Functional neuronal plasticity in the dentate gyrus of freely moving rats

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von Dipl.-Bioch. Jeffrey Lopez Rojas

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Gutachter: Prof. Dr. Julietta Uta Frey

Prof. Dr. Denise Manahan-Vaughan

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Abstract

Long-term potentiation (LTP) is defined as a long-lasting increase in the efficacy of synaptic transmission after a brief high-frequency stimulation of afferent fibers and it has been used as a cellular model of learning and memory. Studies in the dentate gyrus (DG) however, do often only analyze the neuronal output, the population spike amplitude (PSA) instead of the synaptic component, the field excitatory postsynaptic potential (fEPSP). Generally, it was thought that the PSA mimics the synaptic response. This dissertation primarily describes how dentate granule cells' synaptic efficacy relates to the neuronal firing in LTP and which molecular mechanisms underpin these synaptic and excitability changes in freely moving animals. To understand the relation among these two phenomena and its mechanisms is a central question in neuroscience today. We took advantage of a recently developed method in our lab to measure both the fEPSP and the PSA in the proximity of their generation sites. In a first series of experiments we studied the outcome of different tetanization patterns on fEPSP and PSA. We found that the firing output of the granule cells can be upregulated relatively independent of the synaptic efficacy by distinct electrical stimuli and that these excitability changes can endure for at least 24 h. Of the studied tetanization protocols just the strongest one was able to induce a synaptic, that is fEPSP, late-LTP. In a second series through pharmacological manipulations we inquired about the underpinnings of this late-LTP. We found that both fEPSP and PSA potentiation were strongly dependent on NMDA receptor activation at the time of their induction, even when our results suggest that other mechanisms could also be involved in the PSA potentiation induction. We have intrahippocampally injected the PKMzeta inhibitors ZIP (zeta-inhibitory peptide) or chelerythrine chloride 1 h after the induction of LTP. This fully prevented synaptic late-LTP; PSA potentiation was however not affected. In summary our results suggest that in the DG of intact freely moving animals the neural firing does not only depend on the strength of the excitatory synapses, but also on neuronal excitability which can be long-lasting modified in an activity-dependent manner.

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List of abbreviations

A-P	anterior-posterior
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP	action potential
BLA	basolateral amygdala
CA	*cornu ammonis*
cAMP	cyclic adenosine monophosphate
CaMKII	calcium/calmodulin-dependent protein kinase II
CREB	cAMP response element-binding protein
D-V	dorso-ventral
D-AP5	D(-)-2-amino-5-phospho-valeric acid
DG	dentate gyrus
EPSP	excitatory postsynaptic potential
fEPSP	field excitatory postsynaptic potential
fEPSP-hi	field excitatory postsynaptic potential higher stimulation intensity
fEPSP-li	field excitatory postsynaptic potential lower stimulation intensity
GABA	gamma-(γ)-aminobutyric acid
HFS	high-frequency stimulation
I-O	input-output
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
M-L	medio-lateral

NMDA	N-methyl-D-aspartate
PKA	protein kinase A
PKC	protein kinase C
PKMzeta	protein kinase Mzeta
PP	perforant pathway
PRP	plasticity-related protein
PS	population spike
PSA	population spike amplitude
PTP	post-tetanic potentiation
scr-ZIP	scrambled version of the zeta (protein kinase Mzeta)-inhibitory peptide
SEM	standard error of mean
STET	strong tetanus
STP	short-term potentiation
VDCC	voltage-dependent calcium channel
WTET	weak tetanus
ZIP	zeta (protein kinase Mzeta)-inhibitory peptide

1- Introduction

1.1- Learning and memory

The ability to learn and remember is of utmost importance for adaptation and survival of animals, including humans. Learning can be defined as a change in the individual behavior as a result of experience. Memory is the individual ability to store this information and recall it later on (Squire, 2004;Sweatt, 2003).

1.1.1- Memory classification

Memory is generally classified into two principal forms: implicit (or nondeclarative) and explicit (or declarative) (Milner et al., 1998). Implicit memory is the sort of memory in which previous experiences facilitate performance on a task that does not require the conscious recollection of those experiences. It is generally slowly acquired through many repetitions, inflexible and is exteriorized throughout performance. Implicit memory includes the learning of skills, habit formation, priming effects, emotional memories, classical associative memories and nonassociative memories like sensitization and habituation. Depending on the specific case, implicit memory relies on brain structures like the striatum, neocortex, amygdala, cerebellum or even the reflex pathways (Purves, 2004). Explicit memory is the kind of memory that is normally meant as “memory” in our routine language. It is the conscious knowledge of facts and events and allows us to model the external world through mental representations. Explicit memory is strongly dependent on the correct functioning of the medial temporal lobe structures, particularly the hippocampus (Squire, 1998;Squire, 2004;Squire and Zola, 1996).

In understanding memory, the study of patients with some pathological abnormalities in their brain has been of high significance. Especially the case of H.M revolutionized the field. H.M suffered from severe epilepsy that impeded him from a normal life. He had surgery to extirpate the part of his brain responsible for his sickness and indeed this treatment succeeded in relieving the seizures. H.M and even his medical staff did not know that they were about to be part of history. After surgery H.M was as intelligent as ever, but surprisingly he was unable to get certain things into his long-term memory and completely forgot his recent personal history. This weird case produced such enormous interest that H.M. reportedly became the most intensively studied medical subject in history. These studies revealed that because of the removal of the medial temporal lobe structures, including his hippocampi, H.M could not form permanent personal (declarative) memories anymore. (Milner, 1959;Scoville and Milner, 1957).

After this extraordinary case, the hippocampus became the focus of many memory studies in humans (DeJong et al., 1969;Smith and Smith, 1966;Song et al., 2011) and also in animals (Shapiro et al., 1965;Sutherland et al., 1989;Winson, 1978;Zola and Squire, 2001), which stressed the importance of this structure in memory formation. The first experimental proof of the synaptic neuronal plasticity in the hippocampus (Bliss and Gardner-Medwin, 1973;Bliss and Lomo, 1973) was another important factor that gave an extra impulse to the research in this area.

1.1.2- Memory consolidation hypotheses

In addition to the types of memory defined by the nature of what is remembered, memory can also be categorized according to its temporal phases. It has been known for a long time that a person who has suffered a head trauma is susceptible to

experiencing a selective loss of the more recent memory events that occurred before the trauma (retrograde amnesia), whereas the older memories remain more stable and relatively undisturbed. In their classical studies Müller and Pilzecker also found that memories of newly learned information were relatively easy disrupted by the learning of other information shortly after the original learning (Müller and Pilzecker, 1900). Further experimental evidence obtained in animals showed that indeed, recently formed memories are susceptible to enhancement or impairment for a limited time after they are formed (McGaugh and Alpern, 1966;Messing et al., 1979;Moncada and Viola, 2007). The finding that protein synthesis inhibitors were able to disrupt memory when applied during or shortly after learning, without preventing learning (Agranoff et al., 1966), was a major step in this regard and served to classify memory in (at least) two stages: a short-term memory, independent of the synthesis of new proteins, and a long-term memory, protein synthesis dependent.

Subsequent studies revealed that protein synthesis in fact occurred in two phases (Popov et al., 1975). It was suggested that the early phase (occurring minutes after learning) serves to synthesize the regulatory proteins which control the formation of target proteins, responsible for the neuronal connectivity/efficiency remodeling in the latest phase (around 8 h after learning). The separate inhibition of each phase of increased protein synthesis resulted in a significant loss of the long-term memory (Grecksch and Matthies, 1980).

Thus, the general idea about memory consolidation is that initially our memories persist in a fragile state and become stronger (consolidated) over time in a process dependent on the synthesis of new proteins. Once consolidated, memories become less vulnerable to erasure (McGaugh, 2000). However, recently it has been discovered that consolidated memories are not as stable as previously thought.

Numerous experimental evidence points to the susceptibility of different kind of memories to be erased by the inhibition of a subtype of protein kinase C (PKC): the PKMzeta (Serrano et al., 2008;Shema et al., 2009;von Kraus et al., 2010).

1.2- The hippocampus

Deep into the medial temporal lobe lies a group of millions of neurons, named hippocampus, whose special characteristics have attracted the attention of countless researchers. The earliest description of this structure comes from the anatomist Arantius (1587), who also coined the term hippocampus (derived from the Greek word of sea horse), due to its resemblance (or more precisely the DG resemblance) with the sea creature (Walther, 2002).

1.2.1- Anatomy and intrinsic connectivity

Nowadays it is generally accepted that the term hippocampus refers just to the regions comprising the *cornu ammonis* (CA) fields: CA1, CA2 and CA3, whereas the term hippocampal formation is used to refer to the hippocampus proper, DG, subiculum, presubiculum, parasubiculum and entorhinal cortex. The three last fields are also referred to as parahippocampal cortex, because of their multilaminate neocortical-like structure, in contrast to the allocortical (fewer than six layers) organization of the hippocampus proper, the DG and the subiculum (Amaral and Lavenex, 2007).

A characteristic trait of the connections between regions of neocortex is its reciprocity (Felleman and Van Essen, 1991), which is strongly in contrast with the hippocampal intrinsic connectivity that is largely unidirectional (including the parahippocampal cortex), a fact noted since its first description by Cajal in the 1890's. However, that

does not mean that the information flows only in a sequential way within the hippocampus. Because of the divergence of some projections, the information flows also in parallel ways. Indeed, the convergent-divergent organization of hippocampus connections is a feature that fits well for the proposing function of mixing or comparing the highly processed, multimodal sensory information that it receives from a variety of neocortical sources (Amaral and Lavenex, 2007).

In spite of such divergences, it is widely accepted that the information flow in the hippocampus follows a main stream. Much of the neocortical input that enters the hippocampal formation does it through the entorhinal cortex, so this field can be considered the first step in the intrinsic hippocampal circuit. The projections from the entorhinal cortex reach the DG through the perforant path (PP) (the entorhinal cortex, which serves as an important point of divergence, projects also to CA3, CA1 and subiculum but in a lesser extent than to the DG). The DG principal neurons, in turn, give rise to axons called mossy fibers that connect with the pyramidal neurons in the CA3 field. Through the so called Schaffer collaterals, the CA3 pyramidal neurons make synapses with the pyramidal neurons in CA1. The CA1 field then projects to the subiculum, which in turn makes connection with the presubiculum and parasubiculum and also back to the entorhinal cortex closing the hippocampal processing loop. The **Fig. 1** gives a schematic representation of this circuitry and also shows some further connections. Additionally to the described circuitry, there are prominent associational connections (for example in the CA3 and DG) as well as extensive commissural connections between both hippocampi (for example ipsilateral - contralateral: CA3 - CA3, CA3 – CA1, CA1 - CA1, DG – DG,) (Amaral and Lavenex, 2007;O'Keefe and Nadel, 1978;Witter and Amaral, 2004).

1.2.2- Dentate gyrus

As the DG is the main target of projections from the entorhinal cortex and since the entorhinal cortex is the source of much of the cortical sensory information that the hippocampus uses to accomplish its function, the DG is considered to be the first step in the processing of information that ultimately leads to the production of declarative memory; therefore, the high significance of its study.

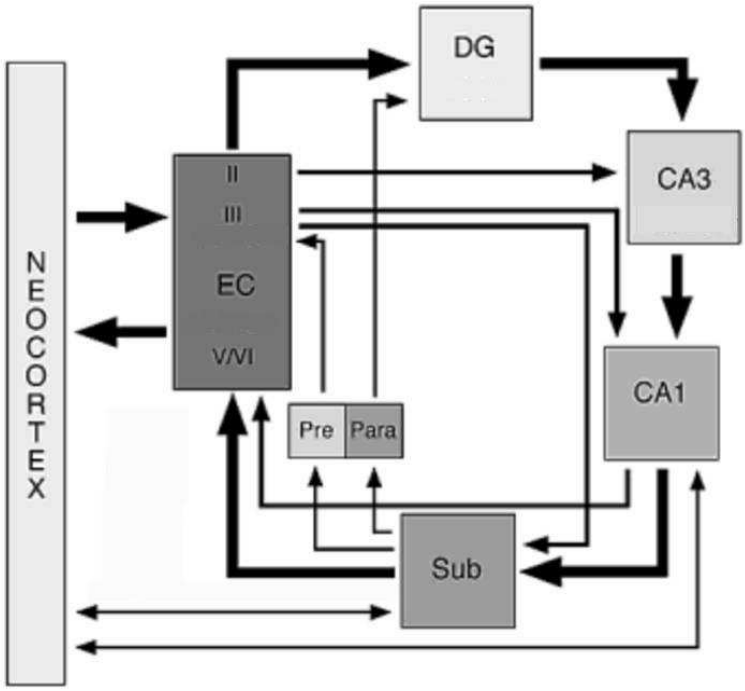

Fig.1. Summary of the ipsilateral pathways through the hippocampal formation. Even though the intrinsic hippocampal circuitry is mainly unidirectional, the information flows in serial and parallel pathways due to highly divergent-convergent pathways. Stronger connections are shown with thicker lines (EC: entorhinal cortex; DG: DG; CA3: cornu ammonis 3; CA1: cornu ammonis 1; Sub: subiculum; Pre: presubiculum; Para: parasubiculum). Adapted from (Amaral and Lavenex, 2007).

The principal neuron type of the DG is the granule cell. The granule cells have an elliptical soma, a typical cone-shaped tree of spiny apical dendrites (absent basal

dendrites in rats) and an unmyelinated axon emerging from the basal surface (Claiborne et al., 1990;Felthauser and Claiborne, 1990).

The DG has a classical allocortical structure with three layers (**Fig. 2**):

1- Molecular layer: a relatively cell-free layer that contains interneurons, the apical dendrites of the granule cells, the PP fibers and other extrinsic input fibers.

2- Granule cell layer: is made up basically of densely packed granule cell bodies, but also contains some interneurons.

3- Polymorphic cell layer (or hilus): contains a number of different cells, including interneurons and the mossy cells, and also the mossy fibers (Amaral et al., 2007).

The PP fibers that reach the DG arise mainly from cells located in the layer II of the entorhinal cortex, though a minor component also originates from layers V and VI (Deller et al., 1996;Steward and Scoville, 1976). The PP fibers are glutamatergic (White et al., 1977). The PP can be divided into lateral PP and medial PP according to the origin in the lateral or medial entorhinal areas, respectively. The lateral PP fibers terminate in the outer third of the molecular layer, whereas the medial PP fibers do it in the middle third. The inner third of the molecular layer lacks PP innervations, but is the location where most commissural/associational connections occur (Amaral and Witter, 1989;Hjorth-Simonsen and Jeune, 1972).

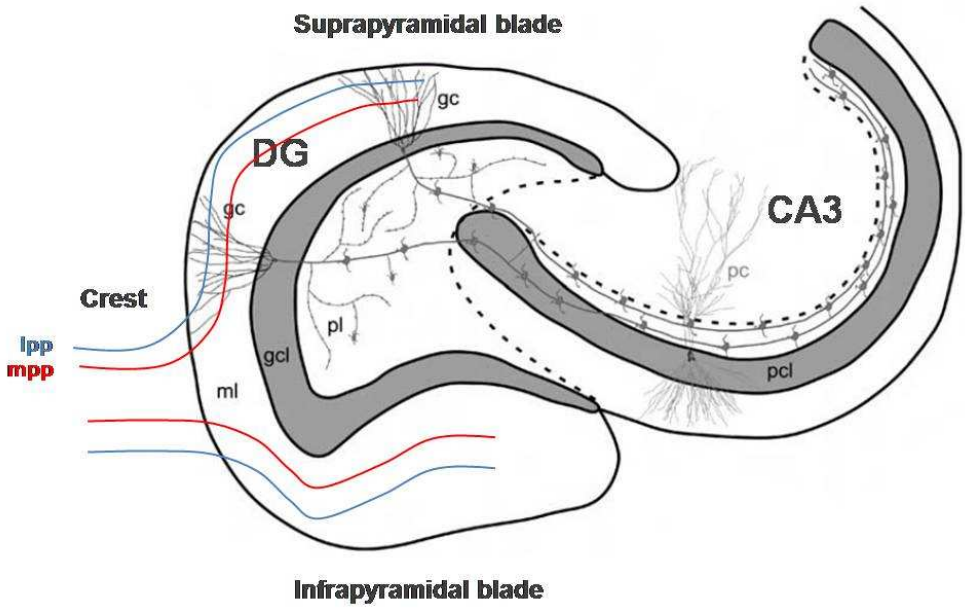

Fig. 2. Schematic representation of the rat DG. The granule cell (gc) is the principal cell in the DG. The DG has three layers containing the different parts of the granule cells. The molecular layer (ml) harbors the dendritic tress of gc, the gc layer (gcl) contains the somata and the polymorphic layer (pl) contains the gc axons on its way to the CA3 field. The tri-laminar DG is folded such that in a cross-section it looks like a V- or C-shaped structure, with two "blades" (suprapyramidal and infrapyramidal blades) and an area where these two blades meet (Crest). The gc receives input from the entorhinal cortex through the lateral PP (lpp, blue line) and the medial PP (mpp, red line). The lpp makes synapses with the gc denrites in the outer third of the ml, whereas the mpp synapses onto the medial third. (pc: pyramidal cell; pcl: pyramidal cell layer) Adapted from (Amaral et al., 2007).

A wide and diverse population of GABAergic neurons abides the DG (Houser, 2007). The basket cells, for example, constitute a group composed of at least five different sorts (Ribak and Seress, 1983). Their somata are located into the base of the granule cell layer. Interestingly, they have a dendritic tree that extends over all the DG layers, making possible for them to potentially access the information at any point inside the DG (Ribak, 1992). Its axon terminals make inhibitory contacts with the soma and proximal dendritic regions of the granule cells, forming a dense terminal plexus distributed so widely that a single basket cell can influence as many as 10 000 (1%)

granule cells (Sik et al., 1997;Struble et al., 1978). Basket cells can mediate feed-forward as well as feed-back inhibition (Kneisler and Dingledine, 1995).

Due to its anatomy, it is relatively easy to obtain extracellular recordings from the DG. In fact, most of the work in the functional plasticity field in freely moving animals has been done in the PP-DG pathway, including the first description of the LTP (Bliss and Gardner-Medwin, 1973). However, most of the work has been done using just a recording electrode located in the hilus for measuring the population spike (PS) and assuming the changes in the PS amplitude (PSA) as representative of the synaptic changes. As we will analyze below (especially in section 1.4 and in the discussion), this is not always the case, so a new approach is needed in this respect.

1.2.3- DG field potentials

Field potentials are extracellular potentials recorded from a group of nerve cells in response to synaptic or antidromic stimulation. The data recorded can only be interpreted in macroscopic terms, rather than microscopic. However, in some cortical laminated structures like the hippocampus, neurons are aligned so regularly, that the current-source-density analysis of the field potentials may elucidate important information about the voltage changes occurring at specific locations of the neurons (Amaral et al., 2007;Andersen et al., 1969;Leung et al., 1995).

Following an electrical stimulus applied to the medial PP, the entorhinal cortex neurons will fire action potentials (APs), causing the release of glutamate from the PP axons making contact with the medial portion of the dendritic tree of the granule cells. This released glutamate, in turn binds to the AMPA receptors on the post-synaptic membrane of the granule cells and allows the influx of ions, especially Na^+ cations. The net influx of positive charged ions causes a local depolarization that turns the

intracellular potential at this dendritic location more positive than other parts of the membrane (but does not reverse the membrane potential). As a result, an intracellular current flows away from this specific dendritic location from high to low membrane potential. Since the current flows in closed loops, the main extracellular current flows in an opposite direction to the intracellular flow: from the cell body layer towards the dendrites, resulting in a negative potential in the synaptic region and a positive potential in the cell body region, relative to a distant electrode (**Fig. 3A**).

When the electrical stimulus applied is high enough to produce a glutamate release able to depolarize the granule cells above the firing threshold (that means, the depolarization wave that travels from the dendrites reaches the initial axonic portion with a magnitude able to open the voltage-dependent Na^+ channels, which are present with the highest density in these area), APs are generated in the DG granule cells. With the opening of the voltage-dependent Na^+ channels a massive influx of Na^+ cations occurs and as a consequence a complete reversion of the membrane potential takes place. That is, the interior of the membrane acquires a net positive charge and the exterior a negative one. The intracellular current, which was initially flowing from the dendrites, starts to flow now from the initial portion of the axon, due to the currently net positive charged membrane interior in this portion of the neuron. With an external electrode a deflection in the opposite direction of the initial current flow can be recorded (**Fig. 3 B**).

The potential recorded in the molecular layer is known as field excitatory postsynaptic potential (fEPSP) and represents the depolarization in the postsynaptic membrane, indicating that the glutamatergic transmission took place at the medial PP-DG synapses. On the other side, the biphasic potential recorded in the proximity of the granule cell layer is known as PS and represents the synchronized firing of the granule cells.

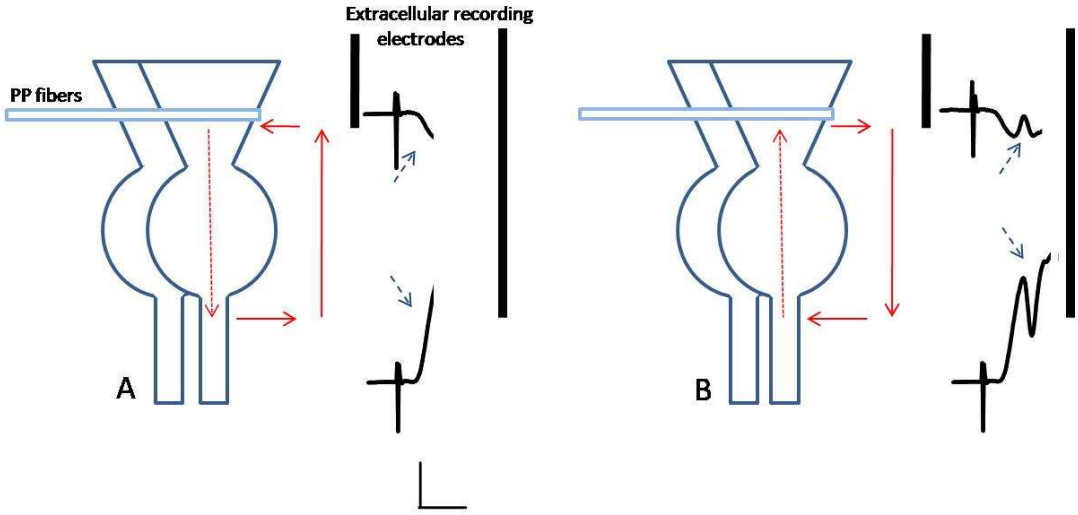

Fig. 3. Schematic representation of the current flow during a synaptic event in a granule cell population. **(A)** When a PP fibers (light blue rectangle) volley produces the release of glutamate that reaches the dendrites of a granule cell population, an influx of positive charges occurs into this region, becoming this zone an extracellular current sink. An extracellular electrode near the dendritic region will record a negative deflection, as positives charges are leaving in the opposite direction. In contrast, an electrode placed near the somatic region, in this case an extracellular current source, will detect a positive potential as positive charges are flowing to it. **(B)** If the stimulus is able to produce the discharge of the granules cells a complete reversion of the membrane potential will occur and a positive deflection will be detected in the formerly negative-going potential recorded close to the dendritic region and a negative deflection in the formerly positive-going potential recorded by an electrode placed near the somatic zone. See the explanation in text for further details. (Calibration 5ms/5 mV).

1.3- LTP

If we learn something, then some things change in our brain to represent that memory. But, which are these changes? Since there are no noticeable changes in the number of neurons that can explain the amount of information stored during a lifetime, the synapse has been a good candidate to mnemonic substrate (Matthies, 1989). The synapse is a highly specialized type of cellular junction. It constitutes the

principal bridge to the flow of information from one neuron to the other, thus allowing all the parts of the system to interact functionally. The importance of synapse in storing information has been postulated since the times of Ramón y Cajal and more recently in Konorski's, Hebb's and Matthies' works (Hebb, 1949;Konorski, 1948;Matthies, 1976).

A significant support to the idea that synaptic changes could underpin memories came with the description of the LTP phenomenon (Bliss and Gardner-Medwin, 1973;Bliss and Lomo, 1973;Lomo, 1966): it was demonstrated that indeed the synapses have the capability to modify their strengths for long periods of time in response to external stimuli. The fact that the LTP was described in the hippocampus, a structure well implicated in the memory processes, gave a further impulse to the supporters of the idea. Using different patterns of repetitive afferent PP stimulation Bliss, Lomo and colleagues were able to induce synaptic plastic changes enduring for hours or even days in the hippocampus, specifically the DG, of rabbits (Bliss and Gardner-Medwin, 1973;Bliss and Lomo, 1973;Richter-Levin et al., 1994). Up to this discovery all other reports of activity-dependent synaptic changes were concerning changes enduring just minutes (Andersen, 1960), hence the significance of the LTP discovery and the enormous attention it drew. During the subsequent years to the earliest 1970's and up to today, the LTP has been intensively studied not only in the hippocampus, but in many brain areas (Calabresi et al., 1992;Clugnet and LeDoux, 1990;Feldman et al., 1999;Kombian and Malenka, 1994;Racine et al., 1986).

1.3.1- LTP basic properties

From the very first report Bliss and colleagues suggested the input-specificity of the LTP. Using a second control input they realized that the plastic changes occurred only in the tetanized pathway, without potentiation in the control input (Bliss and Gardner-Medwin, 1973;Bliss and Lomo, 1973). Additional proofs to the input-specificity came years later with the use of the hippocampal slices and the possibility to better localize and stimulate two independent inputs converging in the same neural population (Andersen et al., 1977). LTP is input-specific since synapses that are inactive at the time of the tetanus do not share in the potentiation induced in the tetanized pathway. Another interesting fact noted also from the very first reports and confirmed later (McNaughton et al., 1978), was the existence of threshold intensity for inducing the potentiation, a property formalized as cooperativity. The “weak” tetani activating relatively few afferent fibers do not trigger LTP, but “strong” tetani able to recruit sufficient amount of fibers do it. Associativity is another characteristic of the LTP, whereby if a weak input (unable by itself to undergo LTP) is activated at the same time when a neighboring pathway onto the same cell is strongly activated, both synaptic pathways undergo LTP (Levy and Steward, 1979;McNaughton et al., 1978). However, this kind of associativity operates just for a short time restraining the effective time of interaction to less than a second. A new LTP property: the late-associativity, was discovered in the late 1990’s in the frame of the synaptic tagging hypothesis and describes a new form of associativity with a much more flexible and longer effective time window (up to 90 min in *in vitro* conditions) (Frey and Morris, 1997;Frey and Morris, 1998b).

The discovery of the LTP induction dependence on the activation of NMDA glutamate receptor (Collingridge et al., 1983) provided explanation for the above described LTP properties, but the late-associativity. At low frequency stimulation the evoked excitatory postsynaptic currents in the hippocampus are largely mediated by the

AMPA glutamate receptors subtype and the NMDA receptors only contribute a small component. That is due to a special property of the NMDA receptor: it is a ligand- and voltage-dependent ionotropic channel. At near-resting membrane potentials, the channel is blocked by Mg^{2+} and a substantial depolarization is needed to expel the Mg^{2+} from the channel (Crunelli and Mayer, 1984;Nowak et al., 1984). In consequence, the NMDA receptor acts as a temporal coincidence detector of activity in the presynaptic (due to the requirement of the glutamate ligand) and the postsynaptic terminal (due to the requirement of the postsynaptic depolarization). When the NMDA receptor opens it is permeable to Ca^{2+}, an indispensable cation for the induction of the LTP (Collingridge et al., 1992;Lynch et al., 1983). Thus, due to the requirement of the NMDA receptor activation for the LTP induction it is clear that: 1- only the input receiving the presynaptic glutamate signal and the postsynaptic depolarization will be potentiated (input specificity), 2- a certain stimulus intensity able to produce the postsynaptic depolarization required to expel the NMDA receptor blockade is needed (cooperativity), and 3- if two inputs are activated in such a way (close enough temporally and spatially) that the depolarization produced for one of them reaches the other input, then both inputs will develop LTP, because both of the requirements for the NMDA receptor activation will be fulfilled (associativity).These properties of the LTP make it an attractive cellular model of learning and memory (Bliss and Collingridge, 1993;Malenka, 2002).

There are also other LTP forms whose induction does not depend on NMDA receptors, like the LTP at the mossy fibers-CA3 synapses (Harris and Cotman, 1986). However, this mossy fiber LTP shows as well input specificity (Zalutsky and Nicoll, 1992), associativity (Derrick and Martinez, Jr., 1994;Kobayashi and Poo, 2004) and cooperativity ((Derrick and Martinez, Jr., 1994), but see (Zalutsky and Nicoll, 1992)).

1.3.2- LTP phases

The finding that protein synthesis was an essential requirement for the maintenance of the late phase of LTP (in freely moving animals (Krug et al., 1984) and also in *in vitro* conditions (Frey et al., 1988)) drew a lot of attention on LTP as a cellular model of memory and also served to classify the LTP in an early phase, dependent on the posttranslational modification of preexisting proteins but independent of the protein synthesis, and a late phase, strictly dependent on protein synthesis. A closer look into both the early- and the late-LTP reveals that even these phases are not unitary phenomena.

Broad spectrum kinase inhibitors can reduce the duration of potentiation to around 1 h (Malenka et al., 1989;Matthies and Reymann, 1993), whereas the protein synthesis inhibitors reduce it to 4 – 6 h (Frey et al., 1988). This leaves an initial LTP kinase-independent component enduring 1 h, referred to as short-term potentiation (STP). The NMDA receptor antagonists, however, are able to reduce this STP to a short-lasting potentiation of just some minutes (Errington et al., 1987), known as post-tetanic potentiation (PTP). The PTP seems to be a presynaptic facilitation process (Tang and Zucker, 1997). The protein synthesis-independent potentiation that develops after 1 h in the early phase of the LTP requires the activity of at least, the PKC and $Ca^{2+/}$calmodulin kinase II (CAMKII) (Malinow et al., 1989;Reymann et al., 1988).

On the other hand, the late-LTP seems also to be not a unitary phenomenon. There is evidence that later stages of the late-LTP require transcription in addition to translation (Frey et al., 1996;Vickers et al., 2005). Thus, the protein synthesis-dependent first stage of LTP seems to be maintained by pre-existing mRNA whereas later stages may require additional gene expression.

1.3.3- Synaptic tagging hypothesis

Due to the specificity of the LTP, its late phase dependency on protein synthesis brings about a big dilemma: how can the proteins required to the plastic change stabilization go to the appropriate synapses and avoid the stabilization of spurious connections if they are not synthesized in the activated synapses? In 1997 Frey and Morris tested the idea that activated synapses could somehow be locally marked or tagged to be recognized by the plasticity related proteins (PRPs) travelling through the dendrites and provided the first experimental evidence that indeed this was the case (Frey and Morris, 1997). In their classical experiments, Frey and Morris showed that a late-LTP can be induced under protein synthesis arrest, or by a weak tetanus unable to induce protein synthesis, if PRPs are available in a specific time window around the LTP induction, for example by the strong tetanization of an independent synaptic input to the same neural population. Where these PRPs come is not of relevance, given that they are accessible to the stimulated input (Frey and Morris, 1997;Frey and Morris, 1998a;Frey and Morris, 1998b).

In *in vitro* conditions the tag was observed to decay after 2 h (Frey and Morris, 1998b) (*in vivo* it seems to decay faster at 30 min (Frey et al., 2001;Frey and Frey, 2008), probably due to the higher temperature), setting a time boundary for the PRPs capturing. Also the PRPs availability is characterized by a specific, relatively short half-life of about 1 - 2 h (Frey et al., 2001;Sajikumar et al., 2005a). Both, synaptic tag and PRPs availability must overlap in time for a late-LTP to be induced at a specific input (**Fig. 4**) (Frey and Frey, 2008;Frey and Morris, 1998b). Interestingly, the synaptic tag deactivation cannot only occur passively through cellular degradation processes, but as well actively through specific electrical stimulation patterns applied within the first minutes of the setting of the tag (Sajikumar and Frey, 2004b).

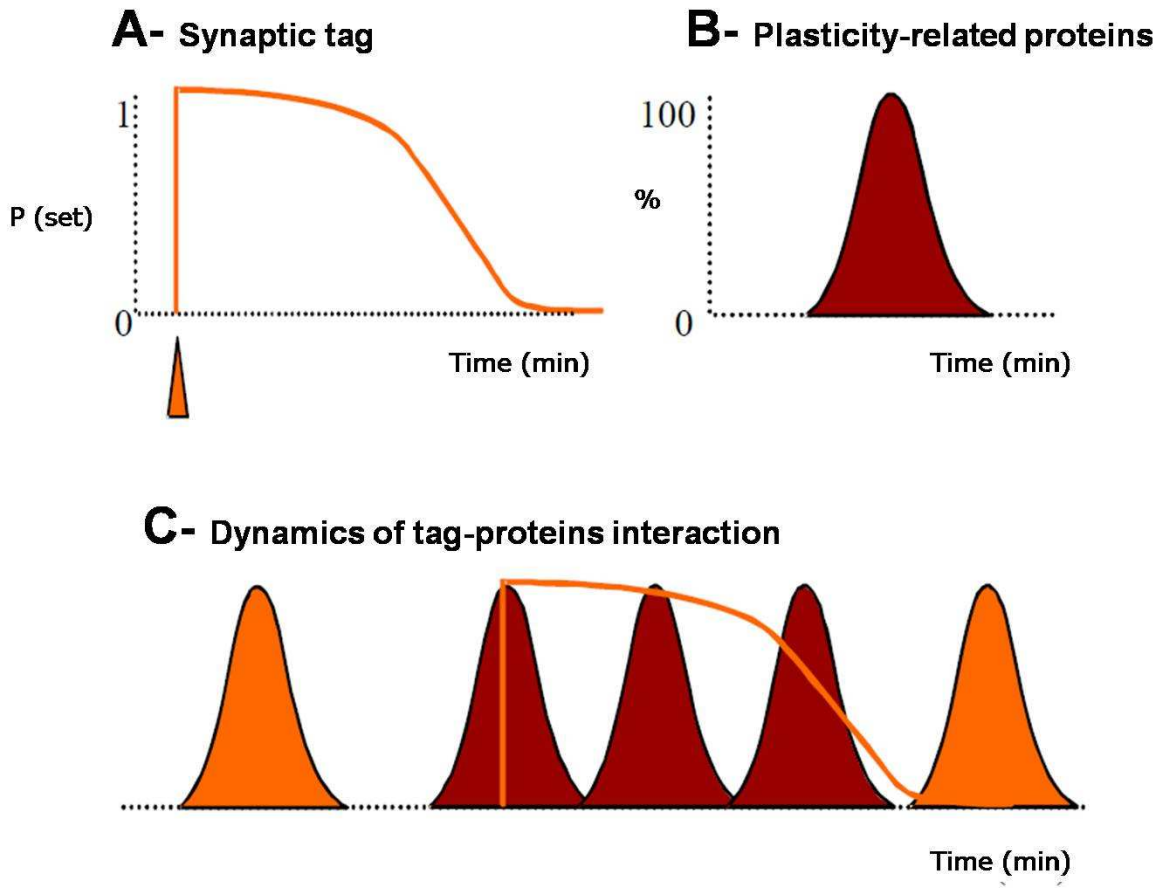

Fig. 4. Diagram showing (**A**) the setting and time decay of the synaptic tag, (**B**) the time course of availability of plasticity-related proteins and (**C**) the potential for tag-protein interactions as a function of the relative times of their induction. For a late-LTP to arise, the tag and plasticity-related proteins availability should overlap in time. The red curves show cases where a successful interaction occurs and a late-LTP is feasible, and the orange's the cases where the tagging and availability of proteins are dissociated and as consequence no late-LTP should occur. Y-axes show, respectively, the probability of a tag being set, and the magnitude of plasticity-proteins available at a synapse; and the x-axis shows time. Modified from (Frey and Morris, 1998b).

The synaptic tagging hypothesis has been verified in many occasions in different labs (Barco et al., 2002;Martin et al., 1997;Young and Nguyen, 2005) and has also been described for long-term depression (LTD) (Kauderer and Kandel, 2000;Sajikumar and Frey, 2004a). Recent work from Frey's lab revealed the identity of synaptic tag molecules in the different compartments of CA1 hippocampal neurons. Thus, CAMKII

and mitogen-activated kinases (MAPKs) mediates the synaptic tagging for LTP and LTD, respectively, in the apical dendrites of CA1 neurons, whereas in the basal dendrites protein kinase A (PKA) and PKMzeta seem to be responsible for LTP tagging (Sajikumar et al., 2007). From Frey's lab came also the first description of the functional interaction between LTD and LTP named 'cross-tagging'. Cross-tagging describes the ability of late-LTP/late-LTD in one synaptic input to convert the opposite, protein synthesis independent early-LTD/early-LTP in an independent synaptic input into its long-lasting form (Sajikumar and Frey, 2004a). Process-specific and process-unspecific PRPs have been identified for LTP and LTD. The phosphodiesterase 4B3 has been proposed as an unspecific PRP (Navakkode et al., 2004;Navakkode et al., 2005) whereas PKMzeta is the first LTP-specific PRP described.

PKC is a family of around 15 different isoforms, each with individual cellular functions. A typical full-length PKC isoform consist of both a catalytic and a regulatory domain. The regulatory domain holds binding sites for second messengers and a pseudosubstrate sequence that inhibits the catalytic domain. Second messengers activate a full-length PKC by binding to the regulatory domain and causing a transient conformational change that releases the inhibition of the pseudosubstrate (Newton, 2001). PKMzeta, in contrast, consists just of a catalytic domain that is constitutively active. It is a brain-specific protein expressed from a unique mRNA, which is produced from an internal promoter within the PKzeta gene (Hernandez et al., 2003;Sacktor et al., 1993). PKMzeta is the only one protein that has been shown to be necessary and sufficient for maintaining synaptic potentiation in the CA1 region of the hippocampus (Ling et al., 2002;Madronal et al., 2010) and is the first specific PRP identified for the LTP (Sajikumar et al., 2005b). Recent studies indicate that PKMzeta regulates the N-ethylmaleimide-sensitive factor (NSF)/glutamate subunit 2 (GluR2)-

dependent AMPA receptor trafficking, increasing the amount of postsynaptic AMPA receptor by preventing their removal from postsynaptic sites (Migues et al., 2010;Sacktor, 2011;Yao et al., 2008). This seems to be the mechanism through which PKMzeta is able to maintain the synaptic potentiation over time.

In agreement with the hypothesis that synaptic changes underlie memory, it has been furthermore shown that PKMzeta activity is needed for the persistence of different kinds of them (Migues et al., 2010;Serrano et al., 2008;von Kraus et al., 2010).

1.4- Plasticity of neuronal excitability

Whereas synapses mediate fast intercellular communication in the nervous system, information is ultimately conveyed by APs. Consequently, not only the study of synaptic plasticity deserves special attention; but also the way how neurons modify its facility to fire APs independently of their synaptic excitatory drive. Indeed, from the point where synapses take place up to the AP initiation zone there is usually quite a distance and the amount of depolarization that finally arrive at that location is highly influenced by many factors, including the inhibitory activity of GABAergic interneurons and the presence of voltage-dependent channels.

Since the very first reports on LTP it was clear that synaptic efficacy was not the only variable modified by the LTP-inducing stimulus; the coupling among the excitatory input: the fEPSP and the neuronal output: the PS was also affected, rendering bigger PS values than could be accounted for by the fEPSP change. Actually, in some cases PS potentiation occurred in the absence of any synaptic change (Bliss and Gardner-Medwin, 1973;Bliss and Lomo, 1973). This phenomenon was later called “E-S potentiation” by Andersen to indicate the left shift in the relation PS *vs.* fEPSP (Andersen et al., 1980). Two main non-exclusive hypotheses have been proposed to

explain the mechanism of the still controversial E-S potentiation: (a) an altered inhibition-excitation balance (Abraham et al., 1987;Chavez-Noriega et al., 1989;Staff and Spruston, 2003) (b) changes in the intrinsic excitability, that is in the properties of the voltage-dependent channels (Daoudal et al., 2002;Jester et al., 1995).

In support of the physiological role of the nonsynaptic plasticity (referred to the excitatory synapse on principal neurons), some reports have recently shown that protein synthesis might not only serve to consolidate the synaptic changes induced by LTP or learning, but also to maintain neuronal excitability changes over several hours (Cohen-Matsliah et al., 2010;Xu et al., 2005). Even the cAMP-response element binding protein (CREB), the classically “memory gene” whose activity is crucial for long-term maintenance of synaptic LTP and memory, has been shown a key regulator of intrinsic excitability in hippocampus, amygdala and striatum (Benito and Barco, 2010;Lopez de et al., 2007;Viosca et al., 2009). Thus, the question arises whether the ability of protein synthesis inhibitors to impair long-term memory is due to its effects on synaptic plasticity, on neuronal excitability or a combination of both. Maybe the proteins that have been assumed to be necessary to consolidate the synaptic changes induced by learning, are also needed for consolidating key excitability changes.

Specificity is one of the traditional arguments used to favor synapses over excitability in its role in learning and memory. The fact is that each neuron receives a huge number of synaptic contacts and that gives synapses an outstanding potential for storing information, due to the input specificity of the synaptic plastic events. As AP are mostly generated in the initial axon segment, an information-storage mechanism relying in excitability changes in the axo-somatic compartment (like a modification of the GABAergic inhibition or voltage-gated ion channels in this area) will result in a general increase in the neuron excitability, irrespective of which synaptic afferents

are bringing the excitatory drive and this will drastically reduce the storing capability of this mechanism. However, there are some things we should consider. First, it might be useful for some kind of memories to be stored, in part, using mechanisms that are not synapse-specific, especially some nondeclarative memories (Zhang and Linden, 2003). Second, nonsynaptic plasticity does not necessarily always involve the axo-somatic region. Dendrites also possess voltage-gated ion channels which can influence the conduction of synaptic signals to the axo-somatic region. If a particular pattern of synaptic activation on a dendrite produce a local change in voltage-gated channels (as has recently been probed can be the case (Frick et al., 2004)), this could enhance the chance of a certain dendritic module (bigger than a single synapse, but smaller than a whole neuron) to evoke an AP. The same could happen if a set of inhibitory interneurons targeting a specific dendritic compartment changes its influence on that dendritic zone. Therefore, the information-storage capacity of nonsynaptic plasticity might be larger than commonly assumed.

In fact, it is now clear that different forms of learning produce profound excitability changes by modulating the inhibitory drive onto the principal neurons (Brioni et al., 1989;Doron and Rosenblum, 2010;Gusev and Alkon, 2001;Jasinska et al., 2010;Lin et al., 2009) and also the voltage-dependent channels (Cohen-Matsliah et al., 2009;Moyer, Jr. et al., 1996;Mozzachiodi et al., 2008;Oh et al., 2003;Thompson et al., 1996). The exact role of these excitability changes in learning and memory is not completely understood and there is actually a debate whether these changes are part of the mnemonic engram itself or just serve a “secondary” role in facilitating the synaptic modifications needed for the memory storage (Mozzachiodi and Byrne, 2010;Sjostrom et al., 2008;Zhang and Linden, 2003).

1.5- Aims of the dissertation

This dissertation is primarily concerned with the study of how DG granule cells' synaptic efficacy, estimated through the fEPSP, relates to the AP firing, estimated through the PS, in LTP and which molecular mechanisms underpin these synaptic and excitability changes in freely moving animals. To understand the relation among these two phenomena and its mechanisms is a central question in neuroscience today.

A common issue for most of the previous "synaptic" plasticity studies in the DG of freely moving animals is the use of a single recording electrode located in the hilus to measure the PS (including preceding own work (Almaguer-Melian et al., 2010;Bergado et al., 2007;Bergado et al., 2009;Kudolo et al., 2010;Lopez et al., 2008)). As we have seen above, this is not always the case that PS changes reveal underlying synaptic changes. We took advantage in this dissertation of the recently developed technique to simultaneously record the fEPSP and PS from the same animal (Frey and Frey, 2009).

In a first series of experiments we studied the influence of different HFS protocols on the fEPSP and PSA. We studied the effects of two weak and two strong HFS patterns. We knew, from previous work of our lab, that the two weak HFS patterns were able to induce transient changes in the PSA whereas the strong protocols induced a PSA potentiation lasting for at least 24 h. We wanted to investigate for each one of these HFS patterns how the PSA time course relates to the fEPSP variations.

In a second series of experiments we decided to explore pharmacologically some of the molecular basis of the fEPSP and PSA potentiation. We chose for this second series the strongest HFS paradigm from the first series, because it was the only one

capable of inducing a late-LTP (fEPSP) additionally to the PSA potentiation. Using intrahippocampal infusion of drugs, through an intrahippocampal cannula placed in the proximity of the recording site, we investigated the role of NMDA receptors as well as the role of PKMzeta in these plasticity processes.

2- Materials and methods

2.1- Laboratory animals

All experimental procedures were performed according to the guidelines of the regional council of Saxony-Anhalt. The principles of laboratory animal care were strictly followed. Every possible effort was made to minimize the number of animals used and their suffering.

Male adult Wistar (Wistar Schönwalde) rats (*Rattus norvegicus*) of our local colony in the Leibniz Institute for Neurobiology were our experimental subjects. At the time of surgery rats were 7-8 weeks old and weighting 270-330 g. They were kept in our departmental animal facility under standard housing conditions, with temperature of 22 ± 2 ℃, humidity of 55 ± 5 % and a 12 h light-da rk cycle with light starting on at 6:00 am. Before surgery, animals were housed in plastic translucent standard breeding cages (55 x 35 x 18) cm in groups of five per cage. After the surgical electrode implantation they were placed in individual cages of (40 x 25 x 18) cm. They had free access to water and food (food pellets, ssniff, R/M-H, Soest) at all times.

2.2- Electrode implantation

Animals received an intraperitoneal injection of pentobarbital (Sigma-Aldrich Chemie GmbH, Munich, Germany) at a dose of 40 mg/kg. The anaesthetized animals were then mounted on a stereotaxic frame (430005-series, TSE Systems, Germany) with bregma and lambda at the same plane. The animals' forehead and neck were shaved. The scalp was incised to expose the skull by cutting part of the skin and removing periosteum. During the surgery, foot withdrawal as well as eye reflexes

were checked to assess the depth of anesthesia. If required, an additional dose of approximately one third of the initial bolus of Nembutal was administered.

All coordinates for the electrode implantation were taken from the atlas of Paxinos and Watson (Paxinos and Watson, 1998). Bregma was used as reference for the anterior-posterior (A-P) and medio-lateral (M-L) coordinates. The dura at the place of insertion was used as the dorso-ventral (D-V) reference. The brain was exposed through small burr holes on the skull made with a trepan. Animals were implanted with a “double recording electrode” in the DG (A-P -4.0 mm, M-L 2.3 and D-V approximately -2.7 mm), an intrahippocampal 33-Ga stainless steel cannula placed nearby the recording site (A-P -4.0 mm, M-L 2.4 and D-V -2.0 mm) and an ipsilateral bipolar stimulation electrode aimed to the medial PP (A-P -7.5 mm, M-L 4.1 mm, D-V approximately -2.6 mm) (**Fig. 5**). The final position of the electrodes, especially the depth, was adjusted under electrophysiological control. Stainless steel miniscrews attached to the skull and welded to silver wires served as ground and indifferent electrodes. The entire assembly, electrodes and miniscrews, was fixed to the skull with dental cement (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) once optimal and stable potentials were achieved.

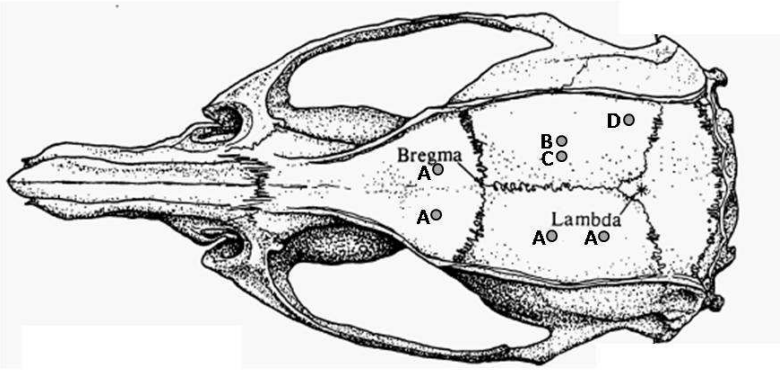

Fig. 5. Skull of a male Wistar rat with the schematic representation of the positioning of (A) ground and indifferent electrodes, (B) intrahippocampal cannula, (C) double recording electrode in the DG, (D) stimulation electrode in the PP. Figure adapted and modified from (Paxinos and Watson, 1998)

Both, the recording electrode and the stimulation electrode, were made of two lacquer isolated stainless steel wires (diameter per wire: 125 µm) straightened close together and fixed with glue to a small piece of paper. The distance of 400 µm between the tips of the recording electrode was achieved by using a special scissor and a scale with the help of the microscope. The so-called "double recording electrode" is a novel tool recently introduced for our lab (Frey and Frey, 2009) for measuring both, the fEPSP and the PS, near of their generation sites. With a 400 µm distance between the two tips of this double recording electrode, it is possible to reach in our animals the hilus with one of the tips and the molecular layer of the suprapyramidal blade of the DG with the other.

2.3- Electrophysiological experiments

2.3.1- Experimental setup

For the different experimental manipulations the animals were placed into large experimental boxes (40 x 40 x 40) cm. There they had free access to food and water at all times and were able to move freely. The animals were connected to the electrophysiological equipment through a swivel connector via a ribbon cable (LEMO Elektronik GmbH, München, Germany) (**Fig. 6**). Thus, the stimulation electrode was connected to an isolated pulse stimulator (Isolated pulse stimulator, Model 2100, A-M System, Sequim, USA) and the recording electrodes were connected to a differential

amplifier (Differential AC Amplifier, Model 1700, A-M System, Sequim, USA). Recorded signals were filtered (0.1 Hz – 5 KHz) and amplified with a gain of 100x. From the amplifier the signals were digitalized through an analogue-digital converter (CED 1401; Cambridge Electronic Design, Cambridge, UK) and visualized on a computer, which also served to trigger the stimulators through the connection with the analogue-digital converter. The computer program used for generating the electrical stimulation and for analyzing the bioelectrical signals was PWIN, a custom-made program (PWIN, Magdeburg, Germany). The EEG of the animals, recorded through the fEPSP electrode, was monitored all the time with an oscilloscope.

Fig. 6. Recording chamber with an animal inside. The rat is connected through a ribbon cable to the swivel; the tube connecting the injector with the microsyringe is also appreciated. The animal had free access to water and food.

2.3.2- Measured variables

We estimated the strength of the synaptic transmission through the slope function calculated from four successive points of the steepest 400 µs segment in the negative flank of the fEPSP recorded with the shorter tip of the double recording electrode. The amount of discharges of the granule cells was evaluated measuring the amplitude between the early positivity and the negative peak of the field potential

PS recorded with the larger tip of the double recording electrode (PSA) (**Fig. 7**). Indeed, intracellular recordings have probed that the fEPSP slope function and the PSA, are reliable measures of a single neuron synaptic depolarization and firing activity respectively, if fEPSP and PS are obtained with electrodes placed in the proximity where these events occur (Andersen et al., 1971;Lomo, 1971).

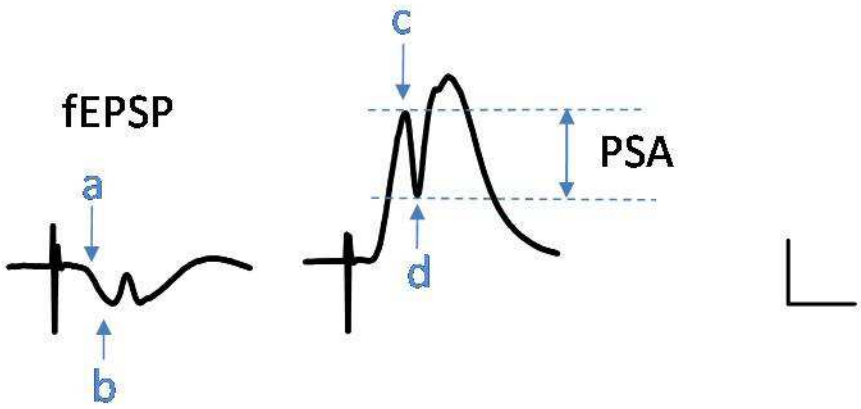

Fig. 7. Evoked field potentials to the stimulation of PP in the DG. The slope function (mV/ms) of the field fEPSP was calculated from four successive points of the steepest 400 µs segment inside the markers “a” and “b”. The PSA (mV) was evaluated by taking the voltage difference between onset (“c”) and peak (“d”). (Calibration 5ms/5 mV).

2.3.3- Different HFS protocols induce different forms of long-term potentiation in the DG of freely moving rats

Animals recovered for at least 10 days after surgery and then were habituated to the recording chamber for 4 h as a minimum before any experimental electrophysiological recordings. After connecting the animals to the swivel via a ribbon cable, a 30 min time interval was allowed for the animals to recover from handling. Apart from the insertion of ribbon cable at the start of the experiment, the disturbance of the animals was kept to an absolute minimum. Ambient conditions

were kept constant to prevent environmental influences on behavioral state that might affect the recordings.

Input-output curve

An input-output (I-O) curve was generated for each animal in this first day of the experiment. Usually the I-O curves are constructed stimulating all animals with the same intensities in a fixed range of values and then plotting the group mean values of the PSA or fEPSP versus the used intensities. However as all animals have different sensibilities to the electrical stimuli, an important variation is introduced with this procedure and also sometimes the full dynamic range is not achieved for various animals. In our lab we have implemented a new method for generating the I-O curves and it consists in having a complete individual representation of the output responses for each animal. That means that in making the I-O curve, the intensity is individually changed in order to have a representation from each animal of the threshold, 25%, 40%, 75% and maximum PSA (100%). The values of the fEPSP evoked using these intensities are also recorded at the same time and expressed as percent values, taking the bigger value registered as 100%. The averaged responses to 3 biphasic current pulses (0.1 ms per cycle) at 0.2 Hz applied to the PP are taken for each one of the stimulus intensities used.

Experimental schedule

On the next day, two intensities were used for recording the evoked potentials as describe previously (Frey and Frey, 2009): one was set to achieve about 50-60 % of the maximal value of the fEPSP (fEPSP recorded at this ***l***ow ***i***ntensity will be referred to as "fEPSP-***li***") and the other intensity, always higher than the low intensity, was set

to evoke about 40 % of the maximal PSA (fEPSP recorded at this ***h***igher ***i***ntensity will be referred to as “fEPSP-***hi***”). This higher intensity was the one used for the HFS. The reason for using two stimulation intensities for recording is that normally with the higher intensity we have an fEPSP of around 80 – 90 % of its maximum value and thus the fEPSP potentiation that could be recorded at this high intensity should be lower than the one that could be registered for the fEPSP-li. Lacking the ceiling effect, fEPSP-li seems to be a better candidate in following the synaptic changes. However, this fEPSP-li is not necessarily in direct relation to the recorded changes in the PSA, which is always recorded with the higher intensity (in most of the cases the reduced intensity is subthreshold). Then to have a better monitoring of the synaptic events, and also of its relation to the excitability changes, we decide to use both intensities.

During baseline recordings animals were stimulated with both intensities alternating each one every 5 min. Each recording consisted of 5 consecutive averaged responses to biphasic impulses (0.1 ms per cycle) at a frequency of 0.2 Hz. After a stable baseline was recorded for 1 h we applied a different HFS pattern for each one of the groups or just continued with the test stimulations in the case of the baseline controls.

The main aim of these experiments was to assess how the synaptic and nonsynaptic properties of the DG granule cells are modulated with the use of different HFS protocols. Four different HFS patterns were used. The weakest (**WkWkTET**): 3 bursts of 10 biphasic impulses at 200 Hz, with 10 s between the bursts and 0.1 ms of impulse width per cycle. Other used (**WkTET**) was similar to WkWkTET, but applying 3 burst of 15 impulses instead of 3 burst of 10 impulses. A stronger tetanus (**StrTET**) was composed of 20 trains of 15 impulses at 200 Hz also with 10 s between the bursts, but every pulse had a width of 0.2 ms per cycle. The strongest tetanizing

protocol (**4xStrTET**) consisted in applying four times the Str TET pattern, with 15 min of inter-train interval.

At 5 min after the HFS, we measured the fEPSP with the reduced intensity and then the PS 5 min later (10 min after HFS) with the higher intensity, also as a mean value of 5 impulses at 0.2 Hz. Afterwards, we recorded the fEPSP and PS with these alternating intensities every 15 min up to 8 h.

The next day, that is the third day of the experiment, we measured the PS and fEPSP values for 1 h (24 h values) and we took a new I-O curve using the same stimulation intensities of the initial I-O. In **Fig. 8** a schematic representation of the experimental sessions is given.

Fig. 8. Electrophysiological experiments consisted in 3 days sessions. On the first day, I-O curves were generated for each one of the animals. On the second day, a baseline was recorded for 1 h and after that a group of animals received no HFS, but just test recordings for 8 h. The other groups of animals received one of the studied HFS patterns and after that test recordings for 8 h. On the third day, test recordings were obtained for 1 h, approximately at a time corresponding to 24 h after the end of the baseline. After waiting for 30 min the I-O relation was checked again.

The PSA and fEPSP values were averaged in 1 h - periods (but the 5 min and 10 min recordings after a HFS) and expressed as percentiles of baseline. In the I-O curves the values are expressed as percentiles, considering the 100% in each animal as the maximum value recorded in the initial I-O curve.

2.3.4- Pharmacology of the late-LTP

In the experiments with intrahippocampal infusion of drugs, the experimental protocol was slightly different. All measures for the experiment were taken on the same single day with the animals having the injector inside their brains. We decided on this procedure to avoid the possible variations in the potentials as a consequence of a mechanic or other unspecific effects of the insertion or removal of the injector.

The protocol was as follow. Early in the morning, between 6:00-7:00 am, the animals were connected to the electrophysiological equipment and the injector was carefully inserted into the implanted guide cannula. The injector was designed 0.5 mm larger than the guide cannula, so that the substances could freely diffuse into the tissue. The injector was connected through a polyethylene tube to a Hamilton microsyringe (CR-700-20, Hamilton Co., Reno, USA), the tube and the syringe were filled with dH2O. The evoked potentials were monitored for around 1-1.5 h after the injector insertion to assure that no disturbances were present at the time of experimental recordings. Once stable values were achieved we generated an I-O curve for each animal and continue after 30 min with the experimental protocol in a similar fashion as explained above for the experiments without intrahippocampal injections. The main differences were that in these experiments an intrahippocampal injection was made and that we recorded only for 6 h after the HFS. A schematic representation of the experimental sessions with pharmacological manipulations is given in **Fig. 9.**

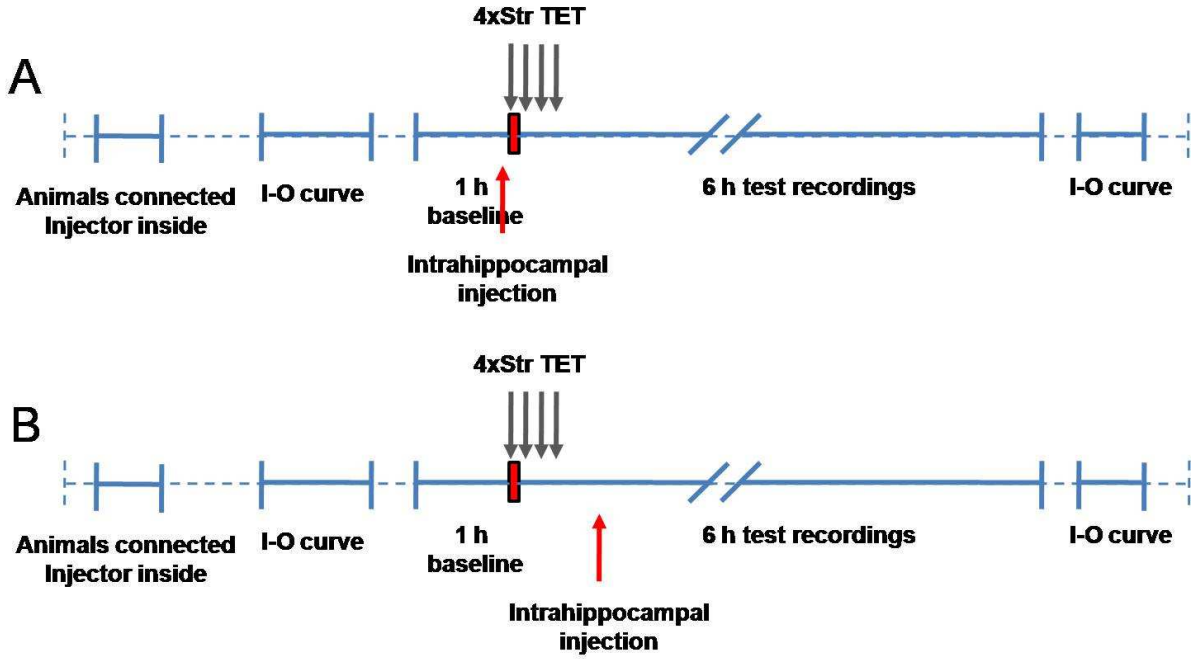

Fig. 9. Pharmacological experiments were carried out in a single day. Depending on the used drug, it was applied either 10 min before the studied HFS (**A**) or 1 h after HFS (**B**). In the control experiments for studying the effect of the used drugs on basal synaptic transmission no HFS stimulation was applied, just test recordings.

At the moment of injection, i.e. 10 min before HFS or 1 h after HFS depending on the drug, each animal received intrahippocampally 0.05 µl of saline plus 1.00 µl of substance within 8-10 min (approximately 0.1 µl in 1 min). This volume of 0.05 µl of physiological saline was always charged to create an interface between the drugs and the tissue, with the purpose of restricting the undesirable diffusion of the drugs to the tissue before the time of injection.

These series of pharmacological experiments were designed to study the mechanisms of the synaptic LTP in the DG as well as its relation to the excitability changes induced. Thus, to test the dependency on NMDA receptors we used the NMDA antagonist D(-)-2-amino-5-phospho-valeric acid (D-AP5, Sigma-Aldrich Chemie GmbH, Munich, Germany) at a concentration of 25 mM (25 nmol in 1 µl) applied 10 min before the HFS. For studying the relation of the LTP induced with the

PKMzeta activity, we used the PKMzeta inhibitors ZIP (myr-SIYRRGARRWRKL-OH, Biosource) and chlelerythrine, both in a concentration of 10 mM (10 nmol in 1 µl). The scrambled ZIP (scr-ZIP, myr-RLYRKRIWRSAGR-OH, Biosource) was used as control for ZIP. Stability control experiments with just test recordings and application of vehicles or drugs at the corresponding time points were also made.

D-AP5 solution in physiological saline was freshly prepared the same day of application. ZIP and scr-ZIP were solved in physiological saline and stored at -20 ℃, whereas chelerythrine was dissolved in dH_2O and also stored at -20 ℃.

The concentration for the different drugs was chosen according to previous behavioral/electrophysiological experiments showing effect of these drugs on memory/synaptic plasticity processes (Pastalkova et al., 2006;Serrano et al., 2008;Szapiro et al., 2003).

2.4- Histology

At the end of the experiments, the placement of the electrodes and cannula was histologically examined. The animals were deeply anesthetized with 2-Bromo-2-chloro-1, 1, 1-trifluoroethane (Sigma–Aldrich Chemie GmbH, Munich, Germany) and after that decapitated. The brains were carefully removed, frozen at approximately –60℃ and stored at –20℃. Using a freezing microtome (Microm HM 560, Microm International GmbH, Walldorf, Germany) coronal sections of 40 µm thick were cut. The slices were air-dried on a heating plate (37℃) an d stained with toluidine blue.

For histological examination, a stereo microscope (Leica Z16 APO, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) was used. Digital images were made with a digital camera if necessary. Only animals with correct electrode and cannula placement (i.e. within the structures of interest) were considered for final

analysis. In **Fig**.**10** examples of correct localization of electrodes and cannula are given.

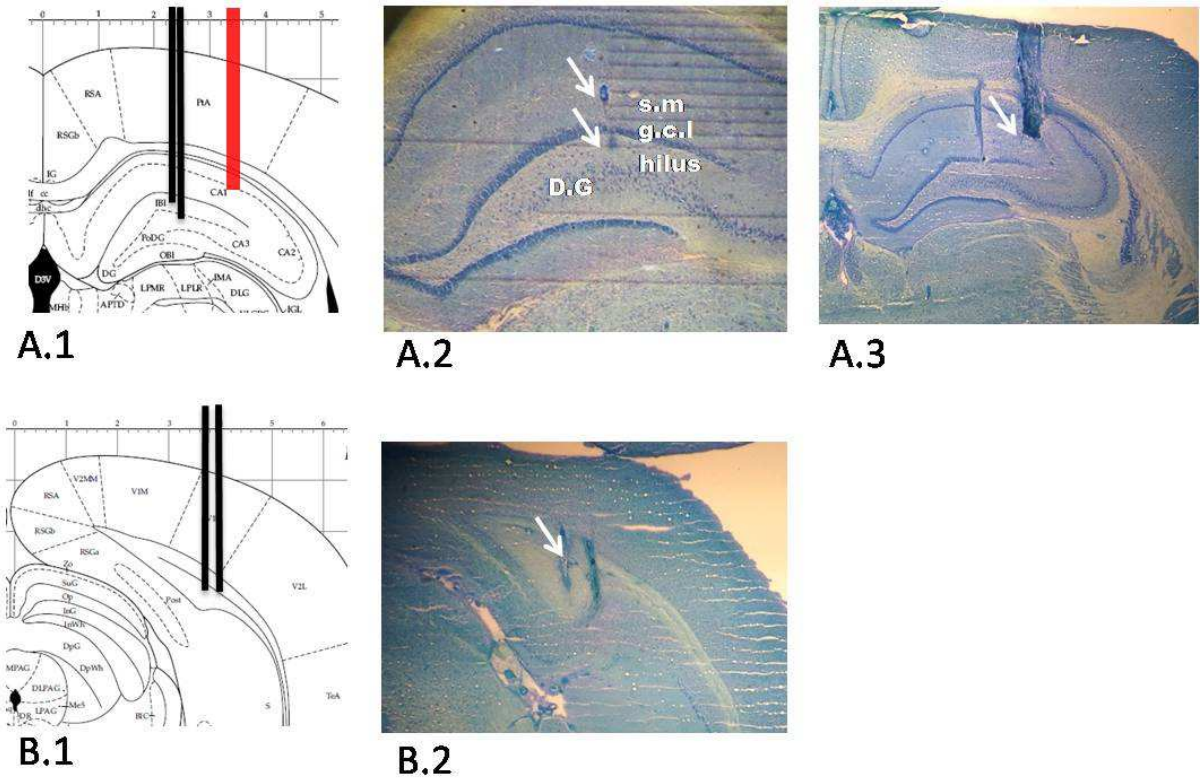

Fig. 10. Histology. **A.1** Schematic representation of the positioning of the double recording electrode in the DG and intrahippocampal cannula (adapted from (Paxinos and Watson, 1998) corresponding to A-P = - 4.16 mm). **A.2** and **A.3** Histological samples of the correct placement of the double recording electrode and the intrahippocampal cannula, respectively. **B.1** Schematic representation of the positioning of the stimulation electrode in the PP (adapted from (Paxinos and Watson, 1998) corresponding to A-P = - 7.04 mm) and **B.2** example of the correct localization of the stimulation electrode.

2.5- Statistics

Statistical analyses were performed using a Wilcoxon matched pairs test for within group comparisons and the Mann-Whitney U test for between group comparisons. A two-tailed $p < 0.05$ was established as statistically significant. All PSA and fEPSP values in graphics are expressed as mean ± standard error of the mean (SEM).

3- Results

3.1- Different HFS protocols induce different forms of long-term potentiation in the DG of freely moving rats

Stability control

In a first series of experiments we recorded the time course of the neuronal responses to test low frequency stimuli over the experimental period (n = 13). The fEPSP values recorded with the low intensity, fEPSP-li, are presented in the graphic **A** of the **Fig. 11**, as will be presented always. The fEPSP values on the right panel (**Fig. 11 B**), correspond to the values obtained with the stimulation intensity used for recording the PSA and as explained before will be referred to as fEPSP-hi. The PSA time course is shown in **Fig. 11 C**. As can be appreciated, no dramatic changes occurred in any of the studied variables as a result of the test stimulation, circadian or other influences, even when a little trend to decrease was observed.

The values of fEPSP-li for the 24 h, 90.1 ± 4.0 % (mean ± SEM), or the fEPSP-hi, 92.2 ± 3.5 %, were not statistically different from its baseline values ($p > 0.05$; Wilcoxon test). Also the other time points were not different from baseline ($p > 0.05$; Wilcoxon test). The PSA as the fEPSP showed a trend to decrease, but a significatively difference from baseline was only detected for PSA at the 8 h value 81.1 ± 4.2 % ($p < 0.05$; Wilcoxon test).

In the same way, no statistically significant differences were detected when comparing the values of the I-O curves obtained the first day of the experiment and

the one on the third day (**Fig. 12** ; p>0.05; Wilcoxon test). Thus, we can conclude that the evoked potentials remained stable over all the recording period.

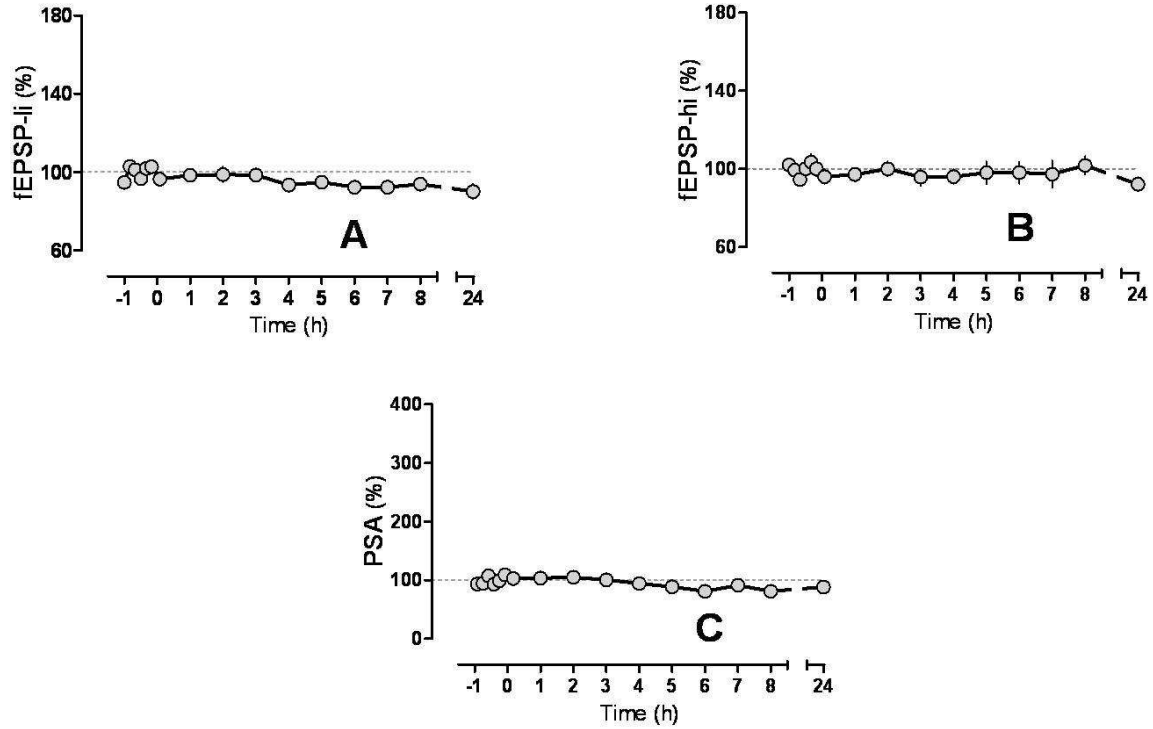

Fig. 11. Time course of the evoked potentials. In **(A)** the fEPSP recorded at a reduced intensity, able to evoke a value of around 50-60 % of the maximum fEPSP. **(B)** and **(C)** were recorded with the same stimulus intensity, able to evoke approximately 40 % of the maximum PSA at baseline. Stable potentials were obtained over all the recording session, only the PSA showed a statistically significant difference at 8 h with respect to baseline ($p < 0.05$ Wilcoxon test).

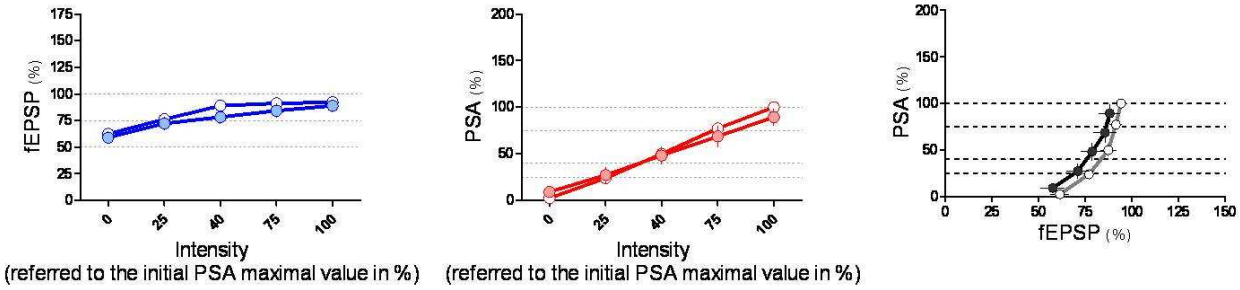

Fig. 12. Input-output curves were generated at the first day of the experiment and also at the third and final day. No statistically significant differences were detected in this group of animals that only

received low frequency test stimuli (n = 13). The values of the first day are represented by the open circles in all graphs and the values corresponding to the third are represented with the filled circles.

Early-LTP

Early LTP is usually induced with a stimulation pattern consisting of 3 trains of 15 pulses at 200 Hz. Former results indicate that this HFS protocol (Wk TET) can be stronger than desired (Almaguer-Melian et al., 2010;Frey et al., 2001). Thus, we decided to study in more detail the changes induced with this HFS pattern, as well as the ones induced with a weaker HFS stimulation consisting of 3 trains of 10, instead of 15, pulses at 200 Hz (Wk Wk TET).

The Wk TET group (n = 15) showed an initial fEPSP potentiation of 137.0 ± 3.1 % when recorded with the reduced intensity 5 min after the HFS ($p < 0.05$ Wilcoxon test) and of 119.7 ± 4.9 % when recorded with the higher stimulus intensity 10 min after the HFS ($p < 0.05$ Wilcoxon test). This Wk TET group showed significant potentiated fEPSP-li values for 8 h, when compared with the corresponding stability control ($p < 0.05$ Mann Whitney U - test), that returned to baseline at 24 h (107.3 ± 7.0; $p > 0.05$ Wilcoxon test). The fEPSP-hi, even when showed a lower initial potentiation than fEPSP-li ($p < 0.05$ Mann Whitney U - test), followed a similar trend. The PSA was potentiated to 230.8 ± 17.8 % at 10 min after Wk TET ($p < 0.05$ Wilcoxon test) and remained potentiated over 8h (122.6 ± 9.8 %; $p < 0.05$ Mann Whitney U - test); returning to its pretetanus baseline values at 24 h (123.5 ± 11.5 %; $p > 0.05$ Wilcoxon test) (**Fig. 13**). When the I-O curves are analyzed (**Fig. 14**), it can be seen that the fEPSP was not modified in this group one day after the Wk TET and also the PSA in most of the range was unchanged; but the PSA threshold was lowered ($p < 0.05$ Wilcoxon test). The stimulus intensity that before the Wk TET was

unable to produce the firing of the granule cells (PSA threshold = 2.1 ± 1.3 %), was able to evoke a PSA of 21.0 ± 8.9 % one day after the HFS.

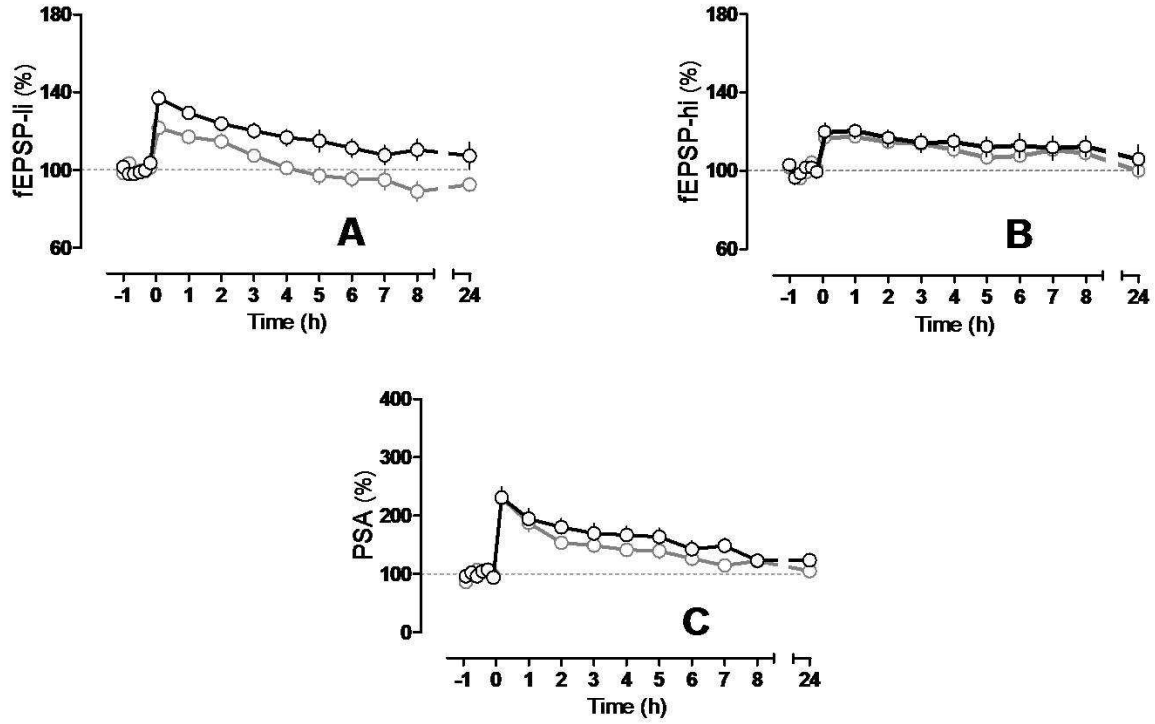

Fig. 13. Different transient forms of potentiation were induced with distinct HFS patterns. The Wk Wk TET (n = 12; gray line, open circles) produced a much milder potentiation than the Wk TET HFS (n = 15; black line, open circles). The fEPSP potentiation was evident for 2 h - 4h in the Wk WK TET in contrast to the Wk TET group in which fEPSP values remained different from control for the first 8 h (Mann Whitney U test) (**A, B**). PSA potentiation was also longer upregulated in the Wk TET, for the first 8 h (Mann Whitney U test), than in the Wk Wk TET group where PSA showed different from control just for the first 5 h (Mann Whitney U test).

On the other hand, the weaker HFS pattern Wk Wk TET (n = 12) induced a lower fEPSP-li potentiation in comparison to the Wk TET group, not only regarding the initial potentiation at 5 min (121.7 ± 3.8 %), but most of the recordings ($p < 0.05$ Mann Whitney U – test for all time points; except 3 h, 7 h and 24 h that $p > 0.05$ Mann Whitney U – test). The induced potentiation lasted only for 2 h ($p < 0.05$ Mann Whitney U – test) and after that the fEPSP-li came back to baseline ($p > 0.05$

Wilcoxon test). The fEPSP-hi initial potentiation was slightly lower (117.0 ± 3.6 %) compared to the fEPSP-li's, but no statistically different from it ($p > 0.05$ Mann Whitney U – test). The fEPSP-hi remained potentiated however for a longer time, its values being different from the corresponding stability control until the 4 h ($p < 0.05$ Mann Whitney U – test). At 5 h the fEPSP-hi reached the baseline (106.7 ± 3.5 %; $p > 0.05$ Mann Whitney U – test). The PSA with and initial potentiation of 230.3 ± 15.4 % ($p < 0.05$ Wilcoxon test), stayed upregulated till the 5 h (139.4 ± 12.5 %, $p < 0.05$ Mann Whitney U – test), coming back to pretetanus baseline at 6h (126.2 ± 12.5 %; $p > 0.05$ Wilcoxon test) (**Fig. 13**). The analysis of the I-O curves reveals that one day after the Wk Wk TET HFS, no fEPSP or PSA changes endured ($p > 0.05$ Wilcoxon test) (**Fig. 14**).

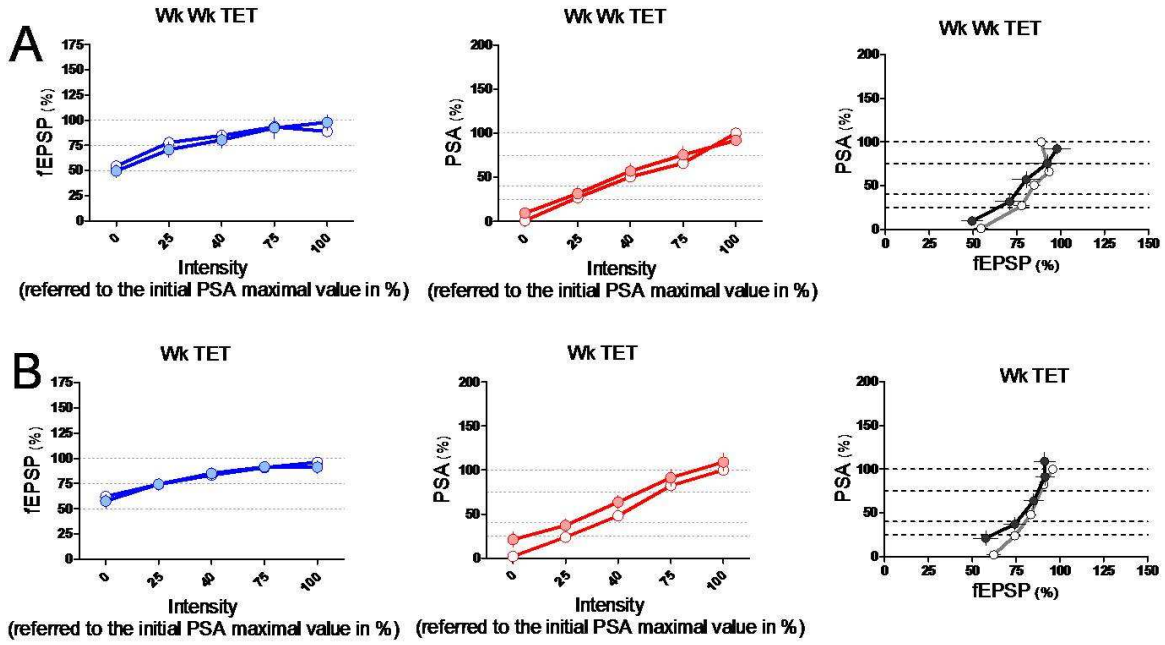

Fig. 14. Input-output curves were generated at the first day of the experiment and also at the third and final day. In the Wk Wk TET group (**A**; n = 12) no statistically significant differences were present after the HFS in respect to the values registered before (Wilcoxon test). The values of the Wk TET group (**B**; n = 15) were also essentially unchanged one day after the HFS, however the PSA threshold was significantly lowered in this group (Wilcoxon test). The values of the first day are represented by the

open circles in all graphs and the values corresponding to the third are represented with the filled circles.

Early-LTP with profound changes in excitability

In the previous groups, Wk Wk TET and Wk TET, we observed a gradient-like effect. With the stronger Wk TET HFS pattern more profound and lasting changes could be induced than with the Wk Wk TET, especially in the excitability as can be appreciated from the I-O curves (**Fig. 14**). We decided to take it a step further and studied a stronger stimulation pattern, the Str TET. The Str TET consisted of 20 trains of 15 pulses at 200 Hz, intertrain interval was 10 sec and pulse width was 0.2 ms.

The Str TET group (n=9) showed an fEPSP-li initial potentiation of 140.4 ± 4.7 % ($p < 0.05$ Wilcoxon test) which differed from the stability control for 2 h after the HFS ($p < 0.05$ Mann Whitney U test) and returned to baseline at 3 h (108.4 ± 4.7 %; $p > 0.05$ Wilcoxon test). In a similar way, the fEPSP-hi even with a lower initial potentiation of 121.3 ± 4.9 %, probed significant differences with the control for the first 2 h ($p < 0.05$ Mann Whitney U test) and after that came to baseline at 3 h (106.1 ± 5.4 %; $p > 0.05$ Wilcoxon test). In contrast to the transient fEPSP potentiation, the PSA with an initial potentiation level of 247.7 ± 12.6 %, which was not statistically different from the initial level of potentiation of the Wk Wk TET and Wk TET groups, remained different from the stability control and from its pretetanus values for all the recording time (PSA 24 h = 173.0 ± 12.0; $p < 0.05$ Mann Whitney U test and Wilcoxon test) (**Fig. 15**).

When the I-O is analyzed (**Fig. 16**), it can be appreciated that no fEPSP potentiation was present in this group ($p > 0.05$ Wilcoxon test), however the PSA was strongly potentiated through the entire studied interval. All points, but the PSA maximum value, were statistically different from its pretetanus values (Wilcoxon test). As a

result a clear left-shift could be observed when plotting the PSA *vs.* fEPSP (the so-called E-S plotting).

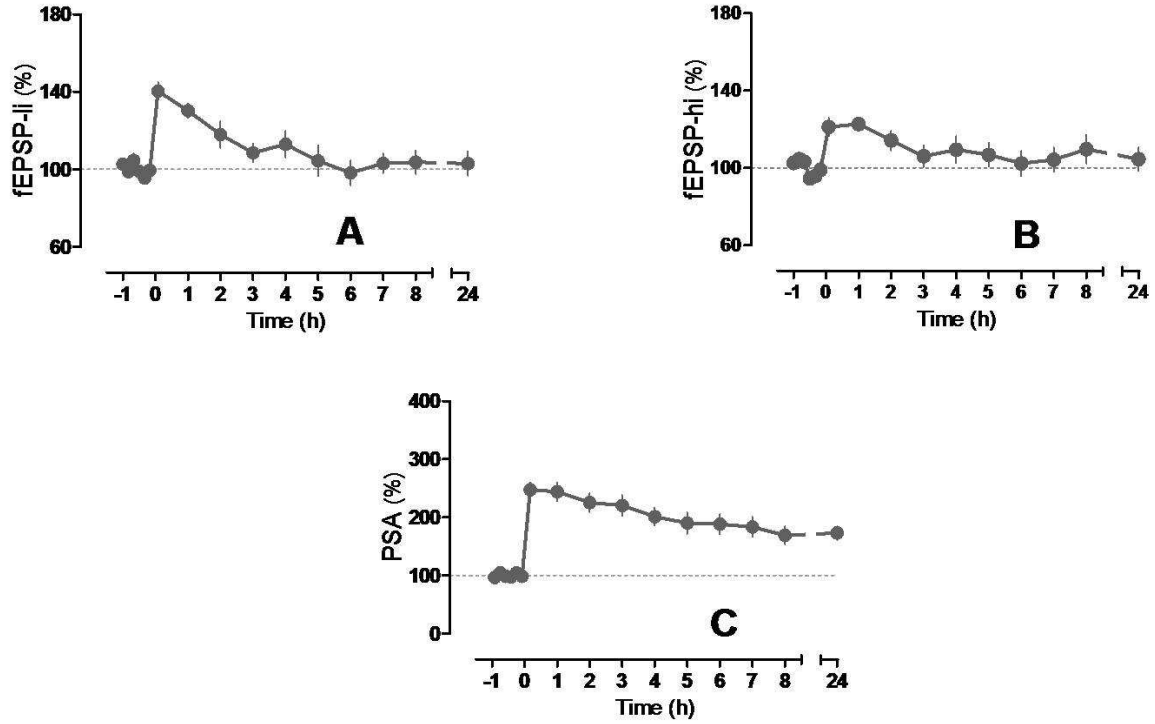

Fig. 15. A long-lasting upregulation of the neuronal excitability occurred after the Str TET HFS. The Str TET produced only a 2 h transient fEPSP potentiation, when compared to the stability control ($p < 0.05$ Mann Whitney U test), that returned to baseline at 3 h ($p > 0.05$ Wilcoxon test). (**A, B).** In spite of just a transitory synaptic potentiation, the PSA (**C**) was potentiated over the entire 24 h recorded period of time ($p < 0.05$ Mann Whitney U test, Wilcoxon test) ($n = 9$).

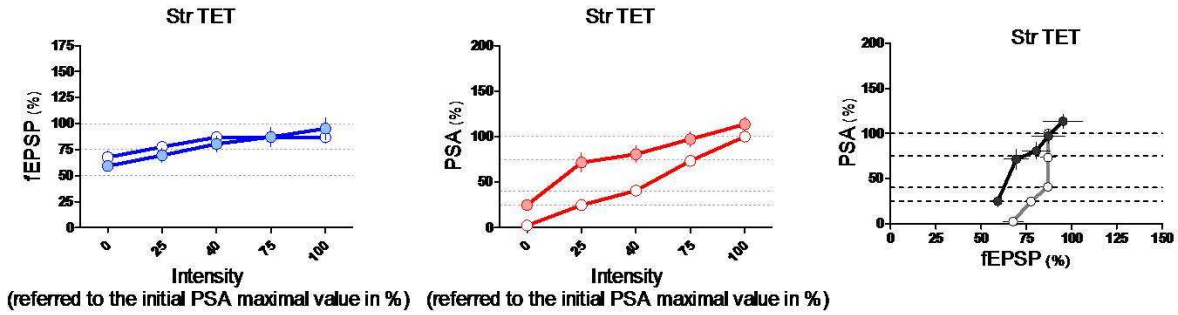

Fig. 16. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). With the Str TET HFS pattern no long-lasting fEPSP potentiation was detected when recording one day after the HFS ($p > 0.05$ Wilcoxon test), however

potentiation of the PSA endured at least until this time at all studied points, except for the PSA maximal value (Wilcoxon test). As a result a clear left-shift can be observed when plotting the PSA *vs.* fEPSP. (n = 9).

Late-LTP

So far, none of the studied HFS patterns (that is, Wk TET, Wk Wk TET or Str TET) was able to induce a long-lasting synaptic potentiation. As has been reported earlier (Frey and Frey, 2009) applying four times the Str TET, with 15 min interval, can result in a synaptic LTP. Thus, we decided to study also this 4xStr TET HFS pattern with the purpose of having a closer look at the induced synaptic potentiation and how it relates to the excitability changes.

The fEPSP-li potentiation 5 min after the last train in the 4xStr TET protocol reached the 153.7 ± 12.3 % value and the fEPSP-hi the 131.6 ± 9.1 % when measured 5 min later (n = 8). These values were not statistically different from the initial potentiation in the Str TET group ($p > 0.05$ Mann Whitney U test); however they remained potentiated over all the 24 h ($p < 0.05$ Mann Whitney U test, Wilcoxon test). The PSA potentiation, initially of 290.9 ± 15.1 %, was also statistically different from its baseline and from the stability control during the entire recording period ($p < 0.05$ Wilcoxon test, Mann Whitney U test) (**Fig. 17**). The I-O curve (**Fig. 18**) showed also an fEPSP potentiation that was statistically significant for the values corresponding to the three highest intensities ($p < 0.05$ Wilcoxon test) and a PSA potentiation for all the range of values ($p < 0.05$ Wilcoxon test). Interestingly, the PSA *vs.* fEPSP plotting showed in this group, as in the Str TET group, a left-shift of the curve corresponding to the values after the HFS regarding the initial curve. This left-shift indicates that the changes occurred in the PSA cannot be fully explained by the synaptic changes that took place in this group.

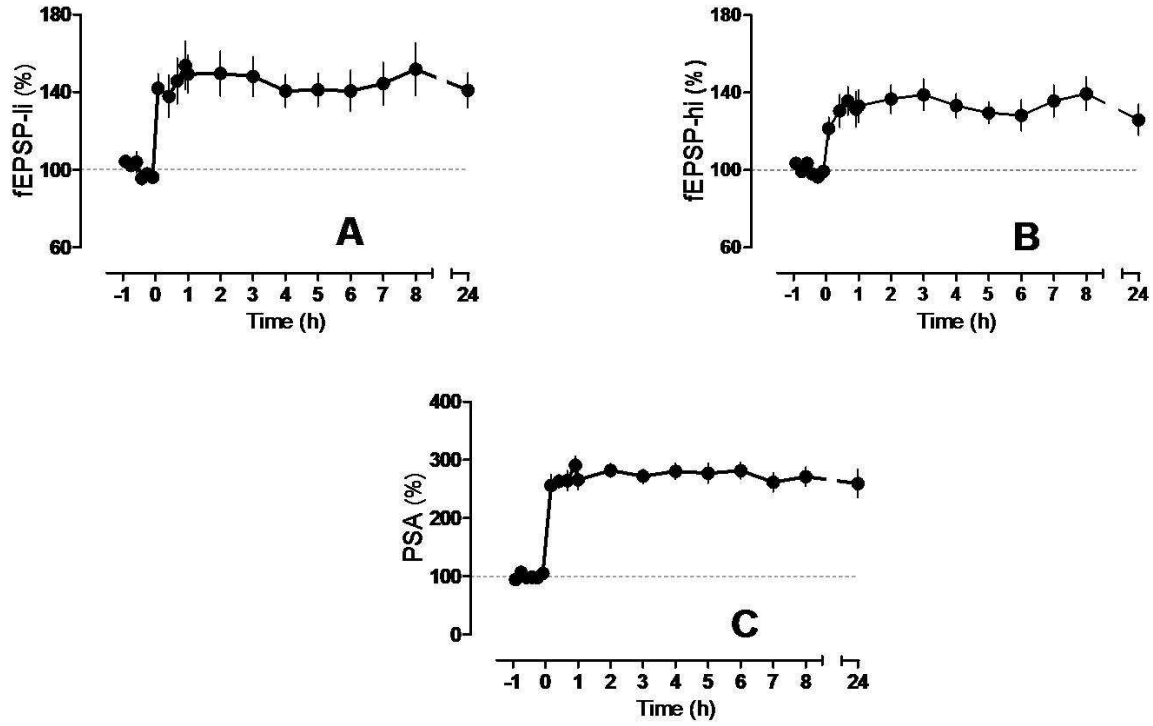

Fig. 17. A long-lasting synaptic potentiation (**A, B)**, for at least 24 h, resulted from the 4xStr TET stimulation pattern, which was accompanied by a PSA potentiation (**C**) that endured also for 24 h. (n = 8, Mann Whitney U test, Wilcoxon test).

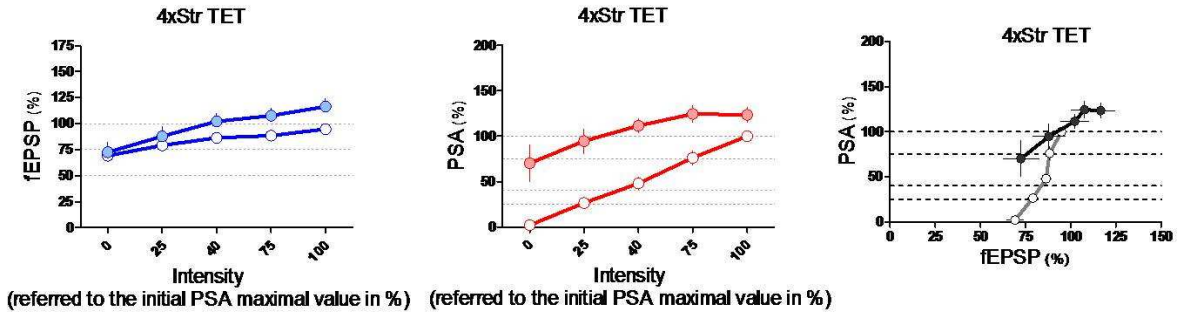

Fig. 18. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). With the 4xStr TET HFS pattern resulted a long-lasting fEPSP potentiation that was statistically significant for the three highest intensities ($p < 0.05$ Wilcoxon test). The PSA was different from its pretetanus values in the entire studied range ($p < 0.05$ Wilcoxon test). A left-shift is appreciated in the PSA *vs.* fEPSP plotting. (n = 8).

It drew our attention the fact that in this 4xStr TET group some animals (n = 2) did not show any long-lasting synaptic potentiation (fEPSP < 105 % at 8 h and/or 24 h), but nevertheless the PSA at 24 h was potentiated up to a level comparable to the PSA potentiation reached in the group of animals where a synaptic potentiation was evident (n = 6). Thus, at 24 h the fEPSP-hi was in the no-fEPSP potentiation-group 103. 6 ± 11.9 % whereas in the fEPSP-potentiated group it reached a value of 133. 4 ± 8.2 % ($p < 0.05$ Mann Whitney U test). However, in spite of this difference the PSA showed similar potentiation in both groups: 237.2 ± 72.0 % and 266.9 ± 27.1 % ($p > 0.05$ Mann Whitney U test) (**Fig. 19**).

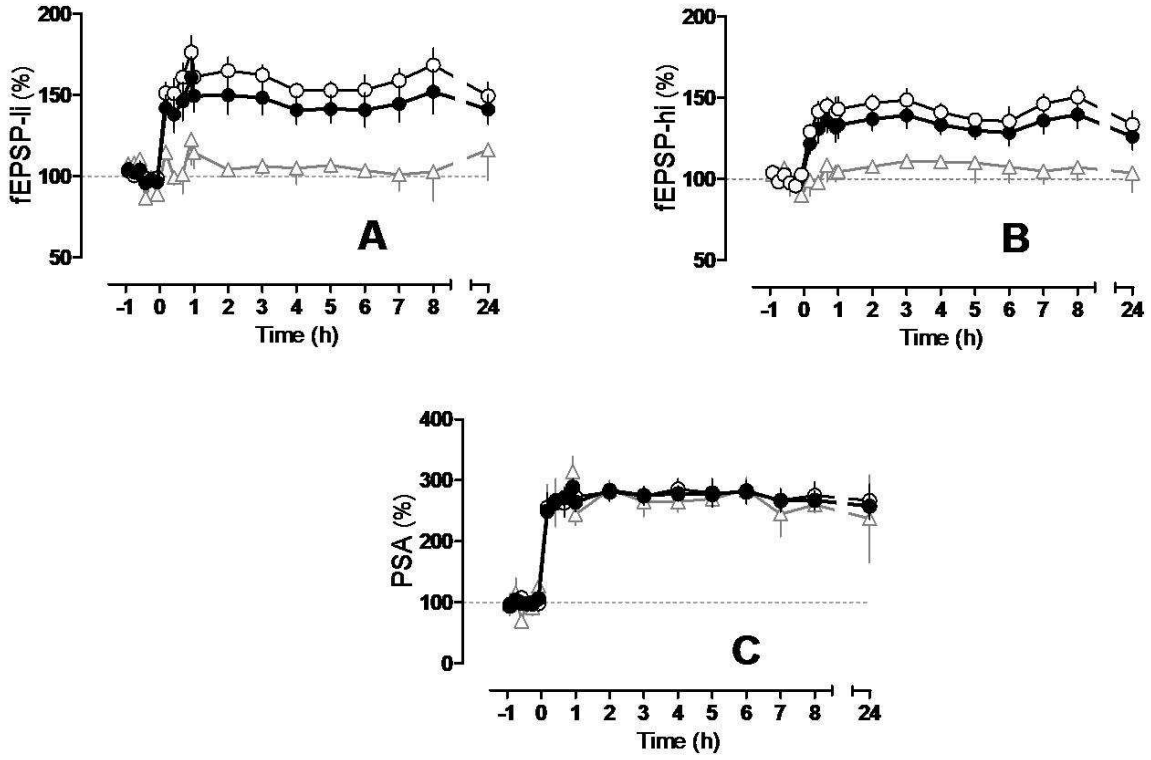

Fig. 19. Two subgroups could be differentiated from the 4xStr TET group, taking into consideration the criteria of fEPSP potentiation < 105 % at 8 h and/or 24 h (**A, B**). From the 8 animals of the 4xStr TET group (black line, filled circles), 2 showed no fEPSP potentiation (gray line, open triangles) however their PSA potentiation was similar to the one showed for the fEPSP potentiated subgroup (black line, open circles) ($p > 0.05$ Mann Whitney U test) (**C**).

3.2- Pharmacology of the late-LTP

The purpose of these series of experiments was to study, with the help of pharmacological manipulations, the synaptic potentiation in the DG of freely moving animals and its relation to the excitability changes to a greater detail. As the 4xStr TET was the only HFS pattern able to induce a reliable long-lasting synaptic potentiation, this was the HFS pattern chosen for the further analysis.

Role of NMDA receptors in the induction of DG neuronal plasticity

Relative stable recordings could be achieved in these pharmacological experiments. Intrahippocampal infusion of physiological saline solution had no significant effects on the evoked potentials; not immediately after injection or at a long term (n = 7) ($p > 0.05$ Wilcoxon test) **(Fig. 20)**. In the I-O curve it is also shown that the fEPSP and PSA values remained stable during the recording period ($p > 0.05$ Wilcoxon test) (**Fig. 21**).

Infusion of the vehicle 10 min before 4xStr TET HFS had no important influence on the LTP induced (n = 11). The fEPSP-li potentiation 5 min after the last train in the 4xStr TET protocol reached the 133.1 ± 8.6 % value and the fEPSP-hi 118.9 ± 3.8 % when measured 5 min later. The fEPSP-li remained potentiated over the first 3 h ($p < 0.05$ Mann Whitney U test), being indistinguishable of its baseline at 4 h ($p > 0.05$ Wilcoxon test), even when a trend to potentiation was observed (fEPSP-li 6 h = 112.7 ± 5.9 % $p = 0.07$ Mann Whitney U test). The fEPSP-hi was however potentiated for all the 6 h ($p < 0.05$ Mann Whitney U test). The PSA potentiation, initially of 258.9 ± 20.4 %, was also statistically different from its baseline and from the stability control during the entire recording period ($p < 0.05$ Wilcoxon test, Mann Whitney U test) (**Fig.**

20). When the I-O curve was examined, the fEPSP was significantly potentiated for the three highest stimulation intensities and the PSA showed increased values over the entire studied range ($p < 0.05$ Wilcoxon test) (**Fig. 21**).

On the other hand, the infusion of the NMDA receptor antagonist D-AP5 10 min before the 4xStr TET HFS abolished any synaptic potentiation (n = 11). None of the recordings differed from the stability control with vehicle infusion or from its baseline pretetatnus values ($p > 0.05$ Mann Whitney U test, Wilcoxon test). Also fEPSP values in this group, both fEPSP-li and fEPSP-hi, differed from 2 - 6 h from the ones of the vehicle plus 4xStr TET group ($p < 0.05$ Mann Whitney U test). The PSA showed a mild potentiation enduring from 1 h until the 5 h ($p < 0.05$ Mann Whitney U test). At 6 h, the PSA value 138 ± 17.1 % was no more statistically different from its baseline or the corresponding stability control ($p > 0.05$ Wilcoxon test, Mann Whitney U test) (**Fig. 20**). The I-O curve showed that indeed no synaptic (fEPSP) potentiation was present and also no PSA potentiation, even when a trend, not statistically significant, was seen to increased PSA values ($p > 0.05$ Wilcoxon test) (**Fig. 21**).

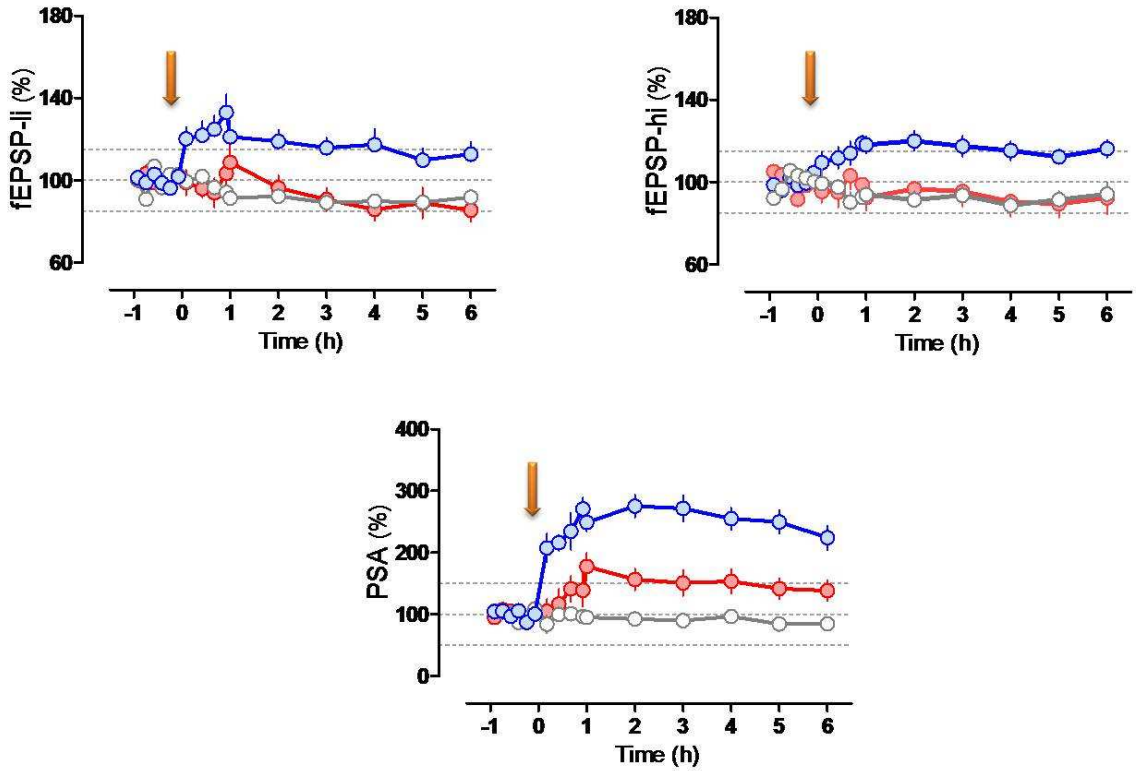

Fig. 20. The intrahippocampal infusion of the NMDA receptor antagonist D-AP5 10 min before the 4xStr TET HFS (red line, filled circles, n = 11) prevented any synaptic potentiation over all the recording time when compared to the stability control with intrahippocampal vehicle infusion (grey line, open circles, n = 7) or its own pretetanus baseline values ($p > 0.05$ Mann Whitney U test, Wilcoxon test). The PSA developed a mild and transient potentiation from 1 h till 5 h ($p < 0.05$ Mann Whitney U test), reaching the baseline values at 6h ($p > 0.05$ Wilcoxon test). The group with vehicle infusion 10 min before the 4xStr TET (blue line, filled circles, n = 11) differed from the D-AP5 plus 4xStr TET group during all the time from 2 – 6 h ($p < 0.05$ Mann Whitney U test). The arrow represents the time point when the intrahippocampal injection occurred.

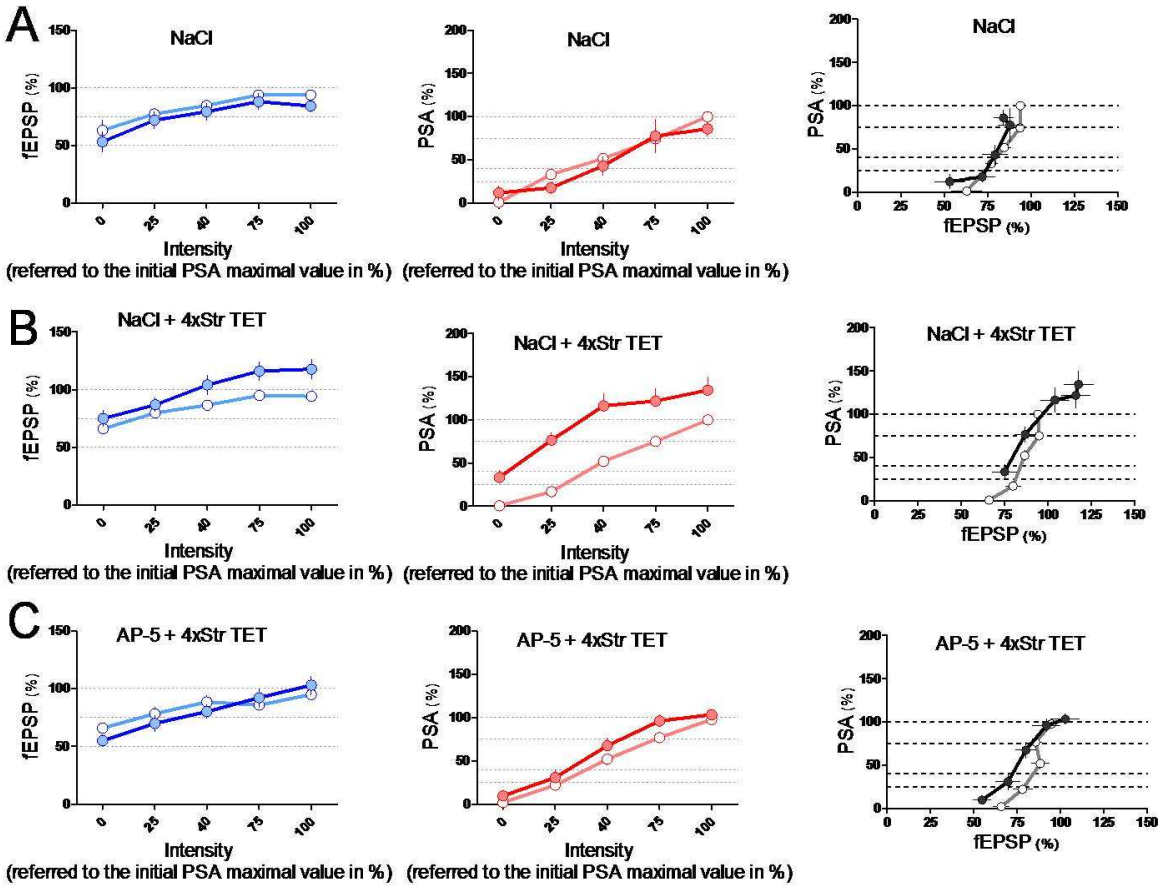

Fig. 21. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). (**A**) Stability control group with vehicle intrahippocampal infusion (n = 7) showed stable fEPSP and PSA values during the experimental time ($p > 0.05$ Wilcoxon test). (**B**) Vehicle injected group 10 min before 4xStr TET HFS (n = 11) exhibited a long-lasting fEPSP potentiation that was statistically significant for the three highest intensities ($p < 0.05$ Wilcoxon test). The PSA was different from its pretetanus values in the entire range that was studied ($p < 0.05$

Wilcoxon test). (**C**) Intrahippocampal injection of the NMDA receptor antagonist D-AP5 10 min before 4xStr TET HFS prevented any statistically significant potentiation of fEPSP or PSA ($p > 0.05$ Wilcoxon test)

Role of PKMzeta in DG late-LTP and its relation to the plasticity of excitability

In our intention to study the synaptic plasticity underpinnings in the DG of freely moving rats and its relation with the excitability changes, the PKMzeta comes to play a key role. Two of the best known PKMzeta inhibitors were used: ZIP and chelerythrine. These inhibitors were intrahippocampally infused 1 h after the LTP induction.

ZIP

First we studied the effects of ZIP (n = 9), scr-ZIP (n = 6) and the vehicle, physiological saline (n = 13), on basal neuronal transmission before performing experiments looking for its effects on synaptic plasticity. As can be recognized from the **Fig. 22**, with none of the three substances deviations were appreciated in the studied evoked potentials over the recording period of 6 h, rather all groups remained stable ($p > 0.05$ Wilcoxon test) and did not show any differences among them ($p > 0.05$ Mann Whitney U test). This result could be corroborated when analyzing the I-O curves ($p > 0.05$ Wilcoxon test) (**Fig. 23**).

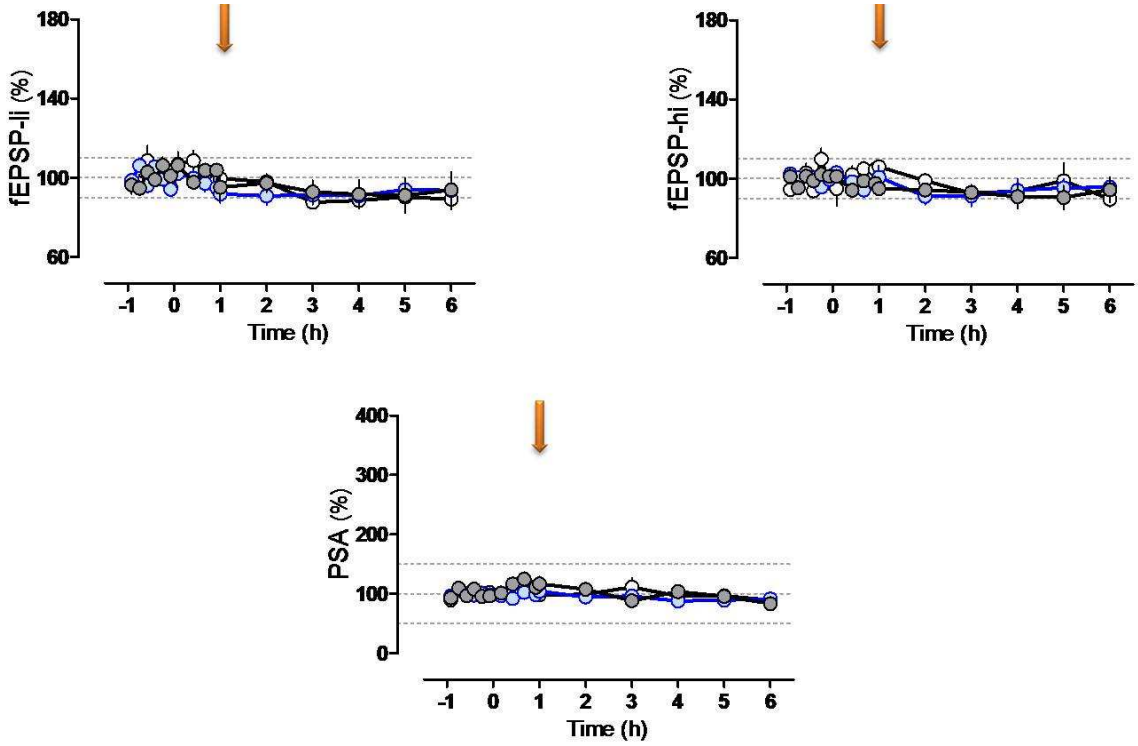

Fig. 22. The intrahippocampal injection of physiological saline (blue line, filled circles; n = 13), scr-ZIP (black line, open circles; n = 7) or ZIP (black line, filled circles; n = 9) had no appreciable effects on basal synaptic transmission or neuronal excitability during the 6 h of recordings ($p > 0.05$ Wilcoxon test). The arrow signals the time point of the intrahippocampal injection.

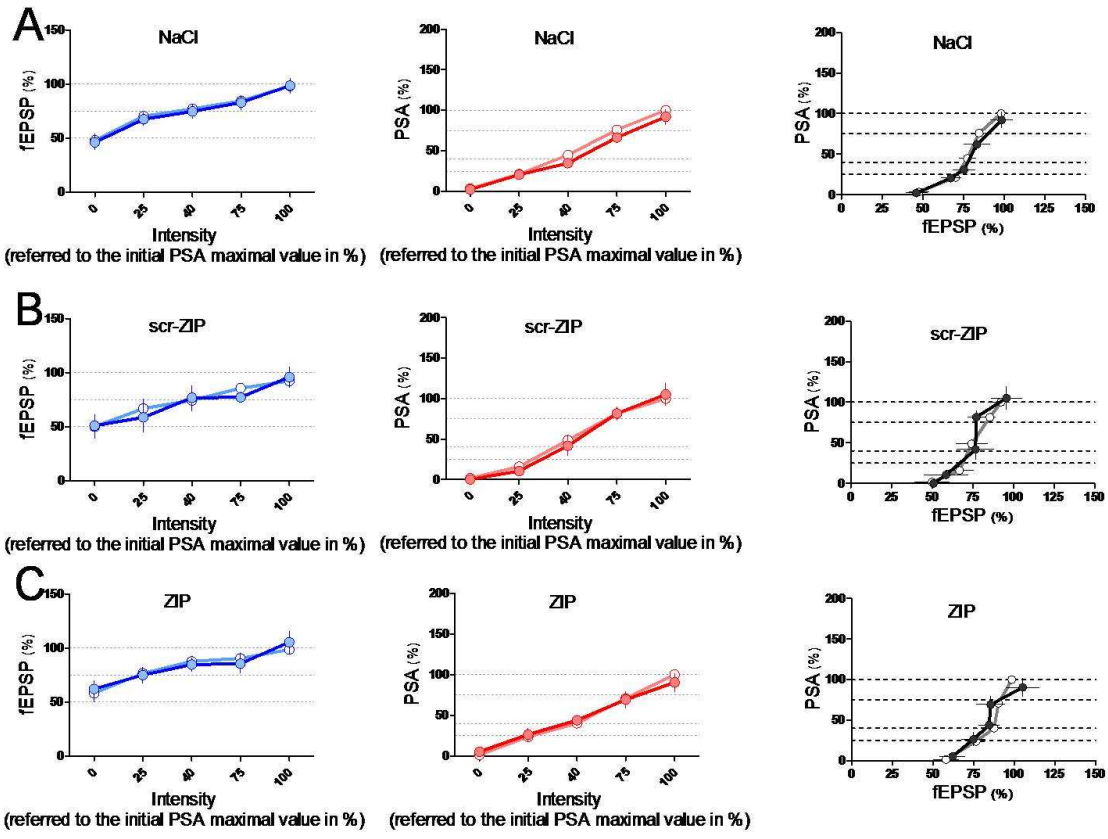

Fig. 23. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). None of the groups (**A**: physiological saline n = 13; **B**: scr-ZIP n = 7; **C**: ZIP n = 9) showed statistically significant differences in the I-O curves; not for the fEPSP, not for the PSA ($p > 0.05$ Wilcoxon test).

In the next series of experiments we applied either saline, ZIP or scr-ZIP 1 h after the initiation of the 4xStr TET HFS protocol (n = 15 in each group). In all the three groups the level of potentiation just before the intrahippocampal injection was similar (in the order saline, ZIP and scr-ZIP; fEPSP-li at 1h: 135.4 ± 5.7 %; 130.1 ± 8.5 %; 136.2 ± 5.7 %; fEPSP-hi at 1h: 119.4 ± 5.0 %; 120.5 ± 5.7 %; 129.4 ± 4.7; PSA at 1h: 237.5 ± 9.2 %; 253.7 ± 15.6 %; 251.5 ± 13.7 %; $p > 0.05$ Mann Whitney U test in all cases). The saline group showed no deviation from the expected behavior and exhibited fEPSP, both fEPSP-li and fEPSP-hi, and PSA significant potentiation over all the 6 h of recording ($p < 0.05$ Wilcoxon test). Furthermore, the intrahippocampal infusion of ZIP had a profound impact on the synaptic potentiation and 2 h after the inhibitor injection, which is 3 h after the 4xStr TET, the fEPSP potentiation was back to its pretetanus baseline ($p > 0.05$ Wilcoxon test). Surprisingly, the PSA potentiation seemed to be unaffected by ZIP and remained stable potentiated over the 6 h ($p > 0.05$ Wilcoxon test), at a level similar to the one of the saline group ($p > 0.05$ Mann Whitney U test). The scr-ZIP group, even when seemed to follow a trend similar to the saline group, displayed just a transient fEPSP potentiation, for both fEPSP-li and fEPSP-hi, until 3 h when compared to the corresponding stability control ($p < 0.05$ Mann Whitney U test). The fEPSP (fEPSP-li and fEPSP-hi) was at 4 h statistically indistinguishable from its own baseline ($p > 0.05$ Wilcoxon test). It is interesting to note that the fEPSP potentiation for this scr-ZIP group was at 6 h, for both the fEPSP-li and fEPSP-hi, not statistically different from the ZIP's group; but was also statistically equivalent to the saline's group ($p > 0.05$ Mann Whitney U test).

Regarding the PSA, in the scr-ZIP it was significantly potentiated for the 6 h, as in the other groups ($p < 0.05$ Wilcoxon; $p > 0.05$ Mann Whitney U test) (**Fig. 24**). When the I-O curves were analyzed the above results were confirmed (**Fig. 25**). All three groups showed potentiated PSA over the entire studied range and the main differences were concentrated on the fEPSP. Whereas in the saline group the fEPSP was upregulated for all the stimulation intensities, no fEPSP potentiation was verified for the two other groups (Wilcoxon test).

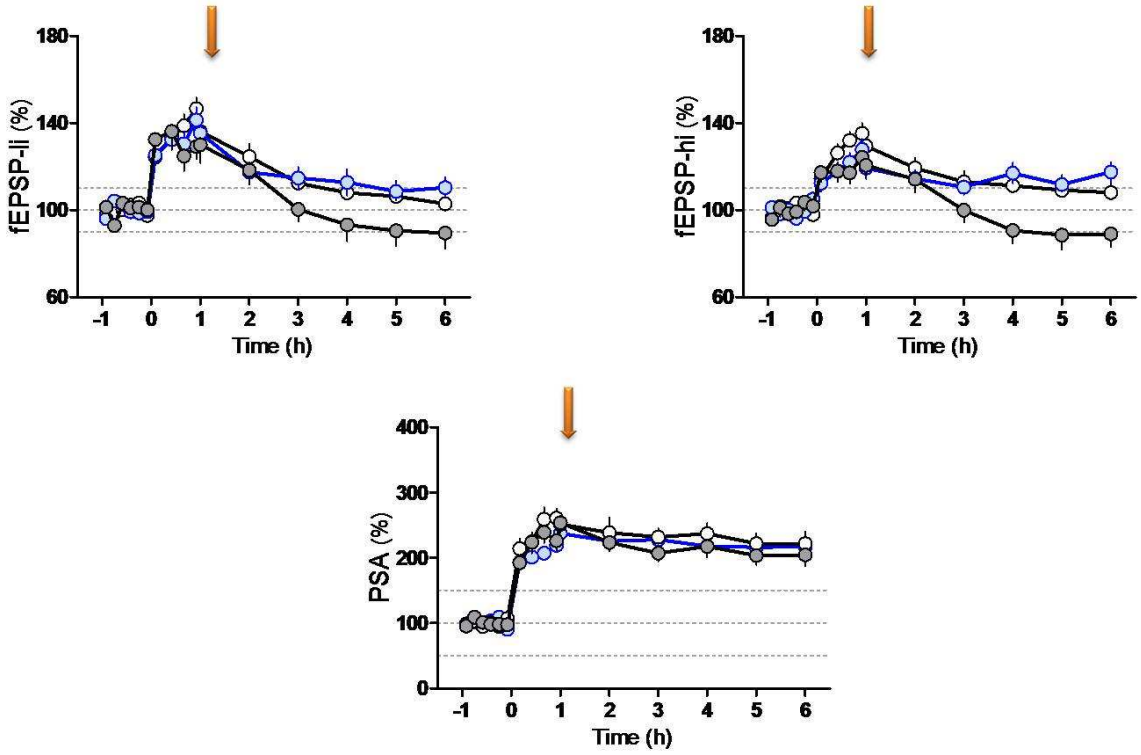

Fig. 24. The intrahippocampal infusion of the PKMzeta inhibitor ZIP 1 h after the 4xStr TET HFS (black line, filled circles, n = 15) completely reversed the fEPSP potentiation to its pretetanus baseline values. However it had no appreciable effects on the PSA, which remained upregulated for at least 6 h (Wilcoxon test). The saline group (blue line, filled circles, n = 15) displayed potentiated fEPSP and PSA during the entire recording period ($p < 0.05$ Wilcoxon test). Scr-ZIP intrahippocampal infusion (black line, open circles, n = 15) did not affect the PSA potentiation, however it provoked an attenuation of the fEPSP potentiation, that even when followed a similar trend to the saline's group, was over at 4 h (Mann Whitney U test, Wilcoxon test). The arrow signals the time point of the intrahippocampal injection.

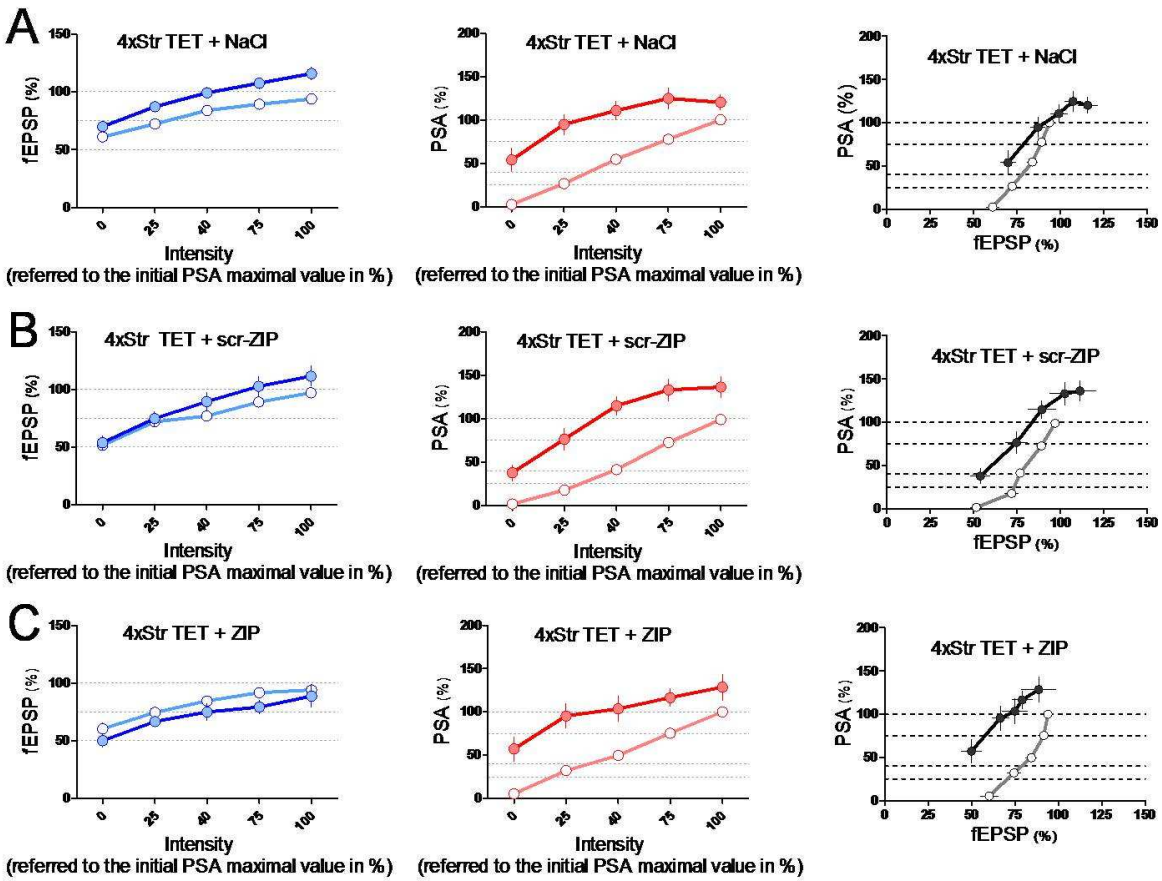

Fig. 25. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). None of the intrahippocampally infused substances (**A**: physiological saline, **B**: scr-ZIP, **C**: PKMzeta inhibitor ZIP; n = 15 in each group) affected the PSA potentiation that occurred as a result of the 4xStr TET ($p < 0.05$ Wilcoxon test). However the fEPSP showed a significant potentiation only for the saline group ($p < 0.05$ Wilcoxon test for each stimulation intensity).

Chelerythrine

We used the PKC catalytic subunit inhibitor chelerythrine (Herbert et al., 1990), as a second PKMzeta inhibitor to verify our results with ZIP.

As ZIP, no evident changes occurred in the basal synaptic transmission or the neuronal excitability with the intrahippocampal infusion of chelerythrine (n = 3) (**Fig. 26**). The I-O curve confirms this result (**Fig. 27**).

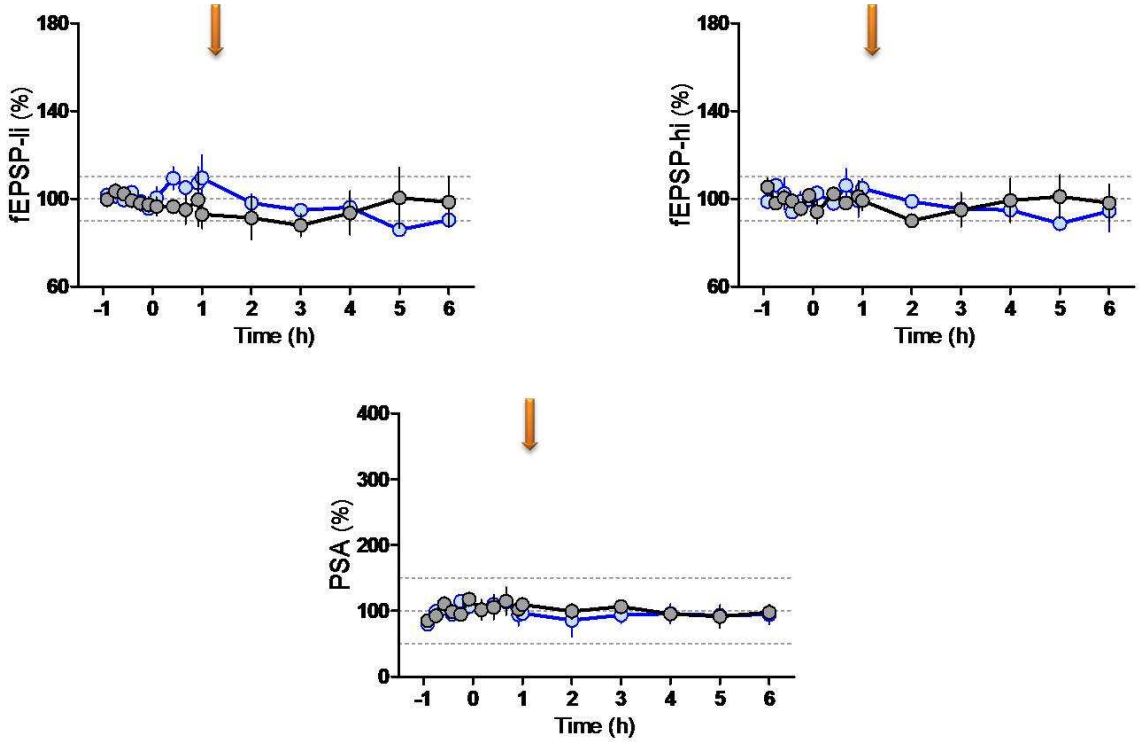

Fig. 26. The intrahippocampal injection of dH2O (blue line, filled circles; n = 3) or chelerythrine (black line, filled circles; n = 3) had no appreciable effects on basal synaptic transmission or neuronal excitability during the 6 h of recordings. The arrow signals the time point of the intrahippocampal injection.

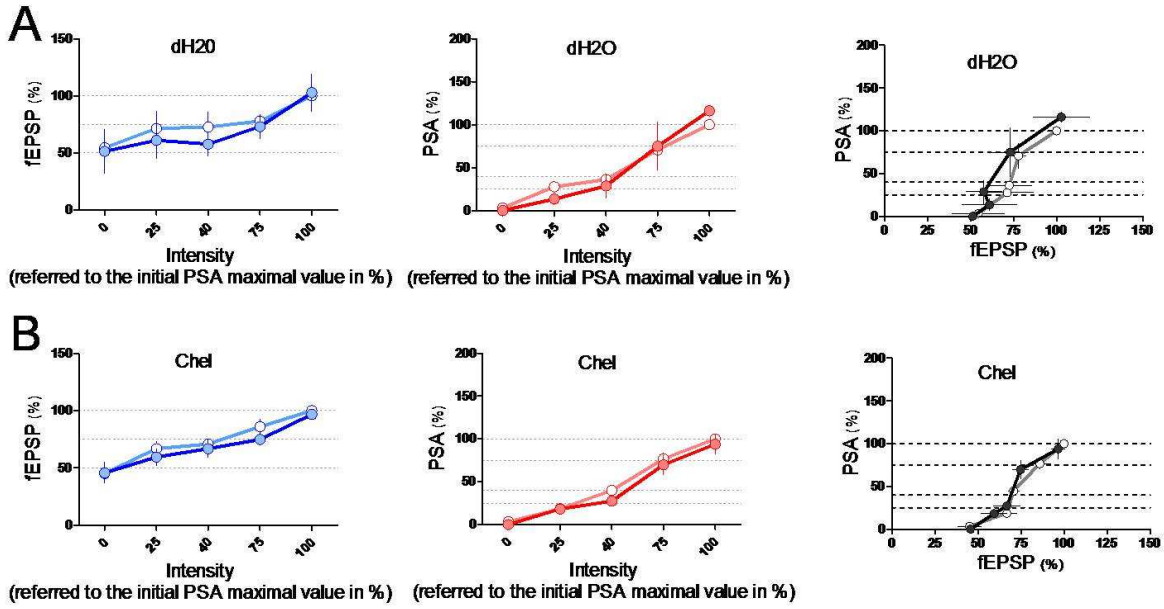

Fig. 27. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). None of the groups (**A**: dH2O n = 3; **B**: chelerythrine n = 3)

showed statistically significant differences in the I-O curves; not for the fEPSP, not for the PSA ($p > 0.05$ Wilcoxon test).

When injected 1 h after the 4xStr TET, chelerythrine completely reversed the fEPSP potentiation back to baseline at 3 h ($n = 11$, $p > 0.05$ Wilcoxon test). The fEPSP potentiation level exhibited by the vehicle infusion group, for both fEPSP-li and fEPSP-hi, ($n = 14$) was statistically different at 6 h from the chelerythrine's group ($p < 0.05$ Mann Whitney U test). Interestingly, the PSA potentiation in the chelerythrine group displayed a trend to decrease after the 3 h, but it was still at 6h statistically different from its baseline ($p < 0.05$ Wilcoxon test) and did not differ statistically from the PSA potentiation exhibited for the vehicle group ($p > 0.05$ Mann Whitney U test). Both, the vehicle and the chelerythrine groups, showed similar potentiation levels just before the injection (in the order vehicle, chelerythrine; fEPSP-li at 1h: 126.4 ± 5.9 %; 132.7 ± 8.8 %; fEPSP-hi at 1h: 122.7 ± 4.5 %; 131.5 ± 8.5 %; PSA at 1h: 234.2 ± 18.8 %; 222.7 ± 18.3 %; $p > 0.05$ Mann Whitney U test in all cases) (**Fig. 28**).

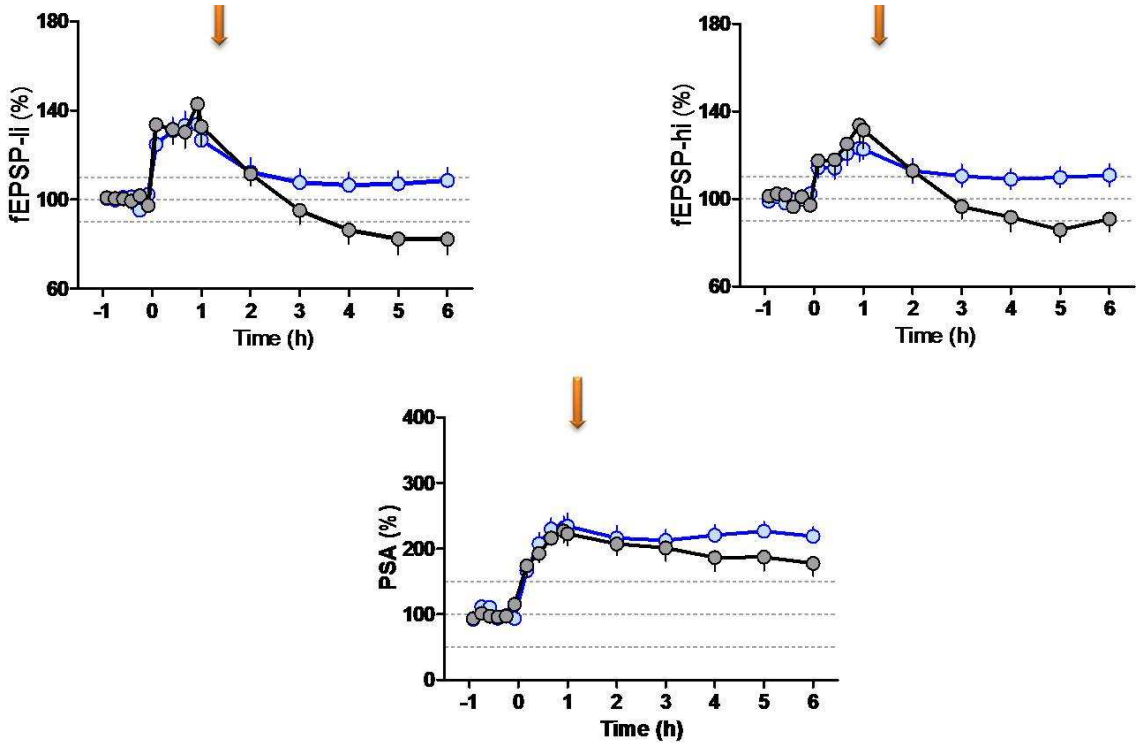

Fig. 28. The intrahippocampal infusion of the PKMzeta inhibitor chelerythrine 1 h after the 4xStr TET HFS (black line, filled circles, n = 11) completely reversed the fEPSP potentiation to its pretetanus baseline values (p > 0.05 Wilcoxon test). However it had no statistically significant effects on the PSA, which remained upregulated for at least 6 h (p > 0.05 Wilcoxon test), even when a trend to decrease was observed. The fEPSP potentiation in the vehicle group (blue line, filled circles, n = 14) was statistically different from the chelerythrine's group at 6 h, for both fEPSP-li and fEPSP-hi (p < 0.05 Mann Whitney U test). The PSA remained potentiated for all the 6 h and was indistinguishable from the chelerythrine's group at 6 h (p < 0.05 Wilcoxon test, p > 0.05 Mann whitney U test). The arrow signals the time point of the intrahippocampal injection.

The I-O curve revealed a significant fEPSP potentiation for the 3 highest intensities and a PSA potentiation over the entire studied range in the vehicle group (p < 0.05 Wilcoxon test). The chelerythrine group showed no fEPSP potentiation (p > 0.05 Wilcoxon test), nevertheless PSA was statistically different from all the pretetanus values, but the maximal PSA (Wilcoxon test))(**Fig. 29**).

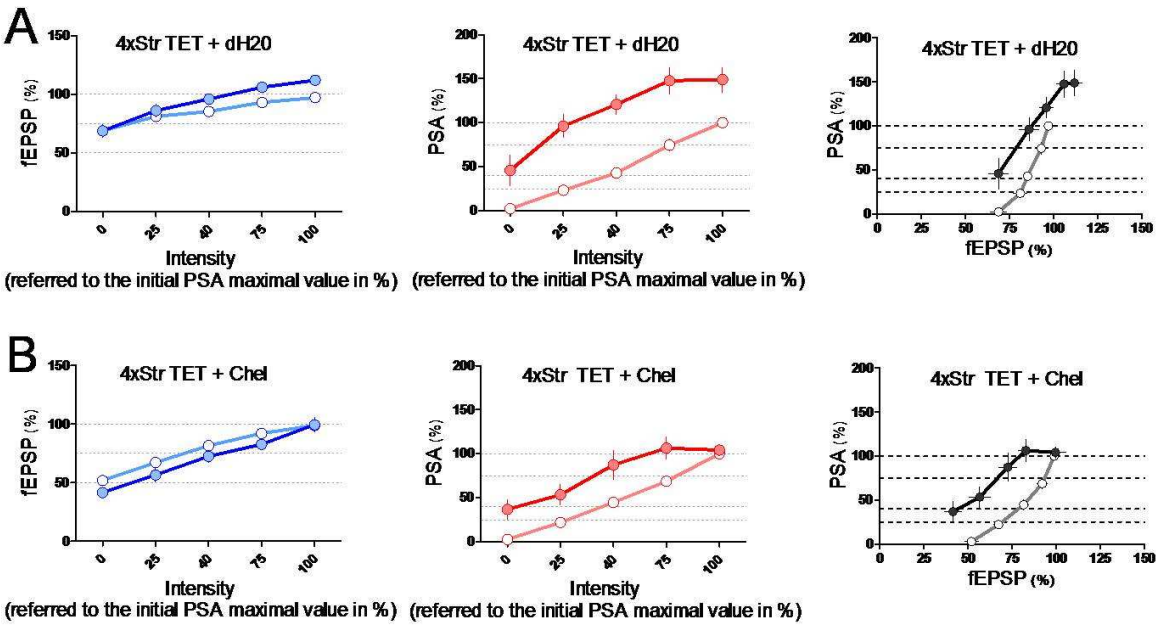

Fig. 29. Input-output curves were generated at the first day of the experiment (open circles) and also at the third and final day (filled circles). None of the intrahippocampally infused substances (**A**: dH2O, n = 14; **B**: chelerythrine, n = 11) affected the PSA potentiation that occurred as a result of the 4xStr

TET ($p < 0.05$ Wilcoxon test), except for the maximal PSA in the chelerythrine group which was no statistically different from its pretetanus value ($p > 0.05$ Wilcoxon test). The fEPSP showed a significant potentiation only for the vehicle group ($p < 0.05$ Wilcoxon test for the three highest stimulus intensities).

4- Discussion

The operations carried out by neurons from the reception of the stimulus until the final firing response, are complex and not completely comprehended. They are highly regulated processes involving, for instance, receptors, ionic channels and yet other neural cells. Studying these processes in freely moving animals is indispensable. Preparations such as *in vitro* slices or anaesthetized animals provide extensive possibilities of experimental manipulations, but they lack some physiological features like the intact neuromodulatory afferents and the integral inhibitory network that can be preserved in freely moving animals.

For some time we have studied the DG LTP in freely moving animals, its mechanisms and how it is regulated by emotional-related manipulations. We have identified that an early form of LTP can be transformed into a late form in the hippocampus by electrical stimulation of neuromodulatory afferents, such as the basolateral amygdala, or by a behavioral appetitive reinforcer if these stimuli are applied in an adequate time window around the LTP induction ((Frey et al., 2001;Seidenbecher et al., 1997) own work: (Almaguer-Melian et al., 2010;Bergado et al., 2007;Bergado et al., 2009)). But an historical and large limitation of these studies in freely moving animals has been the lack of the simultaneous and accurate recording of both, the fEPSP and the PS. For technical reasons, most of the studies involved merely the measurement of the PS through a recording electrode placed in the hilus and a “dirty” estimate of the fEPSP using this same electrode located far away from the site of generation of the triggered response. The hilus is a densely populated area of the DG and many other electrical fields can interfere with the fEPSP, producing a heterogeneous potential difficult to interpret. To overcome these problems a method was used to measure both the EPSP and the PS in the proximity

of their generation sites in the DG of freely moving animals (Frey and Frey, 2009). This allowed us to take a closer look at the relationship between synaptic and excitability changes induced by different paradigms. The current approach also provided a better control over the stimulation used to induce the plastic event.

4.1- Different HFS protocols induce different forms of long-term potentiation in the DG of freely moving rats

The first series of experiments examined the effect of four HFS patterns of different strength on the time course of fEPSP and PS using a double recording electrode technique.

Early-LTP

We know from previous work in our laboratory that the two weakest HFS, i.e. Wk Wk TET and Wk TET, are able to induce transient PSA potentiations with slight differences. With the present work, we confirmed these findings. Both protocols induced a PSA potentiation that returned to baseline after 24 h. However, the Wk TET induced a more endurable potentiation than the Wk Wk TET, which was also evident from the I-O curves: the Wk TET group showed a reduced PS threshold at 24 h. The Wk Wk TET induced a milder potentiation not only regarding the PSA but also fEPSP. However, fEPSP did not show any potentiation at 24 h in any of the groups. From the analysis of these two groups we can conclude that: (i)- very slight variations in the tetanization protocol can have a profound impact on the induced plastic events; (ii)- even when the recording of the evoked potentials at a single stimulation intensity reveals a certain trend, it does not necessarily mean that the neuronal population

responses over a broader range of input intensities follow the same trend; (iii)- even weak HFS patterns can produce durable excitability changes.

It is well documented that tetanization parameters play a significant role in determining the quality and extent of the plastic changes. The amount of postsynaptic depolarization is determined by the specific tetanization protocol, and thus the further opening of certain voltage-gated channels, such as voltage-dependent calcium channels (VDCCs) (Grover and Teyler, 1990;Morgan and Teyler, 1999), the release of neuromodulatory transmitters (Lessmann and Brigadski, 2009) and heterosynaptic plasticity events (for instance in GABAergic interneurons) (Kullmann and Lamsa, 2007;Ross and Soltesz, 2001). This is one of the reasons we chose to construct the I-O curves in a new way, to have a better control for each particular animal over the stimulation used to test recordings and tetanization. In the CA1 region, there is a substantially different outcome when the same HFS pattern is applied to contralateral CA3 using different stimulus intensities (Hassan et al., 2006). With a higher intensity, an initial depression is induced, while using a lower intensity no depression occurs.

Hippocampal principal cells are supplied with afferent input volleys of varying intensities (Buzsaki et al., 1983). Therefore, a description of how neural cells are affected by behavior or LTP must employ the full dynamic range of inputs. For example, in our Wk TET group no PSA potentiation was appreciated for a stimulation intensity evoking 40 % of the maximal value; however we had a significant potentiation for the lowest studied intensity. This kind of intensity-specific potentiation could have a functional meaning. For a given learning paradigm or memory engram, it could be useful to allow just some particular intensity range signals, and not all the information, to pass to the next neural station.

It is interesting to note that both weak HFS patterns studied produced some perdurable excitability changes in the neural population, indicating they are not as innocuous as sometimes they are assumed to be.

Early-LTP with profound changes in excitability

In the Str TET group, only a transient synaptic potentiation occurred in contrast to the long-lasting PSA potentiation. As a consequence, a clear left shift was present in the PSA *vs*. fEPSP plot. With this group, it becomes clearer that the PSA is not always a direct measure of the synaptic change. If only the PSA is considered, it is almost impossible to say whether the observed change is entirely synaptic, entirely a change in the input-output coupling or something in between. Hence the necessity of measuring both, the fEPSP and the PS, to define whether the observed modifications correspond to synaptic and/or excitability reasons. In this regard, it would be important to reinvestigate the effects of some LTP reinforcers, like BLA electrical stimulation, which reinforcing effect has been assessed using a single recording electrode in the hilus (Frey et al., 2001). There is the possibility that BLA stimulation produces an increase in the DG neurons excitability instead of, or in addition to, causing synaptic modifications. The evidence that the BLA reinforcing effect (or others like the appetitive behavioral reinforcement (Bergado et al., 2003)) is dependent on protein synthesis (Frey et al., 2001) does not necessarily support the synaptic hypothesis because, as mentioned before, protein synthesis seems to be a requirement for some excitability changes as well (Cohen-Matsliah et al., 2007;Lopez de et al., 2007;Xu et al., 2005). In fact, in an earlier work (Straube and Frey, 2003) a HFS pattern, similar to the one used in this dissertation (Str TET), was shown to produce a long-lasting PSA potentiation that was dependent on protein synthesis.

This finding supports the idea that excitability changes in the DG are also protein synthesis-dependent.

Late-LTP

The 4xStr TET was the only HFS pattern studied able to induce a fEPSP late-LTP. In the 4xStr TET group, both the fEPSP and the PSA remained potentiated for 24 h. Nevertheless, a left shift was appreciated in the PSA *vs.* fEPSP plotting. This left shift indicates that the fEPSP potentiation cannot completely explain the changes occurred in PSA. There is a component of the PSA change that is due to other causes beyond the synaptic one.

As discussed before, excitability changes can occur at least for two reasons: a change in the excitatory-inhibitory balance or changes in the voltage-dependent ionic channels. Interneurons constitute a very diverse and complex population in the DG. Their function can be drastically affected in activity-dependent ways: the mossy fiber-excitatory input to basket cells is able to undergo LTP with tetanic stimulation (Alle et al., 2001) and a long-lasting depolarization has been reported to occur in DG interneurons located in the granule layer-hilar border as a result of a PP HFS (Ross and Soltesz, 2001). Other hippocampal interneurons also show activity-dependent plasticity. Potentiation and depression has been reported to occur at the excitatory synapses onto interneurons (Lapointe et al., 2004;McMahon and Kauer, 1997;Perez et al., 2001) and at the inhibitory synapses of interneurons on pyramidal cells (Caillard et al., 1999;Shew et al., 2000) in CA1.

Electrical signals in the nervous system are generated by the movement of ions across the nerve cell membranes. Thus, the importance of voltage-dependent ion channels in neuronal physiology is obvious. Their function can be activity-dependent

changed producing a facilitatory effect on the ability of neurons to fire action potentials (Frick et al., 2004;Xu et al., 2005), but also yielding a down-regulation of the excitability and acting thus as a homeostatic mechanism (Campanac et al., 2008;Fan et al., 2005).

From our data we cannot certainly elucidate whether the observed excitability changes in the different groups are due to changes in the inhibition, in the properties of the voltage-gated channels or other reasons. Further studies are required to clarify the contribution of these or other factors affecting the granule cell excitability.

Another interesting finding in the 4xStr TET group was that a subgroup of animals that did not show long-term synaptic potentiation (LTP) exhibited PSA-potentiation levels similar to the subgroup where a synaptic potentiation was verified. Since the very first LTP description, Bliss and coworkers described that: “Increases in amplitude of the population spike and decreases in its latency have sometimes been seen with no change in the synaptic wave” (Bliss and Gardner-Medwin, 1973). Our results add to this the fact that similar PSA potentiation levels were achieved in both subgroups. What we would expect is that in the non-fEPSP potentiation group the PSA were potentiated but to a lesser extent than in the fEPSP potentiation group, showing just the component of the PSA potentiation independent of the synaptic potentiation (see above). The actual finding, however, points to a PSA upregulation that is apparently completely independent of the fEPSP potentiation. With the next series of experiments we pretended to shed light onto the mechanisms of these PSA and fEPSP potentiation with the help of pharmacological tools.

4.2- Pharmacology of the late-LTP

Since we were interested in exploring the mechanisms of synaptic late-LTP in the DG as well as its relation with the neuronal firing outcome, we had chosen the 4xStr TET tetanization paradigm for these series of pharmacological experiments; because it was the only one able to induce a fEPSP late-LTP.

Role of NMDA receptors in the induction of DG neuronal plasticity

As a first step in investigating the mechanisms of the DG late-LTP, we decided to study its induction dependency on the NMDA receptor. Two substances have provided the pharmacological tools to investigate the role of NMDA receptors in discrete synaptic pathways. These are the competitive antagonists AP-5 and 2-amino-7-phosphonoheptanoic acid (AP-7). For both substances, their D isomers are the active forms (Collingridge and Watkins, 1995) . We injected the antagonist D-AP5 intrahippocampally 10 min before 4xStr TET HFS and we found a complete abolishment of the fEPSP LTP and a substantial reduction of the PSA LTP.

Our results are in agreement with previous studies in anaesthetized rats (Errington et al., 1987;Morris, 1989), unanaesthetized rats (Laroche et al., 1989) and hippocampal slices *in vitro* (Colino and Malenka, 1993;Hanse and Gustafsson, 1992) that have shown that NMDA receptor antagonists block synaptic LTP induction in the medial PP. Glutamate binding to the NMDA receptor together with postsynaptic membrane depolarization triggers LTP by allowing calcium entry through the NMDA receptor-linked channel (Lynch et al., 1983;Malenka et al., 1988;Regehr and Tank, 1990). Thus, preventing glutamate binding to the NMDA receptor with D-AP5 blocks the synaptic LTP induction.

Interestingly, the PSA potentiation was also considerably reduced, however not completely abolished for the first hours. This suggests that the induction of the PSA potentiation depends mainly on NMDA receptor activation, but further mechanisms could have a potential role in the induction of the PSA potentiation. There is also the possibility that the threshold activation of NMDA receptors for triggering PSA potentiation is lower than the one for the fEPSP potentiation.

The fact that both fEPSP and PSA potentiation were reduced with D-AP5 does not necessarily tell us that the PSA potentiation was reduced because of the fEPSP potentiation abolishment. PSA and fEPSP potentiations could be independent processes, but both NMDA–dependent. In fact, a number of changes in intrinsic excitability are NMDA receptor dependent (Daoudal et al., 2002;Fink and O'Dell, 2009;Jester et al., 1995).

Role of PKMzeta in DG late-LTP and its relation to the plasticity of excitability

A major challenge in neuroscience has been to understand how our brain is able to store information for long periods of time. Many molecules are important for the formation of long-term memory, but not for its maintenance. Receptors, second messengers and their effectors are involved in the initial stabilization of memory and ultimately regulate the synthesis of new proteins, the hallmark of memory consolidation. However when inhibitors of these molecules or inhibitors of protein synthesis are given after this initial phase of consolidation, none of them disrupts the storage of an established long-term memory (Sanes and Lichtman, 1999). Recently, a lot of attention has come over a single molecule thought to be a key player in this long-term storage process: PKMzeta.

PKMzeta was first described as a potential candidate for memory maintenance in relation to its role in LTP. When analyzing the effects of CA1 LTP induction *in vitro* on various PKC isoforms, Sacktor and colleagues noted that most PKC isoforms were affected just transiently in translocating from the cytosol to the membrane. This effect vanished within 1 min. The only noticeable persistent effect was the upregulation of the PKMzeta expression, which persisted for at least 2 h. Indeed, the increase in PKMzeta correlated with the amount of synaptic potentiation during LTP maintenance (Osten et al., 1996;Sacktor et al., 1993). The first concrete evidence of the role of PKMzeta in memory came from a work on odor avoidance conditioning in *Drosophila*. PKMzeta overexpression during a certain time-window after learning improved memory persistence. Also, blocking the PKMzeta activity prevented long-term memory formation without affecting short-term memory (Drier et al., 2002). Later, several studies have confirmed that PKMzeta plays a key role in the maintenance of different kinds of memories (Madronal et al., 2010;Serrano et al., 2008;Shema et al., 2007;Shema et al., 2009;von Kraus et al., 2010), but see (Parsons and Davis, 2011). The mechanism underlying these profound effects of PKMzeta on memory and LTP maintenance seems to be the reconfiguration of AMPA receptor trafficking to persistently increase their number at postsynaptic sites (Ling et al., 2006;Migues et al., 2010;Yao et al., 2008).

Regarding the DG specifically, the only report of PKMzeta contribution to synaptic plasticity came from a 2006 study (Pastalkova et al., 2006). However, the study was carried out in anaesthetized rats using a single recording electrode in the hilus. Thus, we decided to reinvestigate this issue in freely moving animals to avoid the unspecific influence of anesthetics and using our double recording electrode for a better estimation of the synaptic and excitability changes.

We could show that two PKMzeta inhibitors, ZIP and chelerythrine, when applied intrahippocampally 1 h after the 4xStr TET HFS, are able to reverse the fEPSP potentiation to its pretetanus values. This result coincides with the previous work in anaesthetized animals (Pastalkova et al., 2006) showing the complete reversal of LTP after ZIP infusion. On the other hand, the control peptide scr-ZIP caused an fEPSP potentiation of a value intermediate between the vehicle group's and the ZIP group's levels. Still the effect of scr-ZIP on fEPSP potentiation was weaker than ZIP's effect. This issue, taking into consideration the negligible effects of scr-ZIP on basal synaptic transmission, seems to be related with a possible residual inhibitory activity of scr-ZIP on PKMzeta. The scr-ZIP peptide should be innocuous, at least regarding its PKMzeta inhibitory activity. However scr-ZIP is able to inhibit PKMzeta to some extent (Todd Sacktor, personal communication). Some others have also found problems when using scr-ZIP as a control peptide for ZIP, reporting ZIP-like inhibition for scr-ZIP peptide (Kwapis et al., 2009).

An astonishing result was the apparent lack of effect of PKMzeta inhibition on the PSA potentiation. Indeed, the PSA remained potentiated at the same level of controls with no decrease. Chelerythrine showed a slight effect on the PSA potentiation, unlike ZIP, but it was still evident and non-distinguishable from control at 6 h. This dissociation among the fEPSP and PSA corroborates our previous observation in the 4xStr TET experiments without pharmacological manipulations that regardless of the fEPSP potentiation, PSA was potentiated to a similar level for 24 h (**Fig. 19**). However, these results are not in accordance with the previous work of Pastalkova and colleagues. They found that ZIP application produced a complete reversal of the PSA potentiation. Our work differs in some instances, which could provide an explanation for the different outcomes. First of all, their work was done in urethane anaesthetized animals and urethane can have a profound effect on NMDA receptor

functioning (Hara and Harris, 2002;Rockhold et al., 1994), protein synthesis (Brzeznicka et al., 1987;De Kock and Neethling, 1972), GABAergic inhibitory function (Nicoll, 1972;Shirasaka and Wasterlain, 1995) and other neuronal functional properties (Moore and Appenteng, 1990;Riedel et al., 1994). Second, they used a relatively high number of test recordings (one test stimulus every minute) during a long time (24 h). This huge amount of stimulation could have caused some unspecific effects on the induced plastic changes (Fonseca et al., 2006). Third, their intrahippocampal injection of ZIP was done at a different time point (22 h after tetanization *vs.* 1 h after tetanization in our experiment). It could be the case that at later periods the PSA potentiation dependence on PKMzeta changes.

Chelerythrine, a benzophenanthridine alkaloid rather than a pseudosubstrate peptide like ZIP, is a general inhibitor of the catalytic domain of PKCs that strongly inhibits PKM forms (Herbert et al., 1990;Ling et al., 2002;Thompson and Fields, 1996). We support that the slight effect of chelerythrine on PSA potentiation could be due to some PKMzeta-unrelated inhibition of chelerythrine. The peptide ZIP is much more specific in inhibiting PKMzeta than chelerythrine (Ling et al., 2002) and we did not observe any noticeable effect of ZIP on PSA potentiation.

In general, our results with the PKMzeta inhibitors suggest that the synaptic plasticity in the DG of freely moving animals is dependent on PKMzeta for its maintenance; however the excitability changes co-induced with the synaptic LTP are PKMzeta-independent and can support the PSA potentiation even when the synaptic LTP has already returned to baseline (**Fig. 30**).

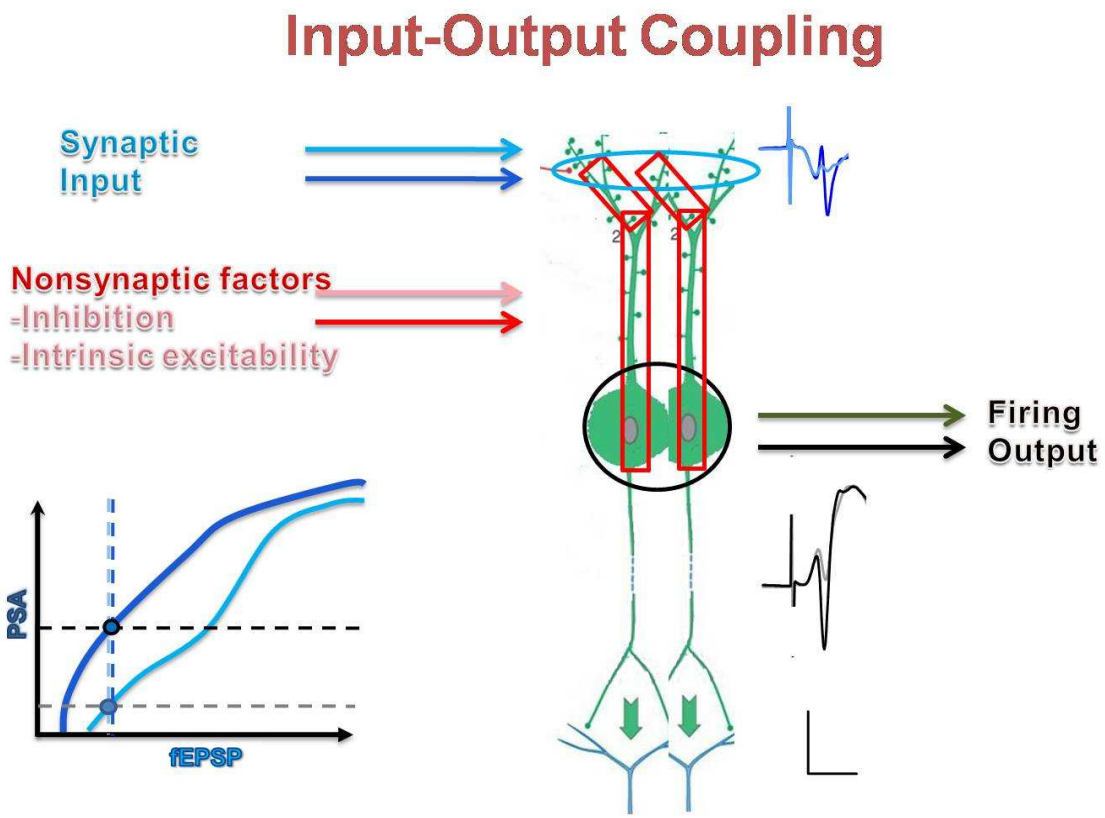

Fig. 30. The figure shows a representation of the input-output coupling in a granule cell population of the dentate gyrus during baseline and 3 - 6 h after LTP induction, having infused a PKMzeta inhibitor 1 h after the tetanization. The inhibition of PKMzeta activity 1 h after LTP induction causes the return of the potentiation to its baseline pretetanus value. After 2 h of the PKMzeta inhibitor infusion the fEPSP potentiation returns and the evoked fEPSP (dark blue) becomes similar to its fEPSP pretetanus baseline value (light blue). In spite of this, the firing of the granule cells, measured through the PSA, remains upregulated. The PSA recorded at 2 h after the PKMzeta inhibitor infusion (black) is considerably larger than its pretetanus baseline value (gray). Even when the neuronal population perceives the same synaptic input depolarization 2 h after PKMzeta inhibitor infusion than before tetanization, its increased excitability causes a larger population discharge, PSA. In other words, from the point where the neurons receive the synaptic input up to the site where the

neuronal discharge is generated, there is a long distance. Some nonsynaptic factors like the GABAergic inhibition or the intrinsic excitability could exert a powerful influence on the way that neurons translate the synaptic input into the final firing output. (Calibration 5mV/5ms).

5- Conclusions

In summary the main findings of this dissertation are:

a- Different forms of LTP can be induced in the DG of freely moving animals.

b- “Transient” forms of fEPSP and PSA potentiations can be induced in the DG of freely moving animals with the Wk Wk TET and Wk TET HFS patterns that slightly differ in their duration and final consequences for the excitability of the granule cells.

c- Even when the recording of the evoked potentials at a single stimulation intensity reveals a certain trend it does not necessarily mean that the neuronal population responses over the entire range of input intensities follow the same trend. It is necessary to evaluate the I-O over the entire dynamic range.

d- Even weak tetanization protocols can produce some perdurable excitability changes in the DG of freely moving animals.

e- With the Str TET an early-LTP was induced, but profound excitability changes accompanied this transient fEPSP potentiation. The PSA values remained significatively upregulated for 24 h.

f- From the studied HFS patterns only the 4xStr TET was able to induce a late-LTP. Both fEPSP and PSA remained potentiated for 24 h.

g- In the 4xStr TET group together with the synaptic, i.e. fEPSP, potentiation some excitability changes were also induced responsible for the left shift in the PSA *vs.* fEPSP relation.

h- In the 4xStr TET group a subgroup of animals did not show fEPSP potentiation however its PSA potentiation was comparable to the one of the subgroup of animals where a fEPSP potentiation occurred.

i- The synaptic and excitability changes induced by 4xStr TET HFS were largely NMDA dependent. NMDA- independent component seems to contribute to the excitability changes, at least partially.

j- The PKMzeta plays a key role in maintaining the synaptic potentiation in the DG of freely moving animals. Unlike the fEPSP potentiation, the PSA remained potentiated after PKMzeta inhibition and seemed to not be affected by this.

k- Given the dissociation that can exist among the PSA and fEPSP potentiations it is of utmost importance to record both variables in studies of functional neuronal plasticity in the DG in freely moving animals. If only the PSA is recorded it is almost impossible to discern whether the registered PSA changes are resulting from synaptic changes, excitability changes or something in between.

l- In the DG of the intact freely moving animals the neural firing does not only depend on the strength of the excitatory synapses, but also depends strongly on neural population excitability which can be long-lastingly modified in an activity-dependent manner.

Future studies

Many questions remain, such as:

a- Which is the nature of the excitability changes induced by the different HFS patterns in the DG of freely moving animals? Are they mainly changes in the inhibitory drive into the neurons, changes in the intrinsic excitability or others?

b- Are the excitability changes dependent on other mechanisms for their induction apart from NMDA receptor activation, for example VDCC activation?

c- Are the long-term excitability changes induced with the studied HFS patterns protein-synthesis dependent? Which are their molecular underpinnings?

d- Is the synaptic potentiation in the DG of freely moving animals protein synthesis dependent?

e- Even when the extracellular field recordings are a very useful tool for studying functional neuronal plasticity in freely moving animals, the use of further techniques like intracellular recordings can contribute to have a better understanding of the processes occurring at a single cell level.

6- Reference List

Abraham,W.C., Gustafsson,B., and Wigstrom,H. (1987). Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. J. Physiol. *394*, 367-380.

Agranoff,B.W., Davis,R.E., and Brink,J.J. (1966). Chemical studies on memory fixation in goldfish. Brain Res. *1*, 303-309.

Alle,H., Jonas,P., and Geiger,J.R. (2001). PTP and LTP at a hippocampal mossy fiber-interneuron synapse. Proc. Natl. Acad. Sci. U. S. A. *98*, 14708-14713.

Almaguer-Melian,W., Bergado,J.A., Lopez-Rojas,J., Frey,S., and Frey,J.U. (2010). Differential effects of electrical stimulation patterns, motivational-behavioral stimuli and their order of application on functional plasticity processes within one input in the dentate gyrus of freely moving rats in vivo. Neuroscience *165*, 1546-1558.

Amaral,D.G., and Lavenex,P. (2007). Hippocampal neuroanatomy. In The hippocampus book, P. Andersen, R.G. Morris, D.G. Amaral, T. Bliss, and J. O'Keefe, eds. Oxford University Press, Inc.), pp. 37-114.

Amaral,D.G., Scharfman,H.E., and Lavenex,P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog. Brain Res. *163*, 3-22.

Amaral,D.G., and Witter,M.P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience. *31*, 571-591.

Andersen,P. (1960). Interhippocampal impulses. III. Basal dendritic activation of CA3 neurons. Acta Physiol Scand. *48*, 209-230.

Andersen,P., Bliss,T.V., Lomo,T., Olsen,L.I., and Skrede,K.K. (1969). Lamellar organization of hippocampal excitatory pathways. Acta Physiol Scand. *76*, 4A-5A.

Andersen,P., Bliss,T.V., and Skrede,K.K. (1971). Unit analysis of hippocampal polulation spikes. Exp. Brain Res. *13*, 208-221.

Andersen,P., Sundberg,S.H., Sveen,O., Swann,J.W., and Wigstrom,H. (1980). Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. J. Physiol. *302*, 463-482.

Andersen,P., Sundberg,S.H., Sveen,O., and Wigstrom,H. (1977). Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature. *266*, 736-737.

Barco,A., Alarcon,J.M., and Kandel,E.R. (2002). Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell *108*, 689-703.

Benito,E., and Barco,A. (2010). CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci. *33*, 230-240.

Bergado,J.A., Almaguer-Melian,W., Kostenko,S., Frey,S., and Frey,J.U. (2003). Behavioral reinforcement of long-term potentiation in rat dentate gyrus in vivo is protein synthesis-dependent. Neurosci. Lett. *351*, 56-58.

Bergado,J.A., Frey,S., Lopez,J., Almaguer-Melian,W., and Frey,J.U. (2007). Cholinergic afferents to the locus coeruleus and noradrenergic afferents to the medial septum mediate LTP-reinforcement in the dentate gyrus by stimulation of the amygdala. Neurobiol. Learn. Mem. *88*, 331-341.

Bergado,J.A., Scherf,T., Almaguer-Melian,W., Frey,S., Lopez,J., and Frey,J.U. (2009). Stimulation of the nucleus raphe medialis modifies basal synaptic transmission at the dentate gyrus, but not long-term potentiation or its reinforcement by stimulation of the basolateral amygdala. Neurosci. Lett. *464*, 179-183.

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

Bliss,T.V., and Gardner-Medwin,A.R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J. Physiol. *232*, 357-374.

Bliss,T.V., and Lomo,T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J. Physiol. *232*, 331-356.

Brioni,J.D., Nagahara,A.H., and McGaugh,J.L. (1989). Involvement of the amygdala GABAergic system in the modulation of memory storage. Brain Res. *487*, 105-112.

Brzeznicka,E.A., Lehman,L.D., and Klaassen,C.D. (1987). Induction of hepatic metallothionein following administration of urethane. Toxicol. Appl. Pharmacol. *87*, 457-463.

Buzsaki,G., Leung,L.W., and Vanderwolf,C.H. (1983). Cellular bases of hippocampal EEG in the behaving rat. Brain Res. *287*, 139-171.

Caillard,O., Ben-Ari,Y., and Gaiarsa,J.L. (1999). Mechanisms of induction and expression of long-term depression at GABAergic synapses in the neonatal rat hippocampus. J. Neurosci. *19*, 7568-7577.

Calabresi,P., Pisani,A., Mercuri,N.B., and Bernardi,G. (1992). Long-term Potentiation in the Striatum is Unmasked by Removing the Voltage-dependent Magnesium Block of NMDA Receptor Channels. Eur. J Neurosci. *4*, 929-935.

Campanac,E., Daoudal,G., Ankri,N., and Debanne,D. (2008). Downregulation of dendritic I(h) in CA1 pyramidal neurons after LTP. J. Neurosci. *28*, 8635-8643.

Chavez-Noriega,L.E., Bliss,T.V., and Halliwell,J.V. (1989). The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. Neurosci. Lett. *104*, 58-64.

Claiborne,B.J., Amaral,D.G., and Cowan,W.M. (1990). Quantitative, three-dimensional analysis of granule cell dendrites in the rat dentate gyrus. J. Comp Neurol. *302*, 206-219.

Clugnet,M.C., and LeDoux,J.E. (1990). Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. J Neurosci. *10*, 2818-2824.

Cohen-Matsliah,S.I., Brosh,I., Rosenblum,K., and Barkai,E. (2007). A novel role for extracellular signal-regulated kinase in maintaining long-term memory-relevant excitability changes. J Neurosci. *27*, 12584-12589.

Cohen-Matsliah,S.I., Motanis,H., Rosenblum,K., and Barkai,E. (2010). A novel role for protein synthesis in long-term neuronal plasticity: maintaining reduced postburst afterhyperpolarization. J Neurosci. *30*, 4338-4342.

Cohen-Matsliah,S.I., Rosenblum,K., and Barkai,E. (2009). Olfactory-learning abilities are correlated with the rate by which intrinsic neuronal excitability is modulated in the piriform cortex. Eur. J Neurosci. *30*, 1339-1348.

Colino,A., and Malenka,R.C. (1993). Mechanisms underlying induction of long-term potentiation in rat medial and lateral perforant paths in vitro. J Neurophysiol *69*, 1150-1159.

Collingridge,G.L., Kehl,S.J., and McLennan,H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol *334*, 33-46.

Collingridge,G.L., Randall,A.D., Davies,C.H., and Alford,S. (1992). The synaptic activation of NMDA receptors and Ca2+ signalling in neurons. Ciba Found. Symp. *164:162-71; discussion 172-5.*, 162-171.

Collingridge,G.L., and Watkins,J.C. (1995). The NMDA receptor Oxford University Press).

Crunelli,V., and Mayer,M.L. (1984). Mg2+ dependence of membrane resistance increases evoked by NMDA in hippocampal neurones. Brain Res. *311*, 392-396.

Daoudal,G., Hanada,Y., and Debanne,D. (2002). Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proc. Natl. Acad. Sci. U. S. A. *99*, 14512-14517.

De Kock,D.H., and Neethling,A.C. (1972). The effect of urethane on protein biosynthesis. S. Afr. Med. J. *46*, 299-300.

DeJong,R.N., Itabashi,H.H., and Olson,J.R. (1969). Memory loss due to hippocampal lesions. Report of a case. Arch. Neurol. *20*, 339-348.

Deller,T., Martinez,A., Nitsch,R., and Frotscher,M. (1996). A novel entorhinal projection to the rat dentate gyrus: direct innervation of proximal dendrites and cell bodies of granule cells and GABAergic neurons. J. Neurosci. *16*, 3322-3333.

Derrick,B.E., and Martinez,J.L., Jr. (1994). Frequency-dependent associative long-term potentiation at the hippocampal mossy fiber-CA3 synapse. Proc. Natl. Acad. Sci. U. S. A *91*, 10290-10294.

Doron,G., and Rosenblum,K. (2010). c-Fos expression is elevated in GABAergic interneurons of the gustatory cortex following novel taste learning. Neurobiol. Learn. Mem. *94*, 21-29.

Drier,E.A., Tello,M.K., Cowan,M., Wu,P., Blace,N., Sacktor,T.C., and Yin,J.C. (2002). Memory enhancement and formation by atypical PKM activity in Drosophila melanogaster. Nat. Neurosci. *5*, 316-324.

Errington,M.L., Lynch,M.A., and Bliss,T.V. (1987). Long-term potentiation in the dentate gyrus: induction and increased glutamate release are blocked by D(-)aminophosphonovalerate. Neuroscience. *20*, 279-284.

Fan,Y., Fricker,D., Brager,D.H., Chen,X., Lu,H.C., Chitwood,R.A., and Johnston,D. (2005). Activity-dependent decrease of excitability in rat hippocampal neurons through increases in I(h). Nat. Neurosci. *8*, 1542-1551.

Feldman,D.E., Nicoll,R.A., and Malenka,R.C. (1999). Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. J. Neurobiol. *41*, 92-101.

Felleman,D.J., and Van Essen,D.C. (1991). Distributed hierarchical processing in the primate cerebral cortex. Cereb. Cortex *1*, 1-47.

Felthauser,A.M., and Claiborne,B.J. (1990). Intracellular labeling of dentate granule cells in fixed tissue permits quantitative analysis of dendritic morphology. Neurosci. Lett. *118*, 249-251.

Fink,A.E., and O'Dell,T.J. (2009). Short Trains of Theta Frequency Stimulation Enhance CA1 Pyramidal Neuron Excitability in the Absence of Synaptic Potentiation. J. Neurosci. *29*, 11203-11214.

Fonseca,R., Nagerl,U.V., and Bonhoeffer,T. (2006). Neuronal activity determines the protein synthesis dependence of long-term potentiation. Nat. Neurosci. *9*, 478-480.

Frey,S., Bergado-Rosado,J., Seidenbecher,T., Pape,H.C., and Frey,J.U. (2001). Reinforcement of early long-term potentiation (early-LTP) in dentate gyrus by stimulation of the basolateral amygdala: heterosynaptic induction mechanisms of late-LTP. J. Neurosci. *21*, 3697-3703.

Frey,S., and Frey,J.U. (2008). 'Synaptic tagging' and 'cross-tagging' and related associative reinforcement processes of functional plasticity as the cellular basis for memory formation. Prog. Brain Res. *169*, 117-143.

Frey,S., and Frey,J.U. (2009). Synaptic plasticity and the analysis of the field-EPSP as well as the population spike using separate recording electrodes in the dentate gyrus in freely moving rats. J. Neurosci. Methods. *184*, 79-87.

Frey,U., Frey,S., Schollmeier,F., and Krug,M. (1996). Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. J. Physiol. *490*, 703-711.

Frey,U., Krug,M., Reymann,K.G., and Matthies,H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res. *452*, 57-65.

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

Frey,U., and Morris,R.G. (1998a). Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. Trends Neurosci. *21*, 181-188.

Frey,U., and Morris,R.G. (1998b). Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. Neuropharmacology. *37*, 545-552.

Frick,A., Magee,J., and Johnston,D. (2004). LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat. Neurosci. *7*, 126-135.

Grecksch,G., and Matthies,H. (1980). Two sensitive periods for the amnesic effect of anisomycin. Pharmacol. Biochem. Behav. *12*, 663-665.

Grover,L.M., and Teyler,T.J. (1990). Two components of long-term potentiation induced by different patterns of afferent activation. Nature *347*, 477-479.

Gusev,P.A., and Alkon,D.L. (2001). Intracellular correlates of spatial memory acquisition in hippocampal slices: long-term disinhibition of CA1 pyramidal cells. J. Neurophysiol. *86*, 881-899.

Hanse,E., and Gustafsson,B. (1992). Long-term Potentiation and Field EPSPs in the Lateral and Medial Perforant Paths in the Dentate Gyrus In Vitro: a Comparison. Eur. J Neurosci. *4*, 1191-1201.

Hara,K., and Harris,R.A. (2002). The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. Anesth. Analg. *94*, 313-318.

Harris,E.W., and Cotman,C.W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. Neurosci. Lett. *70*, 132-137.

Hebb,D.O. (1949). The organization of behaviour New York: Wiley & Sons).

Herbert,J.M., Augereau,J.M., Gleye,J., and Maffrand,J.P. (1990). Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem. Biophys. Res. Commun. *172*, 993-999.

Hernandez,A.I., Blace,N., Crary,J.F., Serrano,P.A., Leitges,M., Libien,J.M., Weinstein,G., Tcherapanov,A., and Sacktor,T.C. (2003). Protein kinase M zeta synthesis from a brain mRNA encoding an independent protein kinase C zeta catalytic domain. Implications for the molecular mechanism of memory. J. Biol. Chem. *278*, 40305-40316.

Hjorth-Simonsen,A., and Jeune,B. (1972). Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. J. Comp Neurol. *144*, 215-232.

Houser,C.R. (2007). Interneurons of the dentate gyrus: an overview of cell types, terminal fields and neurochemical identity. Prog. Brain Res. *163*, 217-232.

Jasinska,M., Siucinska,E., Cybulska-Klosowicz,A., Pyza,E., Furness,D.N., Kossut,M., and Glazewski,S. (2010). Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J. Neurosci. *30*, 1176-1184.

Jester,J.M., Campbell,L.W., and Sejnowski,T.J. (1995). Associative EPSP--spike potentiation induced by pairing orthodromic and antidromic stimulation in rat hippocampal slices. J. Physiol. *484*, 689-705.

Kauderer,B.S., and Kandel,E.R. (2000). Capture of a protein synthesis-dependent component of long-term depression. Proc. Natl. Acad. Sci. U. S. A *97*, 13342-13347.

Kneisler,T.B., and Dingledine,R. (1995). Spontaneous and synaptic input from granule cells and the perforant path to dentate basket cells in the rat hippocampus. Hippocampus *5*, 151-164.

Kobayashi,K., and Poo,M.M. (2004). Spike train timing-dependent associative modification of hippocampal CA3 recurrent synapses by mossy fibers. Neuron *41*, 445-454.

Kombian,S.B., and Malenka,R.C. (1994). Simultaneous LTP of non-NMDA- and LTD of NMDA-receptor-mediated responses in the nucleus accumbens. Nature *368*, 242-246.

Konorski,J. (1948). Conditioned reflexes and neuron organization Cambridge University Press).

Krug,M., Lossner,B., and Ott,T. (1984). Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain Res. Bull. *13*, 39-42.

Kudolo,J., Tabassum,H., Frey,S., Lopez,J., Hassan,H., Frey,J.U., and Bergado,J.A. (2010). Electrical and pharmacological manipulations of the nucleus accumbens core impair synaptic plasticity in the dentate gyrus of the rat. Neuroscience *168*, 723-731.

Kullmann,D.M., and Lamsa,K.P. (2007). Long-term synaptic plasticity in hippocampal interneurons. Nat. Rev Neurosci. *8*, 687-699.

Kwapis,J.L., Jarome,T.J., Lonergan,M.E., and Helmstetter,F.J. (2009). Protein kinase Mzeta maintains fear memory in the amygdala but not in the hippocampus. Behav. Neurosci. *123*, 844-850.

Lapointe,V., Morin,F., Ratte,S., Croce,A., Conquet,F., and Lacaille,J.C. (2004). Synapse-specific mGluR1-dependent long-term potentiation in interneurones regulates mouse hippocampal inhibition. J. Physiol. *555*, 125-135.

Laroche,S., Doyere,V., and Bloch,V. (1989). Linear relation between the magnitude of long-term potentiation in the dentate gyrus and associative learning in the rat. A demonstration using commissural inhibition and local infusion of an N-methyl-D-aspartate receptor antagonist. Neuroscience *28*, 375-386.

Lessmann,V., and Brigadski,T. (2009). Mechanisms, locations, and kinetics of synaptic BDNF secretion: an update. Neurosci. Res. *65*, 11-22.

Leung,L.S., Roth,L., and Canning,K.J. (1995). Entorhinal inputs to hippocampal CA1 and dentate gyrus in the rat: a current-source-density study. J. Neurophysiol. *73*, 2392-2403.

Levy,W.B., and Steward,O. (1979). Synapses as associative memory elements in the hippocampal formation. Brain Res. *175*, 233-245.

Lin,H.C., Mao,S.C., and Gean,P.W. (2009). Block of gamma-aminobutyric acid-A receptor insertion in the amygdala impairs extinction of conditioned fear. Biol. Psychiatry *66*, 665-673.

Ling,D.S., Benardo,L.S., and Sacktor,T.C. (2006). Protein kinase Mzeta enhances excitatory synaptic transmission by increasing the number of active postsynaptic AMPA receptors. Hippocampus *16*, 443-452.

Ling,D.S., Benardo,L.S., Serrano,P.A., Blace,N., Kelly,M.T., Crary,J.F., and Sacktor,T.C. (2002). Protein kinase Mzeta is necessary and sufficient for LTP maintenance. Nat. Neurosci. *5*, 295-296.

Lomo,T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. Acta Physiol Scand. *68*, 128.

Lomo,T. (1971). Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. Exp. Brain Res. *12*, 18-45.

Lopez de,A.M., Jancic,D., Olivares,R., Alarcon,J.M., Kandel,E.R., and Barco,A. (2007). cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons. J. Neurosci. *27*, 13909-13918.

Lopez,J., Almaguer,W., Perez,H., Frey,J.U., and Bergado,J.A. (2008). Opposite effects of shell or core stimulation of the nucleus accumbens on long-term potentiation in dentate gyrus of anesthetized rats. Neuroscience. *151*, 572-578.

Lynch,G., Larson,J., Kelso,S., Barrionuevo,G., and Schottler,F. (1983). Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature *305*, 719-721.

Madronal,N., Gruart,A., Sacktor,T.C., and Delgado-Garcia,J.M. (2010). PKMzeta inhibition reverses learning-induced increases in hippocampal synaptic strength and memory during trace eyeblink conditioning. PLoS. One. *5*, e10400.

Malenka,R.C. (2002). Synaptic Plasticity. In Neuropsychopharmacology: The Fifth Generation of Progress, K.L. Davis, D. Charney, J.T. Coley, and C. Nemeroff, eds. ACNP), pp. 147-158.

Malenka,R.C., Kauer,J.A., Perkel,D.J., Mauk,M.D., Kelly,P.T., Nicoll,R.A., and Waxham,M.N. (1989). An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature *340*, 554-557.

Malenka,R.C., Kauer,J.A., Zucker,R.S., and Nicoll,R.A. (1988). Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science *242*, 81-84.

Malinow,R., Schulman,H., and Tsien,R.W. (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science *245*, 862-866.

Martin,K.C., Casadio,A., Zhu,H., Yaping,E., Rose,J.C., Chen,M., Bailey,C.H., and Kandel,E.R. (1997). Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. Cell *91*, 927-938.

Matthies,H. (1976). Cellular mechanisms of learning processes and the shaping of memory. Z Psychol *184*, 308-328.

Matthies,H. (1989). Neurobiological aspects of learning and memory. Annu Rev Psychol *40*, 381-404.

Matthies,H., and Reymann,K.G. (1993). Protein kinase A inhibitors prevent the maintenance of hippocampal long-term potentiation. Neuroreport *4*, 712-714.

McGaugh,J.L. (2000). Memory--a century of consolidation. Science. *287*, 248-251.

McGaugh,J.L., and Alpern,H.P. (1966). Effects of Electroshock on Memory: Amnesia without Convulsions. Science *152*, 665-666.

McMahon,L.L., and Kauer,J.A. (1997). Hippocampal interneurons express a novel form of synaptic plasticity. Neuron. *18*, 295-305.

McNaughton,B.L., Douglas,R.M., and Goddard,G.V. (1978). Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. Brain Res. *157*, 277-293.

Messing,R.B., Jensen,R.A., Martinez,J.L., Jr., Spiehler,V.R., Vasquez,B.J., Soumireu-Mourat,B., Liang,K.C., and McGaugh,J.L. (1979). Naloxone enhancement of memory. Behav. Neural Biol. *27*, 266-275.

Migues,P.V., Hardt,O., Wu,D.C., Gamache,K., Sacktor,T.C., Wang,Y.T., and Nader,K. (2010). PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. Nat. Neurosci. *13*, 630-634.

Milner,B. (1959). The memory defect in bilateral hippocampal lesions. Psychiatr. Res. Rep. Am. Psychiatr. Assoc. *11*, 43-58.

Milner,B., Squire,L.R., and Kandel,E.R. (1998). Cognitive neuroscience and the study of memory. Neuron *20*, 445-468.

Moncada,D., and Viola,H. (2007). Induction of long-term memory by exposure to novelty requires protein synthesis: evidence for a behavioral tagging. J Neurosci. *27*, 7476-7481.

Moore,J.A., and Appenteng,K. (1990). Contrasting effects of urethane and pentobarbitone anaesthesia on the electrical properties of rat jaw-elevator motoneurones. Brain Res. *523*, 139-142.

Morgan,S.L., and Teyler,T.J. (1999). VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. J. Neurophysiol. *82*, 736-740.

Morris,R.G. (1989). Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. J Neurosci. *9*, 3040-3057.

Moyer,J.R., Jr., Thompson,L.T., and Disterhoft,J.F. (1996). Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. J Neurosci. *16*, 5536-5546.

Mozzachiodi,R., and Byrne,J.H. (2010). More than synaptic plasticity: role of nonsynaptic plasticity in learning and memory. Trends Neurosci. *33*, 17-26.

Mozzachiodi,R., Lorenzetti,F.D., Baxter,D.A., and Byrne,J.H. (2008). Changes in neuronal excitability serve as a mechanism of long-term memory for operant conditioning. Nat. Neurosci. *11*, 1146-1148.

Müller,G.E., and Pilzecker,Z. (1900). Experimentelle Beiträge zur Lehre vom Gedächtnis. Z Psychol *1*, 1-300.

Navakkode,S., Sajikumar,S., and Frey,J.U. (2004). The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. J. Neurosci. *24*, 7740-7744.

Navakkode,S., Sajikumar,S., and Frey,J.U. (2005). Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging. J. Neurosci. *25*, 10664-10670.

Newton,A.C. (2001). Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem. Rev. *101*, 2353-2364.

Nicoll,R.A. (1972). The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J. Physiol *223*, 803-814.

Nowak,L., Bregestovski,P., Ascher,P., Herbet,A., and Prochiantz,A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. Nature *307*, 462-465.

O'Keefe,J., and Nadel,L. (1978). Anatomy. In The hippocampus as a cognitive map., J. O'Keefe, and L. Nadel, eds. (Oxford: Oxford University Press), pp. 102-133.

Oh,M.M., Kuo,A.G., Wu,W.W., Sametsky,E.A., and Disterhoft,J.F. (2003). Watermaze learning enhances excitability of CA1 pyramidal neurons. J Neurophysiol *90*, 2171-2179.

Osten,P., Valsamis,L., Harris,A., and Sacktor,T.C. (1996). Protein synthesis-dependent formation of protein kinase Mzeta in long-term potentiation. J. Neurosci. *16*, 2444-2451.

Parsons,R.G., and Davis,M. (2011). Temporary disruption of fear-potentiated startle following PKMzeta inhibition in the amygdala. Nat. Neurosci. *14*, 295-296.

Pastalkova,E., Serrano,P., Pinkhasova,D., Wallace,E., Fenton,A.A., and Sacktor,T.C. (2006). Storage of spatial information by the maintenance mechanism of LTP. Science *313*, 1141-1144.

Paxinos,G., and Watson,C. (1998). The rat brain in stereotaxic coordinates, 4th ed. Academic Press).

Perez,Y., Morin,F., and Lacaille,J.C. (2001). A hebbian form of long-term potentiation dependent on mGluR1a in hippocampal inhibitory interneurons. Proc. Natl. Acad. Sci. U. S. A. *98*, 9401-9406.

Popov,N., Schulzeck,S., Schmidt,S., and Matthies,H. (1975). Changes in labeling of soluble and solubilized rat brain proteins using (3H)-leucine as precursor during a learning experiment. Acta Biol. Med. Ger. *34*, 583-592.

Purves,D. (2004). Memory. In Neuroscience, Sinauer Associates), pp. 733-753.

Racine,R.J., Wilson,D.A., Gingell,R., and Sunderland,D. (1986). Long-term potentiation in the interpositus and vestibular nuclei in the rat. Exp. Brain Res. *63*, 158-162.

Regehr,W.G., and Tank,D.W. (1990). Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. Nature *345*, 807-810.

Reymann,K.G., Frey,U., Jork,R., and Matthies,H. (1988). Polymyxin B, an inhibitor of protein kinase C, prevents the maintenance of synaptic long-term potentiation in hippocampal CA1 neurons. Brain Res. *440*, 305-314.

Ribak,C.E. (1992). Local circuitry of GABAergic basket cells in the dentate gyrus. Epilepsy Res. Suppl *7*, 29-47.

Ribak,C.E., and Seress,L. (1983). Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. J. Neurocytol. *12*, 577-597.

Richter-Levin,G., Errington,M.L., Maegawa,H., and Bliss,T.V. (1994). Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning. Neuropharmacology. *33*, 853-857.

Riedel,G., Seidenbecher,T., and Reymann,K.G. (1994). LTP in hippocampal CA1 of urethane-narcotized rats requires stronger tetanization parameters. Physiol Behav. *55*, 1141-1146.

Rockhold,R.W., Byrne,M., Sprabery,S., and Bennett,J.G. (1994). Urethane anesthesia reverses the protective effect of noncompetitive NMDA receptor antagonists against cocaine intoxication. Life Sci. *54*, 321-330.

Ross,S.T., and Soltesz,I. (2001). Long-term plasticity in interneurons of the dentate gyrus. Proc. Natl. Acad. Sci. U. S. A. *98*, 8874-8879.

Sacktor,T.C. (2011). How does PKMzeta maintain long-term memory? Nat. Rev. Neurosci. *12*, 9-15.

Sacktor,T.C., Osten,P., Valsamis,H., Jiang,X., Naik,M.U., and Sublette,E. (1993). Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation. Proc. Natl. Acad. Sci. U. S. A. *90*, 8342-8346.

Sajikumar,S., and Frey,J.U. (2004a). Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. Neurobiol. Learn. Mem. *82*, 12-25.

Sajikumar,S., and Frey,J.U. (2004b). Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1 in vitro. Neuroscience. *129*, 503-507.

Sajikumar,S., Navakkode,S., and Frey,J.U. (2005a). Protein synthesis-dependent long-term functional plasticity: methods and techniques. Curr. Opin. Neurobiol. *15*, 607-613.

Sajikumar,S., Navakkode,S., and Frey,J.U. (2007). Identification of compartment- and process-specific molecules required for "synaptic tagging" during long-term potentiation and long-term depression in hippocampal CA1. J. Neurosci. *27*, 5068-5080.

Sajikumar,S., Navakkode,S., Sacktor,T.C., and Frey,J.U. (2005b). Synaptic tagging and cross-tagging: the role of protein kinase Mzeta in maintaining long-term potentiation but not long-term depression. J. Neurosci. *25*, 5750-5756.

Sanes,J.R., and Lichtman,J.W. (1999). Can molecules explain long-term potentiation? Nat. Neurosci. *2*, 597-604.

Scoville,W.B., and Milner,B. (1957). Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20*, 11-21.

Seidenbecher,T., Reymann,K.G., and Balschun,D. (1997). A post-tetanic time window for the reinforcement of long-term potentiation by appetitive and aversive stimuli. Proc. Natl. Acad. Sci. U. S. A. *94*, 1494-1499.

Serrano,P., Friedman,E.L., Kenney,J., Taubenfeld,S.M., Zimmerman,J.M., Hanna,J., Alberini,C., Kelley,A.E., Maren,S., Rudy,J.W., Yin,J.C., Sacktor,T.C., and Fenton,A.A. (2008). PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. PLoS. Biol. *6*, 2698-2706.

Shapiro,M.M., GOL,A., and KELLAWAY,P. (1965). Acquisition, retention, and discrimination reversal after hippocampal ablation in monkeys. Exp. Neurol. *13*, 128-144.

Shema,R., Hazvi,S., Sacktor,T.C., and Dudai,Y. (2009). Boundary conditions for the maintenance of memory by PKMzeta in neocortex. Learn. Mem. *16*, 122-128.

Shema,R., Sacktor,T.C., and Dudai,Y. (2007). Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKM zeta. Science *317*, 951-953.

Shew,T., Yip,S., and Sastry,B.R. (2000). Mechanisms involved in tetanus-induced potentiation of fast IPSCs in rat hippocampal CA1 neurons. J. Neurophysiol. *83*, 3388-3401.

Shirasaka,Y., and Wasterlain,C.G. (1995). The effect of urethane anesthesia on evoked potentials in dentate gyrus. Eur. J. Pharmacol. *282*, 11-17.

Sik,A., Penttonen,M., and Buzsaki,G. (1997). Interneurons in the hippocampal dentate gyrus: an in vivo intracellular study. Eur. J. Neurosci. *9*, 573-588.

Sjostrom,P.J., Rancz,E.A., Roth,A., and Hausser,M. (2008). Dendritic Excitability and Synaptic Plasticity. Physiol. Rev. *88*, 769-840.

Smith,R.A., and Smith,W.A. (1966). Loss of recent memory as a sign of focal temporal lobe disorder. Report of a case. J. Neurosurg. *24*, 91-95.

Song,Z., Wixted,J.T., Hopkins,R.O., and Squire,L.R. (2011). Impaired capacity for familiarity after hippocampal damage. Proc. Natl. Acad. Sci. U. S. A *108*, 9655-9660.

Squire,L.R. (1998). Memory systems. C. R. Acad. Sci. III *321*, 153-156.

Squire,L.R. (2004). Memory systems of the brain: a brief history and current perspective. Neurobiol. Learn. Mem. *82*, 171-177.

Squire,L.R., and Zola,S.M. (1996). Structure and function of declarative and nondeclarative memory systems. Proc. Natl. Acad. Sci. U. S. A *93*, 13515-13522.

Staff,N.P., and Spruston,N. (2003). Intracellular correlate of EPSP-spike potentiation in CA1 pyramidal neurons is controlled by GABAergic modulation. Hippocampus. *13*, 801-805.

Steward,O., and Scoville,S.A. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. J. Comp Neurol. *169*, 347-370.

Straube,T., and Frey,J.U. (2003). Involvement of beta-adrenergic receptors in protein synthesis-dependent late long-term potentiation (LTP) in the dentate gyrus of freely moving rats: the critical role of the LTP induction strength. Neuroscience. *119*, 473-479.

Struble,R.G., Desmond,N.L., and Levy,W.B. (1978). Anatomical evidence for interlamellar inhibition in the fascia dentata. Brain Res. *152*, 580-585.

Sutherland,R.J., McDonald,R.J., Hill,C.R., and Rudy,J.W. (1989). Damage to the hippocampal formation in rats selectively impairs the ability to learn cue relationships. Behav. Neural Biol. *52*, 331-356.

Sweatt,J.D. (2003). The basics of physiological learning and memory theory. In Mechanisms of memory, Academic Press), pp. 3-27.

Szapiro,G., Vianna,M.R., McGaugh,J.L., Medina,J.H., and Izquierdo,I. (2003). The role of NMDA glutamate receptors, PKA, MAPK, and CAMKII in the hippocampus in extinction of conditioned fear. Hippocampus. *13*, 53-58.

Tang,Y., and Zucker,R.S. (1997). Mitochondrial involvement in post-tetanic potentiation of synaptic transmission. Neuron *18*, 483-491.

Thompson,L.J., and Fields,A.P. (1996). betaII protein kinase C is required for the G2/M phase transition of cell cycle. J. Biol. Chem. *271*, 15045-15053.

Thompson,L.T., Moyer,J.R., Jr., and Disterhoft,J.F. (1996). Transient changes in excitability of rabbit CA3 neurons with a time course appropriate to support memory consolidation. J Neurophysiol *76*, 1836-1849.

Vickers,C.A., Dickson,K.S., and Wyllie,D.J. (2005). Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. J. Physiol *568*, 803-813.

Viosca,J., Lopez de,A.M., Jancic,D., and Barco,A. (2009). Enhanced CREB-dependent gene expression increases the excitability of neurons in the basal amygdala and primes the consolidation of contextual and cued fear memory. Learn. Mem. *16*, 193-197.

von Kraus,L.M., Sacktor,T.C., and Francis,J.T. (2010). Erasing sensorimotor memories via PKMzeta inhibition. PLoS. One. *5*, e11125.

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

White,W.F., Nadler,J.V., Hamberger,A., Cotman,C.W., and Cummins,J.T. (1977). Glutamate as transmitter of hippocampal perforant path. Nature *270*, 356-357.

Winson,J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science. *201*, 160-163.

Witter,M.P., and Amaral,D.G. (2004). Hippocampal formation. In The rat nervous system, G. Paxinos, ed. Elsevier), pp. 635-704.

Xu,J., Kang,N., Jiang,L., Nedergaard,M., and Kang,J. (2005). Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. J. Neurosci. *25*, 1750-1760.

Yao,Y., Kelly,M.T., Sajikumar,S., Serrano,P., Tian,D., Bergold,P.J., Frey,J.U., and Sacktor,T.C. (2008). PKM zeta maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. J. Neurosci. *28*, 7820-7827.

Young,J.Z., and Nguyen,P.V. (2005). Homosynaptic and heterosynaptic inhibition of synaptic tagging and capture of long-term potentiation by previous synaptic activity. J. Neurosci. *25*, 7221-7231.

Zalutsky,R.A., and Nicoll,R.A. (1992). Mossy fiber long-term potentiation shows specificity but no apparent cooperativity. Neurosci. Lett. *138*, 193-197.

Zhang,W., and Linden,D.J. (2003). The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat. Rev Neurosci. *4*, 885-900.

Zola,S.M., and Squire,L.R. (2001). Relationship between magnitude of damage to the hippocampus and impaired recognition memory in monkeys. Hippocampus *11*, 92-98.

Appendices

I- Zusammenfassung der Dissertation

Lang-Zeit-Potenzierung (LTP) ist gekennzeichnet als ein lang anhaltender Anstieg in der Wirksamkeit der synaptischen Übertragung nach einer kurzen hochfrequenten Stimulierung der afferenten Fasern. LTP wird verwendet als ein zelluläres Model für Lernen und Gedächtnis. Studien im Gyrus Dentatus (DG) analysieren allerdings oft nur den neuronalen "Output", den Populationsspike (PS), statt der synaptischen Komponente, dem exzitatorischen postsynaptischen Potential (EPSP). Im Allgemeinen wird angenommen, dass der PS die synaptische Antwort imitiert. Diese Arbeit beschreibt hauptsächlich, wie synaptische Erregbarkeit der Granullarzellen mit neuronalem Feuern während LTP in Verbindung stehen und welche molekularen Mechanismen diese synaptischen und Erregbarkeitsänderungen in freibeweglichen Ratten unterstützen. Eine wichtige Frage in der heutigen Neurowissenschaft ist die Beziehung zwischen diesen beiden Phänomenen sowie ihre Mechanismen zu verstehen. Zur Untersuchung dieser Fragestellung benutzten wir eine kürzlich in unserem Labor eingeführte Methode, um den PS und das EPSP an ihren Entstehungsorten abzuleiten.

In einer ersten Serie von Experimenten untersuchten wir die Effekte unterschiedlicher Tetanisierungsprotokolle auf EPSP und PS. Wir fanden heraus, dass das Feuern der Granullarzellen in relativer Unabhängigkeit von der synaptischen Wirksamkeit bei unterschiedlichen elektrischen Reizen verstärkt sein kann. Diese Erregbarkeitsänderung kann bis zu 24 Stunden in freibeweglichen Ratten andauern. Von den verwendeten Tetanisierungsprotokollen war nur das "stärkste“ in der Lage, eine späte Phase der synaptischen LTP zu beeinflussen.

In einer zweiten Serie von Experimenten untersuchten wir pharmakologische Eigenschaften der late-LTP. Wir fanden, dass beide gemessenen Parameter, die EPSP- und PS-Potenzierung stark von der Aktivierung der NMDA Rezeptoren während LTP-Induktion abhängig sind. Wir fanden aber auch, dass zusätzliche, noch zu identifizierende Mechanismen für die PS-Potenzierung verantwortlich gemacht werden müssen.

In einer folgenden Serie untersuchten wir, ob die Proteinkinase Mzeta (PKMzeta) auch im intakten Versuchstier für die langzeitige Aufrechterhaltung der LTP sowie möglicherweise für die PS-Potenzierung verantwortlich ist. Wir injizierten intrahippocampal die PKMzeta-Inhibitoren ZIP (zeta-Inhibitor Peptid) oder Chelerythrine Chlorid jeweils eine Stunde nach Tetanisierung. Synaptische late-LTP konnte dadurch vollständig unterbunden werden, wobei interessanterweise die PS-Potenzierung nicht beeinflusst war.

Insgesamt weisen unsere Ergebnisse daraufhin, das im DG der freibeweglichen Ratte, das neuronale Feuern nicht nur von der Stärke der exzitatorischen Synapsen abhängig ist, sondern auch von neuronalen Erregbarkeitsänderungen, welche aktivitätsabhängig lang andauernd geändert werden kann.

II- Curriculum vitae

Personal information

Name: Jeffrey Lopez Rojas

Date of birth: 23 July 1982

Place of birth: Havana, Cuba

Nationality: Cuban

Marital status: Married

Address: Salbkerstrasse 8, 39120. Magdeburg. Germany.

Telephone: +49 176 2058 9061

Email: jeffrey.yaime@googlemail.com

Education

2008 – 2011	**Doctoral student.** Department of Neurophysiology, Leibniz Institute for Neurobiology. Otto-von-Guericke University. Magdeburg, Germany.
2005 – 2008	**Junior Research Fellow.** Experimental Neurology Department, Neurosurgery and Neurology Institute, Havana, Cuba.
2000 – 2005	**Diplom. Biochemistry.** Havana University, Havana, Cuba.
1997 – 2000	**Pre-university secondary education.** Vocational pre-university Institute of exact sciences “Ignacio Agramonte”, Ciego de Avila, Cuba.

1994 – 1997 **Basic secondary education.** Basic secondary school “Benito Juarez”, Ciego de Avila, Cuba.

Further education

2010 Haifa-Magdeburg Summer School Mechanisms of Learning & Memory, Magdeburg, Germany.

2007 IBRO International Workshop in Cognitive Neurosciences, Havana, Cuba.

Honors and awards

2007 Free Registration Award for the 2nd World Conference of Stress, Budapest, Hungary.

2005 Graduated with honors, Havana University

Reviewing

2007 – 2008 Reviewer of Clinical Biochemistry (Official Journal of the Canadian Society of Clinical Chemists)

III- Scientific publications

Scientific journal publications

1. **López, J**, González,M.E.; Lorigados, L., Morales, L.; Riverón, G.; Bauza, Y. (2007). Oxidative stress markers in surgically treated patients with refractory epilepsy. Clinical Biochemistry 40: 292-298.

2. **López-Rojas, J**.; Almaguer-Melián, W., Bergado-Rosado, J. (2007). Synaptic tagging and memory trace. Rev Neurol 45 (10): 607-614.

3. . Bergado, JA; Frey, S; **López, J**; Almaguer, W; Frey JU. (2007). Cholinergic afferents to the locus coeruleus and noradrenergic afferents to the medial septum mediate LTP-reinforcement in the dentate gyrus by stimulation of the amygdala. Neurobiology of Learning and Memory 88: 331-341.

4. **López, J.;** Almaguer, W.; Pérez, H.; Frey, J.U.; Bergado, J.A. (2008). Opposite effects of shell or core stimulation of the nucleus accumbens on LTP in dentate gyrus of anesthetized rats. Neuroscience 151(2): 572-578.

5. Bergado, J.A.; Scherf, T.; Almaguer-Melian, W.; Frey, S.; **López, J**; Frey, J.U. (2009). Stimulation of the nucleus raphe medialis modifies basal synaptic transmission at the dentate gyrus, but not long-term potentiation or its reinforcement by stimulation of the basolateral amygdala. Neuroscience Letters 464): 179-183.

6. Almaguer-Melián, W.; Bergado, J.A.; **López-Rojas, J**.; Frey, S.; Frey, J.U. (2010). Differential effects of electrical stimulation patterns, motivational-behavioral stimuli and their order of application on functional plasticity processes within one input in the dentate gyrus of freely moving rats in vivo. Neuroscience 165: 1 546-1558.

7. Kudolo, J.; Tabassum, H.; Frey, S.; **López, J**.; Hassan, H.; Frey, J.U.; Bergado, J. (2010). Electrical and pharmacological manipulations of the nucleus accumbens core impair synaptic plasticity in the dentate gyrus of the rat. Neuroscience 168: 723-731.

Published abstracts

1. **López, J**.; Frey, J.U.; Frey, S. (2010) Inhibition of protein kinase Mzeta and its effect on long-term potentiation in the dentate gyrus of freely moving rats. XIIth Magdeburg International Neurobiological Symposium “Learning and Memory: Cellular and Systemic Views”. P53, page 100.

IV- Selbständigkeitserklärung

Erklärung

Hiermit erkläre ich, Jeffrey Lopez Rojas, dass ich die von mir eingereichte Dissertation mit dem Thema:

“**Functional neuronal plasticity in the dentate gyrus of freely moving rats**”

selbständig verfaßt, nicht schon als Dissertation verwendet habe und die benutzten Hilfsmittel und Quellen vollständig angegeben wurden.

Weiterhin erkläre ich, dass ich weder diese noch eine andere Arbeit zur Erlangung des akademischen Grades doctor rerum naturalium (Dr. rer. nat.) an anderen Einrichtungen eingereicht habe

Magdeburg, 28.06.2011 Unterschrift: