# Functional plasticity in the hippocampal slices in vitro

Dissertation

zur Erlangung des akademischen Grades

**doctor rerum naturalium**

**(Dr.rer.nat.)**

genehmigt durch

die Fakultät für Naturwissenschaften

der Otto-von-Guericke-Universität Magdeburg

von M.Sc. Sreedharan Sajikumar

geb. am 10. 05. 1976 in Chingoli, Keralam, India.

Gutachter: Prof. Dr. Julietta U. Frey

Prof. Dr. Denise Manahan-Vaughan

Prof. Dr. Volker Höllt

Eingereicht am: 3.12.2004

Verteidigung am: 04.05.2005

**Dedicated to my M.Sc mentor**

**Prof. Dr. V. K. Sasidharan**

# ACKNOWLEDGEMENTS

I feel extremely fortunate to have the privilege of working under the inspiring supervision of Prof. Dr. Julietta Uta Frey. I sincerely thank her for the constant support, encouragement and constructive criticisms. Her unstinted guidance and monitoring of this work has enabled me to take the right direction.

I am highly grateful to Sabine Opitz for her excellent technical assistance during the initial period of this work.

I am highly indebted to Manuela Homeyer for her timely help, and co-operation extended to me during the entire period of this work.

I wish to acknowledge the help and valuable suggestions given to me by Dr. Maxim Sokolov, Dr. Volker Korz, Dr. Sabine Frey, Dr. Thomas Behnisch, Dr. Tariq Ahmed and Dr. Anna Karpova.

I express my sincere thanks to Gusalija Behnisch, Sybille Tschorn, Diana Koch and Silvia Vieweg for their excellent technical assistance and co-operation during the course of this study.

I am specially thankful to all of my colleagues especially to Sheeja Navakkode for her help and scientific discussions.

The constant support and encouragement extended to me by Prof. Dr. T. Ramakrishna and Prof. Dr. V. K. Sasidharan is acknowledged here with great fondness and sincerity.

Above all I wish to express my deep sense of gratitude and special indebtedness to my parents, brothers and sister for their love, tolerance and moral support, which made me possible to stand at this point of life.

**S. Sajikumar**

# ABSTRACT

Processes of functional plasticity such as hippocampal long-term potentiation (LTP) and long-term depression (LTD) are regarded as cellular mechanisms underlying learning and memory formation. LTP and LTD are used as suitable models for the investigation of the latter processes.

During the last decades LTP was intensively investigated. However, less is known about LTD and its relation to learning. Here, studies were performed to investigate whether electrically-induced LTD within rat CA1 hippocampal slices in vitro shares common cellular features with LTD in the intact animal, with particular emphasis being placed on mechanisms required for its late maintenance. My initial studies have led to the development of stimulation protocols which were able to reliably induce different forms of LTD in vitro. Depending on the induction protocol, either a transient protein synthesis-independent early-LTD (with duration of up to 3-4 h) or a de novo protein synthesis-dependent late-LTD (lasting for at least 8 h) could be induced in the hippocampal slices in vitro. Both forms required NMDA-receptor activation during their induction. Furthermore, LTD was input-specific, i.e., the expression was shown only by those synapses specifically stimulated by a low-frequency protocol. Thus, phenotypically LTD in vitro was characterized by analog induction properties as LTP.

Recently, it was described that the induction of LTP can mark a specifically activated synapse by a ´synaptic tag´ to capture synapse non-specific plasticity-related proteins (PRPs) and thus maintaining input-specific LTP for prolonged periods. My studies show that in rat hippocampal slices in vitro, the induction of protein synthesis-dependent late-LTD is also

characterized by processes of ´synaptic tagging´ and that heterosynaptic induction of either LTD or LTP on two sets of independent synaptic inputs S1 and S2 can lead to late-associative interactions between LTD- and LTP-inputs: early-LTD in a synaptic input S2 was transformed into a late-LTD, if late-LTP was induced in a synaptic input S1 of the same neuronal population within a distinct time interval. The synthesis of process-independent PRPs by late-LTP in S1 was sufficient to transform early- into late-LTD in S2 when process-specific synaptic tags were set. We have named this new late associative property of cellular information processing as 'cross-tagging', since process-unspecific PRPs can be captured by either LTP- or LTD-synpatic tags thus transforming a normally transient LTP or LTD in a long-lasting form.

The 'tag' as well as the PRPs are characterized by a relatively short half-life of several minutes up to a few hours before they degrade most likely by processes such as dephosphorylation. The question now arose whether the ‘tags’ or better: the ‘tag complex’ can also be reset in an activity-dependent manner, thus preventing the processing of PRPs with the result of transient short-lasting plasticity. Early-LTP was used to study this and we found that low-frequency stimulation shortly after early-LTP induction (5 min) reset the 'tag' preventing any lasting forms of LTP and thus, preventing the formation of a cellular memory trace.

Next, we searched for the possible 'tag' candidate or 'PRP'-molecules. The role of a PKC isoform is widely speculated as a candidate molecule involved in the ´synaptic tag-complex’. So we investigated the putative role of protein kinase M-zeta (PKMζ) required for the protein synthesis-dependent

phases of late-LTP/-LTD, 'synaptic tagging', or ‘synaptic cross-tagging’. PKMζ inhibition, after LTP induction reversed late-LTP maintenance, and subsequently depressed tetanized inputs. In contrast, LTD maintenance was unaffected, but its induction was blocked. PKMζ inhibition prevented ´synaptic tagging´ of LTP, but during ´cross-tagging´, the inhibitor reversed late-LTP, while early-LTD of a second, independent synaptic input was converted into late-LTD. Thus PKMζ is specific to the ´synaptic tagging´ mechanism of LTP, but not LTD. Our data provide evidence that PKMζ activity has dual functions: (1) it is specifically involved in LTP-maintenance and LTP-tagging, but not in LTD-maintenance and LTD-tagging and (2) it is required for processes necessary for the induction of both LTP and LTD.

# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS……..3**

**ABSTRACT……..4**

**1.0. Introduction……..10**

1.1. Synaptic plasticity, learning and memory……....10

1.2. The hippocampal formation ……...13

1.2.1. Main hippocampal in- and outputs ……..……..13

1.2.2. Intrahippocampal pathways ……...14

1.2.3. Electrophysiology of the glutamatergic CA3-CA1 synapses ……...16

1.2.4. The CA1 pyramidal neuron…….……..……18

1.3. Temporal phases of LTP and LTD…….……...19

1.4. Basic properties of LTP and LTD……..……21

1.5. Cellular mechanisms of LTP and LTD……..23

1.5.1. NMDA-receptor-dependent LTP and LTD……....23

1.5.2. Role of protein kinases in LTP and LTD……25

1.5.3. Role of protein synthesis and transcription factors in LTP and LTD ……...28

1.5.4. Heterosynaptic requirements: Role of dopamine in hippocampal synaptic plasticity……30

1.6. Synaptic plasticity and subsequent activity at the same input: Depotentiation (DP)……32

1.7. Synaptic tagging during LTP……..……...33

1.8. Aims of the dissertation……...38

**2.0. Materials and methods……..40**

2.1. Brain slice preparation and incubation……....40

2.2. Stimulation protocols: Induction of late-LTD, early-LTD, late-LTP early-LTP and depotentiation……....45

2.3. Pharmacology……..46

2.4. Statistics……47

**3.0. Results……48**

3.1. Protein synthesis and NMDA-receptor-dependent late-LTD…….48

3.2. ‘Synaptic tagging’ during LTD……51

3.3. Conversion of early-into late-LTD: ‘late-associativity’ during LTD and the decay time course of synaptic tagging during LTD……..…...54

3.4. Heterosynaptic requirements for ‘synaptic tagging’……...56

3.5. Late-associative interactions between LTP and LTD: ‘Cross- tagging’……...58

3.6. Depotentiation of early-LTP in S1 by LFS……61

3.7. Depotentiation of early-LTP in S1 and subsequent ´tagging´ by 3 x TET of S2……..……64

3.8. Effects of the PKM-zeta (PKMζ) inhibitor, myr-ZIP, on LTP and LTD……66

3.9. Effects of PKMζ inhibitor on processes of ‘cross-tagging’……69

**4.0. Discussion……72**

4.1. ‘Synaptic tagging’ during late-LTD……73

4.2. The decay time course and the nature of the ‘tag’……76

4.3. Depotentiation and ‘synaptic tagging’……..81

4.4. Heterosynaptic, modulatory requirements……..83

4.5. Late-associative LTP-LTD-interactions……85

4.6. The nature of plasticity-related- proteins (PRPs)……..……88

**5.0. Conclusions……..…92**

**6.0. References……..……..94**

**APPENDICES……109**

I. ZUSSAMMENFASSUNG DER DISSERTATION……..…109

II. SELBSTÄNDIGKEITSERKLÄRUNG……..113

III. PUBLICATIONS……..114

IV. CURRICULUM VITAE……....116

# 1.0. Introduction

## 1.1 Synaptic plasticity, learning and memory

One of the most intriguing of the brain's complex functions is its ability to store information provided by experience. Learning is the process by which new information is acquired by the nervous system, and memory vouches for the mechanisms underlying storage and/or retrieval of that information (Dudai, 2004;Dudai, 2002;Dudai, 1997). Memory, as measured by changes in an animal's behavior some time after learning, reflects many processes including acquisition, consolidation, retention, retrieval and performance.

A fundamental issue in neuroscience is how the environment can modify its representations within the mammalian brain by changing the efficacy of synaptic circuitry to mediate long-lasting cellular processes which might finally underlie such complex phenomena like cognition and specific behaviour (Thomas and Malenka, 2003). To address this issue experimentally, two basic questions have been posed. First, what are the molecular mechanisms activated by specific patterns of neural activity and how do they modify synaptic efficacy? Progress in answering this question has come mainly through the discovery and study of hippocampal long-term potentiation (LTP) and long-term depression (LTD).

Work on LTP began in the early 1970s, when Timothy Bliss and his colleague Terje Lømo discovered that brief high-frequency electrical stimulation can enhance synaptic transmission for days or even weeks in the rabbit hippocampus in vivo (Bliss and Lomo, 1973;Bliss and Gardner-Medwin, 1973). LTP is defined as ‘persistent increase in the synaptic efficacy after a

brief tetanic stimulation in the afferent pathway' (Malenka and Bear, 2004;Malenka, 1994;Malenka and Nicoll, 1999).

In the late 1970's Dunwiddie and Lynch discovered LTD, that was found to occur at the synapses between the Schaffer collaterals and the CA1 pyramidal cells in the hippocampus (Dunwiddie and Lynch, 1978). LTD is defined as 'persistent decrease in synaptic efficacy after a relatively short episode of low-frequency stimulation (LFS)´ (Bear and Malenka, 1994;Nicoll and Malenka, 1997;Braunewell and Manahan-Vaughan, 2001;Malenka, 1994;Malenka and Bear, 2004).

Work on LTP and LTD has greatly expanded our understanding of the molecular mechanisms underlying activity-dependent synaptic function in general. A second challenge is to search for the consequences of these fuctional modifications on neural circuits and behaviour. LTP is a highly popular topic in neuroscience research. The great interest is generated by its properties, making it a useful candidate for cellular processes which may underlie learning and memory.

At the beginning of the 20$^{th}$ century, Cajal (Ramón y Cajal S, 1894) proposed that neuronal networks are not cytoplasmatically continuous, but communicate with each other at distinct junctions, which Sherrington termed ´synapses´ (Sherrington et al., 1897). In human brains, approximately 100 billion of neurons interconnect in vast networks via even more number of synapses (Pakkenberg and Gundersen, 1997;Mouton et al., 1994). The brain accomplishes all of its remarkable activity through networks of neurons. It is thought that information processing and storage is achieved within neuronal networks. A single neuron is unlikely to encode a specific memory, but it is

able to store a memory trace, i.e. part of a specific memory. Hebb (Hebb, 1949) increased our understanding of how networks of neurons might store information with the provocative theory, that memories are represented by reverberating assemblies of neurons. Hebb recognized that a memory, so represented cannot reverberate forever and that some alteration in the network must occur, to provide integrity both to make the assembly a permanent trace and to make it more likely that, the trace could be reconstructed as a remembrance. Because neurons communicate with each other mainly through synapses, the activity of the assembly or network is most easily (perhaps only) altered by changes in synaptic function. Hebb formalized this idea known as Hebb's postulate:

> " When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficacy, as one of the cells firing B, is increased." (Hebb, 1949, pg.62)

> ''….any two cells or systems of cells that are repeatedly active at the same time will tend to become 'associated', so that activity in one facilitates activity in the other '' (Hebb, 1949, pg.70)

Hebb's postulate is very close to the common definition of LTP and LTD.

LTP and LTD has been most thoroughly studied in the mammalian hippocampus, an area of the brain that is specially important in the the formation and/or retrieval of some forms of memory.

### 1.2. The hippocampal formation

The hippocampus, named for its resemblance to the sea horse (*hippo* =horse, kampos= sea monster ; Greek) is one among a group of structures forming the limbic system and is a part of the hippocampal formation, which also includes the dentate gyrus, subiculum, and entorhinal cortex. Different components of the limbic system have been shown to play a critical role in all aspects of emotions, fear, learning and memory (Geinisman, 2000;Geinisman et al., 2000). The initial insights on the role of the hippocampus came from studies of amnesia in human patients following removal of the hippocampus and neighboring medial temporal structures. Extensive evidence implicates the hippocampus and related structures in the formation of episodic memories in humans (Reilly, 2001;Aggleton and Brown, 1999) and in consolidating information into long-term declarative memory (Mumby et al., 1999).

#### 1.2.1. Main hippocampal in- and outputs

Around 1970´s it became clear that, the hippocampus has a lamellar functional organization (Andersen et al., 1969). It has direct connections to the entorhinal cortex (via the subiculum) and the amygdala. Outputs from these structures can then affect many other areas of the brain (Fig. 1). For example, the entorhinal cortex projects to the cingulate cortex, which has a connection to the temporal lobe cortex, orbital cortex, and olfactory bulb. Thus, all of these areas can be influenced by hippocampal output, primarily from CA1.

The entorhinal cortex has a major source of inputs to the hippocampus, collecting information from the cingulate cortex, temporal lobe cortex, amygdala, orbital cortex, and olfactory bulb (Amaral and Witter, 1989). The hippocampus receives inputs via the precommissural branch of the fornix from the septal nuclei.

### 1.2.2. Intrahippocampal pathways

One of the main inputs to the hippocampus (perforant pathway) arises from the entorhinal cortex and passes through the dentate gyrus. From the granule cells of dentate gyrus, connections are made to area CA3 of the hippocampus proper via mossy fibers. CA3 sends connections to CA1 pyramidal cells via the Schaffer collateral (SC) as well as commissural fibers from the contralateral hippocampus (Fig. 1). The major neurotransmitter in these three pathways is glutamate. The final output from the two CA fields passes through the subiculum, entering the alveus, fimbria, and fornix and then to other areas of the brain. Hippocampus is one of the useful structures for brain slice preparation and for investigating synaptic plasticity. The main reason is because of its structure, that allows a slice to be cut whilst preserving a large number of neurons and their interconnecting axons (Andersen et al., 1969;Amaral and Witter, 1989). The dendritic structure of the three main hippocampal cell types and their interconnecting axons lay in a single plane. This plane is oriented normal to the ventricular surface and to the longitudinal axis of the hippocampus. The lamellar structure allows slices to be taken without destroying the neurons together with their dendrites and axons. The highly organized and laminar arrangement of synaptic pathways

makes the hippocampus a convenient model for studying synaptic function in vivo and in vitro (Andersen et al., 1969;Amaral and Witter, 1989).

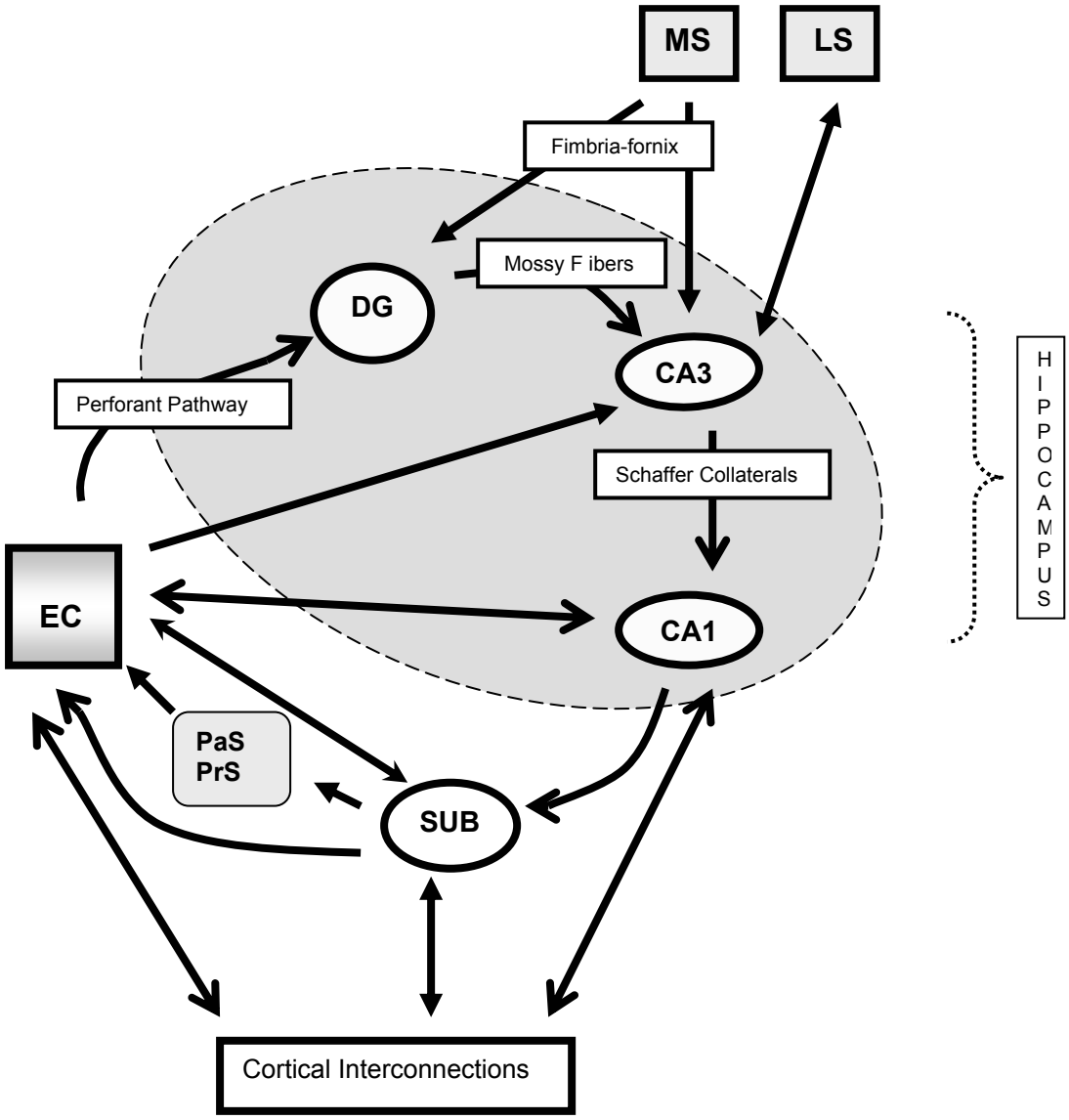


**Figure 1. Schematic representation of major intrinsic connections of the mammalian hippocampal formation** (adapted from Amaral and Witter, 1995). EC, entorhinal cortex; DG, dentate gyrus; MS, medial septum; CA1 and 3, fields of Ammon's horn; SUB, subiculum; PaS, parasubiculum; PrS, presubiculum.

Brain slices offer a variety of novel opportunities, the most obvious being visual inspection. Depending upon the brain region, histological landmarks can be seen with an ordinary dissecting microscope. In many ways the tissue can be seen in a gross microscopic slide. This allows visual control of electrode placement. It is also possible to direct electrodes to known parts of a given cell. For example, in the hippocampus, an electrode may be placed in the apical or basal dendritic tree of pyramidal cells at known distances from the soma to record the activity of a small group of synapses. Hippocampal slice also allows a comparison of the effectiveness of proximal and distal synapses to the same cell to be made. A great advantage is the lack of anaesthesis. This is of obvious importance for many studies on neuronal excitability, but is also invaluable for many pharmacological studies. Furthermore, in the slice preparation the influence of the blood brain barrier is removed. The ability to change the tissue concentration of interesting molecules at will provides good experimental control of the preparation. In addition to the temperature and oxygen concentration, the pH, ionic concentration and hormonal levels can be changed at will. The slice neurons are consequently under less synaptic bombardment than cells in the intact brain. Other modulating influences (neuromodulators, biological clocks, hormones) are also absent.

### 1.2.3. Electrophysiology of the glutamatergic CA3-CA1 synapse

Extracellular field recordings represent the summed responses from a number of neurons in the vicinity of the recording electrode. Because of the orderly polar arrangement of the pyramidal neurons and their dendrites,

electrical field recordings offer valuable information about the temporal arrangement of responses from apical dendrites to cell bodies. Following stimulation of the Schaffer collaterals and commissural fibers, an extracellular recording electrode in the stratum radiatum containing synapses, would record a small negative potential that results from the action potentials generated in the presynaptic fibers, corresponding to the field excitatory postsynaptic potential (fEPSP). The fEPSP represents in general, depolarization at the post synaptic membrane, indicating that glutamatergic transmission took place at the CA3-CA1 synapse. Placing the recording electrode in the stratum pyramidale would allow us to record a positive deflection due to current exciting the basal dendrites near the cell body. If the magnitude of the depolarization is sufficient to drive the membrane potential to a distinct threshold, it will fire one or more action potentials. These simultaneous firing of action potentials from a population of hippocampal neurons generate a field in which we can record the so named population spike (PS) as a negative potential overlapping the positive potential. While the EPSP is affected by changes occurring at the synapse, the PS is affected by combination of 3 factors: 1) the amplitude of the EPSP, 2) the passive properties of the CA1 pyramidal cell (from dendrites to axon- hillock), and 3) the level of inhibition produced by the GABAergic interneurons innervating the CA1 pyramidal neurons. A change in the PS gives great deal of information about the number and excitability of neurons involved in the final output from the hippocampus.

### 1.2.4. The CA1 pyramidal neuron

Activation of the CA3 neuron leads to an increase in glutamate release from the nerve terminals of the Shaffer collaterals. Glutamate released in the stratum radiatum and stratum lacunosum moleculare layer of CA1 activates ionotropic and metabotropic receptors. The ionotropic glutamate receptors are classified into three types AMPA, kainite, and NMDA-receptors, named after the ligand initially used to characterize them. AMPA and kainate receptors mediate the fast EPSP seen following SC stimulation (Karnup and Stelzer, 1999). NMDA- receptors mediate slow-rising EPSP's and are thought to be responsible for some forms of long-term synaptic plasticity (Tsien et al., 1996;Kullmann et al., 1996). Metabotropic glutamate receptors, which are located at both the presynaptic and postsynaptic side act to modulate release of neurotransmitter presynaptically, and modify postsynaptic responses (Manahan-Vaughan et al., 1998;Wilsch et al., 1998;Baskys and Malenka, 1991;Xiao et al., 2001;Behnisch et al., 1998;Manahan-Vaughan and Reymann, 1997;Riedel and Reymann, 1996).

The major inhibitory neurotransmitter in the hippocampus is gamma-aminobutyric acid (GABA) (Dutar and Nicoll, 1988). Eliciting a single evoked potential via stimulation of the SCs results in a characteristic sequence of excitation followed by inhibition when recorded from the stratum pyramidale. In rats, the excitation typically precedes the inhibition by a few milliseconds. The inhibition arises from feed-forward and feedback connections via inhibitory interneurons. The inhibition corresponds to the release of GABA, which initiates two types of inhibitory responses, a fast inhibitory postsynaptic

potential (IPSP) mediated by $GABA_A$-receptors and a slow IPSP brought on by $GABA_B$-receptor activation.

### 1.3. Temporal phases of LTP and LTD

Brief high-frequency stimulation of the the CA3-CA1 synapses can result in LTP, which can be divided into several temporal phases characterized by different underlying mechanisms. In general, it is divided into induction, expression and maintenance. The initial induction phase of LTP i.e. so named ‘posttetanic potentiation’ (PTP) with a duration of several seconds to minutes is characterized by presynaptic mechanisms, i.e. transient increase in transmitter release (Huang, 1998;Dobrunz et al., 1997). PTP is followed by a ‘short-term potentiation’ (STP) with a duration up to one hour. Postsynaptic events like activation of receptors by local protein kinases (e.g.CaMKII, tyrosine kinase) (Huang, 1998;Dobrunz et al., 1997) are responsible for the maintenance of that phase. STP can be followed by at least two further phases: early-and late-LTP (Matthies et al., 1990;Huang, 1998). Early-LTP is a transient form of LTP which lasts 3-4 h in vitro and 7-8 h in vivo, while late-LTP lasts for 8-10 h in vitro and days or even months in intact animals

The different forms of LTP can be specifically induced by distinct stimulus protocols in acute slices in vitro (Frey et al., 1993;Huang and Kandel, 1994). A single high-frequency stimulus train of distinct stimulation strength can induce early-LTP that lasts for up to 3-4 h, but such a protocol is normally not sufficient to induce late-LTP. The induction of late-LTP, on the other hand, requires repeated or stronger trains of high-frequency stimulation. Processes

specifically involved in early- and late- phases of LTP require different cellular signaling pathways (Fig. 2).

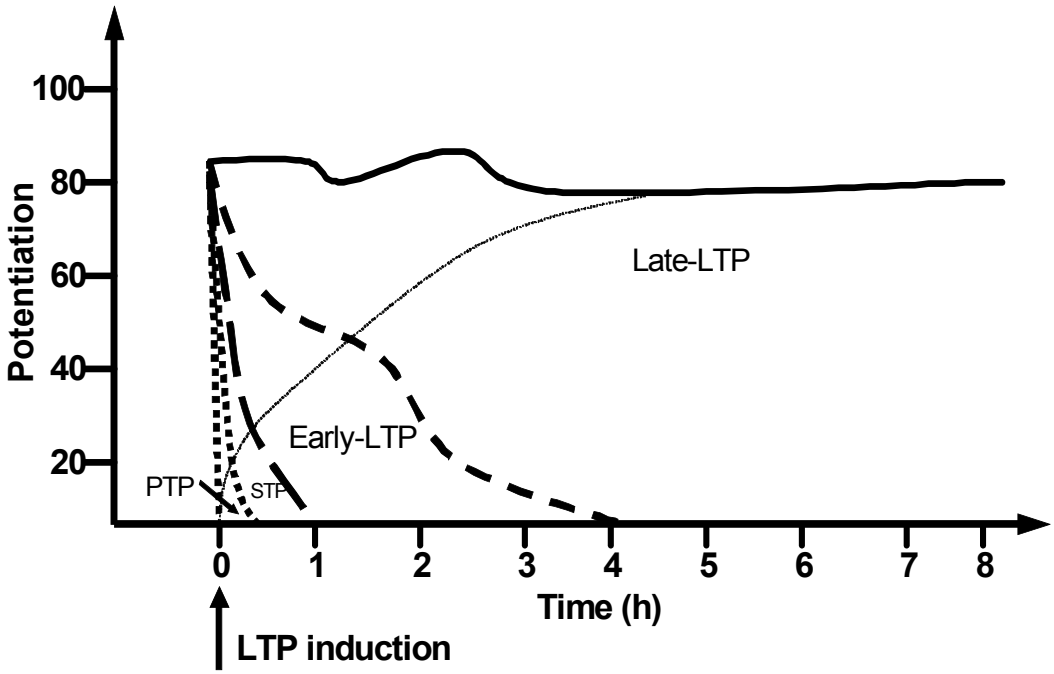


**Figure 2. The multiple phases of LTP**. See text for a detailed description.

The early-phase of LTP is transient and protein synthesis-independent, lasting about 2-4 h, induced by second messenger cascades, activated by $Ca^{2+}$ influx, and maintained by activated kinases like CaMKII, tyrosine kinase, (Malenka and Nicoll, 1999;Soderling and Derkach, 2000). Late-LTP begins gradually during the first 1-3 h and can last for 6-10 h in hippocampal slices in vitro and for days to months in vivo (Krug et al., 1989;Frey et al., 1995;Reymann et al., 1985;Otani et al., 1989;Abraham et al., 2002;Kandel, 2001). A further major difference between early-LTP and late-LTP is that late-LTP requires protein synthesis (Krug et al., 1984;Frey et al., 1988;Otani et al., 1989). Application of suppressors of RNA-translation during LTP-induction resulted in a decremental early-LTP while late-LTP was

prevented (Krug et al., 1984;Stanton and Sarvey, 1984;Deadwyler et al., 1987;Abraham and Kairiss, 1988;Frey et al., 1988;Frey et al., 1996;Mochida et al., 2001).

The phases and mechanisms of LTD are less extensively studied as compared to LTP. However, there is also evidence that LTD in area CA1, like LTP, requires NMDA-receptor activation (Mulkey and Malenka, 1992;Dudek and Bear, 1992) and protein synthesis for stable expression (Kauderer and Kandel, 2000;Manahan-Vaughan et al., 2000)

### 1.4. Basic properties of LTP and LTD

LTP and LTD at the Schaffer collaterals CA1 synapses share several common properties: like input-specificity, co-operativity, associativity and late-associativity (Bliss and Collingridge, 1993;Bear and Malenka, 1994;Malenka and Bear, 2004). LTP/LTD is input-specific, in the sense that it is restricted to the synapses which receive high-frequency stimulation (HFS) or low-frequency stimulation respectively (LFS) (Kelso and Brown, 1986;Lynch et al., 1977). This feature is consistent with its involvement in memory formation. If activation of one set of synapses led to the activation of all other synapses, even inactive ones-being potentiated or depressed, it would be difficult to selectively enhance particular sets of inputs, as is presumably required for learning and memory (Bliss and Collingridge, 1993). Cooperativity refers to the fact that, the probability of inducing LTP/LTD increases or decreases with the number of stimulated or depressed afferents (McNaughton et al., 1978;Malenka and Bear, 2004;Bear and Malenka, 1994;Otmakhova and Lisman, 1998) for instance weak-HFS/-LFS was found to result in weak LTP/-

LTD while strong HFS/-LFS results in late-LTP/-LTD. LTP/-LTD in area CA1 is also associative as shown in preparations, when two distinct axonal inputs converge on the same cell, where stimulation or depression of a weak input (few stimulated/depressed afferents) only evokes weak LTP/-LTD when coupled with stimulation/depression of the strong input (many stimulated /depressed afferents). In other words, strong activation /depression of one set of synapses can facilitate LTP/LTD at synapses on the same cell if both are activated/depressed during a specific time window (Barrionuevo and Brown, 1983;Levy and Steward, 1983). This selective enhancement /depression of conjointly activated sets of synaptic inputs is often considered as a cellular analog of associative or classical conditioning. More generally, associativity is expected in any network of neurons that links one set of information with another.

Late-associativity is a novel property of LTP/LTD. It describes intersynaptic interventions within a time frame of few minutes to few hours (Frey and Morris, 1997;Frey and Morris, 1998a;Frey and Morris, 1998b;Morris and Frey, 1999). More clearly, a weak protein synthesis independent early-LTP/-LTD in one synaptic input can be transformed into a late, protein synthesis-dependent form, if a protein synthesis-dependent late-LTP/-LTD is induced in the second synaptic input preceded by the weak events in the first synaptic input (weak before strong) within a specific time frame (Frey and Morris, 1998b;Kauderer and Kandel, 2000).

### 1.5. Cellular mechanisms of LTP and LTD

#### 1.5.1. NMDA-receptor -dependent LTP and LTD

The N-methyl-D-aspartate (NMDA) receptor is a voltage-dependent glutamate receptor subtype. NMDA-receptors are composed of assemblies of NR1 and NR2 subunits, which can be one of four separate gene products (NR2A-D) (Gomperts et al., 2000;Racca et al., 2000;Robert et al., 2000). Expressions of both subunits are required to form functional channels. The glutamate binding domain is formed at the junction of NR1 and NR2 subunits (Yamakura and Shimoji, 1999). In addition to glutamate, the NMDA-receptor requires a co-agonist, glycine, to bind to allow the receptor to function. The glycine binding site is found on the NR1 subunit. The NR2B subunit also possesses a binding site for polyamines, regulatory molecules that modulate the functioning of the NMDA-receptor (Yamakura and Shimoji, 1999). At resting membrane potentials, NMDA-receptors are inactive. This is due to a voltage-dependent blockade of the channel pore by magnesium ions. In a recent report by Liu et. al., (Liu et al., 2004) and Massey et al., (Massey et al., 2004) showed that selectively blocking NMDA-receptors that contain the NR2B subunit abolishes the induction of LTD, but not LTP. In contrast, preferential inhibition of NR2A containing NMDA-receptors prevents the induction of LTP without affecting LTD. This result demonstrates that distinct NMDA-receptor subunits are critical factors that determine the polarity of synaptic plasticity.

For the induction of LTP/LTD, the NMDA-receptor must be activated by the neurotransmitter glutamate and simultaneously there must be a sufficient depolarization of the postsynaptic membrane to relieve a magnesium block in

the NMDA-receptor-associated ion channel, which allows then the entry of $Ca^{2+}$ into the post synaptic terminal. $Ca^{2+}$ activates a number of $Ca^{2+}$ -sensitive second-messenger processes. Because NMDA-receptors are sensitive to both presynaptic transmitter release and postsynaptic depolarization, they act as Hebbian coincidence detectors (Collingridge, 2003). NMDA-receptor-dependent LTP can be triggered experimentally either by delivering high-frequency tetani to a critical number of presynaptic afferent fibers, or by pairing postsynaptic depolarization with presynaptic stimulation (Gustafsson et al., 1987).

The mechanism of LTD induction are some what similar to those underlying LTP, in that both postsynaptic $Ca^{2+}$ influx and NMDA-receptor activation are necessary. These findings were first reported by Mulky et al., (Mulkey and Malenka, 1992) who found that intracellular injection of BAPTA, a calcium chelator, or suppression of NMDA currents by intracellular hyper-polarization both blocks LTD induction. Although both $Ca^{2+}$ influx and NMDA-receptor activation are necessary for LTP as well as LTD-induction in CA1, the concentration of intracellular $Ca^{2+}$ determines whether LTP or LTD is induced. According to Lisman (Lisman, 1989), a small influx of $Ca^{2+}$, which produces a low intracellular $Ca^{2+}$ concentration, may activate selective protein phosphatases whose action leads to the induction of LTD. Selective inhibitors of protein phosphatases block the induction of LTD in CA1 (Mulkey et al., 1993).

### 1.5.2. Role of protein kinases in LTP and LTD

Protein kinases critically regulate synaptic plasticity in the mammalian hippocampus (Gass et al., 1998;Frey et al., 1993;Rongo, 2002;Sweatt, 1999;Sweatt, 2004). Protein phosphorylation is mediated by protein kinases, and it is a key regulatory mechanism in neurons, enabling and modulating a plethora of important cellular processes, including neuronal development, growth, and plasticity (Walaas and Greengard, 1991). A large number of chemical neurotransmitters, hormones, and other signaling substances use cyclic adenosine 3',5'-monophosphate (cAMP) as an intracellular second messenger (Nguyen and Woo, 2003). The principal target for cAMP in mammalian cells is cAMP-dependent protein kinase A (PKA), which is ubiquitously expressed and mediates intracellular signal transduction and intracellular signal transmission in invertebrates and vertebrates (Frey et al., 1993;Nguyen and Kandel, 1996;Brandon et al., 1995). Late-LTP requires cAMP-dependent PKA activity during LTP induction (Frey et al., 1993;Huang and Kandel, 1994;Abel et al., 1997;Nguyen and Kandel, 1997;Nguyen and Woo, 2003). For example, application of cAMP analogs to synapses in a hippocampal slice induces a slowly expressing, but long-lasting LTP (Frey et al., 1993). Furthermore, application of PKA inhibitors attenuates LTP expression, apparently eliminating the ability of synapses to express LTP (Otmakhova et al., 2000) . These results suggest that PKA activated by cAMP may gate the expression of late-LTP by direct, indirect or permissive activation of transcription factors.

In addition to PKA, numerous signaling molecules that regulate synaptic plasticity have been identified that, include calcium/calmodulin

dependent protein kinases (CaMKII, CaMKIV) and others. (Rongo, 2002; Miyamoto and Fukunaga, 1996; Colbran and Brown, 2004). However, most experiments had investigated only early stages of LTP and LTD. With respect to candidate enzymes involved in maintaining LTP, in addition to PKA, the role of protein kinase C (PKC) has been suggested to be important (Angenstein et al., 1994; Matthies, Jr. et al., 1991;Reymann et al.,1988a;Fedorov et al., 1995;Reymann et al., 1988b;Bliss and Collingridge, 1993;Linden and Routtenberg, 1989;Malenka and Nicoll, 1999;Malinow et al., 1989).

PKC has been shown to exist in the neuron in at least three forms differing with respect to their activation characteristics: free cytosolic, membrane-associated and membrane -inserted (Ohno and Nishizuka, 2002). PKC consists of a family of ~15 different isoforms that has been classified into three second messenger-dependent groups: conventional ($Ca^{2+}$/diacylglycerol (DAG) dependent), novel ($Ca^{2+}$ dependent, DAG independent) and atypical ($Ca^{2+}$/ DAG-independent) (Nishizuka, 1995). Finally, the recently discovered PKC related kinases (PRKs) define a fourth group consisting of at least three members, PRKs 1 to 3. Like atypical PKCs, PRKs are insensitive to $Ca^{2+}$, DAG and phorbol esters. (Hirai and Chida, 2003; Hirai et al., 2003). It has been reported that an isozyme of PKC called PKM-zeta (PKMζ) (Sacktor et al., 1993), is activated during LTP by a mechanism fundamentally different from that of the other kinases implicated in LTP (Osten et al., 1996a;Hernandez et al., 2003) even though its role in LTD is not known except that it is down regulated during its maintenance (Hrabetova and Sacktor, 1996;Hrabetova and Sacktor, 2001) . Atypical full-length PKC

isoform consists of an N-terminal regulatory domain and a C-terminal catalytic domain (Hernandez et al., 2003). The regulatory domain contains binding sites for second messengers and a pseudo substrate 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 pseudo substrates inhibition. PKMζ, in contrast, consists of an independent catalytic domain of the atypical PKCζ isoform, and lacking inhibition from a regulatory domain, is persistently active without continual second messenger stimulation. Although PKM is usually thought of as a cleavage product of full-length PKC (Kishimoto et al., 1983). But recently Hernandez et al., (Hernandez et al., 2003) reported that PKMζ is formed in LTP not by proteolysis, but by a gene expression of a brain-specific PKMζ mRNA, which is generated from an internal promoter within the PKCζ gene. Tetanic stimulation induces protein synthesis from PKMζ mRNA, persistently increasing the amount of the independent, autonomously active ζ catalytic domain during LTP maintenance (Osten et al., 1996b;Hernandez et al., 2003). Phosphorylation by PKMζ potently enhances AMPA-receptor-mediated synaptic transmission (Ling et al., 2002). In addition, inhibition of PKMζ activity 1h after tetanization reverses the maintenance of AMPA-receptor-mediated synaptic potentiation (Ling et al., 2002). PKMζ could potentially mediate synaptic enhancement in the early phase, in the transition from early- to late-, or in the late phases of LTP (Ling et al., 2002).

More recently, the mitogen-activated protein kinase (MAPK) cascade that activates extracellular signal-regulated kinases (ERKs) has been implicated in LTP as well as in some forms of learning and memory (Sweatt,

2001;Sweatt, 2004;Thomas and Huganir, 2004). Tonegawa and colleagues (Kelleher, III et al., 2004a;Kelleher, III et al., 2004b) recently reported that, the conditional expression of a dominant-negative form of mitogen activated extracellular kinase-1 (MEK1) in the postnatal murine forebrain inhibited ERK activation and caused selective deficits in hippocampal memory retention and the translation-dependent, transcription-independent phase of hippocampal late-LTP. Two other kinases deserving of mention are phosphatidylinositol 3-kinase (PI3 kinase) and the tyrosine kinase Src. PI3 kinase appears to be required for a form of LTP that involves the trafficking of AMPA-receptors to synapses in dissociated cultured hippocampal neurons (Man et al., 2003). Src, on the other hand, may serve to enhance NMDA-receptor function during the LTP induction protocol (Salter and Kalia, 2004).

### 1.5.3. Role of protein synthesis and transcription factors in LTP and LTD

Protein synthesis is assumed to be necessary for the cell to maintain synaptic changes over long time periods, which require constant molecular turnover and eventually leads to synaptic growth. It is hypothesized that late-LTP requires the activation of transcription factors for sustaining prolonged periods of synaptic enhancement and finally making the synaptic change relatively permanent. Intraventricular application of anisomycin, a reversible translational inhibitor, prevents the long-term maintenance of LTP in the dentate gyrus, an effect that parallels the block of long-term memory in several learning tasks (Krug et al., 1984;Otani and Abraham, 1989). The application of anisomycin before, during, or immediately after tetanization produced a gradual decrease of potentiation after 4-6 h without affecting

early-LTP. Application of anisomycin 1 h after tetanization had no effect. A similar phenomenon was observed when LTP was induced in dendritic stumps of CA1 pyramidal cells of hippocampal slices in vitro (Frey et al., 1989b). In these experiments the cell-body layer, the major site of protein synthesis was surgically removed from the apical dendrites. The isolated dendrites revealed a pronounced early-LTP in the fEPSP as is found in intact slices. However, the potentiation gradually decreased after about 4 h, thus showing the same lack of late-LTP as observed in complete CA1 neurons after inhibition of protein synthesis with anisomycin.

It has been shown that, the transcription factor cAMP-responsive element binding protein (CREB) differs in its activation following the induction of either short or long form of LTP (Matthies et al., 1997;Impey et al., 1998;Schulz et al., 1999). CREB is a member of the basic leucine zipper super family of transcription factors that modulate the transcription of genes by binding to a regulatory DNA promoter known as cAMP responsive element (CRE) (Brindle and Montminy, 1992;Mayr and Montminy, 2001). Nuclear CREB can be activated by several neural signaling pathways, including the cAMP and $Ca^{2+}$ pathways which are known to be involved in memory and are activated or up-regulated by stimuli that induce LTP (West et al., 2001;Deisseroth et al., 1998). A variety of kinases induced by these pathways have been shown to activate CREB by phosphorylating the Ser 133 site (Gonzalez and Montminy, 1989;Sheng et al., 1991;Bito et al., 1996;Deisseroth and Tsien, 2002;Ying et al., 2002). In contrast, CREB mutant mice showed normal LTP and intact learning in most hippocampus dependent tasks (see (Balschun et al., 2003)).

### 1.5.4. Heterosynaptic requirements: Role of dopamine for synaptic plasticity in hippocampal CA1

In hippocampal slice preparations in vitro, there are many neuromodulatory afferents alongside the Schaffer collaterals from CA3 to CA1. It is likely that the multiple strong tetani used to induce late-LTP also activate these fibres. One candidate modulatory system is the mesolimbic dopaminergic pathway that, in addition to its many other projections, is afferent to the hippocampus from the ventrolateral tegmentum (Gasbarri et al., 1994). Immunohistochemical localization of dopamine D1 and D5 receptors shows that there is heavy staining along pyramidal cells of the CA1 (Huang et al., 1992) and blockade of these receptors with SCH23390 inhibits the maintenance of late-LTP in hippocampal slices (Frey et al., 1990;Frey et al., 1991b). It has been shown, however, that to affect LTP, dopamine antagonist must be present at the time of induction (Frey et al., 1990;Frey et al., 1991b;Frey et al., 1989a); application after induction had no effect. Dopamine has been shown to affect early- and late-LTP in the hippocampal CA1 pyramidal cells (Frey et al., 1990;Frey et al., 1991b;Frey et al., 1993;Huang and Kandel, 1995;Otmakhova and Lisman, 1998;Otmakhova and Lisman, 1996;Otmakhova et al., 2000) and both LTD and LTP in inhibitory striatal neurons (Calabresi et al., 1997).The normal role of this dopaminergic activation might be to activate second-messenger systems, such as the PKA/cAMP cascade or the ERK/MAPkinase pathway, and thereby regulate downstream gene transcription or translation (Frey et al., 1993;Ying et al., 2002).

Dopamine plays an important role in both working (Goldman-Rakic, 1995) and long-term memory. In long-term memory, dopamine is involved specifically in the mechanisms of reinforcement (Cooper et al., 1990;Schultz et al., 1993). Midbrain dopaminergic neurons respond to a reward and deliver dopamine to target brain structures, including the hippocampus (Gasbarri et al., 1994). The pivotal role for the hippocampal dopaminergic system has been demonstrated in several types of learning: intrahippocampal injections of dopamine agonists enhances passive avoidance (Bernabeu et al., 1997) visual discrimination (Jork et al., 1982) and win-shift positive reinforcement learning (Packard and White, 1991). Dopamine depletion in the hippocampus impairs spatial navigation (Gasbarri et al., 1994).

Activation of D1-like receptors enhances LTD, induced by low-frequency stimulation in rat hippocampal CA1 neurons (Chen et al., 1995). Similarly, blockade of either D2- or D1-like receptors decreases the magnitude of late phases of LTP, which seems to involve cAMP-dependent mechanisms (Frey et al., 1990;Frey et al., 1991b;Frey et al., 1993). Slices perfused with high concentrations of D1-like agonists without any tetanus can itself mimic the late phases of LTP; the effect which is blocked by inhibitors of protein synthesis (Huang and Kandel, 1995). Interestingly, dopamine produces a synapse-specific enhancement of early-LTP through D1/D5 receptors and cAMP (Otmakhova and Lisman, 1996).

Recent work has shown that D1/D5 dopamine receptors inhibit depotentiation (DP) at CA1 synapses via cAMP-dependent mechanism (Otmakhova and Lisman, 1998). In an another study, the importance of D2 like receptors are also reported in depotentiation in vivo (Manahan-Vaughan

and Kulla, 2003). Together these data suggest the involvement of different dopamine-dependent mechanisms in the control of synaptic plasticity in various brain areas.

### 1.6. Synaptic plasticity and subsequent activity at the same input: Depotentiation (DP)

Depotentiation refers to the reversal of LTP by the application of low-frequency stimulation (LFS) shortly after LTP-induction (Staubli and Chun, 1996a), first described in CA1-region of the hippocampus and is considered as a mechanism for forgetting at cellular level (Manahan-Vaughan and Kulla, 2003). An important feature of depotentiation is its dependence on time, i.e. LFS is effective only if given within a distinct time window after LTP induction(Staubli et al., 1998;Staubli and Scafidi, 1999). Time-dependent depotentiation can effectively destabilize the putative mechanisms essential for the maintenance of LTP (Woo and Nguyen, 2003). Depotentiation in vivo was shown in the CA1 region as well as in the dentate gyrus (DG) (Staubli and Scafidi, 1999;Straube and Frey, 2003). However, LTP in the DG appears to be more resistant to depotentiation compared to CA1 region. In DG, only delays of 2 or 5 min-but not of 10 min-between LTP induction and LFS led to depotentiation. Brief 7 Hz stimulation (which is having behavioral relevance, since oscillations at a frequency of about 7 Hz are the dominant events in the hippocampal EEG of rats responding to new sensory input and plays an important role for hippocampal information processing) of 100 stimuli repeatedly after LTP induction in DG of freely moving rats also effectively induce depotentiation (Straube and Frey, 2003).

In hippocampal CA1, a one minute episode of 5 Hz (theta frequency) stimulation beginning 1-3 min after LTP had no effect on the degree of potentiation measured 30 min later. However, in the presence of norepinephrine, 5 Hz stimulation reduced LTP by about 30% (Larson et al., 1993). Theta frequency stimulation was only effective when administered with in 10 min of LTP induction and had no long lasting effects on non-potentiated synapses. Stimulation at 1 Hz did not reverse LTP and stimulation at 10 Hz was no more effective than 5 Hz stimulation. LTP could be nearly completely reversed by theta frequency stimulation when potentiation was induced by milder and more naturalistic stimulation patterns. Under these conditions, LTP reversal was blocked by an antagonist of adenosine A1-receptors. These results suggest that the hippocampal theta rhythm promotes both induction of LTP and its subsequent reversal with the latter processes involving activation of adenosine receptors. The importance of D2 like receptors are also reported in depotentiation in vivo (Manahan-Vaughan and Kulla, 2003). Agonist priming of D2 like receptors with a drug concentration which had no effect on synaptic transmission, inhibited depotentiation but did not affect LTP. The agonist effects on depotentiation were prevented by D2 like antagonist remoxipride. Remoxipride itself did not influence basal synaptic transmission or depotentiation. These results suggest a specific role for dopamine D2 like receptors in the regulation of depotentiation in vivo.

### 1.7. ´Synaptic tagging´ during LTP

A significant challenge for the neuroscience community is to fit the stock of ‘learning and memory’ molecules that have been identified so far with

the cellular and physiological observations that are associated with enduring synaptic changes. Single synapses or sets of synapses can undergo selective modifications when stimulated and although any given neuron receives thousands of synaptic contacts, each of them can potentially be modified in an independent manner for longer periods of time. Because this type of synaptic modification requires both transcription and translation, the problem of targeting gene products from the nucleus to the few activated synapses in a vast dendritic tree has been solved by the neuron in ways that we do not yet fully understand. The identity of the proteins responsible for stabilizing LTP has not been established, its occurrence raises a fundamental question: given that macromolecule synthesis occurs mainly in the cell body, how do these proteins find their way through the dendrites to the appropriate synapses which were activated by a weak stimuli ? In general there are considered to be four hypotheses about how the synapse specificity of late-LTP could be achieved (Frey and Morris, 1998a): the ‘mail’ hypothesis; the ‘local’ hypothesis; the ´synaptic tag´ hypothesis and the ‘sensitization’ hypothesis. The ‘mail’ hypothesis involves elaborate intracellular protein trafficking, where proteins, at the time of their synthesis, are given a ‘synaptic address’ to which they are destined. The mail hypothesis is intrinsically unlikely because the requirement of proteins to travel from the soma to a specific synapse in a cell that, in case of CA1 pyramidal cells, might have more than 10,000 synapses (Frey and Morris, 1998a). The local synthesis hypothesis predicts the relevance of local protein synthesis machinery which is activated due to stimulation of nearby synapses. The local synthesis idea is supported by the presence of spine associated poly ribosome. Input specificity is a straight

forward consequence of this cellular architecture. Recent findings by Kang and Schumann (Kang and Schuman, 1996) support local dendritic protein synthesis by neurotrophin-induced potentiation, but this form of plasticity is input non-specific. The 'sensitization' hypothesis entails distribution of plasticity related macromolecules to every synapses of the cell. These would have the effects of altering the threshold at which synaptic activation (or $Ca^{2+}$ influx) gives rise to lasting synaptic changes. When few of these macromolecules are available, a high threshold prevails, and tetanization usually induces only early-LTP; when many macromolecules are available, it is much easier for late-LTP to be induced (Malinow et al., 2000). The sensitization hypothesis is supported by recent findings of a *de novo* protein synthesis dependent formation of protein kinase Mζ (Ling et al., 2002;Hernandez et al., 2003;Muslimov et al., 2004) .

The ´synaptic tagging´ hypothesis describes a mechanism, how input specificity is achieved during a protein synthesis-dependent stage (Frey and Morris, 1997;Frey and Morris, 1998a;Frey and Morris, 1998b;Martin and Kosik, 2002). Key experiments in two systems in rat (Frey and Morris, 1997) and in Aplysia (Martin, 2002) elucidated ´synaptic tagging´ in vertebrate and in invertebrate brain respectively.

The synaptic tag hypothesis (Frey and Morris, 1997;Frey and Morris, 1998a) proposed that the persistence of LTP is mediated by the intersection of two dissociable events. The first event involves the generation of a local 'synaptic tag' at specific synapses in association with and perhaps causally related to the induction of LTP. The second involves the production and diffuse distribution of 'plasticity related proteins' (PRPs) that are captured and

utilized only at those synapses possessing a tag. A paradoxical but logical prediction of this hypothesis is that, in two pathway experiments, it should be possible to induce protein-synthesis-dependent late-LTP following tetanisation that either does not by itself trigger protein synthesis, or may even occur during the inhibition of protein synthesis. Such late-LTP induction could occur if synaptic tags set at the pathway that is tetanised weakly, or tetanised during the inhibition of protein synthesis, capture the PRPs induced by the strong tetanisation of other afferents. This prediction was tested by stimulating two independent inputs to a population of CA1 pyramidal cells in hippocampal slices. Late-LTP was induced on one pathway (S1), and the protein synthesis inhibitor anisomycin then bath applied just before the second pathway (S2) was tetanised. Normally, only early-LTP would be induced and late-LTP inhibited in the presence of anisomycin. However, the LTP induced on S2 remained potentiated for up to 8 h post-tetanus (Frey and Morris, 1997).

In addition to input-specificity, synaptic tagging is characterized by new late-associative properties which have been identified in an elegant study in hippocampal slices. The weak tetanic stimulation that normally induces only early-LTP could be ‘transformed’ into late-LTP heterosynaptically if a strong tetanus was delivered to an independent input to the same population of CA1 pyramidal cells shortly before or shortly after the weak tetanus (Frey and Morris, 1998b). These results indicate that the weak stimulus created a ‘synaptic tag‘ that could ‘hijack’ the proteins from the other synaptic input, resulting in persistent strengthening of synapses that would otherwise express only early-LTP. These experiments show that the products of transcription are delivered through out the cell, and that they persist for a specific period,

during which they can be captured by sub threshold synaptic activity at another site in the cell (Frey and Morris, 1998a).

´Synaptic tagging´ is also elucidated in invertebrate model such as Aplysia (Martin, 2002). In culture system, a single bifurcated Aplysia sensory neuron can form synaptic contacts with two spatially separated motor neurons. Delivery of five puffs of serotonin (5-hydroxy tryptamine or 5HT) to one contact selectively enhances synaptic efficacy at that synapses without altering the efficacy of the other contact (a phenomenon that has been termed branch specific facilitation). This increase in synaptic potency persists for more than 24 h and depends on transcription, as it can be blocked by the transcriptional inhibitor actinomycin D (Martin, 2002). ´Synaptic tagging´ can be shown in this experimental system when a single puff of serotonin is delivered to one contact and five puffs are applied to the other contact, the facilitation produced by single puff is long lasting. Importantly, to produce long-term facilitation (LTF), the single pulse of serotonin must be given within a discrete time window either 1-2 h before or 1-4 h after five pulses of serotonin were applied to the other connection. These observations indicate that long term synaptic changes at one synapse can trigger a cell wide process that is captured by another synapse that has experienced a level of activation that would otherwise produce only short term changes, furthermore they show that this phenomenon has a transient life time (Martin and Kosik, 2002).

The ‘sensitization’ hypothesis shares with the ´synaptic tag´ idea that the persistence of LTP can vary as a function of the recent history of activation of the neuron, but this variability would be strictly dependent on the

past history of activation, and uninfluenced by the immediate future history of activation. It also makes the false prediction that application of protein synthesis inhibitors shortly after LTP induction should have no effect on LTP persistence although effects later than eight hours cannot yet be excluded. The ´synaptic tag´ hypothesis permits greater flexibility and more intracellular co-operativity than any of the other ideas (Frey and Morris, 1998a) .

### 1.8. Aims of the dissertation

The studies which will be presented here investigated: In a first series of experiments I studied whether electrically-induced long-term depression within rat hippocampal slices in vitro shares common cellular features with LTD in the intact animal, with particular emphasis being placed on mechanisms required for its late maintenance. Initial studies have led to the development of stimulation protocols which resulted in reliable induction of different forms of LTD, like protein synthesis-dependent late-LTD or -independent early-LTD, depending on the stimulation protocols. These studies were important pre-requisites for investigating a second series of experiments which includes studying the mechanisms of ‘synaptic tagging’ and ‘late-associativity’ during LTD. So far ‘synaptic tagging’ and processes of ‘late-associativity’ were described only for LTP in vitro. Thus I was interested as to whether LTD is characterized by similar phenomena which also includes the search for possible heterosynaptic requirements for LTD and LTD-related tagging.

A third complex series of studies were undertaken to investigate a very interesting and exciting question: Can LTP and LTD interact in a single

neuronal population. This question was successfully studied in this thesis showing positive interactions of LTP and LTD, a property which we have named ´cross-tagging´. In the fourth series of experiments, I was interested in the search for the molecular nature, the physiological activation/deactivation of the putative 'tag' and plasticity-related proteins (PRPs) required for late-LTP as well as for late-LTD. Thus, I have investigated the role of PKC isotype PKMζ. My results revealed that PKMζ can be considered as an LTP-specific PRP which plays an important role in LTP- and LTD-induction as well as in LTP-tagging but not in LTD-tagging. And finally, I have studied whether activated 'tag' or 'tag complex' can be reset in a time-dependent manner by using depotentiation. Thus, my main goal was to characterize processes of ´synaptic tagging´ during LTD and its associative interactions.

## 2.0. Materials and methods

### 2.1. Brain slice preparation and incubation

All experiments were performed in right hippocampal slices (400 µm thick) prepared from 7 weeks old male Wistar rats (total number of animals: 275). The animal was stunned by a blow behind the foramen magnum and decapitated immediately. Following decapitation, the skin and fur covering the skull were cut away and an incision was made on both sides. The bone covering the brain was prised away and dura removed before transfering the brain into cooled and carbogenated (carbogen: gas consisiting of 95% $O_2$ and 5% $CO_2$) artifical cerebro spinal fluid (ACSF) (about 4°C). Cold solution was used to slow down the metabolism of the tissue, to limit the extent of excitotoxic and other kinds of damage occurring during the preparation of slices (Reymann et al., 1985). The hemispheres were separated mid-sagitally by a deep cut using a scalpel and the hippocampal commissure was cut and the right hippocampus was taken out on to the stage of McIlwain tissue chopper (Cambden,UK), and 400 µm slices were cut at 70° transverse to the long axis from the middle third of the right hippocampus. After sectioning, the slices were picked up by a wet artist's brush, floated in a petri dish containing the cooled and carbogenated ACSF, and immediately transfered to the nylon net in the experimental chamber by a wide bored pipette. One of the critical points which elapses between the removal of the brain and the placing of the slices in the chamber, is that time should not exceed 4 min. Cooling of the brain and slices between the removal of the brain from the skull and the arrival in the chamber improves the viability of the slices. When slices are taken out with proper care the responses, observed on stimulation are similar

to those seen in intact animals. Slices were incubated within an interface chamber at 32°C (carbogenated incubation medium contained 124 mM NaCl, 4.9 mM KCl, 1.2 mM $KH_2PO_4$, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 24.6mM $NaHCO_3$, 10 mM D-glucose). Supply of oxygen was achieved by controlling the gas flow over the surface of the slice (carbogen flow rate: 18 l/h) thus preventing the drying out of the slices (see Fig. 3).

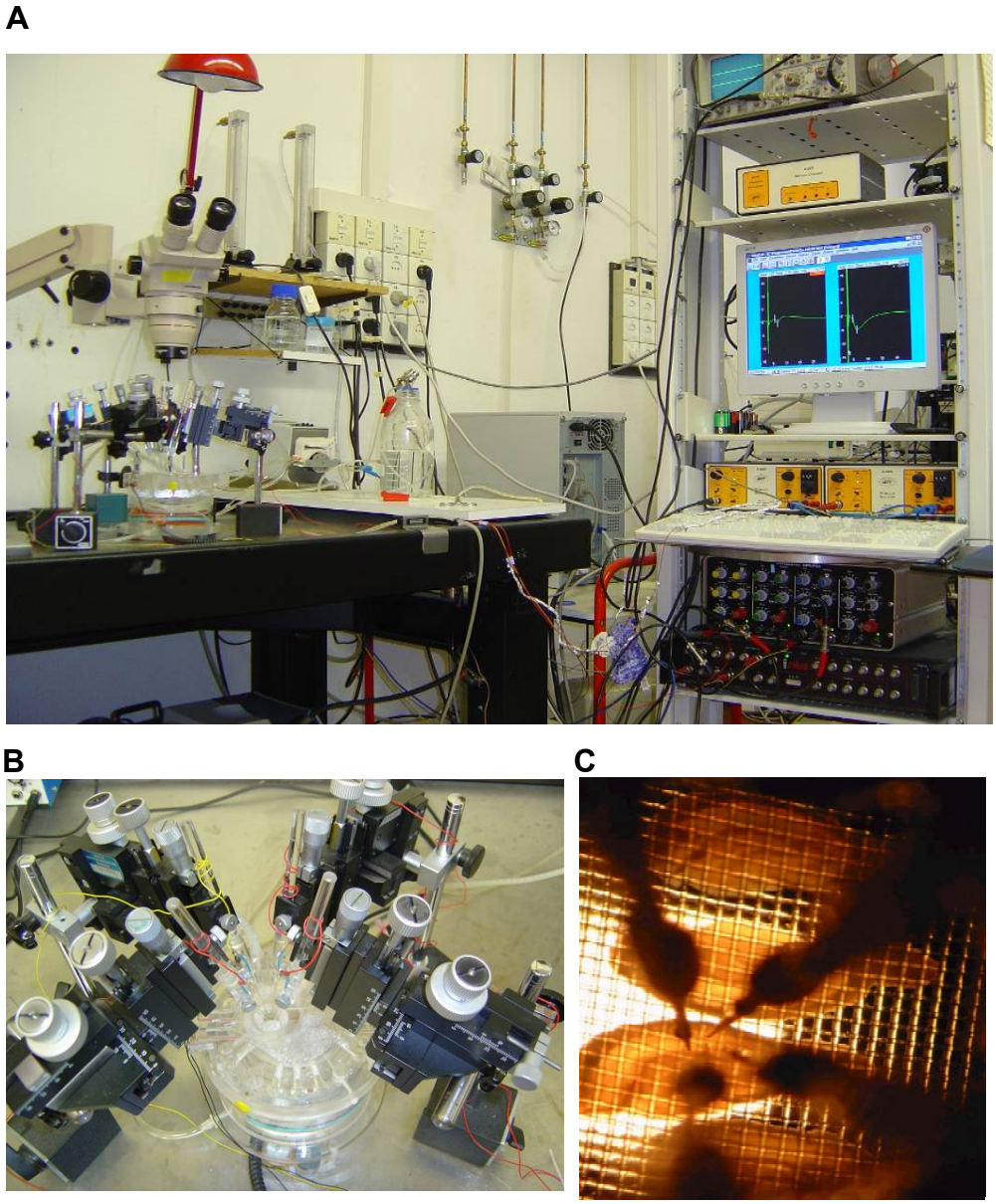


**Figure 3. Interface chamber and electrical set-up for long term extra cellular recording.** **(A)** An overview of recording chamber and its electrical set-up. **(B)** Interface chamber with manipulators. **(C)** Microscopic view of a hippocampal slice located with electrodes.

In all experiments, two monopolar lacquer-coated, stainless steel electrodes (input resistance: 5 MΩ; AM-Systems, USA) were positioned within the stratum radiatum of the CA1 region for stimulating two separate independent synaptic inputs S1 and S2 (Fig. 4).

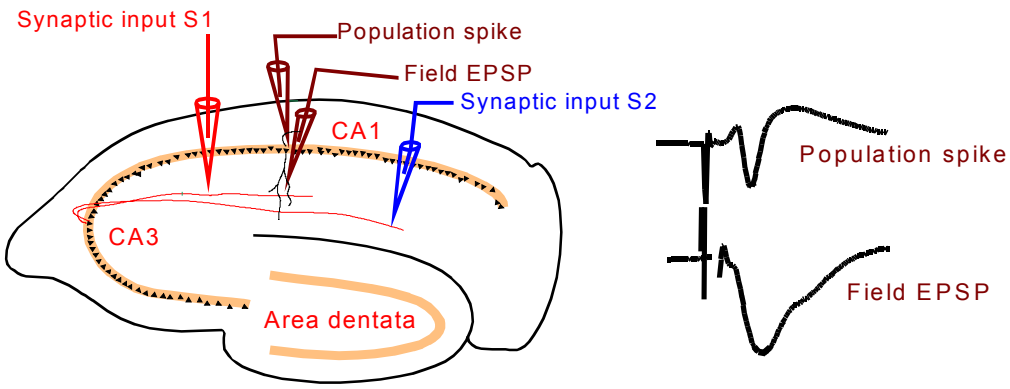


**Figure 4. Transversal hippocampal slice showing the positioning of the electrodes.** Two independent synaptic inputs S1 and S2 to the same neuronal population and the recording sites for the population spike amplitude and the field EPSP as well as analog recording traces as representative examples of them are shown.

For recording, two electrodes (5 MΩ; AM-Systems) were placed in the CA1 dendritic and cell body layer of a single neuronal population. Recorded potentials were amplified by a custom made amplifier (INH, Magdeburg, Germany). The analog signals were then digitized using a CED 1401 A/D converter and analyzed with custom-made software (PWIN, Magdeburg, Germany). Slices were preincubated for at least 4 h, a quite unusual long period, but it has been shown by the following reasons to be critical for a stable long-term recording as well as the study of late plasticity for up to 16 h, under conditions which resemble the functionality of studies in vivo.

This methodical issue of the required prolonged preincubation period for subsequent functional plasticity studies is very often overseen. Hippocampal slices in vitro are characterized by a very low spontaneous activity which may result from an almost ‘absolute rest’ during preincubation. Biochemical studies have shown that metabolic stability is reached in slices after 2-4 h, i.e., metabolite levels require 2-4 h to stabilize, and these levels are then maintained for at least 8 h of incubation (Whittingham et al., 1984). This includes parameters for the activity of enzymes, second messengers, pH, and others. Interestingly, the value for bio-active molecules which stabilizes then at a very low level, if strong electrical stimulation was not delivered to the tissue. We suppose that in addition to processes of the acute slice preparation, low electrical activity may result in the delayed but prolonged metabolic stability at a low level after about 4 h if no stimulation is applied to the tissue. This may lead to a reduction of PRPs to an amount near zero if the half life of the proteins is considered with about 2 h. Thus, starting with functional experiments after a preincubation time of 4-5 h, may rectify all slices and neurons to a low but very comparable basal metabolic and plasticity level. Tetanization for instance, would then activate a machinery of processes ‘from zero’ (a situation never occurring in behaving animals) which is mechanistically more useful to determine time constants during plastic events, than it would be the case by using freely behaving untreated rats. If in intact rats protein synthesis is blocked by a pharmacological reversible inhibitor a similar situation as in slices can be created revealing similar time constants for early-LTP in vitro. Unfortunately, currently available reversible protein synthesis inhibitors reduce the synthesis of macromolecules in the

intact animal for several hours, making this preparation probably unusable to directly study processes of synaptic tagging with the methods used so far. Thus, slice preparations represent an ideal, however also partially artificial model to determine properties of tagging and late-associativity. Although, most of the problems concerning brain slice incubation are known for a long time, most laboratories start their 'physiological' slice experiments after a very short preincubation period of even less than 1 h. Knowing the metabolic instability during that period we prolonged the preincubation of hippocampal slices to at least 4 h to obtain comparable and more physiological results in describing functional processes in slice preparations. This requirement is supported by additional data such as measuring basal endogenous protein phosphorylation patterns and the translocation of different protein kinase C isoenzymes (α, β and γ) to the membrane as markers of their activation in tissue obtained from hippocampal slices in vitro or from intact, untreated rats. Studies revealed that only slices incubated in the same way as described here showed comparable patterns of phosphorylation and enzyme translocation as detected in the intact animal (Angenstein and Staak, 1997). Although one could argue that specific modifications of slice preparation may circumvent distinct problems raised above, to maintain the complex slice physiology at a level which allows reliable studies of functional plasticity favors a more simple method: to wait (Sajikumar and Frey, 2004a).

Following the preincubation period, the test stimulation strength was determined for each input to elicit a population spike of about 40 % (for LTD studies) or 25 % (for studies conducted to investigate LTP and the effect of dopamine application) of its maximal amplitude determined by slice specific

input-output relationship. For stimulation, biphasic constant current pulses were used. The baseline was recorded for at least 60 min before LTP/LTD induction. In the dopamine studies the baseline was recorded for at least 30 min. Four 0.2 Hz biphasic, constant-current pulses (0.1 ms per polarity) were used for testing 1, 3, 5, 11, 15, 21, 25, 30 min post-tetanus or 21, 25, 30 min post-LFS and thereafter once every 15 min up to 8 h (30 min in dopamine series). Since the two recorded parameters showed either similar time course in the experiments (if the population spike was not abolished after induction of LTD at all), for clarity only the fEPSP data are shown.

All experiments were carried out in accordance with the European Communities Council Directive of 24$^{th}$ November 1986 (86/609/EEC). It is also certified that formal approval to conduct the experiments described has been obtained from the animal subjects review board of our institution/local government which can be provided upon request. All efforts were made to minimize the number of animals used and their suffering.

### 2.2. Stimulation Protocols: Inuction of late-LTD, early-LTD, late-LTP, early-LTP and depotentiaton

For inducing late-LTD, a strong low-frequency stimulation protocol (SLFS) which consisted of 900 bursts (one burst consisted of 3 stimuli at a frequency of 20 Hz, interburst interval=1 s, i.e. f=1 Hz, stimulus duration 0.2 ms per half-wave; a total number of stimuli of 2700) was found to be the most effective protocol (Sajikumar and Frey, 2003;Sajikumar and Frey, 2004a). This stimulation pattern produced a stable LTD in vitro for at least 8 h. For inducing a transient early-LTD a weak low-frequency stimulation protocol

(WLFS) consisting of 900 pulses (f=1 Hz, impulse duration 0.2 ms per half-wave, a total number of stimuli of 900) was determined to be the most efficient in inducing early-LTD (Sajikumar and Frey, 2003;Sajikumar and Frey, 2004a). Late-LTP was induced using three stimulus trains of 100 pulses (‘strong’ tetanus: f=100 Hz, stimulus duration 0.2 ms per polarity with 10 min intertrain-intervals) (Frey and Morris, 1997;Frey and Morris, 1998b). In experiments with induction of early-LTP, a single tetanus with 21 pulses was used (`weak’ tetanus: f=100 Hz, stimulus duration 0.2 ms per polarity, population spike threshold stimulus intensity for tetanization) (Frey and Morris, 1997;Frey and Morris, 1998b). A protocol for depotentiation was used, which consisted of a low frequency stimulation of (LFS) of 250 impulses at a frequency of 1Hz (Sajikumar and Frey, 2004b).

In the series investigating the action of dopamine, instead of a threefold tetanization, 3 stimuli were applied with doubled stimulus duration at time points where in LTP-experiments the tetani were applied.

### 2.3. Pharmacology

2-amino-5-phospho-valeric acid (APV; Sigma) was used at a concentration of 50 µM (dissolved in ACSF) to block the NMDA-receptor. Anisomycin (Sigma) (dissolved in ACSF), a reversible protein synthesis-inhibitor, was used at a concentration of 25 µM (a concentration that blocks at least 85% of incorporation of 3H-leucine into hippocampal slices (for details see (Frey et al., 1991a)). A structurally different irreversible protein synthesis inhibitor, emetine (Tocris), was used at a concentration of 20 µM (dissolved in ACSF). Dopamine (10 or 50 µM; Tocris) was applied into the bath medium

three times for three min with an inter-application interval of 10 min. In these, and in the adequate control experiments the anti-oxidant ascorbic acid (1 mM) was added. Dopamine and ascorbic acid were dissolved in bath solution in a dark tube immediately before application. The selective dopaminergic D1/D5-receptor antagonist SCH23390 was used at a concentration of 0.1 µM (Tocris; dissolved in ACSF). The myristoylated ζ pseudosubstrate peptide (myr-SIYRRGARRWRKL-OH, Biosource) was used at a concentration of 1 µM. It was prepared by dissolving it in distilled water as a stock solution (10 µM) which was stored at -20°C. The required volume containing the final concentration of 1 µM was dissolved in ACSF immediately before bath application. The scrambled control peptide (myr-RLYRKRIWRSAGR-OH, Biosource, (Laudanna et al., 1998) was prepared in the same way as mentioned above.

### 2.4. Statistics

The averaged potential recalculated as percentage per time point ± SEM of slope function (mV/ms) of the field EPSP were then subjected to statistical analysis (Wilcoxon-signed-rank-test, when compared within one group or the Mann-Whitney-U-test when data was compared between groups ($P$<0.05 was considered as being statistically significant different)).

# 3.0. Results

## 3.1. Protein synthesis- and NMDA-receptor-dependent late-LTD

LTD induced by low-frequency stimulation can be maintained in the hippocampal structures of the intact animal, with a similar time course as LTP. However, with respect to underlying cellular mechanisms and in contrast to LTP, the long-lasting maintenance of LTD has so far been studied less extensively. The question arises as to whether, the prolonged maintenance of LTP and LTD share common cellular properties? To investigate this question we used hippocampal slices in vitro. First, late-LTD was induced in a synaptic input S1 by using a SLFS which resulted in a long-lasting late-LTD with a duration of up to 8 h (Fig. 5 A; filled circles). Control stimulation of an independent synaptic input S2 revealed stable potentials for the time course investigated (Fig. 5 A; open circles). The next experimental series tested whether the late-LTD requires NMDA-receptor activation and protein synthesis. Application of the NMDA-receptor antagonist AP-5 thirty min before until 30 min after SLFS in S1 prevented the induction of LTD (Fig. 5 E; filled circles). Responses obtained from S2 (open circles) remained stable potentials at control levels. These results support our hypothesis that late-LTD shares common properties like late-LTP. Application of the protein synthesis inhibitors anisomycin (25 µM) or emetine (20 µM), 30 min before SLFS in S1 (Fig. 5 B and 5 C; filled circles) resulted in the prevention of late-phases of LTD and revealed a transient, short-lasting depression, i.e. early-LTD. The control input was not influenced by the drug (open circles; statistically significant difference between S1 and S2 was detected for the first 255 min after anisomycin application; $P<0.05$, *U* test).

In the next series, a transient and protein synthesis-independent form of LTD (early-LTD) was induced by using a WLFS (Fig. 5 D; filled circles). The depression reached the base line within 3 h after its induction (statistically significant different for 185 min when compared with control input S2, open circles, *U*- test, or for 210 min when compared with its baseline before WLFS, Wilcoxon test, $P<0.05$).

In summary, protein syntesis and NMDA-receptor dependent late-LTD and a transient form of early-LTD can be induced in CA1 area of hippocampal slices in vitro by using appropriate stimulation protocols.

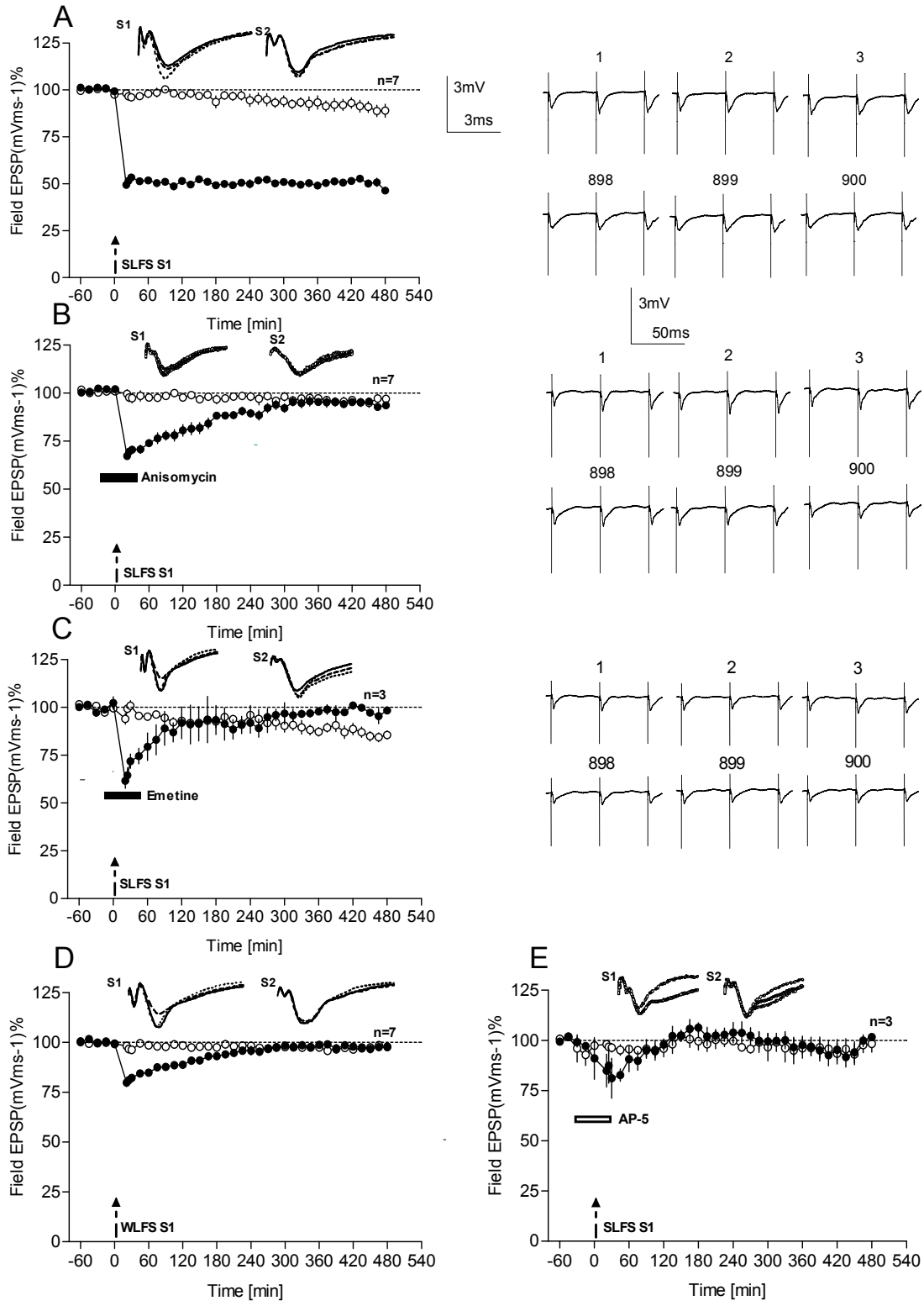


**Figure 5. Protein synthesis and NMDA-receptor dependent late-LTD. (A)** Induction of late-LTD in S1 (filled circles) using the repeated LFS protocol (i.e., strong low-frequency stimulation (SLFS; broken arrow). Late-LTD showed statistically significant difference for the 8 h investigated when compared with the time course of potentials recorded from a control input S2 (open circles;$P<0.05$, *U* test). Control stimulation of S2 revealed relatively stable

potentials for the time course investigated. **(B and C)** Application of the protein synthesis inhibitors (box) anisomycin (25 µM) or emetine (20 µM), as a second structural different inhibitor, 30 min before until 30 min after SLFS in S1 (filled circles) revealed a transient early-LTD. The control input S2 (open circles) was not influenced by the drug. Statistically significant difference between S1 and S2 was detected for the first 255 min after anisomycin application; $P<0.05$, *U*- test). The analog triplets next to the graph illustrate the initial and last three triplets of analog traces during the LTD-inducing trains (i.e., potentials obtained at the 1st, 2nd, 3rd, and 898th, 899th or 900th second of one LFS-train). **(D)** A WLFS protocol (broken arrow) was used which elicited transient early-LTD of the field EPSP with a duration of 185-210 min in S1 (filled circles; statistically significant different for 185 min when compared with control input S2 (open circles)), *U*- test, or for 210 min when compared with its baseline before WLFS, Wilcoxon test, $P<0.05$). **(E).** Application of the NMDA-receptor antagonist AP-5 (box) 30 min before and until 30 min after SLFS in S1 prevented the induction of LTD (filled circles). Responses obtained from S2 (open circles) revealed relatively stable potentials at control levels.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples presented; scale bar for triplets: 3 mV/50 ms)

### 3.2. ´Synaptic tagging´ during LTD

The NMDA-receptor-dependent induction and the protein synthesis-dependent maintenance of late -LTD allow us to speculate that late-LTD may also display common functional properties and consequences for information processing similar to LTP at the cellular level. This may include phenomena like 'synaptic tagging' and 'late-associativity'.

For investigating ´synaptic tagging´ during LTD, late-LTD was induced in S1, 30 min later WLFS was applied in S2. Using such a stimulation protocol of two separate independent synaptic inputs revealed that early-LTD in S2 was transformed into a late-LTD (Fig. 6 A; filled circles) (Wilcoxon test, $P<0.05$). In the next series of experiments, SLFS was applied to both inputs S1 and S2 with in an interval of 60 min, but the S2 was in presence of anisomycin (anisomycin applied 30 min before (a time period which will not

affect the S1) and after the induction of late-LTD in S2) (Fig. 6 B). Under these conditions late-LTD on S2 was still observed suggesting that protein synthesis initiated immediately after induction of LTD on S1 provided macromolecules that were still available for the establishment of late-LTD on S2. LTD in S1 or S2 were always statistically significant different from their adequate baselines (Wilcoxon test; $P<0.05$). Application of anisomycin (25 μM) during the induction of late-LTD in S1 and S2 prevented late-LTD in both synaptic inputs (Fig. 6 C) (anisomycin was applied 30 min before induction of LTD in input S1 and was washed out 30 min after the induction of LTD in the second input S2). The next series investigated whether the early-LTD in S1 has any effect on S2. Subsequent inductions of early-LTD in S1 and S2 alone had no effect on both inputs (Fig. 6 D).

In summary, LTD in CA1 area of hippocampal slices shows the phenomenon of ‘synaptic tagging’.

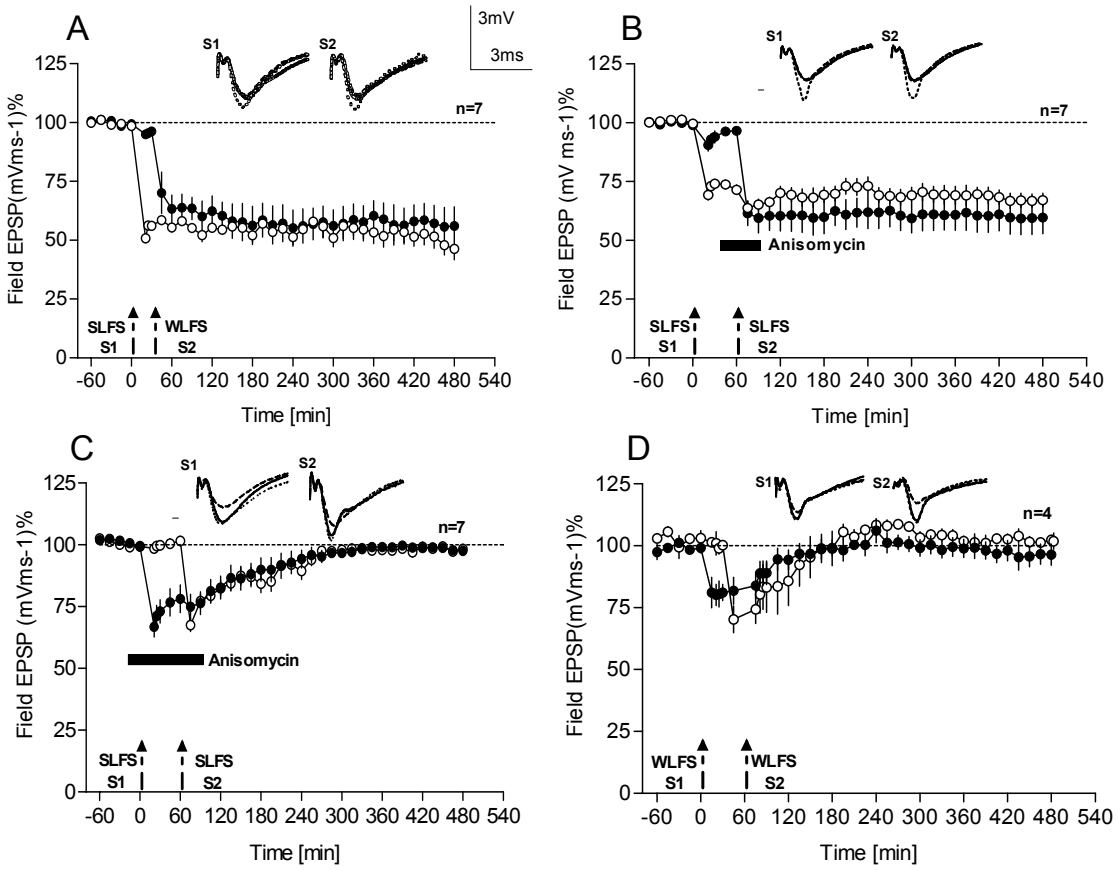


**Figure 6. Synaptic tagging during LTD. (A)** Prior induction of late-LTD in S1 (open circles) 30 min before WLFS in S2 transformed the early-LTD in S2 into a late-LTD (filled circles) (Wilcoxon test, $P<0.05$). Here the 'tag' set due to the early-LTD in input S2 hijacked the proteins synthesized by the synaptic input S1 (late-LTD), showing the paradoxical conversion of early to a late-LTD in input S2 **(B)** Late-LTD was induced in S1 without drug application (open circles), 30 minutes after SLFS in S1 anisomycin was added (box) and 1 h after LTD of S1, input S2 was stimulated by the SLFS protocol but now under inhibition of protein synthesis (filled circles). Under these conditions late-LTD on S2 was still observed suggesting that protein synthesis initiated immediately after induction of LTD on S1 provided macromolecules that were still available for the establishment of late-LTD on S2. The depressions in S1 or S2 were always statistically significant different from their adequate baselines (Wilcoxon test; $P<0.05$). **(C)** Anisomycin (25 µM) (box) was applied 30 min before induction of LTD in input S1 (filled circles) and was washed out 30 min after initiation of LTD in the second input S2 (open circles). In both synaptic inputs late-LTD was prevented due to lack of protein synthesis (because of the presence of anisomycin at the time of the induction of late-LTD in both inputs). **(D)** Subsequent induction of early-LTD in S1 and S2 alone have no effect on both inputs (statistically significant different for 145 min in S1 (filled circles) and 165 min in S2 (closed circles) when compared with its baseline before WLFS, Wilcoxon test, $P<0.05$). All values are presented as the mean ± SEM. Hatched arrows indicate application of SLFS or WLFS for inducing late-LTD or early-LTD on corresponding synaptic inputs.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.3. Conversion of early- into late-LTD: ´late-associativity´ during LTD and the decay time course of the synaptic tagging during LTD.

A weak-before-strong-protocol was used to determine the decay time course of the tag (Frey and Morris, 1998b). Thus, an early-LTD was induced in S1 followed by late-LTD in S2 at distinct time points thereafter, i.e. 30 min, (Fig. 7 A), 1 h (Fig. 7 B),2 h (Fig. 7 C) and 3 h (Fig. 7 D). Early-LTD in S1 (filled circles in Fig. 5 A-D) was transformed into late-LTD if the induction of late-LTD in S2 occurred within 1-2 h after tetanization of S1. Thus, the decay time of the tag during LTD is about 1-2 h (Fig. 7 E), i.e., similar to the decay time for the tag during LTP, when measured in hippocampal slices at a temperature of 32 °C.

In summary, LTD in CA1 area of hippocampal slices shows similar ‘late-associative’ property like LTP and the decay time course of the LTD ´synaptic tag´ is within 30-60 min.

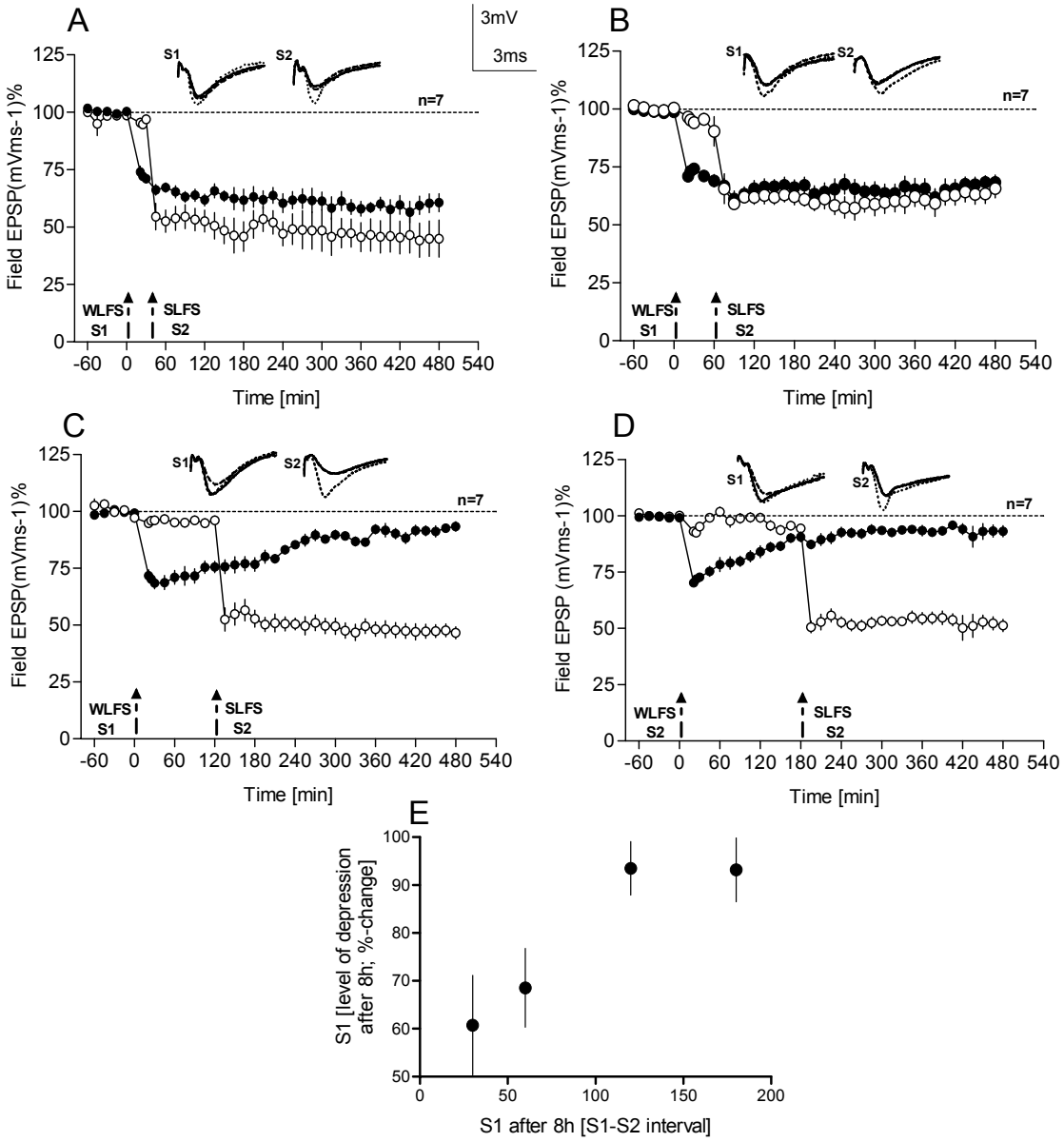


**Figure 7. Weak-before-strong induces late-LTD on the weakly stimulated WLFS input S1:** when repeated SLFS and subsequent induction of late-LTD in input S2 (open circles) followed the weak single WLFS in S1 (filled circles), the early-LTD was transformed into late-LTD in that input when 'weak and strong' LFS occurs within 30 min **(A)** to 60 min **(B)** (Wilcoxon test, $P<0.05$), but it declined as this interval lengthens to 2 h **(C)** (statistically significant difference retained up to 240 min in S1 (filled circles) when compared with its baseline before WLFS, Wilcoxon test, $P<0.05$) and is almost absent at 3 h **(D)**, although a very small remaining depression of the potentials remained over time (statistically significant difference retained up to 125 min in S1 (filled circles) when compared with its baseline before WLFS, Wilcoxon test, $P<0.05$). **(E)** Decay time course of the LTD ´synaptic tag´: analysis of the slope of field EPSP measured 8 h after WLFS of S1 in (A-D) showed the magnitude of late-LTD to be a function of the weak-before-strong interval. Assuming the synthesis and distribution of PRPs to be relatively rapid, this function will approximate the decay time course

of a population of synaptic tags at 32°C in vitro. Hatched arrows indicate application of SLFS or WLFS for inducing late-LTD or early-LTD on corresponding synaptic inputs.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.4. Heterosynaptic requirements for ´synaptic tagging´

Electrical stimulation of afferent fibres with a field electrode always activates modulatory pathways in addition to glutamatergic ones. Thus, one can assume that in studies using field stimulation and recording electrodes, heterosynaptic events will occur. Thus the question arises: What is the role of these modulatory inputs? A three-fold short-lasting application of 10 µM dopamine results in a delayed onset, but long-lasting depression (Fig. 8 A; filled circles), while 50 µM results in a delayed onset long-lasting potentiation (Fig. 8 B; filled circles). Ascorbic acid, an antioxidant which was applied together with dopamine alone had no effect on baseline potentials (Fig. 8 A-B). In the next series of experiments the role of dopamine during LTD induction and ´synaptic tagging´ was investigated. The application of the D1/D5-receptor antagonist SCH23390 (0.1 µM) during LTD-induction prevented its maintenance (Fig. 8 C; filled circles). Late-LTD was induced in both synaptic inputs, S1 and S2 in presence of SCH23390 (0.1 µM) which resulted in the prevention of late-LTD in both inputs (Fig. 8 D). For investigating its role during tagging, late-LTD was induced in input S1 followed by late-LTD in S2 but in presence of SCH23390 (Fig. 8 E). The role of dopamine during synaptic tagging in LTP was investigated by inducing late-LTP in both synaptic inputs (ie, S1 and S2) but S2 was in presence of

SCH23390, paradoxically both inputs maintained a late-LTP showing the tagging interactions (Fig. 8 F).

In summary, all these results supports our hypothesis that dopamine might be directly involved in processes required for late-LTP and late-LTD.

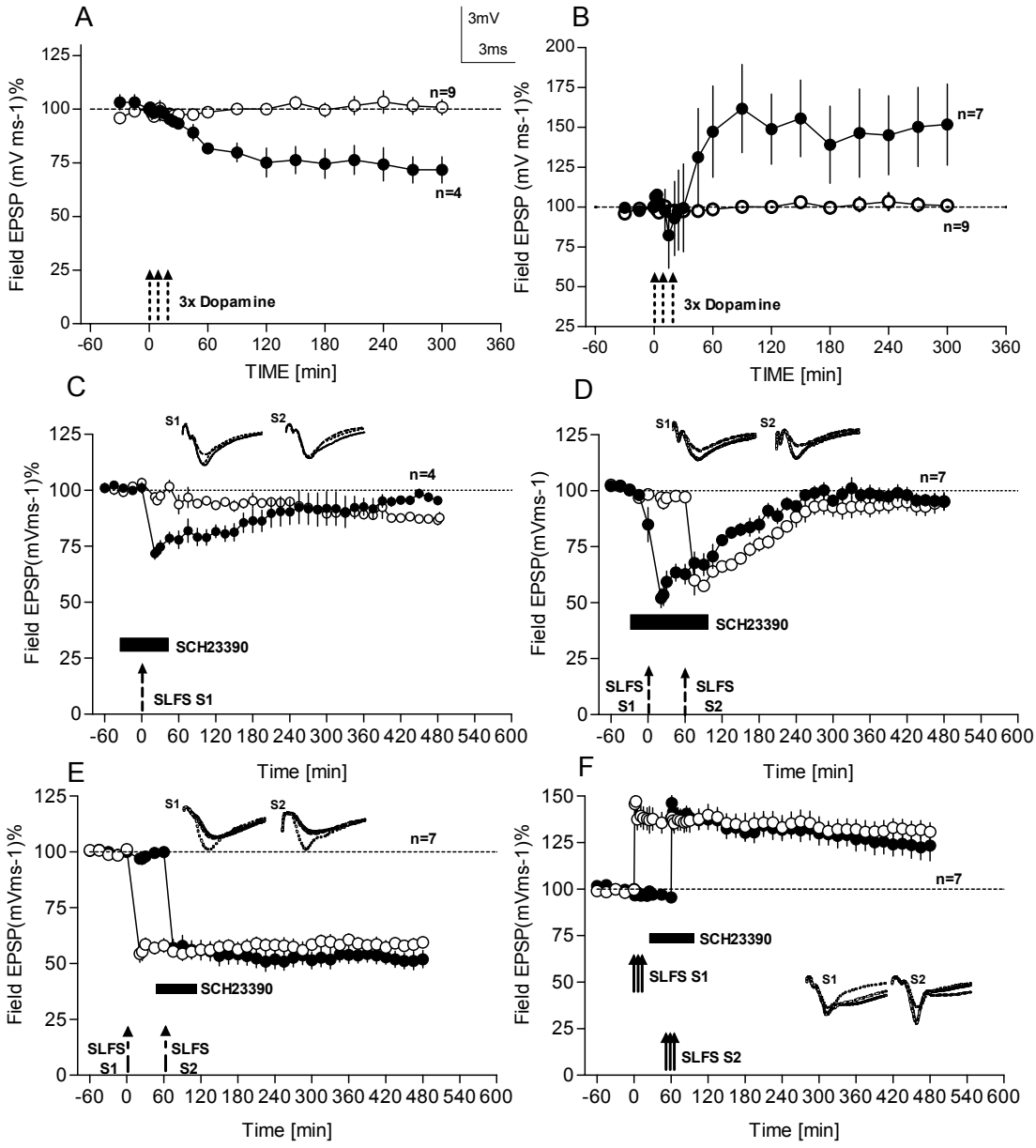


**Figure 8. Heterosynaptic requirements for ´synaptic tagging´. (A)** Threefold application of 10 µM dopamine/1 mM ascorbic acid (arrows) revealed a delayed-onset LTD (filled circles). The anti-oxidant ascorbic acid alone had no effect on baseline potentials (open circles). **(B)** Application of 50 µM dopamine/1 mM ascorbic acid (arrows) resulted in a delayed-onset LTP (filled circles) when compared to the control series with ascorbic acid alone (open circles as in

(A)). **(C)** SLFS in inputs S1 (filled circles) after inhibition of dopaminergic D1/D5-receptors by SCH23390 (box) resulted in a transient early-LTD lasting about 165 min similar to the time course of LTD after the application of protein synthesis inhibitors (see Fig. 5 B and C) (stastiically significant difference in S1 (filled circles) up to 185 min when compared with control input S2, *U* test, and 205 min when compared with its baseline before SLFS, Wilcoxon test, $P<0.05$). Control potentials in S2 remained relatively stable at baseline levels (open circles). **(D)** SCH23390 (box) was applied 30 min before induction of LTD in input S1 (filled circles) and was washed out 30 min after induction of LTD in the second input S2 (open circles). Late-LTD was prevented by SCH23390 in hippocampal CA1 (duration of LTD: in S1 210 and 165 min in S2 when compared to their baseline values before SLFS, Wilcoxon test, $P<0.05$). **(E)** Late-LTD was induced in S1 without drug application (open circles). Thirty minutes after SLFS in S1, SCH23390 was added (box) and 30 min after LTD of S1 SLFS was applied to input S2 but now under inhibition of D1/D5-receptors (filled circles). Under these conditions late-LTD on S2 was still observed suggesting that protein synthesis initiated immediately after induction of LTD on S1 by interaction with the activation of the dopaminergic D1/D5-receptor provided macromolecules that were still available for the establishment of late-LTD on S2 (Wilcoxon test, $P<0.05$). **(F)** The same experiment as in **(E)** was repeated but now by the induction of LTP in the two inputs S1 and S2 (solid arrows + STET). Late-LTP was observed in both inputs suggesting that similar as to LTD, LTP-specific tagging in CA1 requires dopaminergic receptor activation as well (Wilcoxon test, $P<0.05$). Hatched arrows indicate application of SLFS for inducing late-LTD on corresponding synaptic inputs. Triplets of filled arrows indicate a strong, threefold tetanization for inducing late-LTP in that input.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (Solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.5. Late-associative interactions between LTP and LTD: 'Cross-tagging.'

Earlier results show that dopamine-receptor activation in hippocampal CA1 is required for the protein synthesis stage of LTP and LTD. We hypothesize that the role of such modulatory inputs in synergistic action with glutamatergic processes causes the synthesis of PRPs. If so, the question arises as to whether the activation of dopaminergic receptors during late-LTP/-LTD induction regulates a general pool of PRPs with an unspecificity to

the particular process, i.e. LTP or LTD. However, the process-unspecific PRPs could then be used by LTP- or LTD-tags in a process-specific way. For investigating this, early-LTP was induced in input S1 (Fig. 9 A; filled circles) which was followed by the induction of late-LTD in S2 30 min later (open circles). Paradoxically, early-LTP in S1 was transformed into late-LTP by SLFS through a separate synaptic input S2. Similarly, early-LTP in S2 (Fig. 9 B, filled circles) was transformed into late-LTP if the induction of late-LTD in S1 (Fig. 9 B; open circles) preceded WTET in S2. We named this positive late-associative interaction of LTP and LTD: synaptic ´cross-tagging´. The order of induction of LTP or LTD in ´cross-tagging´ is unimportant because induction of late-LTP in S2 (Fig. 9 C) converts the early-LTD in S1 to a late-LTD or induction of late-LTP in S1 converts the early-LTD in S2 to a late-LTD (Fig. 7 D). The prior induction of late-LTP in one input will preclude the expression of late-LTP in that input (Fig. 9 E). Similarly the prior induction of late-LTD in one input will preclude the expression of late-LTP in same input (Fig. 9 E).

In summary, the data provide evidence that LTP and LTD can interact in a positive manner in hippocampal slices in vitro.

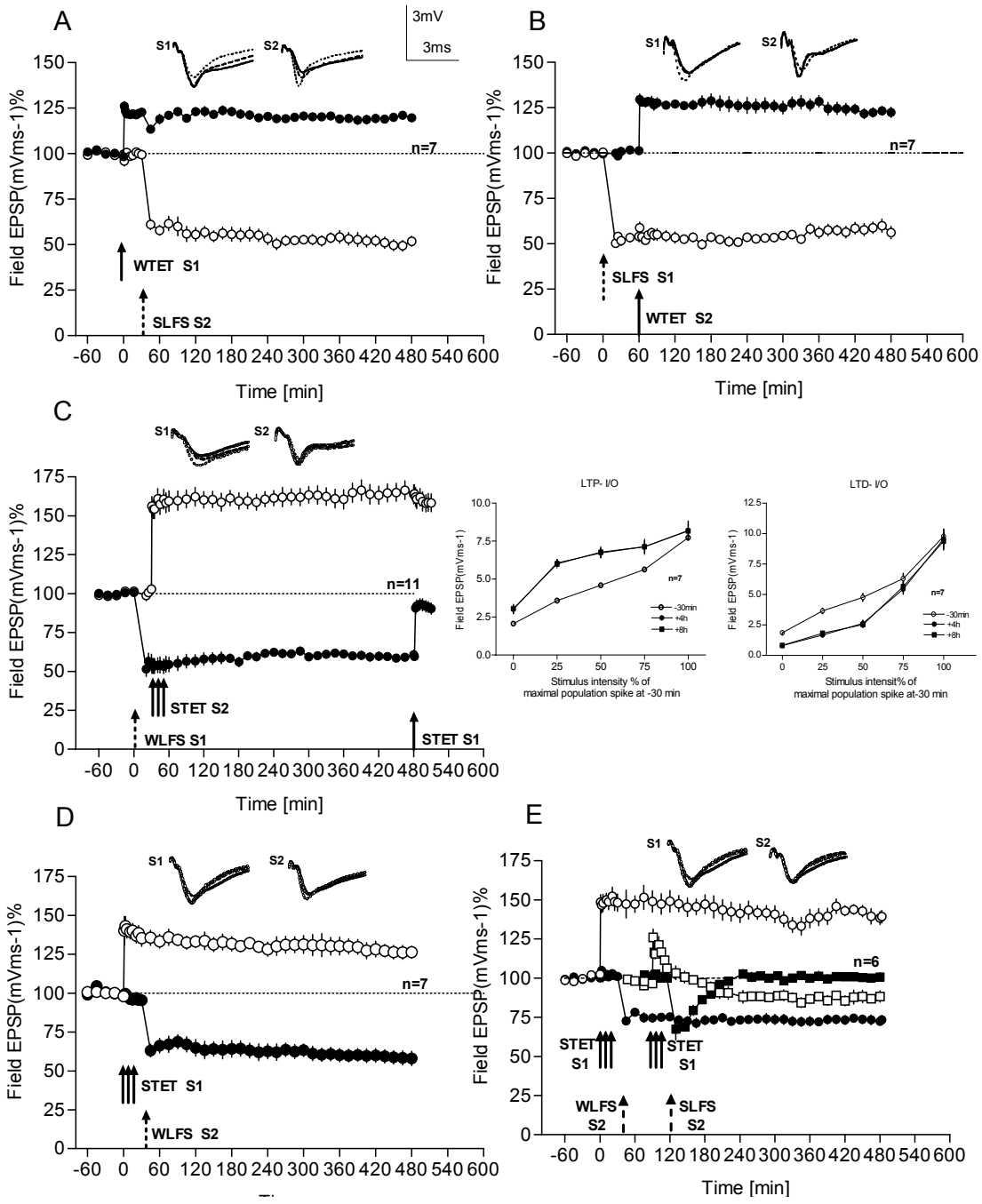


**Figure 9. Positive associative interactions of LTP/LTD: 'Cross-tagging'. (A)** Early-LTP in synaptic input S1 (filled circles) was transformed into a late-LTP, when late-LTD was induced by repeated SLFS in S2 (open circles) 30 min after the induction of early-LTP in S1. **(B)** Late-LTD in S1 was able to affect early-LTP in S2, transforming the latter to late-LTP. **(C)** Early-LTD was transformed into late-LTD in S1 by strong tetanization, i.e., induction of late-LTP in S2. Tetanization of input S1 8 h after LTD induction resulted in normal LTP with a duration of at least 30 min. The graphs on the right illustrate the shift of the input-output curve for LTP and LTD, respectively, 4 and 8 h after induction of the plastic process. **(D)** Induction of late-LTP in S1 was followed by WLFS in S2. Here, early-LTD was transformed into late-LTD in S2. All forms of LTP or LTD in these experiments were statistically significant different from their

adequate baseline control potentials (Wilcoxon test, $P<0.05$). **(E)** Cross tagging experiments, however 1 h after LTP induction in S1 new stimulation parameters were determined for this input (open squares) in addition to test stimuli of LTP (open circles) in S1 which resembled pre-tetanus baseline values. After recording a new baseline for 30 min a STET was applied. Interestingly, the prior induction of late-LTP in this input precluded the expression of late-LTP in S1. Similarly, in input S2 a new baseline was determined (filled squares) 30 min after WLFS in this input. A baseline of 30 min was also recorded with the newly determined stimulus parameters irrespective of testing with the old stimulus intensity in the same input (filled circles). Then a SLFS was applied which normally produces late-LTD, however, the former transformation of early- into late-LTD by mechanisms of cross-tagging prevented the expression of LTD if SLFS was applied 60 min after the induction of early-LTD in this input. Filled arrows indicate the time point of WTET and hatched arrows indicate application of SLFS or WLFS to the corresponding synaptic inputs. Triplets of filled arrows indicate a strong, threefold tetanization for inducing late-LTP in that input.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.6. Depotentiation of early-LTP in S1 by LFS

Control experiments revealed a transient early-LTP of synaptic input S1 decaying to baseline values about 3 h after WTET (Fig. 10 A; filled circles). A separate afferent synaptic input S2 to the same neuronal population remained at stable baseline levels during the investigated time period of 7 h in this set of control experiments (Fig. 10 A; open circles). Previous experiments have demonstrated that the weak tetanus in S1 which resulted in early-LTP activates a synaptic tag at the particular synapse S1. We had shown that the tag is transient and decays within about 1 h. The question arises as to whether the tag can also be reset in an activity-dependent manner. Thus, the next series investigated the effect of a LFS-protocol applied to the tetanized input S1, 5 min after WTET. As seen in Fig. 10 B (filled circles) early-LTP was totally reversed by the LFS whereas posttetanic potentiation was expressed at

normal levels. The control input S2 remained stable at baseline levels (Fig. 10 B; open circles). Prolongation of the time interval between WTET and LFS from 5 to 10 min in S1 (Fig. 10 C; filled circles) resulted in PTP followed by a transient depotentiation to baseline levels and a recovery of early-LTP within 2 h before early-LTP followed its normal transient maintenance, decaying to baseline after about 3 h. A similar transient partial depotentiation was observed when the time interval between WTET and LFS in S1 was prolonged from 10 to 15 min (Fig. 10 D; filled circles). It remains unclear why a tendentious prolonged early-LTP (however statistically significant) was observed under this regimen (early-LTP decayed to baseline only after 5 h and 15 min when compared to the control input S2; U-test). The control input S2 remained stable in the latter two sets of experiments.

In summary, the data provide evidence that, a LFS-protocol can reset early-LTP, if LFS was applied 5 min after WTET. At later time points only a transient depotentiation was observed.

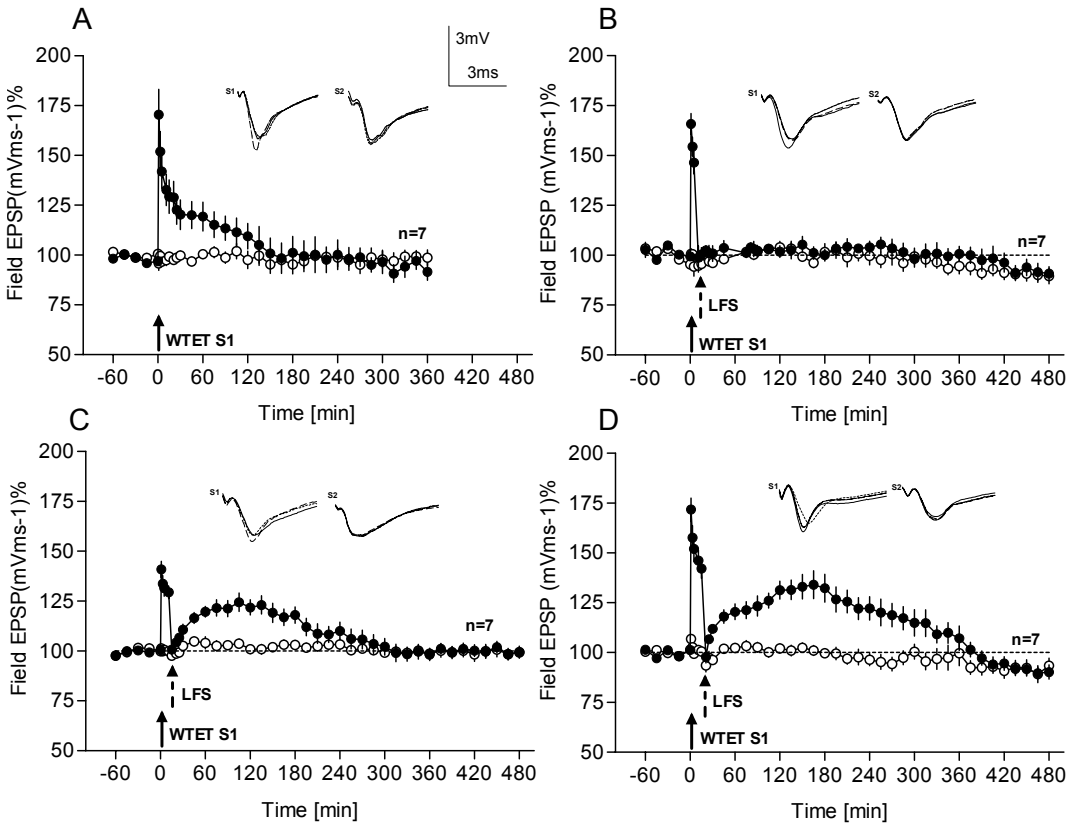


**Figure 10**. **Depotentiation of early-LTP in S1 by LFS of S1**. **(A)** Time course of early-LTP induced by a weak tetanus in S1 (filled circles). A statistically significant potentiation in S1 up to 210 min after the tetanus (U test, $P<0.05$) as compared to control input S2 (open circles). **(B)** LFS 5 min after WTET of S1 resulted in an immediate and complete depotentiation of early-LTP. LFS 10 min **(C)** or 15 min **(D)** after WTET of S1 was only able to transiently depotentiate early-LTP of S1. A transient potentiation recovered after depotentiation from 45 to 180 min (C) or 45-315 min after WTET and LFS in S1 in (D) (compared with the corresponding control input S2; *U* test, $P<0.05$) before reaching baseline levels. Filled arrows indicate the time point of WTET and hatched arrows indicate application of LFS to the corresponding synaptic inputs.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.7. Depotentiation of early-LTP in S1 and subsequent 'tagging' by 3xTET of S2

In the next series of experiments we investigated the hypothesis whether depotentiation of early-LTP by LFS is able to reset the 'synaptic tag' in this input originally set by the WTET. If so, induction of late-LTP in a second input S2 should not be able to influence the initially-induced and depotentiated LTP in input S1. Thus, a WTET protocol in S1 which normally induces early-LTP and sets its 'tag' without the synthesis of PRPs. After 5 (Fig. 11 A), 10 (Fig. 11 B) or 15 min (Fig. 11 D) a LFS was applied to the same input S1, similar as in the experiments represented in Fig. 10 B-D. As seen there LFS 5 min after WTET resulted in a full depotentiation of early-LTP, whereas a time interval of 10 or 15 min was not sufficient to fully depotentiate early-LTP in S1. Using the same time intervals for the application of depotentiating stimuli, we have now applied subsequently 3×TET to S2 (open circles in Fig. 11 A-D), i.e. induced late-LTP in S2, 30 min after WTET of S1-and LFS 5 min thereafter-was ineffective in influencing the time course of the depotentiated event in S1. It seems that the 'tag' in S1 was reset by LFS 5 min later, thus, being unable to bind PRPs provided by late-LTP of S2. In the next experiment this hypothesis is studied more thoroughly and have re-applied a WTET to S1 again (Fig. 11 C; filled circles), i.e. 15 min after 3xTET of S2. Under these conditions the newly established 'synaptic tag' in S1 could benefit from the PRPs provided by late-LTP of S2, which could paradoxically transform its original early-LTP into late-LTP showing re-tagging with a duration of at least 8 h.

In experiments, where the LFS was applied 10 or 15 min after WTET of S1 and which was followed by the induction of late-LTP in S2 thirty min later, a transient depression in S1 was observed (similar as in Fig. 10 C and D) which was then followed by a rescued recovering early- and the subsequent paradoxical development of late-LTP in S1 (Fig. 11 B and D). It can be concluded that the latter two conditions were unable to reset the 'synaptic tag' in S1 which could then benefit from the PRPs provided by late-LTP of S2.

In summary, the resetted ‘tag’ or ‘tag complex’ in a synaptic input can be reactivated by inducing an early-LTP in the same input.

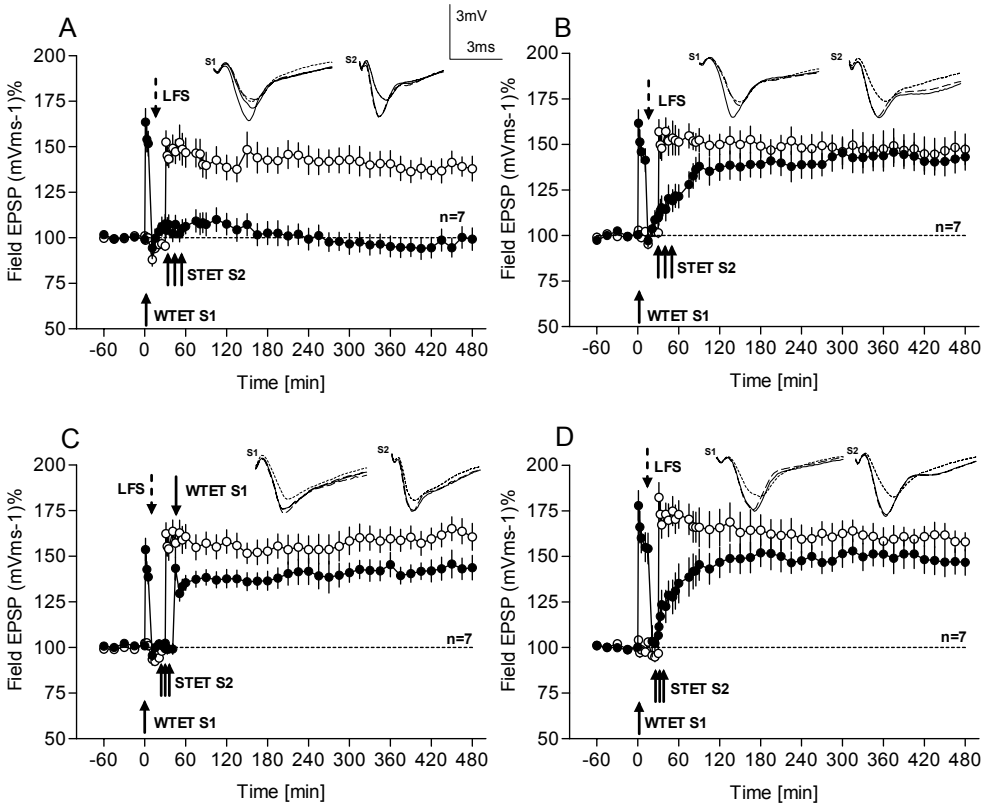


**Figure 11. Depotentiation of early-LTP in S1 and tagging by 3xTET of S2**. Panels **(A)**, **(B)** and **(D)** illustrate the effect of subsequent induction of late-LTP in S2 (open circles) 30 min after WTET of S1 (filled circles) and LFS of S1 either 5 min **(A)**, 10 min **(B)** or 15 min **(D)** after the latter. Fig A shows that LFS 5 min after WTET in S1 completely resets the 'tag' preventing 'synaptic tagging' by induction of late-LTP in S2. Application of depotentiating stimuli to S1 either 10 or 15 min after WTET resulted in a transient depotentiation of LTP in S1

(depotentiated for 5 min after LFS when compared to its own baseline, Wilcoxon-test, $P<0.05$). However, induction of late-LTP in S2 (open circles) 30 min after WTET of S1 resulted in a transformation of recovered early- into late-LTP in S1, 'synaptic tagging' took place. **(C)** represents a novel setting of the 'tag' (re-tagging) in S1 after resetting it by LFS 5 min after WTET (filled circles): On the background of depotentiated early-LTP of S1 (filled circles; using the 5-min-interval of depotentiation) late-LTP was induced in S2 (open circles) followed by a secondary induction of early-LTP in S1 15 min after strong TET of S2. As seen in **(C)** early-LTP in S1 was then paradoxically transformed into late-LTP by processes of 'synaptic tagging'. Filled arrows indicate the time point of WTET and hatched arrows indicate application of LFS to the corresponding synaptic inputs. Triplets of filled arrows indicate a strong, threefold tetanization of S2 for inducing late-LTP in that input.

The analog examples given in the figures represent adequate potentials 30 min before (dotted line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.8. Effects of the PKM-zeta (PKMζ) inhibitor, myr-ZIP, on LTP and LTD

The next series of experiments investigated the putative role of one candidate protein which was assumed to stand as a synaptic tag molecule: PKMζ. Induction of late-LTP in synaptic input S1 (Fig. 12 A; filled circles) resulted in a long-lasting, statistically significant late-LTP ($P<0.05$, Wilcoxon-signed rank test) with a duration of 8 h. A control input S2 remained stable at baseline levels for the entire experimental session (Fig. 12 A; open circles). It has been reported that this type of late-LTP is NMDA-receptor- and protein synthesis-dependent. Application of the myristoylated ζ-pseudosubstrate peptide inhibitor (myr-ZIP) 1h after the induction of late-LTP in S1 (drug application continued up to 5h) (Fig. 12 B; filled circles) prevented its late maintenance, returning to baseline responses 135 min after tetanization ($P<0.05$, Wilcoxon-signed rank test). Interestingly, the potentials further attenuated below baseline levels from 4 h onwards, expressing a lasting depression ($P<0.05$, Wilcoxon-signed rank test). Control recordings of S2

revealed stable potentials throughout the same period (Fig. 12 B; open circles).

In the next series of experiments we investigated whether the myr-ZIP can reverse late-LTD in a similar way as it did during LTP. Fig. 12 C represents the time course of the fEPSP of input S1 in which myr-ZIP was applied 1h after induction of late-LTD. Application of the drug up to 5 h after SLFS did not influence early- or late-LTD, respectively (Fig. 12 C; filled circles) as compared to the control early and late-LTD (Fig. 5 A and D; filled circles). The control input S2 (Fig. 12 C; open circles) remained stable. Thus, in contrast to LTP, the maintenance of late-LTD seems to be independent of PKMζ activity. We then studied whether myr-ZIP has any effect on transient early-LTD induced by WLFS (Fig. 12 D; filled circles). Fig. 12 D shows no effects of myr-ZIP on early-LTD in S1 (filled circles) when compared to the early-LTD without drug application (Fig. 5 D, filled circles). In both cases the control input S2 (open circles) was stable for the entire experimental session.

In summary PKMζ is necessary for the late maintenance of LTP but not for LTD.

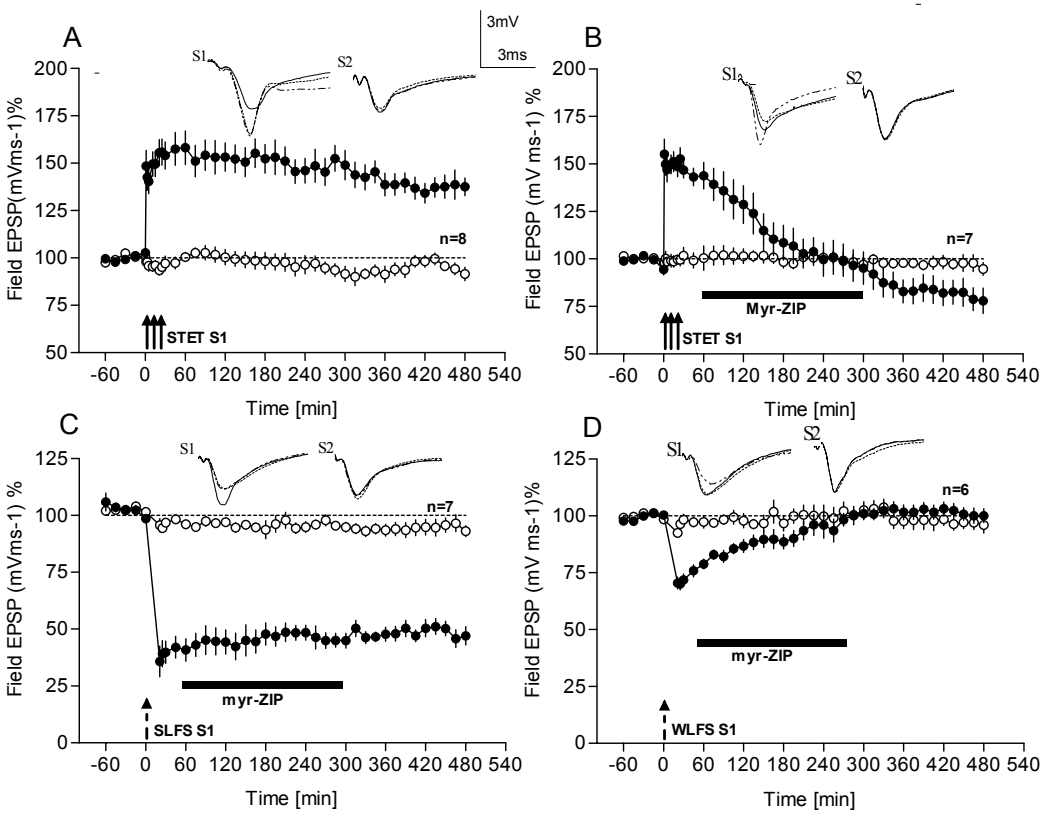


**Figure 12. Effects of the PKM-zeta (PKMζ) inhibitor, myr-ZIP, on LTP and LTD.**

**(A)** Induction of late-LTP in S1 (filled circles) using high-frequency stimulation (HFS) resulted in late-LTP that was significantly different for the 8 h, compared with the control input S2 (*P*<0.05, U-test). Control stimulation of S2 revealed relatively stable potentials for the time course investigated (open circles). **(B)** Induction of late-LTP by applying HFS to S1 (filled circles) and application of the myristoylated -ζ pseudosubstrate peptide inhibitor ( myr-ZIP, 1 μM) 1h after its induction reversed the late phase of LTP, while the potentials of the control pathway S2 remained stable throughout the recording period of 8 h (open circles). **(C)** Application of SLFS to S1 (filled circles) resulted in a significant depression of that input when compared to a control input S2 (open circles, *P*<0.05, U-test), bath application of myr-ZIP (1 μM) 1h after its induction had no effect on the maintenance of late-LTD. **(D)** A WLFS showed a transient early-LTD of the fEPSP with a duration of 185-210 min (185 min when compared with control input S2,and 210 min when compared with its baseline before WLFS, Wilcoxon test, *P*<0.05). This early-LTD was unaffected when the myr-ZIP was applied 1h after its induction. Control stimulation of S2 revealed relatively stable potentials for the time course investigated (open circles). Triplets of filled arrows indicate a strong, threefold tetanization for inducing late-LTP in that input and hatched arrows indicate application of SLFS or WLFS for inducing late-LTD or ealry-LTD on the corresponding synaptic inputs. The analog examples given in the figures represent adequate potentials 30 min before (dotted

line), 30 min after (broken line) as well as 8 h (solid line) after the induction of the event in input S1 and S2, respectively. Scale bar: 3 mV/3 ms (valid for all single analog examples).

### 3.9. Effects of PKMζ inhibitor on processes of 'cross-tagging'

As shown above, PKMζ plays an important role for the maintenance of LTP. In contrast, inhibition of PKMζ did not affect the maintenance of early- or late-LTD (Fig. 12 C and D). These data evoked the following question: Is there a specific role for PKMζ on the processes of ‘synaptic tagging’? And if so, is PKMζ specifically involved in ´synaptic tagging´ during LTP but not LTD? To investigate these questions we studied the effect of PKMζ inhibition on established ‘cross-tagging’. PKMζ inhibition after the application of STET to S1 and WLFS to S2 interferes with the processes of ´cross-tagging´. As shown in Fig. 13 A, late-LTP in S1 (filled circles) was reversed similarly as in the series presented in Fig. 12 B. However, although late-LTP was reversed in S1, ´cross-tagging´ took place since early-LTD was transformed into late-LTD in S2 (Fig. 13 A; open circles). To verify a specific action of myr-ZIP, with an inactive scrambled sequence of ZIP (scr-myr-ZIP, 1µM) in a similar design as shown in Fig. 13 A was used, but with the exception that the drug was used at the time of induction of LTP and LTD in iputs S1 and S2 respectively. As presented in Fig. 13 B, no effects were observed on the induction and maintenance of LTP or LTD and subsequent processes of cross-tagging. In the next series of experiments (Fig. 13 C) STET was delivered in S1 (filled circles) followed by WLFS in S2 (open circles) but now under the influence of myr-ZIP (drug application: 2 h before the induction of late-LTP in S1 up to 2h after tetanization). LTP was blocked by myr-ZIP and additionally, no cross-tagging could be observed, i.e., no transformation of early- into late-LTD

occurred by such an intervention. Interestingly, early-LTD was also somewhat depressed when myr-ZIP was applied in conjunction with LTP-induction in S1. A similar picture emerged when SLFS in S1 was followed by WTET in S2 (Fig. 13 D). Interestingly, inhibition of PKMζ in such an experimental design, i.e., application of SLFS in S1 followed by WTET in S2 under the influence of PKMζ inhibition also prevented late-LTD. Here, all lasting plasticity forms were prevented when myr-ZIP was applied during the induction of the events in S1 and S2.

In summary, PKMζ inhibition prevents the induction of ´cross-tagging´.

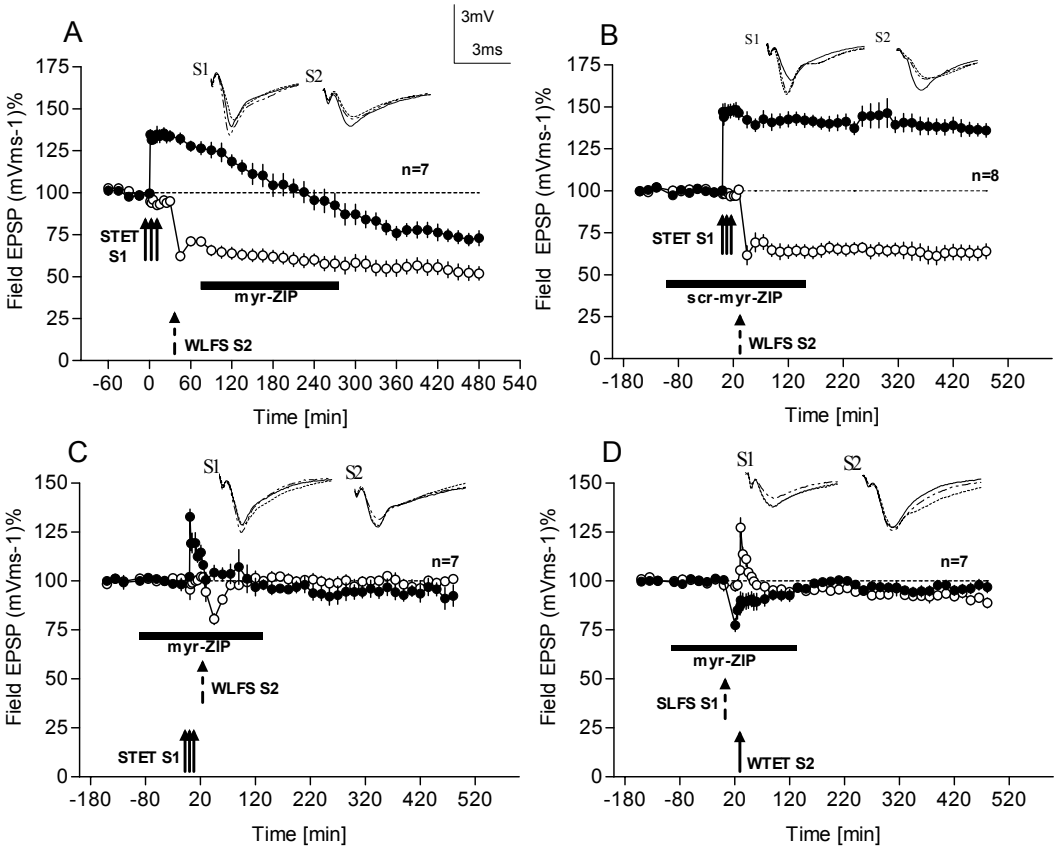


**Figure 13. Effects of PKMζ inhibitor on processes of 'cross-tagging'. (A)** Induction of late-LTP in S1 (filled circles) was followed by early-LTD in S2 (open circles) thirty min after STET of S1 (cross-tagging), bath application of myristoylated-ζ pseudosubstrate peptide inhibitor 1h after its induction prevented the maintenance of late-LTP in S1 (filled circles; a

statistically significant potentiation observed up to 130 min when compared to its base line,*P*<0.05, U-test), the early-LTD in S2 (open circles) was transformed into a late-LTD (a statistically significant depression observed up to 8 h compared to its base line,*P*<0.05, U-test) irrespective of the blockade of late-LTP in S1. **(B)** Similar experiment as Fig. A, but with an inactive scrambled peptide (scr-myr-ZIP, 1 µm) instead of myr-ZIP (but with the exception that the drug was used at the time of induction of LTP and LTD in iputs S1 and S2 respectively). No effect on ´cross-tagging´ was observed. **(C)** Application of myr-ZIP 2 h before and until 2 h after the tetanization of S1 prevented ´cross-tagging´, S1 (filled circles) tetanized with HFS, 30 min later WLFS was applied to S2 (open circles) to induce early-LTD. **(D)** Similar experiment as in **C** but in a reverse manner, i.e., S1 with late-LTD-induction (filled circles) and S2 with early-LTP (open circles). Analog traces as well as symbols similar to Fig. 12.

## 4.0. Discussion

The presented results confirm and extend existing studies on LTP and LTD within the hippocampus and other brain regions and data obtained within the intact animal. Late-LTD depends on ongoing protein synthesis during its induction. Studies within the cerebellum and later in the hippocampus indicated that the prolonged maintenance of LTD may share similar cellular properties as LTP (Levenes et al., 1998;Linden, 1996;Manahan-Vaughan et al., 2000). These results, together with data describing the influence of novelty acquisition and recognition in freely moving animals (Manahan-Vaughan and Braunewell, 1999) allowed us to speculate that electrically-induced LTD may underlie processes of learning and memory formation in a similar manner as LTP (Bear and Abraham, 1996). My initial studies were conducted to investigate whether LTD within the hippocampal CA1 region in vitro shares similar properties as LTP. Different forms of LTD can be induced depending on the induction protocol, i.e. a transient protein synthesis-independent early-LTD (Fig. 5 D) and a late-LTD (Fig. 5 A) which depends on ongoing protein synthesis during its induction. Furthermore, LTD in area CA1 is input-specific, i.e. the expression of LTD was observed only in those synaptic inputs specifically activated using a low-frequency stimulation protocol. Other synaptic inputs can thus react differentially to afferent stimulation, a necessary condition to investigate processes like ‘late-associativity’ and ‘synaptic tagging’.

Early- and late-LTD were blocked if an NMDA-receptor antagonist (50 µM AP-5, Fig. 3 E) was applied during SLFS and late-LTD was prevented

if protein synthesis inhibitors (25 µM anisomycin or 20 µM emetine) were applied during SLFS (Figs. 5 B and C). These data support the hypothesis that NMDA-receptor-dependent late-LTD can also be induced in slices resembling results obtained in intact animals (Bear and Abraham, 1996;Manahan-Vaughan et al., 2000). Unspecific effects could be excluded by using structurally different inhibitors and by analyzing the initial and last three triplets of analog traces during the LTD-inducing trains (i.e., potentials obtained at the 1st, 2nd, 3rd, and 898th, 899th or 900th s of one LFS-train). There was no non-specific action of the drugs used (Figs. 5 B-C) when compared with controls (Fig. 5 A). In addition, with respect to the the adequate and stable control inputs (Figs. 5 B and C, open circles), these data support a specific action of the inhibitors on protein synthesis

### 4.1. 'Synaptic tagging' during late-LTD

´Synaptic tagging´ describes a mechanism how input specificity is achieved during a protein synthesis dependent stage. Consecutive induction of late-LTD to S1 and S2 (at 1 h interval) was prevented by anisomycin, if the protein synthesis inhibitor was applied 30 min before initiation of LTD in input S1 until 30 min after initiation of LTD in S2 had elapsed (Fig. 6 C). The depression of the fEPSP in vitro recovered in both inputs in a similar manner as described in a previous study in vivo (Manahan-Vaughan et al., 2000). In a first key set of experiments, LTD was induced in input S1 (Fig. 6 B, open circles) and anisomycin added to the bath medium 30 min later (a time point where the protein synthesis-inhibitor was ineffective in influencing late-LTD in this input). Thirty minutes later (i.e., 1 h after LTD induction in S1), SLFS was

applied to input S2 under conditions of protein synthesis inhibition (Fig. 6 B, filled circles). Importantly, the establishment of late-LTD in S2 was normal, suggesting that proteins synthesized by LTD in S1 also enable the induction of late-LTD in S2. These results support the validity of the synaptic tag hypothesis established originally for LTP and also for LTD, in two ways: (a) induction of early-LTD appears to be a necessary condition for late-LTD to occur, it will not develop on an input without LFS and (b) protein synthesis-dependent late-LTD can be induced during inhibition of protein synthesis under particular circumstances. While SLFS of a second input in the presence of a protein synthesis inhibitor is unable to induce its 'own' synthesis of macromolecules, it can still create its own local, protein synthesis-independent ´synaptic tag´. This ´tag´ can bind proteins synthesized by the activation of the first input. Thus, the consolidation of LTD involves protein synthesis but this is insufficient to induce synapse-specific late-LTD. A ´synaptic tag´ must also be transiently activated during the induction of early-LTD. LFS must reach some critical threshold for the 'tag' to be activated. These data provide evidence for the selective catching of proteins by heterosynaptically activated synapses enabling late-LTD to have input-specific properties without elaborate protein-trafficking and that the ´synaptic tag´ does not require somatic or local dendritic protein synthesis in the adult hippocampus (Martin et al., 2000;Steward and Falk, 1986). Interestingly, ´synaptic tagging´ takes place during LTP (Barco et al., 2002;Frey and Morris, 1997) and LTD (Kauderer and Kandel, 2000).

Our data on 'synaptic tagging' during LTD in tissues from adult rats are quite similar to the phenomenon recently described in neuronal cultures from

juvenile tissue (Kauderer and Kandel, 2000). The authors had also described tagging during two-input-LTD in their system. However, whether the functionality of plasticity forms and their underlying cellular processes in juvenile versus adult nervous tissue is similar remains to be investigated. It has been shown that late-LTD in the intact adult rat does depend on protein, but not mRNA synthesis during the first 8 h of its maintenance (Manahan-Vaughan et al., 2000). In neurons from juvenile tissue, in the above study a dependence on both-mRNA and protein synthesis-has been demonstrated suggesting severe differences in maintaining LTD in neurons of a different developmental age. However, a cross-synaptic interaction seems to play an important role in neuronal plasticity and its function in information processing in general. Data of behavioral and structural heterosynaptic reinforcement (Frey et al., 2001;Korz and Frey, 2003;Straube et al., 2003) in the intact animal support this hypothesis. Modulatory transmitter systems may result in the activation of various processes, e.g., the induction of the same ( Fig. 14 D) or different PRPs ( Fig. 14 C) in distinct brain structures as well as neuronal sub-regions. Thus, innervation of particular neuronal compartments by a specific modulatory transmitter may exert its explicit action, e.g., induction of distinct PRPs, etc. However, the better understanding of processes underlying the intriguing heterosynaptic induction of the synthesis of a single pool of PRPs in one neuronal population during plastic events in general, i.e., by LTP or LTD, and the putative benefit of the synapses of these PRPs-provided a plasticity-specific synaptic tag is set may result in new ways of thinking of how associative interactions during learning are accomplished between different neurons and structures. Thus, the described processes may also explain why

neuromodulatory events even with an emotional content, such as stress, pain, luck, etc., may lead under distinct circumstances to improved memory formation.

### 4.2. The decay time course and the nature of the tag

If the input-specificity of late-LTD is determined by the ´synaptic tag´ and its persistence by the availability of relevant proteins, the question arises whether early-LTD can be transformed into late-LTD by prior induction of late-LTD in another input to the same neuronal population. Similar to LTP, different temporal forms of LTD can be induced by varying LFS-intensity. As late-LTD normally requires stronger LFS consisting of more impulses (here: 2700), another stimulation paradigm was used (900 impulses, weak LFS (WLFS)) to identify early-LTD. The latter protocol led to LTD with a typical duration of about 2-3 h (Fig. 5 D, filled circles). The subsequent induction of early-LTD in the two inputs S1 and S2 (30 min interval) did not result in a prolongation of the transient depression in either of the inputs (Fig. 6 D). In contrast, prior induction of late-LTD in input S1 (Fig. 6 A, open circles) transformed early-LTD in S2 into late-LTD (Fig. 6 A, filled circles). Using the same protocol, i.e., induction of late-LTD and early-LTD in the two inputs but now in the opposite sequence can be used to determine the decay time course of the ´tag´ (Fig. 7 A-D) (Frey and Morris, 1998a). The decay time of the tag during LTD is about 1 h (Fig. 7 E), i.e., similar to the decay time for the ´tag´ during LTP (Frey and Morris, 1998b). However, it must be considered that the LTP (Frey and Morris, 1998b) as well as LTD-experiments were performed in slices in vitro at an incubation temperature of 32 °C. Thus, it can be assumed that the decay time for the tag in the intact animal, at a

physiological temperature of around 38 °C, is shorter. Studies in vivo in which early-LTP was transformed into late-LTP either by direct stimulation of modulatory inputs or by behavioral manipulations suggest a decay time course for the tag of less than 30 min (Frey et al., 2001;Seidenbecher et al., 1997).

Any candidates with the following criteria are appropriate for fulfilling the function of a synaptic ‘tag’, particularly in the light of the findings that a ´tag´ lasts at most 1-2 h at 32 °C. First, it should be spatially restricted (that is, it must be local). Second, it should be time limited and reversible. Third, it should be able to interact with cell wide molecular events that occur after strong stimulation to produce long-term, synapse specific strengthening. So from a broad perspective, anything that provides a spatially restricted trace of activity is a candidate for the ´synaptic tag´ or better: of the ‘tag complex’. One possibility is a change in spine neck diameter. Synapses which display early-LTP are characterized by wider neck diameter than synapses that had not recently been potentiated. Access to the synaptic apposition zone might then be easier for the large macromolecules that we assume are responsible for stabilizing LTP (Frey and Morris, 1998a). Simulation studies have revealed that although changes in spine shape are not responsible for changes of synaptic efficacy, branching of spines and changes in their geometry could be more significant. A further, possibility is that persistently active kinases meet several of the criteria for a ‘tag’, as they allow a synapse to ‘remember’ previous activity in a spatially restricted and reversible manner. CaMKII, the atypical Protein kinase C isotype, PKMζ, (Martin and Kosik, 2002;Hegde, 2004), Homer mediated insertion of the metabotropic glutamate receptor

mGluR5 into the membrane , and palmitate cycling on postsynaptic density protein 95 (PSD95) (Martin and Kosik, 2002) also may satisfy as a ´tag´ candidate. We have thus investigated one candidate molecule for which it was assumed by others (Martin and Kosik, 2002;Hegde, 2004) that it could fulfill the function of a synaptic tag: PKMζ.

Our results demonstrate that PKMζ activation is essential for the immediate induction of LTP and LTD and for the prolonged maintenance of late-LTP but not late-LTD. Furthermore, it is required for processes of ´cross-tagging´. These results have confirmed and extended the work by Ling and colleagues, who have shown a role of PKMζ on the maintenance of LTP (Ling et al., 2002). In addition the present studies have shown that PKMζ inhibition results in a delayed but long-lasting depression of the potentiated input during very late phases beyond 4 h (Fig. 12 B). Phosphorylation by PKMζ enhances AMPA-receptor-mediated synaptic transmission (Ling et al., 2002), and it was shown that AMPA-receptor function is attenuated during LTD (Gutlerner et al., 2002;Hirai, 2001;Carroll et al., 1999). Thus, it can be suggested that continuous PKMζ inhibition results in a prolonged decrease of AMPA-receptor-mediated synaptic transmission only in a prior tetanized input since the control input without the induction of plastic events remained stable. This suggests that LTP, induced by strong tetanization, may destabilize baseline responses, and that persistently increased PKMζ activity produced by the tetanization may maintain both baseline and enhanced AMPA responses specifically in the activated synaptic input at very late phases. Persistent changes in PKMζ levels triggered by low- or high-frequency stimulation can result in long-term dephosphorylation during LTD and increased

phosphorylation during LTP (Hrabetova and Sacktor, 2001). These results further support the extraordinary plasticity role for PKMζ in AMPA-receptor mediated events and may contribute to the better understanding of processes of metaplastic states of a synapse (Bortolotto and Collingridge, 2000).

Although PKMζ inhibition did affect the induction of LTP and LTD if the inhibitor was present during the initiation of these events (Fig. 13 C and D), it was ineffective in blocking established late-LTD if applied after the induction procedure (Fig. 12 C). This is in contrast to LTP, where established late-LTP was prevented when the inhibitor was applied after strong tetanization (Fig. 12 B). Thus continuous PKMζ activity seems to be required for LTP but not LTD maintenance.

It is known that LTP as well as PKMζ activation can also be inhibited when protein synthesis is prevented (Osten et al., 1996b). Thus the following picture emerges: PKMζ activation is protein synthesis-dependent, its inhibition prevents both LTP and LTD initiation but only prevents late-LTP maintenance but not late-LTD maintenance (although both late forms require protein synthesis). The question now arose as to whether processes of cross-tagging might be influenced by inhibition of PKMζ. Inhibition of PKMζ after establishing the ‘cross-tagging’ (Fig. 13 A) supports the hypothesis that late plasticity forms initiate the synthesis of a pool of different (Fig.14 C) and, indeed, process-specific PRPs. This hypothesis was confirmed by showing that late-LTP was suppressed under the influence of PKMζ inhibition (in Fig. 13 A), while early-LTD in the second input was nonetheless transformed into late-LTD. Since the induction of early-LTD is unable to initiate its synthesis of PRPs but sets its process-specific synaptic tags, the effecter processes most

likely the PRPs captured by the tags must be initiated by the strong tetanization in the separate synaptic input with suppressed LTP. Presumably, PKMζ is not directly involved in the induction of processes resulting in the synthesis of PRPs, but it is itself a PRP specific for LTP but not LTD. So the first process-specific PRP was identified. Thus, the hypothesis of the synthesis of a pool of relatively unspecific PRPs was extensively studied by the notion that PRPs include process-specific proteins. Thus, the pool consists of PRPs specific for either LTP or LTD, as well as process-nonspecific proteins, such as the phosphodiesterase type-4B3 (PDE4B3), for which it has been recently shown a role during both LTP and LTD, without a specificity for that enzyme to a particular plasticity process (Navakkode et al., 2004;Ahmed et al., 2004;Ahmed and Frey, 2003). In the latter case, the different role of PDE4B3 for LTP or LTD is determined by the level of its activation. However it is also involved in the expression of both forms, of late-LTP and late-LTD, by a synergistic interaction of the PDE4B3 with the process-specific tags (Navakkode et al., 2004). Taken together, we can suggest that not only the process-specific tag consists of a complex machinery of molecules (Frey and Morris, 1998a), but also PRPs represent a highly specified pool of proteins expressing their effecter roles only in an unambiguous interaction with the process-specific tag complex. Interestingly, however, inhibition of PKMζ during the induction of either late-LTP or late-LTD also prevented the maintenance of both processes. Thus, one can assume that besides the role of PKMζ as an LTP-specific PRP it must have at least a second plasticity-process-unspecific function in the initiation of processes required for lasting plastic forms in general. One possibility is that PKMζ is

also crucially involved in the interplay of NMDA-receptor function (Hrabetova and Sacktor, 1996) and the induction of immediate processes during LTP and LTD, which maintain these phenomena during their earlier phases and that may be synergistically involved in the initiation of mechanisms required for prolonged maintenance.

### 4.3. Depotentiation and ´synaptic tagging´

It has been studied whether the maintenance of LTP exclusively depends on the availability and the decay time course of 'synaptic tags' and PRPs or whether other processes, such as distinct activity, could also be part of a regulatory machinery of it. One possibility is the presentation of specific homosynaptic activity shortly after LTP-induction. It has been shown that LTP can be depotentiated if LFS is applied to the potentiated input shortly after its induction (Staubli and Chun, 1996b;Staubli et al., 1998;Martin, 1998). This study demonstrates that such a depotentiation could also reset a 'tag' or the synthesis of PRPs. For excluding the latter, it was examined in early-LTP - a transient form of LTP which sets 'synaptic tags' but which is unable to induce its own synthesis of PRPs (Frey and Morris, 1998b;Frey and Morris, 1998a). Recently, Woo and Nguyen (Woo and Nguyen, 2003) reported in mouse hippocampal slices, that multiple trains of high-frequency stimulation, i.e. strong tetanization, caused an immediate synaptic immunity to depotentiation. This immunity to depotentiation of synapse-specific LTP was prevented by inhibitors of protein synthesis. The authors suggested that local translational processes mediated the input-specific synaptic immunity against depotentiation. They also proposed that, in addition to translation, products of transcription can provide cell-wide immunity to depotentiation via a

heterosynaptic transfer of synaptic immunity between distinct pathways in area CA1. Translation and transcription may importantly regulate long-term storage of information by conferring synaptic immunity to depotentiation at previously potentiated synapses. The present study investigated the effect of depotentiation very shortly after early-LTP induction, i.e. 5, 10 or 15 min after a single tetanus. Thus the form of potentiation and the complete depotentiation of early-LTP by applying LFS 5 min after tetanization may be due to the lack of translational or transcriptional products as suggested by Woo.et.al., (Woo and Nguyen, 2003). It has been shown previously that early-LTP is protein synthesis independent, however the rescue of early- and its transformation into late-LTP requires the synthesis of PRPs. Moreover, in contrast to the experiments from Woo and Nguyen (Woo and Nguyen, 2003) which were performed in mice, the present study investigated LTP in rat hippocampal CA1 region.

The present study confirmed results from others (Staubli and Chun, 1996a;Martin, 1998) that LTP can be reversed by LFS within a short time window after its induction. In CA1-neurons of hippocampal slices in vitro this effective period was less than 10 min when early-LTP was investigated. The LFS shortly but beyond 5 min, after LTP-induction resulted always in a transient depotentiation with a subsequent recovery of early-LTP. However, application of LFS 10 or 15 min after tetanization was unable to reset the 'tag' (Fig. 10 C and D). Only a very short time window of 5 min was able to effectively reset the 'tag' by subsequent LFS (Fig. 10 B).

Resetting of the 'tags' or better of the 'tag-complex' (Frey, 2001) in an activity-dependent manner shortly after its induction did not prevent the

activation or setting of the 'tag' by a subsequent tetanization of the same synaptic input. As shown in Fig. 11 A, B, C the 'tag' can be reset by activity and re-activated (Fig. 11 C) by subsequent tetanization providing the system with a very dynamic functional tool in processing of information.

This study provided evidence that the duration of LTP does depend on the decay time course of the 'tag', the availability of PRPs (Frey and Morris, 1998a) and, in addition, on the presence of homosynaptic activity at the synaptic input tetanized at least shortly after its induction. Whether this activity-dependent resetting of the 'tag' contributes to metaplastic states of a neuron/synapse (Abraham and Bear, 1996) remains to be investigated. However, depotentiation with its property of resetting the 'tag' or the ‘tagging machinery’ may be considered as an active process in preventing the creation of a long-lasting memory trace and thus, may play an important role in processes of forgetting.

### 4.4. Heterosynaptic, modulatory requirements

It is known that the activation of dopaminergic receptors are required for late-LTP (Frey et al., 1990;Frey et al., 1991b;Frey et al., 1991a) and it can modulate the magnitude of LTD in area CA1 (Chen and Tonegawa, 1997). Here we conducted experiments to investigate whether dopamine alone can induce a long-lasting plastic changes similar to the direct activation of the D1/D5-receptors (Huang and Kandel, 1995) or after the activation of the PKA-pathway by cAMP-analogs (Frey et al., 1993). Threefold short-lasting application of dopamine revealed a concentration-dependent effect: 10 µM resulted in a delayed-onset, long-lasting depression (Fig. 8 A) whereas a higher concentration, i.e., 50 µM, revealed a delayed-onset potentiation (Fig.

8 B). Application of the dopaminergic D1/D5-receptor antagonist SCH23390 (0.1 µM) during electrically induced LTD induction prevented its maintenance (Fig. 8 C, filled circles) in a similar way as after the application of protein synthesis inhibitors in LTD or LTP studies (Frey et al., 1990;Frey et al., 1991b;Frey et al., 1991a). It can be hypothesized that dopamine might be directly involved in processes required for synthesis of PRPs. It is known that late-LTP depends on the activation of the cAMP/PKA-dependent cascade activated presumably through the D1/D5-receptor in hippocampal CA1 possibly in a complex, synergistic action with the glutamatergic NMDA-receptor and subsequent activation of third messengers (Frey et al., 1993). The results with dopamine application support the hypotheses: (1) that the activation of dopamine receptors initiates processes directly related to the synthesis of plasticity proteins and in addition (2) they suggest that dopaminergic processes are also involved in the setting of and stabilizing the tag-complex (presumably if glutamatergic activity was present at the time of dopamine application) resulting in long-lasting plastic changes (see also (Otmakhova and Lisman, 1998)). Furthermore, a specific regulation of distinct AMPA-receptor phosphorylation sites by bidirectional plasticity (Lee et al., 2000) has been suggested. Thus, the dual action of dopamine, i.e., the induction of either LTP or LTD, might be explained by a concentration-dependent effect on different phosphorylation processes either resulting in LTD or LTP. PKA-activation is a necessary step at least to induce protein synthesis-dependent late-LTP and -LTD (Brandon et al., 1995;Frey et al., 1993;Kameyama et al., 1998). Therefore the influences of the D1/D5-receptor antagonist on processes of tagging were investigated. As shown in Fig. 8 D

late-LTD was prevented in both inputs in presence of SCH23390. In another set of experiments late-LTD in S2 was seen even during the blockade of the D1/D5-receptors (Fig. 8 E), if late-LTD was induced in S1. These sets of experiments resemble the results obtained by anisomycin for LTD (Figs. 6 B and C) as well as for LTP described in the original tagging experiments(Frey and Morris, 1997). In addition, the main concern was, whether tagging during CA1-LTP does also depend on D1/D5-receptor activation. Thus, the experiment shown in Fig. 8 E was repeated, but now by applying a strong tetanization protocol instead of SLFS to the two synaptic inputs (see in(Frey and Morris, 1997)). As shown in Fig. 8 F, LTP is also characterized by heterosynaptic, late-associative interactions. In summary, these data offer strong support for a direct interaction of dopamine-receptor activation required for the synthesis of PRPs.

### 4.5. Late-associative LTP-LTD-interactions

Dopamine mediated heterosynaptic action is required for ´synaptic tagging´ during LTP and in LTD. So, the question now arises as to whether the activation of dopaminergic receptors during late-LTP/-LTD induction influences a general pool of PRPs relevant for the two processes?

For studying the late associative interactions of LTP and LTD, early-LTP was induced in input S1 (Fig. 9 A, filled circles) which was followed by the induction of late-LTD in S2 30 min later (open circles). Paradoxically, early-LTP in S1 was transformed into late-LTP by SLFS through a separate synaptic input, S2. Similarly, early-LTP in S2 (Fig. 9 B, filled circles) was transformed into late-LTP if the induction of late-LTD in S1 (open circles)

preceded WTET in S2. Interestingly, associative interactions of the two inputs occlude the induction of late-LTP or late-LTD 60 min after its induction (Fig. 9 E; squares), i.e., at a time point where the tags were still set or ‘occupied’ by the prior event. Figs. 9 C and D demonstrate that the order of weak or strong stimulation of the two inputs is unimportant if it occurs within a time interval of less than 2 h. In addition, such associative interactions reveal a normal shift in the corresponding input-output curves (Fig. 9 C) and still allows a normal reaction of the slices to further plastic changes at very late time points demonstrating the viability and responsiveness of the preparation to functional events (STET 8 h after WLFS in S2 resulted in a normal potentiation with a duration of at least 30 min, Fig. 9 C). These data shows a positive interaction of LTP and LTD, i.e., LTP can benefit from PRPs synthesized by LTD and vice versa, thus paradoxically expressing late-LTP or -LTD. This phenomenon is named as ‘cross-tagging’. However, as it was described by others (Muller et al., 1995), the two phenomena can also negatively influence each other. Interestingly, the time interval had to be increased between induction of late-LTD in S1 and subsequent weak tetanization in S2 from 30 to 60 min to obtain the transformation of early- into late-LTP in S2 by associative interactions with S1 (Fig. 9 B). If the time interval was 30 min, LTP in S2 was prevented by prior SLFS in S1. The history of the synapses seems to be important for a distinct phosphorylation state of plasticity-related kinases such as calcium calmodulin kinase II (CAMKII), the ζ-isoform of protein kinase C (Hrabetova and Sacktor, 1996;Ling et al., 2002;Hernandez et al., 2003) and/or PKA (Lee et al., 2000) influencing the functional response of the synapse. Different kinetics as well as the specificity of the earlier activation of such cellular key

players may determine the form of plasticity induced by subsequent stimulation.

Summarizing the results regarding the associative LTP-LTD interactions in a given neuron, the data suggest that the induction of a late form of plasticity-either late-LTD or late-LTP-sets its plasticity-specific 'tag' and activates the synthesis of plasticity unspecific PRPs, which can be used by either of the processes. Abraham and colleagues suggested earlier that the induction of homosynaptic LTP and heterosynaptic LTD in one neuronal population may activate process-specific immediate early genes (IEG)(Abraham et al., 1994). It is well known that LTP can cause an increase in IEG expression (Cole et al., 1989). However, whether these IEGs are identical with or fulfill the role of PRPs remains speculative. Similarly, whether PRPs of the same kind or process-specific pools of proteins are synthesized (Fig. 14) also still remains to be determined. Since the induction of early-LTP or early-LTD does not require heterosynaptic, modulatory activation, whereas the late maintenance of the two processes depends on it, so one can assume that early forms are carried by homosynaptic, glutamatergic events. In contrast, the late forms require heterosynaptic, modulatory transmitter activation during initial, early stages. Previous work favors a synergistic action of glutamatergic and non-glutamatergic inputs for the late phases to occur (Frey et al., 1993;Frey and Morris, 1998a). Together with the data presented here, it can be speculated that the setting of the plasticity-specific ´tag´ and the activation of the protein synthesis is due to a synergistic action of glutamatergic and heterosynaptic processes (including the possible interaction with metabotropic glutamate receptors (Fig. 14) (Lee et al., 2000).

### 4.6. The Nature of plasticity-related-proteins (PRPs)

The nature of PRPs is still speculative. One possibility is the increased synthesis of AMPA-receptors 3 h after LTP induction. The synthesis of the AMPA-receptors are PKA-dependent (Nayak et al., 1998). These data are in accordance with the finding that late-LTP, as well as late-LTD, in the hippocampal CA1 requires the activation of dopaminergic D1/D5-receptors which subsequently lead to the activation of PKA. Although increased synthesis of AMPA-receptors could be a necessary step for LTP, it would not explain the expression of LTD. Supposing that the synthesis or the functional activation of AMPA-receptors is specifically regulated by other type of PRPs (other than functional, structural proteins like the AMPA-receptor) different molecules come into consideration. Thus, two possible candidates for process-specific PRPs could be: (1) protein kinases and (2) phosphatases- the first activating whereas the second down-regulating AMPA-function. In the first scenario late-LTP and in the second, late-LTD would be expressed. There is yet at least one further possibility: a single enzyme regulates the activity of the AMPA-receptor (or other ion-channel/receptor complexes). It has been reported recently that an LTP-specific regulation of a phophodiesterase (PDE4B3) occurs during late-LTP (Ahmed et al., 2004;Ahmed and Frey, 2003). Although, it is not yet shown a regulation of that protein during late-LTD, the enzyme could fulfill the function of a general PRP for LTP and LTD. The substrate of the PDE4B3 is cAMP. Therefore, PDE4B3 could regulate the activity of PKA which has been shown to be crucial for late-LTP. Therefore, PDE4B3-dependent regulation of PKA might be responsible for the activation or deactivation of AMPA-receptors (or other effector

proteins) thus expressing late-LTP or late-LTD. However, whether the PKA-dependent regulation of AMPA-receptor synthesis, as a putative effector protein, may be sufficient for the expression of LTP for more than 3-8 h remains unclear.

As already mentioned, modulatory transmitter systems may result in the activation of various processes, e.g., the induction of the same (Fig. 14 D) or different PRPs (Fig. 14 C) in distinct brain structures as well as neuronal sub-regions. Thus, innervation of particular neuronal compartments by a specific modulatory transmitter may exert its explicit action, e.g., induction of distinct PRPs, etc. However, the better understanding of processes underlying the intriguing heterosynaptic induction of the synthesis of a single pool of PRPs in one neuronal population during plastic events in general, i.e., by LTP or LTD, and the putative benefit of the synapses of these PRPs-provided a plasticity-specific ´synaptic tag´ is set-may result in new ways of thinking of how associative interactions during learning are accomplished between different neurons and structures.

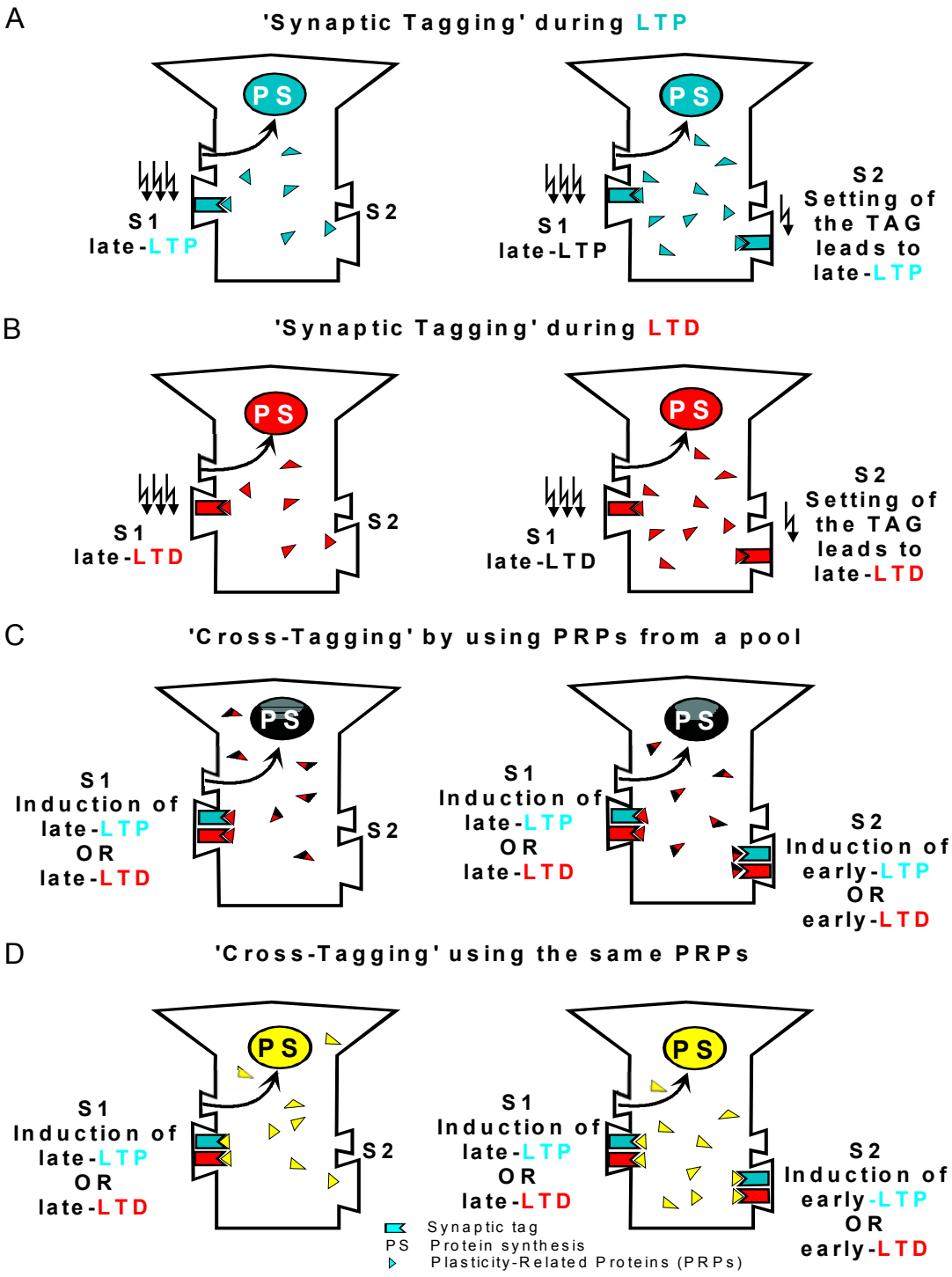


**Figure 14. Schematic illustration of the situation at two separate synaptic inputs S1 and S2 of a CA1-pyramidal neuron during induction of LTP/LTD:** Processes of ´synaptic tagging´ and late-associative interactions approximately 30 min after induction of a plastic event in S1. 'Stimulation of S1 or S2' always represents the heterosynaptic activation of glutamatergic and non-glutamatergic, i.e. dopaminergic receptors.

**(A)** Tagging during LTP: The left cell represents a situation where a repeated strong tetanization in S1 leads to late-LTP by setting its transient ´synaptic tag´ and activating synapse-unspecific protein synthesis. If a weaker tetanization protocol (right neuron) is

applied to a second input S2 (within 1 h after strong tetanization in S1) which sets a ´synaptic tag´ but is unable to induce its own PRPs, it can benefit from PRPs provided by late-LTP induction of S1 thus paradoxically expressing late-LTP. **(B)** A similar situation occurs after the SLFS to input S1 and subsequent WLFS in S1. **(C)** and **(D)**, the combination of the induction of either early-LTP in S1 with late-LTD in S2 or late-LTP in S1 and early-LTD in S2 revealed a new property of ´synaptic tagging´ which is named as ´cross-tagging´: The setting of the ´tag´ is process-specific, i.e. distinct tetanization sets a LTP-specific tag whereas distinct LFS results in setting of a LTD-specific tag (blue or red symbols at the synapses, respectively). However, the induction of the protein synthesis-dependent late phase of either LTP or LTD by dopaminergic D1/D5-receptors in hippocampal CA1 activates the synthesis of process-unspecific PRPs which can be used by either the LTP- or the LTD-specific tags. Whether the same kind of PRPs bind to the different process-specific tags (D) or different LTP- and LTD-specific PRPs are synthesized by the induction of a late phase plastic event, i.e. a pool containing diverse PRPs are synthesized (C).

## 5.0. Conclusions

The principal findings of this dissertation are as follows.

1) A strong low-frequency stimulation protocol (SLFS) can reliably induce input specific late-LTD in hippocampal CA1 lasting at least 8 h and a weak-low frequency stimulation protocol (WLFS) can induce an early-LTD lasting 2-3 h.
2) Late-LTD induced by SLFS is dependent on protein synthesis and activation of D1/D5-receptor, while the early-LTD is independent of protein synthesis.
3) 'Synaptic tagging' and 'late-associative' properties during LTD are similar to that observed for LTP.
4) Dopamine at higher concentration by itself can induce a long-lasting potentiation while a lower concentration can induce a long-lasting depression.
5) Activation of D1/D5-receptor during the induction of a long-lasting plasticity-event is essential for tagging during LTP and LTD in area CA1.
6) The duration of the LTD-'synaptic tag' is 1 h, similar to the time course of the tag during LTP.
7) Induction of LTP and LTD in separate synaptic inputs of one neuronal population can interact in a positive manner, a phenomenon which we named: 'cross-tagging'.
8) Early-LTP in hippocampal slices in vitro can be depotentiated effectively if a LFS is applied after 5 min of its induction. A LFS 10 or 15 min after the induction of early-LTP results in a transient depotentiation.

9) ‘Synaptic tag’ can be reset by using a LFS in a time-dependent manner.
10) The resetted ‘tag’ can again be set by inducing an early-LTP in the same synaptic input.
11) The PKC isotype, PKMζ is essential for the induction of both LTP and LTD.
12) The activity of PKMζ is essential for the maintenance of LTP but not for LTD.
13) The PKMζ seems to stand as an LTP-specific PRP.
14) The PKMζ inhibition prevents the maintenance of LTP in ‘cross-tagging‘ while the early-LTD was transformed to a late-LTD showing the tagging interactions.
15) The PKMζ specifically act as one of the PRPs in LTP tagging but not in LTD tagging.

## 6.0. References


Abel T, Nguyen PV, Barad M, Deuel TA, Kandel ER, Bourtchouladze R (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88: 615-626.

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19: 126-130.

Abraham WC, Christie BR, Logan B, Lawlor P, Dragunow M (1994) Immediate early gene expression associated with the persistence of heterosynaptic long-term depression in the hippocampus. Proc Natl Acad Sci U S A 91: 10049-10053.

Abraham WC, Kairiss EW (1988) Effects of the NMDA antagonist 2AP5 on complex spike discharge by hippocampal pyramidal cells. Neurosci Lett 89: 36-42.

Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J Neurosci 22: 9626-9634.

Aggleton JP, Brown MW (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav Brain Sci 22: 425-444.

Ahmed T, Frey JU (2003) Expression of the specific type IV phosphodiesterase gene PDE4B3 during different phases of long-term potentiation in single hippocampal slices of rats in vitro. Neuroscience 117: 627-638.

Ahmed T, Frey S, Frey JU (2004) Regulation of the phosphodiesterase PDE4B3-isotype during long-term potentiation in the area dentata in vivo. Neuroscience 124: 857-867.

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

Amaral DG, Witter MP (1995) Hippocampal formation. In:The Rat Nervous System, 2nd edition (Paxinos G, ed), pp 443-493. San Diego: Academic Press.

Andersen P, Bliss TV, Lomo T, Olsen LI, Skrede KK (1969) Lamellar organization of hippocampal excitatory pathways. Acta Physiol Scand 76: 4A-5A.

Angenstein F, Riedel G, Reymann KG, Staak S (1994) Hippocampal long-term potentiation in vivo induces translocation of protein kinase C gamma. Neuroreport 5: 381-384.

Angenstein F, Staak S (1997) Receptor-mediated activation of protein kinase C in hippocampal long-term potentiation: facts, problems and implications. Prog Neuropsychopharmacol Biol Psychiatry 21: 427-454.

Balschun D, Wolfer DP, Gass P, Mantamadiotis T, Welzl H, Schutz G, Frey JU, Lipp HP (2003) Does cAMP response element-binding protein have a pivotal role in hippocampal synaptic plasticity and hippocampus-dependent memory? J Neurosci 23: 6304-6314.

Barco A, Alarcon JM, Kandel ER (2002) Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell 108: 689-703.

Barrionuevo G, Brown TH (1983) Associative long-term potentiation in hippocampal slices. Proc Natl Acad Sci U S A 80: 7347-7351.

Baskys A, Malenka RC (1991) Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. J Physiol 444:687-701.: 687-701.

Bear MF, Abraham WC (1996) Long-term depression in hippocampus. Annu Rev Neurosci 19:437-62.: 437-462.

Bear MF, Malenka RC (1994) Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4: 389-399.

Behnisch T, Wilsch VW, Balschun D, Reymann KG (1998) The role of group II metabotropic glutamate receptors in hippocampal CA1 long-term potentiation in vitro. Eur J Pharmacol 356: 159-165.

Bernabeu R, Cammarota M, Izquierdo I, Medina JH (1997) Involvement of hippocampal AMPA glutamate receptor changes and the cAMP/protein kinase A/CREB-P signalling pathway in memory consolidation of an avoidance task in rats. Braz J Med Biol Res 30: 961-965.

Bito H, Deisseroth K, Tsien RW (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. Cell 87: 1203-1214.

Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31-39.

Bliss TV, Gardner-Medwin AR (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 TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232: 331-356.

Bortolotto ZA, Collingridge GL (2000) A role for protein kinase C in a form of metaplasticity that regulates the induction of long-term potentiation at CA1 synapses of the adult rat hippocampus. Eur J Neurosci 12: 4055-4062.

Brandon EP, Zhuo M, Huang YY, Qi M, Gerhold KA, Burton KA, Kandel ER, McKnight GS, Idzerda RL (1995) Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 92: 8851-8855.

Braunewell KH, Manahan-Vaughan D (2001) Long-term depression: a cellular basis for learning? Rev Neurosci 12: 121-140.

Brindle PK, Montminy MR (1992) The CREB family of transcription activators. Curr Opin Genet Dev 2: 199-204.

Calabresi P, Saiardi A, Pisani A, Baik JH, Centonze D, Mercuri NB, Bernardi G, Borrelli E (1997) Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. J Neurosci 17: 4536-4544.

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci 2: 454-460.

Chen C, Tonegawa S (1997) Molecular genetic analysis of synaptic plasticity, activity-dependent neural development, learning, and memory in the mammalian brain. Annu Rev Neurosci 20:157-84.: 157-184.

Chen Z, Fujii S, Ito K, Kato H, Kaneko K, Miyakawa H (1995) Activation of dopamine D1 receptors enhances long-term depression of synaptic transmission induced by low frequency stimulation in rat hippocampal CA1 neurons. Neurosci Lett 188: 195-198.

Colbran RJ, Brown AM (2004) Calcium/calmodulin-dependent protein kinase II and synaptic plasticity. Curr Opin Neurobiol 14: 318-327.

Cole AJ, Saffen DW, Baraban JM, Worley PF (1989) Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. Nature 340: 474-476.

Collingridge GL (2003) The induction of N-methyl-D-aspartate receptor-dependent long-term potentiation. Philos Trans R Soc Lond B Biol Sci 358: 635-641.

Cooper LD, Aronson L, Balsam PD, Gibbon J (1990) Duration of signals for intertrial reinforcement and nonreinforcement in random control procedures. J Exp Psychol Anim Behav Process 16: 14-26.

Deadwyler SA, Dunwiddie T, Lynch G (1987) A critical level of protein synthesis is required for long-term potentiation. Synapse 1: 90-95.

Deisseroth K, Heist EK, Tsien RW (1998) Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. Nature 392: 198-202.

Deisseroth K, Tsien RW (2002) Dynamic multiphosphorylation passwords for activity-dependent gene expression. Neuron 34: 179-182.

Dobrunz LE, Huang EP, Stevens CF (1997) Very short-term plasticity in hippocampal synapses. Proc Natl Acad Sci U S A 94: 14843-14847.

Dudai Y (1997) How big is human memory, or on being just useful enough. Learn Mem 3: 341-365.

Dudai Y (2002) Molecular bases of long-term memories: a question of persistence. Curr Opin Neurobiol 12: 211-216.

Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol 55:51-86.: 51-86.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci U S A 89: 4363-4367.

Dunwiddie T, Lynch G (1978) Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. J Physiol 276:353-67.: 353-367.

Dutar P, Nicoll RA (1988) A physiological role for GABAB receptors in the central nervous system. Nature 332: 156-158.

Fedorov NB, Pasinelli P, Oestreicher AB, DeGraan PN, Reymann KG (1995) Antibodies to postsynaptic PKC substrate neurogranin prevent long-term potentiation in hippocampal CA1 neurons. Eur J Neurosci 7: 819-822.

Frey JU (2001) Long-lasting hippocampal plasticity: cellular model for memory consolidation? Results Probl Cell Differ 34:27-40.: 27-40.

Frey S, Bergado-Rosado J, Seidenbecher T, Pape HC, Frey JU (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, Schwiegert C, Krug M, Lossner B (1991a) Long-term potentiation induced changes in protein synthesis of hippocampal subfields of freely moving rats: time-course. Biomed Biochim Acta 50: 1231-1240.

Frey U, Frey S, Schollmeier F, 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, Hartmann S, Matthies H (1989a) Domperidone, an inhibitor of the D2-receptor, blocks a late phase of an electrically induced long-term potentiation in the CA1-region in rats. Biomed Biochim Acta 48: 473-476.

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 260: 1661-1664.

Frey U, Krug M, Brodemann R, Reymann K, Matthies H (1989b) Long-term potentiation induced in dendrites separated from rat's CA1 pyramidal somata does not establish a late phase. Neurosci Lett 97: 135-139.

Frey U, Krug M, Reymann KG, 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, Matthies H, Reymann KG, Matthies H (1991b) The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. Neurosci Lett 129: 111-114.

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

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

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

Frey U, Schollmeier K, Reymann KG, Seidenbecher T (1995) Asymptotic hippocampal long-term potentiation in rats does not preclude additional potentiation at later phases. Neuroscience 67: 799-807.

Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. Brain Res 522: 69-75.

Gasbarri A, Verney C, Innocenzi R, Campana E, Pacitti C (1994) Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. Brain Res 668: 71-79.

Gass P, Wolfer DP, Balschun D, Rudolph D, Frey U, Lipp HP, Schutz G (1998) Deficits in memory tasks of mice with CREB mutations depend on gene dosage. Learn Mem 5: 274-288.

Geinisman Y (2000) Structural synaptic modifications associated with hippocampal LTP and behavioral learning. Cereb Cortex 10: 952-962.

Geinisman Y, Disterhoft JF, Gundersen HJ, McEchron MD, Persina IS, Power JM, van der Zee EA, West MJ (2000) Remodeling of hippocampal synapses after hippocampus-dependent associative learning. J Comp Neurol 417: 49-59.

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14: 477-485.

Gomperts SN, Carroll R, Malenka RC, Nicoll RA (2000) Distinct roles for ionotropic and metabotropic glutamate receptors in the maturation of excitatory synapses. J Neurosci 20: 2229-2237.

Gonzalez GA, Montminy MR (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59: 675-680.

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

Gutlerner JL, Penick EC, Snyder EM, Kauer JA (2002) Novel protein kinase A-dependent long-term depression of excitatory synapses. Neuron 36: 921-931.

Hebb DO (1949). The Organization of Behavior. New York: Wiley (Interscience), 62, 70.

Hegde AN (2004) Ubiquitin-proteasome-mediated local protein degradation and synaptic plasticity. Prog Neurobiol 73: 311-357.

Hernandez AI, Blace N, Crary JF, Serrano PA, Leitges M, Libien JM, Weinstein G, Tcherapanov A, Sacktor TC (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.

Hirai H (2001) Modification of AMPA receptor clustering regulates cerebellar synaptic plasticity. Neurosci Res 39: 261-267.

Hirai T, Chida K (2003) Protein kinase Czeta (PKCzeta): activation mechanisms and cellular functions. J Biochem (Tokyo) 133: 1-7.

Hirai T, Niino YS, Chida K (2003) PKC zeta II, a small molecule of protein kinase C zeta, specifically expressed in the mouse brain. Neurosci Lett 348: 151-154.

Hrabetova S, Sacktor TC (1996) Bidirectional regulation of protein kinase M zeta in the maintenance of long-term potentiation and long-term depression. J Neurosci 16: 5324-5333.

Hrabetova S, Sacktor TC (2001) Transient translocation of conventional protein kinase C isoforms and persistent downregulation of atypical

protein kinase Mzeta in long-term depression. Brain Res Mol Brain Res 95: 146-152.

Huang EP (1998) Synaptic plasticity: going through phases with LTP. Curr Biol 8: R350-R352.

Huang Q, Zhou D, Chase K, Gusella JF, Aronin N, DiFiglia M (1992) Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. Proc Natl Acad Sci U S A 89: 11988-11992.

Huang YY, Kandel ER (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn Mem 1: 74-82.

Huang YY, Kandel ER (1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. Proc Natl Acad Sci U S A 92: 2446-2450.

Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR (1998) Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 21: 869-883.

Jork R, Grecksch G, Matthies H (1982) Apomorphine and glycoprotein synthesis during consolidation. Pharmacol Biochem Behav 17: 11-13.

Kameyama K, Lee HK, Bear MF, Huganir RL (1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. Neuron 21: 1163-1175.

Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294: 1030-1038.

Kang H, Schuman EM (1996) A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 273: 1402-1406.

Karnup S, Stelzer A (1999) Temporal overlap of excitatory and inhibitory afferent input in guinea-pig CA1 pyramidal cells. J Physiol 516: 485-504.

Kauderer BS, Kandel ER (2000) Capture of a protein synthesis-dependent component of long-term depression. Proc Natl Acad Sci U S A 97: 13342-13347.

Kelleher RJ, III, Govindarajan A, Jung HY, Kang H, Tonegawa S (2004a) Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell 116: 467-479.

Kelleher RJ, III, Govindarajan A, Tonegawa S (2004b) Translational Regulatory Mechanisms in Persistent Forms of Synaptic Plasticity. Neuron 44: 59-73.

Kelso SR, Brown TH (1986) Differential conditioning of associative synaptic enhancement in hippocampal brain slices. Science 232: 85-87.

Kishimoto A, Kajikawa N, Shiota M, Nishizuka Y (1983) Proteolytic activation of calcium-activated, phospholipid-dependent protein kinase by calcium-dependent neutral protease. J Biol Chem 258: 1156-1164.

Korz V, Frey JU (2003) Stress-related modulation of hippocampal long-term potentiation in rats: Involvement of adrenal steroid receptors. J Neurosci 23: 7281-7287.

Krug M, Koch M, Schoof E, Wagner M, Matthies H (1989) Methylglucamine orotate, a memory-improving drug, prolongs hippocampal long-term potentiation. Eur J Pharmacol 173: 223-226.

Krug M, Lossner B, 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.

Kullmann DM, Erdemli G, Asztely F (1996) LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. Neuron 17: 461-474.

Larson J, Xiao P, Lynch G (1993) Reversal of LTP by theta frequency stimulation. Brain Res 600: 97-102.

Laudanna C, Mochly-Rosen D, Liron T, Constantin G, Butcher EC (1998) Evidence of zeta protein kinase C involvement in polymorphonuclear neutrophil integrin-dependent adhesion and chemotaxis. J Biol Chem 273: 30306-30315.

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405: 955-959.

Levenes C, Daniel H, Crepel F (1998) Long-term depression of synaptic transmission in the cerebellum: cellular and molecular mechanisms revisited. Prog Neurobiol 55: 79-91.

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

Linden DJ (1996) A protein synthesis-dependent late phase of cerebellar long-term depression. Neuron 17: 483-490.

Linden DJ, Routtenberg A (1989) The role of protein kinase C in long-term potentiation: a testable model. Brain Res Brain Res Rev 14: 279-296.

Ling DS, Benardo LS, Serrano PA, Blace N, Kelly MT, Crary JF, Sacktor TC (2002) Protein kinase Mzeta is necessary and sufficient for LTP maintenance. Nat Neurosci 5: 295-296.

Lisman J (1989) A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. Proc Natl Acad Sci U S A 86: 9574-9578.

Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304: 1021-1024.

Lynch GS, Dunwiddie T, Gribkoff V (1977) Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. Nature 266: 737-739.

Malenka RC (1994) Synaptic plasticity in the hippocampus: LTP and LTD. Cell 78: 535-538.

Malenka RC, Bear MF (2004) LTP and LTD; An Embarrassment of Riches. Neuron 44: 5-21.

Malenka RC, Nicoll RA (1999) Long-term potentiation--a decade of progress? Science 285: 1870-1874.

Malinow R, Mainen ZF, Hayashi Y (2000) LTP mechanisms: from silence to four-lane traffic. Curr Opin Neurobiol 10: 352-357.

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

Man HY, Wang Q, Lu WY, Ju W, Ahmadian G, Liu L, D'Souza S, Wong TP, Taghibiglou C, Lu J, Becker LE, Pei L, Liu F, Wymann MP, MacDonald JF, Wang YT (2003) Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. Neuron 38: 611-624.

Manahan-Vaughan D, Braunewell KH (1999) Novelty acquisition is associated with induction of hippocampal long-term depression. Proc Natl Acad Sci U S A 96: 8739-8744.

Manahan-Vaughan D, Braunewell KH, Reymann KG (1998) Subtype-specific involvement of metabotropic glutamate receptors in two forms of long-term potentiation in the dentate gyrus of freely moving rats. Neuroscience 86: 709-721.

Manahan-Vaughan D, Kulla A (2003) Regulation of depotentiation and long-term potentiation in the dentate gyrus of freely moving rats by dopamine D2-like receptors. Cereb Cortex 13: 123-135.

Manahan-Vaughan D, Kulla A, Frey JU (2000) Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. J Neurosci 20: 8572-8576.

Manahan-Vaughan D, Reymann KG (1997) Group 1 metabotropic glutamate receptors contribute to slow-onset potentiation in the rat CA1 region in vivo. Neuropharmacology 36: 1533-1538.

Martin KC (2002) Synaptic tagging during synapse-specific long-term facilitation of Aplysia sensory-motor neurons. Neurobiol Learn Mem 78: 489-497.

Martin KC, Barad M, Kandel ER (2000) Local protein synthesis and its role in synapse-specific plasticity. Curr Opin Neurobiol 10: 587-592.

Martin KC, Kosik KS (2002) Synaptic tagging -- who's it? Nat Rev Neurosci 3: 813-820.

Martin SJ (1998) Time-dependent reversal of dentate LTP by 5 Hz stimulation. Neuroreport 9: 3775-3781.

Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. J Neurosci 24: 7821-7828.

Matthies H, Jr., Behnisch T, Kase H, Matthies H, Reymann KG (1991) Differential effects of protein kinase inhibitors on pre-established long-term potentiation in rat hippocampal neurons in vitro. Neurosci Lett 121: 259-262.

Matthies H, Frey U, Reymann K, Krug M, Jork R, Schroeder H (1990) Different mechanisms and multiple stages of LTP. Adv Exp Med Biol 268:359-68.: 359-368.

Matthies H, Schulz S, Thiemann W, Siemer H, Schmidt H, Krug M, Hollt V (1997) Design of a multiple slice interface chamber and application for resolving the temporal pattern of CREB phosphorylation in hippocampal long-term potentiation. J Neurosci Methods 78: 173-179.

Mayr B, Montminy M (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2: 599-609.

McNaughton BL, Douglas RM, Goddard GV (1978) Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. Brain Res 157: 277-293.

Miyamoto E, Fukunaga K (1996) A role of Ca2+/calmodulin-dependent protein kinase II in the induction of long-term potentiation in hippocampal CA1 area. Neurosci Res 24: 117-122.

Mochida H, Sato K, Sasaki S, Yazawa I, Kamino K, Momose-Sato Y (2001) Effects of anisomycin on LTP in the hippocampal CA1: long-term analysis using optical recording. Neuroreport 12: 987-991.

Morris RG, Frey U (1999) Tagging the hebb synapse: reply. Trends Neurosci 22: 256.

Mouton PR, Pakkenberg B, Gundersen HJ, Price DL (1994) Absolute number and size of pigmented locus coeruleus neurons in young and aged individuals. J Chem Neuroanat 7: 185-190.

Mulkey RM, Herron CE, Malenka RC (1993) An essential role for protein phosphatases in hippocampal long-term depression. Science 261: 1051-1055.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9: 967-975.

Muller D, Hefft S, Figurov A (1995) Heterosynaptic interactions between LTP and LTD in CA1 hippocampal slices. Neuron 14: 599-605.

Mumby DG, Astur RS, Weisend MP, Sutherland RJ (1999) Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations. Behav Brain Res 106: 97-107.

Muslimov IA, Nimmrich V, Hernandez AI, Tcherepanov A, Sacktor TC, Tiedge H (2004) Dendritic transport and localization of protein kinase Mzeta mRNA:Implications for molecular memory consolidation. J Biol Chem ..

Navakkode S, Sajikumar S, Frey JU (2004) The Type IV-Specific Phosphodiesterase Inhibitor Rolipram and Its Effect on Hippocampal Long-Term Potentiation and Synaptic Tagging. J Neurosci 24: 7740-7744.

Nayak A, Zastrow DJ, Lickteig R, Zahniser NR, Browning MD (1998) Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. Nature 394: 680-683.

Nguyen PV, Kandel ER (1996) A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. J Neurosci 16: 3189-3198.

Nguyen PV, Kandel ER (1997) Brief theta-burst stimulation induces a transcription-dependent late phase of LTP requiring cAMP in area CA1 of the mouse hippocampus. Learn Mem 4: 230-243.

Nguyen PV, Woo NH (2003) Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. Prog Neurobiol 71: 401-437.

Nicoll RA, Malenka RC (1997) Neurobiology. Long-distance long-term depression. Nature 388: 427-428.

Nishizuka Y (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 9: 484-496.

Ohno S, Nishizuka Y (2002) Protein kinase C isotypes and their specific functions: prologue. J Biochem (Tokyo) 132: 509-511.

Osten P, Hrabetova S, Sacktor TC (1996a) Differential downregulation of protein kinase C isoforms in spreading depression. Neurosci Lett 221: 37-40.

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

Otani S, Abraham WC (1989) Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats. Neurosci Lett 106: 175-180.

Otani S, Marshall CJ, Tate WP, Goddard GV, Abraham WC (1989) Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. Neuroscience 28: 519-526.

Otmakhova NA, Lisman JE (1996) D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. J Neurosci 16: 7478-7486.

Otmakhova NA, Lisman JE (1998) D1/D5 dopamine receptors inhibit depotentiation at CA1 synapses via cAMP-dependent mechanism. J Neurosci 18: 1270-1279.

Otmakhova NA, Otmakhov N, Mortenson LH, Lisman JE (2000) Inhibition of the cAMP pathway decreases early long-term potentiation at CA1 hippocampal synapses. J Neurosci 20: 4446-4451.

Packard MG, White NM (1991) Dissociation of hippocampus and caudate nucleus memory systems by posttraining intracerebral injection of dopamine agonists. Behav Neurosci 105: 295-306.

Pakkenberg B, Gundersen HJ (1997) Neocortical neuron number in humans: effect of sex and age. J Comp Neurol 384: 312-320.

Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P (2000) NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J Neurosci 20: 2512-2522.

Ramón y Cajal S (1894) La fine structure des centres nerveux. Proc R Soc Lond. 55:444-68.

Reilly CE (2001) Hippocampus selectively supports episodic memory retrieval. J Neurol 248: 1014-1015.

Reymann KG, Brodemann R, Kase H, Matthies H (1988a) Inhibitors of calmodulin and protein kinase C block different phases of hippocampal long-term potentiation. Brain Res 461: 388-392.

Reymann KG, Frey U, Jork R, Matthies H (1988b) 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.

Reymann KG, Malisch R, Schulzeck K, Brodemann R, Ott T, Matthies H (1985) The duration of long-term potentiation in the CA1 region of the hippocampal slice preparation. Brain Res Bull 15: 249-255.

Riedel G, Reymann KG (1996) Metabotropic glutamate receptors in hippocampal long-term potentiation and learning and memory. Acta Physiol Scand 157: 1-19.

Robert A, Howe JR, Waxman SG (2000) Development of glutamatergic synaptic activity in cultured spinal neurons. J Neurophysiol 83: 659-670.

Rongo C (2002) A fresh look at the role of CaMKII in hippocampal synaptic plasticity and memory. Bioessays 24: 223-233.

Sacktor TC, Osten P, Valsamis H, Jiang X, Naik MU, 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, Frey JU (2003) Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. Neurosci Lett 338: 147-150.

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

Sajikumar S, Frey JU (2004b) Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1in vitro. Neuroscience 129: 503-507.

Salter MW, Kalia LV (2004) Src kinases: a hub for NMDA receptor regulation. Nat Rev Neurosci 5: 317-328.

Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. J Neurosci 13: 900-913.

Schulz S, Siemer H, Krug M, Hollt V (1999) Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. J Neurosci 19: 5683-5692.

Seidenbecher T, Reymann KG, 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.

Sherrington, C.S. To Schäfer, E.A (1897) letter 27 Nov. 1897, in the Sharpey-Schäfer papers in the Contemporary Medical Archives Centres, the Wellcome Institute for the History of Medicine, reference PP/ ESS/B21/8.

Sheng M, Thompson MA, Greenberg ME (1991) CREB: a Ca(2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252: 1427-1430.

Soderling TR, Derkach VA (2000) Postsynaptic protein phosphorylation and LTP. Trends Neurosci 23: 75-80.

Stanton PK, Sarvey JM (1984) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. J Neurosci 4: 3080-3088.

Staubli U, Chun D (1996a) Factors regulating the reversibility of long-term potentiation. J Neurosci 16: 853-860.

Staubli U, Chun D (1996b) Proactive and retrograde effects on LTP produced by theta pulse stimulation: mechanisms and characteristics of LTP reversal in vitro. Learn Mem 3: 96-105.

Staubli U, Chun D, Lynch G (1998) Time-dependent reversal of long-term potentiation by an integrin antagonist. J Neurosci 18: 3460-3469.

Staubli U, Scafidi J (1999) Time-dependent reversal of long-term potentiation in area CA1 of the freely moving rat induced by theta pulse stimulation. J Neurosci 19: 8712-8719.

Steward O, Falk PM (1986) Protein-synthetic machinery at postsynaptic sites during synaptogenesis: a quantitative study of the association between polyribosomes and developing synapses. J Neurosci 6: 412-423.

Straube T, Frey JU (2003) Time-dependent depotentiation in the dentate gyrus of freely moving rats by repeated brief 7 Hz stimulation. Neurosci Lett 339: 82-84.

Straube T, Korz V, Balschun D, Frey JU (2003) Requirement of beta-adrenergic receptor activation and protein synthesis for LTP-reinforcement by novelty in rat dentate gyrus. J Physiol 552: 953-960.

Sweatt JD (1999) Toward a molecular explanation for long-term potentiation. Learn Mem 6: 399-416.

Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 76: 1-10.

Sweatt JD (2004) Mitogen-activated protein kinases in synaptic plasticity and memory. Curr Opin Neurobiol 14: 311-317.

Thomas GM, Huganir RL (2004) MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci 5: 173-183.

Thomas MJ, Malenka RC (2003) Synaptic plasticity in the mesolimbic dopamine system. Philos Trans R Soc Lond B Biol Sci 358: 815-819.

Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 87: 1327-1338.

Walaas SI, Greengard P (1991) Protein phosphorylation and neuronal function. Pharmacol Rev 43: 299-349.

West AE, Chen WG, Dalva MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 98: 11024-11031.

Whittingham TS, Lust WD, Christakis DA, Passonneau JV (1984) Metabolic stability of hippocampal slice preparations during prolonged incubation. J Neurochem 43: 689-696.

Wilsch VW, Behnisch T, Jager T, Reymann KG, Balschun D (1998) When are class I metabotropic glutamate receptors necessary for long-term potentiation? J Neurosci 18: 6071-6080.

Woo NH, Nguyen PV (2003) Protein synthesis is required for synaptic immunity to depotentiation. J Neurosci 23: 1125-1132.

Xiao MY, Niu YP, Dozmorov M, Wigstrom H (2001) Comparing fluctuations of synaptic responses mediated via AMPA and NMDA receptor channels--implications for synaptic plasticity. Biosystems 62: 45-56.

Yamakura T, Shimoji K (1999) Subunit- and site-specific pharmacology of the NMDA receptor channel. Prog Neurobiol 59: 279-298.

Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci 22: 1532-1540.

## APPENDICES

### I. ZUSAMMENFASSUNG DER DISSERTATION

Prozesse funktionaler Plastizität wie hippokampale Langzeitpotenzierung (LTP) und Langzeitdepression (LTD) werden als zelluläre Mechanismen angesehen, die Lernen und der Gedächtnisformierung unterliegen. LTP und LTD werden daher als geeignete Modelle zur Untersuchung dieser Prozesse herangezogen.

Eine Vielzahl von Eigenschaften der Langzeitpotenzierung wurden in den letzten Jahrzehnten bereits intensiv untersucht, während über Langzeitdepression und ihre Bedeutung für Lernprozesse weniger bekannt ist. In der vorliegenden Studie wurde untersucht, ob elektrisch induzierte LTD in der CA1-Region von Hippokampusschnittpräparaten von der Ratte ähnliche Eigenschaften aufweist wie LTD im intakten Tier, unter besonderer Berücksichtigung der Mechanismen der langfristigen Aufrechterhaltung von LTD. In initialen Experimenten wurden Stimulationsprotokolle entwickelt mit denen es möglich ist, zuverlässig verschiedene Formen von LTD in vitro zu induzieren. In Abhängigkeit vom Stimulationsprotokoll gelang es, entweder eine frühe, proteinsyntheseunabhängige Form (mit einer Dauer von 3 h-4 h) oder eine späte, de novo-proteinsyntheseabhängige langfristige Form (bis zu 8 h) zu induzieren. Beide Formen sind abhängig von der Aktivierung von NMDA-Rezeptoren. Darüber hinaus ist die LTD durch Eingangsspezifität gekennzeichnet d.h. LTD wurde nur an den Synapsen induziert, die eine entsprechende niedrigfrequente Reizung erfuhren. Die Entwicklung dieser Protokolle führte demnach zu einer LTD in vitro, die phänotypisch analoge Induktionseigenschaften wie LTP aufweist.

In früheren Untersuchungen wurde gezeigt, das die Induktion einer frühen LTP einen synaptischen Marker (`synaptic tag') an einer spezifisch aktivierten Synapse setzen kann, der dann in der Lage ist synapsenunspezifische plastizitätsrelevante Proteine, deren Synthese durch die Induktion einer späten LTP an einem zweiten, unabhängigen Eingang induziert wurde, einzufangen und zu prozessieren, so das auf diese Weise die LTP des ersten Eingangs langfristig aufrechterhalten werden kann. Meine Untersuchungen zeigen nun, das in vitro, dieser als `synaptic tagging' bezeichnete Mechanismus ebenso für LTD gültig ist. Darüber hinaus konnte gezeigt werden, das die Induktion entweder von LTD oder LTP an zwei unabhängigen synaptischen Eingängen (S1 und S2) ebenso zu späten assoziativen Interaktionen zwischen den beiden Formen synaptischer Plastizität führen: eine frühe LTD in Eingang S2 wird in eine langfristige LTD überführt, wenn eine späte LTP in Eingang S1 derselben Neuronenpopulation innerhalb eines bestimmten Zeitfensters induziert wird. Die Synthese prozessunabhängiger plastizitätsrelevanter Proteine durch die Induktion einer späten LTP in S1 führte somit zu einer Transformation der frühen in eine späte LTD in S2 wenn prozessspezifische synaptische Marker gesetzt wurden. Wir haben diese neue späte assoziative Eigenschaft zellulärer Informationsverarbeitung `cross tagging' genannt, da prozessunspezifisch plastizitätsrelevante Proteine durch entweder LTD- oder LTP-spezifische synaptische Marker verarbeitet werden können und zur langfristigen Ausprägung der beiden Formen synaptischer Plastizität führen können.

Sowohl der synaptische Marker als auch die plastizitätsrelevanten Proteine sind durch eine relativ kurze Halbwertszeit von einigen Minuten bis

zu wenigen Stunden gekennzeichnet bevor sie, sehr wahrscheinlich durch Dephosphorylierung, zu inaktiven Formen degradieren. Dies führte zu der Frage, ob der Marker oder besser der molekulare `Markerkomplex' in eine inaktive Form zurückgesetzt werden kann und auf diese Weise die Prozessierung plastizitätsrelevanter Proteine verhindert wird. Dies sollte dann zu der Ausbildung nur der frühen Formen synaptischer Plastizität, hier der untersuchten LTP führen. Es ergab sich, das eine niedrigfrequente Reizung sehr kurz (5 min) nach der Induktion einer frühen LTP den synaptischen Marker inaktiviert und zu keinerlei Ausprägung einer langfristigen LTP und somit zu keiner langfristigen zellulären Gedächtnisspur im tagging-Experiment führt.

Der nächste Schritt war die Suche nach möglichen Kandidaten für synaptische Markerkomplexe oder plastizitätsrelevante Proteine. Über die Rolle einer PKC-isoform als ein mögliches Molekül für den Markerkomplex wurde bereits intensiv durch andere Autoren spekuliert. Daher untersuchten wir die Rolle der Proteinkinase M-zeta (PKMζ) für langfristige plastische Veränderungen, d.h. konkret für die Aufrechterhaltung der proteinsyntheseabhängigen Phasen von LTD/LTP, des synaptic tagging oder des cross-tagging Mechanismus. Die Inaktivierung von PKMζ nach Induktion einer späten LTP führte zu einer Umkehrung derselben und nachfolgend zu einer Depression der tetanisierten Inputs. Im Gegensatz hierzu war die Aufrechterhaltung einer induzierten späten LTD nicht beeinträchtigt, jedoch deren Induktion gehemmt. PKMζ-Inhibition verhindert synaptic tagging von LTP. Während cross tagging wird die späte LTP verhindert, während eine frühe LTD an einem unabhängigen zweiten synaptischen Eingang in eine

späte LTD transformiert wird. Dies lässt den Schluß zu, das PKMζ spezifisch in den synaptic-tagging- Mechanismus für LTP, jedoch nicht in LTD involviert ist, aber das die PKMζ sowohl für Induktionsprozesse der LTP als auch der LTD benötigt wird.

II. SELBSTÄNDIGKEITSERKLÄRUNG

**Erklärung**

Hiermit erkläre ich, daß ich die von mir eingereichte Dissertation mit demThema

**Functional plasticity in the hippocampal slices in vitro**.

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.

Förderstedter Str.90, Magdeburg, 1.12.2004

Master of Science - Sreedharan Sajikumar

III. PUBLICATIONS

**Sajikumar S,** Navakkkode S, Frey JU. In search for the specificity of 'synaptic tags' during LTP and LTD. (In Preparation)

Navakkode S, **Sajikumar S** and Frey JU. The effect of rolipram, a type IV-specific phosphodiesterase inhibitor, on distinct forms of long-term depression and ´synaptic tagging´ (Submitted)

**Sajikumar S**, Navakkkode S, Sacktor TC, Frey JU. (2005) Synaptic tagging and Cross-tagging: the role of protein kinase Mζ in maintaining long-term potentiation, but not long-term depression (J. Neuroscience, accepted)

**Sajikumar S**, Frey JU. (2004) Resetting of 'synaptic tags' is time- and activity-dependent in rat hippocampal CA1 in vitro. Neuroscience. 129(2):503-7.

Navakkkode S, **Sajikumar S** Frey JU. (2004) The type IV-specific phosphodiesterase inhibitor, rolipram, and its effect on hippocampal long-term potentiation and synaptic tagging. J. Neurosci.24 (35):7740-4.

**Sajikumar S**, Frey JU. (2004) Late- associativity, synaptic tagging and the role of dopamine during LTP and LTD. Neurobiol Learn Mem.2 (1):12-25.

**Sajikumar S**, Frey JU (2003) Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. Neurosci Lett. 27; 338(2):147-50

**Sajikumar S,** Goel HC (2003) Podophyllum hexandrum prevents radiation-induced neuronal damage in postnatal rats exposed in utero. Phytother Res. 17(7):761-6

Goel HC, **Sajikumar S**, Sharma A. (2002) Effects of Podophyllum hexandrum on radiation induced delay of postnatal appearance of reflexes and physiological markers in rats irradiated *in utero*. Phytomedicine. 2002, 9(5):447-54

PUBLISHED ABSTRACTS

**Sajikumar S,** Navakkkode S, Frey JU.(2005) In search for the specificity of 'synaptic tags' during LTP and LTD. Proceedings of the 30th Göttingen neurobiology conference and the 6th meeting of the German neuroscience society 2005.

Navakkode S, **Sajikumar S** and Frey JU, (2005) The effect of rolipram, a type IV-specific phosphodiesterase inhibitor, on distinct forms of long-term depression and ‘’synaptic tagging’’. Proceedings of the 30th Göttingen neurobiology conference and the 6th meeting of the German neuroscience society 2005.

**Sajikumar S,** Frey JU. (2004) Depotentiation and time-dependent resetting of ‘synaptic tags’ during LTP in rat hippocampal slices in vitro. Socity For Neuroscience. Abstr. 636.11.

**Sajikumar S,** Frey JU. (2004) "Synaptic tagging" during long-term depression and "cross-tagging” in rat hippocampal slices in vitro. 4th Forum of European Neuroscience Society meeting, Lisbon, Portugal. Abstr. 260.

**Sajikumar S,** Frey JU. (2003). "Synaptic tagging" and long-term depression in rat CA1 hippocampal slices in vitro. Socity For Neuroscience. Abstr. 169.13

**Sajikumar S,** Frey JU. ’Synaptic tagging’ and long-term depression in rat hippocampal slices in vitro. Proceedings of the 29th Göttingen neurobiology conference and the 5th meeting of the German neuroscience society 2003. Abstr. 626.

# IV. CURRICULUM VITAE

**Sreedharan Sajikumar**
Birth date: May 10, 1976
Place of Birth: Chingoli, Kerala, India.

**Present adress**

Dept. of Neurophysiology
Leibniz Institute for Neurobiology
Brenneckestr.- 6,
39118, Magedeburg
Germany.
Phone: + 49 391 6263424/405
Fax: 49 391 6263421
E-mail: saji.kumar@ifn-magdeburg.de

**Home adress**

Förderstedter Str.90
39112, Magdeburg
Germany.

**Education**

2001(Oct) - Present Doctoral student

Leibniz Institute for Neurobiology, Otto-von-Guericke Universität, Magdeburg,Germany
Full Scholarship, Leibniz Institute for Neurobiology, Magdeburg, Germany.
Dissertation Title: *Functional plasticity in the hippocampal slices in vitro.*

2000(Aug) -2001(Sept) Junior Research Fellow (JRF**)**

Dept. of Radiation biology, Institute for Nuclear Medicine and Allied Sciences, New Delhi, India.
Full Scholarship, Defence Research Development Organisation (DRDO)
Project Title: *Radio protective role of some herbal extracts on developing nervous system of rats.*

1998- 2000(July) Master of Science

Department of Life sciences, University of Calicut, Kerala, India.

Thesis Title: *Cognitive profiles in developing rats as tested through T- Maze; Effect of Betaine/ Pyridoxine*

1997-1998(May) Bachelor of Education

| | |
|---|---|
| | Faculty of Natural Science, University of Kerala, India. |
| 1992- 1996 | Bachelor of Science<br>Dept. of Zoology, University of Kerala, India.<br>Major degree: Zoology, minor degree: Chemistry and Botany. |

**Research Projects**

| | |
|---|---|
| 2001-Present | Research student, Supervisor: Prof. Dr. Julietta Uta Frey<br>Dissertation Project: Functional plasticity in the hippocampal slices in vitro.<br>*Methods: Hippocampal slice preparation and maintenance up to 14h. Long-term extracellular recording of LTP/LTD.* |
| 2000-2001 | Junior Research Fellow, Supervisor: Dr. H. C. Goel<br>Thesis Project: Radio protective role of some herbal extracts on developing nervous system of rats.<br>*Methods: Histology, behavioural experiments (T-maze, Y-maze , Radial arm maze)* |
| 1998-2000 | Master's student, Supervisor: Prof. Dr. T. Ramakrishna<br>Thesis Project: Cognitive profiles in developing rats as tested through T- Maze; Effect of Betaine /Pyridoxine:<br>Methods: Behavioural experiments and histology. |

**Personal References**

**Prof. Dr. Julietta Uta Frey**
Leibniz Institute for Neurobilogy
Department of Neurophysiology
Brennecke Str.6
39118 Magdeburg, Germany.

Phone: + 49 391 62  63424/405
Email : E-mail: frey@ifn-magdeburg.de

**Prof. Dr. T. Ramakrishna**
Emeritus Professor of Physiology
Department of Life Sciences
University of Calicut
Kerala, India.
Phone: 0091-80-23515885
E-mail: svuniv@eth.net

**Dr. H. C. Goel**
Retired Scientist 'G'
Department of Radiation Biology
Institute for Nuclear Medicine &Allied Sciences
Defence Research Development Organisation.
Delhi, India.
E-mail: goelharish@hotmail.com

S. Sajikumar