1 Introduction

The last 4 decades have put the hippocampus forward, as a brain structure that is highly involved in memory functions. Damage of the hippocampus can lead to severe memory impairment, e.g. patients with hippocampal lesions show deficits in declarative memory functions. Two contending theories consider the hippocampus to be either the locus for temporary storage of to-be-consolidated information [Squire, 1992], or the locus of permanent information storage through multiple memory traces [Riedel & Micheau, 2001, Nadel & Moscovitch, 1997]. Furthermore, a role in episodic as well as semantic information processing has been debated [Squire & Zola, 1998, Vargha-Khadem et al., 1997, Tulving & Markowitsch, 1997]. There is still an ongoing controversy as to whether the hippocampus is involved in spatial, non-spatial or both types of information encoding [Cohen & Eichenbaum, 1991, Morris & Frey, 1997, Muller et al., 1996].

Establishing the neuronal correlate for memory will have a tremendous impact on the search for therapeutic drugs. Information in the brain is believed to be encoded and stored by the strength of synaptic weights within networks of connected neurons [Kandel & Squire, 2000] which may take the form of long-term potentiation (LTP) or depression (LTD) of synaptic efficacy. Shortly after the discovery of LTP [Bliss & Lomo, 1973] it was proposed that the

neural mechanisms of activity dependent hippocampal synaptic plasticity are activated during and necessary for certain kinds of learning and memory. Plasticity comprises the ability to change and adapt the nervous system to new experiences/insights. This reflects the premise for learning and memory. By investigating several forms of synaptic plasticity, one might not find insights into the psychological aspects of memory, but rather determine the neuronal mechanisms that might encode the information. Since its discovery in 1973 [Bliss & Lomo, 1973] the hypotheses of "LTP equals memory" exists. To test this hypothesis one needs to compare the physiological properties and the time course of both phenomenon.
Its highly regular organisation as well as its high impact in memory processes renders the hippocampus a good candidate for long-term physiological investigations.

1.1 Synaptic transmission in the hippocampus

1.1.1 The hippocampus and its structure

The hippocampus is a specialized region of the limbic cortex, located in the temporal lobe, in the inferiomesial part of the parahippocampal gyrus. The cortical structure, hippocampus, derives its name from the sea horse (*ho hippos* - horse, *he kampé* - the bend; Greek). The hippocampal formation is divided into the hippocampus proper, the dentate gyrus and the subiculum. The hippocampus proper is further subdivided into the subfields CA1-CA4. The hippocampus proper as well as the dentate gyrus are three-layered cortices [Bayer, 1985]. The dentate gyrus comprises the molecular layer, in which the perforant path fibres terminate (the lateral perforant path

in the outer molecular layer and the medial perforant path in the inner molecular layer); the granule cell layer, which is populated by the principle cell type, the granule cell; and the polymorphic layer, which contains the granule cell afferents and glia cells. The granule cell layer forms a close and compact 'U' shape around the first CA sector (CA4, hilus) and is tightly packed and quite dense. Dendrites of these granule cells, as well as diverse other cells occupy the molecular layer (*stratum moleculare*) of the dentate gyrus. Granule cell axons are called mossy fibres and emerge from the bottom of the cell body collating in the polymorphic layer before entering the CA3 field.

The trisynaptic pathway is an internal circuit that is unique to the hippocampus. Placing the hippocampus on the coronal plane, the granule cell layer of the dentate gyrus is considered to be the first stage of the trisynaptic pathway. As illustrated (see Fig. 1.1), this circuit includes the perforant path projections to the dentate gyrus, the subsequent mossy fibre projection to the area CA3 and finally the Schaffer collaterals targeting neurons in CA1.

The dentate gyrus is the target for the majority of entorhinal afferents (originating in layer II, [Heinemann et al., 2000]) carrying sensory information of multiple modalities about the external world (olfactory, gustatory, visual and somatosensory). These afferents reach the granule cells via the perforant pathway and terminate in the stratum moleculare. The CA3 subfield represents the second stage of the trisynaptic pathway. Pyramidal cells of this region are the principal targets of granule cell axons. The axons of CA3 pyramidal cells arise from the lower pole of the soma. They give rise to extensive axonal arborisation and project to the CA1 subfield generally known as Schaffer collaterals. Finally, the CA1 subfield represents the third and last stage of the intrahippocampal trisynaptic loop. It is the major target of CA3 pyramidal cell axons. These dendritic processes

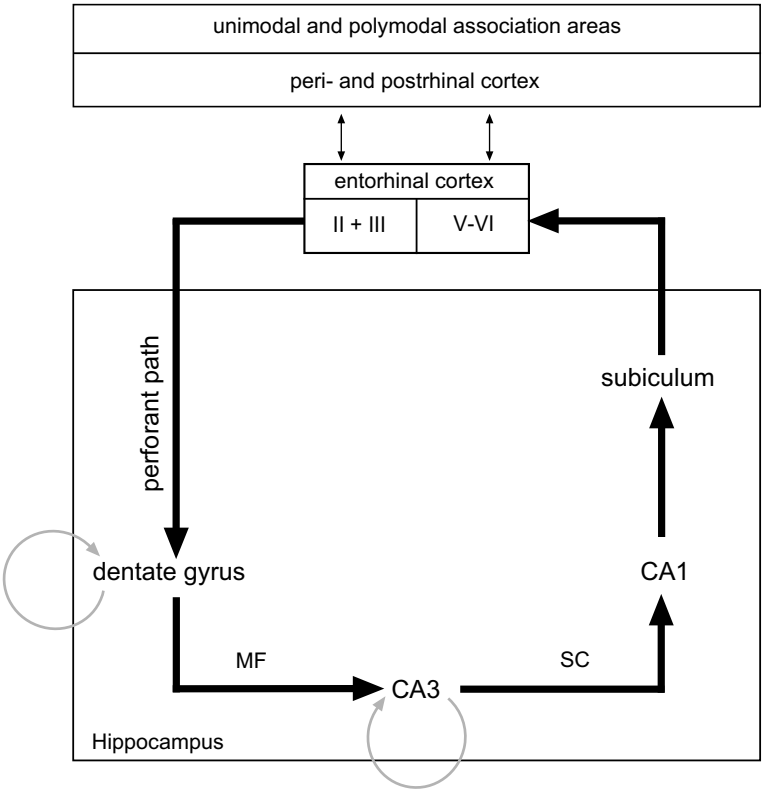

Figure 1.1: Schematic illustration of hippocampal connectivity
The main hippocampal input arises from the entorhinal cortex via the perforant path to the dentate gyrus. The dentate gyrus granule cells project to the subfield CA3, using their axons, the mossy fibres (MF). The CA3 pyramidal cells project with their collateral axons the Schaffer collaterals (SC) to the pyramidal cells of CA1. Main projections: marked as **thick black lines**, contralateral connections marked as grey circuits. Schematic modified from [Menzel, 1996, Witter et al., 2000].

ultimately terminate in a tuft of thin branches in the *stratum lacunosum moleculare* usually reaching the hippocampal fissure. It is important to note that in addition to this circuit, back-propagating

connections and direct inputs exist. Last but not least, associational fibres connect the transverse circuitry (septal-temporal axis).
The following discussions concerning basal synaptic transmission and plasticity in the hippocampus will be restricted to occurrences in the dentate gyrus and the CA1 region. Mossy fibre plasticity in the CA3 region is a phenomenon in its own right which would require substantial discussion.

1.1.2 Basal synaptic transmission in the dentate gyrus

The amino acid glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. It is involved in a vast number of neuronal and glial processes. In addition to its well known involvement in learning and memory processes, glutamate is also known to be a potent neurotoxic agent. In that it plays a critical role in the progression of diverse neurological disorders (epilepsy, ischemia, neurophatic pain and neurodegenerative diseases). For a long time it was believed that glutamate exerts its neurotransmitter action via a number of ionotropic glutamate receptors, namely the NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid) and kainate receptors (for review, see [Koerner & Cotman, 1981]). Later on a new type of glutamate receptor was discovered that is coupled to G-proteins and second messenger systems. These metabotropic glutamate receptors (mGlus) either stimulate phosphatidylinositol hydrolysis and intracellular Ca^{2+} mobilization [Nicoletti et al., 1986], via coupling to phospholipase C, or are negatively linked to adenylate cyclase activity [Tanabe et al., 1992] (see Fig. 1.2).
Fast inhibitory neurotransmission in the hippocampus is mediated by GABAergic interneurons. The excitability of principle neurons is regulated by an interconnected GABAergic network. GABA binds

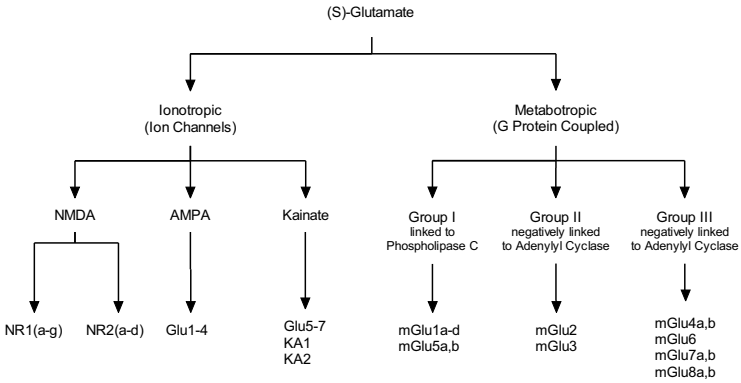

Figure 1.2: Classification of glutamate receptors
Schematic representation of ionotropic and metabotropic glutamate receptor subtypes, subunits and splice variants

to three types of receptors [Chebib & Johnston, 1999]. The $GABA_A$ and $GABA_C$ receptors, ion channels mostly permeable to chloride, and the $GABA_B$ receptors which activate slow potassium currents through a G-protein dependent mechanism.

Metabotropic glutamate receptors

To date, eight different mGlu subtypes have been described, termed mGlu1 to mGlu8, which can be classified into three different groups based on their amino acid sequence homologies, their coupling mechanisms and pharmacological profiles [Nakanishi, 1992]. For schematic representation of ionotropic and metabotropic glutamate receptor subtypes, subunits and splice variants see Fig. 1.2. A major distinction between group I mGlus and group II and III mGlus is that group I couples with $G_{q\text{-}ll}$ proteins, which subsequently activate phospholipase C (PLC). This results in phosphoinositide (PI) hydrolysis, re-

Table 1.1: Transduction mechanisms, agonists and antagonists for mGlu subgroups

	Group I mGlu1/5	**Group II** mGlu2/3	**Group III** mGlu4/6/7/8
Transduction mechanism	$G_{q\text{-}ll}$ $\uparrow PLC \uparrow Ca^{2+}$	G_i/G_o $\downarrow AC \downarrow cAMP$	G_i/G_o $\downarrow AC \downarrow cAMP$
Pharmacology			
Agonists	(1S,3R)-ACPD (S)-3,5 DHPG CHPG (mGlu5)	(1S,3R)-ACPD LY354740	L-AP4
Antagonists	MCPG LY367385 (mGlu1) MPEP (mGlu5) 4CPG	MCPG LY341495	MCPG CPPG

lease of calcium from internal stores and protein kinase C (PKC) activation [Manahan-Vaughan & Reymann, 1996, Schoepp et al., 1999]. In contrast, group II and III mGlus are negatively coupled to adenylyl cylase (AC) through G_i/G_o proteins, thereby inhibiting cyclic AMP (cAMP) formation and cAMP-dependent protein kinase (PKA) activation [Manahan-Vaughan & Reymann, 1996, Schoepp et al., 1999]. The classification of mGlus needs to be expanded to include those that involve activation of other enzymes, such as phospholipase D (PLD) [Schoepp et al., 1999, Pellegrini-Giampietro et al., 1996]. In addition to the described pathways, mGlus can also regulate other signal transduction pathways. For example, group I mGlu activation leads to:

- altered phosphorylation of K^+ and Ca^{2+} channels resulting in modulation of glutamate release [Anwyl, 1999]

- the activation of cAMP, mediated by coupling to AC through G_s [Pellegrini-Giampietro et al., 1996]

- G-protein independent activation of Src-family tyrosine kinase [Heuss et al., 1999]
- phosphorylation of mitogene activated protein (MAP) kinase, such as ERK1/2 (p44/p42) [Peavy & Conn, 1998]
- cyclic GMP accumulation [Gasparini et al., 2002]

It has been shown that phosphorylated group I mGlus uncouple pertussis toxin-sensitive G proteins in exchange for pertussis toxin-insensitive G proteins and thereby inhibit further glutamate release [Rodriguez-Moreno et al., 1998]. Electrophysiological data obtained in the CA1 region demonstrated that the group I mGlu agonist, DHPG, depresses excitatory postsynaptic currents but facilitates synaptic transmission when endogenous glutamate activity is suppressed. Thus, group I mGlus can elicit both enhancing and depressant effects on postsynaptic responses in the hippocampus [Rodriguez-Moreno et al., 1998].
The group II mGlus also appear to couple to PLC under certain conditions and may also stimulate PLD, leading in turn to PKC activation and inositol triphosphate (IP_3) receptor-mediated postsynaptic increases of Ca^{2+} concentration [Otani et al., 2002].
In conclusion, mGlus regulate the phosphorylation of various kinases, ion channels and receptors and, in addition, activate several transcription factors. Their modulation of ion channels as well as their modulation of synaptic transmission is reviewed in Conn & Pinn (1997). While the group I mGlus appears to be predominantly involved in processes of enhanced excitability and synaptic transmission, group II and III mGlus typically produce depressant effects.
The 95kDa postsynaptic density/disc-large/zona occludens-1 (PDZ) domain-containing proteins interact with several mGlus. The protein interacting with C-kinase (PICK1) binds to mGlu7 regulating the expression and phoshorylation of the latter [Dev et al., 2000,

Dev et al., 2001]. Tamalin, another PDZ-binding protein regulates the surface expression of group I mGlus [Kitano et al., 2002]. The Homer family of proteins seem to control the surface expression and the constitutive activity of group I mGlus [Ango et al., 2002]. The Homer-1a isoform, for example, can stimulate group I mGlu activity in the absence of an agonist.

Localisation of mGlus Metabotropic glutamate receptors are localised in all behaviourally relevant brain structures, such as the hippocampus, striatum, amygdala, cerebrellum, cortex and others. In the hippocampal formation, all groups of mGlus are present. Group I mGlus are mainly found in the dentate gyrus, but are also present in lower concentrations in area CA3 and CA1. The group I mGlu subtype mGlu1 is predominantly found in the dentate gyrus, whereas CA1 pyramidal neurons mainly express mGlu5 [Lujan et al., 1996, Fotuhi et al., 1994]. Group II mGlu mRNA levels are high in the rat denate gyrus, but absent on hippocampal pyramidal cells [Neki et al., 1996, Ohishi et al., 1993a, Ohishi et al., 1993b]. Group III mGlus are expressed differently. The subtype mGlu6 is selective to retinal ON-bipolar cells [Nakajima et al., 1993]. Metabotropic glutamate receptor 7 is found in the CA1 and CA3 regions of the hippocampus and, in lower concentrations, also on the medial and lateral perforant path. However, it is only poorly present in the dentate gyrus [Saugstad et al., 1994]. In contrast, mGlu4 has not been found in the dentate gyrus and the CA1 and CA3 regions, but is present in the entorhinal cortex and the medial and lateral perforant path [Saugstad et al., 1994, Koerner & Cotman, 1981]. MGlu type 8 is mainly expressed in the perforant path [Shigemoto et al., 1997] and in the outer molecular layer of the dentate gyrus [Bradley et al., 1996]. The subcellular distribution is strikingly different accross the vari-

ous types of mGlus. Electron microscopic immunogold labeling has revealed that group I mGlus are localized postsynaptically on dendritic spines and are concentrated at perisynaptic sites [Baude et al., 1993, Lujan et al., 1996, Shigemoto et al., 1997]. However, a presynaptic localisation for these receptors has also been suggested [Romano et al., 1995, Gereau & Conn, 1994, Fotuhi et al., 1994]. A predominantely pre- but also a postsynaptic localisation has been proposed for group II mGlus [Lujan et al., 1997, Pin & Duvoisin, 1995, Shigemoto et al., 1997]. Like the group I mGlus, they are localised at the periphery of the synapse, at a preterminal region, far from release site. In contrast, the group III mGlus are typically targeted to presynaptic membranes [Shigemoto et al., 1997] and attenuate excitatory transmission [Nicoll et al., 1998].

1.2 Changes in synaptic efficacy

Synaptic efficacy represents the amplitude of transmembrane voltage of the postsynapse which arises as a consequence of specific stimulation of the presynapse. Synaptic plasticity is a process in which synapses change their efficacy as a consequence of their previous activity. It consists of any changes in synaptic connections between neurons, including strengthening and weakening of synapses, changes in the postsynaptic transduction processes, changes in the distribution of receptor proteins, and changes in the morphology of synapses. These changes of synaptic strength can be short- and long-lasting as well as positive or negative. A common prediction is, that memory can be encoded by changes in synaptic strength.
Bliss and Lomo [Bliss & Lomo, 1973] were the first to present neurophysiological data corresponding to this hypothesis, when they dis-

covered LTP, a long-lasting increase of synaptic strength in the hippocampus, which occurs in response to high-frequency stimulation. Subsequently, a long lasting decrease (LTD) in synaptic strength, induced by low-frequency stimulation was presented [Lynch et al., 1977] and first fully described as a homosynaptic event in vitro in 1992 [Dudek & Bear, 1992]. Thus, the regulation of synaptic strength by activity is bi-directional [Shouval et al., 2002]. In addition to these long-lasting forms of synaptic plasticity, activity could also induce short-term modifications in synaptic strength.

1.2.1 Short-term modifications

Even a single pulse delivered to a neuron can modulate the efficacy of the next pulse delivered to a neuron. If the synaptic transmission is increased on the second pulse, this phenomenon is called paired pulse facilitation (PPF); if it is decreased, the term paired pulse depression is used (PPD). An increase in intracellular Ca^{2+} in the presynaptic terminal due to the first stimulation increases the probability of neurotransmitter release and leads to PPF, which is therefore a presynaptically mediated phenomenon.
Stimulation of the medial perforant path leads to a depression of the second synaptic response to paired stimuli at short- (10–40ms) and long-latency (200–2000ms) interstimulus intervals (ISIs) separated by a period of reduced depression at intermediate interstimulus intervals (50–200ms) [Joy & Albertson, 1993]. The first phase of PPD reflect the activation of $GABA_A$ fast inhibitory post-synaptic potentials (IPSPs) and subsequent inhibition of dentate granule cells [Moser, 1996, DiScenna & Teyler, 1994]. The synaptic depression elicited with pulse intervals of 200–2000ms may reflect activation of late $GABA_B$ IPSPs [Rausche et al., 1988, Rausche et al., 1989], with the facilitatory and inhibitory effects of

these receptors depending tightly on the interstimulus interval of the paired pulses [Kahle & Cotman, 1993]. Opening of calcium-dependent potassium channels which suppress presynaptic glutamate release also contribute to this second phase of paired pulse depression [Thalmann & Ayala, 1982]. Paired pulse facilitation elicited with pulse intervals of 50–200ms reflected the selective increase of NMDA-mediated responses to further release of glutamate from the presynaptic bouton [McNaughton, 1982] and may also be mediated by activation of $GABA_B$ receptors [Kahle & Cotman, 1993].

1.2.2 Long-term modifications

Long-term synaptic plasticity can be NMDA receptor dependent or independent. Numerous publications describes both forms in vitro and in vivo [Bliss & Collingridge, 1993, Manahan-Vaughan et al., 1998, Dudek & Bear, 1992, Mulkey & Malenka, 1992]. This introduction is restricted to the describtion of NMDA receptor dependent forms of synaptic plasticity.

Depending on the induction protocol, several forms of plasticity can be elicited [Teyler et al., 1994]: homosynaptic (modulation of the stimulated pathway), heterosynaptic (modulation of an unstimulated pathway) and associative (modulation of one pathway by co-activation of at least two pathways). Here, focus will be made on the homosynaptic plasticity elicited at the granule cell–perforant path synapses.

NMDA receptor dependent homosynaptic long-term plasticity

The key mechanism underlying LTP and LTD comprises activation of the NMDA receptor [Bliss & Collingridge, 1993, Dudek & Bear, 1992, Mulkey & Malenka, 1992], which is a

Na^+/Ca^{2+} channel that is activated by glutamate only when the postsynaptic cell membrane is depolarised from resting potential, due to the action of AMPA receptors [Bliss & Collingridge, 1993]. Although the affinity of NMDA receptors to glutamate is even higher than for AMPA receptors, activation of NMDA receptors also requires concurrent postsynaptic depolarization to release the voltage-dependent Mg^{2+}-block in the receptor [Ozawa et al., 1998]. The resultant increase in intracellular Ca^{2+} generates the postsynaptic response underlying long-term plasticity.

Multiple factors mediate the long-lasting expression of synaptic plasticity. For example, reversible modulation of AMPA receptor phosphorylation alters post-synaptic responses underlying LTP [Malinow & Malenka, 2002]. LTP and LTD are associated with phosphorylation and dephosphorylation, respectively [Lee et al., 2000].

Of particular interest are the mGlus which are differentially involved in the induction and maintenance of NMDA receptor-dependent LTP and LTD. Whereas group I mGlu activation leads to a facilitated LTP in both dentate gyrus and CA1 regions in vivo [Manahan-Vaughan et al., 1996, Manahan-Vaughan & Reymann, 1996, Manahan-Vaughan, 1997, Manahan-Vaughan et al., 1999b], not much is known about group I mGlu involvement in LTD in the dentate gyrus in vivo [Kemp & Bashir, 2001]. In vitro studies and investigations of the CA1 subfield in vivo suggests that group I mGlu activation induces stable LTD [Camodeca et al., 1999, Huber et al., 2001, Nicoll et al., 1998, Watabe et al., 2002]. Antagonists of this mGlu subgroup fully block LTP in the dentate gyrus in vivo [Manahan-Vaughan et al., 1998] and LTD in vitro [O'Mara et al., 1995]. To date, not much is known about the subtype-specific involvement of the group I mGlu in the induction and maintenance of both forms of synaptic plasticity in the dentate

gyrus in vivo. This study therefore set about to address the role of mGlu1 and mGlu5 in hippocampal plasticity.
Group II mGlus in contrast contribute to the induction of LTD [Manahan-Vaughan, 1997, Huang et al., 1999], but seem to play a lesser role in LTP expression [Manahan-Vaughan et al., 1998, Manahan-Vaughan, 1997].
There is evidence for an modulatory involvement of group III mGlu in synaptic plasticity, where the age and strain of the rat, as well as the hippocampal region play an important role [Manahan-Vaughan & Reymann, 1995c, Manahan-Vaughan, 2000].

Determining the direction of synaptic plasticity

There is a wide agreement that both forms of synaptic plasticity, LTP and LTD, are triggered by different Ca^{2+} levels [Bear & Malenka, 1994, Malenka, 1994]. Thus, large calcium rises in a narrow time-window are associated with LTP (see Fig. 1.3), whereas a smaller calcium rise in a wider time-window is required for induction of LTD (see Fig. 1.4) [Lisman, 1989, Cho & Bashir, 2002]. In this respect, LTP is commonly induced by high-frequency stimulation (classically $100Hz$) and LTD by prolonged ($15min$) low-frequency (1–$5Hz$) stimulation. The possibility of different stimulation paradigms resulting in different concentrations of cytosolic Ca^{2+} is the key argument in the hypotheses of Lisman (1989) and Artola and Singer (1993). In their hypotheses the binding affinities of Ca^{2+}-binding proteins (kinases and phosphatases) mediate the expression of synaptic efficacy. Moderate rises in Ca^{2+} should lead to a predominant activation of phosphatases, while stronger increases would favour activation of kinases.
Experimental data from the developing visual cortex have led to the formulation of a biphasic synaptic modification rule known as

Bienenstock-Cooper-Munro (BCM) model [Bienenstock et al., 1982]. The authors predict that the direction of synaptic gain changes as a function of postsynaptic activity, which is defined as the product between presynaptic activity and synaptic efficacy. A modification threshold known as θ_{m} determines the degree of postsynaptic activity required to generate LTP. This modification threshold is itself dynamically regulated by the average level of postsynaptic activity. Artola, Bröcher and Singer (1990), modified the BCM rule according to data obtained in the mature cortex . The so-called ABS rule predicts, that the direction of synaptic gain changes is dependent on the membrane potential of the postsynaptic cell [Artola et al., 1990]. Artola et al. (1990) redefined θ_{m} into θ^{+} and θ^{-}, where θ^{+} defined the modification threshold for generation of LTP, and θ^{-} defined the modification threshold for LTD.

The term metaplasticity has been introduced to describe the changes in the ability to undergo LTP and LTD [Abraham & Bear, 1996]. It has become apparent from experimental data that the ability to express plasticity can be altered by previous synaptic activity [Holland & Wagner, 1998, Wang & Wagner, 1999], by neuromodulators [Akirav & Richter-Levin, 1999, Kim & Yoon, 1998], or behavioural experience [Manahan-Vaughan & Braunewell, 1999, Seidenbecher et al., 1997]. Thus, the modification threshold is a mechanism that keeps the modifiable synapse within a useful dynamic range [Abraham et al., 2001].

Synaptic potentiation can be separated into several temporal stages that use different mechanisms:

- the induction phase – NMDA receptor dependent.
- short-term potentiation (STP), which lasts only 15 to 30min – due to kinase activity.

- early-phase LTP, which is stable for 2 to $3h$ – needs persistently activated protein kinases.
- and late-phase LTP, which can last for at least $8h$ – requires gene expression and protein synthesis.

Key components of the molecular machinery underlying long-term plasticity

There are only a few molecules for which the evidence of a key role in LTP and LTD is compelling. There is evidence for α-calcium-calmodulin-dependent proteinkinase II (CaMKII) [Lisman et al., 1997, Bliss & Collingridge, 1993], Ca^{2+}/phospholipid-dependent protein kinase (PKC) and several proteinphosphatases (Calcineurin (PP2B), proteinphosphatase 1 (PP1) and protein phosphatase 2A (PP2A)) [Malenka & Nicoll, 1999, Soderling & Derkach, 2000, Dineley et al., 2001].

CaMKII Calcium-calmodulin-dependent protein kinase II is an enzyme of the nervous system, which has been implicated in a variety of neurobiological processes [Lisman et al., 2002]. This kinase has the ability of precise modulation of neuronal functions and plays therefore an important role in several processes underlying synaptic plasticity.
After the triggering of LTP, CaMKII is brought into an autophosphorylated state, and its activity is not longer dependent on Ca^{2+}-calmodulin. This autophosphorylation might be responsible for long-lasting biochemical changes [Lisman & Goldring, 1988, Fukunaga et al., 1995]. Phosphorylated CaMKII induces a biochemical cascade underlying protein synthesis that results in the net addition of AMPA receptors, due to their phosphorylation [Malenka & Nicoll, 1999, Lisman et al., 2002,

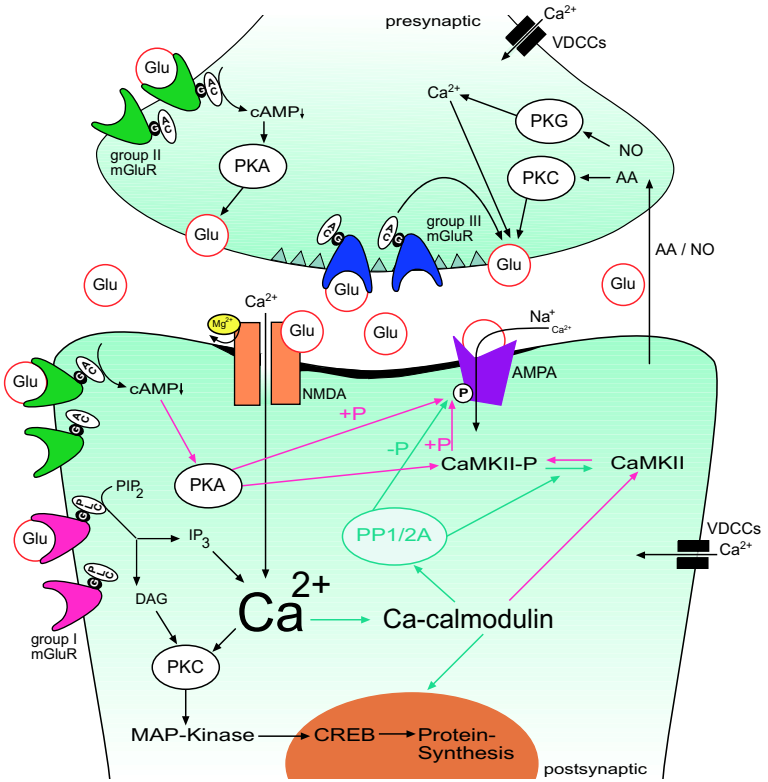

Figure 1.3: Mechanisms underlying LTP expression

Schematic illustration representing the main mechanisms activated by a high calcium peak due to HFT. Abbreviations: AC adenylyl cyclase; AA: arachidonic acid; CaMKII: calcium-calmodulin dependent protein kinase II; CREB: cAMP response element binding protein; DAG: diacylglycerol; Glu: glutamate; IP_3: inositol triphosphate; mGlu: metabotropic glutamate receptor; MAP-kinase: mitogen activated protein kinase; NO: Nitric oxide; P: phosphate; PIP_2: phosphatidyl inositol; PLC: phospholipase C; PKA: cAMP-dependent protein kinase; PKC: protein kinase C; PKG: protein kinase G; PP1/2A: protein phosphatase 1/2A; VDCCs: voltage gated calcium channels. Red arrows indicate activated pathways and green arrows indicate down-regulated pathways. For further information see paragraph 1.2.2. Figure modified from [Kemp & Bashir, 2001].

Malinow & Malenka, 2002, Luscher & Frerking, 2001]. In addition, this constitutively active form serves to translocate the kinase to the postsynaptic density (PSD) [Strack et al., 1997] leading to a further activation of NMDA receptors. It is also described that the autophosphorylated form of CaMKII decreases the dissociation of Ca^{2+}-calmodulin.

A relatively lower Ca^{2+} level, triggers LTD and promotes the formation of Ca^{2+}-calmodulin. Ca^{2+}-calmodulin activates Ca^{2+}-calmodulin dependent phosphatase 2b (PP2B or calcineurin) [Mulkey et al., 1994] which inactivates/dephosphorylates inhibitor I, thereby removing inhibition from protein phosphatase 1 (PP1) [Lisman, 1989, Mulkey et al., 1994]. The increase in PP1 activity cause a net dephosphorylation of CaMKII [Strack et al., 1997] and therefore triggers dephosphorylation or LTD (review: [Lisman & Zhabotinsky, 2001, Lisman et al., 2002]). Since autophosphorylation is controlled by protein phosphatases [Blitzer et al., 1998] and thus requires convergence between several activated signalling routes, CaMKII has been proposed to act as a molecular coincidence detector in synaptic plasticity. Both LTP and LTD are dependent on CaMKII activation [Mayford et al., 1995].

PKC Protein kinase C is a family of serine-threonine kinases, which can be activated by diacylglycerol in a calcium dependent or independent manner and by phorbol esters. PKC has been suggested to play a role similar to that of CaMKII in synaptic plasticity.

PKC activation has been shown to result in the enhancement of Ca^{2+} currents and elevation of cytosolic-free Ca^{2+}, resulting in increased neurotransmitter release [Malenka et al., 1986] and regulation of NMDA receptor activity. The RAS/MAP kinase pathway has been shown to be a target pathway of PKC [Lu et al., 1999, Skeberdis et al., 2001].

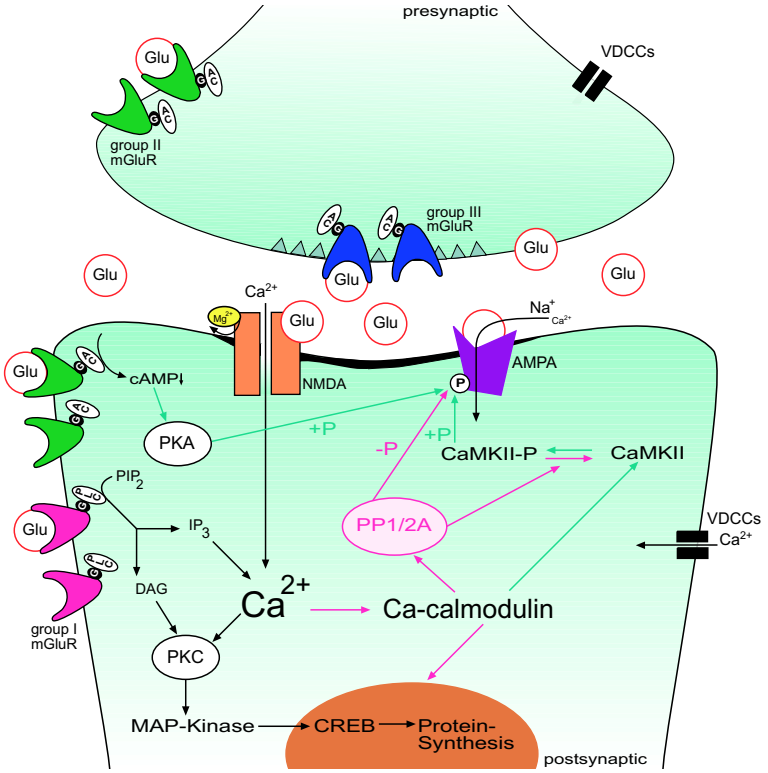

Figure 1.4: Mechanisms underlying LTD expression

Schematic illustration representing the main mechanisms activated by a moderate calcium concentration due to LFS. Abbreviations: AC: adenylyl cyclase; CaMKII: calcium-calmodulin dependent protein kinase II; CREB: cAMP response element binding protein; DAG: diacylglycerol; Glu: glutamate; IP_3: inositol triphosphate; mGlu: metabotropic glutamate receptor; MAP-kinase: mitogen activated protein kinase; P: phosphate; PIP_2: phosphatidyl inositol; PLC: phospholipase C; PKA: cAMP-dependent protein kinase; PKC: protein kinase C; PP1/2A: protein phosphatase 1/2A; VDCCs: voltage gated calcium channels. Red arrows indicate activated pathways and green arrows indicate down-regulated pathways. For further information see paragraph 1.2.2. Figure modified from [Kemp & Bashir, 2001].

This sides of action strongly correlates PKC to processes underlying synaptic plasticity. In this regard it has been shown that PKC activators potentiate synaptic transmission, whereas inhibitors prevent LTP [Carroll et al., 1998]. In addition, PKC can be converted to an autonomously active form which seems to be important for LTP maintenance [Roberson et al., 1999].

Phosphatases The three best-characterized serine/threonine phosphatases in the brain are protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A) and calcineurin (PP2B) [Soderling & Derkach, 2000, Winder & Sweatt, 2001]. These Ca^{2+} sensitive phosphatases downregulate synaptic transmission [Yakel, 1997]. Moreover, phosphatase inhibition can lead to synaptic potentiation and lowers the LTP induction threshold. There is abundant pharmacological, genetic and biochemical evidence for the involvement of PP1/PP2A and/or PP2B activation in the induction of LTD [Winder & Sweatt, 2001]. PP2A has been demonstrated to produce the putative retrograde messenger arachidonic acid [Sweatt, 2001] and thereby influencing presynaptic glutamate release.

Others

PKA A cyclic adenosine monophosphat (cAMP)-dependent protein kinase (PKA)-dependent suppression of PP1 activity participates in the induction of LTP by autophosphorylation of CaMKII and subsequent AMPA receptor modification [Blitzer et al., 1998]. It is hypothesized that increase in presynaptic Ca^{2+} level stimulate adenylyl cyclase, elevate cAMP level and activate PKA.

PLC & PLD Phospholipase C and D (PLC, PLD) mediate the hydrolysation of membrane lipids upon the activation by G-proteins [Pontzer et al., 1990, Exton, 2000, Exton, 2002]. The products of the hydrolysation by PLC function as important intracellular messengers: diacyl-glycerol (DAG) activates PKC, and inositol-triphosphate (IP_3) binds to receptors of intracellular calcium stores, mediating their opening.
Phospholipase D (PLD) is the enzyme in a signal transduction pathway leading to the formation of phosphatidic acid and diacylglycerol. Metabotropic glutamate receptor agonists can stimulate PLD through a PKC-independent mechanism [Pellegrini-Giampietro et al., 1996].

Tyrosine kinase The NMDA receptor mediated rise in intracellular Ca^{2+} levels is enhanced by tyrosine phosphorylation [Wang & Salter, 1994] and the induction of LTP is prevented by tyrosine kinase inhibition [O'Dell et al., 1991]. Pharmacological evidence indicates that the activation of tyrosine kinase is also required for LTD [Boxall & Lancaster, 1998].

1.2.3 Expression of LTD and LTP

As mentioned earlier, the long-lasting forms of synaptic plasticity need to establish synaptic modifications to guarantee their persistence, in addition to the simple activation of kinases and phosphatases. Therefore the final targets of the intracellular cascade are a major subject in the research field of synaptic plasticity. Once determined it will be possible to identify the regulatory machinery in the acquisition or storage of memories.

The site of synaptic modification

The site of synaptic modification is still controversial. The only possibility to strengthen a connectivity is to modify the synapse. These modifications include dynamic changes in the structural characteristics as well as increased transmitter release on the pre-synapse or an increased transmitter affinity on the post-synapse.
Postsynaptic modifications that increase the strength of a synapse may contribute to a decrease in the signal-to-noise ratio of information [Otmakhov et al., 1993]. In contrast, presynaptic modifications may be more related to short-term plasticity, and therefore an important mechanism in gain-control [Chance et al., 2002]. In a not necessarily contradictory view concerning gain-control, LTD serves to reduce the signal to noise ratio and thereby reinforcing the effect of LTP [Braunewell & Manahan-Vaughan, 2001]. Therefore gain-control serves in balancing the resolution of the neuron to adapt its sensitivity. Fast neuronal events are processed with a low resolution whereas slow events are processed with a higher one [Abbott et al., 1997]. With such a gain-control mechanism one can exclude that slow firing inputs might be masked by fast and irregular firing ones. In this respect, synaptic alterations of a short duration are more useful in altering the balance of neuronal interactions in the hippocampal circuit, whereas more lasting alterations might act as temporary or persistence templates [McEachern & Shaw, 1999].

Mechanisms underlying late-phase plasticity

Whereas the early phase of LTP and LTD is dependent on persistent activation of kinases and phosphatases, the late phase requires gene expression and new proteins. Protein synthesis is required for the inclusion of AMPA receptors and the production of new spines [Malinow & Malenka, 2002]. Several activity-regulated genes have

also been identified, and their protein products may contribute to mechanisms maintaining plasticity [Abraham et al., 1991].
In this respect one has to mention the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signalling in the regulation of gene transcription in neurons. MAPK activation by cyclic adenosine monophosphate (cAMP) or PKC in LTP has been demonstrated [Roberson et al., 1999]. In alignment, MAP kinase inhibition effects LTP. It was shown that the induction of LTP leads to a MAP kinase dependent gene expression [Rosenblum et al., 2002]. The mechanisms of substrate phosphorylation by MAPK are complex and a multitude of signal transductions involving transcription factors or the modulation of K^+ channels have been reported [Sweatt, 2001]. It has been shown that MAPK/ERK, cAMP response element-binding protein (CREB) and the immediate early gene (IEG) zif268 are essential components of a signalling cascade required for the expression of late phase plasticity [Davis et al., 2003, Sweatt, 2001, Nguyen et al., 1994, Rosenblum et al., 2002, French et al., 2001, Jones et al., 2001]. Other transcription factors implicated in the late phase signalling include members of the fos and jun family.

1.3 Plasticity and memory

Numerous experiments demonstrate that various manipulations with impact on electrically induced LTP and LTD also effect the execution of spatial memory tasks. There exists evidence that CA1 LTP is associated with forms of declarative memory [Martin et al., 2000]. AP5, a NMDA receptor antagonist, prevents spatial memory and fear conditioning in a concentration, which also blocks LTP. The same is true for general blockade of mGlus, leading to the blockade of LTP

expression and impairment of spatial learning [Riedel et al., 1994]. More specifically, mGlus seem to be critical for the consolidation of memory [Riedel et al., 2003]. As yet, there exists no direct evidence for LTP as amemory mechanism, but correlative data strongly indicate, that a mechanism similar to LTP must underlie learning and memory processes in the brain. Similarly, strong correlative data also support a role for LTD as a memory mechanism. LTP and LTD fulfil three crucial criteria, which justifies their classification as memory mechanisms: input-specificity, associativity (cooperative) and persistence.

Input and synapse specificity means that only the tetanised, and not non-tetanised inputs, express potentiation. This feature might increase accuracy of information storage [Malenka, 1994].

Associativity means that a weak input can be potentiated if it is activated in the same time as a strong but convergent input. From the neuronal view point associativity is a model for classical conditioning, one well-known kind of learning [Debanne & Thompson, 1996].

LTP in the hippocampus is typically decremental, even though in some cases it may not be [Abraham, 2003]. This decremental property of LTP seems to contradict the proposed persistence. The artificial and uninformative strengthening of the synapse underlying LTP induction might explain this controversy. On the other hand, memory could be decremental in general, while it still remains persistent by periodical re-strengthening.

One characteristic of long-term memory as well as of LTP and LTD is its sensitivity to protein synthesis inhibitors during consolidation/induction. Metabotropic glutamate receptors are believed to contribute to the late phase of memory consolidation [Riedel & Micheau, 2001]. Novelty acquisition enhances LTD, indicating an involvement of these mechanisms in this kind of memory [Manahan-Vaughan & Braunewell, 1999][Kemp & Manahan-

Vaughan, personal communication]. Thus, LTD is, like LTP, a form a synaptic plasticity that can be correlated with learning and memory processes in the mammalian brain.
In summary, neurophysiological investigations indicate that the hippocampus plays a key role in information processing and/or memory storage. Consistent with this, pathophsiological changes in the hippocampus lead to several disorders such as Alzheimer's disease and temporal lobe epilepsy. Intensive investigations of the cellular mechanisms underlying the normal and abnormal forms of hippocampal neuronal activity have taken place.

1.4 Aim of this study

Motivated by advances in mGlu agonist and antagonist specificity, this study set about to address the role of mGlus in synaptic plasticity and pathology. Particular focus on group I mGlu function was undertaken. The following question was addressed:

> Do group I mGlu subtypes mGlu1 and mGlu5 contribute differentially on the mechanisms of long-term potentiation and long-term depression in the dentate gyrus of freely moving wistar rats?

The contribution of the hippocampus not only to favourable properties like learning and memory but also to Alzheimer's disease and epilepsy, as well as the fact that most characteristics of synaptic plasticity have a role in neuroplastic and neuropathological events, raises the question as to whether there exists a sliding threshold between plasticity and pathology [McEachern & Shaw, 1999]. Preceding results have demonstrated a connection between one form of chemically induced long-term potentiation, the slow-onset potentiation, and neuronal cell death [Manahan-Vaughan et al., 1999a]. This

slow-onset potentiation is induced by a mass activation of group I mGlus. In vitro studies have successfully demonstrated an induction of long-term depression by activation of the same kind of receptor [Camodeca et al., 1999, Huber et al., 2000]. The benefit of chronically implanted electrodes and a intracerebroventricular (icv) cannula confered the opportunity to address the following question:

> Does chemical activation of group I mGlus cause long-term depression in the dentate gyrus in vivo, and is such kind of plasticity related to neural cell death?

In this thesis, pharmacological activation and deactivation of several mGlus was paired with electrophysiological stimulation paradigms as well as with histological quantification of neural cell death to answer both questions.
The chapter *Material and Methods* will deliver an insight into the materials and methods used. The pharmacological deactivation of mGlu1 and mGlu5 followed by synaptic plasticity inducing stimulation answered the question of the subtype specific involvement of these receptors in LTP and LTD in the dentate gyrus of freely moving rats.
Using group I mGlu agonists it was possible to induce LTD without stimulation. The differences between LTD induced with group I mGlus and with stimulation was investigated concerning their subtype specific involvement of mGlu1 and mGlu5.
Activation of group III mGlus results in a LTD with a quite similar phenotype. This type of LTD was compared with LTD induced by activation of group I mGlus concerning their sides of expression and their protein synthesis dependency. The second question of this thesis work (role of mGlu in synaptic pathology) will be answered by histological investigations of rat brains in which LTD was induced by pharmacological activation of group III mGlus.