Activity-dependent synaptic plasticity processes in the immature rat hippocampus

Thesis submitted for the degree of "***Doctor Philosophiae***"
S.I.S.S.A. – Neurobiology Sector

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Notes

The work described in this dissertation was carried out at the International School for Advanced Studies, Trieste, between November 2002 and August 2006. All work reported here, with the exception of the immunocytochemical experiments described in the second paper (carried out by Paola Zacchi) arise from my own experiments and data analysis.

- **Mohajerani MH**, Cherubini E (2005), Spontaneous recurrent network activity in organotypic rat hippocampal Slices. Eur J Neurosi: 22(1). 107-18

- **Mohajerani MH**, Zacchi P, Sivakumaran S, Aguilera P, Cherubini E, At immature CA3-CA1 synapses GABA-mediated giant depolarizing potentials act as coincident detection signals to persistently enhance glutamate release *via* the activation of the ERK pathway. In preparation.

The following publications, arising from a collaborative project in which I am co-author, are not included in the present thesis:

- Raffaelli G, Saviane C, **Mohajerani MH**, Pedarzani P, Cherubini E (2004) . BK channels control transmitter release at CA3-CA3 synapses in the rat hippocampus. J Physiol 557, 147-57.

- Saviane C, **Mohajerani MH**, Cherubini E (2003). An ID-like current that is down regulated by Ca^{2+} modulates information coding at CA3-CA3 synapses in the rat hippocampus. J Physiol 552, 513-524.

Abbreviations

AMPA	(*R,S*)-α-amino-3-hydroxy-5-methyl-4-isoxadepropionate
AMPAR	AMPA receptor
NMDA	N-Methyl-D-Aspartate
NMDAR	NMDA receptor
Trk	Tyrosine Kinase Receptor
mRNA	Messenger Ribonucleic Acid
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
E	embryonic day (E0 indicates the date of conception)
GABA	gamma-aminobutyric acid
GABA-PSC	postsynaptic $GABA_A$ receptor-mediated current
GDP	giant depolarizing potential
I_h	hyperpolarization-activated cation current
$[K^+]_O$	extracellular potassium concentration
NKCC1	sodium-potassium-2chloride co-transporter isoform 1
KCC2	potassium-chloride co-transporter isoform 2
LTD	long-term depression
LTP	long-term potentiation
P	postnatal day (P0 indicates the date of birth)
sAHP	slow afterhyperpolarization
sI-K(Ca)	slow calcium-activated potassium current
TTX	tetrodotoxin
cAMP	Cyclic Adenosine Monophosphate
MAPK	Mitogen-Activated Protein Kinase
ERK	Extracellular Signal-Regulated Kinase
EPSP	Excitatory Postsynaptic Potential
IPSC	Inhibitory Postsynaptic Current
P_r	Probability of neurotransmitter release

Abstract

During development, synchronized network oscillations constitute a primitive form of synaptic activity. This activity is generated before maturation of sensory inputs and enables a high degree of correlation among immature neurons in spite of the small number of functional synapses. Understanding the function of these oscillations is a challenging problem in Neuroscience since they are thought to be involved in the maturation of neuronal circuitries. In the developing hippocampus neuronal activity is characterized by the so-called 'giant depolarizing potentials' (GDPs). GDPs are network-driven events characterized by recurrent membrane depolarization with superimposed fast action potentials. These are associated with Ca^{2+} transients. In the present study I have investigated the general mechanisms involved in GDP generation and their functional role in regulating synaptic efficacy. To this aim, in a first set of experiments, I have used organotypic hippocampal slice cultures. Cultured slices exhibited from 12 days in vitro spontaneous events which closely resembled giant depolarizing potentials (GDPs) recorded in acute hippocampal slices during the first days of postnatal life. GDP-like events occurred over the entire hippocampus as demonstrated by pair recordings from CA3–CA3, CA3–CA1 and interneurone–CA3 pyramidal cells. As in acute slices, spontaneous recurrent events were generated by the interplay between GABA and glutamate. In the majority of cases the release of GABA preceded that of glutamate. Moreover, $GABA_A$ and ionotropic glutamatergic receptor antagonists blocked GDPs. Precise measurements of the resting membrane potential and the equilibrium potential for GABA showed that in majority of neurons GABA was depolarizing. In spite of its depolarizing action, GABA showed to be excitatory or inhibitory as it enhanced or blocked the firing of principal cells, respectively. The excitatory action of GABA was in line with the bicuculline-induced block of GDPs and with the isoguvacine-induced increase in GDP frequency. Overall these results indicate a crucial role for the depolarizing and excitatory action of GABA in GDPs generation.

In a second set of experiments I have tested the hypothesis that, during the first postnatal week, GDP-associated calcium signals may alter the properties of synaptic transmission at poorly developed Schaffer collateral (SC)-CA1 connections, GDPs were paired with SC stimulation. Pairing GDPs with afferent stimulation resulted in a persistent increase in

frequency of spontaneous AMPA-mediated glutamatergic currents an effect that required calcium influx through postsynaptic L-type calcium channel. Calcium controls many cell functions including gene expression. Pairing afferent stimulation with theta bursts or plateau potentials, to mimic the effects of GDPs, failed to induce similar synaptic potentiation. Activity-dependent plasticity processes depend on electrical activity which in turn switches on particular molecules responsible for the long term effects. I have found that three signaling molecules, PKA, BDNF and ERK are necessary for the induction of long-term potentiation (LTP) at SC-CA1 synapses. Brain-derived neurotrophic factor (BDNF) has emerged as a key regulator of activity-dependent synaptic transmission and plasticity. GDPs-induced increase in frequency of sEPSCs required the activation of TrkB receptors by BDNF. k-252a and k-252b, two alkaloids that inhibit the kinase activity of the Trk receptor family prevented the increase in frequency of sEPSCs. GDPs-induced synaptic potentiation was also blocked by the soluble form of TrkB receptor IgG (TrkB-IgG), which prevents the activation of TrkB receptors by endogenous ligands, but not by TrkA-IgG which prevents the activation of TrkA receptors by endogenous NGF. Moreover, GDPs induced potentiation required cyclic AMP-dependent protein kinase A (PKA). PKA inhibitors (membrane permeable and impermeable) completely blocked the increase in frequency of sEPSCs. Inhibitors of ERK prevented GDPs-induced synaptic potentiation. Similar results were obtained when slices were treated with antibodies against ERK phosphorylation, as revealed by immunocytochemical experiments. The present results suggest that BDNF signaling is "gated" by cAMP, a second messenger responsible for activating PKA which would act as a linker between TrkB receptors and ERK activation. Overall these data support the hypothesis that during a critical period of postnatal development, $GABA_A$-mediated GDPs are involved in the structural remodeling of excitatory glutamatergic synapses and provide new insights into the molecular mechanisms involved in this process.

Introduction

1. The role of activity during the development of central nervous system (Nature and Nurture)

The immature brain is not a small adult brain. Several processes take place primarily or exclusively in the developing brain including cell division (proliferation), migration and differentiation, synapse formation (synaptogenesis) and elimination, programmed cell death and the formation of brain structures. Typically, these neurodevelopmental processes can be broadly divided into two classes: activity-independent mechanisms (Nature), and activity-dependent mechanisms (Nurture). Activity-independent mechanisms are generally believed to occur as hardwired processes determined by genetic programs played out within individual neurons. They control the general organization of the developing brain including differentiation, migration and axon guidance to their target areas (Rakic and Komuro, 1995; Manent *et al.,* 2005). Activity-dependent mechanisms are believed to have an instructive role in synaptic formation or elimination, as well as in synaptic plasticity. Activity-dependent mechanisms are basically responsible for shifting a silent structure with no electrical activity and no synapses to an active one that possesses a highly diversified range of electrical signals and billions of selective synapses (Figure 1). Because activity dependent pattern are present in a wide range of structures and species, this suggests that there is a temporal developmental program that underlies the formation of neuronal circuits which has been conserved throughout evolution.

2. The hippocampus: history, function and anatomy

The hippocampus is one of the most thoroughly studied areas of the mammalian central nervous system. The hippocampus has a distinctive and readily identifiable structure at both the gross and histological level. It is beautifully laminated; both the neuronal cell bodies and zones of the connectivity are arranged in orderly layers (Shepherd, 1998). The unusual shape of the human hippocampus resembles that of a see horse, which is what led to its most common name (in Greek *hippo* means "horse" and *kampos* means "sea

monster") described by Giulio Cesare Aranzi (1529-1589) on his book "*De Humano Foetu liber*" (Rome, 1564).

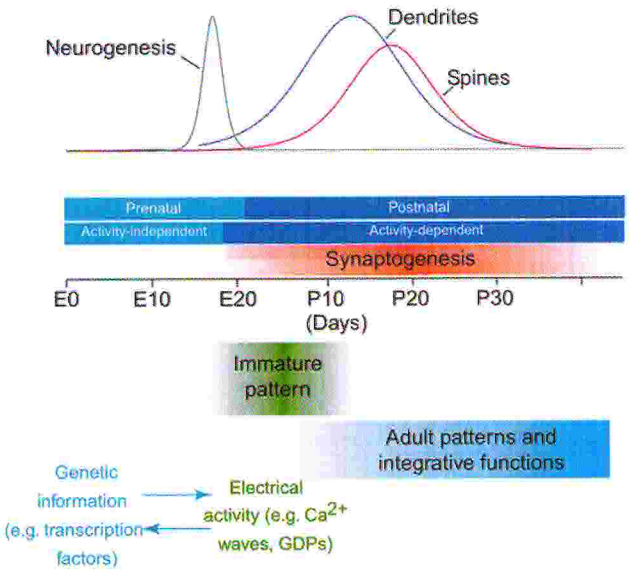

Figure 1. Formation of early neuronal networks relies on genetic information and on electrical activity. Principal events occurring during development of the rat hippocampal network. Principal pyramidal cells are generated before birth and differentiate during the first postnatal month. Hippocampal neurons start to establish synaptic connections around birth (E21) and during the first two postnatal weeks, most synaptic activity is synchronized in immature patterns – GDPs. Adult hippocampal patterns and hippocampal-dependent integrative functions emerge later, during the second and third postnatal week.

According to Walther (2002), a careful reading of the original Aranzi's text leads to the conclusion that it was not the hippocampus in our modern terms but the dentate gyrus, which he compared to a little sea horse or a silkworm. About a century later, Van Diemerbroeck (1609-1674) added a foot to each seahorse and called it "*pes hippocampi*". Jacques Benigne Winslow (1669-1760) in his book "Exposition anatomique de la structure du corps humain" (Paris, 1743) named the hippocampus "cornu arietis" because its coronal section was evocative of the ram's horn shape. René Croissant de Garengeot (1688-1759) changed "cornu arietis" into "cornu ammonis". This name refers to the ancient Egyptian chief god of Theba Amun, who was presented as a ram headed man, or a ram headed sphinx. With the introduction of the Golgi staining method (1843-1926) a

great advancement in understanding the microscopic structure of the nervous system was achieved. Ramon y Cajal (1852-1934) and his talent student Lorente de No' modified the Golgi's method to increase its effectiveness and used it to study the cellular architecture of the hippocampal formation and subdivide it into regions based on the appreciation of the correlation of different connections with the architecture. James Papez (1883-1958) described the circuit which includes hippocampus and suggested that it might constitute the centre of emotion. In 1952, Maclean named this circuit the "limbic circuit".

One of the reasons for the interest in the hippocampus is that since the early 1950s, this structure was recognized to play a fundamental role in some forms of learning and memory. In 1957, Scoville and Milner described the case of a patient known by his initials as H.M., who underwent bilateral removal of the hippocampus for the treatment on an intractable form of epilepsy. HM, probably the most thoroughly studied neuropsychological subject in memory research suffered a permanent loss of the ability to encode new information into long term memories. This anterograde form of memory impairment has been seen in other patients with bilateral damage of the hippocampus. This and subsequent studies have suggested that the deep structures of the temporal lobe, including the hippocampus (Amaral and Witter, 1989), are involved in the storage of long-term memory traces (Milner *et al.,* 1998; Eichenbaum *et al.,* 1999; Kim and Baxter 2001; Burgess *et al.,* 2002). Evidence has been provided that the hippocampus is involved in storing and processing spatial information. Studies on rodents have demonstrated that some neurons in the hippocampus possess spatial firing fields. These cells, called "place cells" (Muller, 1996), fire when the animal finds itself in a particular location in the space regardless the direction of navigation. Place cells have been also seen in humans involved in finding their way around in a virtual reality town. The findings resulted from a research on individuals with electrodes implanted in their brains as a diagnostic part of the surgical treatment for a severe form of epilepsy (Ekstrom *et al.,* 2003).

The discovery of place cells led to the idea that the hippocampus may represent a "*cognitive map*". Recent evidence has cast doubt on this perspective, indicating that the hippocampus may be crucial for more fundamental processes within navigation. Thus, studies on animals have shown that the intact hippocampus is required for simple spatial

memory tasks (for instance, finding the way back to a hidden goal) (Kwok and Buckley, 2006). Pathological states affecting the hippocampus include Alzheimer's disease, depression, temporal lobe epilepsy, febrile seizures, post-traumatic stress disorders, schizophrenia, global ischemia, brain contusion, herpes encephalitis and global amnesia (Morris, 2006).

The hippocampus is an elongated structure located on the medial wall of the lateral ventricle, whose longitudinal axis forms a semicircle around the thalamus. The three dimensional position of rat hippocampal formation in the brain is shown in Figure 2 (Shepherd, 1998). Due to its layered organisation, when the hippocampus is cut across its transverse axis (the septotemporal one), it is possible to identify a particular structure that is preserved in all slices taken with this orientation.

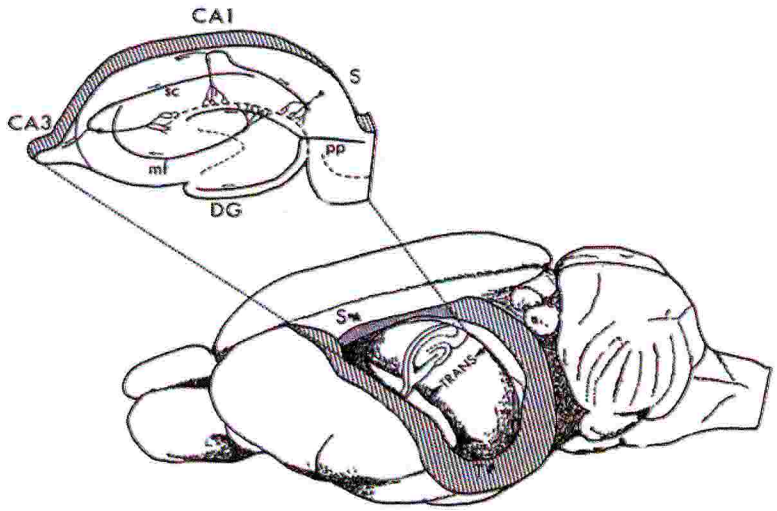

Figure 2. Position of hippocampal formation in the rat brain in which the cortical surface has been removed. The hippocampus is an elongated structure with the septotemporal axis running from the septal nuclei (S) to the temporal cortex (T). A slice cut perpendicular to the long axis shows the well known three synaptic pathway (Shepherd, 1998).

The hippocampus *proper* and its neighbouring cortical regions, the dentate gyrus (DG), subiculum and enthorinal cortex, are collectively termed "hippocampal formation". As shown in Figure 3, the hippocampus *proper* is divided into *stratum oriens*, *stratum pyramidale*, *stratum radiatum* and *stratum lacunosum-moleculare*. In their classical works, Ramón y Cajal (1893) and Lorente de Nó (1933; 1934) grouped excitatory neurons in four regions called CA1-CA4. In general, CA4 is considered the part of the CA3 closed to the dentate gyrus. The CA2 region represents the small portion between the CA3 and the CA1. This part is often ignored but it could have an important role in

epilepsy because of the large amount of recurrent collaterals (Shepherd, 1998). All pyramidal neurones bear basal dendrites that arborise and form the *stratum oriens* and apical dendrites that are radially oriented in the *stratum radiatum* and *lacunosum-moleculare*. In the DG, granule cells represent the principal neurons, while the area between DG and the CA3 region is called the *hilus* (Figure 3).

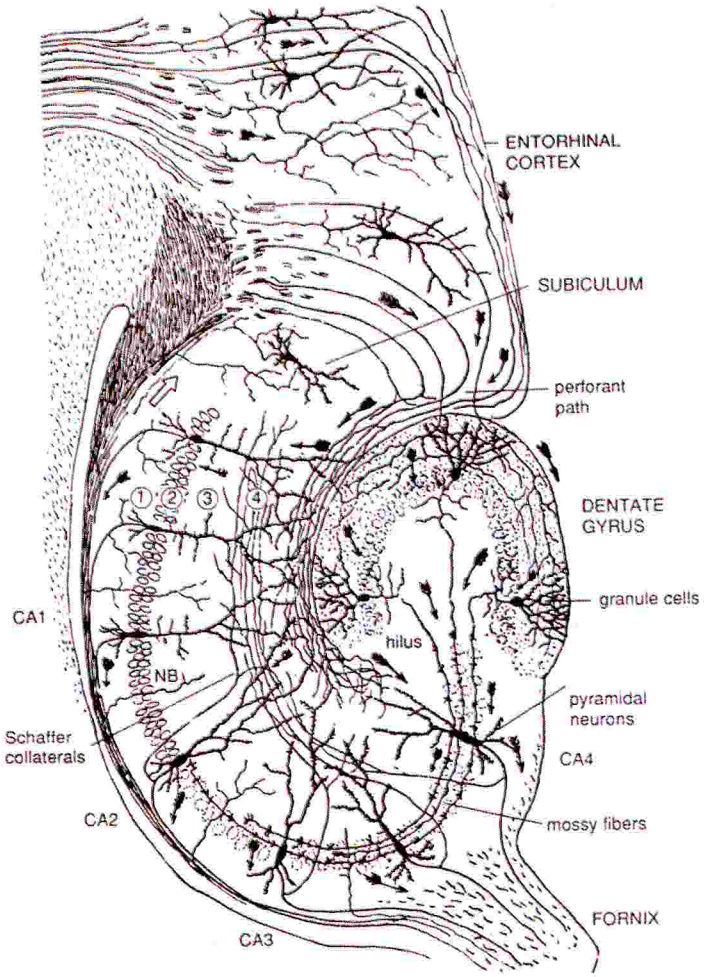

Figure 3. Neuronal elements of the hippocampal formation. Labelled areas include the subiculum, part of the enthorinal cortex, the fornix, the dentate gyrus and the regions CA1 to CA4. The hippocampus *proper* is divided into *stratum oriens*, *stratum pyramidale*, *stratum radiatum* and *stratum lacunosum-moleculare*. (Ramon y Cajal, 1911)

The main inputs to the hippocampus come from the enthorinal cortex, the septum and the contralateral hippocampus, whereas a unique unidirectional progression of excitatory

pathways links each region of the hippocampus, creating a sort of trisynaptic circuit (Figure 3). The perforant path, originating from the enthorinal cortex passes through the subicular complex and terminates mainly in the dentate gyrus, making synapses on granule cells. Then, the distinctive unmyelinated axons of the granule cells (mossy fibres) project to the *hilus* and to the *stratum lucidum* of the CA3 region. Here they make synapses *en passant* on CA3 pyramidal neurones showing the large, presynaptic varicosities typical of mossy fibres-CA3 contacts. These presynaptic expansions form a unique synaptic complex with equally intricate postsynaptic processes called *thorny excrescences* and may contain tens of releasing sites (Jonas *et al.*,, 1993). Information is therefore transferred, through Schaffer collaterals, from CA3 to CA1 pyramidal neurones, which send their axons to the subiculum and the deep layers of the enthorinal cortex (Figure 4).

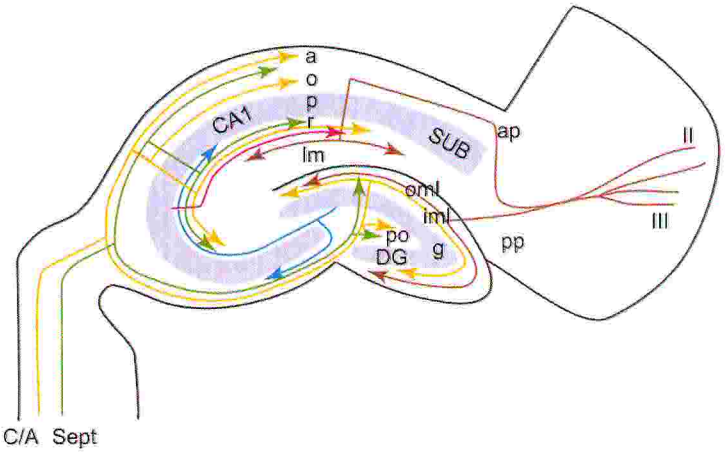

Figure 4. The main axonal pathways of the dentate gyrus and hippocampus. Perforant (pp) and alvear path (ap) projections are shown in brown. Ipsilateral afferents, originating from pyramidal cells and stellate neurons located in layers II and III of the entorhinal cortex (EC), densely innervate the outer molecular layers (oml) of the dentate gyrus (DG) and the stratum lacunosum moleculare (lm) of the cornu ammonis (CA). The excitatory commissural/associational projection (C/A) is shown in yellow. This projection originates mainly from large mossy cells located in the ipsilateral and contralateral polymorphic layer (po) and terminates in the inner molecular layer (iml) of the DG and the *stratum radiatum* (r) of CA. These fibers form a sharp border with axons of the entorhinal projection. The septal projection (Sept) is shown in green. This pathway consists of fibers from the medial septum/diagonal band and terminates in the polymorphic layer underneath the granule cell layer (g) and in both molecular layers of the DG, as well as in the *stratum radiatum* and *stratum oriens* (o) of CA. The mossy-fiber system (Mf) is shown in blue. These fibers specifically connect the DG with CA3. Schaffer collaterals (Sch) are shown in pink. These fibers specifically connect CA3 with CA1. Abbreviations: a, alveus; p, *stratum pyramidale*; SUB, subiculum.

Then, signal is sent back to many of the same cortical areas. Thus, information entering the enthorinal cortex from a particular cortical area crosses the entire hippocampus and returns to the cortical area from which it was originated. The transformations that take place during this process are presumably essential for information storage (Shepherd, 1998). Furthermore, commissural associative fibres provide synaptic contacts between CA3 pyramidal neurones and between the two hippocampi, *via* the fornix. The recurrent connections between pyramidal neurones are particular of the CA3 region and are responsible for making this region quite unstable (Figure 3 and 4). These connections are responsible for generating in particular conditions epileptiform activity, characterised by spontaneous, synchronised and rhythmic firing in a large number of neurones (Miles and Wong, 1986; Traub and Miles, 1991). In contrast to the rather uniform population of excitatory neurones, local inhibitory interneurones, are widely distributed within the entire hippocampus. They have been differently classified according to their morphological, neurochemical and physiological characteristics, which include the intrinsic firing, network properties and activity dependent synaptic plasticity processes. They selectively innervate different domains of pyramidal cells, thus providing the main source of feedback and feed-forward inhibition (Freund and Buzsaki, 1996; Miles *et al.,* 1996). Due to their extensive dendritic and axonal arborisation, GABAergic interneurons can phase the output of principal cells giving rise to a coherent oscillatory activity (Klausberger *et al.,* 2003, 2004; Somogyi and Klausberger, 2005) thus exerting a powerful control on network excitability and information processing in the brain. Although some oscillations can be reproduced *in vitro*, they occur mainly *in vivo* during particular behavioural states of the animal (Buzsaki, 2002; Buzsaki and Draguhn, 2004). Oscillations have been implicated in encoding, consolidation and retrieval of information in the hippocampus (Freund and Buzsaki, 1996). Oscillatory rhythms are facilitated by the intrinsic properties of GABA releasing cells (Maccaferri and McBain, 1996) and by their electrical coupling *via* gap junctions (Hestrin and Galarreta, 2005).

3. Development of the hippocampus

3.1 Proliferation

Both the hippocampus and the neocortex derive from the telencephalic pallium. At early developmental stages, the pallium of the telencephalic vesicles consists of

undifferentiated germinal cells or ventricular zone. Ventricular zone cells produce pluripotent progenitors which generate neuronal and glial precursors. The first postmitotic cells migrate in a radial fashion out of the ventricular zone and form the first recognizable cortical layer, the preplate, also named early marginal zone. In the rat, the preplate is present at day P10–P15 after conception and consists of pioneer neurons. These neurons are the first to be generated and constitute a heterogeneous population of cortical cells. Around 14–17 days, it appears a cell dense cortical plate (CP) which splits the preplate zone and its neuronal populations into a superficial zone named the marginal zone or fetal layer I, and a deep, lower zone called the subplate (Super *et al.,* 1998).

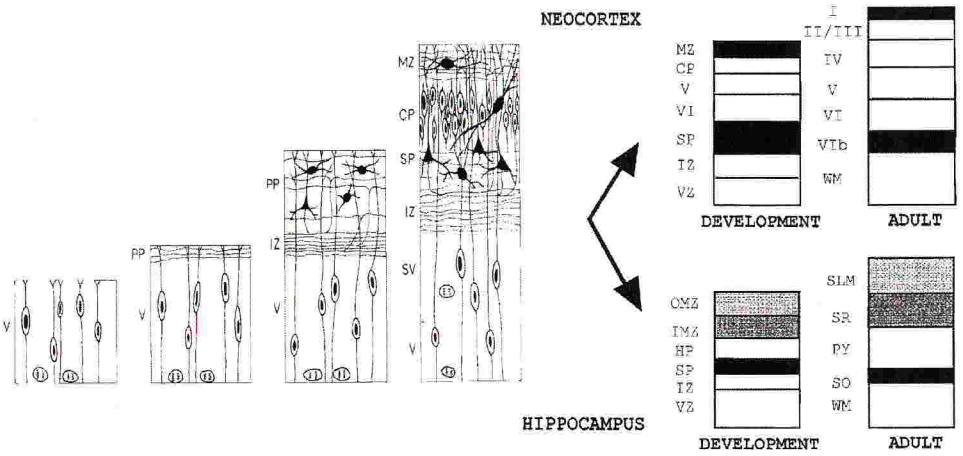

Figure 5. *Scheme showing the laminar organization of the developing and adult neocortex and hippocampus*

In the adult rodent, the marginal zone and subplate of the neocortex transform into the cortical layers I, and VIb, respectively and the cortical plate expands to multi layers and forms layer II/III, IV, V, and VI (Figure 5). In the 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 does not become a multi-laminar layer but remains a cell dense pyramidal layer (PY). In the adult hippocampus, the outer marginal zone becomes the *stratum lacunosum moleculare* (SLM), the inner marginal zone the *stratum radiatum* (SR), and the subplate the *stratum oriens* (SO). See Figure 5.

3.2 Neurogenesis and neuronal migration

In the rat, hippocampal pyramidal neurons are generated between embryonic day 16 (E16) and E20 in the ventricular zone of the pallium (Bayer, 1980a; Bayer, 1980b; Altman and Bayer, 1990a; Altman and Bayer, 1990b). From the ventricular zone, they massively migrate radially towards the hippocampal plate (Nowakowski and Rakic 1981; Rakic, 1995; Nadarajah and Parnavelas, 2002). For their migration they use the radial glial fibers and follow an inside–out sequence where, newly arrived neurons settle in the upper level of already migrated cells. Occasionally pyramidal neurons use for their migration other substrates such as axonal bundles (Nadarajah and Parnavelas, 2002). Radial glial cells are typical long bipolar cells that expand across the whole cerebral wall from the ventricular up to the pial membranes. When neural migration has been completed, most radial glial cells retract their pial endfeet, change their morphology and become astrocytes. The neuronal cell migration along radial glia is believed to be calcium dependent, regulated by NMDA/GABA receptors and various subtypes of voltage-dependent calcium channels (Komuro and Rakic, 1992, 1993, 1998; behar *et al.,* 1998; Spitzer, 2002).

Granule cells in the dentate gyrus begin to be generated at about the same time as pyramidal cells (at E16-E20). However, most dentate gyrus neurogenesis occurs later. 85 % of granule cells are generated during the first postnatal month with a peak between postnatal (P) day P3 and P6 (Bayer 1980a; Bayer 1980b, Altman and Bayer 1990c, Guidi *et al.,* 2005). They are generated mainly in the hilus and in the ventricular zone. Only a small proportion is generated within the dentate gyrus itself. During development, granular cells migrate along glia fibers following an outside-in sequence. Interestingly, neurogenesis of granule cells in the dentate gyrus persists in adulthood (Gage 2002). Hippocampal GABAergic cells 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. Their arrival is more or less concomitant with the appearance of the hippocampal primordium. A small proportion of interneurons migrate postnatally between P2 and P15 (Soriano *et al.,* 1986, Dupuy and Houser, 1996, Pleasure *et al.,* 2000, Morozov and Freund, 2003).

Both GABA and glutamate depolarize neural progenitor cells thus favouring calcium entry *via* voltage-dependent calcium channels. Calcium signalling in turn activates several metabolic pathways necessary for cell functioning including DNA synthesis (Lamantia. *et al.,* 1995; Loturco *et al.,* 1995). Therefore, it is likely that release of neurotransmitters by ingrowing axonal afferents (Demarque *et al.,* 2002) influence developing neurons during their neurogenesis and migration (Heck *et al.,* 2006). Besides neurotransmitters, other factors such as extracellular matrix proteins, neurotrophins and adhesion molecules can regulate neurogenesis and play a critical role in the survival of CNS neurons (Super *et al.,* 1998; Kahn *et al.,* 2002).

Different hippocampal subfields were defined on the basis of differences in cytoarchitecture and connections. The pyramidal cell layer of the hippocampus is divided into two major fields, CA1 and CA3. The CA fields are capped by the dentate gyrus, the third major field of the hippocampus. Molecular markers have been used to determine the field identity of cells in the developing hippocampus. In the mouse, hippocampal CA and dentate gyrus field patterning emerges in the medial telencephalic wall at E14.5-E15.5 (Grove and Tole, 1999). The rat CA fields start to form later between E16 and E20 in area CA1 and CA3, respectively. The dentate gyrus can only be identified at E21-E22 (Bayer, 1980a; Bayer, 1980b; Altman and Bayer, 1990a; Altman and Bayer, 1990c).

3.3 Formation of afferent connections

Development of specific neuronal connections in the CNS requires guidance of outgrowing axons to their proper target areas. Neurons located in distinct brain regions give rise to axons that innervate specific structures and avoid others. This process can be divided into two stages: i). axonal outgrowing and recognition refinement of layer-specific positional and ii). synapse formation.

It has become evident in recent years that specific molecules (molecular cues), which are able to attract or repulse axons to a specific site, are involved in the formation of connectivity maps in various brain regions. Many studies have clarified the functional role of various molecular cues for axonal guidance. These include semaphorins, netrins, the slit proteins, and the family of the ephrin A (Skutella and Nitsch, 2001). The concept of "pioneer neurons" was also proposed to explain the recognition of specific layers and

organisation of proper afferent (McConnell *et al.,* 1989). The pioneer neurons or Cajal-Retzius cells direct the migration of principal neurons and are required for the proper termination of entorhinal afferents on the dendrites of both the pyramidal cells and the dentate gyrus granule cells (Super *et al.,* 1998). These neurons, whose role has been only recently understood, were described about a century ago by Ramón y Cajal (1891) and Retzius (1893). In the rat, Cajal-Retzius cells disappear two weeks after birth. Refinement of ingrowing afferents for their appropriate layers or areas is believed to be achieved by neuronal activity (Krubitzer and Kahn, 2003). The second stage which consists in the formation of synaptic contacts, in rodents occurs essentially during the first postnatal days.

3.4 Synapse maturation

Synaptogenesis is an interactive process which involves both pre and postsynaptic elements (Jessell and Kandel, 1993). This process starts with the initial contact between potential partners and ends up with the formation of complex, functional molecular assemblies. These include active release areas in the presynaptic site, the signal processing machinery at the postsynaptic site and the structured extrasynaptic matrix in the synaptic cleft (Kennedy, 2000).

3.4.1 Neurotransmitter can be released before synapse formation

At early developmental stages, before the establishment of functional synapses, immature hippocampal neurons exhibit a tonic $GABA_A$ (Demarque *et al.,* 2002; Owens and Kriegstein, 2002), NMDA (Valeyev *et al.,* 1993) and kainate (Lauri *et al.,* 2005) mediated conductance. This suggests that $GABA_A$, NMDA and kainite receptors are activated by "ambient" GABA or glutamate, present in the extracellular space and released in a paracrine fashion by axonal growth cones. Like immature neurons, newborn granule cell in the adult dentate gyrus are tonically activated by ambient GABA before being innervated by the pre-existing functional circuitry (Ge *et al.,* 2006). Interestingly, the release of the neurotransmitter may occur in a non vesicular fashion and does not require vesicular release proteins. The paracrine release of a neurotransmitter from axonal growth cones (Gao *et al.,* 2000) of pioneer and migrating cells or glia acts on synaptically

silent neurons to generate large currents prior to synapse formation (Rivera *et al.,* 2004; Represa and Ben-Ari, 2005). The concentration of released glutamate in the extracellular space is controlled by glutamate transporters which are predominantly expressed in axons and presynaptic terminals of pyramidal neurons. Although at early developmental stages the functional role of GABA transporters has been questioned (Demarque *et al.,* 2002), changes in the holding current induced by the GABA transporter blocker NO-711 suggest that the GABA transporter GAT-1 is present and functional already at P2 (Marchionni *et al.,* unpublished). This is in accord with the observation that in the neonatal rat hippocampus GABA uptake limits the action of GABA during GDPs (Sipila *et al.,* 2004) and controls GABAergic transmission at mossy fibre-CA3 synapses (Safiulina *et al.,* 2006).

3.4.2 GABAergic synapses develop before the glutamatergic ones

Studies on rats (Tyzio *et al.,* 1999; Hennou *et al.,* 2002) and non-human primates (Khazipov *et al.,* 2001) have shown that functional GABAergic synapses are formed before glutamatergic ones both in interneurons and in pyramidal cells of the hippocampus. Patch clamp recording from acute hippocampal slices taken from P0 rats allowed morphological reconstruction of recorded neurons (Tyzio *et al.,* 1999; Khasipov *et al.,* 2001; Hennou *et al.,* 2002). Non-innervated "silent" interneurons constituted the 5% of the entire population. 17 % had only GABAergic synapses and 78 % had both GABAergic and glutamatergic synapses (Figure 6). The maturation of synaptic afferents occurred later in pyramidal neurons than in interneurons since 80 % CA1 pyramidal neurons at P0 had small soma and anlage or no apical dendrite and no functional synapses, 10 % had a small apical dendrite restricted to the *stratum radiatum* with only GABAergic synapses on apical dendrite and 10 % hold an apical dendrite that reached the stratum lacunosum moleculare with both GABAergic and glutamatergic synapses. The GABA– glutamate sequence is retained throughout the whole life, as it is maintained in newborn granule cells during adult neurogenesis (Ge *et al.,* 2006). See Figure 6.

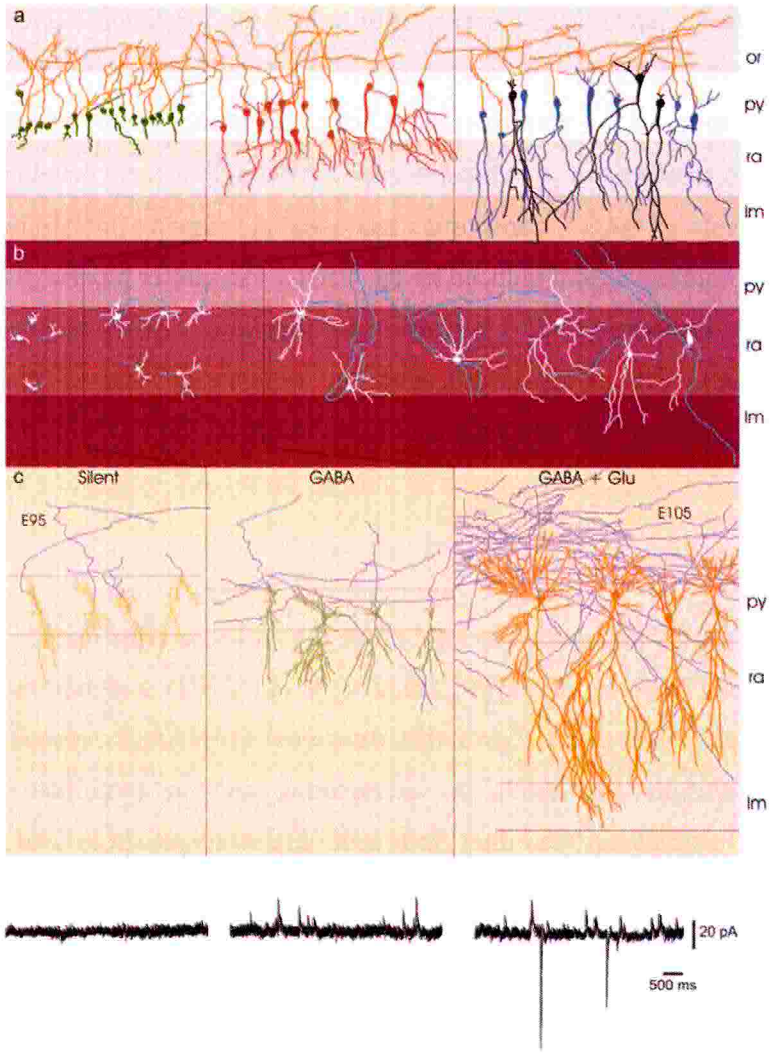

Figure 6. Sequential formation of GABA and glutamate synapses in the developing primate and rodent hippocampus. Rat pyramidal neurons (a) and interneurons (b) were patch-clamp recorded from postnatal-day-0 hippocampal slices, the spontaneous and evoked excitatory postsynaptic currents (PSCs) determined, and the neurons filled with dyes for *post hoc* morphological reconstruction. Note that 80% of pyramidal neurons are silent with no functional PSCs, 10% have only GABA-mediated PSCs, and the remaining 10% have both GABA and glutamate (Glu) PSCs. This correlates with the degree of dendritic and axonal arborization. Interneurons follow a similar developmental gradient, with GABA synapses being established before glutamate synapses, but at an earlier stage. So, at birth, only 5% of the interneurons are silent, and most have both GABA and glutamate PSCs. (c) Similar results were obtained in the primate hippocampus *in utero*. At the mid-embryonic stage, most CA1 pyramidal neurons are silent. Note the difference in the development of pyramidal neurons with no functional synapses, with GABA-only synapses, and with GABA and glutamate synapses. E, embryonic day; lm, *stratum lacunosum moleculare*; or, *stratum oriens*; py, *stratum pyramidale*; ra, *stratum radiatum* (Ben-Ari, 2002).

3.4.3 GABA inhibits adult neurons but excite the immature ones

Although GABA is the major inhibitory neurotransmitter in the adult brain, during development it can have an excitatory action (Cherubini *et al.,* 1991; Ben-Ari, 2002). The excitatory action of GABA was first shown by Obata *et al.,* (1978) in co-cultures of muscle and spinal neurons taken from 6-8 days old chick embryos. In these studies, an inhibitory effect of GABA was seen when culturing was started on day 10 indicating a developmental shift in the effects of GABA. The excitatory effects of GABA (and glycine) on motoneurons was demonstrated by monitoring tetrodotoxin (TTX)-sensitive end-plate potentials in muscle cells. Direct intracellular recordings from the neurons showed that there was a developmental change from depolarizing to hyperpolarizing action of GABA. Subsequent work has shown that the ontogenetic shift in GABA action, from depolarizing and excitatory to inhibitory, is a general feature of developing neurons well preserved during evolution as demonstrated by its persistence in newborn granule cells of the adult dentate gyrus (Ge *et al.,* 2006; Overstreet-Wadiche *et al.,* 2006; Karten *et al.,* 2006). The excitatory action of GABA has been found in different animal species and in different brain structures (Ben-Ari *et al.,* 1989; Luhmann and Prince, 1991; Zhang *et al.,* 1991; Mohajerani and Cherubini, 2005).

$GABA_A$ receptors are permeable to two physiologically relevant anions, Cl^- and $HCO3^-$ (Kaila, 1994). Passive re-distribution of H^+ and $HCO3^-$ ions leads to a more acidic intraneuronal pH (Roos and Boron 1981; Kaila and Ransom 1998). 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 the reversal potential of $GABA_A$ receptor-mediated responses deviates significantly from the equilibrium potential of Cl^- when the intracellular chloride concentration is low (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). Under these conditions, $HCO3^-$ does not play a crucial role in GABA-mediated responses. The intracellular Cl^- concentration of immature neurons 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. 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 intracellular Cl^- concentration decreases during neuronal maturation with a consequent shift in the equilibrium potential for Cl^- to more negative values often beyond the resting membrane potential. The intracellular Cl^- homeostasis is regulated by various transporters (Payne *et al.,* 2003). The main transporter molecule that mediates Cl^- uptake into the cell is the Na-K-2Cl co-transporter isoform 1, NKCC1 (Rohrbough and Spitzer, 1996; Fukuda *et al.,* 1998; Sun and Murali 1999; Chub *et al.,* 2006). The ontogenetic shift of GABA from depolarizing to hyperpolarizing is primarily due to the developmental upregulation of the K-Cl cotransporter isoform 2, KCC2 as demonstrated by changes in the levels of KCC2 messenger RNA in both hippocampal cultures and slices (Rivera *et al.,* 1999). In addition, transfection of the KCC2 gene into hippocampal neurons converts the actions of GABA from excitatory to inhibitory (Chudotvorova *et al.,* 2005). The operation of both NKCC1 and KCC2 transporters is electroneutral. Therefore, the energy for Cl^- transfer derives from the electrochemical gradients of Na^+ and K^+, respectively (Payne *et al.,* 2003).

According to Ganguly and Poo (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 results have not been confirmed by other groups (Ludwig *et al.,* 2003; Titz *et al.,* 2003; also see Delpire and Mount, 2002). Nevertheless, because of the intrinsic heterogeneity of immature neurons (some might have no functional synapses, whereas others already generate network-driven patterns) the expression of KCC2 at birth will vary even between adjacent neurons (Ben-Ari, 2002). Neurotrophins are likely to be involved in this developmental shift (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 (Aguado *et al.,* 2003; but see Rivera *et al.,* 2005). Is GABAergic signalling depolarizing *in vivo*? Direct recordings of GABAergic responses have not been performed in the *in vivo* immature hippocampus. I*n vitro* data from immature CA3 pyramidal neurons show that blockade of $GABA_A$-mediated network activity (GDPs, see chapter 3.4.5) by bumetanide is attributable to inhibition of Cl^- uptake

via NKCC1. In these cells, the effect of bumetanide was mediated by a reduction in the depolarizing driving force for GABA. Since bumetanide blocked sharp positive waves (GDPs recorded in vivo) in the neonate hippocampus, these data suggest that GABAergic signalling is, indeed, depolarizing in vivo (Sipila *et al.,* 2006).

Whether a $GABA_A$ receptor mediated conductance is excitatory under a given condition cannot be strictly inferred from the polarity of the GABAergic voltage response. Neuronal excitation and inhibition are usually defined as the increase or decrease in the probability of firing in response to the activation of a given input. While a hyperpolarizing response to GABA nearly always leads to inhibition of neuronal firing (but see McCormick and Bal 1997; Chen *et al.,* 2001), a depolarizing response does not necessarily lead to excitation since GABA-induced increase in membrane conductance exerts a shunting action (Fatt and Katz, 1953). Thus, shunting would decrease the amplitude and the duration of a voltage response generated by a fixed current pulse (Khalilov *et al.,* 1999; London and Hausser, 2005).

3.4.4 Function of GABAergic depolarization in immature neurons

The depolarizing action of GABA is a general feature of immature neurons as shown in the hippocampus (Mueller *et al.,* 1984; Ben-Ari *et al.,*1989; Zhang *et al.,* 1991; Berninger *et al.,* 1995; Hollrigel *et al.,* 1998), neocortex (Luhmann and Prince, 1991; Owens *et al.,* 1996; Dammerman *et al.,* 2000; Maric *et al.,* 2001), hypothalamus (Chen *et al.,* 1996; Gao *et al.,* 1998; Wang *et al.,* 2001), cerebellum (Eilers *et al.,* 2001) and the spinal cord (Obata *et al.,* 1978; Reichling *et al.,* 1994; Wang *et al.,* 1994; Serafini *et al.,* 1995; Vinay and Clarac 1999). The GABAergic depolarization activates voltage-dependent Ca2+ channels (Yuste and Katz 1991; Leinekugel *et al.,* 1995; Leinekugel *et al.,* 1997; Khazipov *et al.,* 1997; Fukuda *et al.,* 1998; Eilers *et al.,* 2001) and remove the voltage-dependent magnesium block from NMDA receptor channels causing an influx of calcium, which has many consequences on neuronal development (Yuste and Katz 1991;Leinekugel X. *et al.,* 1997; Kasyanov A. *et al.,* 2004).

It has been suggested that, in contrast with glutamate, calcium rise *via* the depolarizing action of GABA is not toxic for the cell because E_{GABA} is closer to the resting membrane potential than $E_{glutamate}$ (-35 mV *vs* 0 mV; (Ben-Ari, 2006)). In addition, in the case of

GABA, the shunting action would inhibit over-excitation produced by both GABA and glutamate (Overstreet Wadiche *et al.,* 2005). Thus, GABA will provide a sufficient degree of excitation necessary to modulate developmental processes (Cherubini *et al.,* 1991; Ben-Ari 2006). In addition, the slow decay of depolarizing GABA-mediated responses would allow the temporal integration of synaptic signalling that early in postnatal days is poorly developed. As glutamatergic synapses reach their full development, GABA shifts from the depolarizing to the hyperpolarizing direction.

3.4.5 Spontaneous network activity in the immature hippocampus

An intriguing feature of developing neurons is the presence, during a limited period of time, of primitive patterns of synchronized activity. These patterns occur before the formation of synapses. In embryonic *Xenopus* spinal cord neurons, spontaneous transient elevations of Ca^{2+} occur before synapse formation and modulate the developmental processes (Spitzer, 1994). Recurrent patterns of activity can be also observed after the formation of the first synapses and in many structures including the hippocampus (Ben-Ari *et al.*, 1989) the spinal cord (O'Donovan, 1989), the retina (Galli and Maffei, 1988), the auditory system (Lippe, 1994), the trigeminal nucleus (Ho and Waite, 1999) and the neocortex (Garaschuk *et al.,* 2000). These patterns provide most of the activity present during a critical period that extends in different species from days to weeks (Ben-Ari, 2002). In the hippocampus such network activity has been called "giant depolarizing potentials" or GDPs (Ben-Ari *et al.*, 1989), "giant GABAergic potentials" (Strata *et al.*, 1997), "early network oscillations" (Garaschuk *et al.*, 1998a) and "population bursts" (Lamsa *et al.*, 2000). In this thesis we will use the term GDPs. GDPs can be recorded in rat hippocampal slices (Ben-Ari *et al.*, 1989), in organotypic slice cultures (Mohajerani and Cherubini, 2005), in the intact hippocampus *in vitro* (Khalilov *et al.,* 1997) and *in vivo* (Leinekugel *et al.*, 2002). In addition they are observed in rabbit hippocampal slices (Menendez de la Prida et al., 1996), mice (Aguado *et al.*, 2003) and macaque monkeys *in utero* (Khazipov *et al.*, 2001). An oscillatory activity similar to GDPs can be recorded in the electroencephalogram of premature babies. The electroencephalogram which consists in a discontinuous pattern of high voltage delta waves alternated with low-amplitude theta activity has been called by Madame Dreyfus Brisac (1999) "*tracé alternante*".

In the immature hippocampus, GDPs can be detected with electrophysiological (cell-attach, whole cell and field potentials recordings) or calcium imaging techniques. They are associated with intracellular bursts of action potentials and Ca^{2+} transients (Leinekugel *et al.*, 1997; Garaschuk *et al.*, 1998; Canepari *et al.*, 2000). GDPs which occur synchronously over the entire hippocampus (Khalilov *et al.*, 1997; Leinekugel *et al.*, 1998; Strata *et al.*, 1997; Mohajerani and Cherubini, 2005) at the frequency of ~0.01-0.3 Hz (Ben-Ari, 2001) consist in membrane depolarizations of ~0.5-2 s duration giving rise to fast action potentials. Along the longitudinal axis and the transverse plane of the hippocampus, the septal pole and the CA3 region, respectively, act as pacemakers for GDPs generation (Leinekugel *et al.*, 1998; Ben-Ari, 2001). At the level of the network, GDPs are associated with an increased firing of principal cells and interneurons as well as high frequency barrages of GABAergic and glutamatergic synaptyic currents (Ben-Ari *et al.*, 1989; Khazipov *et al.*, 1997; Bolea *et al.*, 1999).

3.4.5.1 Role of ionotropic GABAergic transmission in GDPs generation

In neonatal CA3 pyramidal neurons (Ben-Ari *et al.*, 1989) GDPs were shown to reverse at the same potential of the responses evoked by exogenous application of GABA. GDPs were blocked by $GABA_A$ receptor antagonists. $GABA_A$ receptor blockers triggered from the second-third postnatal day interictal discharges (Le Magueresse *et al.*, 2006). These were never observed before P3 despite the prolonged exposure of the slices to $GABA_A$ receptor antagonists (Khazipov *et al.*, 2004). Interictal discharges were similar to those observed in the disinhibited adult hippocampus and differed from GDPs because of their faster onset. Their frequency significantly increases with age (Ben-Ari *et al.*, 1989; Gaiarsa *et al.*, 1991; Le Magueresse *et al.*, 2006; Mohajerani and Cherubini, 2005). Interictal discharges were blocked by glutamatergic receptor antagonists and reversed polarity near 0 mV, suggesting that they were mediated by AMPA/kainate ionotropic glutamate receptors (Ben-Ari *et al.*, 1989; Khalilov *et al.*, 1999; Mohajerani and Cherubini, 2005). They were generated in the CA3 area, since cutting the slice between the CA3 and CA1 regions prevented the propagation of the discharges (Wong and Traub, 1983). Bath application of a low concentration of GABA or $GABA_A$-receptor agonists was shown to increase the frequency of GDPs while a high concentration led to inhibition

of network events (Khalilov *et al.*, 1999; Dzhala and Staley, 2003; Mohajerani and Cherubini, 2005). The ontogeny of GDPs was originally studied with intracellular recordings using sharp electrodes (Ben-Ari *et al.*, 1989). In this work, GDPs were reported to gradually disappear towards the end of the first or the beginning of the second postnatal week in concomitance with the shift of GABA from the depolarizing to the hyperpolarizing direction. Later studies using a different strain of rats (Sprague-Dawley instead of Wistar) and whole cell recordings reported a late disappearance of GDPs at P17 (Khazipov *et al.*, 2004). Using cell-attached recordings or field potentials, the switch of GABA from excitatory to inhibitory was observed ~P14.

3.4.5.2 Role of ionotropic glutamatergic transmission in GDPs generation

Several line of evidence suggests that also glutamate contributes to GDPs: i). blocking $GABA_A$ receptors with intracellular fluoride revealed a glutamatergic component of GDPs which reversed polarity at 0 mV and was reversibly blocked by the AMPA/kainate receptor antagonist DNQX (Khazipov *et al.*, 1997; Bolea *et al.*, 1999). ii). competitive antagonists of AMPA/kainate receptors, when applied at a sufficiently high concentration (50 μM), blocked spontaneous GDPs (Bolea *et al.*, 1999; Lamsa *et al.*, 2000). iii). GDPs frequency was increased by cyclotiazide (20 μM) a selective blocker of AMPA receptor desensitization (Bolea *et at.*, 1999). Although NMDA receptors have been proposed to contribute to GDPs generation (Leinekugel *et al.*, 1997), their role is unclear since, NMDA receptor antagonists either have no effect or merely reduce the frequency of GDPs (Ben-Ari *et al.*, 1989; Bolea *et al.*, 1999). Selective activation of kainite receptors has been recently shown to have a modulatory action on GDPs by affecting both GABA and glutamate release (Lauri *et al.*, 2005 and 2006). These results indicate that glutamate mainly through the activation of AMPA receptors exerts a permissive role in GDPs generation.

3.4.5.3 Role of metabotropic GABAergic and glutamatergic transmission in GDPs generation

While postsynaptic $GABA_A$ receptor-mediated responses are small or absent in neonatal CA3 pyramidal neurons, pre-synaptic $GABA_B$ receptors are clearly functional during the

early postnatal period (Gaiarsa *et al.*, 1995; Caillard *et al.*, 1998). Taking into account the depolarizing, and often excitatory action of $GABA_A$ receptor mediated transmission in the immature hippocampus, a major inhibitory role has been attributed to presynaptic $GABA_B$ receptors (McLean *et al.*, 1996). In particular, $GABA_B$ receptor antagonists have been shown to increase the frequency and the duration of GDPs (Tosetti *et al.*, 2004 and 2005) and of interictal discharges observed in the presence of $GABA_A$-receptor antagonists (De la Prida *et al.*, 2006; Cohen *et al.*, 2006). In addition, $GABA_B$ receptor agonists completely abolished GDPs (Tosetti *et al.*, 2005) suggesting a key role for $GABA_B$ receptors present on GABAergic and glutamatergic terminals in controlling GDPs termination. Also metabotropic glutamate receptors contribute to GDPs as demonstrated by the decrease in GDP frequency after treating immature hippocampal slices with the broad spectrum metabotropic glutamate receptor antagonist, MCPG (Strata *et al.*, 1995). Metabotropic receptors have been shown to affect GABA release by activating cAMP-dependent protein kinase A (Strata et al., 1995).

3.4.5.4 GDPs are modulated by several neurotransmitters

GDPs are regulated by several neurotransmitter and modulators such as acetylcholine acting on muscarinic and nicotinic receptors, adenosine, cannabinoids, etc. Blocking with edrophonium the acetylcholinesterase, the enzyme which hydrolyzes acetylcholine, enhanced the frequency of GDPs, an effect that was antagonized by pirenzepine a M1 muscarinic receptor antagonist indicating that acetylcholine, *via* M1 muscarinic receptors contribute to GDPs (Avignone and Cherubini, 1999). The effect of edrophonium was mimicked by endogenously released acetylcholine by stimulation of the cholinergic fibers in the stratum oriens. In the presence of pirenzepine, the muscarinic agonist carbachol reduced the frequency of GDPs, while in the presence of methoctramine a M1 and M2 receptor antagonist, it increased the frequency of GDPs. This indicates that muscarinic receptors up and down regulate GDPs through M1 and M2 receptors, respectively (Avignone and Cherubini, 1999). Nicotinic acetylcholine receptors modulate GDPs activity via facilitation of GABA and glutamate release (Maggi *et al.*, 2001; Le Magueresse *et al.*, 2006). Adenosine triphosphate has modulatory effects on GDPs via ionotropic (P2X) and metabotropic (P2Y) receptors (Safiulina *et al.*, 2005) while

presynaptic cannabinoid type 1 receptors regulate GDPs activity at presynaptic GABAergic terminals (Bernard *et al.*, 2005).

3.4.5.5 Intrinsic bursts in hippocampal neurons

Intrinsic bursting is a characteristic feature of hippocampal CA3 pyramidal neurons (Kandel and Spencer, 1961; Wong *et al.*, 1979). Depending on the membrane voltage, CA3 pyramids exhibit spontaneous bursts at a rate of ~0.2-5 Hz (Hablitz and Johnston, 1981; Wong and Prince, 1981). Bursts are generated by activation of voltage-dependent calcium channels (Azouz *et al.*, 1996; Chen *et al.*, 2005) or persistent sodium channels (Su *et al.*, 2001). The entry of calcium or sodium would generate a prominent after depolarizing potential which would give rise to 2-8 action potentials at ~100-400 Hz (Kandel and Spencer, 1961; Wong and Prince, 1981). About 40% of immature CA3 pyramidal neurons are able to generate intrinsic bursts already at birth. These cells fire intrinsic bursts at the frequency of 0.2-1.2 Hz when the membrane potential is between -74 and -68 mV (Menendez de la Prida *et al.*, 2000). A membrane depolarization beyond -68 mV switches the firing mode from bursting to repetitive (at 5.2 ± 2.1 Hz). The endogenous nature of the bursts is supported by the finding that they can be observed in the absence of ionotropic glutamatergic and GABAergic signalling as well as by the observation that their frequency is dependent on membrane voltage. The termination of the bursts is associated with a slow membrane after-hyperpolarization (sAHP), due to the activation of slow conductance potassium channels which are activated by the entry of calcium into the cell during the action potentials (Sipila *et al.*, 2005 and 2006; Fernandez de Sevilla *et al.*, 2006). Also interneurons sometimes can generate intrinsic bursts (personal observation). In immature CA3 pyramidal neurons intrinsic bursts are triggered by a slow regenerative depolarization due to the activation of a persistent Na^+ current (Sipila *et al.*, 2006). The issue whether the pacemaker current I_h (Pape, 1996) is involved in intrinsic burst generation is still under debate. Sipila *et al.*, (2006) showed that I_h inhibitors like Cs^+ and ZD 7288 had a negligible effect on intrinsic bursts in immature CA3 pyramidal neurons (but see Bender *et al.*, 2005).

3.4.5.6 Mechanisms of GDPs generation (review of the literature)

In the original GDPs paper Ben-Ari *et al.*, (1989) proposed a simple model to explain the generation of spontaneous GDPs (figure 7).

> *"GABAergic interneurons are thought to undergo oscillatory activity. The cycle might be initiated by a depolarization of GABA-containing cells via activation of their NMDA receptor-channel complex by glutamate released by adjacent pyramidal cells. The NMDA receptor activation will promote a Ca^{2+} influx. This increase in cytosolic Ca^{2+} in turn might activate a Ca^{2+}-dependent K^{+} conductance, which would hyperpolarize the GABAergic cell. The oscillating GABAergic interneuron would release GABA onto pyramidal neurons and participate in a positive feedback loop through which the synchronous discharge of a population of pyramidal cells might be produced. Should the recurrent 'excitatory' connection among pyramidal neurons be functional at this stage, it would then be a suitable mechanism to facilitate synchronization. The cyclic release of GABA could either occur at axo-dendritic synapses or from non-synaptic free endings such as those described in the immature cortex".*

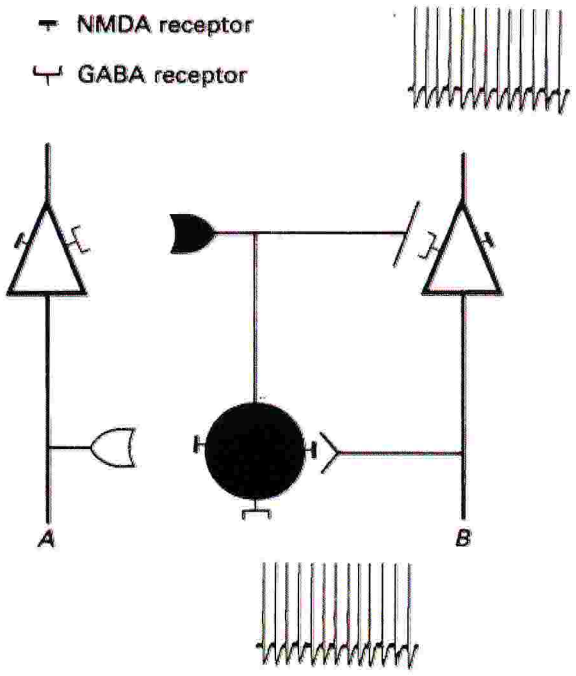

Figure 7. First model of oscillatory behaviour of immature CA3 neurons

It was not clear whether the synchronized activity was driven by pacemaker cells localized within a specific region of the developing hippocampus. Strata *et al.*, (1998)

proposed that hilar interneurons are the pacemaker cells. This hypothesis was based on the observation that surgical isolation of the hilus from the CA3 region abolished GDPs in CA3 principal cells but not in hilar interneurons. In addition, paired recordings from hilar interneurons and CA3 pyramidal cells showed that GDPs in the hilus preceded those recorded in CA3 pyramidal cells by 5-10 ms latency. However, these data have not been confirmed (Garaschuk *et al.*, 1998; Menendez de la Prida *et al.*, 1998; Leinekugel *et al.*, 1998).

In contrast with Strata *et al.*, (1998), Menendez de la Prida *et al.,* (1998) supported the idea that GDPs are not initiated by a single pacemaker structure but by different neuronal populations. Cross correlation analysis showed that GDPs can initiate either from CA1, CA3 or dentate gyrus cells. Leinekugel *et al.*, (1998) used the intact hippocampal preparation to ask whether septal neurons might act as pacemakers for spontaneous GDPs. They found that spontaneous GDPs propagate from the septum to the hippocampus. Moreover, when partially isolated, the septum maintains a higher GDP frequency than the hippocampus. When the hippocampus was isolated from the septum however, it retained the ability to generate GDPs. Leinekugel *et al.*, (1998) proposed that the septum has a pacemaker role. However, according to these authors to maintain its pacemaker function, the septum needs a high level of activity from the hippocampus. It seems likely that the initiation of GDPs is characterized by a build-up period of neuronal excitation which consists in an increase in frequency of post-synaptic potentials (mostly GABAergic) taking place within a 100-300 ms period preceding the GDPs onset (Menendez de la Prida and Sanchez-Andres, 1999, 2000). According to these authors GDPs initiated when the temporal summation of postsynaptic potentials, occurring simultaneously in principal cells and in interneurons, exceeded a threshold level. This suggests that GDP generation is based on an emergent property of the neuronal network. In apparent contrast with this view, Sipila *et al.*, (2005) suggested that, at least in CA3 pyramidal neurons, a $GABA_A$-mediated tonic conductance brings the membrane to the window where intrinsic bursts are generated, thus contributing to synchronized network events. Based on this theory, the depolarizing action of GABA would exert only a permissive role on bursts synchronizing while glutamatergic transmission would accomplish an essential role. However, against this hypothesis is the observation that a

low concentration (0.5 μM) of the $GABA_A$ receptor antagonist gabazine, known to block only phasic $GABA_A$-mediated currents (Semyanov *et al.*, 2003) completely blocks GDPs, indicating that tonic $GABA_A$-mediated conductance may have only a permissive role in GDPs generation (personal observation).

3.2.5.7 Possible mechanisms of GDPs initiation, propagation and termination

Brain function depends on the coordinated activity of large populations of neurons. Distinct behavioral states are associated with the generation of population discharges such as the theta and gamma rhythms (Buzsaki *et al.*, 1983; Traub *et al.*, 1996). The similarity in the mechanisms involved in the generation of GDPs and adult spontaneous large-amplitude irregular activity consisting of sharp positive waves (SPWs) in the hippocampus suggests that the basic features of the hippocampal circuitry are retained throughout development. GDPs were found to disappear during the second or third postnatal week in the rat (Ben-Ari *et al.*, 1989; Khazipov *et al.*, 2004). Similar spontaneous events (termed *in vitro* SPWs) have been observed in adult mouse hippocampal slices (Wu et al., 2005). The similarities between GDPs, SPWs and interictal discharges in the disinhibited hippocampus suggest a common mechanism of generation. Slight differences may be related to the different number of connections and to the different weight (excitatory or inhibitory) of individual elements according to the level of connectivity and stability of the network. Recently, theoretical models have emphasized that a simple plasticity algorithm in a growing network can be made by continuously adding new nodes and adaptive rewiring and weight of the connections according to the dynamic coherence of the activity patterns in the network which is self-organizing (Nakatani *et al.*, 2003; Chao and Chen, 2005; Jeong and Gutkin, 2005). Self-organization is a process in which the internal organization of a system increases in complexity without being guided or managed by an outside source. Hence in the developing hippocampus, intrinsic electrical activity of neurons might self-organize into distinct temporal patterns. During neuronal processing, individual neurons can integrate inputs from thousands of other neurons and, after reaching a threshold, distribute their activity back to the network. This basic process of neuronal integration and redistribution is similar to that seen in many other complex systems in which simple units with

thresholds integrate and then dissipate energy back to the system (Paczuski *et al.*, 1996). Are there analogies between threshold processes in the initiation of a neuronal population bursts and other all-or-none biological events? One obvious parallel is the threshold behavior in the generation of a single action potential. Action potential discharge can occur if Na^+ channels open fast enough to depolarize a cell to threshold before K^+ channels with slower kinetics can prevent further depolarization (Jack *et al.*, 1983). Opening of both Na^+ and K^+ channels depends on membrane potential. Does threshold behavior exist in neuronal population activity? The answer is positive. In this regard population firing may correspond to membrane potential. Excitatory synaptic events would be analogous to Na^+ channel openings in that their frequency depends on population firing and that they act to enhance firing in a positive feedback fashion through their recurrent collateral connections. In contrast, $GABA_B$-mediated synaptic events and slow Ca^{2+}-activated K^+ currents, may be analogous to K^+ channel openings in a single neuron in that they depress population firing with a delay due to i). G protein coupling between receptor activation and ii). channel opening due to increased intracellular calcium concentration. The threshold condition emerges since activity must spread through the network *via* excitatory transmission fast enough that $GABA_B$-mediated inhibition and slow Ca^{2+}-activated K^+ current cannot stop the spread. Clearly cellular and network processes such as connectivity, synaptic efficacy, and EPSP-spike coupling will affect how the population approaches its firing threshold and certainly determine two structurally distinct but functionally similar network pattern during brain maturation. De la Prida *et al.*, (2006) showed that population bursts in the disinhibited CA3 region (epileptic discharges) are initiated at a threshold level of population firing after recovery from a previous event. Each population discharge follows an active buildup period when synaptic traffic and cell firing increases to threshold levels. Similar threshold behavior may generate GDPs (Menendez de la Prida *et al.*, 1999, 2000).

In comparison to adulthood, in the immature hippocampus: i). GABA is depolarizing and often excites the postsynaptic neuron. ii). glutamatergic synapses are poorly developed and they are often silent (Durand *et al.*, 1996; Gasparini *et al.*, 2000). The glutamatergic connections alone would not be able to reach the firing threshold within the network.

Therefore, immature hippocampal circuits take advantage of their excitatory role of GABA to compensate for inefficient glutamatergic connections.

4. Activity-dependent synaptic plasticity processes

4.1 Overview

Since the 20th century scientists believed that memories are not the product of new nerve cells growth. They realized that in the adult brain the number of neurons does not change significantly with age. With this realization came the need to explain how memories are created in the absence of new cells growth. Ramon y Cajal in 1894 was among the first to propose that learning was not the product of new cells growth but was probably dependent on the strengthening of the connections between already existing neurons. Donald Hebb in 1949 reiterated Cajal's ideas and further proposed that, to improve the effectiveness of their communications, cells may develop new connections between each other:

> *Let us assume that the persistence or repetition of a reverberate activity (or "trace") tends to induce lasting cellular changes that add to its stability. When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.*
> *"The general idea is an old one that any two cells or systems of cells that are repeatedly active at the same time will tend to become 'associated', so that activity in one facilitates activity in the other." (Hebb, 1949)*
> *"When one cell repeatedly assists in firing another, the axon of the first cell develops synaptic knobs (or enlarges them if they already exist) in contact with the soma of the second cell." (Hebb, 1949)*

This theory, called "Hebbian" is often summarized as "*cells that fire together, wire together*". This principle has led to the idea that synaptic plasticity should be based on coincidence detection of two separate stimuli within a short time window. Therefore, coincidence detection provides the basis of synaptic plasticity, essential not only for the storage of information during learning and its consolidation into long-term memories but also for fine tuning neuronal network during postnatal development.

This theory was validated in the developing visual system by Hubel and Wiesel who in a series of elegant experiments provided evidence that during a critical period, visual experience shapes and strengthens the pattern of connections that are formed between neurons. After raising kittens under monocular deprivation, neurons in the primary visual cortex became unresponsive to stimulation of the deprived eye (Wiesel and Hubel, 1963a and 1963b). Although it was known that sensory deprivation during development had profound effects on the adult behavior (Feller, 1999), these results showed for the first time at the cellular level that neuronal activity during a 'critical period' of early development was necessary for the normal function of the adult visual cortex. Two years later, in follow-up studies, they reported that, while binocularly deprived animals had surprisingly normal ocular dominance responses, animals raised under artificial squint conditions had no binocular cells in their visual cortices (Hubel and Wiesel, 1965). This implies that the important factor is not the absolute level of activity, but instead the relative level of activity in one eye *versus* the other.

The molecular and cellular correlate of learning and memory is long-term potentiation (LTP) first described by Bliss and Lomo (1973) at glutamatergic synapses in the hippocampus. Bliss and Lomo demonstrated that high-frequency stimulation of input to cells in the dentate gyrus produces a subsequent increase in the amplitude of excitatory postsynaptic potentials that last for hours or even days. There is evidence that long-term potentiation and activity-dependent synaptogenesis share the same underlying cellular mechanisms (Crair, 1999). Indeed correlated activity between the afferent input and postsynaptic neuron strengthens connections through long-term potentiation (LTP), while uncorrelated synaptic inputs are selectively weakened or diminished through long-term depression (LTD). Alterations in synaptic strength can be regulated presynaptically as alterations in the machinery releasing the neurotransmitter, or postsynaptically by changing the number or function of receptors sensing the neurotransmitter signal. Such fast, durable and selective modifications of synaptic strength are necessary for wiring the brain during development (Burrone *et al.,* 2002; Morphy, 2003).

However, Hebbian forms of plasticity operate by positive feedback rules that, if left unchecked, tend to destabilize neuronal networks over time by driving neurons towards maximal and minimal action potential firing frequency ranges, which degrade

propagating signals in the network and render neurons unable to encode subsequent plastic changes by Hebbian mechanisms (Turrigiano and Nelson, 2004). Homeostatic plasticity might provide the global negative feedback necessary to maintain synaptic strength within a functional dynamic range based on the degree of ongoing synaptic activity. This will be achieved by scaling the strength of all synaptic inputs up or down to preserve their relative weights (synaptic scaling) or by altering the ability of synapses to undergo subsequent Hebbian modifications to reduce the effect of plasticity over time (metaplasticity) (Abraham and Tate, 1997; Turrigiano *et al.,* 1998; Perez-Otano and Ehlers, 2005).

In the past years, the temporal specificity in correlated activity required for the induction of LTP/LTD was examined. Levy and Steward (1983) noted that when a weak and a strong input from entorhinal cortex to the dentate gyrus were activated together, the temporal order of activation was crucial. LTP of the weak input was induced when the strong input was activated concurrently with the weak input or following it by as much as 20 ms. Interestingly, LTD was induced when the temporal order was reversed. Later studies have further addressed the importance of the temporal order of pre- and postsynaptic spiking in long-term modification of a variety of glutamatergic synapses and have defined the "critical windows" for spike timing (Debanne *et al.,* 1994; Debanne *et al.,* 1996; Markram *et al.,* 1997; Bi and Poo, 1998; Debbane *et al.,* 1998; Koester and Sakmann, 1998; Egger *et al.,* 1999; Feldman, 2000; Froemke and Dan, 2002; Nevian and Sakmann, 2004; Kaiser *et al.,* 2004; Mu and Poo, 2006, Letzkus *et al.,* 2006; Nevian and Sakmann, 2006).

As illustrated in Figure 8, when a presynaptic action potential precedes postsynaptic spiking within a window of several tens of milliseconds, LTP is induced, whereas spiking of the reverse order (postsynaptic action potential precedes the postsynaptic spiking) leads to LTD. This form of activity-dependent LTP/LTD is now referred to as spike timing-dependent plasticity (STDP). This phenomenon has been observed in various preparations, with some variation in the time-window relevant for plasticity (Dan and Poo, 2006). The rules and mechanisms of learning process in immature brain are not well known, but mutual interactions between early electrical activity and gene expression patterns have been demonstrated in various immature neuronal circuits, and it has been

suggested that the temporal dynamics of the electrical activity (e.g. frequency or duration of calcium transients or action potential bursts) might have distinct effects on different intracellular signaling pathways or transcription of specific genes (Fields *et al.,* 2005; Webb *et al.,* 2005). These genes can be considered as 'thermostats' that are turned up and down by intrinsic electrical activity. As it already mentioned (see setion 3.4.5), spontaneously occurring neuronal oscillations constitute a hallmark of developmental networks.

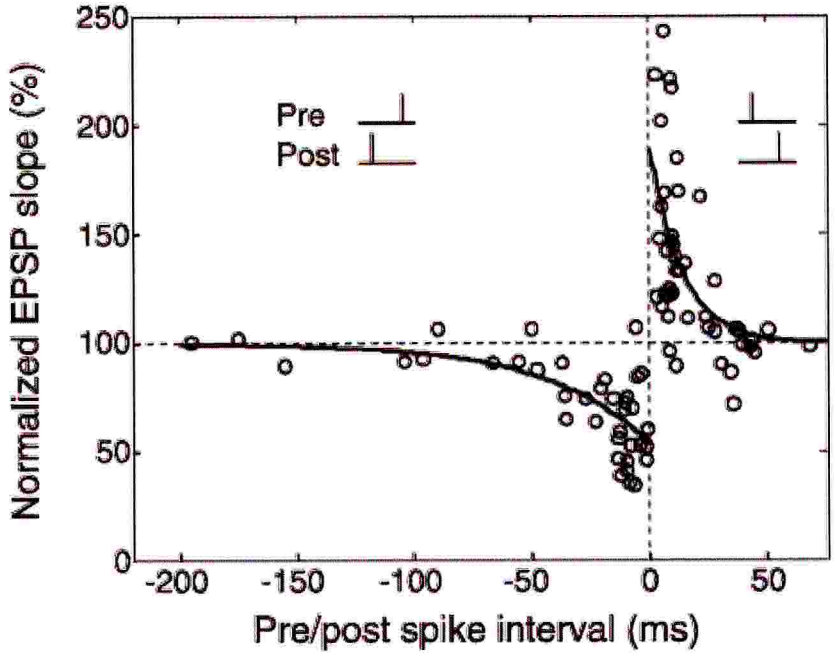

Figure 8. Synaptic modification induced by repetitively paired pre- and postsynaptic spikes in layer 2/3 of visual cortical slices from the rat. Each symbol represents one experiment. Curves are single exponential, least-squares fits of the data. *Insets* depict the sequence of spiking in the pre- and postsynaptic neurons. EPSP, excitatory postsynaptic potential (Dan and Poo, 2006).

Before synaptic circuits are formed, spontaneous electrical activity consists of coordinated calcium transients (calcium potentials) occurring in groups of cells (Spitzer, 1994; Corlew *et al.,* 2004; Moody and Bosma, 2005). When chemical synaptic contacts are established, a distinct network-driven activity emerges in developing brain. Such, intrinsic correlated neuronal activity at early postnatal stages might act as a 'training partner' for sensory-evoked synaptic inputs when these signals from the environment are

available for the first time. In fact this activity-dependent, but experience-independent mechanism might enable gross map formation, whereas map refinement takes place during the subsequent experience-dependent 'critical period' (Khazipov and Luhmann, 2006). In the developing brain, functional synaptic connections seem to be recruited from initially silent synaptic network. This functional synapse induction requires presynaptic action potentials paired with postsynaptic depolarization (Hebb's rule of association). Early recurrent network activities exhibit an endogenous form of neuronal pattern that promotes the activation of large number of pre and postsynaptic neurons. Nevertheless activity-dependent synaptic organization in the developing brain when all the inputs are relatively weak should be dominated by recruitment of new synapses and synapse strengthening (LTP) rather than active elimination and synapse wakening (LTD) of redundant connections. Otherwise elimination or weakening would be greatly favored over recruitment or strengthening (LTP), because correlated presynaptic and postsynaptic activity would be much rarer than uncorrelated activity. If not, LTD would be greatly favored over LTP, because correlated presynaptic and postsynaptic activity would be much rarer than uncorrelated activity and in turn, progressive weakening of all inputs. But this is not the case because early in development, LTP occurs with less specificity (spread of LTP to unstimulated inputs), thus enhancing the synaptic strengthening mechanism (Tao *et al.,* 2001; Engert and Bonhoeffer, 1997; Chen, 2001; Hanse *et al.,* 1997; Bailey *et al.,* 2000; Kasyanov *et al.,* 2004).

As already mentioned, the main hypothesis being investigated is the possibility that in the developing brain spontaneous activity is crucial for the establishment of the adult neuronal circuitry. Studies of glutamatergic silent synapses in the immature hippocampus have provided progressive insights into involvement of synaptic activity in synapse maturation.

4.2 Silent synapses

A common feature of postnatal development is the presence of "silent" synapse. They have been observed in a variety of different structures including the hippocampus (Durand *et al.,* 1996; Liao and Malinow, 1996; Isaac *et al.,* 1997; Rumpel *et al.,* 1998; Losi *et al.,* 2002). Silent synapses are synapses that do not respond at rest but show pure

NMDA-mediated responses at depolarizing potentials. Their conversion into functional synapses is thought to be crucial for stabilization of synaptic contacts and for refinement of neuronal networks (Malenka and Nicoll, 1997; Malinow *et al.,* 2000; Oray *et al.,* 2006). In particular, it has been shown that the percentage of silent synapses decrease during postnatal development (Durand *et al.,* 1996; Liao and Malinow, 1996; Petralia *et al.,* 1999). Conversion of silent synapses into connecting ones occurs in an associated dependent way, such as during long-term potentiation (Isaac *et al.,* 1995; Durand *et al.,* 1996; Montgomery *et al.,* 2001; Poncer and Malinow, 2001).

Different hypothesis have been put forward to explain silent synapses (Voronin and Cherubini, 2003 and 2004; Groc *et al.,* 2006). See Figure 9. The most common interpretation is that these synapses are "postsynaptically" silent, i.e. they do not express functional AMPA receptors on the postsynaptic membrane, but only NMDA receptors which at rest are inactive because of the Mg^{2+} block (Figure 9A). According to this idea, their activation requires insertion of new AMPA receptor proteins on the subsynaptic membrane following NMDA-dependent LTP (Durand *et al.,* 1996; Liao *et al.,* 2001; Poncer and Malinow, 2001). In agreement with this hypothesis is the observation that in pairs of interconnected CA3–CA3 neurons the postsynaptic responsiveness to exogenous application of AMPA increases immediately after LTP induction indicating that synapse unsilencing may occur *via* an increase in postsynaptic AMPAR function (Montgomery *et al.,* 2001). However, exogenous application of AMPA may activate extrasynaptic receptors whose functional properties may differ from those of the synaptic ones. Using immunocytochemical techniques, the presence of AMPA and NMDA receptors in populations of synapses at different developmental stages have been investigated in order to determine whether the two receptor types are recruited simultaneously or sequentially. According to the postsynaptic hypothesis, in some of these studies, immediately after birth few AMPA receptors were found to be expressed on the postsynaptic membrane in comparison with NMDA receptors (Liao *et al.,* 1999; Petralia *et al.,* 1999; Pickard *et al.,* 2000; Liao *et al.,* 2001). In other reports however, the percentage of synapses expressing both AMPA and NMDA receptors was found to be relatively constant over development (Rao *et al.,* 1998; Lissin *et al.,* 1998; Washbourne *et al.,* 2002; Voigt *et al.,* 2005). These differences can be at least in part attributed to the difficulty in using the

immunocytochemical technique in the immature tissue and to clearly distinguish synaptic from extrasynaptic receptors, which constitute the majority of the receptors present on the postsynaptic membrane (Borgdorff and Choquet, 2002; Groc *et al.,* 2006).

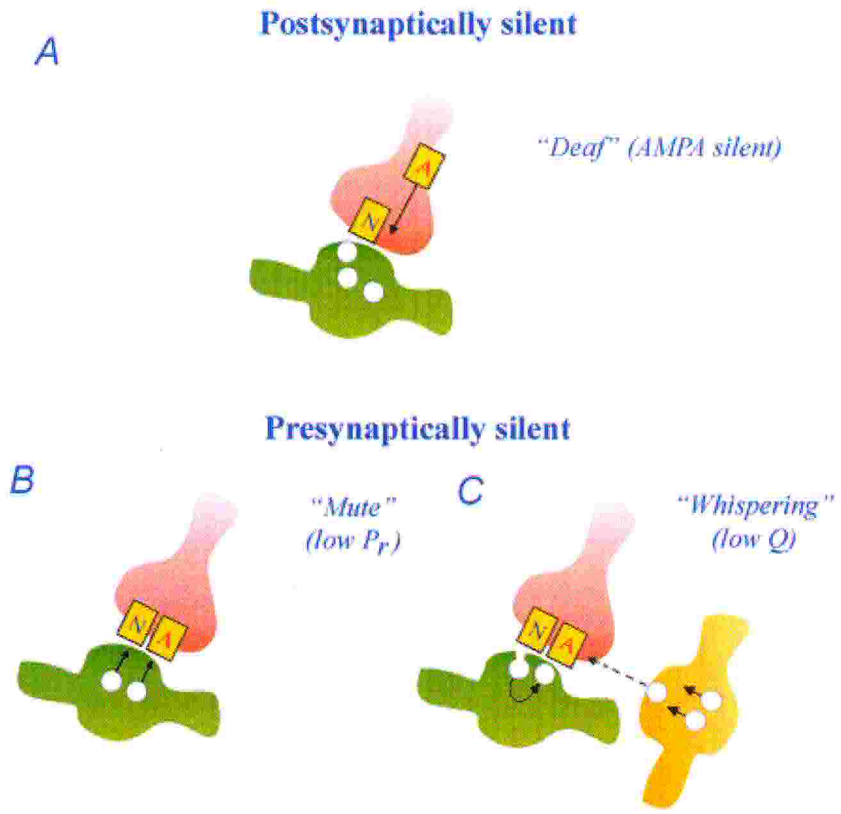

Figure 9. Models of silent synapses and possible mechanisms of their unsilencing during LTP
A, postsynaptically silent (*deaf*) synapse lacking AMPARs, but expressing functional NMDARs (N in the box). Functional AMPARs were delivered to the subsynaptic membrane after LTP induction (A with arrow). *B*, presynaptically silent synapse. In this case, both AMPAR and NMDAR were present on the subsynaptic membrane, but they were activated only rarely because of the very low release probability (P_r) (no docked vesicles). LTP would lead to an increased P_r (arrows). *C*, two types of presynaptic silent synapses. Here, either the amount of glutamate released by one vesicle is too small or the release is too slow to be detected by low affinity AMPARs. This could occur either because of a low conductance fusion pore (green terminal) or because of glutamate spillover from remote synapses (dashed arrow from yellow terminal). A change in the operation mode of the fusion pore from slow to fast adapting (see docked vesicle with black arrow in the green terminal) or an increased P_r (vesicles with arrows in the yellow terminal) from remote synapses would account for synapse unsilencing. (Voronin and Cherubini, 2004)

However, the postsynaptic origin of silent synapses is a matter of debate. An alternative view is that synapses are silent, not because AMPA receptors are not present on the postsynaptic membrane but because the probability of glutamate release from presynaptic nerve endings is too low to activate AMPA receptors. In accord with this hypothesis Groc *et al.,* (2002), have shown that spontaneous AMPAR- and NMDAR-mediated synaptic events are present from birth and that their relative amplitude and frequency remain constant during the first postnatal week. Further evidence in favor of presynaptically silent synapse comes from experiments with minimal paired-pulse stimulation delivered to Schaffer collateral or to mossy fibers (Gasparini *et al.,* 2000; Maggi *et al.,* 2003). Neurones exhibiting responses at +40 mV but only failures at –60 mV occasionally responded to a second pulse delivered 50 ms after the first (Figure 9B). This paired-pulse facilitation phenomenon is known to largely depend on the increase in probability of transmitter release. In line with the initial definition (Redman, 1990) these synapses are presynaptically rather than postsynaptically silent, and increasing probability can lead to their unsilencing. An increase in probability of release can be achieved by other experimental means, such as raising the temperature, applying cyclothiazide (a drug known to block AMPARs desensitization and to enhance transmitter release), activating presynaptic α7 nicotine receptors with nicotine or with endogenously released acetylcholine (Choi *et al.,* 2000; Gasparini *et al.,* 2000; Maggi *et al.,* 2003).

In addition, synapses can be presynaptically silent due to low concentration of released glutamate that would be insufficient to produce a detectable quantal response (Figure 9C). Here, the assumption is that both NMDARs and AMPARs are co-localized and are simultaneously activated. The degree of receptor activation depends on the amount and temporal profile of the transmitter in the cleft. In the case of a low rate of glutamate release (i.e. from a slow adapting fusion pore; Choi *et al.,* 2000; Figure 9*C*, green) or remoteness of the release site from the receptors (spillover from neighboring synapses, Kullmann, 2003; Figure 9*C*, yellow) the amount of glutamate in the cleft would be sufficient to activate high affinity NMDARs but not AMPARs which have an affinity hundred times lower (Kullmann, 2003, Voronin and Cherubini, 2004). Using a fast unbinding NMDA antagonist, Choi *et al.,* (2000) demonstrated LTP-induced increase in

cleft glutamate concentration (from < 170 μM to the millimolar range) able to activate AMPARs. In addition, the idea that AMPAR activation in apparently silent synapses depends on the concentration profile of glutamate in the cleft has been directly tested in cultured hippocampal neurones by slow and fast applications of glutamate (Renger *et al.*, 2001). The former were found to evoke only NMDA currents, while the latter evoked both NMDA and AMPA currents (Kullmann, 2003). In conclusion, during development synapses "unsilencing" can be due not only to insertion of new AMPA receptors at synapses but also to presynaptic changes in release probability.

4.3 Synchronized network activity is necessary for synapse formation

Understanding how early neuronal activity shapes synaptic circuits during postnatal development is a very challenging task. The functional significance of synchronized network activity is largely unknown. The mechanisms generating early network activity rely on intrinsic properties of neurons and their synaptic interactions that are unique to early developmental stages.

In the developing hippocampus, blocking spontaneous activity with TTX induced a significant reduction of miniature $GABA_A$-mediated postsynaptic currents (Coline-Le brun *et al.*, 2004). The effects of TTX were reproduced when the intact hippocampus was incubated in the presence of glutamatergic or GABAergic ionotropic receptor antagonists or in the presence of divalent cations. These findings strengthen the importance of spontaneous synaptic activity in the formation of functional GABAergic synapses, possibly *via* network events which require both glutamatergic and GABAergic receptors. By inhibiting correlated neural activity *in vivo* with injection of tetanus toxin at P1 into the rat hippocampal CA1 area, Groc *et al.*, (2003a) observed few days later a strong reduction in the frequency of spontaneous glutamatergic and GABAergic synaptic currents (but see Lauri *et al.*, 2003). This effect was associated with impairment in the growth of basal dendrites in CA1 pyramidal cells (Groc *et al.*, 2002a and 2003b). During a restricted period of postnatal development, using a conditioning protocol consisting of repetitive depolarizing pulses delivered at the same frequency as GDPs, Caillard et al. (1999) observed an increase in frequency of spontaneous action potential dependent and independent GABAergic synaptic currents. In addition, during the first postnatal week,

pairing GDPs with mossy fiber stimulation induced a persistent increase in synaptic efficacy (Kasyanov *et al.,* 2004). The pairing procedure was also able to switch silent synapses into functional. This effect was dependent on calcium rise in the postsynaptic cell following the activation of voltage-dependent calcium channels during GDPs. Furthermore, recent studies showed that in the adult dentate gyrus, newborn granule cells mature slowly than in the neonate. This might be due to the fact that in neonates but not in adults, neurons are stimulated by network-driven depolarizing events generating calcium transients (GDPs, Overstreet-Wadiche *et al.,* 2006). Network activity may therefore act as coincident detection signal between pre (afferent synaptic input) and post (localized Ca^{2+} influx associated with GDPs) synaptic activity and would provide the Hebbian conditions for LTP. This would lead to functional and structural changes characteristic of the adult circuits.

4.4 Neurotrophins and activity dependent changes

Neurotrophins are not only essential for the survival and the differentiation of several types of neurons but are also involved in the regulation of different forms of synaptic plasticity. Among them, brain-derived neurotrophic factor (BDNF) has been shown to modulate the development and function of synapses in various systems, ranging from the neuromuscular junction to the cortex. The functions of BDNF are mediated through two distinct receptor types: p75 and TrkB which belongs to the family of tyrosine kinases. p75 is a low affinity receptor, to which all neurotrophins bind (Nagappan and Lu, 2005). The highest levels of BDNF and TrkB receptors are found in the hippocampus, cortex and hypothalamus, areas crucial for learning, memory, and higher cognitive functions (Armanini *et al.,* 1995; KatohSemba *et al.,* 1997).

In the hippocampus, BDNF mRNA is localized to pyramidal neurons and granule cells of the dentate gyrus. While the expression of mRNA encoding for BDNF remains constant from birth until 22 months of age, the level of BDNF significantly increases from P1 to P7 and then remains constant up to 10 months when it starts to decline. TrkB are already fully expressed at birth. At P1 the level of BDNF is ~60% of that found at P7 (Silhol *et al.,* 2005). In the hippocampus, the BDNF/TrkB signaling system is thought to play a crucial role in spatial learning. Indeed, the BDNF mRNA expression is upregulated in

response to spatial learning experiments (Gomez-Pinilla *et al.,* 2001; Kesslak *et al.,* 1998; Mizuno *et al.,* 2000) or when the animal is exposed to an enriched-environment (Falkenberg *et al.,* 1992; Ickes *et al.,* 2000). Spatial learning stimulates also the activation of TrkB receptors and downstream signaling pathways (Gooney *et al.,* 2002; Mizuno *et al.,* 2003). Accordingly, alterations in the TrkB receptor function lead to learning deficits. Indeed, TrkB targeted mutants show a dramatic deficit in complex and stressful learning paradigms such as Morris water maze or eight-arm radial maze whereas less demanding learning tests are performed normally (Minichiello *et al.,* 1999). Additional studies in a more naturalistic environment suggest that the TrkB mutant mice could actually learn a spatial task; however, they are unable to rapidly switch to another behavioral strategy (Vyssotski *et al.,* 2002). Similar lack of behavioral flexibility is known to be associated with hippocampal lesions (Terry *et al.,* 1989; Vyssotski *et al.,* 2002).

Secretion and action of BDNF is controlled by spontaneous activity in complex feedback loops. However, BDNF secretion is preferentially stimulated by certain patterns of activity (patterned bursts as opposed to tonic depolarization or steady firing) and requires a rise of intracellular Ca^{2+}. BDNF *via* TrkB receptors induces also elevation of intracellular Ca^{2+} levels by releasing calcium from intracellular calcium stores (Berninger *et al.,* 1993; Canossa *et al.,* 1997; Kaplan and Miller, 1997). The enhanced release of BDNF in turn increases synaptic activity leading to more BDNF released in a positive regulatory mechanism (Canossa *et al.,* 1997; Kruttgen *et al.,* 1998). One of the more intriguing feedback system activated by BDNF during development involves GABAergic transmission. At early stages of development when GABA is depolarizing and excitatory, due to high intracellular chloride concentration (see section 3.4.3 and 3.4.5). GABA can stimulate BDNF expression in a variety of neurons, an effect that disappears as GABA becomes inhibitory (Berninger *et al.,* 1995; Obrietan *et al.,* 2002). During this stage of development, BDNF can acutely increase the frequency of $GABA_A$ mediated miniature currents, thus creating a positive-feedback loop. However, BDNF stimulates also the expression of the KCC2 transporter which, by reducing the intracellular chloride concentration, converts the action of GABA from depolarizing to hyperpolarizing leading to a reduction of BDNF expression (Aguado *et al.,* 2003; but see Rivera *et al.,* 2002).

Like BDNF, also the expression of TrkB is regulated by neuronal activity. Neuronal activity and calcium would increase cAMP levels that would "gate" the action of BDNF (Suzuki *et al.,* 2004), leading to an increased amount of TrkB receptors along dendrites, axons and cell somata (Du *et al.,* 2000; Meyer-Franke *et al.,* 1998). BDNF binding to TrkB triggers autophosphorylation of tyrosine residues in its intracellular domain, leading to the activation of one or more of the three major signaling pathways: the mitogen-activated protein kinase (MAPK), the phosphatidylinositol 3-kinase (PI3K) and the phospholipase Cγ (PLC-γ) (Nagappan and Lu, 2005).

One of the most interesting role of BDNF consists in the regulation of synaptic transmission. BDNF potentiates excitatory synaptic transmission by promoting presynaptic transmitter release. Hence, application of BDNF to developing *Xenopus* neuromuscular synapses rapidly potentiates basal synaptic transmission by increasing neurotransmitter release (Lohof *et al.,* 1993). In the central nervous system, similar enhancement is observed upon BDNF application to hippocampal and cortical slices (Kang *et al.,* 1997) or hippocampal and cortical neurons in cultures (Lessmann, 1998). The site of BDNF action (pre or postsynaptic) is still a matter of debate. Some studies have demonstrated that BDNF acts postsynaptically (Henneberger *et al.,* 2002; Kovalchuk *et al.,* 2002; Levine *et al.,* 1995; Suen *et al.,* 1997) while others support a presynaptic site of action (Frerking *et al.,* 1998; Gottschalk *et al.,* 1998; Lohof *et al.,* 1993; Olofsdotter *et al.,* 2000; Vicario-Abejon *et al.,* 1998). Consistent with a presynaptic site of action is the reduced number of docked vesicles observed at excitatory synapses in the hippocampus of BDNF knockout mice (Pozzo-Miller *et al.,* 1999). Additionally, BDNF mutants have been shown to express a reduced amount of the vesicular proteins synaptobrevin and synaptophysin (Pozzo-Miller *et al.,* 1999; but see Grosse G. *et al.,* 2005). Furthermore, presynaptic but not postsynaptic expression of dominant negative TrkB receptors inhibits synaptic potentiation in cultured neurons (Li *et al.,* 1998). In line with a presynaptic site of action of BDNF, Shen *et al.,* (2006) have demonstrated that a brief burst of action potentials is able to rapidly "awake" silent synapses by increasing the availability of synaptic vesicles for fusion through BDNF-triggered presynaptic actin an effect mediated by the small GTPase Cdc42.

BDNF is involved also in long-term plasticity processes as demonstrated by the impairment of LTP in BDNF knock-out mice. Interestingly, LTP could be rescued by re-expression of BDNF either by virus-mediated gene transfer or exogenous application of BDNF, suggesting that in these mice the impairment is not due to developmental deficits (Korte *et al.,* 1995 and Patterson *et al.,* 1996). Additional evidence in favor of the involvement of BDNF in LTP was provided by studies where LTP was attenuated in the presence of TrkB-IgG proteins which act as BDNF scavengers (Chen *et al.,* 1999). Besides its immediate action, BDNF is essential for the late-phase of LTP which requires new protein synthesis (Korte *et al.,* 1997). This requires CREB activation through the TrkB-ERK signaling pathway (Gooney and Lynch, 2001; Patterson *et al.,* 2001). Interestingly, as demonstrated by Patterson et al (2001) during the late phase of LTP, TrkB signaling seems to regulate the redistribution of activated MAPK towards the nuclear compartment. These data have been validated using genetically modified mice, in which the absence of TrkB in the CA1 region of the hippocampus leads to LTP reduction. Activity-dependent control of TrkB receptors raises the possibility that TrkB could function as a synaptic tag. According to the 'synaptic tagging' hypothesis proposed by Frey and Morris (1997), synapse-specificity of the late form of LTP is achieved by the interaction of plasticity related proteins, which are synthesized in the cell body or dendrites, with the synaptic tags that are generated locally at the stimulated synapses (Nagappan and Lu, 2005).

5. Aim of Study

The aim of this thesis was to study:

i) The general mechanisms involved in GDP generation

ii) How GDPs shape synaptic circuits during postnatal development.

A first set of experiments was undertaken to see whether neuronal membrane oscillations similar to GDPs can be detected in organotypic hippocampal slice cultures. This preparation has the advantage of maintaining morphological and functional features similar to those of native hippocampus even if flattened close to a monolayer. This would allow perturbation of the local circuit by introducing in selected cells, *via* biolistic or viral infection techniques, enhanced green fluorescent protein-tagged genes in order to alter cell excitability and/or the chloride equilibrium potential.

I have studied:

- The time course of emergence and disappearance of GDPs in slice cultures
- How pyramidal and interneurons contributes to GDPs generation
- The functional role of GABA (excitatory or inhibitory) during early postnatal development

Early in development, many glutamatergic connections appear "silent" at rest either because they lack functional AMPA receptors on the subsynaptic membrane or because the probability of neurotransmitter release is very low. Conversion of silent synapses into functional is the most common form of LTP induction. In accord with the Hebb's hypothesis on activity-dependent synaptic strengthening, I have tested the hypothesis that GDPs may act as coincident detector signals between pre and postsynaptic activity for enhancing synaptic strength. This assumption has been verified at Schaffer collateral-CA1 connections that during the first postnatal week are still poorly developed.

Therefore, in a second set of experiments I have investigated:

- Whether the pairing procedure induces short or long term changes in synaptic efficacy.
- Whether similar modifications in synaptic strength can be induced by pairing afferent inputs with theta bursts or plateau potentials.
- The molecular cascade involved in GDPs-induced persistent changes in synaptic strength.

Methods

See the enclosed papers

Results

-Paper 1-

Spontaneous recurrent network activity in organotypic rat hippocampal slices

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Spontaneous recurrent network activity in organotypic rat hippocampal slices

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Keywords: epileptiform discharges, equilibrium potential for GABA, GABAergic interneurones, network activity, pair recordings

Abstract

Organotypic hippocampal slices were prepared from postnatal day 4 rats and maintained in culture for >6 weeks. Cultured slices exhibited from 12 days *in vitro* spontaneous events which closely resembled giant depolarizing potentials (GDPs) recorded in neonatal hippocampal slices. GDP-like events occurred over the entire hippocampus with a delay of 30–60 ms between two adjacent regions as demonstrated by pair recordings from CA3–CA3, CA3–CA1 and interneurone–CA3 pyramidal cells. As in acute slices, spontaneous recurrent events were generated by the interplay of GABA and glutamate acting on AMPA receptors as they were reversibly blocked by bicuculline and 6,7-dinitroquinoxaline-2,3-dione but not by DL-2-amino-5-phosphonopentaoic acid. The equilibrium potentials for GABA measured in whole cell and gramicidin-perforated patch from interconnected interneurones–CA3 pyramidal cells were −70 and −56 mV, respectively. The resting membrane potential estimated from the reversal of *N*-methyl-D-aspartate-induced single-channel currents in cell-attach experiments was −75 mV. In spite of its depolarizing action, in the majority of cases GABA was still inhibitory as it blocked the firing of principal cells. The increased level of glutamatergic connectivity certainly contributed to network synchronization and to the development of interictal discharges after prolonged exposure to bicuculline. In spite of its inhibitory action, in a minority of cells GABA was still depolarizing and excitatory as it was able to bring principal cells to fire, suggesting that a certain degree of immaturity is still present in cultured slices. This was in line with the transient bicuculline-induced block of GDPs and with the isoguvacine-induced increase of GDP frequency.

Introduction

Spontaneously occurring neuronal oscillations constitute a hallmark of developmental networks. They have been observed in several brain structures including the retina (Feller *et al.*, 1997), neocortex (Garaschuk *et al.*, 2000), hippocampus (Ben-Ari *et al.*, 1989) and spinal cord (O'Donovan, 1999). In the developing hippocampus neuronal activity is characterized by the so-called 'giant depolarizing potentials' (GDPs). GDPs are characterized by recurrent membrane depolarization with superimposed fast action potentials. They are generated by the synergistic action of glutamate and GABA that at this developmental stage is depolarizing and excitatory (Ben-Ari *et al.*, 1989; Cherubini *et al.*, 1991). While in the adult brain GABA hyperpolarizes the membrane and inhibits neuronal firing, in immature cells, due to the reverse transmembrane chloride gradient, GABA depolarizes and excites the membrane through an outwardly directed flux of chloride (Cherubini *et al.*, 1991; Owens *et al.*, 1996; Ben-Ari *et al.*, 1997). The intracellular chloride concentration is under the control of two main Cl^- cotransporter systems, NKCC and KCC2, that enhance and lower $[Cl^-]_i$, respectively (Payne *et al.*, 2003). The inbalance between these two transporters is responsible for the high $[Cl^-]_i$ found early in postnatal life. GDPs disappear towards the end of the second postnatal week when GABA becomes inhibitory (Ben-Ari *et al.*, 1989). This results from developmental changes in the expression of the K^+/Cl^- cotransporter KCC2 (Rivera *et al.*, 1999). The molecular basis of the ontogenetic switch in GABA action has been attributed to GABA itself which would regulate the expression of KCC2 (Ganguly *et al.*, 2001). Although this hypothesis has been recently questioned (Ludwig *et al.*, 2003; Titz *et al.*, 2003), the fact that GABA signalling exerts a prominent role in postnatal development cannot be denied.

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). Thus, as in many other developing systems (Ben-Ari, 2002), GDPs constitute a primordial form of synchrony that ensures large calcium oscillations acting as coincidence detector signals for enhancing synaptic efficacy (Kasyanov *et al.*, 2004).

The aim of the present study was to investigate whether neuronal membrane oscillations similar to GDPs can be detected in organotypic hippocampal slice cultures. This preparation has the advantage of maintaining morphological and functional features similar to those of native hippocampus (Gähwiler *et al.*, 1997; De Simoni *et al.*, 2003) even if flattened close to a monolayer. This would allow perturbation of the local circuit by introducing in selected cells, via biolistic or viral infection techniques, enhanced green fluorescent protein-tagged genes in order to alter cell excitability and/or the chloride equilibrium potential. Changes in chloride homeostasis and GABA signalling may affect the expression of other genes essential for the development of the adult hippocampal network. Such an approach could be crucial for understanding the functional role that membrane oscillations have in the refinement of the neuronal connectivity.

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Materials and methods

Organotypic hippocampal slice cultures

The hippocampus was removed from 4-day-old rats killed by decapitation and organotypic cultures were prepared following the method previously described (Gähwiler, 1981; Saviane *et al.*, 2002). The procedure is in accordance with the regulations of the Italian Animal Welfare Act and was approved by the local authority veterinary service (Dr R. Zucca). Transverse 400-μm-thick slices were cut with a tissue chopper and attached to coverslips in a film of reconstituted chicken plasma (Cocalico, Reamstown, PA, USA) clotted with thrombin (Sigma, Milan, Italy). The coverslips were transferred to plastic tubes containing 0.75 mL of medium. The tubes were placed in a roller drum (6 revolutions/h) inside an incubator at 36 °C. The medium contained: basal medium (Eagle with Hanks' salts without L-glutamine; Gibco, 100 mL), Hanks' balanced salt solution (Gibco, 50 mL), horse serum (Gibco, 50 mL), L-glutamine (Gibco, 200 mM, 1 mL) and 50% D-glucose in sterile water for tissue culture (Gibco, 2 mL).

Electrophysiological recordings

After 10–14 days *in vitro* the cultures, which had flattened near monolayer thickness, were transferred to a recording chamber fixed to the stage of an upright microscope. Cultured slices in the recording chamber were superfused at room temperature (22–24 °C) or at 33 °C with artificial cerebrospinal fluid containing (in mM): NaCl, 150; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 1; HEPES, 10; glucose, 10 (pH 7.3, adjusted with NaOH). To test the contribution of HCO_3^- anions to equilibrium potential for GABA (E_{GABA}) additional experiments were performed using an extracellular solution containing (in mM): NaCl, 130; KCl, 3.5; $CaCl_2$, 2; $MgCl_2$, 1.3; $NaHCO_3$, 25; NaH_2PO_4, 1.2; glucose, 10. In this case the solution was saturated with 95% O_2 and 5% CO_2 (pH 7.3).

Electrophysiological experiments were performed from CA1, CA3 pyramidal cells, granule cells of the dentate gyrus and GABAergic interneurones using the cell-attach, the whole-cell configuration of the patch-clamp technique in current- or voltage-clamp mode. When required, gramicidin-perforated patch experiments were also performed.

Recordings were made with a patch-clamp amplifier (Multiclamp 700A; Axon Instruments, Foster City, CA, USA). Patch electrodes were pulled from borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany) which had a resistance of 5–7 MΩ when filled with an intracellular solution containing (in mM): $KMeSO_4$, 135; KCl, 10; HEPES, 10; $MgCl_2$, 1; Na_2ATP, 2; Na_2GTP, 0.4; pH was adjusted to 7.2 with KOH. The stability of the patch was checked by repetitively monitoring the input and series resistance during the experiment. Cells exhibiting 15–20% changes were excluded from the analysis.

Pair recordings were performed from principal cells or principal cells and GABAergic interneurones. GABAergic interneurones, localized in stratum radiutum or in the close vicinity of stratum pyramidale, were identified both visually (using infrared differential interference contrast video microscopy) and on the basis of their firing properties, i.e. their ability to fire repetitively in response to long (400-ms) depolarizing current pulses. Pair recordings were also performed from interconnected GABAergic interneurones and principal cells in the CA3 area. Action potentials evoked in GABAergic interneurones by short (5-ms) depolarizing current pulses at 0.2 Hz elicited monosynaptic currents in principal cells. In some experiments, GABAergic interneurones were stimulated with a glass pipette filled with the extracellular solution. Under our experimental conditions the estimated reversal potential for Cl^- calculated with the Nernst equation was −66 mV. The connected cells were labelled with biocytin (0.2–0.3%; purchased from Sigma) for later identification.

For gramicidin-perforated patch recordings the pipette solution contained the same intracellular solution plus 80 μg/mL of gramicidin D (Sigma). A 20 mg/mL stock of gramicidin in dimethylsulphoxide 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 around 40 min, series resistance decreased and stabilized at around 30 MΩ.

In some experiments, the resting membrane potential was estimated from the reversal potential of NMDA-induced single-channel currents in cell-attach recordings. In these cases pipettes were filled with nominally magnesium-free artificial cerebrospinal fluid plus NMDA (10 μM) and glycine (10 μM). The tip of the pipette was covered with silgard to reduce membrane capacitance. Both multilevel and short (<2 ms) openings were discarded form the analysis. Membrane potential values were also determined as the mean value of the membrane potential obtained during a recording of several minutes in current-clamp mode in the absence of any current. Membrane potential was corrected for the liquid junction potential of 10 mV.

Drugs

All substances were prepared as 1000 times concentrated stock solutions. Tetrodotoxin was purchased from Affinity Research Products (Nottingham, UK). Picrotoxin, bicuculline, SR-95531 (gabazine), 6,7-dinitroquinoxaline-2,3-dione (DNQX), DL-2-amino-5-phosphonopentaoic acid (D-AP5), NMDA and isoguvacine were purchased from Tocris, Cookson (Bristol, UK). All substances were applied through a three-way tap system. Bath volume exchange was completed in less than 2 min.

Data acquisition and analysis

Data were stored on the hard disk of a PC after digitization with an A/D converter (Digidata 1322A; Axon Instruments). Data were sampled at 10 kHz and filtered with a cut-off frequency of 1 kHz. Single-channel experiments were sampled at 50 kHz and filtered at a cut-off frequency of 2 kHz. Acquisition and analysis were performed with Clampfit 9.0 (Axon Instruments). For each cell, GDP frequency and interictal discharges were calculated in control conditions and during drug application (starting 3 min after the onset of drug perfusion). The numerical data are given as mean ± SEM. Significance was assessed using the Student's *t*-test. The differences were considered significant at $P < 0.05$.

Results

Whole-cell patch-clamp recordings in voltage- or current-clamp configuration were performed from 194 cells in organotypic hippocampal slices cultured for periods of 7–48 days. After 2 weeks in culture between 12 and 44 days *in vitro*, spontaneous recurrent network activity similar to GDPs was detected in ~84% of cells examined. Before the second week in culture, GDPs were recorded in only one case out of 13 (from an 8-days *in vitro* slice, Fig. 1A and B). At room temperature, GDPs were characterized by a membrane

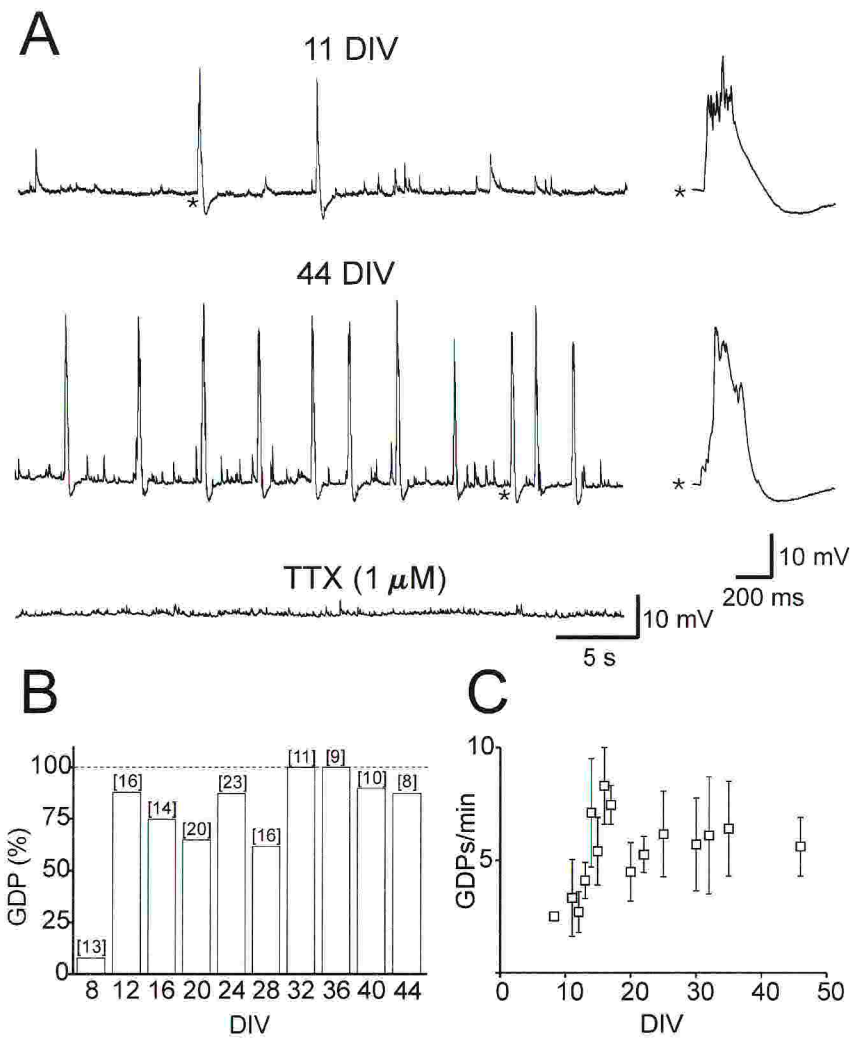

FIG. 1. Giant depolarizing potential (GDP)-like activity is present in hippocampal slice cultures. (A) Representative traces of GDPs (those marked with asterisks are shown on the right at an expanded time-scale) recorded from CA3 neurones (at −68 mV in the upper and −73 mV in the lower trace) in hippocampal slice cultures maintained for 11 or 44 days *in vitro* (DIV). Note the different frequency of GDPs at 11 and 44 DIV. GDPs were generated within the network as they were readily blocked by tetrodotoxin (TTX) (lower trace). (B) Each column represents the number of cells exhibiting GDPs expressed as a percentage of the total number of neurones tested within 4-DIV intervals (numbers in brackets). (C) Each point on the graph represents the mean value of GDPs/min for the cells shown in B. Note the increase in GDP frequency with DIV.

depolarization lasting hundreds of milliseconds (from 80 to 700 ms), often subthreshold for spike generation. As in acute slices, at more physiological temperature (33 °C), GDPs gave rise to several action potentials and were followed by a membrane hyperpolarization. Although a great variability was present between different cells, GDPs occurred at a frequency of 0.1 ± 0.03 Hz ($n = 26$). As shown in Fig. 1A and C, the frequency of GDPs progressively increased during the second week in culture and then remained constant throughout the period examined. This was probably related to the increased glutamatergic synaptic connectivity during the first weeks in culture (De Simoni *et al.*, 2003). As in acute slices, GDP frequency was unaffected by membrane hyperpolarization, suggesting that they were network-driven events. In line with their synaptic origin, GDPs were reversibly abolished by tetrodotoxin (1 μM; $n = 4$) or cobalt (2 mM; $n = 2$; data not shown) which blocks sodium or calcium currents, respectively. Moreover, they were reversibly blocked by the $GABA_A$ receptor antagonists bicuculline (10 μM; $n = 12$; see Fig. 6A), picrotoxin (100 μM; $n = 2$), gabazine (50 μM; $n = 2$) and by the AMPA/kainate receptor antagonist DNQX (20 μM; $n = 8$; data not shown). The NMDA receptor antagonist D-AP5 did not affect the frequency or shape of GDPs (in three cells GDP frequency was 0.09 ± 0.03 and 0.08 ± 0.04 Hz before and after D-AP5, respectively) confirming that, as in acute slices, GDPs were mediated by the synergistic action of GABA acting on $GABA_A$ receptors and glutamate acting on AMPA-type receptors (see also Bolea *et al.*, 1999).

Spontaneous recurrent network activity occurs over the entire hippocampus

In order to see whether, as in acute slices, spontaneous recurrent GDP-like events occurred over the entire hippocampus (Ben-Ari *et al.*, 1989), pair recordings were performed from 34 cells localized in different subfields. As illustrated in the representative examples of Fig. 2, GDPs recorded in either voltage- or current-clamp mode from

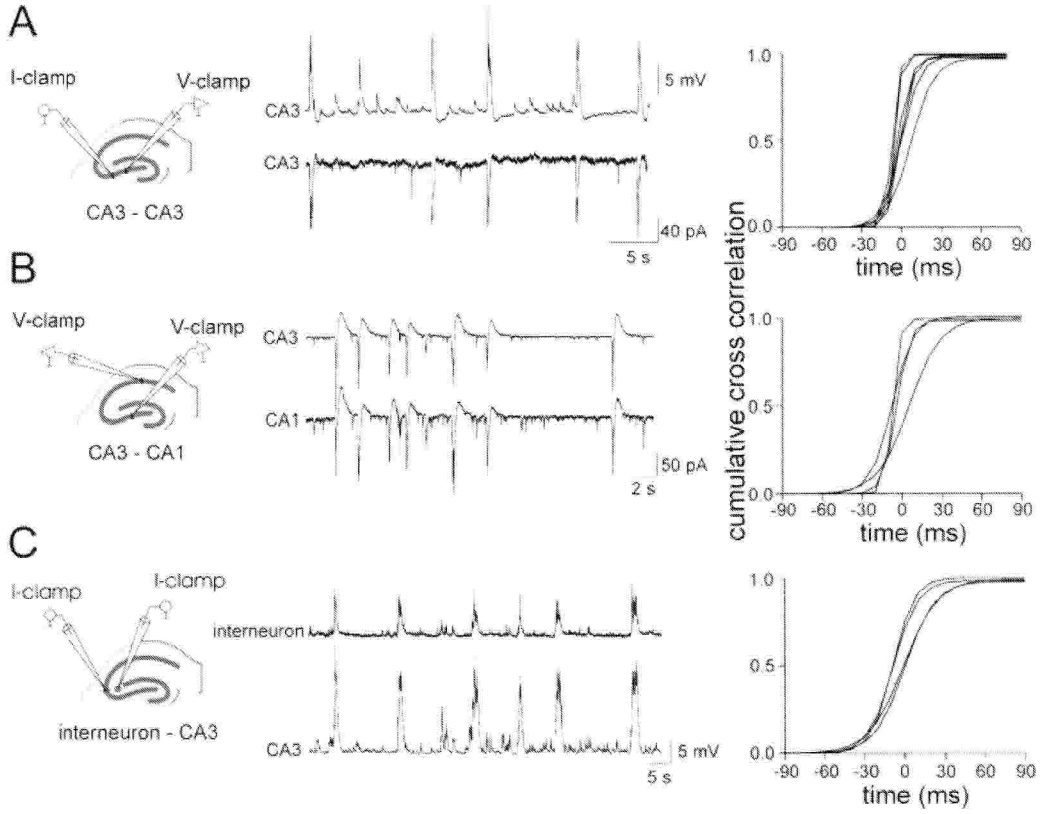

FIG. 2. Giant depolarizing potentials (GDPs) occur over the entire hippocampus. Representative traces showing pair recordings obtained in current-clamp (I-clamp) or voltage-clamp (V-clamp) conditions from CA3–CA3 (A), CA3–CA1 (B) or interneurone–CA3 (C) pairs. On the right cumulative cross-correlation graphs showing the time difference between the onset of GDPs recorded in individual neurones and the closest events in the other neurones.

CA3–CA3 ($n = 7$) and CA3–CA1 pairs ($n = 4$) occurred in the two cells with a minimal delay. Pair recordings were also obtained from CA3 principal cells and interneurones ($n = 4$). In two out of four cases GDPs in interneurones preceded those occurring in pyramidal cells by 18.3 and 17.4 ms, respectively. Cumulative cross-correlation graphs were constructed in order to evaluate quantitatively the time difference between the onset of each GDP recorded in one neurone and the closest event in the other. In the top right graph of Fig. 2, GDPs originating from neighbouring CA3 pyramidal cells occurred in a short interval of 30 ms. The mean latency between the onset of GDPs occurring in CA3 pyramidal cells obtained from seven pairs was 8.5 ± 1.3 ms. The distribution of GDPs originating from distant locations (CA1 and CA3 pyramidal cells) was more scattered (within a time interval of 60 ms). These experiments demonstrate that, as in acute slices or in the intact hippocampal formation (Strata *et al.*, 1997; Leinekugel *et al.*, 1998), in organotypic slice cultures GDPs occur over the entire hippocampus with a minimal delay between adjacent subfields.

GABA depolarizes principal cells through an outward flux of chloride

The contribution of GABA to GDP generation was fully supported by the observation that spontaneous recurrent events were reversibly blocked by the $GABA_A$ receptor antagonists bicuculline, picrotoxin or gabazine. In order to induce a membrane depolarization able to trigger action potentials, GABA should have a depolarizing and excitatory action. GABA released from GABAergic terminals acts on $GABA_A$ and $GABA_B$ receptors. The binding of GABA to $GABA_A$ receptors opens chloride-permeable channels with a substantial permeability also to HCO_3^- (Bormann *et al.*, 1987). The resulting transmembrane currents can either depolarize or hyperpolarize the membrane according to the equilibrium potential of the ions and the resting membrane potential of the cell (V_m). Therefore, in order to better understand the direction of GABA-mediated currents it was crucial to measure the resting V_m and the E_{GABA} as the driving force $E_{GABA} - V_m$ set the conditions for the depolarizing or hyperpolarizing action of this neurotransmitter. To measure the V_m we took advantage of a non-invasive method based on the reversal of NMDA-induced single-channel currents measured in the intact cell via a cell-attached patch. This method has been already used to measure the membrane potential of CA3 pyramidal cells in acute hippocampal slices obtained from newborn rats (Tyzio *et al.*, 2003). The rationale is that current through NMDA channels reverses near 0 mV and therefore in cell-attached recordings NMDA currents should reverse their polarity at a holding potential on the pipette equal to V_m. Figure 3A shows an example of single-channel NMDA currents recorded in cell-attach configuration from a CA3 pyramidal cell at different pipette potential values. The extrapolated reversal potential obtained in six cells by plotting the mean amplitude of single-channel NMDA currents vs. different values of pipette potential was −75 mV (Fig. 3B). This value was very close to that obtained in whole-cell experiments (−72 ± 3 mV; $n = 8$).

In order to study the reversal potential of GABA (E_{GABA}), double patch-clamp recordings were performed from eight pairs of intercon-

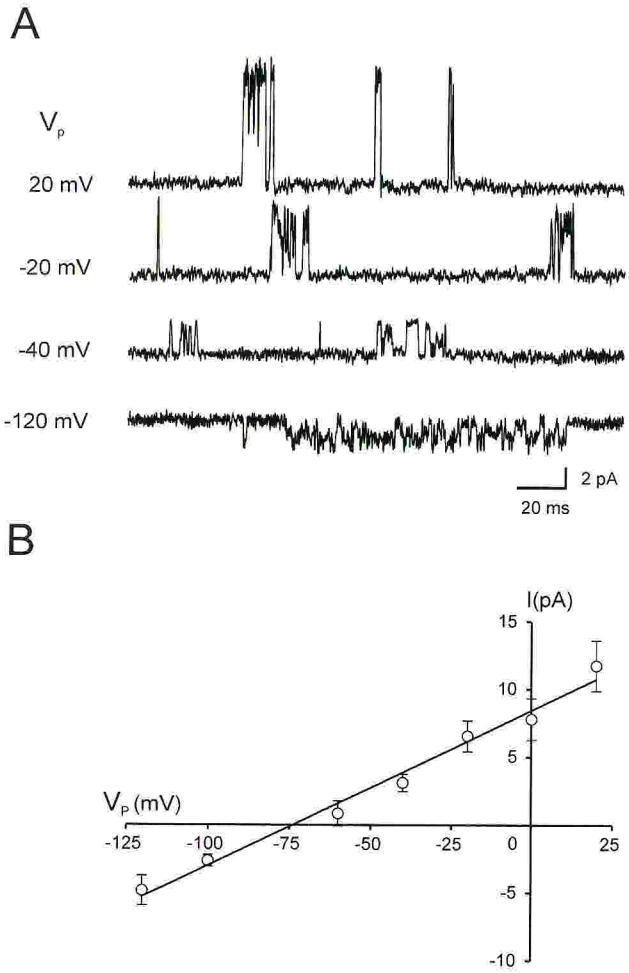

FIG. 3. Resting membrane potential estimated from cell-attach recordings of *N*-methyl-D-aspartate (NMDA)-induced single-channel currents. (A) CA3 hippocampal neurone from an 18 days *in vitro* hippocampal organotypic slice. Cell-attach recordings of NMDA-induced single-channel currents at different pipette potentials (Vp). (B) Current–voltage relationship of NMDA channels recorded in cell-attach mode from six cells recorded during the third week *in vitro*. Note that currents through NMDA channels reversed at −75 mV. Assuming a reversal of NMDA currents equal to 0 mV, the resting membrane potential should be −75 mV.

nected interneurones–CA3 pyramidal cells in the presence of DNQX (10 μM) and D-AP5 (20 μM) to block ionotropic glutamate receptors and polysynaptic activity (Fig. 4A). Interneurones were identified on the basis of their firing properties and later on the basis of their morphology (Fig. 4B). Presynaptic action potentials in interneurones evoked in pyramidal cells monosynaptic currents with a mean latency of 1.9 ± 0.3 ms. Synaptic currents in CA3 principal cells were recorded in two different conditions, whole-cell configuration and gramicidin-perforated patch. The gramicidin-perforated patch allows voltage clamping of the neurones while preserving the anionic conditions of the cell (Kyrozis & Reichling, 1995). Figure 4 illustrates two example of experiments performed in whole cell (left) and perforated patch (right). The extrapolated mean reversal potential of synaptic currents recorded in whole-cell conditions was −75 mV ($n = 4$) while the reversal of those recorded with gramicidin-perforated patch was −56 mV ($n = 4$; Fig. 4C and D). Therefore, in all cells examined with gramicidin-perforated patch the reversal of $GABA_A$-mediated synaptic responses was positive compared with the resting membrane potential. We conclude that, at least in the neurones examined, the value ($E_{GABA} - V_m$) was positive and therefore GABA was still exerting a depolarizing action on target cells.

GABA released from interneurones can inhibit or enhance cell firing in principal cells

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). Therefore, the following experiments were undertaken to see whether GABA-induced depolarization was able to excite target cells. To this aim, in 14 neurones, synaptic $GABA_A$-mediated currents were recorded from CA3 principal cells using gramicidin-perforated patch and an extracellular solution containing bicarbonate (see Materials and methods) plus DNQX (10 μM) and D-AP5 (20 μM). In nine out of 14 cases, stimulation of GABAergic interneurones evoked in CA3 pyramidal cells synaptic currents which reversed polarity at −51.5 ± 1.6 mV (Fig. 5), a value slightly more positive that that found in the absence of bicarbonate (−56 mV) indicating that this anion contributes slightly to the $GABA_A$ receptor permeation. In the remaining five neurones the value of E_{GABA} was more negative (−75.6 ± 1.3 mV). To investigate whether the depolarizing action of GABA observed in five cells was excitatory or inhibitory, GABAergic inputs (in the presence of DNQX and D-AP5) were stimulated 330 ms after the onset of a long (1-s) depolarizing current pulse that caused principal cells to fire. As depicted in Fig. 5, in the majority of cases ($n = 6$) GABA released from the interneurones was able to abolish the firing while in the remaining three it produced an increase in firing rate (from 5 to 27 Hz). Both the decrease and increase in firing rate were prevented by bicuculline indicating that these effects were mediated by the activation of $GABA_A$ receptors.

Further evidence that, at least in some neurones, GABA exerts a depolarizing and excitatory action was given by the experiments in which bicuculline was applied to CA3–CA3 ($n = 6$) or CA3–CA1 ($n = 3$) pairs recorded in whole cell and cell-attach, respectively. As shown in the example of Fig. 6, bath application of bicuculline (10 μM) not only abolished GDPs but also spike activity which occurred simultaneously in the nearby neurone recorded in cell-attach.

In keeping with these results, bath application of the $GABA_A$ receptor agonist isoguvacine (10 μM) induced a small membrane depolarization (3–5 mV) that was associated with an increase of GDP frequency from 0.11 ± 0.05 to 0.2 ± 0.06 Hz ($P = 0.009$; $n = 4$; Fig. 6B and D). This effect was associated with a progressive decrease in amplitude and duration of GDPs (Fig. 6C; see also Khalilov *et al.*, 1999).

Altogether these results suggest that organotypic hippocampal slices maintained in culture for more than 2 weeks retain some degree of immaturity as, at least in some cells, GABA still exerts a depolarizing and excitatory action.

Giant depolarizing potentials result from the synergistic action of GABA and glutamate

We have already shown that GDPs can be blocked by DNQX or bicuculline indicating that they were generated by the synergistic action of glutamate and GABA. In the next series of experiments we measured the reversal potential of GDPs using whole-cell recordings (in current-clamp mode, $n = 10$) or gramicidin-perforated patch ($n = 4$). In these experiments, we used an intrapipette solution

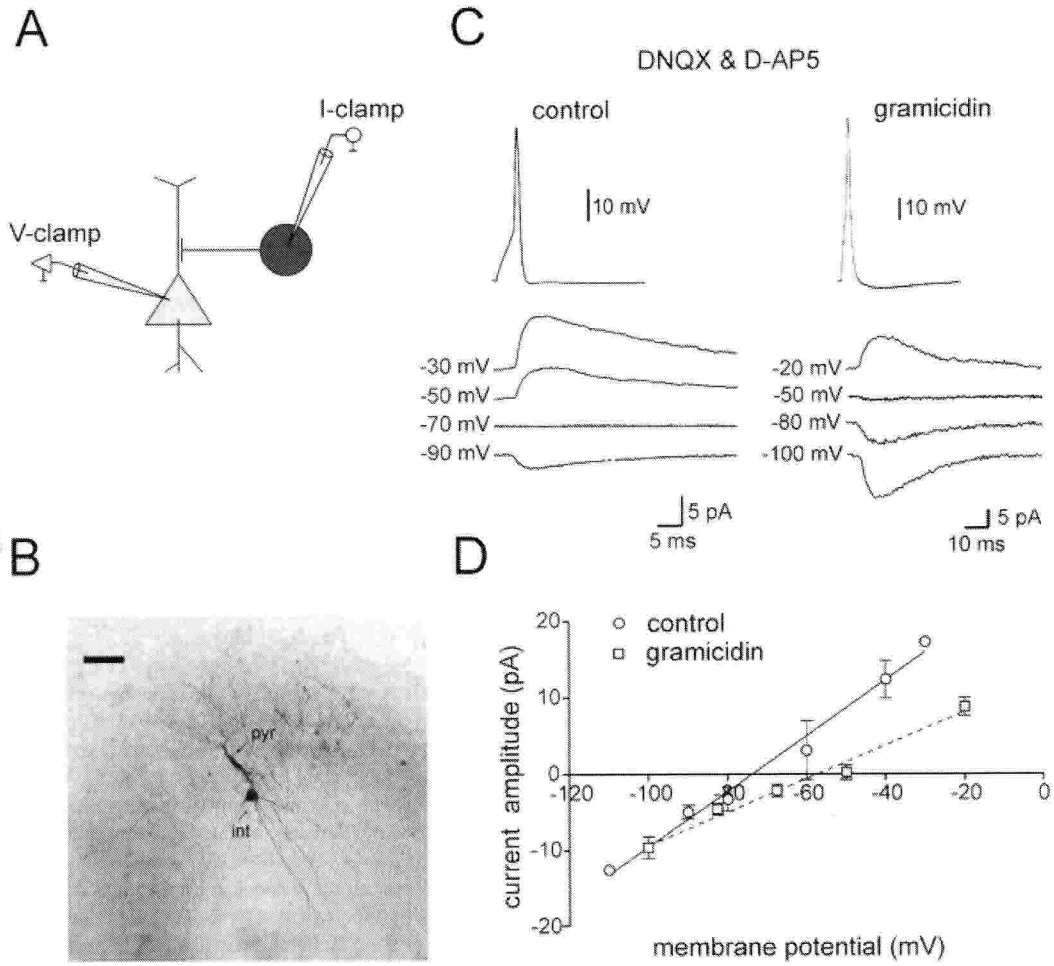

FIG. 4. GABA released from GABAergic interneurones depolarizes principal cells. (A) Schematic representation of a pair of interconnected interneurone–CA3 pyramidal cell revealed in B with biocytin (recordings showing on the left in C). Calibration bar in B equal to 100 μm. (C) Example of two pairs of interconnected interneurones–CA3 pyramidal cells from 20 days *in vitro* recorded in whole cell (left) and gramicidin-perforated patch (right) in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX) (10 μM) and DL-2-amino-5-phosphonopentaoic acid (D-AP5) (20 μM) to block fast glutamatergic connections. Action potentials in interneurones evoked in principal neurones synaptic currents that reversed at −70 mV when recorded in whole-cell configuration (left) and at −50 mV when recorded with gramicidin-perforated patch (right). (D) Reversal of $GABA_A$-mediated synaptic currents recorded in whole-cell conditions (○, $n = 4$) or with gramicidin-perforated patches (□, $n = 4$).

containing N-(2,6-Dimethylphenylcarbomoylmethyl)triethylammonium bromide (QX314, 5mm) to block sodium currents and action potential generation. As shown in Fig. 7, GDPs reversed at −48 mV in whole-cell experiments and at −39 mV in gramicidin-perforated patch. In cells recorded with an extracellular solution containing bicarbonate, GDPs reversed at −44 mV ($n = 3$). These values are both more positive than those obtained in double-patch experiments from interconnected interneurones and principal cells (with or without gramicidin) suggesting, in agreement with previous data from immature hippocamapal slices (Khazipov *et al.*, 1997; Bolea *et al.*, 1999), that a glutamatergic AMPA-mediated component contributes to GDPs.

Giant depolarizing potentials in interneurones precede those occurring in principal cells

As already mentioned, in two pair recordings from interneurones and principal cells (see Fig. 2) GDPs occurred firstly in interneurones. These preceded by a few milliseconds those occurring in principal cells suggesting that the latter could be driven by GABA released from interneurones. Therefore, the following experiments were undertaken to further explore the temporal relationship of isolated GABAergic and glutamatergic inputs to principal cells. In a first set of experiments, simultaneous recordings were performed from two nearby CA3 pyramidal cells. As GDPs result from the dynamic interaction of glutamate and GABA (Khazipov *et al.*, 1997; Bolea *et al.*, 1999), it is possible to selectively reduce the $GABA_A$- or AMPA/kainate-mediated conductance by voltage clamping each cell at the relevant reversal potential. Thus, as shown in Fig. 8 when two neighbouring cells were clamped at −70 mV, a potential close to the reversal of GABA, the glutamatergic currents occurred synchronously. However, when one cell was held at 0 mV (the reversal potential for glutamate), the GABAergic current always preceded the glutamatergic current in the other neurone maintained at −70 mV by ~32 ms. On average, in four out of five cells GABAergic currents preceded the glutamatergic currents by 29.1 ± 6.4 ms. Currents recorded at 0 mV were indeed mediated by GABA as they were readily abolished by bicuculline (10 μM; data not shown). In the remaining cell, the glutamatergic

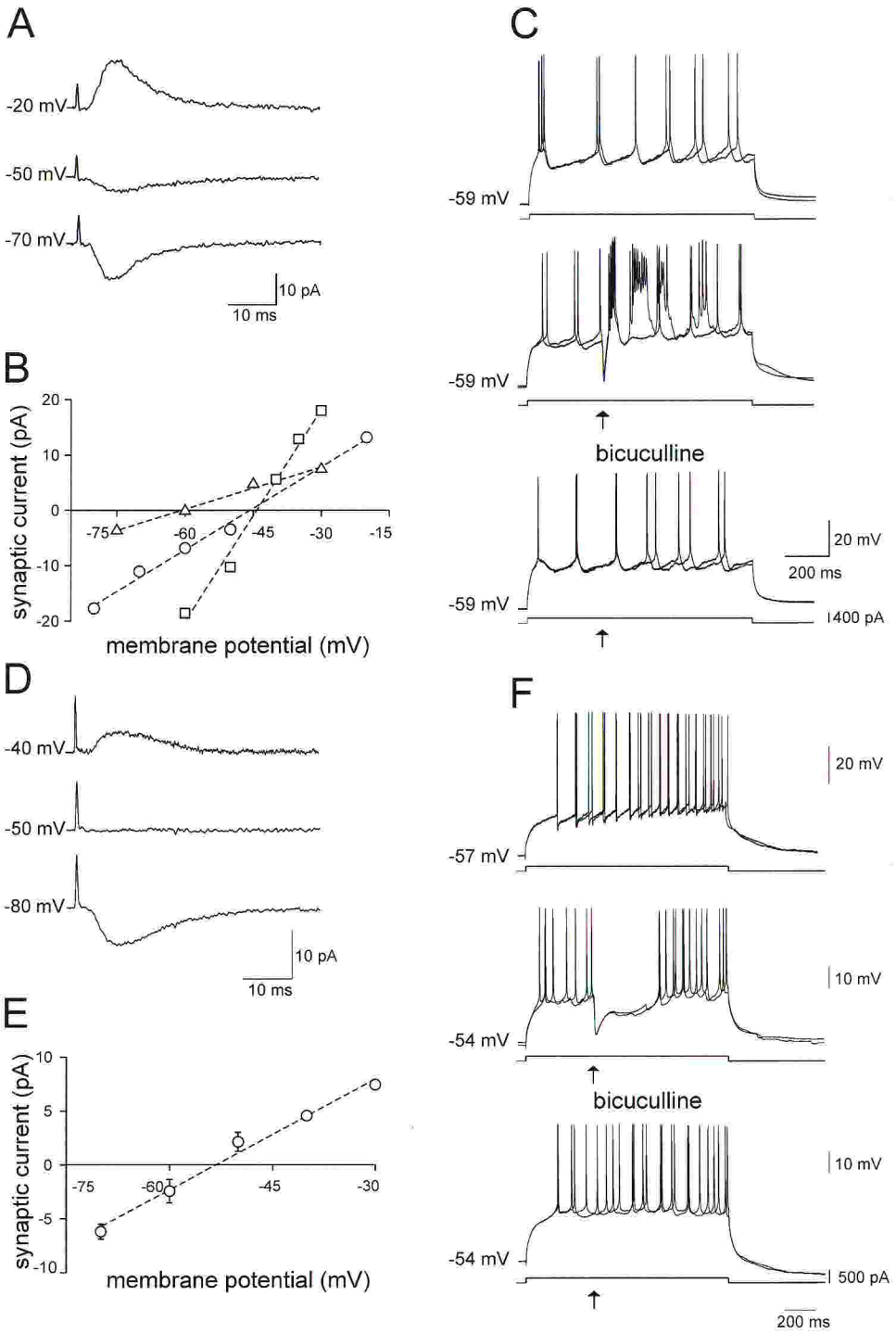

FIG. 5. In spite of its depolarizing action GABA released from interneurones can inhibit or enhance the firing of principal cells. (A) Example of GABAergic synaptic currents evoked at three different holding potentials in a CA3 pyramidal cell by stimulation of a GABAergic input. (B) Current–voltage relation of $GABA_A$-mediated synaptic currents in three neurones in which GABA was depolarizing and excitatory [equilibrium potential for GABA (E_{GABA}) was -50 ± 5 mV]. (C) Firing pattern evoked in a CA3 pyramidal cell by a depolarizing current pulse before (top) and during (arrows in the middle and bottom traces) stimulation of a GABAergic input in the absence or presence of bicuculline (10 μM). In each panel two traces are superimposed. Note that the increase in firing after stimulation of GABAergic input was prevented by bicuculline. (D) Example of GABAergic synaptic currents evoked at three different holding potentials in a CA3 pyramidal cell by stimulation of a GABAergic input. (E) Current–voltage relationship of $GABA_A$-mediated synaptic currents in six neurones in which GABA was depolarizing and inhibitory (E_{GABA}, -53.5 ± 3.4 mV). (F) Firing pattern evoked in a CA3 pyramidal cell by a depolarizing current pulse before (top) and during (arrows in the middle and bottom traces) stimulation of a GABAergic input in the absence or presence of bicuculline (10 μM). In each panel two traces are superimposed. Note that the decrease in firing after stimulation of the GABAergic input was prevented by bicuculline. All recordings were performed in the presence of 6,7-dinitroquinoxaline-2,3-dione (10 μM) and DL-2-amino-5-phosphonopentaoic acid (20 μM).

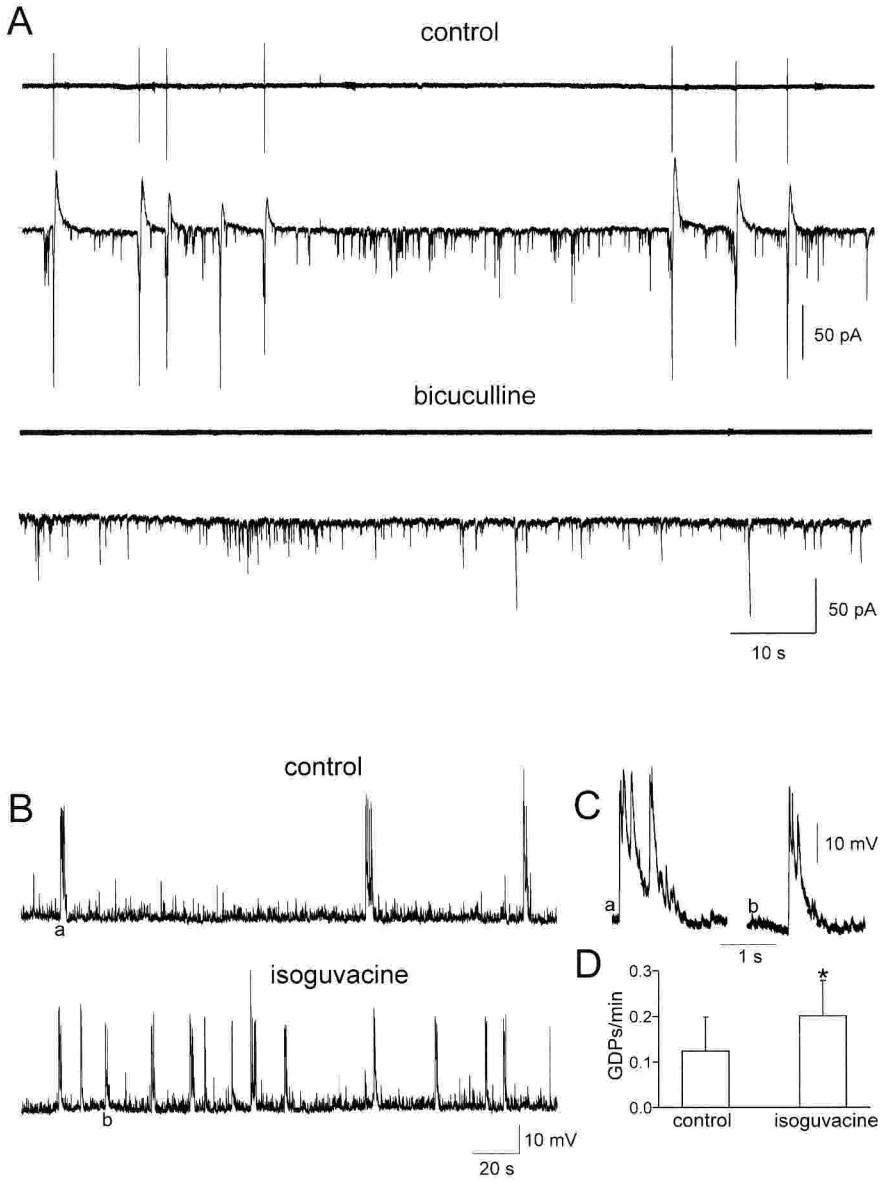

FIG. 6. The excitatory action of GABA contributes to giant depolarizing potential (GDP) generation. (A) Example of pair recordings obtained from neighbouring CA3 pyramidal cells in cell-attach (upper traces) and whole cell (lower traces) before (control) and during bicuculline application. Note disappearance of GDPs and synchronous spikes in the presence of bicuculline. (B) Bath application of the GABA agonist isoguvacine (10 μM) increases the frequency of GDPs. As shown in C, this effect was associated with a decrease in amplitude and duration of GDPs. Compare the shape of a single GDP in control (a) and during superfusion of isoguvacine (b). (D) Each column represents the mean GDP frequency in control and during isoguvacine. $*P = 0.009$.

component preceded the GABAergic component by ~19 ms (not shown).

Development of interictal bursts in the presence of bicuculline

As already mentioned bath application of bicuculline (10 μM) blocked GDPs. However, this drug, after a variable period of time (5.9 ± 2.1 min), induced the occurrence of interictal discharges ($n = 16$). These were reminiscent of recurrent bursts obtained in the newborn intact hippocampus (Khalilov *et al.*, 1999) or in adult hippocampal slices after removal of $GABA_A$ receptor-mediated inhibition (Miles & Wong, 1987). As shown in the representative example of Fig. 9A, interictal bursts developed in the presence of bicuculline after a sustained membrane depolarization with oscillatory activity lasting several seconds. These occurred synchronously over the entire hippocampus where they could be detected in simultaneous whole-cell and cell-attached recordings (Fig. 9B). Interictal bursts were less frequent than GDPs (0.043 ± 0.0041 Hz) and lasted longer (4.38 ± 2.6 s). In comparison with GDPs, interictal discharges had a faster rising phase, were blocked by DNQX (10 μM) and reversed polarity near 0 mV. The extrapolated reversal, calculated in seven neurons, was −6 mV suggesting that they were mediated by AMPA/kainate receptor subtypes (Fig. 9C). Similar effects were produced by picrotoxin (100 μM; $n = 2$) or gabazine (50 μM; $n = 2$). The possibility of inducing interictal discharges after blocking

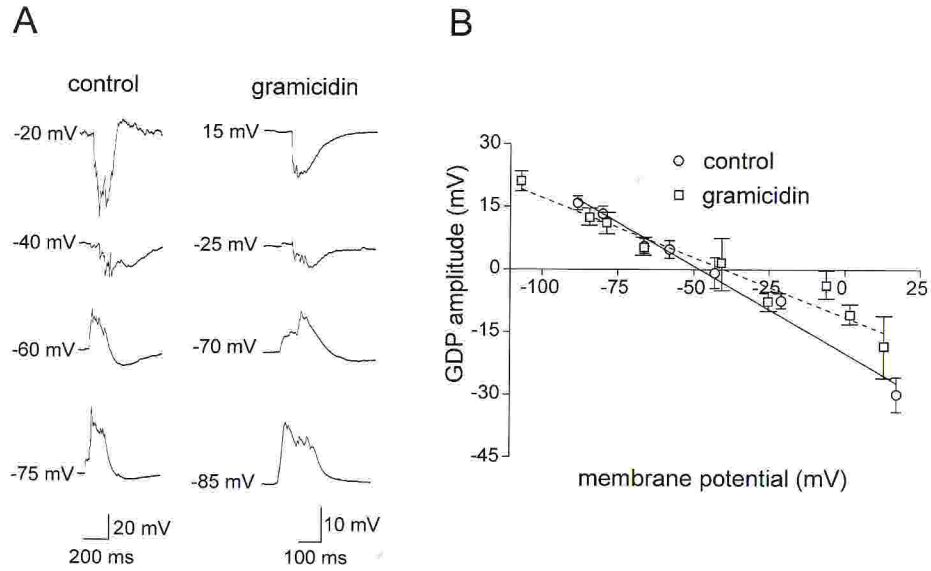

FIG. 7. The giant depolarizing potential (GDP) reversal potential is more positive than equilibrium potential for GABA. (A) Examples of GDPs recorded at different membrane potentials (indicated on the left) from CA3 pyramidal cells in whole-cell conditions (control) and with gramicidin-perforated patch (gramicidin). (B) Reversal of GDPs recorded in whole-cell conditions (○, $n = 10$) and with gramicidin-perforated patches (□, $n = 4$).

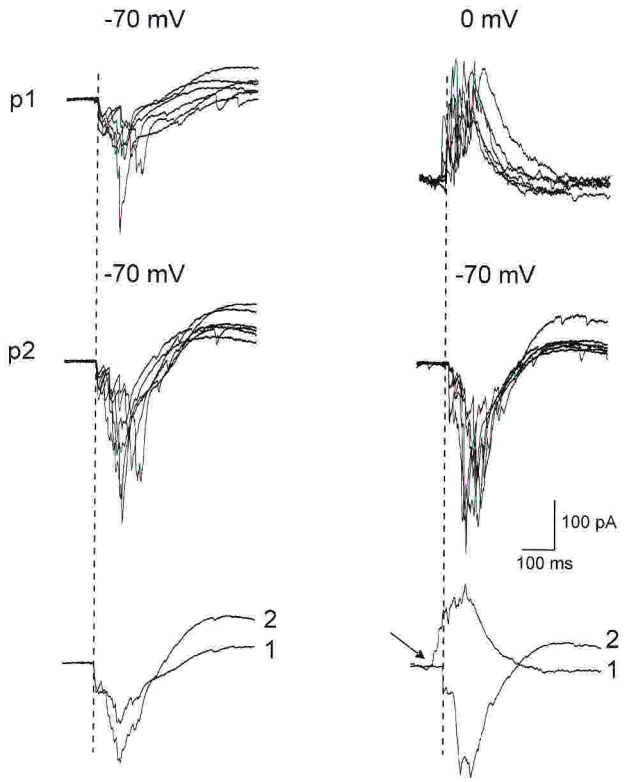

FIG. 8. GABA released from interneurones drives principal cells. Double voltage-clamp recordings from neighbouring CA3 pyramidal cells (p1 and p2) held both close to the reversal potential for GABA (at −70 mV, left) or one to the reversal potential for glutamate (0 mV, upper right) and the other close to the reversal potential for GABA (−70 mV, middle right). Six individual sweeps are superimposed in each condition for each cell (p1 and p2). The averages of individual traces are shown below. Note that the GABAergic component of giant depolarizing potentials (GDPs) precedes the glutamatergic component (see arrow on the lower trace) indicating that GABA released from a GABAergic interneurone drives the pyramidal cell.

$GABA_A$ receptor with bicuculline suggests that in organotypic hippocampal cultures recurrent excitatory glutamatergic connections between principal cells are well developed and functional. As in acute slices from the immature hippocampus (Khalilov *et al.*, 1999; Khazipov *et al.*, 2004), the dual inhibitory and excitatory effects of bicuculline on network activity may result from the complex action of GABA, which on one side excites target cells contributing to GDP generation and on the other inhibits postsynaptic cells through a powerful shunting inhibition due to an increase in membrane conductance as a result of opening of a large number of channels.

Discussion

The present experiments clearly show that membrane oscillations similar to GDPs can be recorded from organotypic hippocampal slices maintained in culture for more than 2 weeks. In previous work from the same preparation GDP-like activity was never observed (Streit *et al.*, 1989). This apparent discrepancy can be attributed to the degree of immaturity of slices at the time of explantation. In comparison with Streit *et al.* (1989) we used slices from 4-day-old (instead of 6-day-old) animals which express GDPs (Ben-Ari *et al.*, 1989). An epileptiform type of activity has been described in organotypic slice cultures obtained from newborn animals (McBain *et al.*, 1989). However, unlike GDPs, epileptiform discharges were associated with a loss of spike afterhyperpolarization and $GABA_A$-mediated IPSPs. As in the acute slice preparation (Ben-Ari *et al.*, 1989), in the present conditions GDPs were generated within the network by the interaction of glutamate and GABA as they were reversibly blocked by the AMPA/kainate and $GABA_A$ receptor antagonists DNQX and bicuculline, respectively, and reversed at a membrane potential more positive than E_{GABA}.

The GDP-like activity reported here closely resembles the spontaneous synchronized bursts generated in the hippocampus of juvenile animals by high potassium (Aradi & Maccaferri, 2004). As in high potassium bursts, glutamatergic activity in principal cells was, in the majority of cases, driven by GABA released from GABAergic

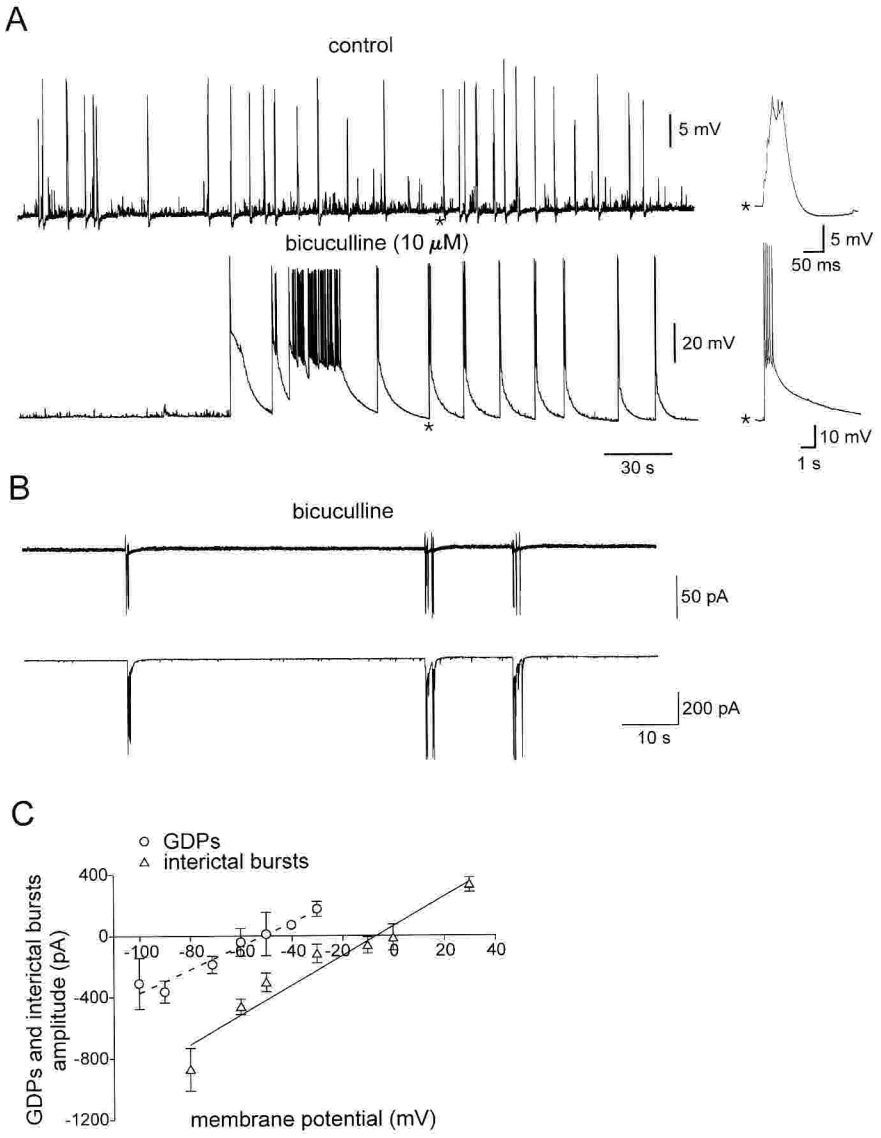

FIG. 9. Bicuculline induces the appearance of ictal and interictal epileptiform discharges. (A) Representative trace of a CA3 neurone from a 14 days *in vitro* cultured slice showing giant depolarizing potentials (GDPs) (on the right at an expanded time-scale, asterisk) before (control) and during application of bicuculline. Note that bicuculline first blocked GDPs and then after 5 min induced the appearance of interictal bursts. One of these (marked by an asterisk) is shown on the right at an expanded time-scale. (B) An example of pair recordings obtained from neighbouring CA3 neurones in cell-attach (upper trace) and whole cell (lower trace) before (control) and 6 min after bicuculline application. (C) Reversal of GDPs (○, $n = 5$) and interictal discharges (△, $n = 7$) recorded in whole-cell conditions. The epileptiform bursts reversed at −6 mV indicating that they were mediated by glutamate acting on AMPA/kainate receptors.

interneurones. However, in contrast with the high potassium model of epilepsy, in which removal of $GABA_A$-mediated conductance with gabazine changed burst strength and frequency, blockade of $GABA_A$ receptors with bicuculline produced here a transient block of GDPs followed after a delay of several minutes by interictal discharges. This can be attributed to the increased connectivity and number of glutamatergic synapses in cultured slices (Gutierrez & Heinemann, 1999; De Simoni *et al.*, 2003). Hippocampal slice cultures indeed retain the ability to generate, throughout the cultivation period, new neurones from neural progenitors present in the dentate gyrus. These new neurones would be incorporated into the hippocampal local circuit leading to an increased neuronal connectivity (Kamada *et al.*, 2004; Raineteau *et al.*, 2004).

Similarly to GDPs, spontaneous recurrent network activity present in organotypic hippocampal cultures occurred over the entire hippocampus as demonstrated by pair recordings from cells in different hippocampal subfields. Although GDPs could be generated anywhere in isolated portions of the hippocampal network, the more scattered cross-correlation values observed when recording from distant locations suggest a preferential propagation along the septo-temporal axis as in the intact hippocampal formation (Leinekugel *et al.*, 1998).

A critical point that should be addressed is the role of GABA in the generation of GDP-like events. Although with gramicidin-perforated patch E_{GABA} was more positive than the resting membrane potential this does not mean *per se* that GABA brings pyramidal cells to fire. Indeed, GABA-induced membrane depolarization may shunt the

membrane thus reducing the firing of principal cells (Lamsa *et al.* 2000). The observation that, in the majority of cells, GABA released from interneurones blocked cell firing in CA3 pyramidal cells supports this hypothesis. Network synchronization would be facilitated by the dense connectivity of recurrent glutamatergic connections in which organotypic slices are particularly enriched (De Simoni *et al.*, 2003). This would also account for interictal bursts (observed after prolonged exposure to bicuculline) which strictly depend on the strength of glutamatergic inputs, particularly those arising from recurrent collaterals of CA3 principal cells (Miles & Wong, 1987).

The hyperpolarizing or depolarizing action of GABA closely depends on the intracellular chloride concentration. In central neurones this is mainly under the control of two cation-chloride cotransporters, NKCC and KCC2, which are involved in Cl^- accumulation and extrusion, respectively (Payne *et al.*, 2003). The low expression of KCC2 at birth would be responsible for the intracellular accumulation of chloride in immature neurones and for the depolarizing action of GABA. Developmental changes in KCC2 expression would promote the switch of GABA from the depolarizing to the hyperpolarizing direction (Rivera *et al.*, 1999) leading to GDP disappearance. However, in apparent contradiction to the above-mentioned data, western blot analysis of homogenates from organotypic hippocampal cell cultures has shown that the KCC2 protein is already maximally expressed at 15 days *in vitro*. This suggests that, in organotypic hippocampal slices, either other cotransporters involved in intracellular chloride accumulation prevail over those required for chloride extrusion or KCC2 is present but not fully functional. Although we cannot exclude the first hypothesis we favour the possibility that, in our preparation, the increased number of glutamatergic synapses leads to an increased firing rate and K^+ accumulation outside the neurones (De Simoni *et al.*, 2003). This would reduce the depolarization needed to trigger the action potentials and neuronal chloride extrusion by reducing the driving force for the K^+-Cl^- cotransporter. Moreover, the accumulation of K^+ in the cytoplasm would contribute to shift E_{GABA} towards more positive values (Thompson & Gähwiler, 1989; Jensen *et al.*, 1993).

In previous work from the same preparation, the reversal potential for GABA was found to be at least 10 mV more negative than that found in the present experiments (Streit *et al.*, 1989; Thompson & Gahwiler, 1989). According to Streit *et al.* (1989) when recording with potassium acetate-filled electrodes, GABA-mediated synaptic currents were hyperpolarizing and therefore inhibitory. It should be stressed, however, that a possible source of errors could be introduced when recording with intracellular microelectrodes. In particular, an alteration in the resting membrane potential, due to modifications of the intracellular composition of the neurone (that may change the ionic gradients and the activity of ion channels), may affect the value of $E_{GABA} - V_m$ which set the direction of GABA action (see Chavas & Marty, 2003). In our case the membrane potential was measured using the non-invasive method of the cell-attach technique which does not disrupt the cytoplasmic environment.

In spite of the main inhibitory role of GABA, the present results clearly demonstrate that, in some cells, this neurotransmitter still exerts a depolarizing and excitatory action as it is able to depolarize principal neurones bringing them to fire. This suggests that some degree of immaturity is still present in organotypic slices even if cultured for several weeks. In addition, the blockade of GDPs with bicuculline and their increase in frequency with isoguvacine are difficult to reconcile with $GABA_A$-mediated inhibition (see also Khalilov *et al.*, 1999; Ben-Ari, 2001; Dzhala & Staley, 2003). Alternatively, we cannot exclude the possibility that ectopic spikes, generated by the excitatory action of endogenously released GABA or exogenously applied $GABA_A$ agonists on $GABA_A$ receptors localized on the axons of principal cells, contribute to network activity. A $GABA_A$-mediated increase of axon terminal excitability has been shown to facilitate the occurrence of epileptiform bursts in kindling (Stasheff *et al.*, 1993) and in the 4-aminopyridine model of epilepsy (Avoli *et al.*, 1998) and to contribute to gamma-frequency oscillations (Traub *et al.*, 2003). However, it should be stressed that, in these cases, activation of presynaptic $GABA_A$ receptors became detectable only after augmenting GABA release with kindling and 4-aminopyridine or kainate application. Although appealing, this hypothesis seems unlikely because in the present experiments we never observed ectopic spikes in principal cells. Moreover, while DNQX was ineffective in blocking ectopic spikes (Stasheff *et al.*, 1993; Avoli *et al.*, 1998; Traub *et al.*, 2003), the AMPA/kainate receptor antagonist readily blocked GDPs. In contrast to ectopic spikes, which were blocked by bicuculline, in cultured hippocampal slices this alkaloid first blocked GDPs and later induced the appearance of interictal epileptiform discharges.

Whatever the mechanisms are which underlie recurrent GDP-like events in cultured hippocampal slices, the depolarizing and excitatory effects of GABA observed in some neurones may certainly contribute to network activity.

Acknowledgements

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Abbreviations

D-AP5, DL-2-amino-5-phosphonopentaoic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; E_{GABA}, equilibrium potential for GABA; GDP, giant depolarizing potential; NMDA, *N*-methyl-D-aspartate; V_m, membrane potential.

References

Aradi, I. & Maccaferri, G. (2004) Cell type-specific synaptic dynamics of synchronized bursting in the juvenile CA3 rat hippocampus. *J. Neurosci.*, **24**, 9681–9692.

Avoli, M., Methot, M. & Kawasaki, H. (1998) GABA-dependent generation of ectopic action potentials in the rat hippocampus. *Eur. J. Neurosci.*, **10**, 2714–2722.

Ben-Ari, Y. (2001) Developing networks play a similar melody. *Trends Neurosci.*, **24**, 353–360.

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

Ben-Ari, Y., Cherubini, E., Corradetti, R. & Gaiarsa, J.L. (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol. (Lond.)*, **416**, 303–325.

Ben-Ari, Y., Khazipov, R., Leinekugel, X., Caillard, O. & Gaiarsa, J.L. (1997) $GABA_A$, NMDA and AMPA receptors: a developmentally regulated 'menage à trois'. *Trends Neurosci.*, **20**, 523–529.

Bolea, S., Avignone, E., Berretta, N., Sanchez-Andres, J.V. & Cherubini, E. (1999) Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. *J. Neurophysiol.*, **81**, 2095–2102.

Bormann, J., Hamill, O.P. & Sakmann, B. (1987) Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones. *J. Physiol. (Lond.)*, **385**, 243–286.

Chavas, J. & Marty, A. (2003) Coexistence of excitatory and inhibitory GABA synapses in the cerebellar interneuron network. *J. Neurosci.*, **23**, 2019–2031.

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

De Simoni, A., Griesinger, C.B. & Edwards, F.A. (2003) Development of rat CA1 neurones in acute versus organotypic slices: role of experience in synaptic morphology and activity. *J. Physiol. (Lond.)*, **550**, 135–147.

Dzhala, V.I. & Staley, K.J. (2003) Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. *J. Neurosci.*, **23**, 1840–1846.

Feller, M.B., Butts, D.A., Aaron, H.L., Rokhsar, D.S. & Shatz, C.J. (1997) Dynamic processes shape spatiotemporal properties of retinal waves. *Neuron*, **19**, 293–306.

Gähwiler, B.H. (1981) Organotypic monolayer cultures of nervous tissue. *J. Neurosci. Meth.*, **4**, 329–342.

Gähwiler, B.H., Capogna, M., Debanne, D., McKinney, R.A. & Thompson, S.M. (1997) Organotypic slice cultures: a technique has come of age. *Trends Neurosci.*, **20**, 471–477.

Ganguly, K., Schineder, A. F., Wong, S. T. & Poo, M. M. (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell*, **105**, 521–532.

Garaschuk, O., Linn, J., Eilers, J. & Konnerth, A. (2000) Large-scale oscillatory calcium waves in the immature cortex. *Nat. Neurosci.*, **3**, 452–459.

Gutierrez, R. & Heinemann, U. (1999) Synaptic reorganization in explanted cultures of rat hippocampus. *Brain Res.*, **815**, 304–316.

Jensen, M.S., Cherubini, E. & Yaari, Y. (1993) Opponent effects of potassium on GABAA-mediated postsynaptic inhibition in the rat hippocampus. *J. Neurophysiol.*, **69**, 764–771.

Kamada, M., Li, R.Y., Hashimoto, M., Kakuda, M., Okada, H., Koyanagi, Y., Ishizuka, T. & Yawo, H. (2004) Intrinsic and spontaneous neurogenesis in the postnatal slice culture of rat hippocampus. *Eur. J. Neurosci.*, **20**, 2499–2508.

Kasyanov, A.M., Safiulina, V.F., Voronin, L.L. & 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.

Khalilov, I., Dzhala, V., Ben-Ari, Y. & Khazipov, R. (1999) Dual role of GABA in the neonatal rat hippocampus. *Dev. Neurosci.*, **21**, 310–319.

Khazipov, R., Leinekugel, X., Khalilov, I., Gaiarsa, J.L. & Ben Ari, Y. (1997) Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *J. Physiol. (Lond.)*, **498**, 763–772.

Khazipov, R., Khalilov, I., Tyzio, R., Morozova, E., Ben-Ari, Y. & Holmes, G.L. (2004) Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur. J. Neurosci.*, **19**, 590–600.

Kyrozis, A. & Reichling, D.B. (1995) Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. *J. Neurosci. Meth.*, **57**, 27–35.

Lamsa, K., Palva, J.M., 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) Ca^{2+} oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. *Neuron*, **18**, 243–255.

Leinekugel, X., Khalilov, I., Ben-Ari, Y. & Khazipov, R. (1998) Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septohippocampal complex in vitro. *J. Neurosci.*, **18**, 6349–6357.

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**, 3199–3206.

McBain, C.J., Boden, P. & Hill, R.G. (1989) Rat hippocampal slices 'in vitro' display spontaneous epileptiform activity following long-term organotypic culture. *J. Neurosci. Meth.*, **27**, 35–49.

Miles, R. & Wong, R.K. (1987) Latent synaptic pathways revealed after tetanic stimulation in the hippocampus. *Nature*, **329**, 724–726.

O'Donovan, M.J. (1999) The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr. Opin. Neurobiol.*, **9**, 94–104.

Owens, D.F., Boyce, L.H., Davis, M.B.E. & Kriegstein, A.R. (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated–patch recordings and calcium imaging. *J. Neurosci.*, **16**, 6414–6423.

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

Raineteau, O., Rietschin, L., Gradwohl, G., Guillemot, F. & Gähwiler, B.H. (2004) Neurogenesis in hippocampal slice cultures. *Mol. Cell. Neurosci.*, **26**, 241–250.

Rivera, C., Voipio, J., Payne, J. A., 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**, 251–255.

Saviane, C., Savtchenko, L.P., Raffaelli, G., Voronin, L.L. & Cherubini, E. (2002) Frequency-dependent shift from paired-pulse facilitation to paired-pulse depression at unitary CA3-CA3 synapses in the rat hippocampus. *J. Physiol. (Lond.)*, **544**, 469–476.

Stasheff, S.F., Mott, D.D. & Wilson, W.A. (1993) Axon terminal hyperexcitability associated with epileptogenesis in vitro. II. Pharmacological regulation by NMDA and GABAA receptors. *J. Neurophysiol.*, **70**, 976–984.

Strata, F., Atzori, M., Molnar, M., Ugolini, G., Tempia, F. & Cherubini, E. (1997) A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. *J. Neurosci.*, **17**, 1435–1446.

Streit, P., Thompson, S.M. & Gähwiler, B.H. (1989) Anatomical and physiological properties of GABAergic neurotransmission in organotypic slice cultures of rat hippocampus. *Eur. J. Neurosci.*, **1**, 603–615.

Thompson, S.M. & Gähwiler, B.H. (1989) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on ECl- in hippocampal CA3 neurons. *J. Neurophysiol.*, **61**, 512–523.

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. (Lond.)*, **550**, 719–730.

Traub, R.D., Cunningham, M.O., Gloveli, T., LeBeau, F.E., Bibbig, A., Buhl, E.H. & Whittington, M.A. (2003) GABA-enhanced collective behavior in neuronal axons underlies persistent gamma-frequency oscillations. *Proc. Natl Acad. Sci. U.S.A.*, **100**, 11 047–11 052.

Tyzio, R., Ivanov, A., Bernard, C., Holmes, G.L., Ben-Ari, Y. & Khazipov, R. (2003) Membrane potential of CA3 hippocampal pyramidal cells during postnatal development. *J. Neurophysiol.*, **90**, 2964–2972.

-Paper 2-

At immature CA3-CA1 synapses GABA-mediated giant depolarizing potentials act as coincident detection signals to persistently enhance glutamate release *via* the activation of the ERK pathway

Article in preparetion

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Abstract

During postnatal development, spontaneous and experience driven electrical activity exerts a critical control on the structural refinement of neuronal networks. This effect is controlled by several factors including neurotrophins which are powerful regulators of synaptic transmission and plasticity. Here we show that in rat hippocampal slices, between postnatal day 2 (P2) and P6, correlated neuronal activity such as giant depolarizing potentials (GDPs) act as coincident detection signals for strengthening CA3-CA1 connections. Pairing GDPs with afferent stimulation resulted in a persistent increase in frequency of spontaneous AMPA-mediated glutamatergic currents an effect that required calcium influx through postsynaptic L-type calcium channel and the activation of three signaling molecules, PKA, BDNF and ERK. Thus, PKA inhibitors, blockers of TrkB receptors or scavengers of endogenous BDNF as well as inhibitors of the ERK pathway prevented GDPs-induced synaptic potentiation.

These results support the hypothesis that during a critical period of postnatal development, $GABA_A$-mediated GDPs are involved in the structural remodeling of excitatory synapses and provide new insights into the molecular mechanisms involved in this process.

Introduction

Spontaneously occurring neuronal oscillations constitute a hallmark of developmental networks[45]. They have been observed in several brain structures including the retina, the neocortex, the hippocampus, the thalamus and the spinal cord[2]. In the immature hippocampus the so-called 'giant depolarizing potentials' (GDPs) are network-driven synaptic events generated by the synergistic action of glutamate and GABA which, at early developmental stages, is depolarizing and excitatory [3,10,2]. They are characterized by a recurrent membrane depolarization with superimposed fast action potentials occurring at the frequency of < 0.5 Hz and separated by long and variable intervals of several seconds. GDPs are generated when synaptic traffic and cell firing within the network increase to a threshold level[12] (but see[55]). GDPs can be recorded also *in vivo* in rat pups where they occur during immobility periods, sleep and feeding [36,32]. In this respect GDPs can be seen as a primordial form of synchrony between neurons, which precedes more

organized forms of activity such as the theta and the gamma rhythms[7]. The depolarizing action of GABA during GDPs results in the activation of voltage-dependent calcium channels and N-methyl-D- aspartate receptors[37]. GDPs associated calcium waves are thought to be crucial for the structural refinement of the neuronal connectivity and for the establishment of the adult neuronal circuit, during a critical period of synapse formation[15,63]. Therefore, rewiring would depend on electrical activity and would involve cooperative and competitive interaction between converging inputs.

As suggested by the Hebb postulate of learning[23] GDPs allow associative modification of coincident signals. We have previously demonstrated that at mossy fiber-CA3 connections, which during the first postnatal week are mainly GABAergic[21,51], pairing GDPs with mossy fibers stimulation induced a persistent increase in synaptic efficacy which was usually restricted to the activated synapse[30].

In the present work we have examined whether excitatory glutamatergic synapses can be associatively modified by pairing $GABA_A$-mediated GDPs with Schaffer collateral stimulation. Our findings suggest that "paring" persistently enhances synaptic efficacy, an effect that like for mossy-fibres CA3 synapses depends on the rise of calcium in the postsynaptic cell. The intracellular cascade mediating this event involves the activation of cAMP-dependent PKA, TrkB receptors by BDNF and the activation of the ERK pathway. These events might underlie the persistent effect on synaptic potentiation in response to neuronal activity.

Results

Pairing GDPs with Schaffer collateral stimulation enhances synaptic efficacy

Whole cell recordings in current clamp mode from 160 CA1 pyramidal neurons in hippocampal slices from P2-P6 old rats revealed the presence of GDPs (**Fig. 1A and B**) whose frequency varied from 0.016 to 0.28 Hz (on average 0.069 ± 0.012 Hz; mean ± S.E.M. here and below). In accord with a previous work[30] from CA3 pyramidal cells, we used a *"pairing"* procedure to correlate GDPs with Schaffer collateral activation. For this purpose, the rising phase of GDPs was used to trigger the stimulation of the Schaffer collateral in *stratum radiatum* in such a way that synchronized network activity was coincident with presynaptic activation of the afferent input (see enlargement of **Fig. 1B**).

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 (*R,S*)-α-amino-3-hydroxy-5-methyl-4-isoxadepropionate (AMPA)-receptor types since they were readily blocked by the AMPA receptor antagonist DNQX (20μM; n= 6; data not shown).

After response stabilization (5-10 min), the patch was switched from voltage-clamp to current-clamp mode and Schaffer collateral responses were paired for 7 min with GDPs (dashed line in **Fig. 1D**). In this period, the mean number of GDPs was 26.1 ± 3.4. As illustrated in **Fig. 1C** and **D**, the pairing procedure produced a strong and persistent potentiation of Schaffer collateral-mediated synaptic currents (from 1.9 ± 1.2 pA in control to 10.2 ± 1.3 pA 20 min after pairing). Average data from 7 cells are shown in **Fig. 1E and F**. In these cells, 25 min after pairing, the mean increase in amplitude of synaptic currents was 214.2 ± 48.1 % of controls; $p<0.01$ (from 8.2 ± 1.6 pA to 17.6 ± 4.1 pA; $p<0.01$). The amplitude enhancement 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 associated with a significant reduction of the paired-pulse ratio (from 5.7 ± 1.6 to 2.1 ± 0.7; $p<0.05$; **Fig. 1F**) and a significant increase in the inverse squared value of the coefficient of variation (CV^{-2}) of response amplitude (from 0.08 ± 0.03 to 0.94 ± 0.2; $p<0.05$; n=8), suggesting that an increased probability of glutamate release accounts for LTP expression. Pairing-induced potentiation of synaptic currents was not related to the spikes riding on the top of GDPs since all experiments were performed with an intra pipette solution containing QX 314 known to block sodium channels. In the absence of pairing no significant changes in synaptic efficacy could be detected "No pairing" consisted in switching the patch for 7 min from voltage clamp to current clamp mode. In this period GDPs occurred randomly in the absence of afferent stimulation. In these cells, the mean peak amplitude current was 7.9 ± 2.1 pA and 7.1 ± 1.9 pA in control and 20 min after switching from current-clamp 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).

Pairing GDPs with Schaffer collateral stimulation enhances the frequency of spontaneous EPSCs.

One interesting question is whether the pairing procedure may affect spontaneous action potential dependent and independent AMPA-mediated synaptic currents (sEPSCs). At immature CA3-CA1 synapses, the amplitude distribution of spontaneous action potential dependent glutamatergic events matches very closely that of miniature events recorded in the presence of tetrodotoxin (TTX, 1 μM; see **suppl. Fig. 1**), since these synapses bear only a single functional release site[26,22]. The multiquantal variability and the skewed distribution of individual events are compatible with a multivesicular model of release in which a single action potential is able to evoke from single release site multiquanta events each of them being far from saturation[49]. Therefore in the following experiments we analyzed the potentiating effects of GDPs on sEPSCs. Spontaneous EPSCs were recorded at the reversal potential of GABA (-53 mV). They had a mean frequency of 0.11 ± 0.03 Hz and a mean amplitude of 14.3 ± 2.4 pA (n = 21). They were reversibly blocked by DNQX (20 μM), indicating that they were mediated by glutamate acting on AMPA receptor types (n=10; data not shown). As illustrated in the representative example of **Fig. 2A and B**, pairing GDPs with afferent stimulation significantly and persistently (for at least 20 min) enhanced the frequency but not the amplitude of individual events (n=6). On average, 20 min after pairing, the frequency of sEPSCs was 80 % higher than controls (it changed from 0.1 ± 0.03 Hz to 0.18 ± 0.05 Hz; n=10; $p<0.01$; Fig. 2C and D). This effect was not associated to significant changes in amplitude of individual events (13.3 ± 1.6 pA and 14.8 ± 1.7 pA, before and after pairing, respectively; $p>0.5$; **Fig. 2C and D**). 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 the frequency of sEPSCs were observed (the frequency of sEPSCs was 0.12 ± 0.02 Hz and 0.11 ± 0.03 Hz, in control and 20 min after switching from current-clamp to voltage-clamp conditions, respectively; $p>0.5$; n= 7; data not shown).

Pairing-induced persistent enhancement of synaptic activity requires the rise in calcium in the postsynaptic cell through voltage-dependent calcium channels.

At glutamatergic synapses, a common trigger for activity-dependent long-lasting modifications of synaptic strength is a postsynaptic rise in intracellular calcium concentration[4, 40]. In the following experiments we tested whether a rise of postsynaptic calcium during GDPs is responsible for pairing-induced potentiation of sEPSCs frequency. In cells loaded with the calcium chelator BAPTA (20 mM; n=6) the pairing procedure did not cause any persistent increase in frequency or amplitude of sEPSC (n=6; $p>0.1$; Fig. 3). A tendency towards depression of both these parameters was observed, although this did not reach a significant level (in the presence of intracellular BAPTA, the frequency of sEPSCs measured in control and 20 min after pairing was 0.08 ± 0.03 Hz and 0.06 ± 0.03 Hz while the amplitude was 13.1 ± 1.9 pA and 10.1 ± 3.9 pA, before and after pairing respectively). These experiments suggest that at CA3-CA1 synapses, pairing-induced LTP depends on calcium rise in the postsynaptic cell.

A rise of intracellular calcium concentration during GDPs may occur *via* voltage-dependent calcium channels or *via* NMDA receptors[37,14]. To identify the source of calcium responsible for GDPs-induced LTP, additional experiments were performed in the presence of the NMDA receptor antagonist D-AP5 (50 μM; n=6) or the voltage-dependent calcium channel (VDCC) blocker nifedipine (10 μM; n=7). D-AP5 failed to prevent pairing-induced persistent changes in sEPSCs frequency (this reached 208.1 ± 34.8 % of control; $p<0.05$; **Fig. 3**) while nifedipine blocked it (this was 96.9 ± 10.5 % of control; $p>0.5$; **Fig. 3**). It is worth noting that nifedipine and D-AP5 did not modify the shape or the amplitude of GDPs[5] (data not shown). These results indicate that, early in postnatal life, calcium rise through VDCC is the common trigger for activity-dependent changes in synaptic strength.

Postsynaptic calcium rise through theta bursts or plateau potentials fails to persistently increase the frequency of sEPSCs at CA3-CA1 connections

In the following experiments, we tried to mimic the effects of GDPs by repeatedly depolarizing the postsynaptic neuron with bursts of action potentials or plateau potentials evoked in the presence of the NMDA receptor blocker D-AP5 (20 μM). Early in postnatal development, elevation of postsynaptic calcium by repeated depolarizing

potentials was reported to enhance spontaneous occurring GABAergic events[20]. Bursts of five action potentials (at 100 Hz) evoked in the postsynaptic cell by brief depolarizing current pulses repeated at the theta frequency every 10 s for 10 times did not modify the amplitude or the frequency of spontaneous events. Moreover, the frequency and amplitude of sEPSCs did not change when theta bursts were paired with Schaffer collateral stimulation. The frequency of sEPSCs were 0.1 ± 0.02 Hz and 0.09 ± 0.02 Hz before and after pairing, respectively ($p>0.5$); the amplitude 16.6 ± 1.7 pA and 16.2 ± 2.3 pA ($p>0.5$), before and after pairing, respectively (n=8; **Fig. 4A, C and E**). Similar results were obtained when 20 plateau potentials at 0.1 Hz were evoked in the postsynaptic cells loaded with caesium to block most of the potassium conductances. However, unlike theta bursts, when plateau potentials were "paired" with afferent stimulation, they triggered a transient increase (165.8 ± 14.7 % of control) in frequency of sEPSC (from 0.1 ± 0.02 Hz to 0.17 ± 0.02 Hz; $p< 0.05$; n=10) which lasted for 2 ± 1 min and then declined to control levels **Fig. 4B and D**). This effect was not associated with modifications of sEPSCs amplitude. Twenty min after pairing both the frequency and the amplitude of sEPSCs were similar to controls. The frequency values were 0.1 ± 0.04 Hz and 0.11 ± 0.02 Hz, while the amplitude values were 13.6 ± 1.9 pA and 13.4 ± 2.3 pA, before and 20 min after pairing, respectively ($p>0.5$ for both frequency and amplitude; **Fig. 4E**). These experiments demonstrate that calcium rise in the postsynaptic cell through VDCC is not *per se* sufficient to persistently enhance glutamate release.

GDPs-induced increase in frequency of sEPSCs requires the activation of cAMP-dependent PKA, BDNF and the ERK signalling pathway

At immature CA3-CA1 synapses, during the first postnatal week, LTP requires the activation of cyclic AMP-dependent protein kinase A (PKA)[65]. To test the hypothesis that this signaling molecule is also crucial for persistently enhancing sEPSCs frequency following GDPs pairing, we first incubated P2-P6 rat pup slices (for 1 hour) with a membrane permeable form of PKA inhibitor (PKI 14-22, 1 μM). Slices were also perfused with PKI 14-22 during recordings. PKI 14-22 completely blocked the increase in frequency of sEPSCs (frequency values were 0.07 ± 0.03 Hz and 0.06 ± 0.02 Hz, before and after pairing, respectively; $p>0.5$; n=6; **Fig. 5A**). To exclude the possibility of

a direct presynaptic effect of PKA activity on glutamate release, we loaded postsynaptic neurons with the membrane impermeable form of PKI (PKI 6-22, 20 μM). Also in these conditions the pairing procedure failed to produce any persistent change in sEPSCs frequency (frequency values were 0.16 ± 0.04 Hz and 0.15 ± 0.02 Hz before and after pairing respectively; $p>0.5$; n=10; **Fig. 5A**). PKA inhibitors did not modify the amplitude of sEPSCs (in the presence of PKI 14-22, amplitude values were 14.5 ± 1.6 pA and 12.7 ± 3.2 pA before and after pairing, respectively; while in the presence of PKI 6-22, 15.9 ± 4.3 pA and 16.5 ± 3.9 pA before and after pairing, respectively (**Fig. 5A**). Both PKI 6-22 and PKI 14-22 *per se* did not modify the frequency or the amplitude of individual AMPA-mediated synaptic events. These experiments indicate that postsynaptic PKA activity plays a critical role for persistently enhancing glutamate release following GDPs pairing.

At developing neuromuscular synapses it has been demonstrated that cyclic AMP-dependent PKA "gate" brain-derived neurotrophic factor (BDNF)-induced synaptic potentiation[6]. BDNF belongs to the neurotrophins family, which not only promotes neuronal survival and differentiation but also regulates activity-dependent synaptic plasticity processes[48]. In particular, in the hippocampus endogenous BDNF is required for inducing LTP at both glutamatergic[44,13] and GABAergic[20] synapses. On the contrary, the lack of BDNF in BDNF knock out mice, causes an impairment of LTP[34]. BDNF acts on tropomyosin-related kinase receptor B (TrkB) and this interaction activates different signalling pathways. To assess the involvement of protein kinases coupled to Trk receptor family (PTKs) in pairing-induced potentiation of sEPSCs we used k-252a, a specific inhibitor of PTKs[33,50]. k-252a, applied in the bath at the concentration of 150 nM for 15 min, fully prevented pairing-induced enhancement of sEPSCs frequency. Mean frequency values were 0.14 ± 0.03 Hz and 0.16 ± 0.04 Hz before and after pairing, respectively ($p>0.5$; n=9; **Fig. 5B),** while mean amplitude values were 14.9 ± 1.1 pA and 15.7 ± 2.3 pA, before and after pairing, respectively ($p>0.5$; **Fig. 5B**). It should be stressed that k-252a alone (in the absence of pairing) did not modify sEPSCs frequency and amplitude (frequency: 0.11 ± 0.03 Hz versus 0.12 ± 0.02 Hz; amplitude: 14.2 ± 4.2 pA versus 12.7 ± 3.4 pA, in control and in the presence of k-252a, respectively). Similar results were obtained with k-252b (150 nM), which is a weaker inhibitor of Trk receptor

coupled PTKs[58]. On average, in 6 cells, before and after pairing the frequency of sEPSCs was 0.08 ± 0.02 Hz and 0.07 ± 0.02 Hz ($p>0.5$), while the amplitude was 15.9 ± 2.5 pA and 14.8 ± 1.4 pA, respectively ($p>0.5$; **Fig. 5B**). To better assess the contribution of endogenous TrkB ligands to GDPs-induced potentiation, we incubated the slices for at least 3 hours with a soluble form of TrkB receptor (TrkB IgG) engineered as immunoadhesin to prevent TrkB activation[53,20]. TrkB IgGs penetrates into the slice tissue and scavenges endogenous BDNF[53,20]. In slices preincubated with TrkB IgG (1μg/mL) the pairing procedure did not affect neither the frequency nor the amplitude (mean frequency values: 0.08 ± 0.03 Hz in control and 0.08 ± 0.02 Hz after pairing; $n= 6$; $p>0.5$; mean amplitude values: 18.9 ± 2.7 pA in control and 17.7 ± 2.2 pA after pairing; $p>0.5$; **Fig. 5B**). TrkB IgG *per se* did not alter the frequency or amplitude of synaptic events (frequency: 0.09 ± 0.02 Hz *versus* 0.08 ± 0.03 Hz; amplitude: 15.8 ± 2.7 pA *versus* 12.1 ± 1.6 pA, in the absence or in the presence of TrkB IgG, respectively). In contrast, when slices were incubated with TrkA IgG[47] which sequester endogenous nerve growth factor but not endogenous BDNF, pairing GDPs with afferent stimulation produced a persistent increase in frequency of sEPSCs (**Fig. 5B**). On average, twenty min after pairing, the frequency of sEPSCs was 115 % higher than control (mean frequency values were 0.07 ± 0.02 Hz and 0.16 ± 0.03 Hz, before and after pairing, respectively; $n= 5$; $p<0.05$; **Fig. 5B**) while the amplitude was slightly reduced to 82.2 ± 15 % of control (mean amplitude values were 16.3 ± 3.9 pA and 12.9 ± 2.9 pA, before and after pairing, respectively; $p>0.5$; **Fig. 5B**). Also in the case of TrkA IgGs no changes in the frequency or amplitude of spontaneous synaptic activity were detected in the absence of pairing (frequency: 0.12 ± 0.03 Hz *versus* 0.13 ± 0.03 Hz; amplitude: 14.8 ± 1.7 pA *versus* 13.6 ± 2.1 pA, in the absence or in the presence of TrkA IgG, respectively). Therefore, it seems likely that endogenous TrkB, but not TrkA ligands, are required for the persistent enhancement in frequency of sEPSCs triggered by GDPs pairing.

A previous study from the hippocampus[46] has demonstrated that some forms of experience-dependent modifications of synaptic strength require cAMP-dependent PKA which causes the activation of the TrkB signaling pathway *via* released BDNF. This pathway in turn activates ERK (extracellular signal-regulated kinase) a subfamily of

mitogen-activated protein kinase (MAPK)[57], which by interfering with the transcription factor cAMP response element binding protein (CREB) into the nucleus contribute to the persistent effects of synaptic plasticity[41,17,61,66,1,31]. To assess whether ERK was involved in pairing-induced persistent changes in frequency of spontaneous synaptic events we tested the effects of inhibitors of the ERK pathway, U0126 and PD98059[11]. Bath application of U0126 (20 μM, for 10 min) to 6 neurons did not modify the frequency and amplitude of sEPSCs which remained at pre-drug levels (before and after U0126 application, the mean frequency values were 0.12 ± 0.02 Hz and 0.11 ± 0.01 Hz, while the mean amplitude values were 14.9 ± 2.2 pA and 15.6 ± 1.5 pA; $p>0.5$ for both frequency and amplitude). However, after pairing, U0126 induced a persistent and significant reduction in sEPSCs frequency (on average, 20 min after pairing the frequency of sEPSCs was 71 % of controls (frequency varied from 0.21 ± 0.04 Hz before to 0.15 ± 0.04 Hz after pairing; ($p<0.05$) while the amplitude was not significantly modified (16.6 ± 2.2 pA and 14.9 ± 0.9 pA, before and after pairing, respectively; $p>0.5$; **Fig. 6A**). Similar effects were obtained with PD98059, another ERK inhibitor. To this purpose, slices were preincubated (for 10 min) with PD98059 (50 μM). While PD98059 *per se* did not modify the frequency or amplitude of synaptic events (frequency: 0.11 ± 0.02 Hz and 0.09 ± 0.02 Hz; amplitude: 14.9 ± 2.7 pA 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 sEPSCs frequency (57 % of controls; $p<0.05$) (mean frequency values were 0.15 ± 0.04 Hz and 0.08 ± 0.02 Hz, before and after pairing, respectively) but not in the amplitude (mean amplitude values were 15.2 ± 2.7 pA and 14.3 ± 3.1 pA, before and after pairing, respectively; $p>0.5$) of sEPSCs (n= 6; **Fig. 6A**).

To test whether ERK activation occurred into the postsynaptic cell, PD98059 was applied directly into the patch pipette[54]. Also in this conditions, PD98059 (50 μM) prevented the effects of ERK on paired-induced potentiation of EPSCs frequency. (mean frequency values were 0.09 ± 0.03 Hz and 0.07 ± 0.02 Hz, before and after pairing, respectively; $p>0.1$; mean amplitude values: 16.1 ± 1.3 pA and 13.8 ± 1.9 pA, before and after pairing, respectively; $p>0.1$; n=5; **Fig. 6A**).

The involvement of ERK in GDPs-induced changes in synaptic efficacy was further validated by immunocytochemical experiments which demonstrated that the pairing procedure was able to induce ERK phosphorylation, an effect that was prevented by U0126 (10 μM; **Fig. 6B)**. In the absence of pairing no ERK phosphorylation was observed. These data suggest that the persistent changes in frequency of sEPSCs following GDPs pairing require the activation of the ERK pathway.

DISCUSSION

The present data clearly show that, during the first week of postnatal life, correlated pre (Schaffer collateral) and post synaptic (GDPs) activity induced a persistent enhancement of synaptic efficacy at CA3-CA1 connections. This required a transient rise of calcium in the postsynaptic cell. In addition, our findings indicate that three signaling molecules cAMP, BDNF and ERK, known to play a crucial role in long-term synaptic plasticity and long-term memory storage in the adult hippocampus[57] are essential for pairing-induced persistent modifications of synaptic activity early in postnatal life.

This study further strengthens and extends to glutamatergic synapses previous observations made on immature mossy fiber-CA3 connections[30], known to be mainly GABAergic[21,51]. The pairing procedure not only produced a strong potentiation of the EPSCs evoked by the Schaffer collateral stimulation but also persistently enhanced the frequency of spontaneous glutamatergic events. In keeping with the late development of glutamatergic[25,62,24] connections, the frequency of sEPSCs was very low[18]. However, their amplitude distribution matched very well that of miniature events obtained in the presence of TTX[18] suggesting in agreement with other studies[26, 22] that, early in postnatal life, CA3-CA1 synapses bear a single release site.

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[8,19,20]. 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 was dependent on calcium rise through VDCC 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 produce LTP. When paired with afferent stimulation, plateau potentials only briefly enhanced sEPSCs frequency that declined to control level few min 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[64]. However, unlike the present case, transient changes in synaptic strength observed in the adult were primarily dependent on postsynaptic mechanisms, since membrane depolarization not only enhanced the frequency of miniature events but also their amplitude as well as the amplitude of responses to exogenously applied AMPA[64]. Whatever the mechanisms, our results indicate that the way by which calcium enters into the cell is crucial for pairing-induced LTP. We can not exclude however, that in the present study, GDPs may concomitantly stimulate presynaptic terminals further strengthening synaptic currents.

Like mossy fiber-CA3 synapses[30] the induction of LTP at CA3-CA1 connections was clearly dependent on postsynaptic events while its expression was mainly presynaptic as suggested by pairing-induced increase in frequency but not in amplitude of spontaneous glutamatergic events. Moreover, the decrease in failure rate, in paired pulse facilitation and the increase in the coefficient of variation of evoked responses are consistent with a presynaptic change in release probability. Although changes in failure rate and coefficient of variation may result from the insertion of new receptors on the subsynaptic membrane of previously silent synapses[35], they are difficult to reconcile with the reduction in the paired pulse ratio.

If induction occurs at the postsynaptic site and maintenance at the pre synaptic site we shall admit that the postsynaptic cell provides a transcellular retrograde signal to the presynaptic neuron. One attractive candidate molecule is BDNF which can be released in a calcium-dependent way by membrane depolarization of the postsynaptic cell[16,38]. Like other neurotrophins, BDNF plays a crucial role not only in survival and differentiation of many types of neurons[60] but also in synaptic transmission and plasticity [34,13,48]. In the present experiments the involvement of BDNF in GDPs induced-potentiation was demonstrated by the observation that scavengers of endogenous BDNF or blockers of Trk receptors coupled PTKs were able to prevent pairing-induced persistent enhancement of EPSCs frequency.

The present results also suggest that BDNF signaling is "gated" by cAMP, a second messenger responsible for regulating a wide range of neuronal functions as shown by the experiments in which inhibitors of PKA prevented GDPs-induced persistent changes in sEPSCs frequency[6,42]. As in adulthood, gating would occur at the receptor level and would involve BDNF-induced TrkB tyrosine phosphorylation[29]. This in turn would lead to structural changes such as dendritic growth and spine formation[29]. In line with our data, it has been recently shown that, during a critical period of synapse formation before P9, at CA3-CA1 connections LTP critically depends on the activation of cAMP dependent PKA[65]. The levels of cAMP are influenced by calcium through the activity of calcium dependent adenylate cyclase[43]. Although we can not exclude a concomitant presynaptic effect of BDNF, the finding that the effect of pairing was blocked when the patched cell was loaded with the membrane impermeable form of PKI may indicate that cAMP controls the release of BDNF in the postsynaptic cell. Once released, BDNF would affect Trk B receptors localized on both pre and postsynaptic membranes. At presynaptic level, BDNF-induced activation of TrkB receptors would enahnce transmitter release[39,13,59,17].

One of the most common signaling pathways activated by BDNF after binding to Trk B receptor is the MAPK/ERK cascade[17,66,27]. Interestingly, this pathway can be synergistically activated by cAMP-dependent PKA[28,9] which would act as a linker between TrkB receptors and ERK activation. Activation of ERK would lead to transcriptional regulation and new protein synthesis[28,57] required for the enduring forms of synaptic plasticity[31]. As in the late phase of LTP[31], also in the present case the activation of ERK was specifically targeted to the postsynaptic neuron as demonstrated by electrophysiological and immunocytochemical experiments. 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[52,1]. Therefore, although BDNF can act as a presynaptic modulator of transmitter release, it can also act postsynaptically to produce morphological modifications necessary for the formation of new synapses and for the refinement of the adult neuronal hippocampal circuit.

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

in a previous study from the immature hippocampus it was shown that activation of presynaptic metabotropic glutamate receptors through the cAMP signaling pathway was able to up regulate GDPs[56]. The cAMP-dependent increase in GDPs frequency would further enhance the probability of occurrence of coincident detection signals for strengthening synaptic activity.

METHODS

Slice preparation

Experiments were performed on acute hippocampal slices obtained from P2-P6 Wistar rats as previously described[30]. Briefly, animals were decapitated after being anaesthetized with an i.p. injection of urethane (2g/kg). 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. The brain was quickly removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 130, KCl 3.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, $MgCl_2$ 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 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 33-34°C.

Electrophysiological recordings

Electrophysiological experiments were performed from CA1 pyramidal cells 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 with bipolar twisted NiCr-insulated electrodes localized in *stratum radiatum.* They were recorded in voltage clamp conditions from a holding potential of –53 mV (the reversal potential of $GABA_A$ receptor). In most cases, paired stimuli were applied at 50 ms interval. 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): K-gluconate 135, KCl 20, HEPES 10, MgATP 4, GTP 0.3, EGTA

0.5 and QX-314 5. In some experiments recordings were performed with patch pipettes containing the calcium chelators 1,2-bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA 20 mM, purchased from Sigma, Milan, Italy). When BAPTA (20 mM) was added to the intrapipette 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 Oregon Green 488 BAPTA-1 (100 μM; Molecular Probes).

Recordings were made with a patch clamp amplifier (Axopatch 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 20-25% changes were excluded from the analysis.

Drugs used were: D-(-)-2-amino-5-phosphonopentaoic acid (D-AP5), 6,7-dinitroquinoxaline-2,3-dione (DNQX) all purchased from Tocris Cookson Ltd, Bristol, UK); nifedipine (from Signa, Milan, Italy); Protein Kinase A Inhibitor 6-22 Amine (PKI 6-22), Protein Kinase A Inhibitor 14-22 Amine (PKI 14-22), k-252a and k-252b, U0126 and PD 98059 all obtained from Calbiochem (La Jolla, CA). Immunoadhesine TrkA-IgG and TrkB-IgG were gifts of Dr. Antonino Cattaneo (SISSA, Trieste, Italy).

All drugs were dissolved in dimethylsulphoxide (DMSO), except D-AP5, PKI 6-22 and PKI 14-22 that were dissolved in water. 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 2 min.

Immunofluorescence staining

Hippocampal slices containing paired or unpaired neurons were collected and fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer. A basic buffer containing PBS, 3% fetal calf serum (from Sigma, Milan, Italy), 0.3% Triton X-100 and 3% BSA was used for labeling of intracellular antigens. After two hours preincubation in basic buffer, primary antibody to doubly phosphorylated ERK1/2 (1:200, Cell

Signaling Technology, Danvers, MA) was applied overnight at 4°C. After thorough washing, the resulting immune complexes were visualized with Alexa 594 labeled goat anti-rabbit antibody (Invitrogen, Carlsbad, CA). Slices were imaged with the Leica (DM-IRE2) confocal system by using sequential dual-channel recording of the Oregon Green injected cells.

Data acquisition and analysis

Data were stored on the hard disk of a PC after digitization with an A/D converter (Digidata 1322A; Axon Instruments). Data were sampled at 20 kHz and filtered with a cut-off frequency of 2 kHz. Data acquisition was done using pClamp 9 (Axon Instruments, Foster City, CA). Spontaneous and evoked EPSCs were analyzed offline with Clampfit 9 program. Spontaneous EPSCs were first collected using the template function of Clampfit and then reviewed by visual inspection. Mean EPSC amplitude was obtained by averaging successes and failures. Paired-pulse ratio was calculated as the ratio between the mean amplitude of EPSC2 and EPSC1. The coefficient of variation (CV) of response amplitude was determined as the ratio between standard deviation and mean. Values are given as mean ± SEM. Significance of differences was assessed by Student's *t*-test, Wilcoxon signed rank test or Kolmogorov-Smirnoff test. Significance level: $p<0.05$.

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COMPETING INTERESTS STATEMENT

The authors declare that they have not competing financial interests.

References

1. Alonso, M., Medina, J.H. & Pozzo-Miller, L. ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons. *Learn Mem* **11**, 172-178 (2004).

2. Ben-Ari, Y. Excitatory actions of gaba during development: the nature of the nurture. *Nat Rev Neurosci* **3**, 728-739 (2002).

3. Ben-Ari, Y., Cherubini, E., Corradetti, R. & Gaiarsa, J.L. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol. (Lond.)* **416**, 303-325 (1989).

4. Bliss, T.V. & Collingridge, G.L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39 (1993).

5. Bolea, S., Avignone, E., Berretta, N., Sanchez-Andres, J.V. & Cherubini, E. Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. *J Neurophysiol* **81**, 2095-2102 (1999).

6. Boulanger, L. & Poo, M. Gating of BDNF-induced synaptic potentiation by cAMP. *Science* **284**, 1982-1984 (1999).

7. Buzsaki, G. & Draguhn, A. Neuronal oscillations in cortical networks. *Science* **304**, 1926-1929 (2004).

8. Caillard, O., Ben-Ari, Y. & Gaiarsa, J.L. Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. *J. Physiol. (Lond.)***518 (Pt 1)**, 109-119 (1999).

9. Cancedda, L., *et al.* Patterned vision causes CRE-mediated gene expression in the visual cortex through PKA and ERK. *J Neurosci* **23**, 7012-7020 (2003).

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

11. Davies, S.P., Reddy, H., Caivano, M. & Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* **351**, 95-105 (2000).

12. de la Prida, L.M., Huberfeld, G., Cohen, I. & Miles, R. Threshold behavior in the initiation of hippocampal population bursts. *Neuron* **49**, 131-142 (2006).

13. Figurov, A., Pozzo-Miller, L.D., Olafsson, P., Wang, T. & Lu, B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature* **381**, 706-709 (1996).

14. Garaschuk, O., Hanse, E. & Konnerth, A. Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J. Physiol. (Lond.)***507 (Pt 1)**, 219-236 (1998).

15. Garaschuk, O., Linn, J., Eilers, J. & Konnerth, A. Large-scale oscillatory calcium waves in the immature cortex. *Nat Neurosci* **3**, 452-459 (2000).

16. Goodman, L.J., *et al.* Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Mol Cell Neurosci* **7**, 222-238 (1996).

17. Gooney, M. & Lynch, M.A. Long-term potentiation in the dentate gyrus of the rat hippocampus is accompanied by brain-derived neurotrophic factor-induced activation of TrkB. *J Neurochem* **77**, 1198-1207 (2001).

18. Groc, L., Gustafsson, B. & Hanse, E. Spontaneous unitary synaptic activity in CA1 pyramidal neurons during early postnatal development: constant contribution of AMPA and NMDA receptors. *J Neurosci* **22**, 5552-5562 (2002).

19. Gubellini, P., Ben-Ari, Y. & Gaiarsa, J.L. Activity- and age-dependent GABAergic synaptic plasticity in the developing rat hippocampus. *Eur J Neurosci* **14**, 1937-1946 (2001).

20. Gubellini, P., Ben-Ari, Y. & Gaiarsa, J.L. Endogenous neurotrophins are required for the induction of GABAergic long-term potentiation in the neonatal rat hippocampus. *J Neurosci* **25**, 5796-5802 (2005).

21. Gutierrez, R., *et al.* Plasticity of the GABAergic phenotype of the "glutamatergic" granule cells of the rat dentate gyrus. *J Neurosci* **23**, 5594-5598 (2003).

22. Hanse, E. & Gustafsson, B. Quantal variability at glutamatergic synapses in area CA1 of the rat neonatal hippocampus. *J. Physiol. (Lond.)***531**, 467-480 (2001).

23. Hebb, D.O. *The organization of behavior; a neuropsychological theory* (Wiley, New York,, 1949).

24. Hennou, S., Khalilov, I., Diabira, D., Ben-Ari, Y. & Gozlan, H. Early sequential formation of functional GABA(A) and glutamatergic synapses on CA1 interneurons of

the rat foetal hippocampus. *Eur J Neurosci* **16**, 197-208 (2002).

25. Hosokawa, Y., Sciancalepore, M., Stratta, F., Martina, M. & Cherubini, E. Developmental changes in spontaneous GABAA-mediated synaptic events in rat hippocampal CA3 neurons. *Eur J Neurosci* **6**, 805-813 (1994).

26. Hsia, A.Y., Malenka, R.C. & Nicoll, R.A. Development of excitatory circuitry in the hippocampus. *J Neurophysiol* **79**, 2013-2024 (1998).

27. Huang, E.J. & Reichardt, L.F. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem* **72**, 609-642 (2003).

28. Impey, S., *et al.* Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* **21**, 869-883 (1998).

29. Ji, Y., Pang, P.T., Feng, L. & Lu, B. Cyclic AMP controls BDNF-induced TrkB phosphorylation and dendritic spine formation in mature hippocampal neurons. *Nat Neurosci* **8**, 164-172 (2005).

30. Kasyanov, A.M., Safiulina, V.F., Voronin, L.L. & Cherubini, E. 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 (2004).

31. Kelleher, R.J., 3rd, Govindarajan, A. & Tonegawa, S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* **44**, 59-73 (2004).

32. Khazipov, R. & Luhmann, H.J. Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. *Trends Neurosci* **29**, 414-418 (2006).

33. Knusel, B. & Hefti, F. K-252 compounds: modulators of neurotrophin signal transduction. *J Neurochem* **59**, 1987-1996 (1992).

34. Korte, M., *et al.* Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A* **92**, 8856-8860 (1995).

35. Kullmann, D.M. Silent synapses: what are they telling us about long-term potentiation? *Philos Trans R Soc Lond B Biol Sci* **358**, 727-733 (2003).

36. Leinekugel, X., *et al.* Correlated bursts of activity in the neonatal hippocampus in vivo. *Science* **296**, 2049-2052 (2002).

37. Leinekugel, X., Medina, I., Khalilov, I., Ben-Ari, Y. & Khazipov, R. Ca2+ oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA

receptors in the neonatal hippocampus. *Neuron* **18**, 243-255 (1997).

38. Lessmann, V., Gottmann, K. & Malcangio, M. Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol* **69**, 341-374 (2003).

39. Lohof, A.M., Ip, N.Y. & Poo, M.M. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363**, 350-353 (1993).

40. Malenka, R.C. & Nicoll, R.A. Long-term potentiation--a decade of progress? *Science* **285**, 1870-1874 (1999).

41. McAllister, A.K., Katz, L.C. & Lo, D.C. Neurotrophins and synaptic plasticity. *Annu Rev Neurosci* **22**, 295-318 (1999).

42. Nagappan, G. & Lu, B. Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends Neurosci* **28**, 464-471 (2005).

43. Nguyen, P.V. & Woo, N.H. Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. *Prog Neurobiol* **71**, 401-437 (2003).

44. O'Dell, T.J., Kandel, E.R. & Grant, S.G. Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Nature* **353**, 558-560 (1991).

45. O'Donovan, M.J. The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr Opin Neurobiol* **9**, 94-104 (1999).

46. Patterson, S.L., *et al.* Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* **32**, 123-140 (2001).

47. Pesavento, E., Margotti, E., Righi, M., Cattaneo, A. & Domenici, L. Blocking the NGF-TrkA interaction rescues the developmental loss of LTP in the rat visual cortex: role of the cholinergic system. *Neuron* **25**, 165-175 (2000).

48. Poo, M.M. Neurotrophins as synaptic modulators. *Nat Rev Neurosci* **2**, 24-32 (2001).

49. Ricci-Tersenghi, F., Minneci, F., Sola, E., Cherubini, E. & Maggi, L. Multivesicular release at developing Schaffer collateral-CA1 synapses: an analytic approach to describe experimental data. *J Neurophysiol* **96**, 15-26 (2006).

50. Ross, A.H., McKinnon, C.A., Daou, M.C., Ratliff, K. & Wolf, D.E. Differential biological effects of K252 kinase inhibitors are related to membrane solubility but not to permeability. *J Neurochem* **65**, 2748-2756 (1995).

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

52. Segal, R.A. & Greenberg, M.E. Intracellular signaling pathways activated by neurotrophic factors. *Annu Rev Neurosci* **19**, 463-489 (1996).

53. Sermasi, E., Margotti, E., Cattaneo, A. & Domenici, L. Trk B signalling controls LTP but not LTD expression in the developing rat visual cortex. *Eur J Neurosci* **12**, 1411-1419 (2000).

54. Shen, M.R., Chou, C.Y., Browning, J.A., Wilkins, R.J. & Ellory, J.C. Human cervical cancer cells use Ca2+ signalling, protein tyrosine phosphorylation and MAP kinase in regulatory volume decrease. *J. Physiol. (Lond.)***537**, 347-362 (2001).

55. Sipila, S.T., Huttu, K., Soltesz, I., Voipio, J. & Kaila, K. Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. *J Neurosci* **25**, 5280-5289 (2005).

56. Strata, F., Sciancalepore, M. & Cherubini, E. Cyclic AMP-dependent modulation of giant depolarizing potentials by metabotropic glutamate receptors in the rat hippocampus. *J. Physiol. (Lond.)***489 (Pt 1)**, 115-125 (1995).

57. Sweatt, J.D. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* **14**, 311-317 (2004).

58. Tanaka, T., Saito, H. & Matsuki, N. Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J Neurosci* **17**, 2959-2966 (1997).

59. Tao, H.W. & Poo, M. Retrograde signaling at central synapses. *Proc Natl Acad Sci U S A* **98**, 11009-11015 (2001).

60. Thoenen, H. The changing scene of neurotrophic factors. *Trends Neurosci* **14**, 165-170 (1991).

61. Tyler, W.J., Alonso, M., Bramham, C.R. & Pozzo-Miller, L.D. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem* **9**, 224-237 (2002).

62. Tyzio, R., *et al.* 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**, 10372-10382 (1999).

63. Voigt, T., Opitz, T. & de Lima, A.D. Activation of early silent synapses by spontaneous synchronous network activity limits the range of neocortical connections. *J Neurosci* **25**, 4605-4615 (2005).

64. Wyllie, D.J., Manabe, T. & Nicoll, R.A. A rise in postsynaptic Ca2+ potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron* **12**, 127-138 (1994).

65. Yasuda, H., Barth, A.L., Stellwagen, D. & Malenka, R.C. A developmental switch in the signaling cascades for LTP induction. *Nat Neurosci* **6**, 15-16 (2003).

66. Ying, S.W., *et al.* Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. *J Neurosci* **22**, 1532-1540 (2002).

Figure legends

Figure 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 due to 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. Top: 12 superimposed individual responses (successes and failures) evoked by Schaffer collateral stimulation, obtained in control and 20 min after "pairing". Bottom: average of 18 trials (successes and failures) obtained in the same experimental conditions. D. The amplitude of synaptic responses (dots) evoked by stimulation of Schaffer collateral before and after pairing (arrow at time 0) are plotted against time for the experiment shown in C. E. Summary plot of mean EPSC amplitude *versus* time for 8 cells. F. Mean EPSC amplitude (left) and mean percentage of successes (right) for 8 P2-P6 CA1 pyramidal neurons examined before (white column) and 20 min after pairing (black column). **$p<0.01$.

Figure 2. The pairing procedure enhances the frequency but not the amplitude of spontaneous AMPA-mediated EPSCs.

A. Consecutive traces from a P4 neuron showing spontaneous AMPA-mediated EPSCs obtained in control and 20 min after pairing. B. Cumulative distribution of inter event interval (IEI, left) and amplitude (right) for the cell shown in A before (thin line) and after pairing (thick line). C. Summary plot showing the mean frequency (left) and amplitude (right) of sEPSCs obtained before and after pairing (arrows at the time 0; n=6). D. Mean frequency (left) and amplitude (right) of spontaneous EPSCs obtained in control (white columns) and 20 min after pairing (black columns). **$p<0.01$; n=10.

Figure 3. 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 spontaneous EPSCs expressed as percentage of controls (dashed lines) obtained 20 min after pairing in the presence of intracellular BAPTA (20 mM, white columns), extracellular nifedipine (10 μM, grey columns) or D-AP5 (50 μM, black columns). Cells tested with BAPTA, n=6; nifedipine, n=7; D-AP5, n=6; * $p < 0.05$.

Figure 4. Pairing theta bursts or plateau potentials with Schaffer collateral stimulation fails to persistently enhance synaptic strength.
A and B. Theta bursts (A) and plateau potentials (B) evoked in CA1 pyramidal cells held in current clamp mode. The rising phase of theta bursts or plateau potentials were used to trigger Schaffer collateral stimulation (stim). C and D. Summary plots showing the mean frequency of spontaneous EPSCs 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 spontaneous EPSCs after pairing the Schaffer collateral with plateau potentials (*$p<0.05$). E. Mean frequency (left) and amplitude (right) of spontaneous EPSCs expressed as percentage of controls (dashed lines) obtained 20 min after pairing with theta bursts (white columns) or plateau potentials (black columns).

Figure 5. Pairing-induced increase in frequency of sEPSCs requires the activation of PKA and TrkB receptors by BDNF.
A. Mean frequency (left) and amplitude (right) of spontaneous EPSCs (normalized to pre-pairing control values, dashed lines) recorded 20 min after pairing in the presence of the PKA inhibitors, PKI 14-22 (1 μM, n=6, white columns) added to the extracellular medium or PKI 6-22 (20 μM, black columns) added to the intra pipette solution. B. Mean frequency (left) and amplitude (right) of spontaneous EPSCs (normalized to pre-pairing control values, dashed lines) recorded 20 min after pairing in the presence of K252a (150 nM, white, n=9), K252b (150 nM, light grey, n=6), TrkA-IgG (1 μg/mL, dark gray, n=5) and TrkB-IgG (1 μg/mL, black, n=6). Note the increase in sEPSCs frequency in the presence of TrkA-IgG.

Figure 6. Pairing-induced increase in frequency of sEPSCs requires the activation of the ERK pathway.

A. Mean frequency (left) and amplitude (right) of spontaneous EPSCs (normalized to pre-pairing control values, dashed lines) recorded 20 min after pairing in the presence of the ERK inhibitors U0126 (20 μM, n=5, white), PD98059 (50 μM) added to the extracellular medium (n=6, grey) or PD98059 (50 μM) added to the intra pipette solution (n=5, black). B. The polyclonal antibody recognizing the doubly phosphorylated form of ERK1/2 (pERK) in the paired neuron. Immunofluorescence images showing the selective ERK pathway activation (in red, middle panels) in Oregon Green-BAPTA1 (OGB-1) injected neuron (in green, first panels) belonging to paired (lower panel) as compared to unpaired neuron (upper panel). Scale bar: 10μm.

Suppl. Figure 1. Immature CA3-CA1 connections express a single release site.

A. Superimposed amplitude distribution histograms of spontaneous EPSCs recorded from a P3 pyramidal neuron in control conditions (light grey) and during application of TTX (1 mM, dark grey). Note the presence of a single peak in both conditions. B. Cumulative amplitude histograms of all spontaneous glutamatergic events recorded from nine cells in control (black line) and during application of TTX (1 mM, grey line). The difference between the two distributions was not significant ($p>0.5$; Kolmogorov-Smirnoff test).

Figure 1

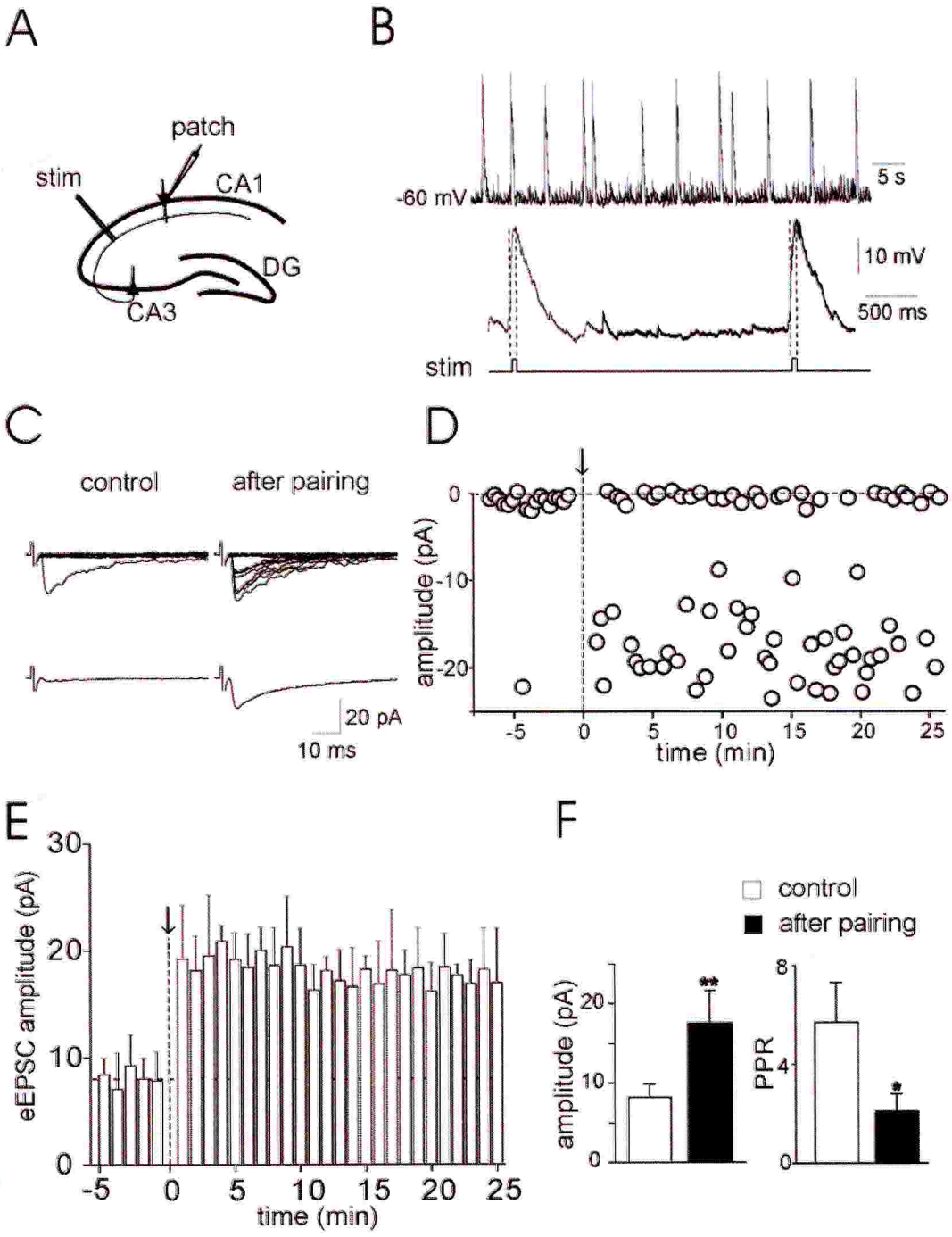

A
patch
stim
CA1
DG
CA3
B
-60 mV
5 s
10 mV
500 ms
stim
C
control
after pairing
20 pA
10 ms
D
amplitude (pA)
0
-10
-20
-5
0
5
10
15
20
25
time (min)
E
eEPSC amplitude (pA)
30
20
10
0
-5
0
5
10
15
20
25
time (min)
F
control
after pairing
amplitude (pA)
20
10
0
**
PPR
8
4
0
*

Figure 2

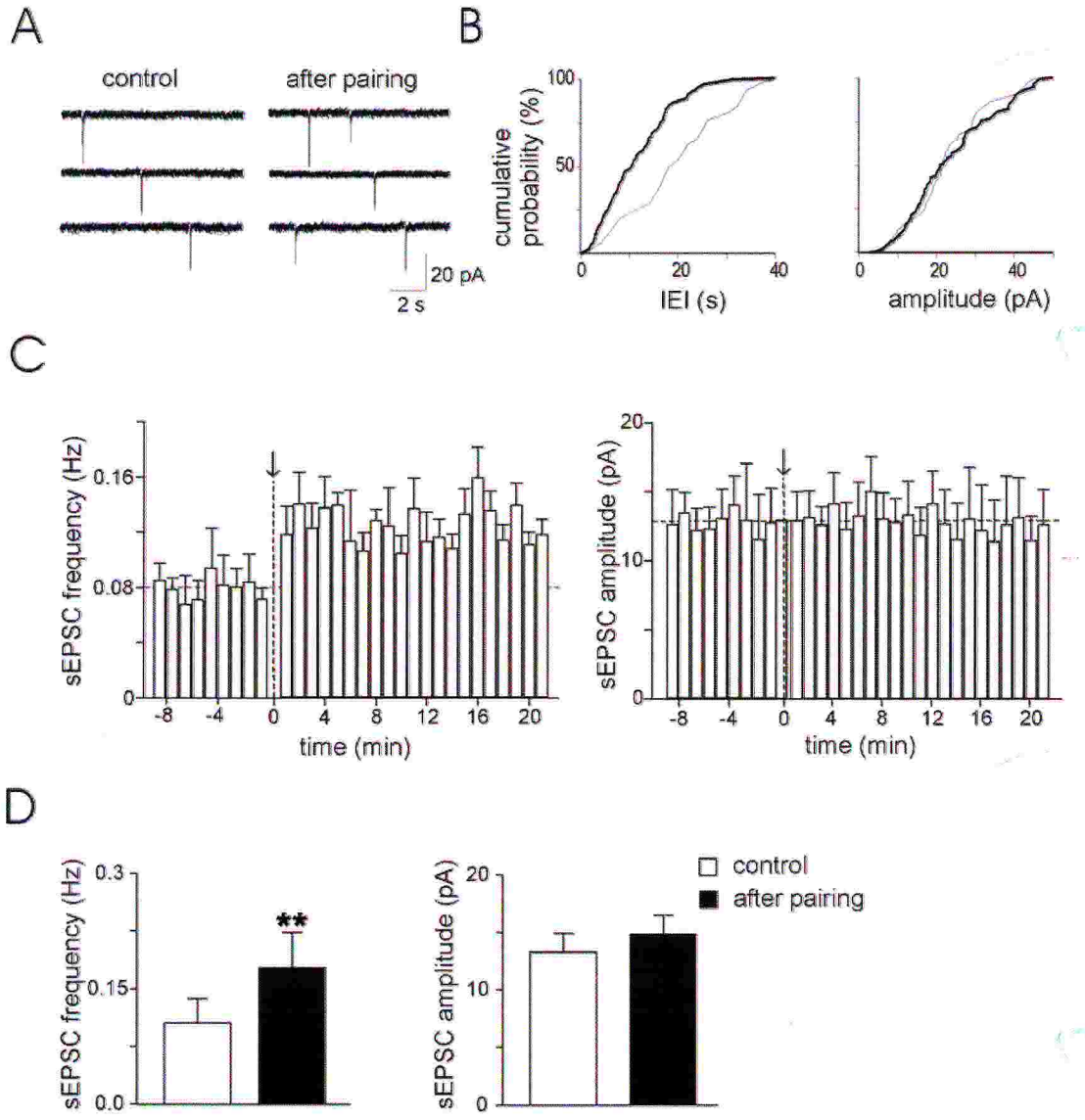

A
control
after pairing
20 pA
2 s
B
cumulative probability (%)
100
50
0
20
40
IEI (s)
amplitude (pA)
C
sEPSC frequency (Hz)
0.16
0.08
0
-8
-4
0
4
8
12
16
20
time (min)
sEPSC amplitude (pA)
20
10
D
0.3
0.15
0.0
**
control
after pairing

Figure 3

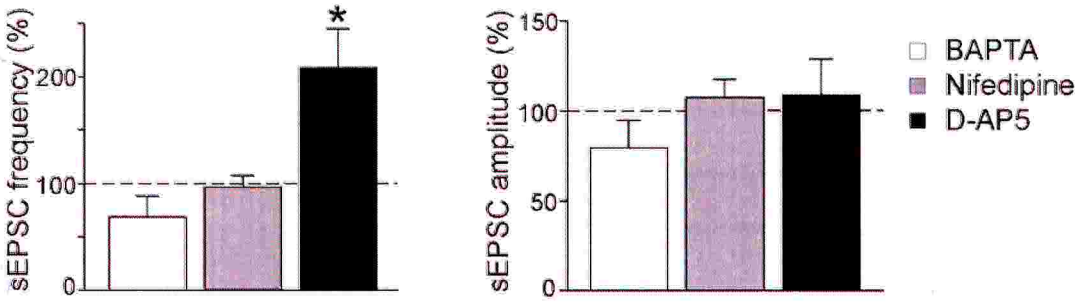

sEPSC frequency (%)
200
100
0
*
sEPSC amplitude (%)
150
100
50
0
BAPTA
Nifedipine
D-AP5

Figure 4

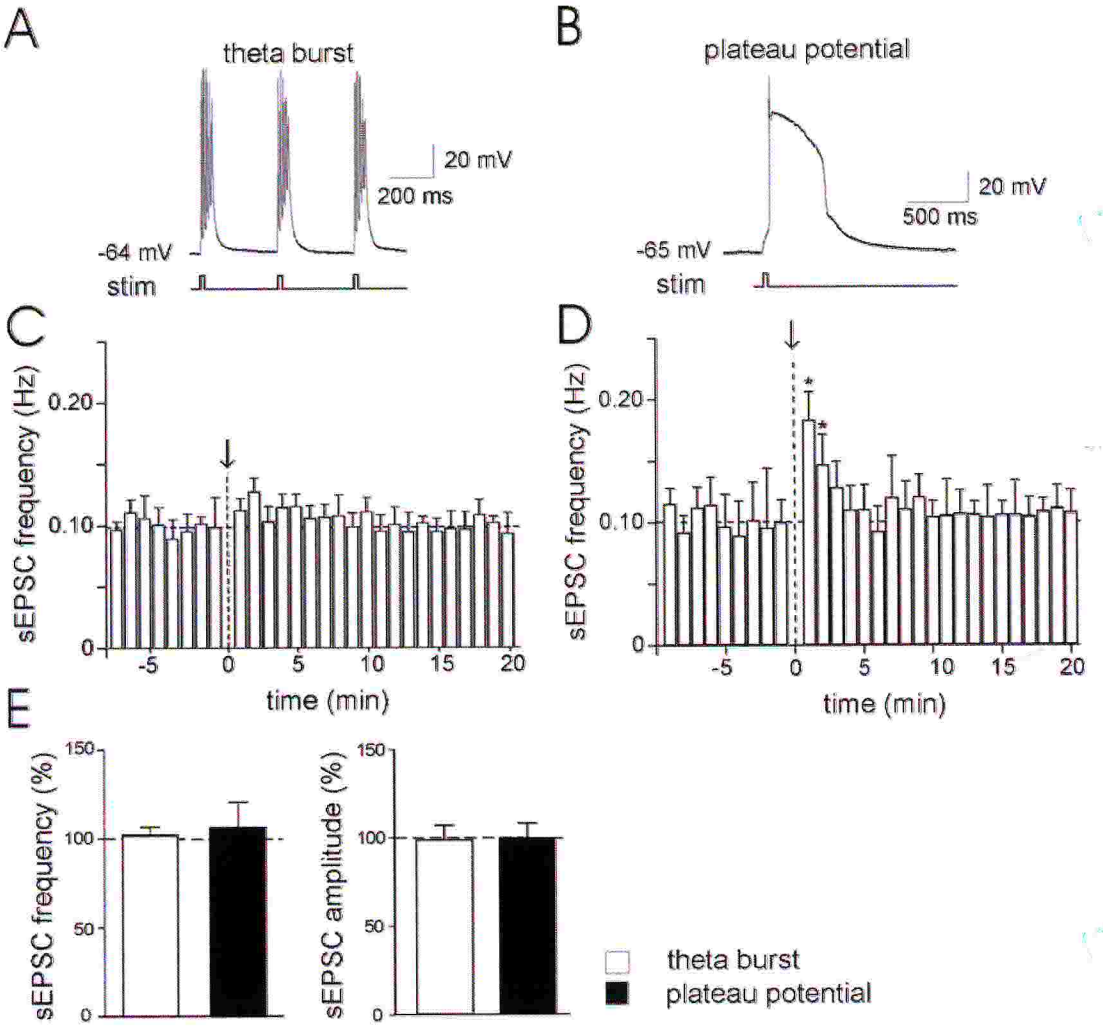

A
theta burst
20 mV
200 ms
-64 mV
stim
B
plateau potential
20 mV
500 ms
-65 mV
stim
C
sEPSC frequency (Hz)
0.20
0.10
0
-5
0
5
10
15
20
time (min)
D
sEPSC frequency (Hz)
0.20
0.10
0
-5
0
5
10
15
20
time (min)
E
sEPSC frequency (%)
150
100
50
0
sEPSC amplitude (%)
150
100
50
0
theta burst
plateau potential

Figure 5

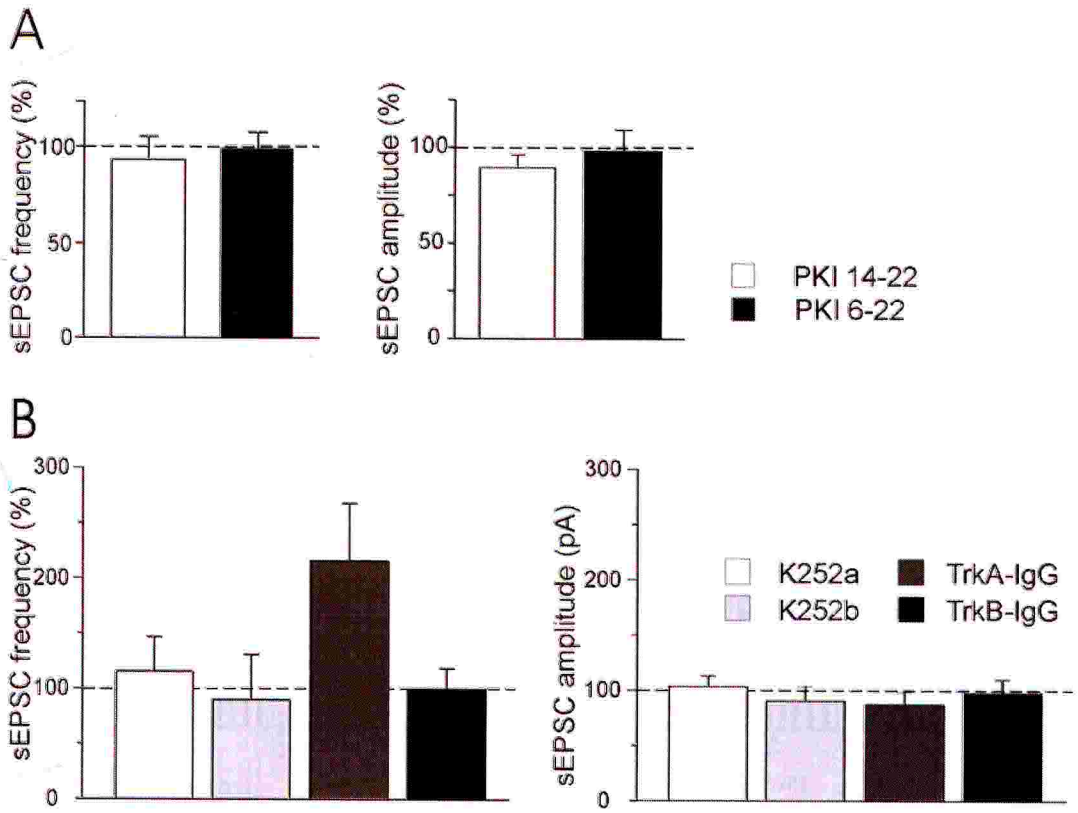
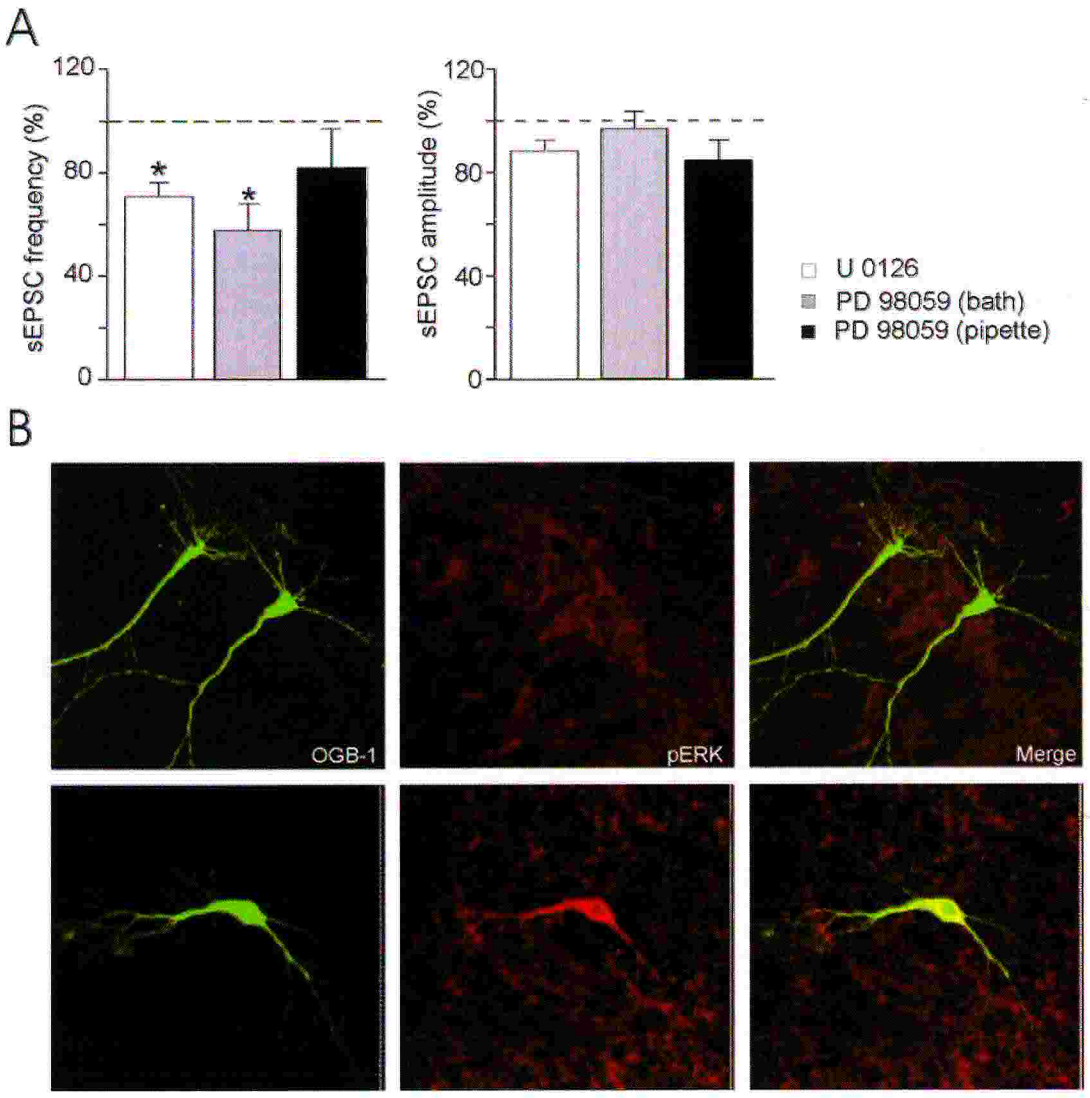
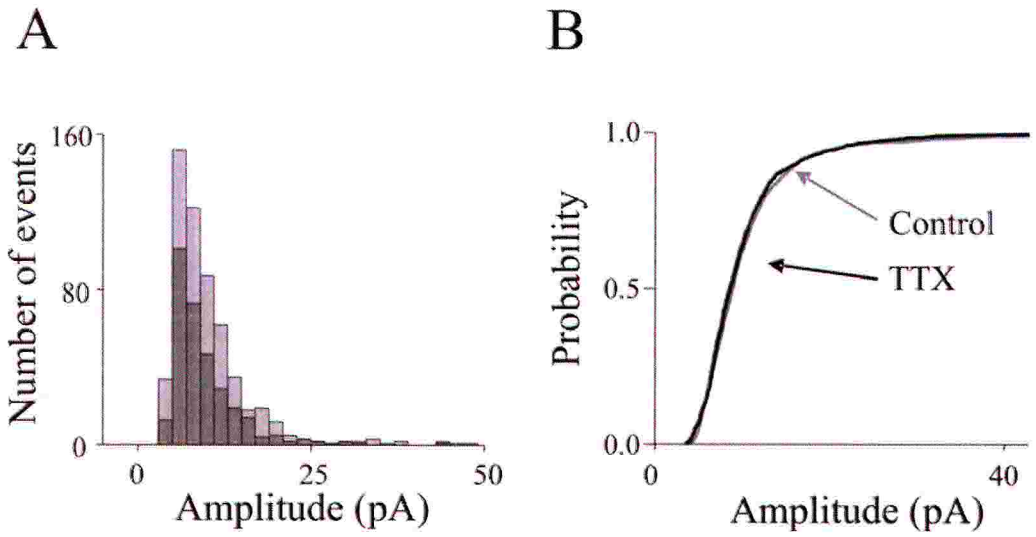

A
sEPSC frequency (%)
100
50
0
sEPSC amplitude (%)
100
50
0
PKI 14-22
PKI 6-22
B
sEPSC frequency (%)
300
200
100
0
sEPSC amplitude (pA)
300
200
100
0
K252a
K252b
TrkA-IgG
TrkB-IgG

Figure 6


A
120
80
40
0
sEPSC frequency (%)
*
*
120
80
40
0
sEPSC amplitude (%)
U 0126
PD 98059 (bath)
PD 98059 (pipette)
B
OGB-1
pERK
Merge

Supplementary Figure 1



Conclusions and future perspectives

A fundamental question in developmental neuroscience is how activity can shape synaptic connections and modify neuronal networks. In the hippocampus, GDPs reflect the synchronized activity of populations of neurons, expressed during a critical period of postnatal development when GABA exerts a depolarizing and excitatory action. In the present study, I have demonstrated that neuronal membrane oscillations similar to GDPs could be detected in organotypic hippocampal slices which, in comparison with acute slices, allow following GDPs expression for several weeks (Gahwiler et al., 1997; Noraberg et al., 2005). As in acute slices, in cultured slices, GDPs resulted from the synergistic action of GABA and glutamate. GABA was still depolarizing since E_{GABA} was positive comparing to the resting membrane potential. However, GABA-induced membrane depolarization does not necessarily mean that GABA excites the target cells bringing them to fire. Thus, GABA may also shunt the membrane reducing cell firing. In the case of excitation, GABA, released from GABAergic interneurons, may drive principal cells. The observation that GDPs are generated by both GABA and glutamate and that the release of GABA precedes that of glutamate further strengthen the idea that, at least in some neurons, GABA is depolarizing and excitatory. As a whole these data indicate a crucial role for the depolarizing and excitatory GABAergic transmission in the generation of GDPs.

In addition I found that GDPs act as coincident detection signals for enhancing synaptic efficacy. Thus, at glutamatergic Schaffer collateral–CA1 connections, which during the first postnatal week express a single release site, pairing GDPs with Schaffer collateral stimulation, induced a persistent potentiation of glutamate release assessed as spontaneous AMPA-mediated glutamatergic synaptic events. This required a transient rise of calcium in the postsynaptic cell. Calcium controls many cell functions including gene expression. I found that the molecular cascade activated by GDPs pairing involves three signaling molecules, PKA, BDNF and ERK. Furthermore, my results indicate that the way by which calcium enters into the cell is crucial for pairing-induced LTP. These results support the idea that, during a critical period of postnatal development, $GABA_A$-mediated GDPs are involved in structural remodeling of excitatory synapses and provide new insights into the molecular mechanisms responsible for this process.

Although several studies from our and other laboratories have shed light on the mechanisms underlying GDPs generation, their function role has been only partially elucidated.

As already mentioned, GDPs are generated stochastically when the frequency of spontaneous synaptic events exceeds a certain threshold. Combinations of single-cell and multiunit records with computational analysis may allow comparing the selective contributions of pyramidal cells and interneurons to GDPs initiation. Cross-correlation analysis will help determining the specific region or layer which drive synchronous bursts.

The relation of GDPs with synaptic potentiation and associated structural change may be evaluated using the two photon laser scanning microscopy. In the adult brain, changes in synaptic strength have been shown to be strictly correlated with morphological modifications (Maletic-Savatic et al., 1999). Refinement of neuronal circuits is a peculiar characteristic of developmental network. For instance, it has been suggested that dendritic filopodia are crucial for the formation of early synapses or regulation of dendritic branching and growth (Bonhoeffer and Yuste, 2002; Yuste and Bonhoeffer, 2001). The question than rise: do GDPs affect dendritic filopodia and/or spines? Two photon laser scanning microscopy may allow monitoring wide range of morphological modifications associated with GDPs pairing. EGFP tagged neurons or cells filled with fluorescent dyes could be analyzed before and after pairing to detect either axonal sprouting or filopodia movements. With this powerful technique it would be also possible to sense changes in shape and/or density of dendritic spines.

Further investigations may allow identifying the source of calcium responsible for activating the cascade of events leading to synaptic potentiation and structural modifications. In particular, selective blockers of calcium induced calcium release from intracellular stores following calcium entry *via* voltage-dependent calcium channels or NMDA receptors would allow evaluting whether this mechanism is involved in activity-dependent processes.

Moreover, we shall investigate whether GDPs control phosphorylation of transcription factor such as cyclic AMP response element-binding protein (CREB). While phosphorylated CREB is highly expressed in all hippocampal layers, after P12 its

expression is drastically reduced (Bender et al., 2001). This may suggest that CREB could be a critical component of the regulatory machinery that coordinates genes transcription which contributes to activity dependent synaptic refinement. CREB phosphorylation could be monitored in immunocytochemical experiments with selective antibodies (see Wu et al., 2001).

Introducing into selected cells *via* virus infection, particular genes coupled to EGFP may allow neutralizing GDPs. This perturbation would allow studying how the circuit develops in the absence of synchronized activity. A similar technique has been successfully used to study how changes in cell excitability control circuit development (Komai et al., 2006, Margrie et al., 2003, Dittgen et al., 2004., Ge et al., 2006).

Finally, microarrays techniques may help determining at the single cell level genes which are up or down regulated in an activity dependent way (after GDPs pairing).

CREB may regulate the coordinated transcription of genes which contribute to the differentiation of granule cells and their integration into the dentate gyrus network.

References

Aguado F, Carmona MA, Pozas E, Aguilo A, Martinez-Guijarro FJ, Alcantara S, Borrell V, Yuste R, Ibanez 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 (Cambridge, England)* **130,** 1267-1280.

Altman J & Bayer SA. (1990c). Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *The Journal of comparative neurology* **301,** 365-381.

Altman J & Bayer SA. (1990b). Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. *The Journal of comparative neurology* **301,** 325-342.

Altman J & Bayer SA. (1990a). Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pyramidale. *The Journal of comparative neurology* **301,** 343-364.

Amaral DG & Witter MP. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience* **31,** 571-591.

Aranzi GC. (1564). *De Humano Foetu liber*. Rome.

Armanini MP, McMahon SB, Sutherland J, Shelton DL & Phillips HS. (1995). Truncated and catalytic isoforms of trkB are co-expressed in neurons of rat and mouse CNS. *The European journal of neuroscience* **7,** 1403-1409.

Avignone E & Cherubini E. (1999). Muscarinic receptor modulation of GABA-mediated giant depolarizing potentials in the neonatal rat hippocampus. *The Journal of physiology (London)* **518 (Pt 1),** 97-107.

Azouz R, Jensen MS & Yaari Y. (1996). Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *The Journal of physiology (London)* **492 (Pt 1),** 211-223.

Bailey CH, Giustetto M, Huang YY, Hawkins RD & Kandel ER. (2000). Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory? *Nature reviews* **1,** 11-20.

Bayer SA. (1980a). Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. *The Journal of comparative neurology* **190,** 87-114.

Bayer SA. (1980b). Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. *The Journal of comparative neurology* **190,** 115-134.

Behar TN, Schaffner AE, Scott CA, O'Connell C & Barker JL. (1998). Differential response of cortical plate and ventricular zone cells to GABA as a migration stimulus. *J Neurosci* **18,** 6378-6387.

Ben-Ari Y. (2001). Developing networks play a similar melody. *Trends in neurosciences* **24,** 353-360.

Ben-Ari Y. (2002). Excitatory actions of gaba during development: the nature of the nurture. *Nature reviews* **3,** 728-739.

Ben-Ari Y. (2006). Basic developmental rules and their implications for epilepsy in the immature brain. *Epileptic Disord* **8,** 91-102.

Ben-Ari Y, Cherubini E, Corradetti R & Gaiarsa JL. (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. *The Journal of physiology (London)* **416,** 303-325.

Bender RA, Galindo R, Mameli M, Gonzalez-Vega R, Valenzuela CF & Baram TZ. (2005). Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function. *The European journal of neuroscience* **22,** 2669-2674.

Bender RA, Lauterborn JC, Gall CM, Cariaga W & Baram TZ. (2001). Enhanced CREB phosphorylation in immature dentate gyrus granule cells precedes neurotrophin expression and indicates a specific role of CREB in granule cell differentiation. *The European journal of neuroscience* **13,** 679-686.

Bernard C, Milh M, Morozov YM, Ben-Ari Y, Freund TF & Gozlan H. (2005). Altering cannabinoid signaling during development disrupts neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America* **102,** 9388-9393.

Berninger B, Garcia DE, Inagaki N, Hahnel C & Lindholm D. (1993). BDNF and NT-3 induce intracellular Ca2+ elevation in hippocampal neurones. *Neuroreport* **4,** 1303-1306.

Berninger B, Marty S, Zafra F, da Penha Berzaghi M, Thoenen H & Lindholm D. (1995). GABAergic stimulation switches from enhancing to repressing BDNF expression in rat hippocampal neurons during maturation in vitro. *Development (Cambridge, England)* **121,** 2327-2335.

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.

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. *The Journal of physiology (London)* **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. *Journal of neurophysiology* **81,** 2095-2102.

Bonhoeffer T & Yuste R. (2002). Spine motility. Phenomenology, mechanisms, and function. *Neuron* **35,** 1019-1027.

Borgdorff AJ & Choquet D. (2002). Regulation of AMPA receptor lateral movements. *Nature* **417,** 649-653.

Brown AM, Fern R, Jarvinen JP, Kaila K & Ransom BR. (1998). Changes in [Ca2+]0 during anoxia in CNS white matter. *Neuroreport* **9,** 1997-2000.

Burgess N, Maguire EA & O'Keefe J. (2002). The human hippocampus and spatial and episodic memory. *Neuron* **35,** 625-641.

Burrone J, O'Byrne M & Murthy VN. (2002). Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* **420,** 414-418.

Buzsaki G. (2002). Theta oscillations in the hippocampus. *Neuron* **33,** 325-340.

Buzsaki G & Draguhn A. (2004). Neuronal oscillations in cortical networks. *Science* **304,** 1926-1929.

Buzsaki G, Leung LW & Vanderwolf CH. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Res* **287,** 139-171.

Caillard O, Ben-Ari Y & Gaiarsa JL. (1999). Long-term potentiation of GABAergic synaptic transmission in neonatal rat hippocampus. *The Journal of physiology (London)* **518 (Pt 1),** 109-119.

Caillard O, Ben-Ari Y & Gaiarsa 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.

Caillard O, McLean HA, Ben-Ari Y & Gaiarsa JL. (1998). Ontogenesis of presynaptic GABAB receptor-mediated inhibition in the CA3 region of the rat hippocampus. *Journal of neurophysiology* **79,** 1341-1348.

Canepari M, Mammano F, Kachalsky SG, Rahamimoff R & Cherubini E. (2000). GABA- and glutamate-mediated network activity in the hippocampus of neonatal and

juvenile rats revealed by fast calcium imaging. *Cell calcium* **27,** 25-33.

Canossa M, Griesbeck O, Berninger B, Campana G, Kolbeck R & Thoenen H. (1997). Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 13279-13286.

Chao TC & Chen CM. (2005). Learning-induced synchronization and plasticity of a developing neural network. *Journal of computational neuroscience* **19,** 311-324.

Chen C. (2001). Heterosynaptic LTP in early development. *Neuron* **31,** 510-512.

Chen G, Kolbeck R, Barde YA, Bonhoeffer T & Kossel A. (1999). Relative contribution of endogenous neurotrophins in hippocampal long-term potentiation. *J Neurosci* **19,** 7983-7990.

Chen G, Trombley PQ & van den Pol AN. (1996). Excitatory actions of GABA in developing rat hypothalamic neurones. *The Journal of physiology (London)* **494 (Pt 2),** 451-464.

Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ & Soltesz I. (2001). Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nature medicine* **7,** 331-337.

Chen S, Yue C & Yaari Y. (2005). A transitional period of Ca2+-dependent spike afterdepolarization and bursting in developing rat CA1 pyramidal cells. *The Journal of physiology (London)* **567,** 79-93.

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

Choi S, Klingauf J & Tsien RW. (2000). Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nature neuroscience* **3,** 330-336.

Chub N, Mentis GZ & O'Donovan M J. (2006). Chloride-sensitive MEQ fluorescence in chick embryo motoneurons following manipulations of chloride and during spontaneous network activity. *Journal of neurophysiology* **95,** 323-330.

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. *The Journal of physiology (London)* **566,** 671-679.

Cohen I, Huberfeld G & Miles R. (2006). Emergence of disinhibition-induced synchrony in the CA3 region of the guinea pig hippocampus in vitro. *The Journal of physiology (London)* **570,** 583-594.

Colin-Le Brun I, Ferrand N, Caillard O, Tosetti P, Ben-Ari Y & Gaiarsa JL. (2004). Spontaneous synaptic activity is required for the formation of functional GABAergic synapses in the developing rat hippocampus. *The Journal of physiology (London)* **559,** 129-139.

Corlew R, Bosma MM & Moody WJ. (2004). Spontaneous, synchronous electrical activity in neonatal mouse cortical neurones. *The Journal of physiology (London)* **560,** 377-390.

Crair MC. (1999). Neuronal activity during development: permissive or instructive? *Current opinion in neurobiology* **9,** 88-93.

Dammerman RS, Flint AC, Noctor S & Kriegstein AR. (2000). An excitatory GABAergic plexus in developing neocortical layer 1. *Journal of neurophysiology* **84,** 428-434.

De la Prida LM, Huberfeld G, Cohen I & Miles R. (2006). Threshold behavior in the initiation of hippocampal population bursts. *Neuron* **49,** 131-142.

Debanne D, Gahwiler BH & Thompson SM. (1994). Asynchronous pre- and postsynaptic activity induces associative long-term depression in area CA1 of the rat hippocampus in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **91,** 1148-1152.

Debanne D, Gahwiler BH & Thompson SM. (1996). Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3-CA1 cell pairs in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **93,** 11225-11230.

Debanne D, Gahwiler BH & Thompson SM. (1998). Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *The Journal of physiology (London)* **507 (Pt 1),** 237-247.

Delpire E & Mount DB. (2002). Human and murine phenotypes associated with defects in cation-chloride cotransport. *Annual review of physiology* **64,** 803-843.

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,** 1051-1061.

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M & Osten P. (2004). Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **101,** 18206-18211.

Du J, Feng L, Yang F & Lu B. (2000). Activity- and Ca(2+)-dependent modulation of

surface expression of brain-derived neurotrophic factor receptors in hippocampal neurons. *The Journal of cell biology* **150,** 1423-1434.

Dupuy ST & Houser CR. (1996). Prominent expression of two forms of glutamate decarboxylase in the embryonic and early postnatal rat hippocampal formation. *J Neurosci* **16,** 6919-6932.

Durand GM, Kovalchuk Y & Konnerth A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* **381,** 71-75.

Dzhala VI & Staley KJ. (2003). Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. *J Neurosci* **23,** 1840-1846.

Egger V, Feldmeyer D & Sakmann B. (1999). Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. *Nature neuroscience* **2,** 1098-1105.

Eichenbaum H, Dudchenko P, Wood E, Shapiro M & Tanila H. (1999). The hippocampus, memory, and place cells: is it spatial memory or a memory space? *Neuron* **23,** 209-226.

Eilers J, Plant TD, Marandi N & Konnerth A. (2001). GABA-mediated Ca2+ signalling in developing rat cerebellar Purkinje neurones. *The Journal of physiology (London)* **536,** 429-437.

Ekstrom AD, Kahana MJ, Caplan JB, Fields TA, Isham EA, Newman EL & Fried I. (2003). Cellular networks underlying human spatial navigation. *Nature* **425,** 184-188.

Engert F & Bonhoeffer T. (1997). Synapse specificity of long-term potentiation breaks down at short distances. *Nature* **388,** 279-284.

Falkenberg T, Mohammed AK, Henriksson B, Persson H, Winblad B & Lindefors N. (1992). Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. *Neuroscience letters* **138,** 153-156.

Fatt P & Katz B. (1953). The effect of inhibitory nerve impulses on a crustacean muscle fibre. *The Journal of physiology (London)* **121,** 374-389.

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.

Feller MB. (1999). Spontaneous correlated activity in developing neural circuits. *Neuron* **22,** 653-656.

Fernandez de Sevilla D, Garduno J, Galvan E & Buno W. (2006). Calcium-Activated after Hyperpolarizations Regulate Synchronization and Timing of Epileptiform Bursts in Hippocampal Ca3 Pyramidal Neurons. *Journal of neurophysiology*.

Fields RD, Lee PR & Cohen JE. (2005). Temporal integration of intracellular Ca2+ signaling networks in regulating gene expression by action potentials. *Cell calcium* **37,** 433-442.

Frerking M, Malenka RC & Nicoll RA. (1998). Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. *Journal of neurophysiology* **80,** 3383-3386.

Freund TF & Buzsaki G. (1996). Interneurons of the hippocampus. *Hippocampus* **6,** 347-470.

Frey U & Morris RG. (1997). Synaptic tagging and long-term potentiation. *Nature* **385,** 533-536.

Froemke RC & Dan Y. (2002). Spike-timing-dependent synaptic modification induced by natural spike trains. *Nature* **416,** 433-438.

Fukuda A, Muramatsu K, Okabe A, Shimano Y, Hida H, Fujimoto I & Nishino H. (1998). Changes in intracellular Ca2+ induced by GABAA receptor activation and reduction in Cl- gradient in neonatal rat neocortex. *Journal of neurophysiology* **79,** 439-446.

Gage FH. (2002). Neurogenesis in the adult brain. *J Neurosci* **22,** 612-613.

Gahwiler BH, Capogna M, Debanne D, McKinney RA & Thompson SM. (1997). Organotypic slice cultures: a technique has come of age. *Trends in neurosciences* **20,** 471-477.

Gaiarsa JL, Corradetti R, Cherubini E & Ben-Ari Y. (1991). Modulation of GABA-mediated Synaptic Potentials by Glutamatergic Agonists in Neonatal CA3 Rat Hippocampal Neurons. *The European journal of neuroscience* **3,** 301-309.

Gaiarsa JL, Tseeb V & Ben-Ari Y. (1995). Postnatal development of pre- and postsynaptic GABAB-mediated inhibitions in the CA3 hippocampal region of the rat. *Journal of neurophysiology* **73,** 246-255.

Galli L & Maffei L. (1988). Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. *Science* **242,** 90-91.

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,** 521-532.

Gao XB, Chen G & van den Pol AN. (1998). GABA-dependent firing of glutamate-evoked action potentials at AMPA/kainate receptors in developing hypothalamic neurons. *Journal of neurophysiology* **79,** 716-726.

Gao XB & van den Pol AN. (2000). GABA release from mouse axonal growth cones. *The Journal of physiology (London)* **523 Pt 3,** 629-637.

Garaschuk O, Hanse E & Konnerth A. (1998). Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *The Journal of physiology (London)* **507 (Pt 1),** 219-236.

Garaschuk O, Linn J, Eilers J & Konnerth A. (2000). Large-scale oscillatory calcium waves in the immature cortex. *Nature neuroscience* **3,** 452-459.

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? *Proceedings of the National Academy of Sciences of the United States of America* **97,** 9741-9746.

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,** 589-593.

Gomez-Pinilla F, So V & Kesslak JP. (2001). Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. *Brain Res* **904,** 13-19.

Gonzalez-Islas C & Wenner P. (2006). Spontaneous network activity in the embryonic spinal cord regulates AMPAergic and GABAergic synaptic strength. *Neuron* **49,** 563-575.

Gooney M & Lynch MA. (2001). Long-term potentiation in the dentate gyrus of the rat hippocampus is accompanied by brain-derived neurotrophic factor-induced activation of TrkB. *Journal of neurochemistry* **77,** 1198-1207.

Gooney M, Shaw K, Kelly A, O'Mara SM & Lynch MA. (2002). Long-term potentiation and spatial learning are associated with increased phosphorylation of TrkB and extracellular signal-regulated kinase (ERK) in the dentate gyrus: evidence for a role for brain-derived neurotrophic factor. *Behavioral neuroscience* **116,** 455-463.

Gottschalk W, Pozzo-Miller LD, Figurov A & Lu B. (1998). Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. *J Neurosci* **18,** 6830-6839.

Groc L, Gustafsson B & Hanse E. (2006). AMPA signalling in nascent glutamatergic synapses: there and not there! *Trends in neurosciences* **29,** 132-139.

Groc L, Gustafsson B & Hanse E. (2003a). Early establishment of multiple release site connectivity between interneurons and pyramidal neurons in the developing hippocampus. *The European journal of neuroscience* **17,** 1873-1880.

Groc L, Gustafsson B & Hanse E. (2003b). In vivo evidence for an activity-independent maturation of AMPA/NMDA signaling in the developing hippocampus. *Neuroscience* **121,** 65-72.

Groc L, Gustafsson B & Hanse E. (2002b). Spontaneous unitary synaptic activity in CA1 pyramidal neurons during early postnatal development: constant contribution of AMPA and NMDA receptors. *J Neurosci* **22,** 5552-5562.

Groc L, Petanjek Z, Gustafsson B, Ben-Ari Y, Hanse E & Khazipov R. (2002a). In vivo blockade of neural activity alters dendritic development of neonatal CA1 pyramidal cells. *The European journal of neuroscience* **16,** 1931-1938.

Grosse G, Djalali S, Deng DR, Holtje M, Hinz B, Schwartzkopff K, Cygon M, Rothe T, Stroh T, Hellweg R, Ahnert-Hilger G & Hortnag H. (2005). Area-specific effects of brain-derived neurotrophic factor (BDNF) genetic ablation on various neuronal subtypes of the mouse brain. *Brain Res Dev Brain Res* **156,** 111-126.

Grove EA & Tole S. (1999). Patterning events and specification signals in the developing hippocampus. *Cereb Cortex* **9,** 551-561.

Guidi S, Ciani E, Severi S, Contestabile A & Bartesaghi R. (2005). Postnatal neurogenesis in the dentate gyrus of the guinea pig. *Hippocampus* **15,** 285-301.

Hablitz JJ & Johnston D. (1981). Endogenous nature of spontaneous bursting in hippocampal pyramidal neurons. *Cellular and molecular neurobiology* **1,** 325-334.

Hanse E, Durand GM, Garaschuk O & Konnerth A. (1997). Activity-dependent wiring of the developing hippocampal neuronal circuit. *Seminars in cell & developmental biology* **8,** 35-42.

Hanse E & Gustafsson B. (2001). Quantal variability at glutamatergic synapses in area CA1 of the rat neonatal hippocampus. *The Journal of physiology (London)* **531,** 467-480.

Hebb DO. (1949). *The organization of behavior; a neuropsychological theory*. Wiley, New York,.

Heck N, Kilb W, Reiprich P, Kubota H, Furukawa T, Fukuda A & Luhmann HJ. (2006). GABA-A Receptors Regulate Neocortical Neuronal Migration In Vitro and In Vivo. *Cereb Cortex*.

Henneberger C, Juttner R, Rothe T & Grantyn R. (2002). Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior

colliculus. *Journal of neurophysiology* **88,** 595-603.

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. *The European journal of neuroscience* **16,** 197-208.

Hestrin S & Galarreta M. (2005). Electrical synapses define networks of neocortical GABAergic neurons. *Trends in neurosciences* **28,** 304-309.

Ho SM & Waite PM. (1999). Spontaneous activity in the perinatal trigeminal nucleus of the rat. *Neuroreport* **10,** 659-664.

Hollrigel GS, Ross ST & Soltesz I. (1998). Temporal patterns and depolarizing actions of spontaneous GABAA receptor activation in granule cells of the early postnatal dentate gyrus. *Journal of neurophysiology* **80,** 2340-2351.

Hubel DH & Wiesel TN. (1965). Binocular interaction in striate cortex of kittens reared with artificial squint. *Journal of neurophysiology* **28,** 1041-1059.

Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH & Granholm AC. (2000). Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Experimental neurology* **164,** 45-52.

Isaac JT, Crair MC, Nicoll RA & Malenka RC. (1997). Silent synapses during development of thalamocortical inputs. *Neuron* **18,** 269-280.

Isaeva E, Isaev D, Khazipov R & Holmes GL. (2006). Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. *The European journal of neuroscience* **23,** 1559-1566.

Jack JJB, Noble D & Tsien RW. (1983). *Electric current flow in excitable cells.* Clarendon Press, Oxford.

Jeong H & Gutkin B. (2005). Study on the role of GABAergic synapses in synchronization. *NEUROCOMPUTING* **65,** 859-868

Jessell TM & Kandel ER. (1993). Synaptic transmission: a bidirectional and self-modifiable form of cell-cell communication. *Cell* **72 Suppl,** 1-30.

Jonas P, Major G & Sakmann B. (1993). Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. *The Journal of physiology (London)* **472,** 615-663.

Kahn DM & Krubitzer L. (2002). Massive cross-modal cortical plasticity and the emergence of a new cortical area in developmentally blind mammals. *Proceedings of the National Academy of Sciences of the United States of America* **99,** 11429-11434.

Kaila K. (1994). Ionic basis of GABAA receptor channel function in the nervous system. *Progress in neurobiology* **42,** 489-537.

Kaila K, Voipio J, Paalasmaa P, Pasternack M & Deisz RA. (1993). The role of bicarbonate in GABAA receptor-mediated IPSPs of rat neocortical neurones. *The Journal of physiology (London)* **464,** 273-289.

Kaiser KM, Lubke J, Zilberter Y & Sakmann B. (2004). Postsynaptic calcium influx at single synaptic contacts between pyramidal neurons and bitufted interneurons in layer 2/3 of rat neocortex is enhanced by backpropagating action potentials. *J Neurosci* **24,** 1319-1329.

Kandel ER & Spencer WA. (1961). Electrophysiology of hippocampal neurons. II. After-potentials and repetitive firing. *Journal of neurophysiology* **24,** 243-259.

Kang H, Welcher AA, Shelton D & Schuman EM. (1997). Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron* **19,** 653-664.

Kaplan DR & Miller FD. (1997). Signal transduction by the neurotrophin receptors. *Current opinion in cell biology* **9,** 213-221.

Karten YJ, Jones MA, Jeurling SI & Cameron HA. (2006). GABAergic signaling in young granule cells in the adult rat and mouse dentate gyrus. *Hippocampus* **16,** 312-320.

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. *Proceedings of the National Academy of Sciences of the United States of America* **101,** 3967-3972.

Katoh-Semba R, Takeuchi IK, Semba R & Kato K. (1997). Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. *Journal of neurochemistry* **69,** 34-42.

Katz LC & Shatz CJ. (1996). Synaptic activity and the construction of cortical circuits. *Science* **274,** 1133-1138.

Kennedy MB. (2000). Signal-processing machines at the postsynaptic density. *Science* **290,** 750-754.

Kesslak JP, So V, Choi J, Cotman CW & Gomez-Pinilla F. (1998). Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? *Behavioral neuroscience* **112,** 1012-1019.

Khalilov I, Dzhala V, Ben-Ari Y & Khazipov R. (1999). Dual role of GABA in the

neonatal rat hippocampus. *Developmental neuroscience* **21,** 310-319.

Khalilov I, Esclapez M, Medina I, Aggoun D, Lamsa K, Leinekugel X, Khazipov R & Ben-Ari Y. (1997). A novel in vitro preparation: the intact hippocampal formation. *Neuron* **19,** 743-749.

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,** 9770-9781.

Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben-Ari Y & Holmes GL. (2004). Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *The European journal of neuroscience* **19,** 590-600.

Khazipov R, Leinekugel X, Khalilov I, Gaiarsa JL & Ben-Ari Y. (1997). Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *The Journal of physiology (London)* **498 (Pt 3),** 763-772.

Khazipov R & Luhmann HJ. (2006). Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. *Trends in neurosciences* **29,** 414-418.

Kim JJ & Baxter MG. (2001). Multiple brain-memory systems: the whole does not equal the sum of its parts. *Trends in neurosciences* **24,** 324-330.

Kirov SA, Goddard CA & Harris KM. (2004). Age-dependence in the homeostatic upregulation of hippocampal dendritic spine number during blocked synaptic transmission. *Neuropharmacology* **47,** 640-648.

Klausberger T, Magill PJ, Marton LF, Roberts JD, Cobden PM, Buzsaki G & Somogyi P. (2003). Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. *Nature* **421,** 844-848.

Klausberger T, Marton LF, Baude A, Roberts JD, Magill PJ & Somogyi P. (2004). Spike timing of dendrite-targeting bistratified cells during hippocampal network oscillations in vivo. *Nature neuroscience* **7,** 41-47.

Koester HJ & Sakmann B. (1998). Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 9596-9601.

Komai S, Licznerski P, Cetin A, Waters J, Denk W, Brecht M & Osten P. (2006). Postsynaptic excitability is necessary for strengthening of cortical sensory responses during experience-dependent development. *Nature neuroscience* **9,** 1125-1133.

Komuro H & Rakic P. (1992). Selective role of N-type calcium channels in neuronal

migration. *Science* **257,** 806-809.

Komuro H & Rakic P. (1993). Modulation of neuronal migration by NMDA receptors. *Science* **260,** 95-97.

Komuro H & Rakic P. (1998). Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca2+ fluctuations. *Journal of neurobiology* **37,** 110-130.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H & Bonhoeffer T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proceedings of the National Academy of Sciences of the United States of America* **92,** 8856-8860.

Kovalchuk Y, Hanse E, Kafitz KW & Konnerth A. (2002). Postsynaptic Induction of BDNF-Mediated Long-Term Potentiation. *Science* **295,** 1729-1734.

Krubitzer L & Kahn DM. (2003). Nature versus nurture revisited: an old idea with a new twist. *Progress in neurobiology* **70,** 33-52.

Kruttgen A, Moller JC, Heymach JV, Jr. & Shooter EM. (1998). Neurotrophins induce release of neurotrophins by the regulated secretory pathway. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 9614-9619.

Kullmann DM. (2003). Silent synapses: what are they telling us about long-term potentiation? *Philosophical transactions of the Royal Society of London* **358,** 727-733.

Kwok SC & Buckley MJ. (2006). Fornix transection impairs exploration but not locomotion in ambulatory macaque monkeys. *Hippocampus* **16,** 655-663.

LaMantia AS. (1995). The usual suspects: GABA and glutamate may regulate proliferation in the neocortex. *Neuron* **15,** 1223-1225.

Lamblin MD, Andre M, Challamel MJ, Curzi-Dascalova L, d'Allest AM, De Giovanni E, Moussalli-Salefranque F, Navelet Y, Plouin P, Radvanyi-Bouvet MF, Samson-Dollfus D & Vecchierini-Blineau MF. (1999). [Electroencephalography of the premature and term newborn. Maturational aspects and glossary]. *Neurophysiologie clinique = Clinical neurophysiology* **29,** 123-219.

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. *Journal of neurophysiology* **83,** 359-366.

Lauri SE, Lamsa K, Pavlov I, Riekki R, Johnson BE, Molnar E, Rauvala H & Taira T. (2003). Activity blockade increases the number of functional synapses in the hippocampus of newborn rats. *Molecular and cellular neurosciences* **22,** 107-117.

Lauri SE, Segerstrale 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, Segerstrale 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.

Le Magueresse C, Safiulina V, Changeux JP & Cherubini E. (2006). Nicotinic modulation of network and synaptic transmission in the immature hippocampus investigated with genetically modified mice. *The Journal of physiology (London)* **576,** 533-546.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y & Buzsaki G. (2002). Correlated bursts of activity in the neonatal hippocampus in vivo. *Science* **296,** 2049-2052.

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.

Leinekugel X, Tseeb V, Ben-Ari Y & Bregestovski P. (1995). Synaptic GABAA activation induces Ca2+ rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *The Journal of physiology (London)* **487 (Pt 2),** 319-329.

Lessmann V. (1998). Neurotrophin-dependent modulation of glutamatergic synaptic transmission in the mammalian CNS. *General pharmacology* **31,** 667-674.

Letzkus JJ, Kampa BM & Stuart GJ. (2006). Learning rules for spike timing-dependent plasticity depend on dendritic synapse location. *J Neurosci* **26,** 10420-10429.

Levine ES, Dreyfus CF, Black IB & Plummer MR. (1995). Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proceedings of the National Academy of Sciences of the United States of America* **92,** 8074-8077.

Levy WB & Steward O. (1983). Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. *Neuroscience* **8,** 791-797.

Li YX, Xu Y, Ju D, Lester HA, Davidson N & Schuman EM. (1998). Expression of a dominant negative TrkB receptor, T1, reveals a requirement for presynaptic signaling in BDNF-induced synaptic potentiation in cultured hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 10884-10889.

Liao D & Malinow R. (1996). Deficiency in induction but not expression of LTP in

hippocampal slices from young rats. *Learning & memory (Cold Spring Harbor, NY* **3,** 138-149.

Lippe WR. (1994). Rhythmic spontaneous activity in the developing avian auditory system. *J Neurosci* **14,** 1486-1495.

Lissin DV, Gomperts SN, Carroll RC, Christine CW, Kalman D, Kitamura M, Hardy S, Nicoll RA, Malenka RC & von Zastrow M. (1998). Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 7097-7102.

Lohof AM, Ip NY & Poo MM. (1993). Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363,** 350-353.

London M & Hausser M. (2005). Dendritic computation. *Annual review of neuroscience* **28,** 503-532.

Losi G, Prybylowski K, Fu Z, Luo JH & Vicini S. (2002). Silent synapses in developing cerebellar granule neurons. *Journal of neurophysiology* **87,** 1263-1270.

LoTurco JJ, Owens DF, Heath MJ, Davis MB & Kriegstein AR. (1995). GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* **15,** 1287-1298.

Ludwig A, Li H, Saarma M, Kaila K & Rivera C. (2003). Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. *The European journal of neuroscience* **18,** 3199-3206.

Luhmann HJ & Prince DA. (1991). Postnatal maturation of the GABAergic system in rat neocortex. *Journal of neurophysiology* **65,** 247-263.

Maccaferri G & McBain CJ. (1996). The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. *The Journal of physiology (London)* **497 (Pt 1),** 119-130.

Maggi L, Le Magueresse C, Changeux JP & Cherubini E. (2003). Nicotine activates immature "silent" connections in the developing hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **100,** 2059-2064.

Maggi L, Sher E & Cherubini E. (2001). Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus. *The Journal of physiology (London)* **536,** 89-100.

Malenka RC & Nicoll RA. (1997). Silent synapses speak up. *Neuron* **19,** 473-476.

Maletic-Savatic M, Malinow R & Svoboda K. (1999). Rapid dendritic morphogenesis in

CA1 hippocampal dendrites induced by synaptic activity. *Science* **283,** 1923-1927.

Malinow R, Mainen ZF & Hayashi Y. (2000). LTP mechanisms: from silence to four-lane traffic. *Current opinion in neurobiology* **10,** 352-357.

Manent JB, Demarque M, Jorquera I, Pellegrino C, Ben-Ari Y, Aniksztejn L & Represa A. (2005). A noncanonical release of GABA and glutamate modulates neuronal migration. *J Neurosci* **25,** 4755-4765.

Margrie TW, Meyer AH, Caputi A, Monyer H, Hasan MT, Schaefer AT, Denk W & Brecht M. (2003). Targeted whole-cell recordings in the mammalian brain in vivo. *Neuron* **39,** 911-918.

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,** 2343-2360.

Markram H, Lubke J, Frotscher M & Sakmann B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275,** 213-215.

McConnell SK, Ghosh A & Shatz CJ. (1989). Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* **245,** 978-982.

McCormick DA & Bal T. (1997). Sleep and arousal: thalamocortical mechanisms. *Annual review of neuroscience* **20,** 185-215.

McLean HA, Caillard O, Khazipov R, Ben-Ari Y & Gaiarsa JL. (1996). Spontaneous release of GABA activates GABAB receptors and controls network activity in the neonatal rat hippocampus. *Journal of neurophysiology* **76,** 1036-1046.

Menendez de la Prida L, Bolea S & Sanchez-Andres JV. (1996). Analytical characterization of spontaneous activity evolution during hippocampal development in the rabbit. *Neuroscience letters* **218,** 185-187.

Menendez de la Prida L, Bolea S & Sanchez-Andres JV. (1998). Origin of the synchronized network activity in the rabbit developing hippocampus. *The European journal of neuroscience* **10,** 899-906.

Menendez de la Prida L & Sanchez-Andres JV. (1999). Nonlinear transfer function encodes synchronization in a neural network from the mammalian brain. *Physical review* **60,** 3239-3243.

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,** 227-241.

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.

Miles R, Toth K, Gulyas AI, Hajos N & Freund TF. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16,** 815-823.

Miles R & Wong RK. (1986). Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *The Journal of physiology (London)* **373,** 397-418.

Milner B, Squire LR & Kandel ER. (1998). Cognitive neuroscience and the study of memory. *Neuron* **20,** 445-468.

Minichiello L, Korte M, Wolfer D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T & Klein R. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* **24,** 401-414.

Mizuno M, Yamada K, He J, Nakajima A & Nabeshima T. (2003). Involvement of BDNF receptor TrkB in spatial memory formation. *Learning & memory (Cold Spring Harbor, NY* **10,** 108-115.

Mizuno M, Yamada K, Olariu A, Nawa H & Nabeshima T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J Neurosci* **20,** 7116-7121.

Mohajerani MH & Cherubini E. (2005). Spontaneous recurrent network activity in organotypic rat hippocampal slices. *The European journal of neuroscience* **22,** 107-118.

Montgomery JM, Pavlidis P & Madison DV. (2001). Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation. *Neuron* **29,** 691-701.

Morozov YM & Freund TF. (2003). Postnatal development and migration of cholecystokinin-immunoreactive interneurons in rat hippocampus. *Neuroscience* **120,** 923-939.

Morris RG. (2006). Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *The European journal of neuroscience* **23,** 2829-2846.

Mu Y & Poo MM. (2006). Spike timing-dependent LTP/LTD mediates visual experience-dependent plasticity in a developing retinotectal system. *Neuron* **50,** 115-125.

Mueller AL, Taube JS & Schwartzkroin PA. (1984). Development of hyperpolarizing inhibitory postsynaptic potentials and hyperpolarizing response to gamma-aminobutyric

acid in rabbit hippocampus studied in vitro. *J Neurosci* **4,** 860-867.

Muller R. (1996). A quarter of a century of place cells. *Neuron* **17,** 813-822.

Murphy TH. (2003). Activity-dependent synapse development: changing the rules. *Nature neuroscience* **6,** 9-11.

Nadarajah B & Parnavelas JG. (2002). Modes of neuronal migration in the developing cerebral cortex. *Nature reviews* **3,** 423-432.

Nagappan G & Lu B. (2005). Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends in neurosciences* **28,** 464-471.

Nakatani H, Khalilov I, Gong P & van Leeuwen C. (2003). Nonlinearity in giant depolarizing potentials. *PHYSICS LETTERS A* **319** 167-172

Nevian T & Sakmann B. (2004). Single spine Ca2+ signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. *J Neurosci* **24,** 1689-1699.

Nevian T & Sakmann B. (2006). Spine Ca2+ signaling in spike-timing-dependent plasticity. *J Neurosci* **26,** 11001-11013.

Noraberg J, Poulsen FR, Blaabjerg M, Kristensen BW, Bonde C, Montero M, Meyer M, Gramsbergen JB & Zimmer J. (2005). Organotypic hippocampal slice cultures for studies of brain damage, neuroprotection and neurorepair. *Current drug targets* **4,** 435-452.

Nowakowski RS & Rakic P. (1981). The site of origin and route and rate of migration of neurons to the hippocampal region of the rhesus monkey. *The Journal of comparative neurology* **196,** 129-154.

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,** 179-184.

Obrietan K, Gao XB & Van Den Pol AN. (2002). Excitatory actions of GABA increase BDNF expression via a MAPK-CREB-dependent mechanism--a positive feedback circuit in developing neurons. *Journal of neurophysiology* **88,** 1005-1015.

O'Donovan MJ. (1989). Motor activity in the isolated spinal cord of the chick embryo: synaptic drive and firing pattern of single motoneurons. *J Neurosci* **9,** 943-958.

Olofsdotter K, Lindvall O & Asztely F. (2000). Increased synaptic inhibition in dentate gyrus of mice with reduced levels of endogenous brain-derived neurotrophic factor. *Neuroscience* **101,** 531-539.

Oray S, Majewska A & Sur M. (2006). Effects of synaptic activity on dendritic spine

motility of developing cortical layer v pyramidal neurons. *Cereb Cortex* **16,** 730-741.

Overstreet Wadiche L, Bromberg DA, Bensen AL & Westbrook GL. (2005). GABAergic signaling to newborn neurons in dentate gyrus. *Journal of neurophysiology* **94,** 4528-4532.

Overstreet-Wadiche LS, Bensen AL & Westbrook GL. (2006). Delayed development of adult-generated granule cells in dentate gyrus. *J Neurosci* **26,** 2326-2334.

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,** 6414-6423.

Owens DF & Kriegstein AR. (2002). Is there more to GABA than synaptic inhibition? *Nature reviews* **3,** 715-727.

Paczuski M, Maslov S & Bak P. (1996). Avalanche dynamics in evolution, growth, and depinning models. *Physical Review E Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics* **53,** 414-443.

Pape HC. (1996). Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annual review of physiology* **58,** 299-327.

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC & Kandel ER. (1996). Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* **16,** 1137-1145.

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

Perez-Otano I & Ehlers MD. (2005). Homeostatic plasticity and NMDA receptor trafficking. *Trends in neurosciences* **28,** 229-238.

Petralia RS, Esteban JA, Wang YX, Partridge JG, Zhao HM, Wenthold RJ & Malinow R. (1999). Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature neuroscience* **2,** 31-36.

Pickard L, Noel 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,** 7922-7931.

Pleasure SJ, Anderson S, Hevner R, Bagri A, Marin O, Lowenstein DH & Rubenstein JL. (2000). Cell migration from the ganglionic eminences is required for the development of hippocampal GABAergic interneurons. *Neuron* **28,** 727-740.

Poncer JC & Malinow R. (2001). Postsynaptic conversion of silent synapses during LTP

affects synaptic gain and transmission dynamics. *Nature neuroscience* **4,** 989-996.

Ponten H, Sonniksen K, Abrahamsson T, Waters N, Gustafsson B, Hanse E & Groc L. (2005). Behavioral and neurochemical repercussions of hippocampal network activity blockade during the neonatal period. *Brain Res Dev Brain Res* **155,** 81-86.

Pozzo-Miller LD, Gottschalk W, Zhang L, McDermott K, Du J, Gopalakrishnan R, Oho C, Sheng ZH & Lu B. (1999). Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. *J Neurosci* **19,** 4972-4983.

Rakic P. (1995). A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends in neurosciences* **18,** 383-388.

Rakic P & Komuro H. (1995). The role of receptor/channel activity in neuronal cell migration. *Journal of neurobiology* **26,** 299-315.

Rao A, Kim E, Sheng M & Craig AM. (1998). Heterogeneity in the molecular composition of excitatory postsynaptic sites during development of hippocampal neurons in culture. *J Neurosci* **18,** 1217-1229.

Redman S. (1990). Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiological reviews* **70,** 165-198.

Reichling DB, Kyrozis A, Wang J & MacDermott AB. (1994). Mechanisms of GABA and glycine depolarization-induced calcium transients in rat dorsal horn neurons. *The Journal of physiology (London)* **476,** 411-421.

Renger JJ, Egles C & Liu G. (2001). A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation. *Neuron* **29,** 469-484.

Represa A & Ben-Ari Y. (2005). Trophic actions of GABA on neuronal development. *Trends in neurosciences* **28,** 278-283.

Rivera C, Voipio J & Kaila K. (2005). Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. *The Journal of physiology (London)* **562,** 27-36.

Rivera C, Voipio J & Kaila K. (2005). Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. *The Journal of physiology (London)* **562,** 27-36.

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,** 251-255.

Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipila 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,** 4683-4691.

Rohrbough J & Spitzer NC. (1996). Regulation of intracellular Cl- levels by Na(+)-dependent Cl- cotransport distinguishes depolarizing from hyperpolarizing GABAA receptor-mediated responses in spinal neurons. *J Neurosci* **16,** 82-91.

Roos A & Boron WF. (1981). Intracellular pH. *Physiological reviews* **61,** 296-434.

Rumpel S, Hatt H & Gottmann K. (1998). Silent synapses in the developing rat visual cortex: evidence for postsynaptic expression of synaptic plasticity. *J Neurosci* **18,** 8863-8874.

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

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

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. *The Journal of physiology (London)* **565,** 981-992.

Semyanov A, Walker MC & Kullmann DM. (2003). GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nature neuroscience* **6,** 484-490.

Serafini R, Valeyev AY, Barker JL & Poulter MO. (1995). Depolarizing GABA-activated Cl- channels in embryonic rat spinal and olfactory bulb cells. *The Journal of physiology (London)* **488 (Pt 2),** 371-386.

Shen W, Wu B, Zhang Z, Dou Y, Rao ZR, Chen YR & Duan S. (2006). Activity-induced rapid synaptic maturation mediated by presynaptic cdc42 signaling. *Neuron* **50,** 401-414.

Shepherd GM. (1998). *The synaptic organization of the brain.* Oxford University Press, New York.

Silhol M, Bonnichon V, Rage F & Tapia-Arancibia L. (2005). Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. *Neuroscience* **132,** 613-624.

Sipila S, Huttu K, Voipio J & Kaila K. (2004). GABA uptake via GABA transporter-1 modulates GABAergic transmission in the immature hippocampus. *J Neurosci* **24,** 5877-5880.

Sipila 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,** 5280-5289.

Sipila ST, Huttu K, Voipio J & Kaila K. (2006). Intrinsic bursting of immature CA3 pyramidal neurons and consequent giant depolarizing potentials are driven by a persistent Na+ current and terminated by a slow Ca2+ -activated K+ current. *The European journal of neuroscience* **23,** 2330-2338.

Sipila ST, Schuchmann S, Voipio J, Yamada J & Kaila K. (2006). The cation-chloride cotransporter NKCC1 promotes sharp waves in the neonatal rat hippocampus. *The Journal of physiology (London)* **573,** 765-773.

Sjostrom PJ, Turrigiano GG & Nelson SB. (2001). Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron* **32,** 1149-1164.

Skutella T & Nitsch R. (2001). New molecules for hippocampal development. *Trends in neurosciences* **24,** 107-113.

Somogyi P & Klausberger T. (2005). Defined types of cortical interneurone structure space and spike timing in the hippocampus. *The Journal of physiology (London)* **562,** 9-26.

Soriano E, Cobas A & Fairen A. (1986). Asynchronism in the neurogenesis of GABAergic and non-GABAergic neurons in the mouse hippocampus. *Brain Res* **395,** 88-92.

Spitzer NC. (1994). Spontaneous Ca2+ spikes and waves in embryonic neurons: signaling systems for differentiation. *Trends in neurosciences* **17,** 115-118.

Spitzer NC, Kingston PA, Manning TJ & Conklin MW. (2002). Outside and in: development of neuronal excitability. *Current opinion in neurobiology* **12,** 315-323.

Stent GS. (1973). A physiological mechanism for Hebb's postulate of learning. *Proceedings of the National Academy of Sciences of the United States of America* **70,** 997-1001.

Strata F, Atzori M, Molnar M, Ugolini G, Tempia F & Cherubini E. (1997). A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. *J Neurosci* **17,** 1435-1446.

Strata F, Sciancalepore M & Cherubini E. (1995). Cyclic AMP-dependent modulation of giant depolarizing potentials by metabotropic glutamate receptors in the rat hippocampus. *The Journal of physiology (London)* **489 (Pt 1),** 115-125.

Su H, Alroy G, Kirson ED & Yaari Y. (2001). Extracellular calcium modulates persistent sodium current-dependent burst-firing in hippocampal pyramidal neurons. *J Neurosci* **21,**

4173-4182.

Suen PC, Wu K, Levine ES, Mount HT, Xu JL, Lin SY & Black IB. (1997). Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 8191-8195.

Sun D & Murali SG. (1998). Stimulation of Na+-K+-2Cl- cotransporter in neuronal cells by excitatory neurotransmitter glutamate. *The American journal of physiology* **275,** C772-779.

Super H, Soriano E & Uylings HB. (1998). The functions of the preplate in development and evolution of the neocortex and hippocampus. *Brain research* **27,** 40-64.

Tao HW, Zhang LI, Engert F & Poo M. (2001). Emergence of input specificity of ltp during development of retinotectal connections in vivo. *Neuron* **31,** 569-580.

Terry P, Herbert BA & Oakley DA. (1989). Anomalous patterns of response learning and transfer in decorticate rats. *Behavioural brain research* **33,** 105-109.

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. *The Journal of physiology (London)* **550,** 719-730.

Tosetti P, Bakels R, Colin-Le Brun I, Ferrand N, Gaiarsa JL & Caillard O. (2004). Acute desensitization of presynaptic GABAB-mediated inhibition and induction of epileptiform discharges in the neonatal rat hippocampus. *The European journal of neuroscience* **19,** 3227-3234.

Tosetti P, Ferrand N, Colin-Le Brun I & Gaiarsa JL. (2005). Epileptiform activity triggers long-term plasticity of GABA(B) receptor signalling in the developing rat hippocampus. *The Journal of physiology (London)* **568,** 951-966.

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. *Annals of the New York Academy of Sciences* **627,** 277-290.

Traub RD, Whittington MA, Stanford IM & Jefferys JG. (1996). A mechanism for generation of long-range synchronous fast oscillations in the cortex. *Nature* **383,** 621-624.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC & Nelson SB. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391,** 892-896.

Turrigiano GG & Nelson SB. (2004). Homeostatic plasticity in the developing nervous system. *Nature reviews* **5,** 97-107.

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,** 10372-10382.

Valeyev AY, Cruciani RA, Lange GD, Smallwood VS & Barker JL. (1993). Cl- channels are randomly activated by continuous GABA secretion in cultured embryonic rat hippocampal neurons. *Neuroscience letters* **155,** 199-203.

Vicario-Abejon C, Collin C, McKay RD & Segal M. (1998). Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J Neurosci* **18,** 7256-7271.

Vinay L, Brocard F, Fellippa-Marques S & Clarac F. (1999). Antidromic discharges of dorsal root afferents in the neonatal rat. *Journal of physiology, Paris* **93,** 359-367.

Voigt T, Opitz T & de Lima AD. (2005). Activation of early silent synapses by spontaneous synchronous network activity limits the range of neocortical connections. *J Neurosci* **25,** 4605-4615.

Voronin LL & Cherubini E. (2003). "Presynaptic silence" may be golden. *Neuropharmacology* **45,** 439-449.

Voronin LL & Cherubini E. (2004). 'Deaf, mute and whispering' silent synapses: their role in synaptic plasticity. *The Journal of physiology (London)* **557,** 3-12.

Vyssotski AL, Dell'Omo G, Poletaeva, II, Vyssotsk DL, Minichiello L, Klein R, Wolfer DP & Lipp HP. (2002). Long-term monitoring of hippocampus-dependent behavior in naturalistic settings: mutant mice lacking neurotrophin receptor TrkB in the forebrain show spatial learning but impaired behavioral flexibility. *Hippocampus* **12,** 27-38.

Walther C. (2002). Hippocampal terminology: concepts, misconceptions, origins. *Endeavour* **26,** 41-44.

Wang J, Reichling DB, Kyrozis A & MacDermott AB. (1994). Developmental loss of GABA- and glycine-induced depolarization and Ca2+ transients in embryonic rat dorsal horn neurons in culture. *The European journal of neuroscience* **6,** 1275-1280.

Wang YF, Gao XB & van den Pol AN. (2001). Membrane properties underlying patterns of GABA-dependent action potentials in developing mouse hypothalamic neurons. *Journal of neurophysiology* **86,** 1252-1265.

Washbourne P, Bennett JE & McAllister AK. (2002). Rapid recruitment of NMDA receptor transport packets to nascent synapses. *Nature neuroscience* **5,** 751-759.

Wasling P, Hanse E & Gustafsson B. (2004). Developmental changes in release properties of the CA3-CA1 glutamate synapse in rat hippocampus. *Journal of neurophysiology* **92,** 2714-2724.

Webb SE, Moreau M, Leclerc C & Miller AL. (2005). Calcium transients and neural induction in vertebrates. *Cell calcium* **37,** 375-385.

Wiesel TN & Hubel DH. (1963a). Effects of Visual Deprivation on Morphology and Physiology of Cells in the Cats Lateral Geniculate Body. *Journal of neurophysiology* **26,** 978-993.

Wiesel TN & Hubel DH. (1963b). Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. *Journal of neurophysiology* **26,** 1003-1017.

Wong RK & Prince DA. (1979). Dendritic mechanisms underlying penicillin-induced epileptiform activity. *Science* **204,** 1228-1231.

Wong RK, Prince DA & Basbaum AI. (1979). Intradendritic recordings from hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **76,** 986-990.

Wong RK & Traub RD. (1983). Synchronized burst discharge in disinhibited hippocampal slice. I. Initiation in CA2-CA3 region. *Journal of neurophysiology* **49,** 442-458.

Wu C, Asl MN, Gillis J, Skinner FK & Zhang L. (2005). An in vitro model of hippocampal sharp waves: regional initiation and intracellular correlates. *Journal of neurophysiology* **94,** 741-753.

Wu GY, Deisseroth K & Tsien RW. (2001). Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proceedings of the National Academy of Sciences of the United States of America* **98,** 2808-2813.

Yuste R & Bonhoeffer T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual review of neuroscience* **24,** 1071-1089.

Yuste R & Katz LC. (1991). Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron* **6,** 333-344.

Zhang L, Spigelman I & Carlen PL. (1991). Development of GABA-mediated, chloride-dependent inhibition in CA1 pyramidal neurones of immature rat hippocampal slices. *The Journal of physiology (London)* **444,** 25-49.

Zhou Q & Poo MM. (2004). Reversal and consolidation of activity-induced synaptic modifications. *Trends in neurosciences* **27,** 378-383.

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