipettes containing the calcium chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), they observed LTD of Mf-GPSCs instead. Although the nature of LTD obtained with BAPTA and with nifedipine is not clear, it could simply result from blockade of a calcium-dependent protection mechanism against a decrease in Cl^- conductance as proposed by others (Woodin et al., 2003). The authors stated that the persistent synaptic depression obtained by blocking TrkB receptors was also of presynaptic origin because of the reduced number of successes and the significant increase in paired-pulse ratio. It might suggest the presence of a second pathway of postsynaptic/presynaptic communication that apparently does not depend on postsynaptic calcium rise and BDNF as a retrograde messenger. An interesting possibility would be to test the role of mGluRs, since this synapse can also release glutamate at this age (Safiulina et al., 2006).

An important point that we would also like to underline concerns the induction protocol of STDP. Immature Mf–CA3 synapses are normally "whispering" synapses (i.e., no postsynaptic response is triggered at the first stimulus). How is it then possible to explain that isolated presynaptic stimulations used during the pairing protocol, which should not trigger a postsynaptic response, give rise to these forms of plasticity? Indeed STDP at immature Mf–CA3 synapses requires both presynaptic and postsynaptic activity. A possible explanation is that another (a second) neurotransmitter is released by mossy fiber stimulation during the pairing protocol. Again, glutamate seems like a good candidate. An intriguing possibility is that glutamate released from Mfs during the pairing protocol would activate postsynaptic mGluRs, inducing increase of intracellular calcium levels and in turn BDNF release. In their experiments the authors had blocked ionotropic glutamate receptors, but not mGluRs, leaving this possibility open. It would be of great importance to verify the possible role of these receptors in STDP.

In conclusion, the work of Sivakumaran et al. (2009) describes for the first time a Hebbian-like form of synaptic plasticity at immature hippocampal mossy fiber synapses. This form of plasticity may contribute to the shaping of neuronal connectivity before the establishment of the adult neuronal circuits. Interestingly this new form of plasticity can result in both LTP and LTD, depending on the precise timing of presynaptic versus postsynaptic activity, which are both postsynaptically induced and presynaptically expressed. The current work raises also the need to readdress the role of the postsynaptic component in modulating synaptic plasticity induction at mossy fiber synapses in adult animals.

References

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a Hebbian learning rule. Annu Rev Neurosci 31:25–46.

Goda Y, Davis GW (2003) Mechanisms of synapse assembly and disassembly. Neuron 40:243–264.

Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546–558.

Mellor J, Nicoll RA (2001) Hippocampal mossy fiber LTP is independent of postsynaptic calcium. Nat Neurosci 4:125–126.

Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24–32.

Safiulina VF, Cherubini E (2009) At immature mossy fibers-CA3 connections, activation of presynaptic GABAB receptors by endogenously released GABA contributes to synapses silencing. Front Cell Neurosci 3:1.

Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597–608.

Sivakumaran S, Mohajerani MH, Cherubini E (2009) At immature mossy-fiber–CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA. J Neurosci 29:2637–2647.

Urban NN, Barrionuevo G (1996) Induction of Hebbian and non-Hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. J Neurosci 16:4293–4299.

Voronin LL, Cherubini E (2004) 'Deaf, mute and whispering' silent synapses: their role in synaptic plasticity. J Physiol 557:3–12.

Williams S, Johnston D (1989) Long-term potentiation of hippocampal mossy fiber synapses is blocked by postsynaptic injection of calcium chelators. Neuron 3:583–588.

Woodin MA, Ganguly K, Poo MM (2003) Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl- transporter activity. Neuron 39:807–820.

Yeckel MF, Kapur A, Johnston D (1999) Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. Nat Neurosci 2:625–633.

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