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Chemical and stimulus-induced NMDA-dependent synaptic plasticity in hippocampus and the possible involved mechanisms

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To my family

Abstract

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Long-term potentiation (LTP) and long-term depression (LTD) are considered as the most important forms of synaptic plasticity involved in learning and memory. The traditional way to induce LTP and LTD is by electric stimulation leading to activation of NMDA-R. Later research also revealed a form of NMDA-dependent plasticity induced by direct application of NMDA. I have investigated the involvement of AMPA-R and NMDA-R in NMDA-induced LTD as well as their contribution to early and late phases of stimulus-induced LTP; examined the roles of different NMDA-R subunits in several forms of synaptic plasticity; and tried to elucidate the possible mechanisms underlying NMDA-induced plasticity.

The experiments were performed in hippocampal slices, 400 μm thick, from 12 to 20 days old Sprague-Dawley rats. Extracellular recording was used to study field excitatory postsynaptic potential (EPSPs) in the CA1 apical dendritic layer. Isolated AMPA EPSPs were obtained in standard solution or in low Mg^{2+} containing NMDA-R blocker AP5. Composite EPSPs were recorded in low Mg^{2+} whereas isolated NMDA EPSPs were expressed in low Mg^{2+} solution with AMPA-R blocker CNQX. LTP was elicited by HFS trains (100 impulses, 100 Hz) or in some cases theta-burst stimulation (10 times 4 impulses, 100 Hz, 200 ms interval). NMDA-induced LTD was achieved by brief (4 min) bath application of NMDA.

The results showed that 20-50 μM NMDA application persistently depressed both AMPA and NMDA responses to a nearly equal extent. In addition, a waveform prolongation of AMPA but not of NMDA EPSPs occurred 15-25 min after NMDA application. On the contrary, stimulus-induced LTP potentiated AMPA and NMDA responses to a different degree, with about two-fold larger increase of AMPA than NMDA component at both 1h and 4 h after induction. Different tests revealed noninvolvement of voltage dependent channels as well as GABAa-ergic inhibition in the NMDA-induced prolongation of AMPA EPSPs. However, EPSP prolongation was occluded by a similar change induced by AMPA-R modulator cyclothiazide (CTZ); and facilitated by AMPA-R modulator aniracetam.

The comparison of NMDA-induced effects in pathways with and without prior LTP demonstrated a larger depression and smaller waveform change in the LTP pathway. LTP and NMDA-induced LTD, as well as a form of stimulus-induced LTD, were all largely prevented by NVP-AAM077, a blocker of NMDA receptors that contain subunits of type NR2A. However, the blockade in these cases could be counteracted by general amplification of NMDA responses by lowering the perfused Mg^{2+} concentration. Blockers of NR2B-containing NMDA receptors by Ro25-6981 or Ifenprodil had no effect on the two forms of LTD whereas LTP was partially blocked. It was found that subunits NR2A and NR2B contributed to about 80% and 20%, respectively, of isolated NMDA EPSPs.

Our results demonstrate that NMDA applied on brain tissue induces multiple synaptic plasticity. Modifications underlying NMDA-induced LTD differ from those in LTP in several respects; however, these two forms of plasticity also interact, suggesting a possible relation. This is also supported by the fact that both LTP and NMDA-induced LTD require activation of NR2A containing NMDA receptors, and that both LTP and NMDA-induced waveform changes interact with AMPA receptor modulators. Notably, NR2A subunits play an essential role in all types of synaptic plasticity examined in this study whereas NR2B also contribute under certain circumstances. We therefore believe that the final magnitude and temporal pattern of Ca^{2+} influx in the spine is a key factor determining the induced synaptic plasticity.

Keywords: synaptic plasticity, hippocampus, LTP, LTD, AMPA, NMDA, NR2A, NR2B

List of articles

This thesis is based on the following papers:

I. Characterization of NMDA-induced depression in rat hippocampus: involvement of AMPA and NMDA receptors. *Neurosci Lett. 2004 Mar 4;357(2):87-90.* (Li R, Dozmorov M, Hellberg F, Tian Y, Jilderos B and Wigström H)

II. Contribution of AMPA and NMDA receptors to early and late phases of LTP in hippocampal slices. *Neurosci Res. 2006 Jun;55(2):182-8.* (Dozmorov M, Li R, Abbas A-K, Hellberg F, Farre C, Huang F-S, Jilderos B and Wigström H)

III. Possible involved mechanisms of NMDA-induced synaptic plasticity. *Manuscript.* (Li R, Abbas A-K, Huang F-S and Wigström H)

IV. Role of NMDA receptor subtypes in different forms of NMDA-dependent synaptic plasticity. *Manuscript.* (Li R, Huang F-S, Abbas A-K and Wigström H)

Other collaborations:

1. Slowly developing depression of N-methyl-D-aspartate receptor mediated responses in young rat hippocampi. *BMC Neurosci. 2004 Aug 3;5(1):26.* (Dozmorov M, Li R, Xu H-P, Jilderos B, Wigström H)
2. Active decay of composite excitatory postsynaptic potentials in hippocampal slices from young rats. *Brain Res. 2003 May 23;973(1):44-55.* (Dozmorov M, Niu Y-P, Xu H-P, Xiao M-Y, Li R, Sandberg M and Wigström H)

Contents

Abbreviations

AChR: acetylcholine receptor
ACPD: (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid
AMP: adenosine monophosphate
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR: AMPA receptor
AP5: D(-)-2-amino-5-phosphonopentanoic acid
CA: cornu ammonis
CaM: calmodulin
CaMKII: calcium/calmodulin-dependent protein kinase II
cAMP: cyclic 3,5-adenosine monophosphate
CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione
CREB: calcium/cAMP response element binding protein
CTZ: cyclothiazide
DG: dentate gyrus
DHPG: (S)-3,5-dihydroxyphenylglycine
E-LTP: early long-term potentiation
EPSC: excitatory postsynaptic current
EPSP: excitatory postsynaptic potential
GABA: γ-aminobutyric acid
GluR: glutamate receptor
HFS: high-frequency stimulation
Ife: Ifenprodil
LFS: low-frequency stimulation
LTD: long-term depression
LTP: long-term potentiation
MAPK: mitogen-activated protein kinase
MF: mossy fiber
mGluR: metabotropic glutamate receptor
MK-801: (+)-MK-801 hydrogen maleate
mRNA: messenger RNA
NMDA: N-methyl-D-aspartate
NMDAR: NMDA receptor
NO: nitric oxide
NVP: NVP-AAM077
PKA: protein kinase A
PKC: protein kinase C
PP: perforant path
PPF: paired-pulse facilitation

PSD: postsynaptic density
PTP: post-tetanic potentiation
PTX: picrotoxin
RNA: ribonucleic acid
Ro: Ro25-6981
Ser: serine
STD: short-term depression
TBS: theta-burst stimulation
TEA: Tetraethylammonium
Thr: threonine
VDCC: voltage-dependent calcium channel

1. Introduction

One hallmark function of human brain is its capability for cognitive processes, including learning and memory, thinking and reasoning, as well as perception and attention. These processes are brought about by complex computational networks within the brain. The basic computing unit is the neuron, which is responsible for receiving, processing and transmitting signals within the nervous system, both inside and outside the brain. The communication between neurons occurs at specialized structures, synapses, believed to play essential roles in neuronal computation as well as information storage. The efficacy of synaptic transmission is activity-dependent and highly modifiable and lasting modifications of synaptic efficacy are regarded as the biological substrate for learning and memory. Many possible mechanisms have been suggested to regulate the efficacy. For instance, changes in transmitter release and the amount of transmitter in a vesicle may influence it presynaptically, while the changes in receptor number, sensitivity and composition may work postsynaptically.

The two classic forms of synaptic plasticity are stimulus-induced long-term potentiation (LTP) and long-term depression (LTD), implying a long-lasting increase and decrease, respectively, of the synaptic efficacy. Substantial efforts have been made to investigate the mechanisms underlying LTP and LTD and the debates have been accompanying the studies for over 30 years; it is now even 40 years since the first account of LTP. Whereas LTP and LTD are traditionally induced by certain patterns of electric stimulation, later work has also demonstrated that synaptic plasticity can also be induced "chemically" by applying certain compounds directly on brain tissue. Such chemically induced plasticity involves a large proportion of the available synapses and hence provides an advantageous experimental model for detecting the chemical and/or structural changes responsible for memory at the synaptic level. In the present project we focused on a type of synaptic plasticity induced by direct application of NMDA, the specific agonist of NMDA receptors, a key type of glutamate receptor. We also paid attention to some types of stimulus-induced synaptic plasticity and tested the interaction between chemical and stimulus-induced changes.

1.1 Hippocampal circuits as a memory model

The ability for memory is most likely dispersed throughout the brain and the kinds of synaptic plasticity in various brain regions can be imagined as subserving different types of memory. Special attention has been paid to neocortex and hippocampus, brain structures which are important in cognitive functions and display both LTP and LTD in experimental studies. Hippocampus is thought to be the place where episodic

memories (memories of experienced events) are laid down, at least to begin with. This idea was prompted by the discovery that bilateral hippocampal lesions in a human patient severely impaired memory for events occurring after the time of the lesion (Scoville and Milner 1957). The hippocampus has widespread connections with other brain regions via the adjacent entorhinal cortex and a nerve bundle called fimbria. A famous trisynaptic excitatory pathway within the hippocampus has been commonly used for experimental studies of synaptic plasticity (Fig. 1). Other excitatory pathways are less well studied and little is known about plasticity in inhibitory circuits.

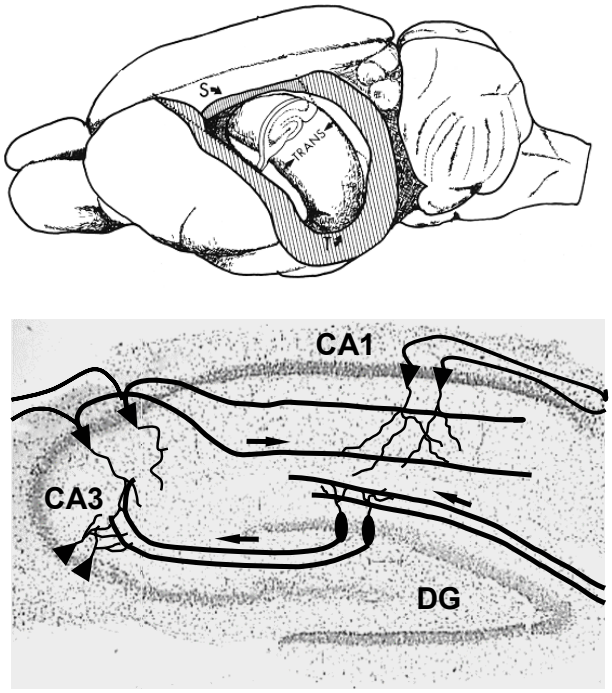

Fig. 1 The hippocampus and trisynaptic circuit. Upper panel shows the anatomical location of hippocampus in rat brain. Lower panel is the simplified diagram describing the neuronal connections within hippocampus.

The trisynaptic circuit (Fig. 1) starts with the perforant pathway, consisting of axons from enthorinal cortex that project to granule cells of dentate gyrus. These cells give rise to axons that form synapses on the pyramidal cells in the CA3 region, known as mossy fiber pathway. Two branches of axons extend from CA3 region. One branch leaves hippocampus via the fimbria and the other, called the Schaffer collateral pathway, forms synapses on the cells of the CA1. Actually the axons in this pathway not only come from CA3 neurons in the same hippocampus, but also consist of the projection from CA3 neurons in the opposite hemisphere. Therefore it is also named as Schaffer collateral-commissural pathway. Finally, the CA1 neurons send axons out to the entorhinal cortex and subiculum, forming the main output from the hippocampus.

All of the components of the trisynaptic circuit display both LTP and LTD. In this present study all the experiments were performed on synapses of the Schaffer collateral pathway.

An often used preparation for studying plasticity in the hippocampus is the rat hippocampal slice, a half-millimeter-thick transverse section of hippocampus, which is maintained in a nutritional solution. It provides for mechanical stability and allows accurate positioning of stimulating and recording electrodes under direct visual guidance. The trisynaptic circuit is well preserved within the slice.

1.2 The synapse – a key element of plasticity

A chemical synapse consists of pre- and postsynaptic terminals, separated by a 20-50 nm space, called synaptic cleft (Fig. 2). Synaptic transmission begins when the nervous impulse reaches the presynaptic axon, leading to depolarization of the presynaptic membrane. The voltage gated Ca^{2+} channels open and result in Ca^{2+} ions flowing into the axon terminal, which initiates a sequence of events leading to neurotransmitter release into the synaptic cleft. Synaptic transmitter binds to the receptors at the postsynaptic membrane. The activation of receptors mediates the specific ion entry into the postsynapse and generates the response, an excitatory postsynaptic potential (EPSP) or inhibitory postsynaptic potential (IPSP). Much basic knowledge about synaptic function was obtained from studies of the neuromuscular junction, as well as other easily accessible synapses such as the calyx of Held and the squid giant synapse. Most central synapses, e.g. within the hippocampus, operate in a similar manner except that the amount of released transmitter at each synapse is usually quite small (Allen and Stevens 1994).

In the following, I consider some essential types of receptors involved in hippocampal synaptic transmission, on both the presynaptic and postsynaptic sides. Postsynaptic receptors will be reviewed in greater detail due to the fact that synaptic plasticity, such as LTP and LTD, is thought to be induced postsynaptically and many researchers believe that the persistent modification is also largely a postsynaptic matter.

1.3 Glutamate receptors

Glutamate mediates the majority of excitatory neurotransmission in the mammalian central nervous system (CNS). It is considered a prerequisite for normal brain function and plays a crucial role in synaptic plasticity. It is also important for clinical neurology

since the elevation of its concentration caused by brain injury is toxic to neurons. In hippocampus, most of cells use glutamate as synaptic transmitter. There are two major families of glutamate receptors at the postsynaptic membrane: ionotropic receptors and metabotropic receptors (mGluRs). Ionotropic receptors combine both transmitter-binding and channel function into a single molecular entity. Their activation mediates the rapid flux of ions across the neuronal membrane. Therefore, they are responsible for the fast synaptic transmission. Metabotropic receptors do not have an ion channel as a part of their structure; instead, they affect channels by one or more metabolic steps activated by the mediating molecules called G-proteins. Both types of glutamate receptor have been reported to take part in synaptic plasticity processes, either NMDA-dependent plasticity or NMDA-independent plasticity. In this study, we focus on the NMDA-dependent type.

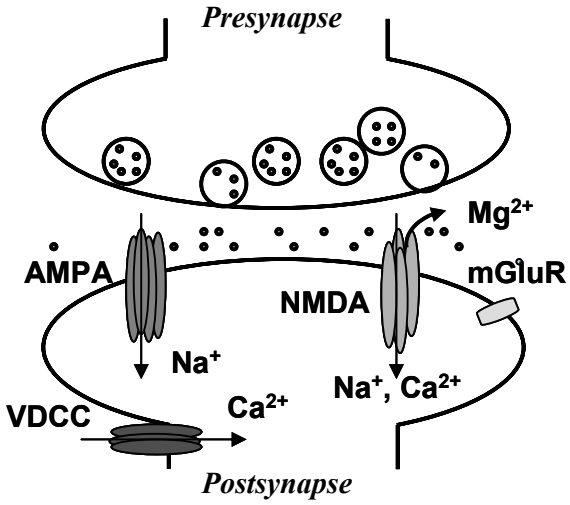

Fig. 2 The synapse – a schematic view: illustrating the structure of a glutamatergic synapse in hippocampus and the main receptors on the postsynaptic terminal.

1.3.1 Functional properties of AMPA and NMDA receptors

Three types of glutamate ionotropic receptors have been found in the postsynaptic membrane, named by their agonists, AMPA, kainate and NMDA receptors (Seeburg 1993; Hollmann and Heinemann 1994). Most of the studies on synaptic transmission focus on AMPA and NMDA receptors. These two receptors, with distinct physiological properties, often coexist in the same synapse in hippocampus. The AMPA receptor is the dominant form in that it generates the majority of electrical postsynaptic response. The NMDA receptor normally contributes less to the response, but rather acts as a triggering mechanism for synaptic plasticity. Both receptors are

permeable to Na^+ and K^+ but NMDA receptors are also permeable to Ca^{2+} ions (Seeburg 1993). In addition, the receptors are equipped with different conditions for bringing about their opening, as explained below.

Weak electrical stimulation of presynaptic axons causes release of glutamate from the axon terminal and this glutamate binds to both AMPA and NMDA receptors. However, under resting membrane potential conditions, say -75 mV, mainly AMPA receptors open. Ion movement across NMDA receptor channels remains minimal even when the cell becomes partially depolarized to a about -35 mV. The low conductance is because the channel is clogged by Mg^{2+}, the so-called Mg^{2+} block. While the Mg^{2+} ion in the NMDA channel prevents ions from passing, the AMPA channel permits ion flow and depolarizes the postsynaptic membrane. Given a stimulus with sufficient strength or frequency, AMPA receptors can depolarize the postsynaptic membrane beyond -35 mV, the Mg^{2+} is expelled from NMDA receptor channels and the latter now open in response to glutamate, not only mediating Na^+ but passing a large amount of Ca^{2+} as well. The increase of intracellular Ca^{2+} concentration via NMDA receptors has been thought to be the key that triggers NMDA-dependent synaptic plasticity (Lynch, Larson et al. 1983; Malenka, Kauer et al. 1988).

The involvement of these postsynaptic glutamate receptors in mediating the change of synaptic efficacy has been well accepted. Moreover, there is growing evidence for the existence of presynaptic glutamate receptors. So far, AMPA, kainate, NMDA and mGluR have all been identified in hippocampus as presynaptic autoreceptors. Those receptors can modulate the transmitter release in different ways and may also contribute to lasting forms of synaptic plasticity (Breukel, Besselsen et al. 1998; Manahan-Vaughan, Herrero et al. 1999; Suarez and Solis 2006).

1.3.2 Subgroup composition of AMPA and NMDA receptors

AMPA receptors are assembled by 4 types of subunits, GluR1-4 (Boulter, Hollmann et al. 1990). Various hetero-oligomeric combinations of these subunits contribute to the distinct properties of different subtype receptors. GluR1 subunits have been suggested to undergo a redistribution during activity-dependent synaptic plasticity (Carroll, Beattie et al. 1999; Shi 2001). The presence of GluR2 renders the AMPA receptor impermeable to Ca^{2+} and loss of this subunit leads to a Ca^{2+}-permeable receptor. Endogenous AMPA receptors in hippocampus are composed of mainly GluR1 with GluR2, and GluR3 with GluR2. It has been reported that GluR2-GluR3 receptors constitutively recycle in the synapse, whereas the delivery of GluR1-GluR2 receptors is highly regulated to modify the synaptic strength (Passafaro, Piech et al. 2001).

AMPA subunits comprise a critical splicing exon, named flip/flop domain, which regulates the recovery from and the rate of AMPA receptor desensitization (Sommer, Keinanen et al. 1990; Johansen, Chaudhary et al. 1995). By alternative splicing of this exon, two types of variant of each AMPA subunit can be generated, flip and flop variant. Drugs that modulate AMPA receptor kinetics seem to affect flip and flop type receptors differently. For example, both aniracetam and cyclothiazide (CTZ), modulators used in the present work, are supposed to influence the desensitization and deactivation of AMPA receptor, but aniracetam is more potent at flop type and CTZ is more efficacious at flip type of AMPA receptor (Partin, Fleck et al. 1996).

NMDA receptors consist of NR1, NR2(A-D) and NR3A subunits. Functional NMDA receptors are combinations of NR1 with at least two NR2 subunit. NR1 subunits which are present ubiquitously in the brain, make up the channel and provide a glycine binding site. NR2 subunits, containing the glutamate binding site, are distributed in restricted areas, mainly in cerebral cortex, hippocampus, and cerebellar granule cells. The subunits in this family have been shown to determine the physiological function of NMDA receptors (Liu and Zhang 2000). Specifically, NR2A- and NR2B-containing NMDA receptors are the predominant forms in hippocampus. It has been suggested that NR2A receptors are located synaptically, whereas NR2B receptors are usually found extrasynapticlly (Stocca and Vicini 1998; Rumbaugh and Vicini 1999; Tovar, Sprouffske et al. 2000; Massey, Johnson et al. 2004). NR2A and NR2B receptors have been thought to be specifically involved in certain forms of synaptic plasticity. Accordingly, it has been shown that LTP requires NR2A-containing NMDA receptors, while LTD is dependent on NR2B-containing NMDA receptors (Liu, Wong et al. 2004; Massey, Johnson et al. 2004). The composition of the NMDA receptor changes during developement, NR2B being gradually replaced by NR2A.

1.4 Other receptors

1.4.1 GABA and glycine receptors

Compared with glutamate, GABA plays an opposite role. It mediates most of the inhibitory neurotransmission in the brain. Ionotropic GABA receptors are chloride-selective ion channels and two types have been identified, GABAa and GABAc. These receptors mediate fast synaptic inhibition whereas the GABAb type receptor is of a metabotropic type and mediates a kind of slow inhibition (Johnston 1996). It is worth noting that LTP and LTD have also been found at GABAergic synapses in certain brain areas. In addition, GABA receptors, via their effect on membrane potential, play

essential roles in controlling the induction conditions leading to LTP/LTD at glutamatergic synapses (Wigström and Gustafsson 1983; Wigström and Gustafsson 1985; Jerusalinsky, Kornisiuk et al. 1997; Bernard, Cossart et al. 2000).

Glycine is another type of inhibitory neurotransmitter that exists in the CNS. Like GABA, it also acts through chloride channels and exerts its inhibition by causing hyperpolarization (Torsney and MacDermott 2005). As mentioned above, glycine also acts on the NMDA receptor, being necessary as a cofactor for glutamatergic activation. In that sense, it has excitatory mode of action but little is known about functional implications. Both GABA and glycine, acting at their "own" receptors, may also have excitatory action at early developmental stages.

1.4.2. Receptors for neuromodulators

In addion, signalling between neurons involves a variety of other neuroactive substances and their associated receptors. Many of these belong to the class of neuromodulators, involving important receptor subclasses, such as monoamininergic and cholinergic receptors. In most cases, the signalling involves activation via G-proteins (metabotropic receptors) but some of the involved messengers can also act directly on ion channels. For instance, cholinergic receptors, which are activated by acetylcholine (ACh), can be divided into two subgroups: muscarinic ACh receptor (mAChR, metabotropic) and nicotinic ACh receptor (nAChR, ionotropic). Acetylcholine as well as the monoamine noradrenaline (norepinephrine) have been implied in memory processes and may also be important in relation to synaptic plasticity. It is thought that ACh can influence LTP by a possible effect on the spike after-hyperpolarization (Hasselmo and Barkai 1995) and noradrenaline (norepinephrine) has been reported to induce a form LTP on its own (Stanton, Mody et al. 1989). Moreover, adenosine agonists attenuate transmitter release via an effect on presynaptic receptors. Although this process resembles LTD it appears to be distinct (Xiao, Karpefors et al. 1995a).

1.5 Functional versus silent synapses

Not all of the structurally present synapses execute a physiological function. A synapse that is lacking any synaptic component or deficient in synaptic transmission has been termed "silent synapse". Both pre- and postsynaptic mechanisms can produce silent synapses. For instance, the absence of presynaptic molecules that are responsible for docking and fusion of synaptic vesicles causes a presynaptically silent synapse,

whereas lack of functional postsynaptic receptors renders a postsynaptically silent synapse (Atwood and Wojtowicz 1999). A special type of silent synapse has been shown in the mammalian central CNS which is lacking of functional AMPA receptors, but expressing NMDA receptors on the postsynaptic membrane (Kullmann 1994; Isaac, Nicoll et al. 1995; Liao, Hessler et al. 1995). This kind of synapse exhibits no postsynaptic response at resting membrane potential, but only NMDA mediated response during depolarization. The conversion between silent synapses and functional ones has been considered as one of the mechanisms underlying the expression of synaptic plasticity.

1.6 Synaptic plasticity

Many types of synaptic plasticity can be triggered according to different induction protocols, depending on brain area and type of synapse involved.

1.6.1 Stimulus-induced plasticity

LTP and LTD are the two most important forms of synaptic plasticity that are believed to underly learning and memory. The traditional way to induce LTP and LTD is by electrical stimultion.

1.6.1.1 LTP

LTP was first discovered in 1973 (Bliss and Gardner-Medwin 1973; Bliss and Lomo 1973). A few seconds of high-frequency electrical stimulation (referred to as a tetanus in the following) of the perforant path in rabbit hippocampus was found to enhance synaptic transmission between the stimulated axons and the postsynaptic granule cells, as revealed by an increase in the size of the recorded field EPSP. This change persisted for hours, and under certain conditions, even for days and weeks. Therefore, this phenomenon has been called long-term potentiation. Like memories, LTP can be generated rapidly and is strengthened with repetition.

There are also other stimulation protocols that have been used to evoke LTP. For instance, pairing a low frequency presynaptic stimulation with postsynaptic depolarization has been reported as an effective way to induce LTP which can last for several hours (Gustafsson and Wigström 1986; Wigström, Gustafsson et al. 1986; Gustafsson, Wigström et al. 1987; Liao, Hessler et al. 1995; Chen, Otmakhov et al. 1999). The time interval for pairing the pre- and postsynaptic stimuli appears to be

critical. Intervals longer than about 50 ms did not produce LTP (Gustafsson and Wigström 1986) and multiple pairing events had to be given within a 10 s interval or else only short-term potentiation (STP) was produced (Colino, Huang et al. 1992).

Theta burst stimulation has been considered as a more natural way to induce LTP due to the occurrence of theta electroencephalogram rhythm (about 5 Hz) in the normal brain. For instance, 10 trains consisting of 4 pulses at 100 Hz, 200 msec interval, at test pulse-intensity effectively induces LTP in CA1 area of hippocampus, a result already shown in the 1980's (Larson, Wong et al. 1986; Staubli and Lynch 1987). However, the same stimulation at higher intensity failed to induce either LTP or LTD; instead it induced depotentiation in a pathway previously subjected to LTP (Barr, Lambert et al. 1995).

1.6.1.2 LTD

Depression of an unspecific type, heterosynaptic depression, was found to accompany LTP in an untreated pathway (Dunwiddie and Lynch 1978). A pathway-specific, persistent decrease in the synaptic efficiency was first found on the basis of prior LTP in 1980. A period of low frequency stimulation in the hertz range (LFS) reversed a potentiated input to its initial level and this phenomenon was termed as "depotentiation" (Barrionuevo, Schottler et al. 1980). Several years later a homosynaptic, de novo LTD was induced by LFS applied for a relatively long period of 10-15 minutes (Dudek and Bear 1992). Both size and slope of field EPSPs were depressed for several hours. Similar to LTP, the induction of this LTD depends on NMDA receptor activation.

The existence of LTD has been suggested to increase the storage capacity of a neural circuit. It could also efficiently prevent LTP saturation and so prevent memory overload, and might be used to actively erase unwanted messages from the brain. It has also been indicated that memories are more efficiently stored by synaptic modifications that occur in both directions.

Protocols employed for inducing LTD vary among investigators, partly due to general differences in experimental design, including the use of different brain areas and animal ages. For instance, in presence of certain neuromodulators, LTD can be effectively induced by a reduced number of stimuli (Kirkwood, Rozas et al. 1999; Scheiderer, Dobrunz et al. 2004). In the adult rat, where LTD is not readily induced by low frequency stimulation, a protocol with paired pulses (1 Hz, 900 pulses) appears to be more optimal (Thiels, Xie et al. 1996; Kemp, McQueen et al. 2000; Lee, Takamiya

et al. 2003). In our lab, we evoked a reliable LTD by a test rate stimulation (0.1 Hz) for 1-2 h in low Mg^{2+} solution (Dozmorov, Niu et al. 2003). It is referred as 'Slow LTD' in the following. Since this slow LTD is larger and more stable than the conventional LTD induced by LFS, we used it as a representative of LTD in this study.

1.6.2 Chemically induced LTP/LTD

Since standard stimulus-induced plasticity only accesses a fraction of available synapses, many research works have shown a great interest in chemically induced synaptic plasticity. Such plasticity involves in a larger population of synapses and therefore has become a desirable model for biochemical and morphological studies of synaptic plasticity. There have been many ways to induce chemical plasticity, for instance, iontophoresis, direct delivery by a cannula, pressure pulse ejection via a micropipette and bath application. Among them bath application has become the favorable method because it is easy to manipulate and would efficiently affect all the cells in the slice.

Since the postsynaptic Ca^{2+} entry through NMDA receptors has been considered as the key point in inducing synaptic plasticity (Collingridge, Kehl et al. 1983; Collingridge, Herron et al. 1988; Malenka, Kauer et al. 1989; Cummings, Mulkey et al. 1996), the NMDA receptor has received much attention from neuroscience researchers. NMDA, as a specific agonist of NMDA receptors, has been widely used to induce different forms of synaptic plasticity since long (Collingridge, Kehl et al. 1983; Kauer, Malenka et al. 1988; Thibault, Joly et al. 1989; Asztely, Hanse et al. 1991; Lee, Kameyama et al. 1998; Kamal, Ramakers et al. 1999; Broutman and Baudry 2001; van Dam, Ruiter et al. 2002; Li, Dozmorov et al. 2004).

In addition to NMDA application, there are many other pharmacological methods to induce chemical synaptic plasticity. In the early 1990's, a form of LTP was induced by application of TEA, a K^+ channel blocker, in CA1 region of the hippocampal slice (Ben-Ari and Represa 1990; Aniksztejn and Ben-Ari 1991). This form of LTP is believed to be NMDA-independent and requires the activation of L-type voltage dependent calcium channels (VDCCs) (Aniksztejn and Ben-Ari 1991; Huang and Malenka 1993). However, other studies reported that TEA-induced potentiation consisted of two distinct potentiations: one activated via NMDA receptors and the other depending on VDCCs (Hanse and Gustafsson 1994; Huber, Mauk et al. 1995).

Metabotropic glutamate receptors also play an important role in modulating synaptic efficacy. A form of mGluR-dependent LTD has been widely studied by application of

a group I mGluR agonist, DHPG, for 10 min (Palmer, Irving et al. 1997; Fitzjohn, Kingston et al. 1999; Huber, Kayser et al. 2000; Rouach and Nicoll 2003). This DHPG-induced LTD does not depend on the activation of NMDA receptors, and was therefore considered to be different from standard LTD. Instead, activation of G-protein, protein tyrosine phosphatases, mitogen-activated protein kinase (MAPK) cascades, and postsynaptic protein synthesis have been suggested to be involved in this form of LTD. Bath perfusion of the group I/II mGluR agonist, ACPD, was also capable of inducing LTP/LTD (Collins, Scollon et al. 1995; Breakwell, Rowan et al. 1996).

Moreover, BDNF (brain-derived neurotrophic factor), glycine and ATP have all been shown to induce synaptic plasticity in different brain areas (Wieraszko and Seyfried 1989; Kang and Schuman 1995; Levine, Black et al. 1998; Lu, Man et al. 2001).

1.6.3 Synaptic scaling and metaplasticity

It has been conceived that the neuron has the ability to adjust all of its synapses proportionally in order to counteract over- or under-activation of the cell. This principle, referred to as "synaptic scaling", is often used in theoretical neural network models to improve performance and has also been found experimentally (Lisman and Raghavachari 2006). Another form of scaling operates on the distance-dependency of synaptic inputs in order to equalize the potency of synapses on distal versus proximal dendrites (London and Segev 2001; Smith, Ellis-Davies et al. 2003; Raghavachari and Lisman 2004). In still another variant, the contributions of AMPA and NMDA receptors are gradually equalized in order to maintain a constant ratio of AMPA versus NMDA EPSPs over time (Watt, Sjöström et al. 2004).

Normalization of total synaptic impact can also be achieved by modulating the induction of synaptic plasticity processes. A cherished model is the "BCM rule", which implies that the degree of postsynaptic activity regulates the direction of the synaptic change, moderate activity leading to LTD and strong activity leading to LTP (Bienenstock, Cooper et al. 1982). The key point is that the LTD/LTP threshold is activity dependent and is slowly adjusted during development. This is an example of plasticity of plasticity, generally referred to as metaplasticity (Abraham and Bear 1996). It is not necessary for the prior activity to induce a change in synaptic efficacy. Rather it changes the ability to produce a future synaptic plasticity. It keeps plasticity working in a proper working range and maintains its "homeostatic status". A number of phenomena have been described as metaplasticity in the hippocampus and

elsewhere, but the relation among them is not fully clear (Fujii, Saito et al. 1991; Huang, Colino et al. 1992; Izumi, Clifford et al. 1992; O'Dell and Kandel 1994).

1.7 Mechanisms for LTP and LTD

1.7.1 Induction mechanism

1.7.1.1 NMDA-dependent synaptic plasticity

Most of LTP and LTD in CA1 region and dentate gyrus of hippocampus require the activation of postsynaptic NMDA receptors since in presence of AP5, a specific antagonist of NMDA receptors, both LTP and LTD are prevented. Whereas AMPA receptors are activated by glutamate binding alone, NMDA receptors require sufficient postsynaptic activity as well as glycine-binding as a cofactor to be activated. The dependency on glutamate accounts for the input specificity of LTP/LTD whereas the dependency on postsynaptic depolarization accounts for the property of cooperativity as observed for LTP, i.e. that a sufficient total activation is needed. Taken together, the two dependencies can explain the fact that LTP induction is associative and follows Hebb's rule (Hebb 1949; Wigström and Gustafsson 1986); a popular description of the latter is that "cells that fire together wire together" (Fig. 3).

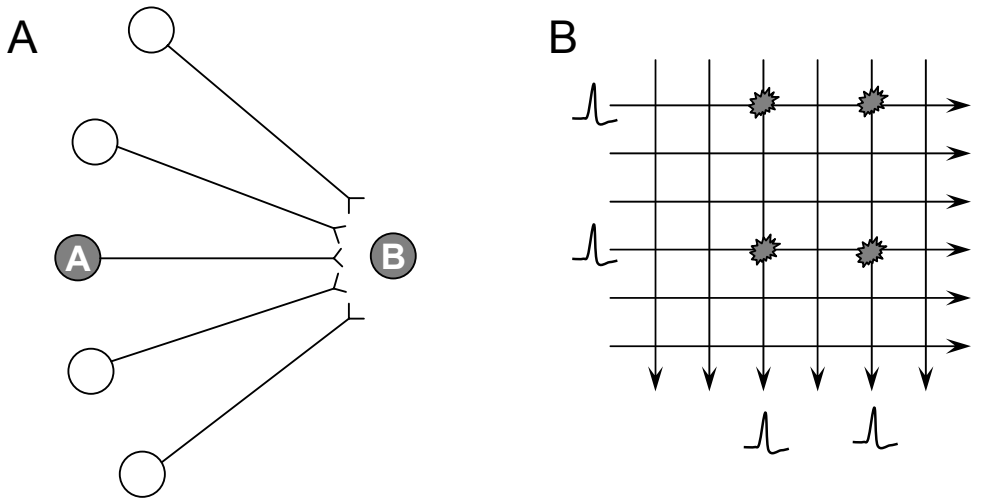

Fig. 3 Hebb's rule for strengthening of synapses. A: If cell "A" and cell "B" fire together, the connection between these two cells will be strengthened. B: Visualizing Hebb's rule as a "learning matrix", in line with the arrangement of axons and dentrites in CA1 area.

It is believed that the opening of the NMDA receptor channels results in an increase of Ca^{2+} concentration in the postsynaptic cell. Ca^{2+} acts as an important second messenger and plays an essential role in inducing synaptic plasticity (Collingridge, Kehl et al. 1983; Lynch, Larson et al. 1983). A rise in Ca^{2+} within the dendritic spine can trigger synaptic plasticity in different directions dependent on different induction conditions and experimental manipulations. It is commonly believed that a large amount of increase of Ca^{2+} within a short period causes LTP and a moderate elevation of Ca^{2+} with a longer duration leads to LTD. Many studies have provided evidence showing the importance of intracellular Ca^{2+}. For instance, pharmacologically buffering a rise in Ca^{2+} abolishes both types of plasticity (Mulkey and Malenka 1992; Bliss and Collingridge 1993; Malenka and Nicoll 1999).

Many protein enzymes have been considered as the targets of increased intracellular Ca^{2+} and their activation is believed to trigger LTP/LTD. Among them, calcium/calmodulin-dependent protein kinase II (CaMKII) is well known to mediate LTP (Lisman 1994; Malenka and Nicoll 1999; Lisman, Schulman et al. 2002), and serine-threonine protein phosphatase is important for LTD. There are also other enzymes worth mentioning. For example, activation of cAMP-dependent protein kinase (PKA) (Yasuda, Barth et al. 2003), protein kinase C (PKC) (Bliss and Collingridge 1993; Malenka and Nicoll 1999) and mitogen-activated protein kinase (MAPK) have been implicated in LTP (Sweatt 2004); the dephosphorylation of PKA and PKC substrates is suggested for LTD (Kameyama, Lee et al. 1998; Lee, Kameyama et al. 1998; van Dam, Ruiter et al. 2002). PKA may be especially important in late (>3 h) phases of LTP, and might be a link to activation of protein synthesis via the CREB enzyme (Abraham and Williams 2003; Lynch 2004). Synthesis of new proteins is considered to be important for the long-term maintenance of LTP as well as memory processes studied in intact animals.

Additionally, an "odd form" of LTP has been reported as a presynaptic NMDA-dependent synaptic plasticity (Humeau, Shaban et al. 2003).

1.7.1.2 NMDA-independent synaptic plasticity

Although most of the studies on synaptic plasticity focus on NMDA-dependent forms, there is also substantial work on NMDA-independent LTP/LTD. Thus, Ca^{2+} entry through VDCC has been considered as another important pathway that can mediate synaptic plasticity. For instance, high frequency tetanic stimulation (e.g. 200-Hz) activates VDCCs and induces NMDA-independent LTP in the presence of NMDA receptor blockers. This NMDA-independent LTP is prevented by application of L-type

VDCC blocker nifedipine (Grover and Teyler 1990; Grover and Teyler 1992; Grover and Teyler 1994; Grover and Teyler 1995; Cavus and Teyler 1996). The availability of Ca^{2+} in the extracellular environment and the release of Ca^{2+} from intracellular stores have also been suggested to be involved in this form of LTP (Grover and Teyler 1990; Bortolotto, Nistico et al. 2005).

Mossy fiber LTP that occurs at the synapse formed by axons of granule cell in dentate gyrus and pyramidal cells in CA3 region has also been found to be NMDA-independent (Harris and Cotman 1986). Different mechanisms have been proposed to explain this type of LTP. Certain studies suggest that mossy fiber LTP does not need any postsynaptic activation but is triggered by an activity-dependent increase of Ca^{2+} in the presynaptic terminal, and so does not obey Hebb's rule (Zalutsky and Nicoll 1990; Katsuki, Kaneko et al. 1991; Maccaferri, Toth et al. 1998; Yeckel, Kapur et al. 1999). Others report a requirement for activation of kainate receptors (Bortolotto, Clarke et al. 1999; Bortolotto, Nistico et al. 2005).

Certain forms of chemically induced plasticity do not require the activation of NMDA receptors. For example, bath application of TEA induced VDCC- and mGluR-dependent LTP (Aniksztejn and Ben-Ari 1991; Huang and Malenka 1993; Hanse and Gustafsson 1994). A transient application of a specific mGluR-agonist, ACPD, in CA1 of rat hippocampus causes a form of LTP that can be prevented by mGluR antagonist MCPG (Bortolotto and Collingridge 1993). There are also other forms of LTP that are partially NMDA dependent and partially dependent on other induction pathways. For example, LTP in the lateral nucleus of the amygdala (LA) was found to be induced by the activation of postsynaptic NMDA receptors together with L-type VDCCs, and expressed presynaptically by increasing the probability of transmitter release (Pare 2004).

1.7.2 Expression mechanisms

To solve the question of how LTP and LTD are expressed is a key step to understanding the mechanisms of memory at the cellular level. Substantial work has been devoted to it and both pre- and postsynaptic mechanisms have been proposed for the expression of LTP and LTD.

1.7.2.1 Presynaptic mechanisms

Any change of the process of transmitter release from the presynaptic terminal could change the efficacy of the synapse. In an early study, Bliss and coworkers used a special perfusion probe and detected an increase of glutamate release after LTP, claiming a presynaptic change (Dolphin, Errington et al. 1982). However, it appears to be problematic to explain LTP/LTD by such a presynaptic mechanism since activation of NMDA receptors and the consequent increase of Ca^{2+} concentration both take place in the postsynaptic cell. It is therefore unclear how the necessary signal could reach the presynaptic terminal. As a possible explanation, it has been postulated that a retrograde signal, such as NO or CO, is the missing link transforming postsynaptic into presynaptic events. Indeed, pharmacological data seem to support this idea (Williams, Li et al. 1993; Arancio, Kiebler et al. 1996; Alkadhi, Al-Hijailan et al. 2001), although the idea has now lost some of the initial momentum.

1.7.2.2 Postsynaptic mechanisms

A process that modify postsynaptic receptors is suggested for a postsynaptic mechanism. Phosphorylation and dephosphorylation of AMPA receptors has become a most popular hypothesis for the maintenance of LTP and LTD. It has been commonly believed that a large increase of Ca^{2+} in the postsynaptic cell activates protein kinases, including Calcium-calmodulin dependent protein kinase type II, or CaMKII. The activated CaMKII can undergo autophosphorylation and keep itself staying at active state (Lisman, Schulman et al. 2002). It affects AMPA receptors in two ways. First it phosphorylates the AMPA receptors that are already present in the postsynaptic membrane, thereby increasing the conductance for ions such as Na^{+}. CaMKII also promotes the movement of AMPA receptors from intracellular stores to the membrane, making more receptors available to be activated (Fig. 4).

As a result of a change of AMPA receptor composition and number, the response to a stimulus at a given strength will be stronger than it was before the NMDA receptors were activated. In this way, the synaptic efficacy is enhanced and this physiological change is thought to be one of the mechanisms underlying the expression of LTP. On the other hand, a small or modest rise of Ca^{2+} is thought to activate calcineurin which causes a dephosphorylation of protein phosphatase 1 (PP1). This process increases the activity of PP1 and results in dephosphorylation of AMPA receptors, so leading to LTD (Mulkey, Endo et al. 1994).

There are many phosphorylation and dephosphorylation sites located on the AMPA receptor. Previous studies have shown that the phosphorylation and dephosphorylation of Ser831 and Ser845 site of GluR1 play a critical role in the expression of LTP and LTD respectively (Stanton 1996; Barria, Muller et al. 1997; Lee, Kameyama et al. 1998). The phosphorylation of Ser863 and Ser880 in GluR2 by PKC is important for the regulation of synaptic targeting of AMPA receptors. The induction of LTD in hippocampus was found to be associated with a change of GluR2 phosphorylation at Ser880 site (Matsuda, Launey et al. 2000). Two phosphorylation sites, Ser830 and Ser842 have also been shown in GluR4, although the associated function is not well known (Gomes, Cunha et al. 2004).

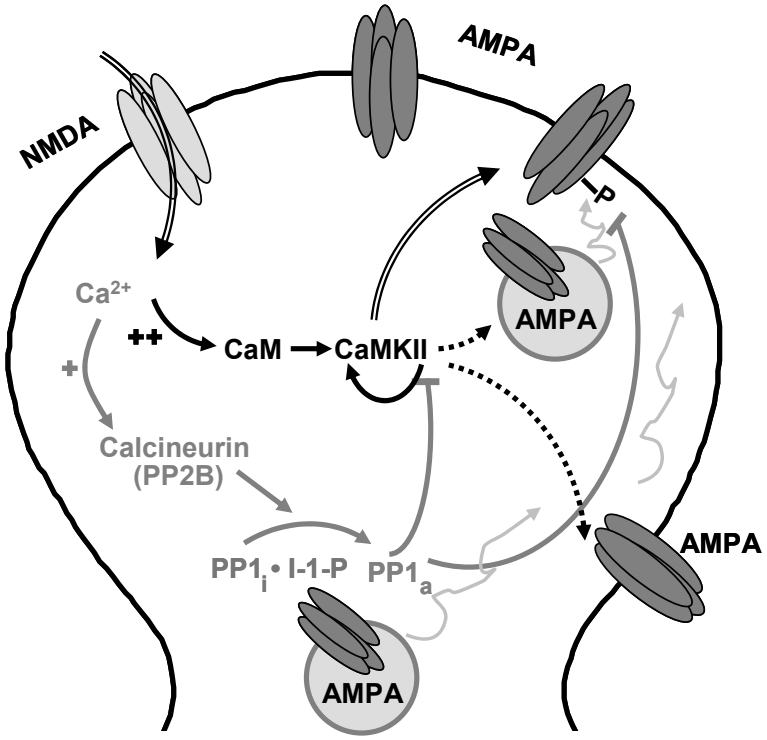

Fig. 4 Biochemical pathways leading to LTP and LTD: the opening of NMDA receptors mediates the entry of Ca^{2+} into the postsynaptic neuron. A large increase of Ca^{2+} activates the CaMKII pathway, resulting in LTP. A moderate increase of Ca^{2+} activates another pathway, triggering LTD.

Additionally, activation of mGluRs can modulate NMDA receptors and accelerate their cycling (externalization and internalization), which may play an important role in LTP/LTD (Pin and Duvoisin 1995; Conn and Pin 1997; Nicoletti, Bruno et al. 1999; Lan, Skeberdis et al. 2001).

1.7.2.3 The pre-post controversy: in search for decisive experiments

The overweight of studies describing postsynaptic mechanisms lends credibility to a postsynaptic scenario; however, the issue is far from settled. It should be realized that several of the above-mentioned biochemical studies were carried out in slice preparations where the proportion of potentiated synapses was small, or using cell cultures subjected to nonstandard induction protocols. In attempts to pinpoint a pre- versus postsynaptic locus, certain critical tests have been performed during the years, and some examples of these tests are presented in the following. As will be seen there are frequent contradictions and possibly some, remaining consensus.

Early work aiming to collect released glutamate by a perfusion probe was mentioned above. Later work in the Bliss group has improved the probe design and confirmed the presynaptic theory (Errington, Galley et al. 2003). On the other hand, the Nicoll lab found a constant glutamate concentration in the vicinity of the synapse by measuring transporter currents in glial cells, indicating a postsynaptic change (Luscher, Malenka et al. 1998). In a straight-forward attempt to test the postsynaptic sensitivity to transmitter, the response to iontophoretically applied AMPA was shown to increase slowly after LTP (Davies, Lester et al. 1989). This imples that (after the postsynaptic induction) LTP is expressed presynaptically and then postsynaptically, an idea lacking support in later work.

Changes of the coefficient of variation (CV) is one important parameter that has been used to distinguish between pre- and postsynaptic changes. Some early studies on the change of CV after LTP showed a decreased value, supporting a presynaptic modification (Bekkers and Stevens 1990; Malinow and Tsien 1990; Kullmann and Nicoll 1992). However, in the context of the idea of silent synapses, later studies suggested that an alteration of CV is mostly due to postsynaptic changes (Isaac, Nicoll et al. 1995; Liao, Hessler et al. 1995).

Some research groups have tested the change of paired pulse facilitation (PPF) after synaptic plasticity, believed to be an index of presynaptic change. For instance, an unchanged value of PPF was found following LTP, thus favoring the postsynaptic theory (McNaughton 1982; Muller and Lynch 1989; Asztely, Xiao et al. 1996). On the other hand, some later studies displayed a decrease of PPF, adding evidence to a presynaptic modification (Schulz 1997; Volianskis and Jensen 2003).

Another possibility is to use the activity-dependent NMDA-receptor blocker MK-801 to detect changes in presynaptic transmitter release (Hessler, Shirke et al. 1993). Both with LTP and LTD, the results were negative (Manabe and Nicoll 1994; Xiao, Niu et

al. 1997). However, the sensitivity of this method in the LTD situation appeared to be lower than expected (Xiao, Niu et al. 1997).

Comparing the relative changes of AMPA versus NMDA receptor mediated responses after LTP/LTD is another test that has been extensively used. In that AMPA and NMDA receptors coexist in the same postsynaptic cell, presynaptic changes would affect these two receptors to the same extent. A postsynaptic change could influence the components either unequally or equally. In the latter case, one could imagine coordinated AMPA and NMDA receptor changes to be responsible.

LTD is the least controversial plasticity as most studies seem to agree that AMPA and NMDA components change equally during LTD, at least under standard conditions (O'Connor, Rowan et al. 1995; Selig, Hjelmstad et al. 1995; Xiao, Karpefors et al. 1995a). Previous work in our lab considered the components to be coupled to each other (Xiao, Karpefors et al. 1995a). However, other work has suggested that the contributions of AMPA and NMDA are due to independent processes (Selig, Hjelmstad et al. 1995; Morishita, Marie et al. 2005). Depending on experimental conditions, the components may then either be equal or one of them can prevail. Whereas unequal AMPA-NMDA supports a postsynaptic mechanism, equal contributions is compatible with both pre- and postsynaptic changes. All of the results are therefore most easily accounted for by a postsynaptic expression mechanism despite the differences in details.

Regarding LTP, the results on AMPA-NMDA involvement differ between researchers. Thus it was shown that LTP is associated with equal changes of both components during LTP (Clark and Collingridge 1995) whereas other studies revealed a predominant potentiation of the AMPA component (Kauer, Malenka et al. 1988; Muller and Lynch 1988; Isaac, Nicoll et al. 1995). Still other studies revealed a preferential increase of AMPA early on, gradually converting to more equal changes later on (Xiao, Karpefors et al. 1995b; Watt, Sjöström et al. 2004). Taken together, most of the studies agree on a predominant change of AMPA during the early part of LTP whereas the AMPA versus NMDA contribution during the later stage is controversial. Interpreted in terms of pre versus post, this implies that at least the early part of LTP is likely to be postsynaptic.

1.8 Unsolved questions

The initial steps of NMDA-dependent LTP and LTD are relatively well understood, including types of enzymes involved, but certain details are missing. One important

question at early induction stage concerns the role of different NMDA receptor subunits. When it comes to expression mechanisms, i.e. the type of persistent modification involved, there is less certainty and the pre-post controversy still remains. As we have just seen, even the basic issue of AMPA- versus NMDA-receptor involvement is only partly settled. The field of temporal phases is an essential matter due to possible relation to memory consolidation. How do early and late expression mechanisms relate to each other? Which particular proteins are needed in an early and a late perspective. If structural changes are involved, what are they?

Apart from standard LTP/LTD, a number of other plasticities also exist, NMDA-dependent and NMDA-independent ones. Further characterization remains in many of the cases with respect to the relation to standard LTP/LTD as well as underlying biochemistry. Research on chemically induced plasticity may lead to improved methods to analyze synaptic plasticity. However, even when restricting the case to the seemingly simple one of NMDA-induced plasticity, there appears to be a multitude of changes that are triggered by the application of NMDA. Bringing order among those different forms of plasticity and relating them to the simulus-induced variants is an essential task.

2. Aim of this study

The present study makes use of the brain slice technique combined with electric recording and pharmacological manipulation to interrogate the complex biological events involved in hippocampal synaptic plasticity. The general aim is to increase our knowledge about several forms of NMDA-dependent synaptic plasticity believed to be particularly relevant for memory processes. Emphasis is put on chemically induced plasticity and its relation to plasticity induced by electrical stimulation. It is my belief that this approach will pave the way for future progress in cellular memory research based on chemical induction methods, which are able to impact a large proportion of synapses.

Specific goals:

1) To investigate the involvement of AMPA-R and NMDA-R in different types of synaptic plasticity, mainly focusing on NMDA-induced LTD as well as stimulus-induced LTP

2) To elucidate the possible mechanisms underlying NMDA-induced waveform prolongation

3) To explore the roles of different NMDA-R subunits in several forms of synaptic plasticity

4) To examine the interaction between NMDA-induced LTD and stimulus-induced LTP

3. Materials and Methods

Experiments were carried out in submerged hippocampal slices using extracellular electrophysiological technique with capability for studying two independent pathways. The hippocampal slice is a popular experimental model, which provides for well controlled stimulation and recording as well as easy exchange of extracellular solutions. In order to increase performance of the system, I commenced my Ph.D. studies by participating in a project to design a novel set of recording chambers with associated electronics. The final system allows up to 4 slices to be studied in parallel in an automated manner. Although this work by itself did not result in any published records and no further details are described in this thesis, it greatly facilitated my subsequent electrophysiological work.

3.1 Hippocampal slice preparation

Sprague-Dawley rats, 12-20 days old, were decapitated after being anesthetized by isoflurane (Forene) for a short period. The brain was rapidly removed and placed into ice-cold medium, referred to as artificial cerebrospinal fluid (ACSF), containing NaCl 119 mM; KCl 2.5 mM; KH_2PO_4 1 mM; $MgCl_2$ 2 mM; $CaCl_2$ 2 mM; $NaHCO_3$ 26 mM; and glucose 10 mM, bubbled with a mixture of 95% O_2/5% CO_2. The hippocampus was dissected out and sectioned in the transverse plane into 400 μm thick slices by a vibratome or tissue chopper. The slices were stored in an incubator with ACSF at room temperature for at least 60 min before transferred to one or several submerged type recording chambers. During the experiment a similar oxygenated ACSF solution but with calcium and magnesium concentrations being at 2.5 mM and 1.3 mM, respectively, was perfused at 1.5 ml/min and maintained at 30-32 °C. After initial positioning of electrodes and starting recording, the solution was often modified with respect to Ca^{2+} and Mg^{2+} content as well as addition of pharmacological agents. Stable baseline was obtained at least 45 min before the actual experiments. All animal experiments were approved by the Göteborg Ethical Committee for Laboratory Animals.

3.2 Stimulation and recording

Field excitatory postsynaptic potentials (EPSPs) were recorded from the CA1 dendritic layer (stratum radiatum) by a glass micropipette filled with ACSF or 1-3 M NaCl (2-5 MΩ resistance). Two monopolar tungsten stimulating electrodes were placed on both sides of the recording electrode (Fig. 5A). Negative, constant current pulses, 100 μs,

10-50 µA were alternately delivered to the two stimulating electrodes, providing access to a pair of separate sets of afferents. The interval between successive stimuli ranged between 5 and 15 s (10 and 30 s for each pathway) depending on experimental design features.

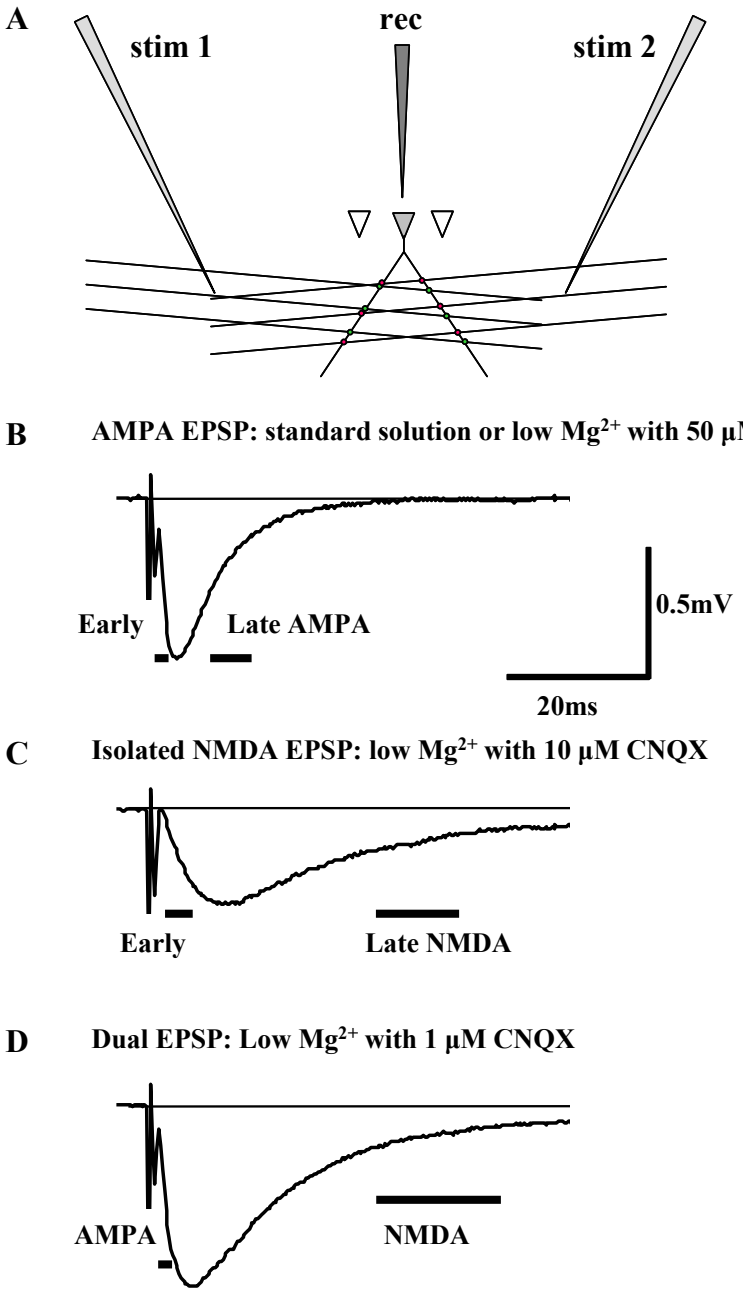

Fig. 5 Electrode placement and recorded potentials. A. Two stimulating electrodes are positioned on each side of the recording electrode, activating two independent bunches of axons that form synapses on dendrites from the same group of neurons. B, C, D. Different types of EPSPs and how they were measured.

Prefabricated stimulating electrodes were obtained from World Precision Instruments, FL, USA, type TM33B. Glass capillaries for making recording electrodes were from World Scientific Instruments, USA.

3.2 Recording of different types of EPSPs

AMPA receptor mediated EPSPs were recorded in the standard perfusion solution (2.5 mM Ca^{2+}, 1.3 mM Mg^{2+}), or in some cases low Mg^{2+} solution (2 mM Ca^{2+}, 0.1 mM Mg^{2+}) in the presence of NMDA receptor blocker AP5 (50 μM). Recording of composite AMPA-NMDA EPSPs was performed in low Mg^{2+} solution (2.0 mM Ca^{2+}, 0.1 mM or in some cases 0.01 mM Mg^{2+}) to facilitate the NMDA response. In this case, a low concentration of CNQX (0.5-1 μM) was also present to partially block the AMPA response, leading to a balanced mixture of AMPA and NMDA components. Isolated NMDA EPSPs were obtained with 2.0 mM Ca^{2+}, 0.1 mM Mg^{2+}, and 10 μM CNQX to fully block the AMPA component (Fig. 5B, C, D).

3.3 Induction protocols for LTP and LTD

LTP was induced by high frequency stimulation consisting of 1-3 stimulus trains, each containing 100 pulses at 100 Hz. Theta burst stimulation was also used, consisting of one or two sequences of 10 brief 100 Hz trains, 4 impulses each, repeated at 5 Hz. In some cases, LTP was induced in low Mg^{2+}. A low concentration of AP5, 5-10 μM was then present to optimize conditions for LTP induction without too much subseqent decay; a substantial decay of LTP is otherwise seen in low Mg^{2+} solution (Xiao, Niu et al. 1996).

Due to the usually small magnitude of LTD induced by conventional 1-2 Hz stimulation in our experimental situation, we used an alternative depression model referred to as ‘slow LTD’. In principle, normal test stimulation was applied for 1-2 h while slices were kept in low Mg^{2+} solution. AP5 (50 μM) was initially present to obtain a baseline of isolated AMPA EPSPs. AP5 was also applied afterwards, resulting in isolated AMPA EPSPs that were compared to the baseline responses. In both LTP and LTD experiments, one of the two pathways was generally used as control.

3.3.1 Drug treatment

NMDA was used in concentrations of 5-60 μM and was applied over a 4 min period. High potassium concentration (50 mM) solution was made specially, containing (in

mM): 71.5 NaCl, 50 KCl, 1 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose and was applied over a 5 min period to compare its effect with that of NMDA. Concentrations of 15 mM and 25 mM potassium were also used in some experiments. Application of AP5 (50 μM) was done to clarify the dependency of the different forms of plasticity on NMDA receptors and to stabilize recordings by preventing NMDA-dependent decay. NMDA receptors with different subunit compositions were selectively blocked by NVP (0.4 μM; NR2A blocker) or Ro/Ife (0.5 μM/3 μM; NR2B blockers). To consider possible involvement of VDCCs, L-type channel blocker nifedipine (50-100 μM), and/or T-type channel blocker Ni^{2+} (30-75 μM) were used. Inhibition was blocked in some experiments by application of 100 μM picrotoxin (PTX), a GABAa receptor antagonist. To suppress the heightened neuronal excitability accompanying PTX we used a modified ACSF (4 mM Ca^{2+}, 4 mM Mg^{2+}). To address the question of possible changes in AMPA receptor kinetics, AMPA receptor modulators aniracetam (1.5-3 mM) and CTZ (50-100 μM) were applied for at least 1 h before NMDA application. At the end of each experiment, the synaptic response was entirely blocked by using a solution containing 10 μM of CNQX and 50 μM of AP5. This non-synaptic response, consisting of stimulus artifact and presynaptic volley, was used to define "true zero".

Drugs were purchased from Tocris Cookson (UK) or Sigma Chemicals (St. Louis, MO). Aniracetam was a generous gift by Nippon-Roche (Japan) and NVP-AAM077 was generously supplied by Novartis Pharmaceuticals.

3.3.2 Data analysis

The signals were amplified, filtered, digitized and transferred to a computer for on-line and off-line analysis by specially designed electronic equipment (based on an Eagle Instruments multifunction board) and own developed computer software. AMPA EPSPs, as well as the AMPA component of composite EPSPs, were measure using a time window of 0-1 or 0-1.5 ms after the fiber volley. The NMDA component of composite EPSPs was measured with a 35-45 ms window whereas isolated NMDA EPSPs were measured using either the same window or one located 0-5 ms after the fibre volley. Measurements were calculated by integrating the EPSP curve along the specified time window after subtraction of the prestimulus baseline. Values were corrected by subtracting the corresponding measurements of the nonsynaptic potential, obtained after total blockage of the EPSP by 10 μM CNQX and 50 μM AP5. Synaptic plasticity was generally estimated as ratio between post- and preinduction values after averaging responses for 5-10 min periods. In two-pathway experiments, the ratio between pathways was sometimes used. In some experiments with NMDA component

recording, there was no preinduction baseline available to be used as 100%. In this case the maximum level was generally used as 100%. Additional scaling of this level was performed in experiments with NMDA subunit blockers to include the effect of the blocker. Data are presented as mean ± S.E.M. Statistical comparisons were made using Student's *t*-test. Statistical significance was set at $p<0.05$.

4. Results

4.1 Involvement of AMPA and NMDA receptors in different types of synaptic plasticity (Paper I, II, IV)

It is commonly believed that LTP mainly involves changes of AMPA receptors, while LTD engages a nearly equal change of the two types of receptors. Here we investigated the involvement of these receptors in chemically induced plasticity as well as some cases of stimulus-induced LTP and LTD in our experimental condition.

4.1.1 NMDA-induced plasticity (Paper I)

4.1.1.1 About equal changes of AMPA and NMDA components

Field EPSPs mediated via AMPA receptors were recorded in standard perfusion solution. It was found that brief bath application of NMDA (30 μM for 4 min) resulted in an initial extinction of EPSPs, a recovery phase (20-30 min) and a stable LTD at 60 min after the application, down to 75-80% of the initial baseline level. In experiments with recording of isolated NMDA EPSPs, a similar depression was observed, but the recovery time after NMDA application was longer than with AMPA EPSPs. NMDA treatment of composite EPSPs mediated by both AMPA and NMDA receptors confirmed the faster recovery of the AMPA component as well as the nearly equal depression of the two components at the late stage after NMDA application.

4.1.1.2 Waveform change of AMPA but not NMDA EPSPs

In addition to the NMDA-induced LTD, the late but not the early measurement of AMPA EPSPs showed a peak at 15-25 min after NMDA application, indicating a transient waveform change. This discrepancy of the two measurements was lacking for isolated NMDA EPSPs. By analyzing EPSP traces obtained before, 20 min after and 60 min after NMDA application, we found that there was a prolongation of the late portion of AMPA EPSPs at 20 min after NMDA treatment. However, the waveform was unaltered at the late stage. On the contrary, NMDA EPSPs kept uniform throughout the whole experiment, showing that the NMDA-induced waveform change only involves the AMPA component. All of these results were confirmed in terms of specific waveform characteristics such as rise time and decay time. Accordingly, at 20

min after NMDA application, there were significant increases of both rise and decay times of isolated AMPA EPSPs. Using a simple computational model, primarily based on linear superposition, we estimated the waveform change of the AMPA component as part of composite EPSPs. The model revealed a waveform prolongation of the calculated AMPA component that was similar to that observed with isolated AMPA EPSPs at the early stage after NMDA application.

4.1.2 Stimulus-induced LTP (Paper II)

Previous work has shown that even small activation of NMDA receptors weakens LTP with time. Hence, our study of NMDA receptor involvement in LTP used a method with minimal exposure of synapses to NMDA current. Experiments were carried out in a low Mg^{2+} solution allowing expression of both AMPA and NMDA responses but the latter were blocked for most of the time by the presence of 50 μM AP5. To assess the NMDA component, AP5-free solution was briefly applied before LTP induction, at 1h after and at 4-5 h after induction. To induce LTP, tetanization was delivered while slices were transiently perfused by low Mg^{2+} solution with 10 μM AP5, leading to partial activation of NMDA receptors and suitable conditions for LTP induction. During LTP, AMPA responses increased by about 60 % at both 1 h and 4 h after the induction event. In contrast, NMDA responses increased by only about 25%, similarly at 1 h and 4 h. Thus, in our experimental sitation there was a significant LTP of not only the AMPA component but also of the NMDA component. However, the relative increase of the NMDA component amounted to only about half of the that of the AMPA component.

We also carried out experiments where LTP was induced in normal Mg^{2+} solution. The NMDA component was then measured at the late stage of the experiment (> 4h) after switching to low Mg^{2+} solution. Using three variants of the low Mg^{2+} solution, the NMDA component was determined in three different ways. (1) First, it was determined from composite EPSPs similar to the sitation in the first set of experiment. (2) Next, increasing the concentration of CNQX to 10 μM, an isolated NMDA response was examined and measured in different ways. (3) To rule out any inhibitory effects on the NMDA response that might contaminate the result, we applied the GABAa receptor blocker PTX (100 μM) as a following step, and measured NMDA EPSPs in the disinhibited state. The results were similar to those above with a larger potentiation of the AMPA component.

All of the above experiments were performed with LTP induced by “standard tetanization” using one or several 100 Hz trains of 1 s duration. In a final set of

experiments we also used thetaburst stimulation, i.e. a sequence of brief 100 Hz trains repeated at "theta frequency", 5 Hz. This way to induce LTP has been proposed to be specially effective, confirmed in our case by a potentiation amounting to about 80% above baseline, which is larger than we obtained with tetanization in this study. When the NMDA component was later assessed in low Mg^{2+} solution, the increase was found to be around 40%. Thus, our data are all consistent, with similar results obtained independently of details in experimental design or measurements. Accordingly, there was a significant potentiation of the NMDA component; however the potentiation of the AMPA component dominated over that of the NMDA component by a factor of about two.

Although the question of the first hour of LTP is not addressed in the published study (Paper II), its Fig. 1 suggests that potentiation of the NMDA component might have been small right after tetanization. We therefore did supplementary work to test this issue (not included in the paper), yielding support for the view that NMDA-receptor mediated LTP is small early on and grows during the first hour.

4.1.3 Stimulus-induced LTD – 'slow LTD' (Paper IV)

Slow LTD was induced by a test rate stimulation (0.1 Hz) for 1-2 h in low Mg^{2+} solution. High concentration of AP5 (50 μM) was initially present to establish a baseline of AMPA responses; the solution was then switched to AP5-free, leading to expression of composite EPSPs which gradually decayed. After 1-2 h, AP5 was washed in again and the resulting isolated AMPA EPSPs were compared with the baseline ones. The primary goal of studying such slow LTD was to quantify the depression of isolated AMPA EPSPs in this case and to compare the results of a standard test situation with those obtained in the presence of subunit-specific NMDA receptor blockers. Less attention was therefore payed to the quantitative aspects of the depression of NMDA responses. Still, we measured and plotted both AMPA and NMDA components during the AP5-free period. It can be appreciated from the illustrated standard case that once NMDA receptors were unblocked, the time courses of AMPA and NMDA components were virtually identical with a depression down to about 60% of the initial value for both components (see Fig. 2 of Paper IV). The data for isolated AMPA EPSPs revealed a slightly larger depression, down to 50% of baseline. These results are in accord with previous work in this lab demonstrating about equal depressions of AMPA and NMDA components during 'slow LTD' (Dozmorov, Niu et al. 2003).

4.2 Possible mechanisms of NMDA-induced waveform change (Paper I, III)

Many previous studies have been carried out to investigate the mechanisms of NMDA-induced LTD, but little is known about the prolongation of EPSP waveform induced by NMDA application. Therefore we performed different tests to explore the possible processes involved in this waveform change.

4.2.1 NMDA-dependent induction but not expression

NMDA applied in the presence of AP5 (50 μM) revealed a total block of the waveform change as well as all other components of NMDA-induced plasticity. If AP5 was applied after the NMDA treatment, or AP5 was present all the time except during NMDA application, all plasticity components remained intact, the AMPA EPSPs showing a similar waveform change as reported before. Our data therefore demonstrate dependency on NMDA receptors in the process of induction but not the expression.

4.2.2 Noninvolvement of voltage-dependent channels

4.2.2.1 Comparison with potassium-induced plasticity

Since NMDA application is likely to cause a big depolarization of the postsynaptic cells, as indicated by the initial extinction of EPSPs, we tested whether depolarization without initial NMDA receptor activation could cause plasticity similar to that evoked by NMDA. Application of 50 mM K^+ for 5 min was used to transiently depolarize hippocampal neurons. Similar to the case with NMDA application, there was an initial elimination of the EPSPs followed by a recovery, and ending up at a somewhat depressed level. However no waveform change could be observed in this type of experiment and with AP5 present, there was even no remaining depression. Varying the concentration of K^+ (15 mM or 25 mM) to change the degree of depolarization also failed to induce a waveform change. Moreover, applying K^+ (50 mM) together with Ca^{2+} (10 mM) to increase the induction strength did not elicit a waveform change either.

4.2.2.2 Role of voltage-dependent calcium channels

To further rule out the possibility of voltage dependent factors, we also did experiments in the presence of blockers of L/T type VDCCs, using either nifedipine (L-type blocker) or Ni^{2+} (T-type blocker). Waveform changes were not affected by those blockers, which indicated noninvolvement of L/T type VDCCs, supporting our previous finding. In fact, all of the NMDA-induced plasticity components seemed normal in the presence of VDCC blockers, although some experiments indicated a slightly faster recovery in nifedipine solution.

4.2.2.3 Varying the stimulus strength

Additionally, we varied the electrical stimulus strength by repeatedly sequencing four differently sized stimuli to the same electrode. When scaling and superimposing the time course plots of successive EPSP measurements, the early and late measurement curves overlapped perfectly within each group. Analysis of EPSP traces showed that the AMPA EPSP waveform was prolonged in a similar way under the different strengths, implying that depolarization-related factors were unimportant for the NMDA-induced waveform change.

4.2.3 Role of inhibition

Since disinhibition of slices by blocking GABAa receptors was shown to prolong EPSP waveforms in previous studies (Wigström and Gustafsson 1983), we wanted to test if the waveform change induced by NMDA in our case was related to a parallel change in the level of inhibition. Therefore we examined the effect of the GABAa antagonist PTX on NMDA-induced plasticity. Adding PTX to the perfusion solution caused a waveform prolongation of AMPA EPSPs by itself, but it did not prevent the waveform change caused by NMDA application, suggesting the involvement of separate processes.

4.2.4 Effect of AMPA receptor modulators

In order to test whether a change of postsynaptic AMPA kinetics might account for the waveform prolongation after NMDA application, we employed two types of AMPA modulators, aniracetam and CTZ. Perfusion with aniracetam at sufficient concentration caused a saturating waveform prolongation, the duration of the EPSP

(halfwidth) increasing by about 50%. Still, the subsequent application of NMDA resulted in a further change of EPSPs. Comparing the NMDA effect obtained in aniracetam experiments and standard ones, we found that prior treatment with aniracetam did not diminish the NMDA effect. On the contrary, it even facilitated the waveform change. This can be illustrated by the fact that the tail of the EPSP that was subjected to increases during aniracetam experiments extended well beyond the duration of EPSPs in normal experiments. We next tested the effect of CTZ which has been suggested to work in a somewhat different way on AMPA receptors as compared to aniracetam. Interestingly, the pretreatment with CTZ prolonged AMPA EPSPs about two-fold, and completely occluded the waveform prolongation caused by the following NMDA application.

4.3 Interaction between LTP and NMDA-induced plasticity (Paper III, IV)

The argument about whether a common synaptic process governs the two most important forms of synaptic plasticity, LTP and LTD, has been a top issue in the neuroscience area for many years. Since some previous work showed an equivalence between NMDA-induced and stimulus-induced LTD, here we tested the interaction between LTP and NMDA-induced plasticity, allowing us to reveal the underlying relationships among some different types of synaptic plasticity.

4.3.1 Comparison of NMDA-induced depression in LTP and naive pathways

Field EPSPs (AMPA responses) were recorded from two pathways, and LTP in one pathway caused an about two-fold increase of EPSP size. Subsequent NMDA application led to a depotentiation in the LTP pathway, down to nearly baseline level, and a depression in the naive one as usual, down to 70-80% of the baseline. Expressing the values of these two types of depression as the ratio before and long after NMDA application, we found that there was a strong correlation between the two types of depression and that the depotentiation dominated over the depression.

4.3.2 Comparison of NMDA-induced waveform change in LTP and naive pathways

Plotting the time course of late measurements for LTP and naive pathways together, we observed a peak on both curves shortly after NMDA application, indicating that a transient waveform change occurred in both pathways. However, further comparison

of the EPSP traces obtained from LTP and naive pathways at 20-25 min after NMDA treatment revealed a different degree of waveform change on the two pathways, the EPSP of the LTP pathway being less prolonged than that of the naive one.

4.4 Contribution of NMDA subunits to isolated NMDA EPSPs (Paper IV)

The NMDA receptor is composed of different NMDA subunits, with at least one NR1 and two or more NR2 subunits. The composition varies during development and different units, especially NR2A and NR2B, have been reported to be selectively involved in different types of synaptic plasticity. Therefore, the contribution of different NMDA subunits to the NMDA response in our experimental animals is a basic question for us to solve. Isolated NMDA EPSPs were obtained as usual in low Mg^{2+} solution during blockade of AMPA receptors by CNQX. We tested the effects of selective blockers of NR2A- and NR2B-containing NMDA receptors, NVP and Ro/Ife respectively. In one set of experiments, NVP was applied first and it reduced the NMDA EPSP down to about 19% of the baseline. Subsequent application of Ro (0.5 μM) or ifenprodil (3 μM) further depressed the response to near zero. As a supportive experiment, we applied the same drugs in a different order, Ro/Ife first and then NVP. It was noted that Ro/Ife blocked NMDA response down to 67% of the baseline and addition of NVP suppressed nearly all the remaining responses. Our results tell that NR2A and NR2B type NMDA receptors make up most of the NMDA receptor mediated response in our 12-18 days old rats, with NR2A contributing to 70-80% and NR2B contributing to 20-30%.

4.5 Involvement of NR2A and NR2B in different types of synaptic plasticity (Paper IV)

It is commonly believed that NR2A subunits are involved in LTP while NR2B subunits mainly contribute to LTD. Here we investigated the role of NR2A and 2B subunits in different types of synaptic plasticity in our experimental condition.

4.5.1 NMDA-induced LTD

Application of NVP (NR2A blocker) or Ro/Ife (NR2B blocker) did not affect the baseline level, indicating that those drugs did not influence the basic synaptic transmission mediated by AMPA receptors. NMDA was applied at least 1 h later than the pretreatment with subunit-specific NMDA receptor blockers. We found that 0.5

μM Ro or 3 μM ifenprodil did not prevent NMDA-induced LTD, EPSPs being depressed down to about 70% of baseline which is similar to the control situation. On the other hand, NVP (0.4 μM) largely blocked all the NMDA-induced LTD, resulting in a depression downt to 92% of the baseline. In addition, we found that the duration of the initial extinction of EPSPs after NMDA application was shorter in NVP experiments than in control ones, with about 10 min and 30 min to half recovery respectively.

Facilitation experiment: Due to the large contribution of NR2A subunits to NMDA receptors in our experimental animal, we could not rule out the possibility that the blocking effect by NVP was because the total size of the NMDA response was reduced to an under-threshold level. To test this hypothesis, we carried out a type of "facilitation experiment" where lower concentration of Mg^{2+} (0.1 instead of 1.3 mM) was used to boost the induction of NMDA-induced LTD. Under such conditions a significant depression was induced in presence of NVP, down to about 60% of baseline level (this result is not yet implemented in the paper, but other facilitation experiments are; see paper IV Fig. 5, and below).

4.5.2 Stimulus-induced LTD

Since the conventional, stimulus-induced LTD (1-2 Hz) was relatively small in our hands, we used test rate stimulation (0.1 Hz for a period of 1-2 h) in low Mg^{2+} solution as an alternative means to induce LTD. AP5 was present initally and at the end of the experiment, allowing comparison of isolated AMPA EPSPs. In this way, we were able to induce a stable LTD of down to 40-50% of baseline. To investigate the role of NR2A and 2B subnits in this type of LTD, NVP or Ro/Ife was introduced to the whole induction period, i.e. when AP5 was absent. Our results showed that Ro/Ife did not affect this stimulus-induced LTD (45% of baseline); however, NVP largely blocked it (93% of baseline).

Facilitation experiment: Lowering the Mg^{2+} concentration from 0.1 to 0.01 μM helped the induction of LTD, yielding a depression down to about 60% of baseline under the blockade of NR2A receptors.

4.5.3 Stimulus-induced LTP, depotentiation and repotentiation

4.5.3.1 LTP

LTP induced by three successive tetani (100 Hz, 100 impulses each) was fully blocked by NR2A blocker NVP (0.4 μM), consistent with previous findings (Liu, Wong et al. 2004). The treatment with Ro/Ife also reduced LTP but only to about half normal size (125% of baseline), suggesting that both NR2A and NR2B subunits contribute to LTP in our experimental conditions.

Facilitation experiment: Using low Mg^{2+} concentration, 0.1 mM instead of 1.3 mM as the medium solution, LTP could be elicited by the same tetani in presence of NVP, but was smaller than in the normal case, amounting to about 120% of baseline.

Synaptic plasticity	AMPA	NMDA	NR2A	NR2B
NMDA-induced LTD	++	++	++	+ (in extra low Mg^{2+})
NMDA-induced waveform change	++			
Stimulus-induced LTP	++	+	++	+
Stimulus-induced LTD	(++	++)	++	+ (in extra low Mg^{2+})
Depotentiation			++	
Repotentiation			++	

Table 1 Summary of results of the present study about the involvement of AMPA and NMDA components and the role of NMDA receptorsubunits in different types of synaptic plasticity. Plus sign indicates "involvement".

4.5.3.2 Depotentiation

NMDA application on the previously potentiated pathway led to a depotentiation, bringing the potentiated level back to baseline. While this was true for averages, individual experiments showed considerable variation, some cases showing incomplete depotentiation whereas others showed depression down to below the original baseline. NVP substantially blocked depotentiation, whereas Ro/Ife had no effect on it.

4.5.3.3 Repotentiation

Based on the depotentiation, a second set of tetani restored the EPSPs to a new potentiated level, which is termed as repotentiation. Under normal conditions, repotentiation reversed the depotentiation caused by NMDA application to a level of about 140% compared to the original LTP of about 180%. In presence of NVP, repotentiation was totally abolished. Compared to the control situation, Ro/Ife seemed to partially block repotentiation.

A summary of important data in the present thesis is given in Table 1.

5. Discussion

In the present thesis, I have dealt with both stimulus-induced and chemically induced synaptic plasticity, with emphasis on the latter. The results demonstrate similarities as well as discrepancies between these forms of plasticity. In both cases, components or temporal phases with specific properties can be defined and are currently examined. A key point is the relative involvement of AMPA and NMDA type glutamate receptors; another one concerns the specific change of EPSP waveform observed after NMDA application and its relation to AMPA receptor kinetics. In all cases, the induction was primarily related to activation of NMDA receptors, possibly via passing of calcium ions, whereas other calcium sources seemed to be uninvolved. Additionally, my examination of NMDA receptor subtypes revealed a dominant behavior of one of the types in all of the tested plasticities, a result that challenges some previous data.

5.1 Why the same NMDA causes different synaptic plasticities

Previous works have shown that NMDA application can cause both potentiation and depression. In the early 1980's, Collingridge and coworkers applied NMDA by iontophoresis but only found a short lasting potentiation (Collingridge, Kehl et al. 1983). A similar result was obtained by the Nicoll group several years later (Kauer, Malenka et al. 1988). At about the same time Gary Lynch and collaborators described a long-lasting form of potentiation induced in field CA1 of rat hippocampal slices by bath application of NMDA (Thibault, Joly et al. 1989). In a previous study in our laboratory, application of NMDA locally via short pressure pulses produced a stable potentiation that was partly the same as LTP (Asztely, Hanse et al. 1991). It was later discovered that "chemical LTD" could be induced by brief bath application of NMDA to hippocampal slices (Lee, Kameyama et al. 1998; Kamal, Ramakers et al. 1999; Li, Dozmorov et al. 2004). However, another recent publication found again that NMDA-induced an LTP-like potentiation in hippocampus (Broutman and Baudry 2001). In the present thesis, we found that bath application of NMDA not only induced an LTD, but also caused a transient prolongation of the waveform of the recorded field EPSP, revealing an additional complexity.

Table 2 summarizes the results from different research groups. We find that the major differences among these works are the method for NMDA application and the composition of the perfusion solution during the experiments regarding to Mg^{2+} concentration. However, the latter does not seem to be critical since in "normal Mg^{2+}" (1-4 mM) solution both potentiation and depression have been reported (Collingridge, Kehl et al. 1983; Kauer, Malenka et al. 1988; Thibault, Joly et al. 1989; Asztely,

Hanse et al. 1991; McGuinness, Anwyl et al. 1991a,b; Lee, Kameyama et al. 1998; Kamal, Ramakers et al. 1999; Li, Dozmorov et al. 2004) and similarly, in low (0.1 mM) Mg^{2+} solution, one earlier study shows a potentiation (Thibault, Joly et al. 1989) and my previous master thesis work shows a depression (Li 2000). On the other hand, the way of applying NMDA appears to be an important factor that could account for the different results, because fast application (e.g. direct pressure injection, iontophoresis and high concentration of NMDA for a few seconds) of NMDA mostly induced potentiation (Collingridge, Kehl et al. 1983; Kauer, Malenka et al. 1988; Thibault, Joly et al. 1989; Asztely, Hanse et al. 1991; McGuinness, Anwyl et al. 1991a,b). Relatively longer application (e.g. bath application for several minutes) tends to induce depression (Lee, Kameyama et al. 1998; Kamal, Ramakers et al. 1999; Li, Dozmorov et al. 2004). This explanation is also consistent with the fact that the electrical stimulation used for triggering LTP is usually brief and with high frequency, while a lower frequency for a longer period is needed for LTD. In further support of this idea, several studies have demonstrated the importance of temporal factors in determining the direction of the synaptic change (Mizuno, Kanazawa et al. 2001; Dozmorov, Niu et al. 2003). For instance, LTD was not induced unless the duration of the stimulation was long enough. LTP could be induced by either short or long duration of the stimulation and required higher induction intensity than LTD.

In addition, there are also studies showing that application of subthreshold concentrations of NMDA (1 μM, 5 min) does not affect baseline synaptic transmission but causes "metaplastic" changes by preventing the induction of LTP later on (Izumi, Clifford et al. 1992; Izumi, Clifford et al. 1992). In the present study, no NMDA concentrations were below 5 μM and, in most cases, concentrations of at least 30 μM were used.

5.2 NMDA-induced waveform change – a new discovery?

My thesis presents the first comprehensive analysis of waveform prolongation of AMPA EPSPs after NMDA application. But what about the result in itself, is it a new one? If we look in retrospect at previous work done on NMDA application, we conclude that a similar result was obtained already fifteen years ago in this laboratory (Asztely et al., 1991). It was then reported that during NMDA-induced LTP there was a relatively greater increase of the amplitude of the EPSP than of the initial slope. This result demonstrated a waveform change caused by NMDA application but no attempts were made to elucidate its mechanisms. It appeared to be more long-lasting than our present waveform prolongation and was superimposed on LTP as compared to LTD in the present case. Nevertheless, it seems likely that it represents the same phenomenon

	Method	Perfusion solution	Results
GL Collingridge 1983	Iontophoresis 100 mM, 50 nA, 10-120 sec	Normal Mg^{2+}	STP
JA Kauer 1988	Iontophoresis 100 mM, 0.4 µA, 5-20 sec	Normal Mg^{2+}	STP
O Thibault 1989	Fast bath appli. by cannula	Low Mg^{2+}, glycine+spermine	LTP
F Asztely 1991	Pressure pulse ejection, 5 mM, 10-100 msec	Normal Mg^{2+}	LTP and wave-form change
N McGuinness 1991	Bath appli. 130 µM, 10 sec	Normal Mg^{2+}	STP
H-K Lee 1998	Bath appli. 20 µM, 3 min	Normal Mg^{2+}	LTD
A Kamal 1999	Bath appli. 20 µM, 3 min	Normal Mg^{2+}	LTD
G Broutman 2001	Bath appli. 50 µM, 5 min	Normal Mg^{2+}	LTP
R Li 2001 master thesis	Bath appli. 30 µM, 4 min	Low Mg^{2+}	LTD and wave-form change
R Li 2004	Bath appli. 30 µM, 4 min	Normal Mg^{2+}	LTD and wave-form change

Table 2 Comparison of previous studies dealing with NMDA application.

and the mentioned discrepancies are not surprising in view of differences in experimental design. While the present thesis work is based on bath application of NMDA for several minutes, the prior study used application via the recording pipette by means of a brief (10-100 ms) pressure pulse. Also the experimental animals differed, young rats being used in this thesis whereas the prior study employed adult guinea pigs.

It might well be that the waveform phenomenon under consideration actually occurred in some of the previous works but without being noticed. In one case of early studies of NMDA application, McGuinness showed that very short-term perfusion of high concentration of NMDA (130 μM, 10 s) resulted in an STP lasting around 15 min (McGuinness, Anwyl et al. 1991a; McGuinness, Anwyl et al. 1991b). EPSP traces were taken before and after NMDA application to demonstrate the increase of the amplitude caused by NMDA. Although the waveform change did not receive any attention (the text actually mentions that EPSPs underwent a uniform change), an effect similar to ours is visible by inspecting the traces shown (McGuinness, Anwyl et al. 1991a; McGuinness, Anwyl et al. 1991b). Similarly, other prior studies of NMDA application did not detect a waveform change (Collingridge, Kehl et al. 1983; Kauer, Malenka et al. 1988; Thibault, Joly et al. 1989; Lee, Kameyama et al. 1998; Kamal, Ramakers et al. 1999). However, as will be detailed in the following, several previous studies dealing with different forms of stimulus-induced potentiation have reported altered waveforms of EPSPs.

5.3 Waveform changes – different induction, same expression?

In the present study, we carried out several tests to investigate the mechanism of NMDA-induced waveform change. We suggest that NMDA receptor activation mediates an increase of Ca^{2+} in the postsynaptic neuron which results in the change of AMPA receptor kinetics, especially involving flip type AMPA receptors. Reviewing previous studies about waveform changes, we found out that in addition to NMDA application, many other methods are also able to induce similar changes. For instance, LTP in CA1 area that was caused by a strong, prolonged induction was accompanied by a pronounced increase of the peak and later portion of the EPSP (Hess and Gustafsson 1990). Similar to normal LTP, this phenomenon depended on NMDA-receptor activation but, in contrast, it was not specific to the tetanized synapses. Another study showed that a brief tetanization at a moderate intensity was sufficient to generate a similar, unspecific waveform change in the dentate gyrus (Hanse, Asztely et al. 1991). Waveform changes also seem to be present in connection with “NMDA receptor independent LTP” where intense tetanization was applied during blockade of

NMDA receptors (Grover and Teyler 1992; Kleschevnikov and Routtenberg 2001). Moreover, intracellularly applied voltage pulses induced a short-term potentiation that seemed to be associated with a prolongation of the synaptic current (Kullmann, Perkel et al. 1992; Chen, Hanse et al. 1998).

The unspecific or heterosynaptic nature of the above stimulus-induced plasticities might be explained in different ways. In the case of NMDA-dependent effects, the influxed calcium ions may spread from one dendritic spine to adjacent ones. Alternatively, the large depolarization caused by NMDA receptor activation could trigger long-range changes via the associated depolarization (Xie, Liaw et al. 1997). In our case, we did several tests to explore the possible involvement of depolarization-mediated factors and found that they were unlikely to be related with NMDA-induced plasticity. Similar tests were not performed for the observed stimulus-induced, NMDA-dependent effects (Hess and Gustafsson 1990; Hanse, Asztely et al. 1991). However, blocking hyperpolarizing K^+ currents by 4-amino-pyridine or TEA also resulted in prolongation of EPSPs, suggesting that depolarization could play a role (Hanse, Asztely et al. 1991). Blocking L-type VDCCs by nifedipine eliminated the effect in some of the cases (Hanse, Asztely et al. 1991) suggesting that associated waveform changes are mediated via an unspecific calcium influx. A strong case for involvement of VDCCs also comes from the above-mentioned studies using intracellularly applied depolarization (Kullmann, Perkel et al. 1992; Chen, Hanse et al. 1998).

In conclusion, there are in the literature a number of cases of AMPA EPSP waveform change induced in different situations. Additionally, it has been reported that a small waveform change is also a characteristic of normal LTP. The protocols for the induction are obviously different, but could it be the same expression mechanism that is involved in all the cases? First, consider that despite the different induction protocols, there may be a common element in terms of postsynaptic Ca^{2+} influx, either via NMDA receptor channels or via VDCCs. With respect to the expression mechanism, it can be noted that the reported effects are of both short-term and long-term type, and so can not be fully equivalent. Some of the cited studies mention changes of K^+ channels as possible mediators whereas in our case we have strong support for involvement of changes in AMPA channel kinetics. This conception agrees with ideas about the associated minor effect of a normal LTP on EPSP waveform (Ambros-Ingerson, Larson et al. 1991; Ambros-Ingerson, Xiao et al. 1993; Stricker, Field et al. 1996). In absence of solid evidence, my tentative guess is that the expression mechanism could be the same in some but not all of the cases. To further resolve this issue, it would be useful to test the interaction between AMPA receptor

modulators, such as aniracetam and CTZ that we used, and the various forms of waveform change.

5.4 The reasons for the initial extinction and its gradual recovery

Almost all the studies about NMDA application revealed an initial extinction of responses immediately after NMDA treatment. Following the extinction there is a gradual recovery. Although there is still no clear-cut explanation for these early phenomena, both presynaptic and postsynaptic factors might be involved. In the following discussion, we will focus on processes that might cause the extinction. The recovery phase could be imagined as due to the ceasing of these processes but other possibilities exist.

Among possible explanations for the extinction of EPSPs, loss of the driving force in the postsynaptic neuron has been considered as the main reason. NMDA application leads to a large activation of NMDA receptors on the postsynaptic membrane, mediating ion influx and depolarization of the postsynaptic neuron. If the depolarization lifts the membrane potential to nearly zero, there will be no driving force for net current of ions through glutamate activated channels, resulting in a disappearance of postsynaptic responses.

The most direct evidence for a presynaptic effect after NMDA application is the observation of a change in the presynaptic volley during the application time: disappearing for a short period, growing up to a potentiated level, and returning to its original size (Li 2000; Suarez and Solis 2006). But what causes the change of presynaptic volley? It is likely that NMDA application not only activates NMDA receptors located on the postsynaptic membrane, but also presynaptic NMDA receptors. The latter may lead to depolarization of axons and possible failure of action potentials on the presynaptic side due to sustained Na^+ channel inactivation, so resulting in the presynaptic volley disappearing. On the other hand, since NMDA application would cause depolarization all along the axons, it would also influence conditions around the stimulating electrode. It might be that following a period of strong depolarization and associated volley failure, a moderate level of depolarization would facilitate the setting up of action potentials, and so the same stimulation would become more effective. More fibres would be recruited, leading to a temporary increase in volley amplitude as observed.

A way in which presynaptic depolarization could lead to loss of synaptic transmission is via excessive release and resulting depletion of presynaptic transmitter stores. The

depletion is expected to persist for some time after ceasing of depolarization, in line with the observation that extinction of EPSPs outlasted the effect on the presynaptic volley. However, presynaptic depolarization could also occur secondarily to postsynaptic depolarization via K^+ signalling. Thus, large depolarization of the postsynaptic cell causes K^+ to efflux and so increases the concentration of this ion in the extracellular space. This elevation of extracellular K^+ may lead to depolarization of presynaptic elements, affecting the volley as well as leading to depletion of transmitter as described above.

Actively down-regulating transmitter release via a "homeostatic" post-to-presynaptic interaction might also be possible. Large activation of NMDA receptors causes a major Ca^{2+} influx into the postsynaptic cell, and one may speculate on the existence of a feedback process to protect the cell from over-activation ("the postsynaptic neuron does not know that the agonist is exogeneous"). Sending a retrograde messenger, e.g. NO, from the postsynaptic to the presynaptic side might then lead to a decrease in transmitter release. In agreement with the idea of a presynaptic mechanism operating during and shortly after NMDA application, a recent study showed an alteration of paired-pulse facilitation during the period of NMDA treatment (Mallon, Auberson et al. 2005). It would be interesting to test for possible changes in paired pulse facilitation during the recovery phase in our experimental situation. However, the stable phase of NMDA-induced LTD appears to occur without changes in paired pulse facilitation (Lee, Kameyama et al. 1998).

In supplementary experiments, we applied NMDA with different concentrations, 5-60 μM and found that the duration of the extinction phase is concentration-dependent, with 5 μM NMDA hardly inducing any change and 60 μM being accompanied by a longer disappearance of EPSPs. We did not try concentrations higher than 60 μM, considering that cell death would otherwise contaminate our results.

While the gradual recovery might be due to the ceasing of early processes responsible for extinction, it is also possible that the recovery, which is sometimes remarkably slow, is related to the late phase of NMDA-induced plasticity. In analogy with LTP that is often accompanied by an early transient phase, referred to as STP (Kauer, Malenka et al. 1988; Hanse and Gustafsson 1994), stimulus-induced LTD is often seen with a more pronounced depression early on, interpreted as STD (Artola and Singer 1993). It could be that a similar STD accompanies NMDA-induced LTD. Additionally, the observed EPSP prolongation during the recovery phase seems to suggest that the issue of recovery may be a complex one.

5.5 Debate about the involvement of AMPA and NMDA components

The relative contribution of AMPA and NMDA components to different types of synaptic plasticity is a key question, which is not yet resolved, despite long and intensive research. Our results provide some new pieces of information that contribute to clarifying this issue. Efforts were primarily directed to stimulus-induced LTP and NMDA-induced LTD but with somewhat different questions in mind. In the LTP case, we searched for evidence for a transition between early and late plasticity phases, paying special attention to the 1-4 h period. With some exceptions, previous studies on AMPA-NMDA contribution to LTP have a much shorter time perspective. The main reason for studying AMPA-NMDA contribution to NMDA-induced LTD was to compare with previous data on stimulus-induced LTD. Here, we were mainly interested in the first 1-2 h. The results may help to compare the two forms of LTD, considered in some previous work to be equivalent. Additionally we made on-the-fly observations of AMPA-NMDA involvement in a form of 'slow LTD', confirming previous results. In addition to the cases of LTP and LTD, we also consider the transient, NMDA-induced EPSP waveform change.

5.5.1 LTP

During the years, we have seen all kinds of results regarding the AMPA-NMDA issue in LTP. Some previous studies demonstrate a selective AMPA change by the induction of LTP (Kauer, Malenka et al. 1988; Muller, Joly et al. 1988; Muller, Arai et al. 1992; Perkel and Nicoll 1993). Others describe an equal or variable involvement of AMPA and NMDA receptors (Clark and Collingridge 1995; O'Connor, Rowan et al. 1995; Xiao, Karpefors et al. 1995b; Xiao, Niu et al. 1996; Watt, Sjöström et al. 2004). There are also studies that support the idea of a prevailing change of the NMDA component after LTP (Bashir, Alford et al. 1991; Berretta, Berton et al. 1991). Other work has even described a decrease of the NMDA response after LTP (Zhao, Toyoda et al. 2005). In the present work we found that stimulus-induced LTP involves both AMPA and NMDA components, but the increase of AMPA is about twofold larger than that of NMDA. The ratio keeps constant as measured both at 1 h and 4 h after LTP induction.

The constant AMPA/NMDA ratio in the 1-4 h period was somewhat unexpected since several other data suggest that LTP undergoes a phase transition during this time. For instance, it has been shown that new LTP can be induced 4 h after a first saturated LTP, but not after 2 h (Frey, Schollmeier et al. 1995). Moreover, there is common agreement that LTP develops from a protein synthesis independent phase to a protein

synthesis dependent one during the first 3 hours (Frey, Huang et al. 1993; Nguyen and Kandel 1996). Our AMPA-NMDA results do not substantiate the idea of a transition/consolidation of LTP around 3 h. However, such a phenomonon can not be excluded as it may involve other biochemistries, not reflected in terms of altered AMPA-NMDA ratio.

The present data do not answer the question about what happens to AMPA/NMDA during the first hour after LTP induction. Previous results in our lab suggest that the NMDA component is initially quite small and increases during the first hour, eventually becoming similar in size to the AMPA component. A problematic issue is that those experiments were probably carried out on depressed responses due to prolonged recording of NMDA currents. In recent supportive experiments with an improved technique, we largely confirmed the result of a growing NMDA component during the first hour (not included in Paper II). However, due to the use of a partially blocked NMDA response, the final AMPA/NMDA ratio was hard to quantify. As shown by the present data, the NMDA component does not catch up with the AMPA component but the potentiation of it remains at about half the value for AMPA. In line with the idea of stabilization of the AMPA/NMDA ratio, a recent study suggests a "homeostatic" regulation, showing a gradual return to the baseline value after an initial 1-2 h of varying value following LTP (Watt, Sjöström et al. 2004). A similar regulatory process might also function in our case except that the ratio after LTP is different from the pretetanus value.

It is possible that LTP results in both pre- and postsynaptic changes: by increasing transmitter release it potentiates both AMPA and NMDA receptors; and by inserting new AMPA receptors or "AMPAfication" it causes further change of the AMPA response. Taking the pre- and postsynaptic effects together, the AMPA component so displays a larger increase than the NMDA component. According to a recent model (Lisman and Raghavachari 2006), a parallel increase of AMPA and NMDA responses can also be accounted for by a parallel growth of pre- and postsynaptic elements, similar to some early suggestions (Xiao, Karpefors et al. 1995a).

A range of experimental designs have been used in experiments on AMPA versus NMDA receptor involvement. Factors, such as recording type (extracellular or intracellular; acute or cultured slices) and induction protocol (strength, paring, tetanization, or chemical) might influence the degree of the contribution of NMDA receptors to LTP (Anikstejn and Ben-Ari 1995; Xie, Barrionuevo et al. 1996; Bayazitov and Kleschevnikov 2000; Lisman 2003; Watt, Sjöström et al. 2004). Differencies at this level could explain some of the inconsistencies among investigations. For example, some of the works about LTP were performed on

composite EPSPs (Xiao, Karpefors et al. 1995a,b; Xiao, Niu et al. 1996), and in this case NMDA activation occurred throughout the experiment. Even a small activation of NMDA receptors can weaken LTP (Xiao, Niu et al. 1996) and cause depression in naive inputs (Dozmorov, Niu et al. 2003). This might lead to a contamination of the results. In contrast, we designed a set of experiments with minimal exposure of synapse to NMDA currents, providing a better model to study LTP in a long time range. It was found that LTP induced by different protocols (tetanization or TBS) caused a similar 2-to-1 potentiation of AMPA and NMDA components, implying that different conditions may also result in a similar mechanisms involved.

5.5.2 LTD

Compared to the variable results on LTP, the involvement of AMPA and NMDA receptors in LTD appears to be less problematic. Thus, there is a consensus that LTD changes the two receptors in a similar way (Perkel and Nicoll 1993; Malenka 1994; Selig, Hjelmstad et al. 1995; Xiao, Karpefors et al. 1995a). In addition to conventional LTD induced by 1-5 Hz stimulation, we have examined another form of LTD induced by subhertz stimulation in low Mg^{2+} solution, here referred to as 'slow LTD' due to its long induction time (1-2 h). Since this slow LTD can occlude the subsequent LTD induced by traditional way, it may be equivalent to conventional LTD (Dozmorov, Niu et al. 2003). In accord with this view, the slow LTD was found to be associated with equal relative involvement of AMPA and NMDA components, i.e. similar to conventional LTD. This result was also observed in the present thesis work.

There are also studies showing that prior conventional LTD can prevent NMDA-induced LTD (Lee, Kameyama et al. 1998), suggesting an agreement of these two types of LTD. However, synaptic plasticity induced by NMDA application appears to be a complex issue involving a multitude of plasticities. Not only can depression as well as potentiation be produced, possibly depending on mode of application. Moreover, as being a main finding of this thesis, NMDA application caused changes in both size and waveform of the EPSP. Consistently, in this study we showed that the size change, i.e. NMDA-induced LTD, was associated with nearly equal, stable depressions of AMPA and NMDA components, supporting the idea of a close relation to stimulus-induced LTD (both conventional and 'slow' type). The issue of AMPA-NMDA in the NMDA-induced waveform change will be dealt with in the following section.

The equal change of AMPA and NMDA components during several types of LTD, induced by stimulation as well as NMDA application, could be due to a reduction of

transmitter release from the presynaptic terminal, or a coordinated postsynaptic change of the two receptors. Since previous work has observed dephosphorylation of the GluR1 subunit of AMPA receptors in NMDA-induced LTD (Lee, Kameyama et al. 1998), we also suggest a similar process on NMDA receptors. Further investigation is needed to understand the mechanism underlying the depression of the NMDA component. As discussed above, a parallel decrease of AMPA and NMDA responses can also be due to parallel pre- and postsynaptic changes (Lisman and Raghavachari 2006).

5.5.3 NMDA-induced waveform change

Different from NMDA-induced LTD, NMDA-induced waveform prolongation is transient and only occurs for AMPA EPSPs. The NMDA EPSPs remained uniformly changed during the whole experiment. Such inconsistent behavior of the receptor types seems to support a postsynaptic expression mechanism. This is also in agreement with the finding that the magnitudes of AMPA and NMDA components recovered at different speeds after NMDA application. A similar conversion of AMPA-NMDA ratio of EPSPs from unequal to equal was shown for LTP during the first hour after induction but no waveform changes seemed to be present for any of the components (Xiao, Karpefors et al. 1995b; Xiao, Niu et al. 1996; Watt, Sjöström et al. 2004).

Since conventional LTD has been considered as a uniform change of EPSPs (Xiao, Niu et al. 1997), similar to NMDA-induced LTD at the late stable phase, the transient NMDA-induced waveform change seems to involve a different mechanism from LTD. Although it is currently not possible to arrive at a complete resolution to this issue, our study succeeds in tracing some basic mechanisms involved. Considering that the AMPA component is the one that selectively undergoes a waveform change, one could imagine several reasons, either an effect directly at AMPA receptor level or changes of other ion channels, here referred to as non-AMPA sources. Using a variety of pharmacological as well as other tools allowed us to exclude the non-AMPA sources and pinpoint the effect as mediated via changes of AMPA channel kinetics. The fact that the waveform was less prolonged in an LTP-treated pathway also provides a clue that these two plasticities might share some common mechanisms, supporting the popular idea of specific AMPA-receptor involvement. LTP itself seems to be associated with only a minor change of the EPSP waveform; however, this change was amplified by applying an AMPA receptor modulator, CTZ (Lin, Brucher et al. 2002). It might be interesting to test the effect of some NMDA receptor modulators (glycine, spermine, spermidine) in a similar manner on both stimulus-induced and NMDA-induced plasticity.

5.6 Composition of NMDA receptors: is a two-week-old rat still young?

The composition of NMDA receptors in the central nervous system changes during development, with the NR2A subunit in receptor complexes being replaced by NR2B subunits. It has been shown that at an early stage, NMDA receptors are largely NR2B type and from 6-10 days postnatal age the expression of NR2A subunit starts (Monyer, Burnashev et al. 1994; Sheng, Cummings et al. 1994). This kind of change gives rise to alterations of synaptic physiology and therefore is very important for the synaptic plasticity as well as its behavioral counterparts. An early study described that NR2A and NR2B subtype NMDA receptors are related with LTP, while NR2C and NR2D subunits are needed for LTD (Hrabetova and Sacktor 1997; Hrabetova, Serrano et al. 2000). Some recent studies focus on the difference between NR2A and NR2B subunits. It has been shown that LTP requires the activation of NR2A subtype NMDA receptors, while hippocampal LTD was shown to rely on NR2B subunits (Liu, Wong et al. 2004; Massey, Johnson et al. 2004). However, there are also studies showing that both NR2A and NR2B subunits are important for LTP or LTD in hippocampus and other brain areas, such as anterior cingulated cortex (ACC) (Berberich, Punnakkal et al. 2005; Toyoda, Zhao et al. 2005; Zhao, Toyoda et al. 2005).

In view of the complex interaction of developmental changes and possible subunit specificity in various forms of plasticity, a basic question for us to solve is what is the composition of NMDA receptors in our test situation. The experimental rats we usually use to investigate synaptic plasticity are around 12-18 days old, a developmental stage where NR2A expression is just turned on. Two selective blockers of NR2A and NR2B subunits were examined here, and the result shows that NR2A and NR2B subgroups make up most of the NMDA response. We also found that NR2A contributes to around 80% and NR2B to only about 20% of the total NMDA-receptor mediated response. It was a bit unexpected for us since we considered two-week rats as young ones, supposed to have more NR2B than NR2A subunits. It seems that once the shift from NR2B to NR2A starts at 6-10 days (Monyer, Burnashev et al. 1994; Sheng, Cummings et al. 1994), it goes rather quick. Similar tests on one week postnatal rats would be needed for future studies. Nevertheless, from other developmental considerations, the rats under study ought to be considered as youngsters; the corresponding entity in humans would be a child. The age used is just after eye opening at 10-11 days (own observations) and puberty in our rats occurs long later, starting at about 5 weeks.

5.7 Why so different results about involvement of NR2A versus NR2B?

In line with our data on NMDA receptor mediated responses, discussed above, this present study also displayed an overweight for NR2A involvement in stimulus-induced LTP and LTD as well as NMDA-induced LTD and depotentiation. Table 3 summarizes some of the previous studies reported in the literature with respect to the involvement of NR2A and NR2B subunits in different forms of synaptic plasticity. It can be noted that there is substantial variation. Though we do not have any in-depth explanation for the differences among results, it can be noted that experimental parameters often varied among the research groups. Those parameters include animal species and age, brain area, type of preparation and recording type, selection of subunit blockers, usage of drugs and composition of perfusion solution, temperature and protocols for inducing synaptic plasticity.

In order to structure the issue a bit and facilitate discussion about possible underlying mechanisms, we propose two alternative working principles, unified and diversified triggering (Fig. 6A, B). In unified triggering mode, we conceive that there is a common pathway, e.g. the final concentration and/or pattern of Ca^{2+} in the spine, which decides the direction of synaptic plasticity. No matter where the sources for the elevated Ca^{2+} are, either through NR2A or NR2B, it is the summation of effects that is taken into account. If one type of receptor is blocked, it is possible then that the other one takes over its role and generates the situation for triggering the synaptic plasticity. On the other hand, in diversified triggering mode, NMDA subunits and target enzymes are supposed to have a specific distribution in the spine, linking certain subunits to certain enzymes. Assuming that the activation of each subtype of receptor occurs locally, only those enzymes that are linked to the activated receptors can be involved, resulting in a certain form of synaptic plasticity.

It seems that our present data fit better with the principle of unified triggering, in view of the finding of a dominant role of NR2A in all types of synaptic plasticity tested in this study. As a supportive result, we found under facilitated induction conditions, where the concentration of Mg^{2+} was lowered, that all the examined types of plasticity could be induced by NR2B subtype receptors alone. In view of these results, it is likely that there is a threshold for the number of NMDA receptors needed to trigger LTP/LTD (see Fig. 6C). When an NR2A inhibitor, NVP, is applied to the slice, about 80% of NMDA receptors are blocked in our case (in terms of their contribution of current). The number of remaining NR2B receptors might not be large enough to induce any plasticity, accounting for the fact that all types of synaptic plasticity viewed in this study were prevented in presence of NVP. However, adding NR2B blocker, Ro/Ife, only partially blocked LTP and no effect on LTD was observed, suggesting

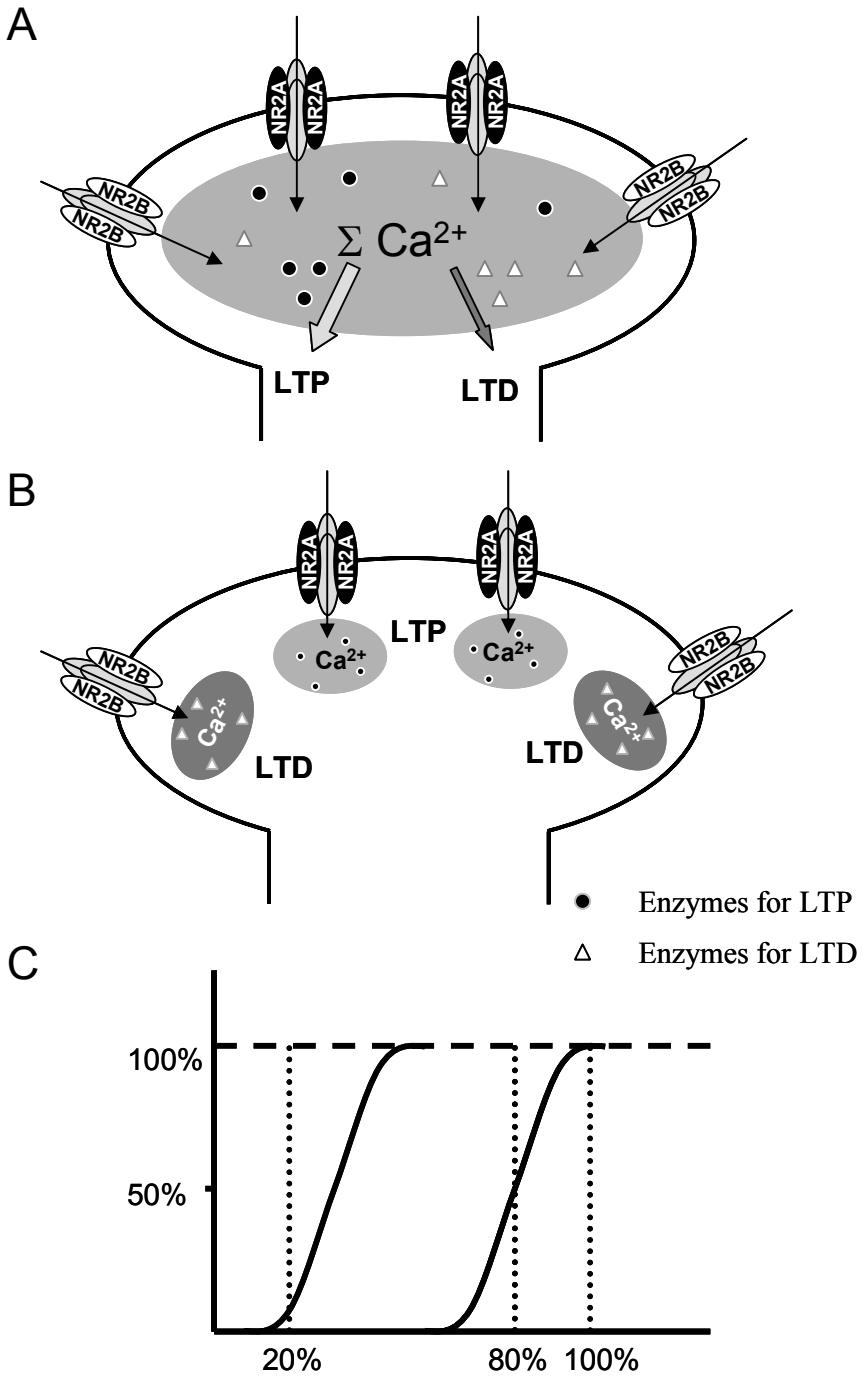

Fig. 6 Two hypotheses about how NMDA receptor subunits contribute to LTP and LTD: A, B. Unified versus Diversified model. C. Induction threshold for LTP and LTD as percentage of the full induction strength. Further description in text.

that the induction threshold for these two forms of synaptic plasticity is different, with more receptors required for LTP than LTD. By lowering the concentration of Mg^{2+} in the perfusion solution, leading to facilitated activation of NMDA receptors by removal of the Mg^{2+} block, we found that NR2B subtype receptors managed to support induction of plasticity. A similar result has also been described by a study where the facilitating condition was created by depolarizing the postsynaptic cell (Toyoda, Zhao et al. 2005).

Those studies showing that different synaptic plasticities require activation of specific NMDA subunits favor the idea of diversified triggering (Liu, Wong et al. 2004; Massey, Johnson et al. 2004). In this case, NR2A and NR2B subunits each have a unique function in which the two types are unable to substitute for each other. Thus, brief high frequency stimulation activates NR2A subtype receptors and induces LTP, while stimulation with lower frequency for a longer period leads to LTD via activation of NR2B type receptors. The possibility was considered that the brief high frequency stimulation can activate both receptor types, but the function of NR2A overcomes that of NR2B due to a larger contribution of NR2A. As a net effect, only LTP would be displayed while LTD would be hidden. As shown in one study, in presence of NVP, the stimulation that usually triggers LTP induced LTD (Liu, Wong et al. 2004).

We have no clear idea about the difference between our results and those above, leading to entirely opposite conclusions about mechanisms. It is also a bit hard to explain that the prior study (Liu, Wong et al. 2004) found a critical role of NR2B in LTD in 3-4 week rats, which are slightly older than the animals we used (2-3 weeks). One could expect that the contribution of NR2B in their experimental rats would be even less than that in our situation. We are inclined to believe our own results and therefore wish to advertise the idea of unified triggering, conceptualized in Fig. 6A and compared with diversified triggering in Fig. 6B.

The debate about the selectivity of NR2A inhibitor NVP-AAM077 brings some uncertainty as to our results since some studies demonstrated that NVP at the concentration we used can also partially block receptors containing NR2B subunits and it may even have some effects on NR2C and NR2D subtype receptors (Feng, Tse et al. 2004; Berberich, Punnakkal et al. 2005; Neyton and Paoletti 2006). Even so, those problems do not challange our main result, i.e. about a predominance of NR2A receptors in both synaptic transmission and plasticity of 12-18 days old rats.

Two very recent publications support our results on NR2A versus NR2B involvement in one or more important aspects (Bartlett, Bannister et al. 2006; Morishita, Lu et al. 2006).

Animal study	LTP	Learning or Memory
Sakimura K 1995	NR2A	Spatial learning
Sprengel 1998	NR2A	Contextual memory
Tang YP 1999	NR2B	Learning and memory
Clayton DA 2002	NR2B	Spatial learning

Slice study	LTP	LTD	Age of animal and brain region
Hrabetova S 1997	NR2A and NR2B	NR2C and NR2D	16-21 days rats hippocampus
Hrabetova S 2000	NR2A and NR2B	NR2C and NR2D	16-21 days rats hippocampus
Bauer EP 2002		NR2B inhibits LTD	12-20 days rats hippocampus
Hendricson AW 2002	NR2B		3-5 weeks rats Hippocampus
Köhr G 2003	NR2A in P>42 NR2B in P14		2/6 weeks rats hippocampus
Liu LD 2004	NR2A	NR2B	3-4 weeks rats hippocampus
Massey PV 2004	NR2A	NR2B	7-12 weeks rats cortex
Toyoda H 2005		NR2A and NR2B	8-12 weeks mice ACC
Zhao MG 2005	NR2A and NR2B		6-8 weeks mice ACC
Berberich S 2005	NR2A and NR2B		P28 mice hippocampus
Mallon AP 2005	NR2A		35-45 days rats LA
Li R 2006	NR2A and NR2B	NR2A (NR2B)	2-3 weeks rats hippocampus

Table 3 Summary of previous results on the role of NMDA receptor subunits in synaptic plasticity and memory.

5.8 NMDA-induced LTD, depotentiation and still another depression?

Long after NMDA application, for instance at one hour or later, the level of EPSPs is largely constant and the depression so revealed is generally considered to be equivalent with stimulus-induced LTD (Lee, Kameyama et al. 1998). As shown in this thesis, the contributions of AMPA and NMDA components in NMDA-induced LTD are nearly equal, similar to the case with standard LTD (Perkel and Nicoll 1993; Malenka 1994; Selig, Hjelmstad et al. 1995; Xiao, Karpefors et al. 1995a). NMDA-induced depotentiation is another stable process. It remains to find out, however, whether LTP and NMDA-induced depotentiation are complementary in terms of the underlying changes of AMPA versus NMDA components. In our case, the relative degree of depression was consistently larger in a previously potentiated pathway (depotentiation) than in a naive one (LTD). This was the case despite large variation in both cases, suggesting a possible relation between depotentiation and LTD. The larger quantiatative value of depotentiation than that of LTD could be viewed in terms of "more room for depression". Still, several prior studies suggest that the two processes differ from each other with respect to underlying mechanisms. Thus, LTP and depotentiation were shown to involve phosphorylation or dephosphorylation of AMPA receptor subunits at specific sites, found to be different from the sites involved in LTD and its reversal de-depression (Malenka and Nicoll 1993; Stanton 1996; Barria, Muller et al. 1997; Lee, Barbarosie et al. 2000). In our opinion, it is hard to know how these sites could be involved in an entirely exclusive manner. We therefore conceive of LTP and depotentiation as related processes, which are complementary at least within some limited working range. With respect to the induction, it can be noted that our results demonstrated that NR2A subunits are predominantly involved in both depotentiation and LTD.

Previous results (Lee, Kameyama et al. 1998) as well as our present findings show that reinstatement of LTP after depotentiation by NMDA was not complete. There may be at least two explanations for this impairment of repotentiation. One possibility is that the second instance of LTP was less successful because of weakened induction conditions related to the NMDA application, a possible case of metaplasticity (Abraham and Bear 1996). Another possibility is that NMDA affects the potentiated pathway not only by depotentiation, which is complementary to LTP, but also by another form of depression which is not complementary. Such a non-complementary depression would also be expected to be induced in a naive pathway. Of the two explanations, we tentatively favor the latter although it involves an additional component and hence additional complexity. While I initially had hoped to arrive at a definite conclusion on the issue, the fact is that no hard evidence is yet available, and the following reasoning provides only circumstantial evidence for the existence of an

unspecific (non-complementary) component of NMDA-induced depression. In any case the proposed model simply accounts for the impairment of repotentiation after depotentiation by NMDA. Possibly, the rather constant depotentiation/depression ratio despite the large span of depressions is also supportive (see regression line in Fig. 5C of Paper III). In paper IV, the similarity between recovery time courses of depressed and depotentiated pathways was taken to indicate a transient form of unspecific depression, later converting into stable form (see Fig. 5A of Paper III; Fig. 6A of paper IV). Further repotentiation experiments will be needed to resolve the issue of an unspecific depression component. These experiments should involve further LTP inductions in both pathways, allowing the saturation level to be assessed.

5.9 Concluding remarks

The understanding of mechanisms for different forms of synaptic plasticity is a key point in exploring the function of our brain. In this present work we have investigated several forms of synaptic plasticity in 2-3 weeks old rats, both chemically induced and stimulus-induced variants. Special attention has been paid to the mechanism underlying NMDA-induced waveform prolongation of AMPA EPSPs and a change of AMPA receptor kinetics has been suggested for it. Our work adds a useful reference to the studies about waveform changes induced by different induction protocols. It would be interesting to see if other waveform changes could also interact with AMPA receptor modulators, and therefore share similar mechanisms as we found here. Regardless of prior work and opinions about the involvement of AMPA and NMDA receptors in LTP/LTD, our study for the first time deals with this issue on a type of chemically induced LTD. It is also one of the few studies that deal with this problem for LTP in a long-term range (> 3h). Although in the latter case we did not provide data for the first hour after LTP induction, our study is likely to provide an accurate account of the changes during the later part since we took measures to minimize confounding factors. A careful future study dealing with this event for the first hour would be appreciated. In this thesis work we also tried to find out the role of different NMDA receptor subunits in some types of synaptic plasticity. Although our result is a bit contradictory to some of other previous studies, it is reasonable as it forms a consistent picture of the two types of subunits and their roles in mediating plasticity. Our results support the idea of a “unified” triggering mode for synaptic plasticity, implying summation of induction events within the dendritic spine, regardless of their origin. Still, a similar study on younger rats (e.g. 1 week), which are supposed to have a larger proportion of NR2B subunits, might provide a better view of this issue.

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APPENDIX (PAPERS I - IV)