Department of Physiology
Institute of Neuroscience and Physiology
at Sahlgrenska Academy
University of Gothenburg

DEVELOPMENTAL PLASTICITY OF THE GLUTAMATE SYNAPSE: ROLES OF LOW FREQUENCY STIMULATION, HEBBIAN INDUCTION AND THE NMDA RECEPTOR

JOAKIM STRANDBERG

UNIVERSITY OF GOTHENBURG

2010

ISBN: 978-91-628-7882-5
http://hdl.handle.net/2077/21195

Printed by Geson Hylte Tryck, Göteborg, Sweden 2010

DEVELOPMENTAL PLASTICITY OF THE GLUTAMATE SYNAPSE: ROLES OF LOW FREQUENCY STIMULATION, HEBBIAN INDUCTION AND THE NMDA RECEPTOR

Joakim Strandberg
Department of Physiology, Institute of Neuroscience and Physiology, Univeristy of Gothenburg, Sweden, 2010

Abstract
The glutamate synapse is by far the most common synapse in the brain and acts via postsynaptic AMPA, NMDA and mGlu receptors. During brain development there is a continuous production of these synapses where those partaking in activity resulting in neuronal activity are subsequently selected to establish an appropriate functional pattern of synaptic connectivity while those that do not are elimimated. Activity dependent synaptic plasticities, such as Hebbian induced long-term potentiation (LTP) and low frequency (1 Hz) induced long-term depression (LTD) have been considered to be of critical importance for this selection. However, in the neonatal brain the glutamate synapse displays a seemingly distinct plasticity in that even very low frequency stimulation (0.05-0.2 Hz) results in depression of the AMPA receptor mediated signaling and hence to possible synaptic elimination. The aim of this thesis was to investigate the relationship and interaction between this very low frequency induced plasticity and the more conventional forms of synaptic plasticity, such as mGlu receptor dependent LTD and NMDA receptor dependent LTP and LTD, using the neonatal rat hippocampal CA3-CA1 synapse as a model synapse.

This thesis shows that very low frequency induced depression is related to NMDA receptor dependent LTD. While elicited even during NMDA receptor blockade, this plasticity is facilitated and stabilized by NMDA receptor activity and largely occludes NMDA receptor dependent LTD. Surprisingly, considering their role in conventionally induced LTD, mGlu receptors were not found to participate in either the very low frequency induced depression or in low frequency induced long-lasting depression. A preceding LTP-inducing Hebbian stimulation was found to only partially stabilize against the very low frequency induced depression, and possibly also only in a temporary manner.

In conclusion; during brain development glutamate activated AMPA receptors are very easily lost upon activation rendering these synapses AMPA silent, and Hebbian activity will only temporarily rescue them from AMPA silence. Thus, synapses in the developing brain will maintain their AMPA signaling only by more or less continuous participation in cooperative neuronal activity, synaptic activity outside this context leading to AMPA silencing and possible elimination.

Keywords: AMPA receptor, development, glutamate, hippocampus, long-term depression, long-term potentiation, NMDA receptor, synaptic depression

POPULÄRVETENSKAPLIG SAMMANFATTNING

Den mänskliga hjärnan består av cirka 100 miljarder nervceller som skickar signaler till och aktiverar varandra via kopplingar som kallas synapser. Genom dessa synapser är våra nervceller sammankopplade i ett stort antal funktionella nätverk inom vilka samtidig aktivering av de ingående nervcellerna utgör det neuronala underlaget för våra tankar och beteende. Under hjärnans utveckling måste således inte bara nervceller bildas och hamna på sin rätta position i hjärnan utan de måste också kopplas ihop på ett korrekt sätt. Att detta sker är av yttersta vikt, och störningar kan ge upphov till sjukdomar såsom autism och olika typer av mentala funktionshinder. Under hjärnans utveckling bildar först varje enskild nervcell enstaka synapser med många andra nervceller. Därefter förstärks synapser mellan de nervceller som är aktiva tillsammans (funktionell aktivering), medan de synapser som inte ingår i samtidig aktivitet (icke-funktionell aktivering) försvinner. Denna aktivitetsberoende kontroll av synapserna kallas för synaptisk plasticitet. Det är också via denna synaptiska plasticitet som hjärnan senare skapar minnen genom bildandet av nya nätverk av nervceller.

I avhandlingen har jag undersökt en form av synaptisk plasticitet som specifikt påvisats i hjärnan hos nyfödda. Denna plasticitet består i att signaleringen i synapserna även vid mycket sparsam icke-funktionell aktivering tystas, en plasticitet som möjligen är ett första steg i elimineringen av en synaps. Målet med avhandlingen har varit att skapa bättre kunskap om egenskaperna hos denna form av plasticitet. I dessa studier har jag använt mig av ett in vitro preparat, tunna skivor av hjärnvävnad från neonatala råttor, tagna från hippocampus, en del av hjärnan som är viktig för bland annat minne och inlärning. I detta preparat har jag elektriskt stimulerat och registrerat aktivitet från synapser med glutamat som signalsubstans. I motsats till tidigare fann jag att i stort sett inga av dessa synapser var immuna mot denna nedtystning, även om denna process kan ta längre tid för en del av synapspopulationen. Jag fann vidare att nedtystningen kan bli långvarig om inte synapsen utsätts för ett funktionellt aktiveringsmönster. Att en synaps genom funktionell aktivering reaktiveras ger dock bara ett kortvarigt skydd då en sparsam icke-funktionell aktivering återigen kan tysta den.

Min slutsats är att synapser under hjärnans tidiga utveckling uppvisar en särskild sårbarhet för icke-funktionell aktivering, vilket leder till en nedtystning av synapsen och i slutändan till dess eliminering. För att en nybildad synaps skall fortleva behöver den kontinuerligt aktiveras tillsammans med andra synapser och kan då fortsätta leda signaler och bli en del i ett funktionellt nätverk av nervceller.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Strandberg J., Wasling P. and Gustafsson B.
Modulation of low frequency induced synaptic depression in the developing CA3-CA1 hippocampal synapses by NMDA and metabotropic glutamate receptor activation.
Journal of Neurophysiology (2009) 101:2252-2262
Used with permission from The American Physiological Society

II. Strandberg J. and Gustafsson B.
Lasting activity-induced depression of previously non-stimulated CA3-CA1 synapses in the developing hippocampus; critical and complex role of NMDA receptors.
In manuscript

III. Strandberg J. and Gustafsson B.
Hebbian activity does not stabilize synaptic transmission at CA3-CA1 synapses in the developing hippocampus.
In manuscript

TABLE OF CONTENTS

ABBREVIATIONS

ACSF	Artificial cerebrospinal fluid
AIDA	(RS)-1-aminoindan-1,5-dicarboxylic acid
AKAP 79/150	A-kinase anchoring protein 79/150
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP2	Adaptor protein 2
Arc/Arg3.1	Activity-regulated cytoskeleton-associated protein/Activity-regulated gene of 3.1 kb
CA	Cornu ammonis
CaMKII	Calcium-calmodulin-dependent kinase II
CNS	Central nervous system
CV	Coefficient of variation
D-AP5	D(-)-2-amino-5-phosphonopentanoic acid
DHPG	(RS)-3,5-dihydroxyphenylglycine
EPSP	Excitatory postsynaptic potential
ER	Endoplasmatic reticulum
fEPSP	Field excitatory postsynaptic potential
FMRP	Fragile X mental retardation protein
GABA	γ-aminobutyric acid
GKAP	Guanylate kinase-associated protein
GluA	AMPA receptor subunit
GluK	Kainate receptor subunit
GluN	NMDA receptor subunit
GRIP/ABP	Glutamate receptor interacting protein/AMPA receptor binding protein
IPSP	Inhibitory postsynaptic potential
KAR	Kainate receptor
LFS	Low-frequency stimulation
LFS-LTD	LFS induced LTD
LTD	Long-term depression
LTP	Long-term potentiation
mGluR	Metabotropic glutamate receptor

MAPK	Mitogen-activated protein kinase
MSPG	(RS)-a-methyl-4-sulfonophenylglycine
NMDA	N-methyl-D-aspartate
NP	Neuronal pentraxin
NSF	N-ethylmaleimide sensitive fusion protein
P	Postnatal day
P_r	Probability of release
P_{ves}	Probability of release for a single vesicle
PICK-1	Protein interacting with C kinase
PKA	Protein kinase A (cAMP-dependent protein kinase)
PKC	Protein kinase C
PLC	Phospholipase C
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2A
PP2B	Protein phosphatase 2B (calcineurin)
PSD	Postsynaptic density
PSD-MAGUK	Postsynaptic density-membrane associated guanylate kinases
STEP	Striatal-enriched protein tyrosine phosphatase
TARP	Transmembrane AMPAR regulatory protein
VGCC	Voltage gated Ca^{2+} channel

INTRODUCTION

During human brain development some 100 billion neurones (nerve cells) are formed, these neurones thereafter making direct physical contacts, synapses, with each other. Each neurone will make synapses with thousands of other neurones thereby forming neuronal networks that ultimately will be capable of handling everything from simple reflexes to higher cognitive functions. These neuronal networks are formed in two distinct phases. During the first phase neurones send axonal branches towards each other governed by guidance molecules ensuring that synapses are established in appropriate brain regions (reviewed by O'Donnell et al., 2009). During the second phase mechanisms dependent on the neuronal activity will form the more precise pattern of neuronal connectivity, linking neurones that are active together within a functional context (reviewed by Lu et al., 2009a). Synaptic connectivity is thus not only genetically pre-determined, but also determined by the operation of processes that ensures the survival of functionally relevant synapses while functionally irrelevant synapses are removed (Waites et al., 2005). While in general much is now known about activity dependent modification of synapses, so called synaptic plasticity, the specific nature of the activity dependent processes operating in the developing brain and their role in synapse selection are still largely unknown. Kandel and O'Dell (1992) suggested over a decade ago that the synaptic plasticity, described in the more mature brain as involved in learning and memory, can be seen as a model for synaptic plasticity also in the developing brain. In line with this notion, experimental studies on synaptic plasticity have only to a lesser degree been concerned with the possible relevance of the age of the experimental animal. Results obtained from animals at different ages have thus often been lumped together when explaining the nature and mechanisms of synaptic plasticity. However, while both brain development and learning involve brain organization and reorganization, there may well be different requirements for synaptic organization/reorganization requiring different synaptic plasticities during brain development and during learning, respectively. In fact, more recent studies have shown forms of plasticity that only exist in the developing brain, as well as shown that activity patterns not resulting in synaptic modification in the mature brain do so in the developing brain (Kidd and Isaac, 1999; Xiao et al., 2004; Lauri et al., 2006; Abrahamsson et al., 2007).

In this thesis I will specifically focus on a recently described form of plasticity which is predominantly found in the developing brain and which may be a key player in synapse

selection (elimination/stabilization). Thus, in the developing hippocampus weak synaptic activation at very low frequencies, such as 0.05-0.2 Hz, easily results in a large depression explained by a total loss of postsynaptic receptors from activated synapses (Xiao et al., 2004; Abrahamsson et al., 2005). This result suggests that synaptic transmission in the developing brain can be particularly labile so that synapses even sporadically active can lose their signaling. As shown by prolonged (tens of minutes) interruption of synaptic stimulation, this activity-evoked depression is in itself labile in that synaptic transmission re-appears during such an interruption (Abrahamsson et al., 2007). This very low frequency induced depression is thus not equal to synaptic elimination. However, such a loss of postsynaptic receptors may be the initial necessary step towards elimination if occurring too frequently during a certain period of time, for example by leaving the synapse exposed to complement factor binding to pentraxin (Perry and O'Connor, 2008) and subsequent physical elimination (Stevens et al., 2007). On the other hand, when a synapse is active in conjunction with many other synapses the transmission can be regained and seemingly stabilized (Xiao et al., 2004; Abrahamsson et al., 2008). Synapses active in a functional context will thus keep, or regain, its signaling. Based on the now existing data concerning this form of plasticity, one can envisage a scenario in which synapses are born with a labile transmission that can result either in loss of transmission and later elimination, or to a stable transmission and survival. Loss of postsynaptic receptors and subsequent elimination is thus the fate suffered by synapses that do not properly participate in network activity together with a sufficient number of other synapses, whereas synapses that do so retain their receptors and survive (Hanse et al., 2009). Such activity-driven synapse stabilization should thus be an ongoing process in the developing brain as indicated by the fact that already by the 2nd postnatal week of the rat only 30-40% of the synaptic response is depressed, i.e., that only 30-40% of the synapses remain labile (Abrahamsson et al., 2007).

A problem however with this scenario is that it is still based on a restricted set of data. For example, while the very low frequency induced depression seemingly reaches a plateau level after some 100 synaptic activations, in a case where more prolonged activation was given, further depression was observed (Abrahamsson et al., 2007). To what extent the majority of the synapses by the 2nd postnatal week have become resistant towards such very low frequency activation is thus unclear. Likewise, the stabilization induced by participation in conjunctive synaptic activity has also only been examined over a restricted time period, which

casts doubt on the idea that such activity actually results in long-term synaptic stability. Whether this very low frequency induced depression actually constitutes a unique plasticity process or forms part of previously known synaptic plasticity also remains unsettled. In this thesis I will put the above scenario to a more extensive experimental test, by examining synaptic transmission using electrophysiological recordings from slices of rat hippocampus, a brain structure commonly used for this kind of investigation. I will specifically examine the glutamatergic monosynaptic connection between CA3 and CA1 pyramidal cells (the CA3-CA1 synapse), an often used model for the study of brain synapse function. I will also focus my experiments on 8-12 days old rats, a time period that corresponds roughly to the last months of the pregnancy in human terms.

In the remaining part of the INTRODUCTION I will summarize the basic characteristics of the glutamate synapse in terms of its composition and physiology, specifically with respect to the postsynaptic aspects of AMPA signaling and focusing on results obtained using the CA3-CA1 synapse as a model. In doing this I first wish to emphasize the fact that essentially none of these results has been obtained on the CA3-CA1 synapse in situ in the living brain. Instead, these results have been obtained using acute slices from rats, or mice, at different ages, or cultures of hippocampus, or on dissociated hippocampal cell cultures. Moreover, these experiments have been performed at temperatures varying from room temperature (~ 20° C) to close to the physiological condition. This variation in type, age and temperature of the experimental preparation may contribute to the often multifaceted, at times even contradictory, results obtained regarding the workings of the CA3-CA1 synapse. In the summary below I will generally disregard this variability in experimental condition with the exception of results obtained in neonatal (≤ 12 postnatal day) rats. Thus, when not directly specified the experimental data can have been obtained in any experimental situation except from a neonatal preparation.

The glutamate synapse

The amino acid glutamate is the major excitatory transmitter in the CNS and glutamate synapses constitute ~ 90% of all synapses onto CA1 pyramidal cells (Megias et al., 2001). Glutamate is released from the presynaptic terminal and mainly acts postsynaptically through four different kinds of receptors, three of which are an integral part of the channel protein (ionotropic receptors) and one which is a G-protein-coupled (metabotropic) receptor. At the

synapse the ionotropic glutamate receptors, the AMPA receptor (AMPAR), the NMDA receptor (NMDAR), and at some synapses, the kainate receptor (KAR), named after their respective main agonists, are highly accumulated in clusters at the postsynaptic density (PSD), a structure which in addition contains a large number of proteins, such as kinases, phosphatases, scaffolding proteins and adaptor proteins associated with each other and with the cytoskeleton (for review see; Okabe, 2007; Feng and Zhang, 2009). AMPARs, NMDARs and KARs can also be expressed at the presynaptic terminal (Schenk and Matteoli, 2004; Corlew et al., 2008; Jane et al., 2009) where they can act by altering transmitter release. The metabotropic glutamate receptors are located both presynaptically and postsynaptically depending on the receptor subtype. Postsynaptically the metabotropic glutamate receptors are mainly located perisynaptically, outside of, but close to the PSD (Ferraguti and Shigemoto, 2006; Okabe, 2007).

Glutamate release

Glutamate is stored in small vesicles in the presynaptic terminal and released by Ca^{2+}-induced exocytosis. This exocytosis takes place at a specific site of the presynaptic terminal directly opposite to the PSD at the so called active zone, at which voltage-gated Ca^{2+} channels (VGCC), vesicles and a number of vesicle- and presynaptic membrane-attached proteins, including the so called SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, are assembled. To be released a vesicle must, with the assistance of the attached proteins, be mobilized from cytoplasmic vesicle pools, be docked to the active zone and thereafter primed for release. This means that at each instant of time there are very few vesicles ready for release of which only at most one is released when an action potential arrives. The probability of release (P_r) is decided by the number of vesicles ready for release and the release probability of the individual vesicle (P_{ves}). Among the CA3-CA1 synapses there is a substantial heterogeneity in P_r with most terminals exhibiting values < 0.2, presumed to reflect either a variation in vesicle number (Dobrunz and Stevens, 1997) or in P_{ves} (Hanse and Gustafsson, 2001c) among the synapses. This heterogeneity is observed already within the first postnatal days and remains fairly constant throughout development (Hanse and Gustafsson, 2001c; Wasling et al., 2004). The P_r at a given synapse is however not a fixed entity but may be modulated by a series of events, including the recent activity of the presynaptic terminal itself, by the release of its own transmitter acting on presynaptic glutamate receptors, by the release of transmitters from other terminals acting on their

ionotropic or metabotropic receptors on the presynaptic membrane, as well as by the release of other transmitters and molecules, such as NO, from the postsynaptic cells or from glia.

The AMPA receptor

The synaptic transmission to be studied in this thesis is mediated by glutamate acting on the AMPAR and represents the most common and basal form of excitatory transmission in the brain. When the AMPAR is activated by glutamate it becomes permeable to Na^+ and K^+ ions and thereby allows for a large influx of Na^+ ions that depolarizes the membrane and creates an excitatory postsynaptic potential (EPSP). The AMPAR is a tetramer, i.e., built up of four subunits. There are four different AMPAR subunits that have variously been referred to as GluRA-D, or GluR1-4. Recently, however, a new terminology for the glutamate receptor subunits has been introduced in which these AMPAR subunits are referred to as GluA1-A4 (Collingridge et al., 2009). In the following I will therefore use this new terminology. Based on the length of their cytoplasmic tail (their C-terminal) the subunits can be divided into two groups, GluA1 and GluA4 having long C-terminals and GluA2 and GluA3 having short C-terminals (Dingledine et al., 1999). AMPARs can be composed of different combinations of these subunits which allows for the existence of AMPARs with different functional characteristics. For example, AMPARs containing only short C-terminal subunits (e.g. GluA2A3) can constitutively traffic into and out of the membrane while those containing a long C-terminal subunit (e.g. GluA1A2) can only do so in an activity dependent manner (Shi et al., 2001). Moreover, AMPARs lacking the GluA2 subunit are permeable not only to Na^+ and K^+ ions but also to Ca^{2+} ions (Isaac et al., 2007). There are also different splice variants of the subunits as all these four subunits are expressed as either a “flip” or a “flop” isoform (Sommer et al., 1990). Receptors containing “flip” variants of the subunits do not desensitize as fast and as much as receptors containing the “flop” variants (Sommer et al., 1990; Dingledine et al., 1999). In addition, the GluA2 subunit also has a splice variant with a longer C-terminal, hence called $GluA2_{long}$ (Köhler et al., 1994) which is thus functionally equivalent to GluA1 and GluA4 subunits.

The subunit composition of AMPARs undergoes changes during development. Thus, while the expression of GluA4 peaks at postnatal day 2 (P2) and is completely abolished by P20 (Zhu et al., 2000), the expression of GluA1-A3 increases throughout the first couple of postnatal weeks. Also the different splice variants are expressed differently during

development such that the “flip” variants are expressed from early on in development while the “flop” variants start to be expressed at significant levels from the 2nd postnatal week (Monyer et al., 1991). Furthermore, the expression of $GluA2_{long}$ peaks during the 2nd postnatal week thereafter being reduced to a lower level (Kolleker et al., 2003).

While studies of receptor expression have shown that GluA1 and GluA2 are the predominating subunits in more adult hippocampal pyramidal neurones (Geiger et al., 1995; Wenthold et al., 1996; Sans et al., 2003), the synaptically located AMPARs were long thought to be dominated by GluA2A3 heteromers (Shi et al., 2001). Even though GluA1A2 heteromers were found to be inserted in an activity dependent manner they were thought to be subsequently replaced by the GluA2A3 heteromers (Shi et al., 2001). However, when recently examined in 2-4 week old rats using a single-cell genetic approach coupled with electrophysiology, principally all AMPARs were found to consist of GluA2-containing heteromers of which the vast majority (80%) actually were GluA1A2 heteromers, and only a small minority (< 20%) were GluA2A3 heteromers (Lu et al., 2009b). GluA2-lacking Ca^{2+} permeable AMPARs are thus at that time essentially absent from the CA1 pyramidal cells. As noted above, in this thesis I have studied rats of age P8-P12 at which GluA4 is still expressed (although the absolute level of this subunit should be quite low) (Zhu et al., 2000) and at which age quite a large fraction of GluA2 at the synapse is of the $GluA2_{long}$ isoform (Kolleker et al., 2003). In what manner these $GluA2_{long}$ subunits preferentially form receptors is not known.

The properties of AMPARs are not only decided by their subunits, but also by auxiliary subunits that when binding to the AMPARs may alter for example the conductance, desensitization, deactivation of the channels as well as the pharmacology of the receptors. One such auxiliary subunit belongs to the TARP (transmembrane AMPAR regulatory proteins) family (Nicoll et al., 2006), and another to the cornichon proteins (Schwenk et al., 2009). As will be described below (see section on Glutamate receptor trafficking), such auxiliary subunits are also involved in the trafficking of AMPARs to and from the synapse.

In the hippocampus the AMPARs can also be expressed presynaptically but not at the CA3-CA1 synapse. Thus, presynaptic AMPARs can be found at the mossy fiber-CA3 synapse

(Fabian-Fine et al., 2000) and might there regulate glutamate release both via Na^+ influx and via metabotropic processes (Wang et al., 1997; Schenk and Matteoli, 2004).

The NMDA receptor

While also the NMDAR is involved in excitatory synaptic transmission per se, its main impact on synaptic transmission is its pivotal importance in the induction of several forms of plasticity of the AMPAR mediated synaptic transmission (see section on Synaptic plasticity). Similar to the AMPAR the NMDAR is a tetramer and there are seven different NMDAR subunits, GluN1, GluN2A-2D and GluN3A-3B (Collingridge et al., 2009). The NMDAR always consists of two GluN1 subunits combined with two other subunits (GluN2 or GluN3). In addition to Na^+ and K^+ ions all NMDAR channels are permeable to Ca^{2+} ions. The NMDAR channel has the very unusual property for a ligand-gated ion channel of a strong voltage dependence of its opening. This is because at normal resting membrane potential levels the ion channel is blocked by extracellular Mg^{2+}. This block is voltage dependent and relieved by membrane depolarization. Thus, in addition to glutamate binding, the membrane needs to be depolarized for the NMDAR channel to open up for ion flux. Further, the NMDAR requires binding of a co-agonist (such as glycine or D-serine) for its activation (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). When activated by synaptically released glutamate the current generated from NMDAR activation rises slower than the AMPAR mediated current, and decays slower (Dzubay and Jahr, 1996), enabling a large Ca^{2+} influx. The slower decay of the NMDAR mediated current is due to the higher affinity for glutamate to the NMDAR than to the AMPAR (Dingledine et al., 1999). NMDARs containing GluN2A and GluN2B subunits are expressed in most regions of the brain. While GluN2B is expressed early at the embryonic stage and is maintained throughout development (Monyer et al., 1994; Sheng et al., 1994; Wenzel et al., 1997), the expression of GluN2A starts just before birth (in the rat) and its expression increases during development. Thus, the GluN2A/GluN2B ratio increases during development, conferring with age faster kinetics to the NMDAR mediated currents (Flint et al., 1997), and smaller Ca^{2+} net influx upon NMDAR activation (Sobczyk et al., 2005). Recent studies have also shown an activity dependent control of the GluN2A/GluN2B ratio (Bellone and Nicoll, 2007; Xu et al., 2009b) (see section on Synaptic plasticity). These developmental and activity dependent changes in the GluN2A/GluN2B ratio are of clear significance since this ratio has been suggested to affect the type of synaptic plasticity to be induced (see section on Synaptic plasticity). NMDARs

containing the GluN3A subunit have a much reduced Ca^{2+} permeability and a reduced sensitivity to Mg^{2+} block compared to the GluN2 containing receptors (Pérez-Otaño et al., 2001; Sasaki et al., 2002). The GluN3A subunit is widely expressed in the CNS, including the CA1 region (Ciabarra et al., 1995; Sucher et al., 1995; Wong et al., 2002), its expression (in the rat) however reaching a peak at P8 and thereafter decreasing into adulthood. This down-regulation of GluN3A during development seems to be important for synaptic maturation, synaptic plasticity and cognitive functions (Das et al., 1998; Roberts et al., 2009).

NMDARs containing the GluN2B subunit have also been observed presynaptically at the CA3-CA1 synapse and facilitate axon excitability (Suárez et al., 2005; Suárez and Solís, 2006). Moreover, in rats < P5 presynaptic NMDARs containing the GluN2D subunit can facilitate glutamate release (Mameli et al., 2005).

The kainate receptor

The KAR is a tetramer and five different KAR subunits, GluK1-K5 (Collingridge et al., 2009) have been identified. The KAR channel is permeable to Na^{+} and K^{+} ions and is located at presynaptic terminals of both glutamate and GABA synapses onto CA1 pyramidal cells (Jane et al., 2009). Activation of the presynaptic KARs at glutamate synapses depresses synaptic transmission, presumably by reducing the probability of vesicular release (Vignes et al., 1998). Early in development (in the P3-P6 rat) glutamate tonically activates these KARs, providing a tonic inhibition of the CA3-CA1 excitatory transmission. Interestingly, this tonic inhibition, which disappears with synaptic maturation, can be modulated in the long term in an activity dependent manner (Lauri et al., 2006; Sallert et al., 2007) (see section on Synaptic plasticity). There are also postsynaptic KARs, but while their contribution is quite substantial at the mossy fiber-CA3 pyramidal cell synapse and at interneurone synapses onto CA3 and CA1 pyramidal cells, their contribution at the CA3-CA1 synapse is quite small (Jane et al., 2009).

The metabotropic glutamate receptor

Through its metabotropic glutamate receptors (mGluRs) glutamate modulates presynaptic release and the postsynaptic excitability as well as induce synaptic plasticity (see section on Synaptic plasticity). The mGluRs are G-protein-coupled receptors, i.e., they are coupled to an effector system through a GTP-binding protein. There are eight different subtypes of mGluRs

(mGlu1-8), divided into three different groups (mGluR group I-III) based on their structure and function (Ferraguti and Shigemoto, 2006). The group I mGluRs consists of mGlu1 and mGlu5 and activates phospholipase C (PLC) resulting in the production of diacylglycerol, an activator of protein kinase C (PKC), and of inositol trisphosphate that releases Ca^{2+} from intracellular stores. Group II receptors consisting of mGlu2 and mGlu3, and group III receptors, consisting of mGlu4 and mGlu6-8, are negatively coupled to adenylate cyclase and thus inhibit cAMP formation (Ferraguti and Shigemoto, 2006; Kim et al., 2008). As indicated by immunohistochemical studies in the CA1 region group I mGluRs are primarily located postsynaptically on either pyramidal cells (primarily mGlu5) or on interneurones (primarily mGlu1) (Martin et al., 1992; Baude et al., 1993; Luján et al., 1996; Shigemoto et al., 1997). In contrast, group II receptors can be located both pre- and postsynaptically, mGlu2 primarily in the stratum lacunosum moleculare and mGlu3 mostly in glial cells (Neki et al., 1996; Petralia et al., 1996; Shigemoto et al., 1997; Ohishi et al., 1998; Tamaru et al., 2001), while group III receptors (predominantly mGlu7) are presynaptic (Shigemoto et al., 1997). Thus, with respect to my study of the CA3-CA1 synapse the most relevant mGluRs are mGlu5 and mGlu7 receptors which are expressed in the rat CA1 stratum radiatum throughout the entire neonatal development (Bradley et al., 1998; Lopez-Bendito et al., 2002). However, in the neonatal rat the mGlu8 receptor as well as group II receptors may be present presynaptically (Li et al., 2002; Ayala et al., 2008). Postsynaptically, also the mGlu1 receptor may be expressed in the CA1 pyramidal cells, at least in rats older than two weeks (Mannaioni et al., 2001; Ireland and Abraham, 2002; Berkeley and Levey, 2003; Rae and Irving, 2004; Volk et al., 2006).

The postsynaptic density

As noted above, scaffolding and adaptor proteins together with a variety of enzymes give rise to an electron dense subsynaptic structure referred to as the postsynaptic density (PSD). The scaffolding proteins in the PSD hold the glutamate receptors in place and thus ensure that the postsynaptic membrane contains a high density of receptors. AMPARs and NMDARs are anchored such that they are positioned directly opposite the presynaptic active zone, while mGluRs are anchored to the PSD such that they are positioned perisynaptically but still very close to the synapse (Newpher and Ehlers, 2008). Several hundred proteins have been identified in the PSD with calcium calmodulin dependent kinase II (CaMKII), the synaptic GTPase activating protein and the postsynaptic density-membrane associated guanylate kinases (PSD-MAGUKs) among the most abundant (Cheng et al., 2006). The PSD-MAGUKs

are a family of scaffolding proteins, consisting of PSD-93, PSD-95, SAP-97 and SAP-102 (Elias and Nicoll, 2007) of which PSD-95 is the most plentiful in the PSDs of adult animals (Cheng et al., 2006). PSD-95 plays a central role in the organization of the PSD, binding directly or indirectly via other scaffolding proteins to a wide variety of membrane proteins, cytoplasmic enzymes, cell adhesion molecules and the cytoskeleton (Kim and Sheng, 2004; Feng and Zhang, 2009). While PSD-95 seems to be the predominating PSD-MAGUK in adult animals, SAP-102 seems to be the predominating PSD-MAGUK during the first couple of postnatal weeks (Sans et al., 2000). Shank is another central scaffolding protein in the PSD, which associates PSD-95 to the actin cytoskeleton via the binding of GKAP (guanylate kinase-associated protein) and cortactin, respectively (Sheng and Kim, 2000). As mentioned above, AMPARs are bound to auxiliary subunits (TARPs and cornichon proteins), and the C-terminals of TARPs bind to the PDZ domains of PSD-95 (as well as PSD-93, SAP-97 and SAP-102), anchoring the AMPARs to the PSD (Chen et al., 2000; Nicoll et al., 2006). On the other hand, the NMDAR is anchored to the PSD by a direct interaction of the GluN2 subunits and PSD-95 (or PSD-93/SAP-97/SAP-102) (Kornau et al., 1995; Niethammer et al., 1996; Sans et al., 2000; Kim and Sheng, 2004). The mGluR group I receptors are associated with the PSD via its binding to Homer, Homer in its turn associates with the PSD scaffolding protein Shank (Ferraguti and Shigemoto, 2006; Okabe, 2007). The ionotropic glutamate receptors seem to have differential location in the PSD membrane area, where AMPARs are concentrated in the periphery or evenly spread out, while NMDARs are concentrated to the center (Newpher and Ehlers, 2008).

Another scaffolding protein localized to the PSD is the A-kinase anchoring protein 79/150 (AKAP79/150), which binds protein kinase A (PKA), PKC and protein phosphatase 2B (PP2B), enzymes which are crucial for synaptic plasticity (Carr et al., 1992; Coghlan et al., 1995; Klauck et al., 1996; Oliveria et al., 2003). AKAP79/150 also binds to PSD-95 and SAP97, thereby positioning PKA, PKC and PP2B close to their substrates GluN2B and GluA1 (Colledge et al., 2000; Oliveria et al., 2003). It seems that the PKA bound to AKAP79/150 acts as a “guard” at the synapse holding AMPARs constrained there by continuously keeping them phosphorylated (Snyder et al., 2005).

Glutamate receptor trafficking

The glutamate receptors are however not statically bound to the PSD but can move in and out of the synapse. This receptor trafficking occurs both via diffusion within the membrane between the synaptic and extrasynaptic membrane (lateral diffusion) and via transport between intracellular organelles and surface membrane coupled with exocytosis/endocytosis (Groc and Choquet, 2006). The receptor subunits are synthesized in the endoplasmic reticulum (ER) not only in the soma but also locally close to the synapse (Kennedy and Ehlers, 2006). In the lumen of the ER and in the Golgi apparatus the subunits are subjected to post-translational modifications, such as glycosylation and palmitoylation, and are assembled into functional receptors together with their auxiliary subunits (Kennedy and Ehlers, 2006; Nicoll et al., 2006; Hayashi et al., 2009). The TARPs are important for the trafficking of the AMPARs between the ER and the Golgi apparatus, as well as for the trafficking to the cell membrane and to the synapse where the TARPs interact with PSD-95 (Nicoll et al., 2006), an interaction that seems very important not least also for the retention of AMPARs in the synapse (Schnell et al., 2002; Bats et al., 2007). The binding of GluA1-containing AMPARs to SAP-97 has also been implicated in the trafficking of these AMPARs from the ER and Golgi apparatus to the cell membrane and to the synapse (Sans et al., 2001; Waites et al., 2009). Possibly due to this GluA1-SAP-97 interaction, GluA1A2 heteromers exit the ER more efficiently than GluA2A3 heteromers (Greger et al., 2002). The insertion of GluA1-containing AMPARs also seems dependent on the interaction between GluA1 and the actin-binding protein 4.1N, which is mediated by depalmitoylation of GluA1 and a PKC mediated phosphorylation at ser-816 and ser-818 (Lin et al., 2009).

While the endocytosis of AMPARs for AMPAR internalization takes place just outside the PSD (Blanpied et al., 2002; Petralia et al., 2003; Rácz et al., 2004), the location of AMPAR exocytosis is a contentious issue. It was initially described that while the exocytosis of AMPARs not containing GluA1 takes place in close proximity to the synapse, the activity dependent exocytosis of GluA1-containing AMPARs takes place extrasynaptically (Passafaro et al., 2001). However, it was thereafter reported that all AMPAR exocytosis should take place at the soma membrane (Adesnik et al., 2005), the AMPARs only reaching the synapse by lateral diffusion from the soma membrane. In contrast, it was also reported that all AMPAR exocytosis takes place directly at the synapse (Gerges et al., 2006). More recently, new techniques enabling the visualization of individual GluA1 insertions have indicated that

exocytosis of at least GluA1-containing AMPARs does take place extrasynaptically, in the soma as well as in the dendrites (Yudowski et al., 2007; Lin et al., 2009). The inserted AMPARs will then constitute a large extrasynaptic pool of AMPARs that can enter the synapse by lateral diffusion (Triller and Choquet, 2005).

AMPARs are retained at the synapse by the interaction of TARPs with PSD-MAGUKs, as mentioned above. In addition, they are retained by GluA2 and GluA3 subunits binding to GRIP/ABP (glutamate receptor interacting protein/AMPA receptor binding protein) (Chung et al., 2000; Osten et al., 2000). The GluA2-GRIP/ABP binding site can also interact with PICK-1 (protein interacting with C-kinase-1) such that phosphorylation of the GluA2 binding site results in dissociation of the GluA2-GRIP/ABP binding allowing for GluA2-PICK1 binding and AMPAR internalization (Chung et al., 2000; Lu and Ziff, 2005). Thus, while GluA2-GRIP/ABP binding retains the AMPARs at the synapse, GluA2-PICK-1 binding results in mobile AMPARs. In addition to phosphorylation of the GluA2 binding site, GluA2-PICK-1 binding can be directly altered by cytoplasmic Ca^{2+} in a biphasic manner. Thus, while modest increases in Ca^{2+} result in an increased GluA2-PICK-1 binding and an increased AMPAR mobility, large increases in Ca^{2+} rather reduces the GluA2-PICK-1 interaction and stabilizes AMPARs at the synapse (Hanley and Henley 2005). AMPARs, if internalized, may thereafter either be recycled back to the cell membrane, or they will be degraded by the lysosome (Ehlers, 2000).

In contrast, it has been reported that neither PICK-1 nor phosphorylation of the GluA2 site play a critical role in AMPAR internalization but rather regulates the AMPAR recycling from the endosomes (Daw et al., 2000; Lin and Huganir, 2007). In this case, phosphorylation of the GluA2 site and the GluA2-PICK-1 binding results in greater retention of AMPARs at the endosomal membrane. N-ethylmaleimide sensitive fusion protein (NSF) (an ATPase involved in membrane fusion) can dissociate the GluA2-PICK-1 binding and facilitate the recycling and synaptic targeting of the AMPARs. In fact, when blocking the NSF-GluA2 interaction and thereby preventing NSF-mediated recycling, the AMPAR mediated synaptic response shows a stimulation dependent run-down (Duprat et al., 2003). This result was taken to suggest that synaptic activation regularly results in ligand-induced AMPAR internalization that is normally masked by a NSF-mediated recycling back to the membrane (Lee et al., 2002; Duprat et al., 2003). Importantly, however, later studies have indicated that the NSF-PICK1-

GluA2 interactions may rather take place at the surface membrane (Gardner et al., 2005). The stimulation-induced run-down following blockade of the NSF-GluA2 interaction may therefore represent a ligand-induced AMPAR lateral diffusion out of the synapse normally masked by a NSF-mediated recycling (within the surface membrane) back to the synapse.

NMDARs also traffic from the ER to the cellular membrane in association with PSD-MAGUKs (Lau and Zukin, 2007). The NMDARs are thought to be less dynamic than the AMPARs (i.e. the anchoring of NMDARs to the PSD is thought to be more stable than that of AMPARs), and NMDARs do indeed have a lower rate of lateral diffusion in the membrane (Groc et al., 2004). Furthermore, GluN2A-containing NMDARs are less mobile than GluN2B-containing NMDARs (Groc et al., 2006b).

Synaptic plasticity

As noted in the introductory paragraph, the activity dependent modification of synapses is commonly referred to as synaptic plasticity. There are several forms of synaptic plasticity, ranging in duration from ms to at least several weeks (and probably years). Short-term plasticity (≤ minutes) is often explained by a direct effect of activity itself on the presynaptic release, for example a Ca^{2+}-induced change in the release probability or a depletion of transmitter vesicles. The mechanisms involved in long-term plasticity (≥ hours) are still hotly debated and both presynaptically and postsynaptically located mechanisms have been suggested to play an important role. Synaptic plasticity can be either homosynaptic, i.e., occurring in only the activated synapses, or heterosynaptic, i.e., occur in nearby inactive synapses as well. In this thesis, the term plasticity will refer solely to homosynaptic plasticity. There are basically two forms of long-term synaptic plasticity, long-term potentiation (LTP) in which there is a sustained increase in synaptic strength, and long-term depression (LTD) in which there is a sustained reduction in synaptic strength. Whether synaptic activity results in LTP or LTD depends upon the strength and the temporal characteristics of synaptic activation, strong brief high frequency activation giving rise to LTP and weaker low frequency activation giving rise to LTD. LTP and LTD share one important characteristic in that they both depend on a postsynaptic Ca^{2+} elevation, the amplitude and temporal pattern thereof determining the direction of plasticity (Yang et al., 1999).

Long-term potentiation

LTP was the first form of long-term synaptic plasticity to be discovered (Bliss and Lømo, 1973), and is induced by correlated pre- and postsynaptic activity, so-called Hebbian activity. Such Hebbian activity can experimentally be achieved in a number of ways, for example by trains of high frequency stimulation involving many synapses or by pairing a weaker synaptic stimulation, even at low frequency, with a large postsynaptic depolarization induced via current injection from an intracellular microelectrode. The most common form of LTP studied critically depends on NMDAR activation for its induction, the NMDAR acting as an associative device requiring both presynaptic activity, in the form of released glutamate, and postsynaptic activity, in the form of postsynaptic depolarization for its activation (Wigström and Gustafsson, 1986). It was reported that the NMDAR subunit composition should affect LTP in that blockade of GluN2A-containing but not GluN2B-containing NMDARs impaired LTP induction (Liu et al., 2004; Massey et al., 2004). However, later studies have subsequently shown that LTP can be induced by activation of GluN2B-containing as well as GluN2A-containing NMDARs (Berberich et al., 2005; Bartlett et al., 2007; Li et al., 2007). Nonetheless, the specific subunit composition of NMDARs does play a role in LTP induction in that a low GluN2A/GluN2B ratio lowers the induction threshold for LTP and vice versa (Xu et al., 2009b). Interestingly LTP itself increases the GluN2A/GluN2B ratio (Bellone and Nicoll, 2007; Xu et al., 2009b), thereby raising the induction threshold for any subsequent LTP, and at the same time decreasing the induction threshold for any subsequent LTD. However, other studies have suggested that LTP can actually inhibit the induction of LTD, at least temporarily (Peineau et al., 2007), because of phosphorylation and inactivation of the ser/thr kinase GSK3β involved in LTD induction (see section on NMDAR-LTD).

While it is well established that the Ca^{2+} influx through the NMDAR channels provides the initial step in LTP induction, which the following steps are remain uncertain. Nonetheless, a number of studies have indicated that a Ca^{2+} mediated activation of CaMKII may be the important next step in the generation of LTP (Malinow et al., 1989; Silva et al., 1992; Fukunaga et al., 1993; Otmakhov et al., 1997). Which other biochemical steps that subsequently occur, or whether these steps result in mainly postsynaptic events such as altered receptor trafficking, or to the production of retrograde messengers affecting presynaptic function, is still unclear. However, since the findings in the 1990s that synapses can be AMPA silent and of the lively AMPAR trafficking (see sections on Glutamate receptor

trafficking, and AMPA silent synapse and AMPA silencing), most researchers have favored a postsynaptic expression for LTP. Nevertheless, some recent studies suggest that LTP at the CA3-CA1 synapse is at least partly expressed as an increased P_r (Zakharenko et al., 2001; Bayazitov et al., 2007). Moreover, at the perforant path-CA1 synapse (but not at the CA3-CA1 synapse) LTP has recently been explained as recruitment of presynaptic N-type Ca^{2+} channels resulting in an increased P_r (Ahmed and Siegelbaum, 2009). In addition, a recent study of the CA3-CA1 synapse measuring P_r at individual synapses using two-photon imaging of transmitter-evoked Ca^{2+} transients suggests that LTP can be expressed solely as an increased P_r (Enoki et al., 2009). In contrast, using this technique on neonatal CA3-CA1 synapses indicated a postsynaptic explanation for LTP expression in these synapses (Ward et al., 2006).

As indicated above, since the late 1990s a large number of studies have suggested that LTP is a postsynaptic enhancement of AMPA signaling, mediated by insertion of AMPARs into the postsynaptic membrane (e.g. Shi et al., 1999; Hayashi et al., 2000; Heynen et al., 2000; Bagal et al., 2005; reviewed by Kerchner and Nicoll, 2008). Thus, CaMKII and/or possibly PKC phosphorylates GluA1 at ser-831 (Roche et al., 1996; Barria et al., 1997a; Lee et al., 2000) resulting in a synaptic delivery of GluA1 and LTP (Hayashi et al., 2000; Lee et al., 2003). In addition, PKC phosphorylation of GluA1 at ser-818 also seems required for synaptic delivery and LTP (Boehm et al., 2006). Studies using electrophysiological recordings combined with cellular imaging reported that GluA1A2 heteromers are inserted in an activity dependent manner (Shi et al., 1999; Shi et al., 2001). More recently it was reported that the AMPARs inserted upon LTP induction are Ca^{2+}-permeable GluA2-lacking GluA1 homomers (Plant et al., 2006), and that the Ca^{2+} influx through these receptor channels should be important for later stabilization of LTP. However, this specific result has not been replicated by others (Adesnik and Nicoll, 2007; Gray et al., 2007). Although the AMPAR delivery to the synapses upon LTP induction has been reported to be GluA1-dependent (Shi et al., 2001), LTP can also be observed in GluA1 knock-out mice, even in adults (Romberg et al., 2009). The GluA1 independent LTP however requires a strong induction protocol and the initial rapidly decaying part of the LTP is impaired (Jensen et al., 2003; Romberg et al., 2009).

LTP maintenance

During LTP induction there is not only an activation of CaMKII but also a recruitment of this enzyme to the PSD (Otmakhov et al., 2004). Following its activation CaMKII can undergo autophosphorylation (at the thr-286 site) and thereby remain active even when the Ca^{2+} concentration has returned to baseline levels (Miller and Kennedy, 1986). Importantly, when recruited to the PSD, CaMKII cannot any longer be dephosphorylated at this site by protein phosphatase 1 (PP1) or 2A (PP2A) (Strack et al., 1997; Mullasseril et al., 2007). Inhibition of this autophosphorylation also greatly attenuates LTP (Barria et al., 1997b; Giese et al., 1998). Taken together, these CaMKII properties could therefore make this enzyme a key player in LTP maintenance and, consistent with this notion, when CaMKII is inhibited after the LTP induction this accordingly reverses the LTP (Sanhueza et al., 2007). The maintenance of LTP is also thought to depend on de novo protein synthesis (Stanton and Sarvey, 1984; Frey et al., 1988; Huang and Kandel, 1994) providing for new proteins to stabilize the potentiation both functionally and morphologically. For example, protein kinase Mζ, a constitutively active kinase, is synthesized upon LTP induction and by enhancing NSF/GluA2 dependent insertion of AMPARs to the postsynaptic membrane contributes to maintain LTP (Osten et al., 1996; Ling et al., 2002; Kelly et al., 2007; Yao et al., 2008). LTP induction also results in de novo synthesis of Arc/Arg3.1 which by inducing local actin polymerization also helps to stabilize LTP (Guzowski et al., 2000; Plath et al., 2006; Messaoudi et al., 2007). This is because such local actin polymerization is crucial for the LTP-associated enlargement of dendritic spines (Matsuzaki et al., 2004; Okamoto et al., 2004; Otmakhov et al., 2004) driven by CaMKII mediated phosphorylation (Steiner et al., 2008; Yamagata et al., 2009) and GluA1 insertion into the synapse (Kopec et al., 2007). Surprisingly, however, if not only protein synthesis is blocked but also protein degradation, LTP can persist (Fonseca et al., 2006). Moreover, in a very recent study (Abbas et al., 2009) LTP was found to persist for many hours despite a protein synthesis blockade. The importance of de novo protein synthesis for LTP maintenance is thus open to question.

LTP in neonatal rats

In contrast to LTP in older rats, LTP in neonatal rats (P7-8) is not blocked by CaMKII inhibition but by PKA inhibition (Yasuda et al). For the neonatal rat there is also good evidence that LTP is expressed by the unsilencing of AMPA silent synapses (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). However, since these AMPA silent synapses are

created by preceding test pulse stimulation (Xiao et al., 2004) LTP in the neonatal animals may rather be seen as a de-depression than as a potentiation of synaptic transmission (Abrahamsson et al., 2008). Nonetheless, the NMDAR mediated Ca^{2+} influx can, via activation of calcium/calmodulin sensitive adenylyl cyclase (Chetkovich and Sweatt, 1993; Roberson and Sweatt, 1996) activate PKA and result in PKA mediated phosphorylation of GluA4 at ser-842 and/or $GluA2_{long}$ at ser-841. This phosphorylation can subsequently result in synaptic delivery of AMPARs containing such subunits (Esteban et al., 2003; Qin et al., 2005). In addition to AMPA unsilencing, there is in the 1st postnatal week a presynaptic LTP, expressed as a relief of KAR induced inhibition of vesicle release as well as a postsynaptic LTP expressed as an increased strength of the synapse (Palmer et al., 2004; Lauri et al., 2006).

Long-term depression

While LTP was discovered in the early 1970s, it took an additional twenty years before the first experimental demonstration of a predicted complementary activity dependent long-term decrease in synaptic efficacy, a long-term depression (LTD), in the hippocampus (Dudek and Bear, 1992). LTD was induced using prolonged (15 min) low frequency (1 Hz) stimulation of the CA3-CA1 synapses, and similar to LTP its induction was blocked by NMDAR antagonists. Later studies have revealed that in addition to NMDAR-LTD there are forms of LTD that are induced by either mGluR activation (mGluR-LTD) or endocannabinoids (Bolshakov and Siegelbaum, 1994; Gerdeman et al., 2002; Chevaleyre and Castillo, 2003). These various forms of LTD can be differentially expressed in different brain regions but can also be found in the same region and even within the same synapse population dependent on the stimulus protocol, specific experimental conditions and developmental stage (Oliet et al., 1997; Kemp et al., 2000; Malenka and Bear, 2004; Pavlov et al., 2004; Nosyreva and Huber, 2005). For the CA3-CA1 synapse NMDAR-LTD and mGluR-LTD are the two forms best characterized.

NMDAR-LTD

LTD at the CA3-CA1 synapse can be induced by prolonged low frequency stimulation (LFS) (1-5 Hz) that activates postsynaptic NMDARs (Dudek and Bear, 1992; Mulkey and Malenka, 1992) and results in a subsequent influx of Ca^{2+} (Mulkey and Malenka, 1992; Cummings et al., 1996). An LTD that is occluded by, and that occludes LFS-LTD, can also be induced by

brief application of NMDA (Lee et al., 1998; Kamal et al., 1999). It was reported that NMDARs containing GluN2B were specifically involved in LTD induction (Liu et al., 2004; Massey et al., 2004). However, recent studies from a number of other laboratories have failed to confirm this finding but have instead demonstrated both that specific blockade of GluN2B-containing NMDARs does not alter LTD (Bartlett et al., 2007; Li et al., 2007; Morishita et al., 2007) and that LTD is blocked by a specific blockade of GluN2A-containing NMDARs (Bartlett et al., 2007; Li et al., 2007). This discrepancy might be explained by the fact that these studies were performed on rats of different ages. Similar to the induction of LTP, the GluN2A/GluN2B ratio also plays a role for the induction of LTD, a low GluN2A/GluN2B ratio raising the induction threshold for LTD and vice versa (Xu et al., 2009b). LTD also alters this ratio, the induction of LTD lowering the ratio and thereby raising the induction threshold for additional LTD and subsequently lowering the induction threshold for LTP (Xu et al., 2009b).

While the exact sequence of events between the NMDAR mediated Ca^{2+} influx and the decrease in synaptic strength is still not clarified, experimental data points to an initial involvement of Ca^{2+}-activated phosphatases ultimately resulting in AMPAR removal by endocytosis. Early studies on LTD using enzyme inhibitors indicated a sequence of events in which Ca^{2+} activation of calcineurin (PP2B) results in dephosphorylation and inactivation of inhibitor-1 which in its turn results in activation of PP1 (Mulkey et al., 1993; Mulkey et al., 1994; Li et al., 2002). The activation of PP1 will thereafter lead to dephosphorylation of ser-845 (a PKA phosphorylation site) of the GluA1 subunit of the AMPAR (Lee et al., 1998; Lee et al., 2000; Lee et al., 2003) and to an AMPAR endocytosis (Carroll et al., 1999; Beattie et al., 2000; Ehlers, 2000; Heynen et al., 2000; Man et al., 2000). However, the endocytosis also requires interaction between the GluA2 subunit of the AMPAR and AP2, a clathrin adaptor protein linking the cytoplasmic domain of the AMPAR to clathrin (Lee et al., 2002). Hippocalcin, a high-affinity Ca^{2+} binding protein, acts here as the Ca^{2+} sensor and binds directly to the AP2 complex to regulate the endocytosis (Palmer et al., 2005). Furthermore, the binding of the PKA and PP2B binding protein AKAP150 to PSD-95 seems to be important for the endocytosis, possibly by positioning PP2B to the appropriate location for its action (Bhattacharyya et al., 2009). The decrease in AMPAR surface expression also seems to depend on the disruption of PKA's interaction with AKAP150 (Snyder et al., 2005) (see section on The postsynaptic density). However, dephosphorylation of PKA substrates other

than the GluA1 subunit has also been implicated in LTD (Kameyama et al., 1998). In addition, the ser/thr kinase GSK3β, which is inactivated by PKA mediated phosphorylation, is activated by PP1-, or PP2A-, mediated dephosphorylation and is involved in LTD induction (Fang et al., 2000; Peineau et al., 2007).

PKC activation, via phosphorylation of the ser-880 site of the GluA2 subunit, can also result in LTD (Kim et al., 2001). This phosphorylation disrupts the GluA2-ABP-GRIP1 interaction and promotes a GluA2-PICK1 interaction resulting in AMPAR internalization (Kim et al., 2001; Perez et al., 2001; Seidenman et al., 2003; Jin et al., 2006). However, as described above (see section on Glutamate receptor trafficking), other studies suggest that these interactions rather play a role in preventing synaptic delivery of AMPARs previously internalized upon LTD induction (Daw et al., 2000; Lin and Huganir, 2007). The interaction between GluA2 and NSF has also been suggested to play a role in LTD (Lüthi et al., 1999), but may instead be needed to maintain the supply of synaptic AMPARs and not be required for LTD (Lee et al., 2002). The p38 mitogen-activated protein kinase (p38 MAPK) has also been suggested to induce AMPAR internalization, where p38 MAPK is activated by NMDAR mediated Ca^{2+} influx via the activation of the small GTPase Rap (Zhu et al., 2002). Additionally it seems that the endocytosis of AMPARs is partly dependent on ubiquitin regulated degradation of PSD-95 (Colledge et al., 2003).

With respect to the maintenance of NMDAR-LTD, it was observed that while the activation of PP1 is transient the activation of PP2A is more persistent (Thiels et al., 1998). This is probably because PP2A can undergo autodephosphorylation allowing PP2A to remain active for a longer time (Chen et al., 1992) and thus be involved in sustaining the LTD (Pi and Lisman, 2008). NMDAR-LTD has been shown to be associated with a shrinkage of dendritic spines (Zhou et al., 2004), and spine retraction (Nägerl et al., 2004), these effects possibly being mediated by a shift in the F-actin/G-actin equilibrium toward depolymerization (Okamoto et al., 2004). However, a later report showed that there was no causal connection between spine shrinkage and LTD (Wang et al., 2007).

mGluR-LTD

At the CA3-CA1 synapse the induction of mGluR-LTD often requires prolonged paired pulse stimulation (PP-LFS) rather than single pulse stimulation at low frequency (Kemp and Bashir,

1997; Huber et al., 2000). However, in the neonatal rat prolonged single pulse stimulation at 1-5 Hz can also be used to induce mGluR-LTD (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Li et al., 2002; Pavlov et al., 2004). Application of the mGluR group I agonist DHPG also induces LTD (Palmer et al., 1997) that occludes synaptically induced mGluR-LTD (Huber et al., 2001), indicating a major involvement of group I mGluRs in mGluR-LTD. In fact, DHPG-induced LTD is now the most common protocol used to study mGluR-LTD.

In the neonatal rat (P3-11) mGluR-LTD depends on activation of postsynaptic mGlu5 receptors (Feinmark et al., 2003; Nosyreva and Huber, 2005) as well as a postsynaptic Ca^{2+} elevation mediated by VGCCs (Bolshakov and Siegelbaum, 1994). These actions results, via the activation of the p38 MAPK pathway, in a postsynaptic production of the arachidonic acid metabolite 12-(S)-HETE (Bolshakov et al., 2000; Feinmark et al., 2003) which is thereafter released from the postsynaptic neurone (Feinmark et al., 2003) to act on the presynaptic terminal, presumably by lowering P_r (Bolshakov et al., 2000; Nosyreva and Huber, 2005). In contrast, a more recent study in neonatal rats of DHPG-induced LTD found evidence for a postsynaptic expression of this LTD, tentatively explained by lateral diffusion of AMPARs from the synaptic region (Moult et al., 2006). Moreover, yet another report has linked neonatal mGluR-LTD to activation of presumably presynaptic group II mGluRs, this mechanism being down-regulated during development by an increased expression of PP2B inhibiting this LTD presynaptically (Li et al., 2002). There is thus little agreement about the nature of neonatal mGluR-LTD.

In the older rats mGluR-LTD seems to be induced by a postsynaptic activation of mGlu1 and mGlu5 receptors independently, both receptors by themselves being sufficient for the induction (Volk et al., 2006). The mGluR-LTD in these rats depends on postsynaptic Ca^{2+} elevations and results in the endocytosis of GluA1- and GluA2-containing AMPARs (Oliet et al., 1997; Huber et al., 2000; Snyder et al., 2001). It also depends on rapid protein synthesis such as increased translation of the tyrosine phosphatase STEP (striatal-enriched protein tyrosine phosphatase) via PI3K-mTor and MEK-ERK signaling pathways (Gallagher et al., 2004; Banko et al., 2006; Ronesi and Huber, 2008; Zhang et al., 2008) and of Arc/Arg3.1. STEP mediates tyrosine dephosphorylation of GluA2, and Arc/Arg3.1 forms a complex with endophilin 2/3 and dynamin, both these effects contributing to the AMPAR endocytosis (Chowdhury et al., 2006; Huang and Hsu, 2006; Moult et al., 2006; Park et al., 2008; Zhang et

al., 2008). The latter effect is mediated partly via the inactivation of fragile X mental retardation protein (FMRP) (Park et al., 2008; Waung et al., 2008), a repressor of translation of Arc/Arg3.1 mRNAs at the synapse (Zalfa et al., 2003). Knock-out of the gene expressing FMRP (*Fmr1*-KO) also enhances mGluR-LTD (Huber et al., 2002). Surprisingly, DHPG actually gives a transient upregulation of FMRP, the FMRP subsequently being rapidly degraded by the ubiquitin-proteasome pathway, and blockade of this degradation abolishes the mGluR-LTD (Hou et al., 2006).

As can be noted from the above, NMDAR-LTD and in older animals mGluR-LTD have been linked to endocytosis of postsynaptic AMPARs, and do not occlude each other (Oliet et al., 1997). These findings could indicate that these forms of LTD are expressed in two independent populations of synapses. In support of this, a recent study using two-photon imaging and un-caging to study activation of individual synapses showed that mGluR-LTD is restricted to large spine synapses containing parts of the ER (Holbro et al., 2009).

Building of a functional network

The building of a functional network is basically a process in which the neurones evolve from a state of having few and weak connections with numerous other neurones to a state where they instead have formed strong connections with a restricted number of other neurones (Hsia et al., 1998). This process involves intense synaptogenesis and at the same time an elimination/stabilization of synapses that shapes and prunes the network. The timing and specificity of this pruning is crucial for the shaping of normal brain function; deficiencies in this process may lead to diseases such as Autism spectrum disorders (including typical autism and Aspergers syndrome) and different kinds of mental retardation, such as fragile X syndrome and Rett Syndrome (Bourgeron, 2009; Yoshihara et al., 2009).

Synaptogenesis

In the rat hippocampus basically all synaptogenesis takes place during the first postnatal month (Steward and Falk, 1991) and is thought to proceed in two principal steps. First, the presynaptic axon and the postsynaptic dendrite will make physical contact, and provided they express the complementary surface molecules they will attach. Second, the presence of synchronous action potential firing, i.e. presynaptic firing coinciding with postsynaptic firing, will result in synaptic stabilization. If the surface molecules are non-complementary there will

be no synapse formation, and if there is no synchronous firing the synapse will disassemble and become eliminated (Lu et al., 2009a).

The postsynaptic dendrite repeatedly makes contact with surrounding axons through small highly motile protrusions, so called dendritic filopodia. Most of these contacts are very short lived, and only very few of them develop into a more lasting connection (Yoshihara et al., 2009). Several cell adhesion molecules such as cadherins, catenins, ephrins, neuroligins and neurexins are important for the initial trans-synaptic interactions (Bourne and Harris, 2008; Yoshihara et al., 2009). These molecules are also responsible for recruiting the pre- and postsynaptic specializations such as scaffolding proteins, receptors and synaptic vesicle proteins (for review see; Arikkath and Reichardt, 2008; Lai and Ip, 2009). Whether a contact between filopodia and axons will become more lasting appears to depend not only on the glutamatergic signaling but also on local Ca^{2+} transients independent of such signaling (Lohmann and Bonhoeffer, 2008). Synapse formation seems in addition to be modulated by glial cells since cultured neurones form few synapses unless co-cultured with astrocytes (Ullian et al., 2004). This glial nodulation of synapse formation seems to be mediated by soluble signals secreted by the astrocytes (Ullian et al., 2004). The matrix protein thrombospondin which is secreted from astrocytes promotes the formation of synapses by binding to neuronal receptors such as gabapentin and neuroligins (Christopherson et al., 2005; Eroglu et al., 2009; Xu et al., 2009a). Synapses formed in the presence of thrombospondin are however postsynaptically silent (see section on AMPA silent synapse and AMPA silencing), indicating that the recruitment of AMPARs to the synapse requires additional, as yet unidentified components secreted by astrocytes (Christopherson et al., 2005). Following the establishment of a more lasting connection the filopodia either remain but transform into spine synapses or withdraws resulting in shaft synapses. These shaft synapses may either protrude again at a later stage and result in spine synapses, or eventually become eliminated (reviewed by Bourne and Harris, 2008). During the 2nd postnatal week there is a shift in the proportion of shaft vs spine synapses, with the proportion of shaft synapses decreasing from ~ 50% to ~ 30% of the synapse population from P6 to P12 while the proportion of spine synapses increases from ~ 15% to ~ 35% during the same time period (Fiala et al., 1998). Thus, the synapses under study in the thesis (P8-P12) will consist of a varying mixture of shaft and spine synapses. In addition, some synapses during this period are either on filopodia or on so called stubby spines (Fiala et al., 1998).

Synapse elimination

A synapse that fails to become part of a functional circuit, i.e. does not participate in synchronous firing, is redundant and will suffer elimination. LTD induced either electrically by low frequency stimulation or pharmacologically by NMDA or DHPG application can result in the elimination of hippocampal synapses (Nägerl et al., 2004; Shinoda et al., 2005; Kamikubo et al., 2006; Bastrikova et al., 2008; Becker et al., 2008). However, the mechanisms behind the actual disassembly of hippocampal synapses are largely unknown, especially as regards the presynaptic terminal. Semaphorins, involved in axon guidance and cell migration, seem also to play a role in elimination of synapses. The semaphorin 3A/F is required for pruning hippocampal axons and the semaphorin 5B promotes the elimination of synaptic contacts in hippocampal neurone cultures (Bagri et al., 2003; O'Connor et al., 2009). Communication between glial cells and neurones seems also to play a role in synapse elimination (Murai et al., 2003). Thus, ephrins released from astrocytes activates Eph receptors on the postsynaptic membrane, which results in the activation of RhoA (a small Rho GTPase) and in subsequent re-organization of the actin cytoskeleton (Tada and Sheng, 2006; Fu et al., 2007). Studies of synaptic refinement in the retino-geniculate system suggest that the actual elimination/disassembly of synapses might also involve the major histocompatibility complex class I molecules, the complement cascade proteins C1q and C3 and neuronal pentraxins (NPs) and microglial phagocytosis of the synapse (Huh et al., 2000; Bjartmar et al., 2006; Stevens et al., 2007; Perry and O'Connor, 2008).

AMPA silent synapse and AMPA silencing

In the mid-90s it was described that many CA3-CA1 synapses in the neonatal rat hippocampus were functionally silent, i.e., activation at resting membrane potential levels did not result in any postsynaptic response. However, activation at positive membrane potentials resulted in an NMDAR mediated response indicating an intact presynaptic function (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). Later studies have shown that the presence of silent synapses are not restricted to the hippocampus, but they seem to be found during development in most brain regions (reviewed by Kerchner and Nicoll, 2008). While such silence of AMPAR mediated transmission can be explained by glutamate spillover from neighboring synapses or by a slow release of glutamate from the presynaptic terminal (Kullmann et al., 1996; Choi et al., 2000; Gasparini et al., 2000; Renger et al., 2001), most evidence favors an absence of postsynaptic AMPARs (Malinow and Malenka, 2002;

Kerchner and Nicoll, 2008). In fact, a recent study using two-photon laser uncaging of glutamate to directly activate postsynaptic receptors has demonstrated the existence of postsynaptically silent synapses with functional presynaptic terminals (Busetto et al., 2008).

It is generally thought that the glutamate synapse is born without AMPARs, i.e., AMPA silent, and that it acquires its AMPA signaling through NMDAR dependent LTP (reviewed by Kerchner and Nicoll, 2008). While several histochemical studies support this idea (Gomperts et al., 1998; Liao et al., 1999; Petralia et al., 1999; Pickard et al., 2000; Liao et al., 2001), a number of contradictory histochemical studies indicate that the glutamate synapse acquires its AMPARs as early as its NMDARs (Lissin et al., 1998; Rao et al., 1998; Hohnke et al., 2000; Washbourne et al., 2002; Voigt et al., 2005). The electrophysiological finding that the frequency of spontaneous AMPAR and NMDAR mediated EPSCs in the neonatal rat was essentially equal also argues for an early acquirement of AMPARs (Groc et al., 2002). Moreover, experiments in which the evoked synaptic response was followed from the very first evoked stimulation (naïve synaptic response) demonstrated that the AMPA signaling of neonatal synapses is quite labile with the synapses easily losing their AMPAR mediated response while retaining the NMDAR mediated response (Xiao et al., 2004). An electrophysiological analysis demonstrated that this loss of AMPA signaling is explained by AMPA silencing of a subpopulation of the activated synapses (Xiao et al., 2004). Interestingly, this AMPA silencing was induced at stimulation frequencies normally used as test frequencies (0.05-1 Hz), appeared independent of NMDAR and mGluR activation and was down-regulated with age (Xiao et al., 2004; Abrahamsson et al., 2007, 2008). When stimulation was interrupted this silencing slowly reversed so that the initial synaptic amplitude was recovered within 20 minutes (Abrahamsson et al., 2007). Considering the low spontaneous synaptic activity in the slice preparation (~ 1 Hz for > 1000 synapses on a given cell) a reversal implies that most synapses would be AMPA signaling in the absence of evoked stimulation and therefore result in essentially equal frequency of spontaneous AMPAR and NMDAR mediated EPSCs. The process of AMPA silencing is not confined to the CA3-CA1 synapses but has now also been described in the developing perforant path-granule cell synapse in the dentate gyrus as well as in glutamatergic inputs to interneurones in the CA1 stratum radiatum (Abrahamsson et al., 2005; Riebe et al., 2009).

Relationship between AMPA silencing and LTP/LTD

AMPA silent synapses are converted to AMPA signaling synapses by the induction of NMDAR dependent LTP (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). Similarly, AMPA silenced synapses become unsilenced by LTP-inducing stimulation and seemingly stabilized (Xiao et al., 2004; Abrahamsson et al., 2008). Thus, LTP in the neonatal brain appears to turn an AMPA labile synapse into a mature AMPA stable synapse, thereby establishing a synapse within a neural network. Since the induction of AMPA silencing does not rely on NMDAR and mGluR activation its induction obviously differs from that of NMDAR-LTD and mGluR-LTD. On the other hand, since LTD also may rely on AMPAR removal, AMPA silencing and LTD should interact, a possibility that will be investigated in this thesis.

OBJECTIVE

The formation of a functional neuronal network in the developing brain is an intricate process of balancing neuronal proliferation and cell death, migration, synaptogenesis and synaptic elimination. At first, coarse patterns of synaptic connectivity are formed and thereafter refined and remodeled by neuronal activity. It is commonly thought that synapses that are highly active in a functional context are stabilized and strengthened while those that are not active in this manner are weakened and subsequently removed. However, little is still known about the synaptic plasticity specifically involved in neural network refinement. The objective of this thesis is to describe the properties of a particular form of synaptic plasticity, specifically present in developing synapses and to test current ideas of how this plasticity may participate in events leading to synapse stabilization or elimination.

Specific aims

i) To clarify the extent of lability of AMPA signaling at developing CA3-CA1 synapses when exposed to continuous sparse synaptic activation, and the long-term effects of this activation.

ii) To understand the relationship between the lability of AMPA signaling to sparse synaptic activation and the low frequency induced LTD.

iii) To elucidate the role of NMDARs and mGluRs in the lability of AMPA signaling.

iv) To clarify the extent of stabilization of AMPA signaling that can be imparted to synapses participating in Hebbian activity.

METHODOLOGICAL CONSIDERATIONS

Brief outline of hippocampal anatomy

As noted in the INTRODUCTION, the thesis work was performed using the rat hippocampus and specifically the glutamatergic monosynaptic connection between CA3 and CA1 pyramidal cells (the CA3-CA1 synapse). The popularity of the hippocampus in the study of brain physiology, apart from it being a structure of great importance for learning and memory (Scoville and Milner, 1957; Stepien and Sierpinski, 1964; Morris et al., 1982), is due to its well-defined structure that is easily dissected from the brain, and its relatively simple intrinsic circuitry. The hippocampal formation consists of four regions; the dentate gyrus, the hippocampus proper (or the *cornu ammonis*, which consists of the subfields, CA3, CA2 and CA1), the subicular complex and the entorhinal cortex (*Fig. 1A*). The main intrinsic circuit of the hippocampal formation consists of unidirectional excitatory projections starting with neurones in the entorhinal cortex that via the perforant path project to the granule cells of the dentate gyrus. The granule cells in their turn project via the mossy fibers to CA3 pyramidal cells whose axons, the so called Schaffer collaterals, make contact with CA1 pyramidal cells. The CA1 pyramidal cells project to neurones of the subicular complex that finally project back to neurones in the entorhinal cortex. The circuitry is however somewhat more complex than just described in that there are projections also within the CA3 region and dentate gyrus (associational projections), as well as a direct projection from the entorhinal cortex to the CA1 region, the entorhinal cells making contact with the distal dendrites of the CA1 pyramidal cells. It should also be noted that the CA1 pyramidal cells, in contrast to neocortical pyramidal cells, are constrained to a single cell layer with basal dendrites extending into the stratum oriens and apical dendrites extending into the stratum radiatum and stratum lacunosum-moleculare (*Fig. 1B)*. Their axons pass through the stratum oriens and form a superficial layer (alveus) that projects to the subiculum.

In addition to the pyramidal cells, the hippocampal circuitry contains a diverse collection of GABAergic interneurones located in the pyramidal cell layer as well as in the dendritic layer. These interneurones mediate feed-forward as well as feedback inhibition to the pyramidal cells and are of great importance for temporally and spatially shaping the action potential output from the hippocampus. There are also within the hippocampus axons from other parts

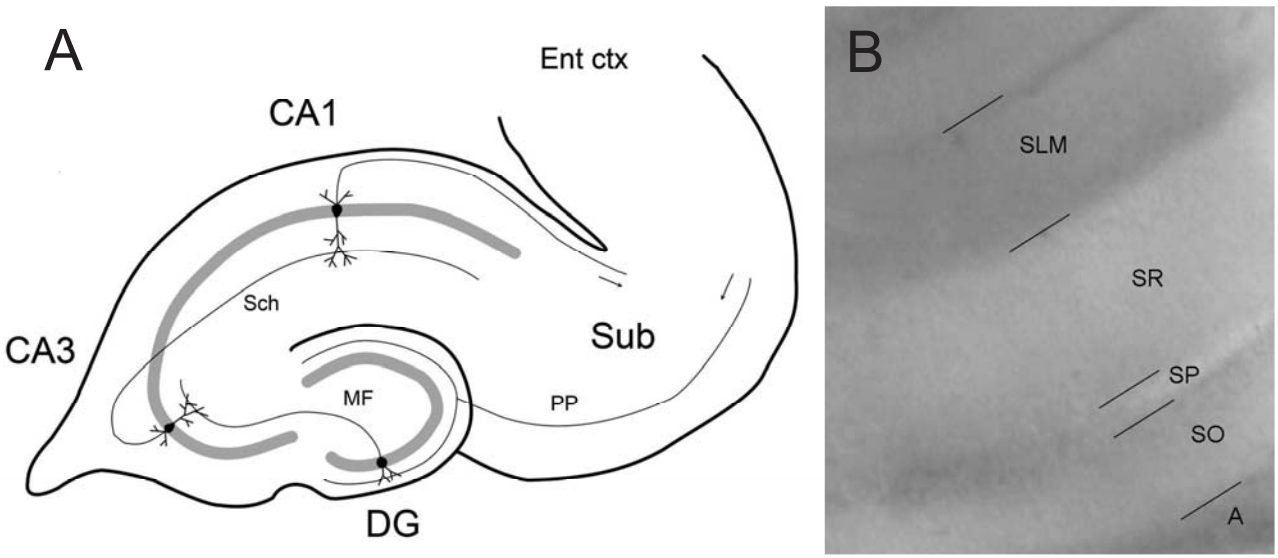

Figure 1. *The hippocampal slice. A, a schematic drawing of the hippocampal slice. The major regions and projections of the hippocampal formation are indicated. Abbreviations: CA – cornu ammonis, DG – dentate gyrus, Ent ctx – entorhinal cortex, MF – mossy fibers, PP – perforant path, Sch – Schaffer collaterals, Sub – subiculum. B, a microscopic picture of the CA1 with the different layers indicated. Note that this picture is rotated approximately 180° compared to the schematic drawing. Abbreviations: A – alveus, SLM – stratum lacunosum moleculare, SO – stratum oriens, SP – stratum pyramidale, SR – stratum radiatum.*

of the brain modulating hippocampal activity through the release of transmitters such as acetylcholine, noradrenaline, and serotonin.

The hippocampal slice preparation

For this thesis I have used the in vitro hippocampal transverse slice preparation taken from postnatal day 8-12 (P8-12) rats. Electrophysiological studies on slices from the hippocampus were pioneered by Skrede and Westgaard (1971) and have thus now been performed for almost 40 years. Early electrophysiological studies suggested that the circuitry of the hippocampus described above is organized in a transverse lamellar fashion (Anderson et al., 1971) but this only seems to hold true for the mossy fibers (Amaral and Witter, 1989). Nonetheless, the Schaffer collateral projections from CA3 to CA1 pyramidal cells are lamellar enough to allow, within a slice preparation, for strong synaptic activation of CA1 pyramidal cells by stimulation of these collaterals in the CA1 stratum radiatum. However, these projections are also non-lamellar enough and/or sufficiently non-parallel to the cell body layer to allow for the stimulation of two completely separate populations of synapses to the

same CA1 pyramidal cells by placing two stimulating electrodes in the stratum radiatum on either side of the recording electrode (Wigström and Gustafsson, 1985).

I limited my study of the CA3-CA1 synapse in the developing hippocampus to P8-12 rats. As outlined in the INTRODUCTION, there are at the end of the 2nd postnatal week changes in e.g. the PSD composition, the enzymatic machinery involved in LTP, and in the LTP itself that indicates a transition to a more adult pattern. Mostly for practical purposes I also excluded rats < P8 because of the greater difficulty to obtain field EPSPs in slices from rats that young. This is not to say that the synapses do not change during the 1st and 2nd postnatal weeks. The paired-pulse ratio, an indicator of release probability, changes from an average value ~ 1 during most of the 1st postnatal week to a value ~ 1.5 at the end of the 2nd postnatal week (Wasling et al., 2004). Much of this switch occurs however just before P8 (Wasling et al., 2004), indicating that the synapses from P8-12 rats should on the average be fairly homogenous with respect to this parameter. There are nevertheless several changes occurring during the P8-12 time period. As noted in the INTRODUCTION, the relative fraction of shaft synapses decreases from P6 to P12 while that of spine synapses increases. In addition, during this time period there is a shift in $GABA_A$ receptor mediated transmission from depolarizing to hyperpolarizing (Ben-Ari et al., 2007), a shift that seems gradual and with an exact timing depending on the species and sex of the animal (Ben-Ari et al., 2007). Therefore, this latter source of heterogeneity in the material was avoided by using picrotoxin in all experiments to block the $GABA_A$-ergic transmission.

The *in vitro* slice, its preparation and storage

The technique to prepare slices of brain tissue was developed during the 1950's (Li and Mc Ilwain, 1957), and it was later described that these slices could be used for electrophysiology (Yamamoto and McIlwain, 1966). The advantages of using the *in vitro* slice compared to *in vivo* studies are that you can easily control the position of stimulating and recording electrodes as well as the extracellular milieu (e.g. ion concentrations), and that drugs to manipulate different receptors can be applied without having to deal with the blood-brain-barrier. The disadvantages are that modulatory inputs from other brain regions are lost, and that the slicing of the tissue may cause injury per se. The trauma elicited by the slicing seems however less when using young than adult animals possibly because of the less extensive dendritic trees of the young pyramidal cells.

The experiments in this thesis were performed in accordance with the regulations of the Swedish Animal Welfare law and approved by the local ethical committee for animal research at the University of Gothenburg. In brief, prior to decapitation the rats were anaesthetized via inhalation of isoflurane. After decapitation the brain was removed and put in an ice-cold (0-3° C) solution containing (in mM): 140 cholineCl, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 ascorbic acid and 7 D-glucose. This low temperature lowers tissue metabolism and thereby promotes neuronal survival (Kataoka and Yanase, 1998). To avoid neuronal activity during slicing Na^+ was substituted by the impermeant choline ion. Transverse slices from the dorsal hippocampal (400 μm thick) were cut with a vibratome and were subsequently stored in artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 4 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 0.5 ascorbic acid, 3 myo–inositol, 4 D,L–lactic acid, and 10 D–glucose. The antioxidants ascorbic acid, myo–inositol and D,L–lactic acid were added to reduce oxidative cellular damage (Pellmar, 1995). After 1-8 hours of storage (typically 2-5 hours) at 25° C, a hippocampal slice was transferred to a recording chamber. In the recording chamber the slice was kept submerged in a constant flow (~2 ml/min) of ACSF at ~ 30° C. The ACSF used for perfusion contained (in mM): 124 NaCl, 3 KCl, 4 $CaCl_2$, 4 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4 , 10 D–glucose and 0.1 picrotoxin.

As noted above my experiments were performed in the presence of picrotoxin to block the $GABA_A$-ergic transmission. When adding picrotoxin to adult slices 4 mM Ca^{2+} and Mg^{2+} is used in the perfusion fluid to lower the excitability of the neurones and thus prevent spontaneous activity (Wigström and Gustafsson, 1983; Fink et al., 2007). While such a high divalent ion concentrations may seem superfluous early in the 2nd postnatal week when the $GABA_A$ receptor mediated transmission is still depolarizing, it may well be required for the slices from the P11-12 rats. As a precaution I therefore used 4 mM Ca^{2+} and Mg^{2+} for the P11-12 slices, and thus by necessity also for the P8-10 slices. As argued by Oliet et al. (1997), this Ca^{2+}/Mg^{2+} ratio may in fact be more physiological than the 2.5 mM/1.3 mM commonly used. As a further precaution against spontaneous activity a surgical cut was made between the CA3 and CA1 regions. However, since most of the CA3 pyramidal cell axons innervating the CA1 pyramidal cells will already have been cut during the slicing (Amaral and Witter, 1989), this procedure only means that all, rather than the vast majority, of the presynaptic terminals activated in these slice experiments are disconnected from their cell body.

It has been reported that certain forms of plasticity are very sensitive to the exact experimental conditions. For example, to induce mGluR-LTD in P11-35 rats Ca^{2+} and Mg^{2+} concentrations have to be high (e.g. 4 mM) and $GABA_A$ receptors cannot be blocked (Oliet et al., 1997). In contrast, Feinmark et al. (2003) had no trouble to induce mGluR-LTD in slices from P4-10 mice during $GABA_A$ receptor blockade. Nevertheless, I also examined whether the very low frequency depression could be induced also at lower Ca^{2+} and Mg^{2+} concentrations and in the absence of picrotoxin, showing that this kind of plasticity is also present under these conditions (unpublished observations). With respect to temperature, in the literature slice experiments can be found to be performed at any temperature between room temperature and close to physiological temperature. In my experiments the temperature in the recording chamber (~ 30° C) was somewhat lower than the physiological temperature of the brain of the rat pup (estimated to be 35° C (Conradi et al., 1984)). This lower temperature was chosen because the solubility of gases in solutions is higher at lower temperature, thereby ensuring a high concentration of O_2 reaching the slice, and also served to slightly lower the metabolism of the slice. During preparation as well as during recordings all solutions were continuously bubbled with 95% O_2 and 5% CO_2 keeping the tissue oxygenated and buffered to a pH of ~ 7.4. Teflon tubes were used between the container holding the ACSF (where it was bubbled) and the recording chamber, in order to minimize O_2 diffusion through the tube walls.

Recording and analysis

In this study extracellular field recordings were used to measure evoked synaptic transmission (*Fig. 2*). On the slice level this is a non-invasive method in that all the postsynaptic neurones that generate the synaptic response are left intact. In simple terms, the field potential is the extracellular component of a circuit produced by current that enters the neurones at the dendritically located synaptic sites, flows intracellularly towards the soma where it exits and returns via the extracellular space to the synaptic sites. Since the CA1 pyramidal cell somas are positioned as a single layer with their apical dendrites projecting at a right angle from that layer, all synaptic currents will flow in the same general direction and thereby summate to generate the field potential. The field EPSP recorded in a hippocampal slice is generated from the postsynaptic currents of hundreds of neurones, and thus the variability of the field EPSP is quite small. In addition to the field EPSP, a presynaptic volley produced by the action potentials of the electrically stimulated Schaffer collaterals will also be recorded (*Fig. 2*). The size of this volley is proportional to the number of presynaptic axons activated, and thus

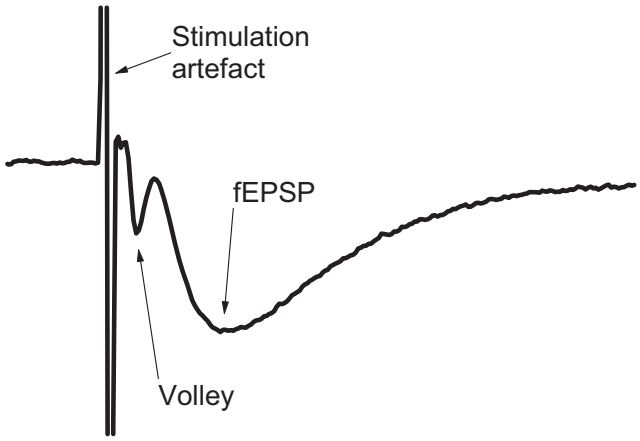

Figure 2. *Example of a field potential recording.*

serves as an internal control of the actual presynaptic activity that is evoked by each stimulus. In my experiments the field recordings were made in the stratum radiatum of the CA1 subfield by means of a glass micropipette (resistance: ~ 2 MΩ, filled with 1 M NaCl). The stimulating electrodes (tungsten electrodes insulated except at the very tip, resistance: ~ 0.1 MΩ) were placed in the stratum radiatum, on either side of the recording electrode, to activate two separate sets of Schaffer collaterals (*Fig. 3*). The stimuli consisted of biphasic constant current pulses (200 + 200 μs, 20-50 μA). Such stimulation would normally set up synaptic activity in the CA1 pyramidal neurones consisting not only of monosynaptic excitation but also of mono- and/or disynaptic inhibition mediated through direct activation of GABAergic interneurone axons and/or synaptic activation of such interneurones, respectively, resulting in a field potential of mixed excitation/inhibition. However, since GABAergic transmission has been blocked in my experiments, the field potential I observe consists solely of a field EPSP.

A very special feature of my experiments was that contrary to common practice I did not wait for a "stable baseline" before applying the experimental stimulation protocol whose effect was to be studied. Instead, "baseline" stimulation per se constituted the experimental stimulation pattern, and the very first field EPSP to be evoked (the naïve level) was also the first synaptic response to be included in the analysis. Before evoking this response I therefore used a specified procedure in all the experiments in that following the placement of the slice in the recording chamber I waited 10 minutes before placing the stimulating and recording electrodes at their respective sites and thereafter an additional 10 minutes before I commenced the stimulation protocol. This procedure was made in order to ensure that the slices as well as the electrodes had equilibrated to their new surroundings in an equal manner among the experiments. That this procedure does indeed result in stable conditions when stimulation was applied was demonstrated by the fact that in control experiments the field EPSP remained

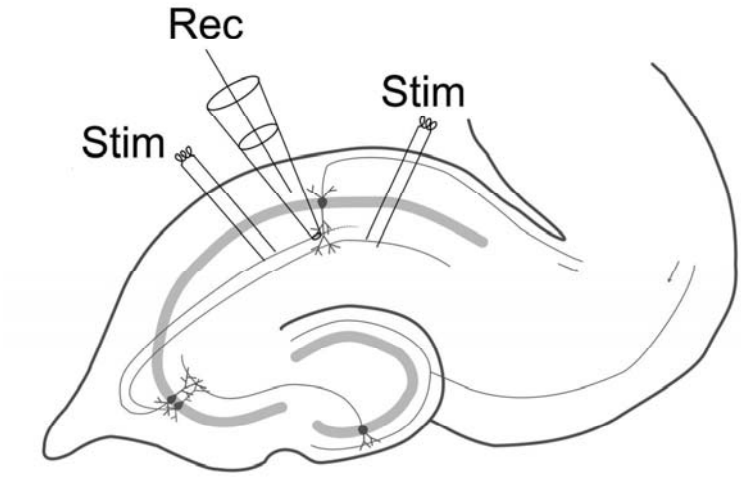

Figure 3. *A schematic drawing of the positioning of the recording electrode and stimulation electrodes in the stratum radiatum of the CA1 subfield.*

unaltered after 2 hours of stimulus interruption following a few initial stimuli to establish the naïve level (**Paper I**, Fig. 1B).

Since the low frequency stimulation used in these experiments (0.05-1 Hz) was aimed to functionally simulate sparse synaptic activity not resulting in network activity, the stimulation was pre-set at a strength that, based on my experience, should produce synaptic activity not resulting in CA1 pyramidal cell action potential activity. Therefore, whenever the pre-set stimulation strength resulted in a field EPSP showing clear signs of population spike activity the experiment was discontinued.

The tetanization protocol that I have used to induce LTP in **Paper III** consisted of three trains of 20 impulses at 50 Hz. To optimize the depolarization of the postsynaptic cells during the tetanization this protocol was applied simultaneously to both the stimulating electrodes on either side of the recording electrode. Moreover, since after a single 20-impulse tetanus the potentiation takes ~ 20 s to reach its peak (Gustafsson et al., 1989), these tetani were given 20 seconds apart. These tetanizations were repeated three times approximately 7.5 minutes apart, because such spaced repeated stimulation has been shown to result in a more persistent activation of enzymes involved in synaptic plasticity (Wu et al., 2001), and results in a more stable LTP (Huang and Kandel, 1994).

Volley compensation

Since my aim with this thesis was to investigate synaptic plasticity it was imperative that the number of axons, and thereby synapses, activated during a given experiment remained

constant either with time per se or by the given stimulation. As indicated above, the size of the presynaptic volley is a measure of the number of activated presynaptic axons, and thus serves as an indicator of the stability of presynaptic activation. The size of the presynaptic volley, measured as the slope of the initial positive-negative deflection by linear regression, was thus measured in parallel with the field EPSP slope in each experiment. While in a number of experiments the volley changed only marginally (< 5%) during the recording period, in other experiments larger changes were observed. In order not to discard all these latter experiments I made an arbitrary cut-off in which I accepted volley changes up to 20% (**Paper I-II**), or for the more prolonged recordings in **Paper III** up to 30%. Since a 20-30% change in size of the axonal volley, if representing a change in the number of activated synapses, will significantly affect the amount of synaptic depression/potentiation observed, I linearly adjusted field EPSP magnitude with the change in the size of the volley in each experiment. To avoid introducing more noise to the field EPSP measurements the individual volley data points were averaged, using a bin width of 20 volleys. Such a compensation assumes a linear relationship between volley and field EPSP size, which has been demonstrated previously (Wigström and Gustafsson, 1985) and which I also observed when examined by lowering the stimulation strength (**Paper I**). Furthermore, for a set of experimental data I also compared uncompensated and compensated field EPSP changes with those observed in the experiments in which the volley was particularly stable (**Paper I**). While a discrepancy between the average field EPSP changes was observed in the uncompensated data compared to that in the "stable" experiments, this discrepancy was no longer present using the compensated data.

An increase in volley size reflecting the 'accidental' recruitment of additional presynaptic fibers however imposes a problem that would be particularly problematic for this compensation for the evaluation of the amount of reversible and lasting depression following stimulus interruption. As these synapses recruited during the stimulus interruption would be previously non-stimulated they would thus add a disproportionate component to the synaptic strength upon renewed stimulation. However, in most of the experiments the volley size was found to decrease, not to increase. In the experiments with wash-out of D-AP5 in control solution (**Paper II**, Fig. 4C), the maximum individual volley increase between the end of the 900 stimuli and the restart of stimulation after the interruption was only 6%, and experiments in which a volley increase was found did not express a larger recovery than those with no

increase or a decrease (after volley compensation). This same result was also observed in the experiments in which D-AP5 was washed-out in the presence of LY 341495 (**Paper II**, Fig. 3C).

SUMMARY OF RESULTS

How labile is the AMPA signaling in the neonatal rat?

A previous field recording study of CA3-CA1 synapses in the 2nd postnatal week rat indicated that ~35% of the naïve (previously non-stimulated) AMPAR mediated synaptic response was readily lost upon sparse stimulation (~ 100 stimuli at 0.05-0.2 Hz) (Abrahamsson et al., 2007). However, this study also indicated that the depression might not have been saturated with this degree of stimulation. Indeed, as I show in **Paper I** (Fig. 1D), after further 0.05-0.2 Hz stimulation (900 stimuli) ~ 60% of the naïve synaptic response was lost, and yet the depression was still not saturated (**Paper I,** Fig. 1B) (see also *Fig, 4*). In **Paper III (**Fig. 1C**)** I used even more prolonged 0.2 Hz stimulation (2700 stimuli) resulting in a loss of ~ 80% of the naïve response. Thus, at most only a small fraction of the synaptic response seems stable in the long run under circumstances of such very low frequency synaptic activation. Assuming that all of the depression evoked in this way is explained by AMPA silencing (Xiao et al., 2004), the present results thus demonstrate that although a clear gradient of lability does exist among the synapses, the vast majority of them can indeed lose their AMPA signaling in response to very low frequency stimulation. In my experiments the fields EPSPs were always subthreshold for spike generation and in the experiments reported in **Paper I** (Fig. 2B) I found no relation between the amount of depression and field EPSP magnitude over a 5-fold range. Moreover, I observed no relation between the amount of depression and an initial NMDAR dependent potentiation induced by this stimulation (**Paper I**, Fig. 2, C and D). The synaptic lability in response to very low frequency activation is thus not critically dependent on the amount of depolarization induced by the synaptic activation.

Next, I examined the depression of the naïve AMPAR mediated response to the “classical" LTD-inducing stimulation of 900 stimuli at 1 Hz. This procedure failed to produce a more potent depression than the corresponding number of stimuli at 0.05-0.2 Hz **(Paper I**, Fig. 2A), a result supporting previous observations in whole-cell recording using a briefer number (~ 100) of stimuli (Xiao et al., 2004). When even more prolonged 1 Hz stimulation was used (2700 stimuli) ~ 90% of the synaptic response was lost. Importantly, however, this amount was not significantly larger than that obtained using the same amount of stimuli at 0.2 Hz (**Paper III**, Fig. 1C and Fig. 3C). Thus, the 1 Hz stimulation is not more potent in inducing

depression during on-going stimulation than stimulation within the test pulse frequency range (0.2-0.05 Hz).

Lability during KAR blockade

KARs have been shown to induce a tonic depression of synaptic depression in the first postnatal week in the rat (Lauri et al., 2006; Sallert et al., 2007). To examine whether KAR activation could play a role in low frequency induced depression in the 2nd postnatal week, I added the KAR antagonist UBP-302 after having established the naïve level by a few 0.2 Hz stimuli, and 20 min later applied 900 stimuli at 0.2 Hz. I did not find any effect on the naïve level or on the amount of induced depression (**Paper III**, Fig. 6). Thus, under my experimental conditions there was neither any tonic KAR mediated depression nor any such depression induced during the stimulation.

Lability during combined blockade of NMDARs and mGluRs

In previous reports using briefer stimulation the low frequency induced depression was found to be unaffected by blockade of NMDARs and mGluRs (Xiao et al., 2004; Abrahamsson et al., 2007). In contrast, I observed that 900 stimuli at 0.2-1 Hz in the combined presence of the NMDAR antagonist D-AP5 (50 μM) and the broad spectrum mGluR antagonist LY 341495 (100 μM) resulted in a somewhat smaller loss of the synaptic response compared to in the absence of these drugs, 45-50% (**Paper I,** Fig. 3B) vs ~ 60% (see above), this difference being statistically significant. This discrepancy vis-à-vis previous reports might be explained by a lingering effect of an initial NMDAR dependent potentiation that counteracts the depression during the first 100 stimuli (**Paper I**, Fig. 2E). Thus, while the synaptic lability to low frequency stimulation largely exists also in the absence of NMDAR and mGluR activation, it is indeed significantly facilitated by such receptor activations.

Lability during NMDAR blockade

Next, I blocked NMDARs and mGluRs separately. Starting with NMDAR blockade, 900 stimuli at both 0.2 Hz and 1 Hz surprisingly resulted in a significantly smaller depression than that observed during combined NMDAR/mGluR blockade. This effect was more pronounced at 0.2 Hz stimulation and resulted in a loss of only ~ 30% of the synaptic response (**Paper I,** Fig. 5C) compared to the ~ 45% observed during a combined blockade, clearly suggesting that mGluR activity results in a decreased lability of the AMPA signaling. To examine which

subtype of mGluRs that mediates this effect, experiments were repeated in the presence of more selective mGluR antagonists. These experiments showed that 900 stimuli at 0.2 Hz resulted in a loss of ~ 50 % and ~ 40% of the synaptic response when selective group I and group II/III antagonists were added, respectively (**Paper I,** Fig. 6A). While these results rule out group II/III mGluRs as mediators of this stabilizing action they do not clearly establish group I mGluRs as sole mediators but rather indicate the contribution of several subtypes.

Lability during mGluR blockade

Application of 900 stimuli at 0.2 Hz and 1 Hz during mGluR blockade resulted in a loss of ~ 60% and ~ 70 %, respectively, of the synaptic response (**Paper I**, Fig. 5, C and D). The lability was thus at least as great as in control solution (the absence of the receptor antagonists), showing that NMDARs alone account for the added lability compared to when both NMDARs and mGluRs are blocked. In fact, 1 Hz stimulation resulted in an even significantly larger depression during mGluR blockade than in control solution, indicating that mGluRs actually oppose the 1 Hz induced depression under these latter conditions. Experiments using selective mGluR antagonists showed that this opposing action was largely explained by group I mGluR activation (**Paper I**, Fig. 6B).

To summarize, when exposed to prolonged low frequency stimulation the AMPA signaling of neonatal CA3-CA1 synapses can in time become almost extinguished, this effect observed to much the same extent at very low frequencies such as 0.05-0.2 Hz as at the conventional LTD-inducing stimulation of 1 Hz. While the induction of this depression is largely NMDAR and mGluR independent, it is, however, facilitated by NMDARs and impaired by mGluRs

Reversibility of the low frequency induced depression

When examined using whole-cell recording the depression induced by the low frequency stimulation was found to remain unaltered when stimulation was resumed after an 8-20 min stimulus interruption (Xiao et al., 2004; Abrahamsson et al., 2007). However, using field recordings and perforated-patch recordings the depression was found to fully reverse with a time constant of ~ 7 min when stimulation was interrupted after ~ 100 stimuli at 0.2 Hz (Abrahamsson et al., 2007). Taken together, these results suggest that the lack of reversal observed in the whole-cell experiments reflects a “wash-out” of cytoplasmic components necessary for reversal. In my experiments, however, the depression induced by 900 stimuli

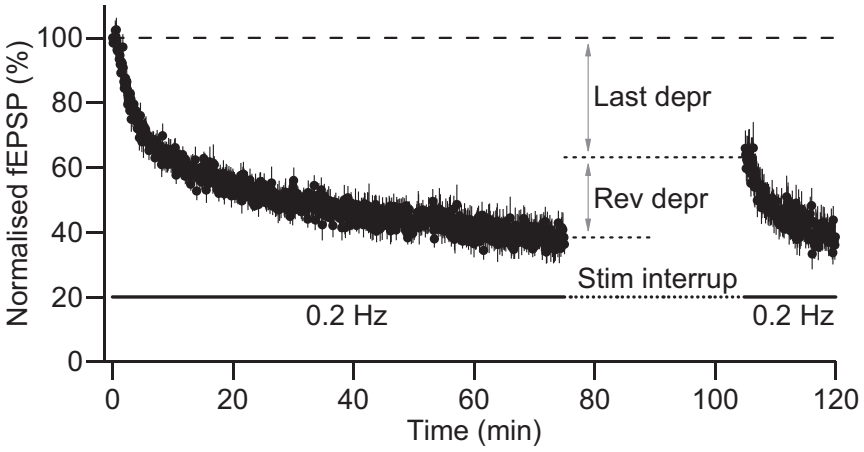

Figure 4. *Representation of the lasting depression (Last depr) and reversible depression (Rev depr).*

only partially reversed by stimulus interruption and thus contains both reversible and more lasting components (**Paper II**, Fig. 1A) (*Fig. 4*). I did not observe any reversal when the stimulus interruption was very brief (1-2 min), and the reversal (of the reversible component) developed slowly but was essentially complete after a 30 min stimulus interruption as no further reversal was observed when a 60 min stimulus interruption was used (**Paper II,** Fig. 1B). The time course of this reversal was thus roughly similar to that described previously (Abrahamsson et al., 2007). Following 900 stimuli the reversible part of the depression was small, representing around one-third of the total depression (**Paper II,** Fig. 1A). After 2700 stimuli at 0.2 Hz this proportion was reduced even further to only one-fifth of the total depression (**Paper III**, Fig. 1C), indicating less and less reversibility of the depression the longer the stimulation. However, surprisingly, this was not true with respect to the 1 Hz stimulation in that almost half the depression induced by 2700 stimuli reversed by stimulus interruption (**Paper III**, Fig. 3C). Thus, the reduction in reversibility with increased number of stimuli cannot be explained by the number of stimuli per se.

Depression reversing by spontaneous NMDAR activity

I found that 900 stimuli at 0.2 Hz and 1 Hz resulted in only a partial reversal also during combined NMDAR/mGluR blockade, indicating that the depression also under these circumstances contains both reversible and lasting components (**Paper II**, Fig. 2). However, in experiments in which I washed-out the NMDAR antagonist as soon as the stimulation was over, stimulus interruption resulted in a complete reversal (**Paper II**, Fig. 3A). This result shows that stimulation in the absence of NMDAR/mGluR activity results in a depression component that reverses as a consequence of spontaneous NMDAR activity during the

stimulus interruption. In fact, this component constituted the major part of both the 0.2 Hz- and 1 Hz-induced depression during combined NMDAR/mGluR blockade (*Fig. 5*). I next examined to which extent stimulation in the control solution results in such a depression component by washing-in an NMDAR antagonist after stimulation was over. While a small such component was still observed after 0.2 Hz stimulation, none was observed after 1 Hz stimulation (*Fig. 5A*). This depression component induced by stimulation in the absence of NMDAR/mGluR activity thus appears to be an unstable version of the lasting depression induced in control solution. Experiments performed when NMDARs or mGluRs were separately blocked suggested that this reversible depression was impaired by mGluR activity (**Paper II**, Fig. 8) (*Fig. 5A*).

Depression reversing in an NMDAR independent manner

My finding that the depression partly reverses also when NMDARs were blocked during the stimulus interruption means that there must be a second form of reversible depression. As noted above, this depression will constitute the major part of the reversible depression obtained when stimulation is given in the control solution and thus be ~ one-third of the total depression induced under these conditions (**Paper II,** Fig. 1) (*Fig. 5*). If expressed relative to the naïve level, this component was similar (15-20% of the naïve level) irrespective of whether it was induced by 0.2 Hz or 1 Hz stimulation, or whether induced in the presence or absence of a combined NMDAR/mGluR blockade (**Paper II**, Fig. 8) (*Fig. 5*). Assuming that the reversal after 2700 stimuli at 0.2 Hz (**Paper III**, Fig. 1C) also mainly reflects this depression, its magnitude relative to the naïve level stays much the same also after this very prolonged stimulation.

Lability of the reversed depression

Following the stimulus interruption I applied 180 stimuli at 0.2 Hz to examine the lability of the reversed AMPA signaling (**Paper II and III**) (*Fig. 4*). These 180 stimuli were sufficient to depress the synaptic response back to the level of depression induced by the preceding 900 stimuli whether given at 0.2 Hz or 1 Hz. This result suggests that the reversed transmission represents a more labile fraction of the AMPA signaling than the naïve AMPA signaling.

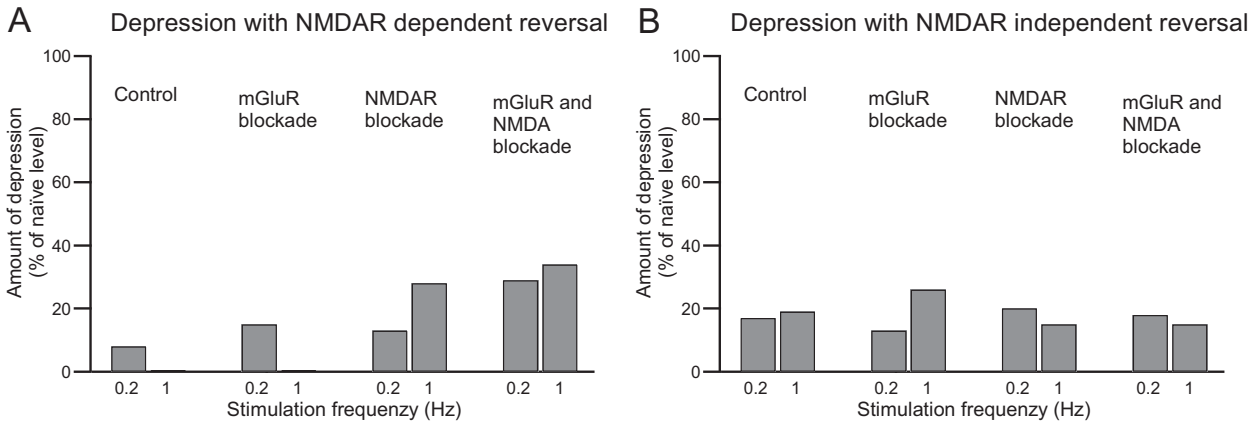

Figure 5. *Summary bar histograms of the two forms of reversible depressions, during the different pharmacological conditions and stimulation frequencies.*

To summarize, when the depression is induced in the absence of NMDAR and mGluR activation it reverses slowly within 30 min when stimulation is interrupted, but via two separate paths. Thus, while ~ one-third of the depression reverses also when NMDARs are blocked during the stimulus interruption the remaining depression requires NMDAR activity during the interruption to reverse. Interestingly, this latter path for reversal is essentially absent when depression was induced in the control solution.

Low frequency induced long-lasting depression

In control solution 900 stimuli at 0.2 Hz and 1 Hz resulted also in a depression that lasted longer than 60 min. By blocking NMDARs and mGluRs separately I found that while stimulation during NMDAR blockade failed to result in long-lasting depression, stimulation during mGluR blockade resulted in long-lasting depression of similar magnitude as in the control solution (**Paper II**, Figs. 4 and 5) (*Fig. 6*). Thus, NMDAR activation during the stimulation seems both necessary and sufficient for the induction of a long-lasting component of the depression. Following 900 stimuli this long-lasting component constituted ~ two-thirds of the total depression and did not differ significantly between 0.2 Hz and 1 Hz stimulation. Surprisingly, this was not the case when 2700 stimuli were given in that 0.2 Hz stimulation was more efficient than 1 Hz stimulation (**Paper III**). Thus, while 0.2 Hz stimulation resulted in long-lasting depression that constituted four-fifths of the total depression and two-thirds of the naïve synaptic response (**Paper III**, Fig. 1C), the long-lasting depression induced by 1 Hz stimulation comprised only ~ one-half of both the total depression and of the naïve synaptic response (**Paper III**, Fig. 3A). Nonetheless, these results show that in the 2nd postnatal week a

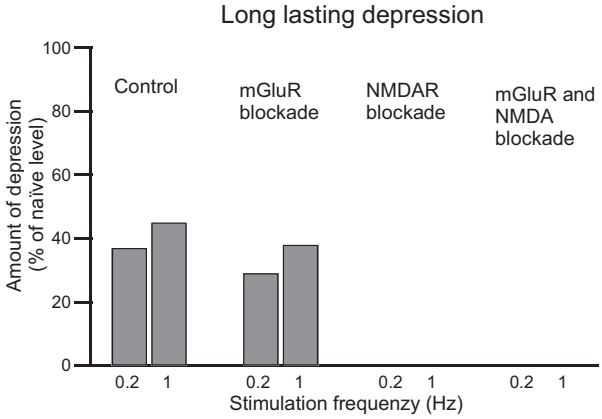

Figure 6. *Summary bar histogram of the lasting depression, during the different pharmacological conditions and stimulation frequencies.*

stimulation that is not expected to induce any lasting depression, like 0.2 Hz stimulation, in the long run can result in a stable loss of most of the naïve synaptic response.

I next examined whether this loss was permanent by applying high frequency tetanization to depressed synapses. These experiments showed that after when low frequency stimulation had resulted in a long-lasting depression to ~ 30% of the naïve synaptic response, three bouts of high frequency tetanization re-established the synaptic response to its naïve level (**Paper III**, Fig. 5). Hence the long-lasting depression evoked by low frequency stimulation is reversible but a reversal requires Hebbian activity.

In contrast to my results described above showing that NMDARs but not mGluRs are critical for long-lasting depression, it has earlier been found that LTD induced in the 2nd postnatal week relies to a higher degree on mGluRs than on NMDARs (Li et al., 2002; Feinmark et al., 2003). When making conventional LTD experiments, i.e., applying 900 stimuli at 1 Hz after having established a stable baseline by applying a 0.1 Hz test pulse stimulation, I also found the resulting LTD to rely more on mGluRs than on NMDARs (**Paper I**, Fig. 5, A and B). These results indicate that the discrepancy between the long-lasting depression of previously naïve synapse and LTD is likely explained by the difference in stimulation protocols rather than in experimental conditions.

To summarize, low frequency induced NMDAR activity is both necessary and sufficient for this stimulation to result in a long-lasting depression, and is induced at least as efficiently at 0.2 Hz as at 1 Hz stimulation. Surprisingly, low frequency induced mGluR activity, while being important for LTD induction, does not result in any long-lasting depression (*Fig. 6*).

Does Hebbian activity reduce lability of AMPA signaling?

A previous key observation was that Hebbian activity, either evoked by pairing low frequency activity with postsynaptic membrane depolarization (Xiao et al., 2004) or evoked by high frequency tetanization (Abrahamsson et al., 2008), appeared to stabilize the synaptic response at its naïve level. This observation led to the hypothesis that the main impact of Hebbian plasticity in the neonatal brain is not to potentiate but instead to stabilize AMPA signaling (Groc et al., 2006a). In my experiments I applied repeated strong high frequency tetanization to a naïve synaptic response and thereafter examined the lability/stability of AMPA signaling by prolonged 0.2 Hz stimulation (~ 4 hours). These experiments showed that after the Hebbian activity had been elicited the 0.2 Hz stimulation resulted in a continuous decline of the synaptic response with a reduction to 37% of its naïve magnitude after 4 hours, i.e., to a depression of 63% from the naïve level (**Paper III**, Fig. 1B). Contrary to what was previously put forward (Groc et al., 2006a; Abrahamsson et al., 2008) Hebbian activity is in fact unable to stabilize AMPA signaling at the naïve level vis-à-vis test pulse stimulation such as that at 0.2 Hz. Nonetheless, since as described above, the synaptic response of naïve synapses declined to ~ 20% of the naïve level following 4 hours of 0.2 Hz stimulation, Hebbian activity seems to result in some stabilization of the AMPA signaling, the depression being reduced from ~ 80% to ~ 60 % from the naïve level, i.e. to ~ three quarters of the depression of naïve synapses.

A comparison between the low frequency induced depression of the AMPA signaling of naïve and tetanized synapses is, however, not straightforward. Abrahamsson et al. (2008) described that tetanization resulted in an initial transient potentiation above the naïve level that declined within 5-15 min after tetanization in a stimulation dependent manner. In fact, I observed that not even when stimulation was interrupted for two hours following the tetanization was the potentiation found to have declined when stimulation was resumed (**Paper III**). Therefore, the time course of the test pulse induced depression of tetanized synapses will not only depend upon the effect of Hebbian activity on the lability of the pre-existing naïve AMPA signaling but also of the lability of the potentiated AMPA signaling above the naïve level. The increased stability following Hebbian activity indicated by the results described above rests therefore upon the assumption that the potentiated AMPA signaling, in contrast the pre-existing AMPA signaling, is fully declined at 4 hours after the tetanization. A scenario that would avoid to result in a possibly exaggerated view of the stabilizing action of Hebbian

activity is that following tetanization the potentiated and the previously naïve AMPA signaling are labile to the same degree with respect to the 0.2 Hz stimulation. In order to compare the lability of AMPA signaling before and after tetanization the depression of tetanized synapses was thus normalized by setting the peak level of the initial potentiation to 100%, as a post-tetanus naïve level. This procedure has also the advantage that it compares the overall lability of AMPA signaling before and after tetanization.

Adopting this procedure for comparison I found that tetanized synapses in the long run (i.e., after 4 hours of stimulation) were not more stable than the naïve synapses were. However, when examined at earlier time points, e.g., within the 1st hour after tetanization, the depression was substantially less for the tetanized synapses indicating a temporary but not permanent partial stabilization of AMPA signaling (**Paper III**, Fig, 2B). Using the same protocol I also applied 1 Hz stimulation and found a similar result (**Paper III**, Fig. 3C). In order to examine whether the temporary stabilization indicated by the above comparison is a function of time after tetanization, 900 stimuli at 1 Hz were applied after tetanization after either 15 min or 2 hours of stimulus interruption. This experiment showed that the depression induced by this stimulation was independent of its timing relative to the tetanization. I also found that the depression induced by this stimulation was less at tetanized than at naïve synapses, particularly with respect to the long-lasting depression. My results thus show that Hebbian activity results in at least a partial stabilization of AMPA signaling, most evident as a reduced ability to induce a long-lasting depression. Moreover, importantly, this partially stabilizing effect is not linked to time after tetanization but to the amount of stimuli given.

To summarize, high-frequency tetanization of naïve synaptic responses results in a potentiated post-tetanus naïve level that will remain unaltered, at least for two hours, unless the synapses are exposed to low frequency stimulation. The AMPA signaling of tetanized synapses normalized to this post-tetanus naïve level is in the long run (4 hours) no more stable than the AMPA signaling of naïve synapses. However, within the first hour of low frequency stimulation irrespective of whether this stimulation is applied 15 min or 2 hours following tetanization the induction of low frequency induced depression, in particular long-lasting depression, is impaired.

GENERAL DISCUSSION

My thesis supports and extends previous observations (Xiao et al., 2004; Abrahamsson et al., 2007) that AMPA signaling at developing CA3-CA1 synapses becomes depressed, i.e., is labile, in the face of very low frequency activation (≤ 0.2 Hz). In fact, while previous work using field recordings suggested that the major fraction of the AMPA signaling by the 2nd postnatal week was stable (Abrahamsson et al., 2007), my present work using a longer duration stimulation protocol suggests that nearly all AMPA signaling in the 2nd postnatal week is labile. In keeping with this result I also found that Hebbian activity in the long run failed to alter this lability. Thus while my study, in agreement with previous observations (Abrahamsson et al., 2008), suggests that Hebbian activity may exert a stabilizing action on the AMPA signaling, this effect is in fact only partial and possibly also only temporary. My thesis does therefore not support the scenario in which synapses that participate in functional network activity, i.e., together with a sufficient number of other synapses, become stabilized (Groc et al., 2006a; Abrahamsson et al., 2008). Instead, my results suggest a scenario in which participation in Hebbian activity is the means for a synapse to temporarily keep, or regain, its AMPA signaling. If so, synapses in the developing brain will maintain their AMPA signaling only by more or less continuous participation in cooperative neuronal activity while synaptic activity outside this context leads to loss of AMPA signaling and to possible elimination.

My thesis also provides a more complex picture of the depression process underlying the lability to very low frequency stimulation. Thus, previous work suggested that this very low frequency induced loss of AMPA signaling is explained by a unique NMDAR and mGluR independent process resulting in AMPA silencing in a subpopulation of the activated synapses. In contrast, my work shows that a large part of the depression in fact is most likely equivalent to the NMDAR-LTD observed in conventional LTD experiments, implying that in the 2nd postnatal week NMDAR-LTD can be induced at much lower frequencies of synaptic activation than previously thought. NMDAR-LTD at these synapses will therefore be substantially underestimated when evaluated in conventional LTD studies as it has already been induced by the initial test pulse stimulation. Importantly, my work suggests that NMDAR-LTD, or rather a mechanistic equivalent to NMDAR-LTD, can be induced in the absence of NMDAR activation but in a labile form that reverses within 30 min when stimulation is discontinued. In the 2nd postnatal week the major role for NMDARs may

therefore not be to induce depression but to stabilize it. Surprisingly, considering that conventionally induced LTD in the first part of the 2nd postnatal week is found to rely more on mGluRs than on NMDARs (**Paper I**) (Li et al., 2002; Feinmark et al., 2003), I did not find any form of depression that relied on mGluRs, not even when 1 Hz stimulation was used. Together with my other results mentioned above this lack of mGluR-related depression calls into question the validity of conventional LTD experiments for analyzing depression at developing synapses.

Finally, my thesis showed that the Hebbian induced transient potentiation above the naïve level did not decay for at least 2 hours after its induction unless the synapses were activated. Thus, not only the naïve AMPA signaling but also the Hebbian induced potentiation is labile in the face of very low frequency stimulation.

In my thesis I have not directly addressed the mechanistic aspects of the observed changes in AMPA signaling experimentally, e.g. by establishing which intracellular signaling systems are involved and in what manner the synaptic function is altered. In the following I will discuss this issue from the perspective of my own results. I will discuss to what extent the presently described depression, de-depression (or reversal), and potentiation can be contained within the single expression mechanism of AMPA silencing/unsilencing, and the possible mechanistic aspects of this silencing/unsilencing.

Mechanistic aspects of the observed changes in AMPA signaling

Expression – postsynaptic

The starting point for any consideration of the expression mechanism(s) for the very low frequency induced depression is the previous demonstration of AMPA silent synapses in the neonatal CA1 region (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996), and the finding that low frequency stimulation can create such AMPA silent synapses (Xiao et al., 2004). Depression of AMPA signaling can in principle be the result of a number of different, both pre- and postsynaptically located, modifications. Below I will argue that there is good reason to exclude a presynaptic modification. Postsynaptically, the AMPARs may be removed either completely or partially from the synapse, or changes in the AMPARs themselves may be induced that alter e.g. their conductance. If the AMPARs are totally removed from a subpopulation of the synapses making them AMPA silent this will be seen

electrophysiologically as an increased variance in the evoked synaptic response (or a decreased $1/CV^2$), denoted as a decrease in quantal content. On the other hand, if the AMPARs are only partially removed from the synapse, or if the AMPAR channel conductance is decreased, leaving the synapses still AMPA signaling albeit at a reduced level, this will be seen as a decrease in quantal amplitude, also denoted as a decreased potency. Xiao et al. (2004) observed that the average change in synaptic magnitude was well matched with the average decrease in quantal content ($1/CV^2$) indicating that the depression was explained by AMPA silencing. A silencing rather than a potency change is also supported by earlier studies examining the quantal properties of neonatal CA3-CA1 synapses (Hanse and Gustafsson, 2001a; Groc et al., 2002). In these studies, in which evoked (Hanse and Gustafsson, 2001a) and spontaneous (Groc et al., 2002) synaptic responses of single synapses were examined, the quantal amplitudes of the evoked responses (see Fig. 3D, Hanse and Gustafsson (2001a)) did not differ from those of the spontaneous responses (see Fig. 5A, Groc et al. (2002)). That is, the quantal amplitude at synapses exposed to stimulation presumed to result in synaptic depression was found to be the same as that of synapses not exposed to such stimulation. This result cannot be reconciled with a depression explained by an overall decrease in potency among the synapses, but can be expected from an all-or-none removal of AMPA signaling in a subpopulation of the synapses.

In the whole-cell experiments by Xiao et al. (2004) NMDAR blockade did not significantly affect the amount of depression observed, and hence most of this silencing must have been induced in an NMDAR independent manner. This seems also reasonable considering that the synaptic responses in the whole-cell experiments were "voltage-clamped" and therefore less likely to result in NMDAR channel opening compared to experiments using field recordings. These whole-cell data would therefore suggest that the NMDAR independent component of the depression observed in my study is explained by AMPA silencing. It is worth noting that in the whole-cell experiments the average depression (using 120 stimuli) was to ~ 60% from the naïve level, i.e., similar to the depression obtained by 900 stimuli using field recordings. It would thus appear that a capacity for AMPA silencing exists in the 2nd postnatal week that includes the majority of the synapses.

I found the low frequency depression to be facilitated by NMDAR activity which, as noted in the INTRODUCTION (section on NMDAR-LTD) can result in AMPAR removal. However,

to what extent NMDAR-LTD is explained by a reduced quantal content and/or reduced quantal amplitude (potency) is unclear. For example, Selig et al. (1995) using 2-6 week old rats showed a decrease in $1/CV^2$ almost matching that of the reduced amplitude, a result compatible with AMPA silencing, whereas Oliet et al. (1996) using 2-4 week old rats found changes in potency only. In the thalamocortical synapse, the NMDAR-LTD was found to be associated with a potency change only (Feldman et al., 1998). The question is however, how relevant these results obtained at CA3-CA1 synapses in older rats, or from other developing synapses, are for the 2nd postnatal week CA3-CA1 synapses. For example, as was noted in the INTRODUCTION (see sections on AMPARs, The postsynaptic density, and Synaptogenesis) the CA3-CA1 synapses change considerably during these first few weeks in morphology (shaft vs spine), in AMPAR subunit composition and in the PSD composition. In fact, the properties of NMDAR-LTP change considerable from P8 to P15 (see section on Long-term potentiation in the INTRODUCTION). Thus data as the above regarding NMDAR-LTD may not contribute to resolving whether NMDARs in the 2nd postnatal week facilitate AMPA silencing rather than induce a decrease in potency. A more important study in this context may instead be that of Adesnik et al. (2008) where NMDARs in individual CA1 pyramidal cells in the developing hippocampus were genetically deleteted. This study showed that CA1 cells devoid of NMDARs exhibited a greater number of AMPA signaling synapses than control cells despite the same overall number of spines, i.e., of existing morphological synapses, indicating that NMDAR activity in the developing hippocampus assists in depressing synapses by maintaining an AMPA silent state.

Expression – presynaptic

The change in $1/CV^2$ observed by Xiao et al. (2004) is also compatible with a presynaptic modification. However, Xiao et al. (2004) did not find any depression of NMDAR mediated transmission as would have been expected from a presynaptic modification in release probability. Other presynaptic scenarios based on a reduced amount of transmitter released, such as kiss and run exocytosis, was also excluded using a low affinity NMDAR antagonist that would have detected a lowered amount of transmitter. Nevertheless, as I used more prolonged stimulation in my experiments than that used by Xiao et al. (2004), a presynaptic component could have been added. During prolonged low frequency stimulation the vesicle release rate could eventually exceed the rate of mobilization of new vesicles resulting in depletion of the number of synaptic vesicles available for release. Depression resulting from

depletion is not necessarily associated with a change in paired-pulse ratio and may therefore mistakenly be thought to be explained by a postsynaptic modification (Hanse and Gustafsson, 2001b). Stimulation of the perforant path-granule cell synapse in the dentate gyrus at 0.2 Hz, but not 0.03 Hz, has in fact been found to result in such a depletion (Abrahamsson et al., 2005). The resulting depression reverses, however, within < 1min after switching to 0.03 Hz, as would also be expected from the kinetics of vesicle recruitment (Stevens and Wesseling, 1999). In contrast, in the present experiments I did not find any reversal from the depression after 1-2 min of stimulus interruption, and no difference in the amount of depression produced by 0.2 and by 0.05 Hz stimulation was observed. Another possible candidate mechanism is the KAR mediated presynaptic depression which has been observed in the first, but not third, postnatal week (Sallert et al., 2007). While this depression is thought to be tonic, and not activity dependent, it is blocked by Hebbian activity resulting in a presynaptic LTP (Lauri et al., 2006). Considering this effect of Hebbian activity it may be presumed that low frequency stimulation might strengthen this depression resulting in LTD. However, using a KAR antagonist, I neither found evidence for a tonic KAR depression nor for any appearance of it during the low frequency stimulation (**Paper III**, Fig. 6).

Induction

My thesis work confirmed that low frequency stimulation depresses neonatal CA3-CA1 synapses without engaging NMDARs and mGluRs. In fact, during combined NMDAR/mGluR blockade almost half of the synaptic response was lost after 900 stimuli (**Paper I**, Fig. 3B), and more prolonged stimulation (2700 stimuli) resulted in a loss of as much as ~ two-thirds of the field EPSP (unpublished observation). However, in contrast to the previous studies (Xiao et al., 2004; Abrahamsson et al., 2007), I found that NMDAR and mGluR activation did affect the amount of depression obtained. Thus, the amount of depression increased when NMDARs were active and was reduced when mGluRs were active, compared to when both receptors were blocked. These results raise the question of how low frequency stimulation in the absence of NMDAR/mGluR activation can result in such a substantial depression of AMPA signaling. They further raise the question of whether the actions of NMDARs and mGluRs represent a modulated induction of the NMDAR/mGluR independent depression, or whether they represent separate processes just adding to or subtracting from the NMDAR/mGluR independent depression.

With respect to the first question it has previously been demonstrated that agonist-binding to the AMPAR can by itself result in AMPAR removal and AMPA silencing. Thus, bath application of AMPA itself has been found to result in AMPAR removal and AMPAR internalization that at least partly is explained by the AMPA binding per se (Ehlers, 2000) (see further below). Furthermore, when disrupting the NSF-GluA2 interaction, low frequency stimulation resulted in depression of the evoked synaptic potential (Lüthi et al., 1999). This depression was explained by a ligand-induced AMPAR internalization, ultimately resulting in AMPA silencing which is normally compensated by a rapid AMPAR recycling (Duprat et al., 2003). However, since the NSF-GluA2 interaction may also regulate the lateral movement of AMPARs from the extrasynaptic to the synaptic membrane (Gardner et al., 2005), this depression can alternatively be explained by a ligand-induced AMPAR lateral diffusion into the extrasynaptic membrane that is normally compensated by a rapid NSF dependent lateral diffusion back (recycling) into the synaptic membrane. Nevertheless, the above results suggest that a ligand-induced AMPAR removal from the synapse can explain an NMDAR/mGluR independent low frequency induced depression, assuming for the neonatal CA3-CA1 synapses a mismatch between such ligand-induced AMPAR removal and the recycling capacity. Such a mismatch could exist either because of a facilitated AMPAR removal or an impaired recycling, and could possibly be a direct consequence of the AMPAR interaction with the PSD proteins existing in the neonatal period. However, Abrahamsson et al. (2008) described that application of the PKA activator forskolin could reverse the very low frequency induced depression and seemingly stabilize AMPA signaling at the naïve level. Moreover, the application of a phosphatase inhibitor considerably slowed the induction time course of the depression (Xiao et al., 2004). These results would suggest that the depression depends on the relative degree of phosphorylation/dephosphorylation of certain synaptic proteins, possibly including the AMPAR itself, whereby a dephosphorylated state promotes depression. Taken together with the data presented above, it thus seems possible that the NMDAR/mGluR independent depression is explained by glutamate binding to dephosphorylated AMPARs resulting in excessive AMPAR removal and/or impaired recycling.

A complicating factor vis-à-vis this interpretation is that the low frequency induced depression seemingly depends on Ca^{2+} for its induction since no depression was observed when the Ca^{2+} chelator BAPTA was present in the whole-cell pipette (Xiao et al., 2004). The

depression could therefore arise as a consequence of a postsynaptic increase in Ca^{2+} rather than because of ligand-binding per se even in the absence of NMDAR and mGluR activation. However, since voltage-gated Ca^{2+} channels are unlikely to contribute (see Xiao et al. (2004)), it is not obvious from where the Ca^{2+} should come. A possible scenario would be that the neonatal CA1 pyramidal cells express GluA2-lacking Ca^{2+}-permeable AMPAR channels. However, the absence of GluA2-lacking AMPARs in synapses onto CA1 pyramidal cells noted by Lu et al. (2009b) seems also to extend back into the 2nd postnatal week. Thus, a study using P8-17 rats, combining electrophysiological recordings with Ca^{2+}-imaging, showed a very low Ca^{2+} permeability of the activated AMPARs (Garaschuk et al., 1996). Moreover, an electrophysiological examination of AMPAR channel properties in P5-13 rats failed to find evidence for GluA2-lacking AMPARs in CA1 pyramidal cells (Isa et al., 1996). In view of these results the effect of BAPTA might not be related to the buffering of Ca^{2+} influx but be explained by a BAPTA mediated lowering of the basal cytoplasmic level of Ca^{2+} below that needed to maintain certain reactions. For example, a ligand-induced AMPAR removal may require ubiquitin-proteasome activation which is Ca^{2+}-sensitive (Patrick et al., 2003). In support of an effect of BAPTA that is more complex than that of direct buffering of Ca^{2+} influx the induction of the depression is not completely blocked unless the stimulation is commenced later than 30 min after the whole-cell break-in (P. Wasling, personal communication)

Accepting ligand-binding to dephosphorylated AMPARs to be the explanation for the NMDAR/mGluR independent depression, NMDAR activation is likely both to facilitate depression during low frequency activation and to reverse it during Hebbian activity. Thus, during low frequency stimulation NMDAR activation will result in additional dephosphorylation of PKA sites (see section on NMDAR-LTD in the INTRODUCTION) such as ser-841 and ser-842 on $GluA2_{long}$ and GluA4 subunits, respectively, resulting in a greater number of dephosphorylated AMPARs. If so, mGluR activation should increase phosphorylation at this PKA site, and, by decreasing the number of phosphorylated AMPARs, impair the depression. However, the mGluR effect was mainly attributed to mGluR group I activation which is not supposed to result in PKA activation. In what manner mGluR activity impairs the depression thus remains unclear. However, NMDAR activation might also facilitate depression in a completely different manner to that described above, namely by restricting AMPAR mRNA availability and translation, thus impairing surface membrane

expression of AMPARs (Grooms et al., 2006). Interestingly, mGluR activation also acts at this level by facilitating dendritic transport of AMPAR mRNA and thus facilitating surface membrane expression of AMPARs (Grooms et al., 2006). As suggested by these authors, by acting in this manner mGluR activation may contribute to the stabilization of LTP induced by a weak tetanization, an effect on LTP reminiscent of that on the low frequency induced depression (Raymond et al., 2000). To what extent such an effect may participate in the mGluR induced impairment of depression is presently unclear. Nonetheless, NMDARs and mGluRs can exercise a bidirectional control of AMPARs reminiscent of their bidirectional control of the low frequency induced depression.

Reversal of depression

Above I have argued that the low frequency induced depression can be explained by a basically NMDAR/mGluR independent postsynaptic AMPA silencing that is facilitated and impaired by NMDAR and mGluR activation, respectively. Nevertheless, when examined after a prolonged stimulus interruption (30-60 min) I found that the depression is dissociated into three different forms, each with distinct minimal requirements for its reversal. Thus, while part of the depression reversed by stimulus interruption alone, another part of the depression was found to require spontaneous NMDAR activity and thus likely an associated AMPAR phosphorylation (cf. Zhu et al. (2000) during the stimulus interruption to reverse. When NMDAR activity was not blocked during the stimulation, a substantial part of the depression failed completely to reverse by stimulus interruption but required Hebbian activity in order to reverse. While these seemingly distinct forms of dissociation may point to the existence of three separate depression processes, it should be noted that stimulus interruption did not result in any reversal whatsoever using whole-cell recording unless perforated-patches were used (Abrahamsson et al., 2007), This result indicates a common sensitivity to postsynaptic wash-out for all these three tentatively separate forms of depression. Moreover, all three of these depressions appear to be momentarily reversed by Hebbian activity (**Paper III**, Fig. 5) (Abrahamsson et al., 2008). The question therefore arises what these three separate requirements for reversal represent?

Reversal of the depression induced during combined NMDAR/mGluR blockade

If the NMDAR/mGluR independent depression is explained by a ligand-induced AMPAR removal could such a depression reverse within the presently observed time span and in this

dual manner? The synaptic depression elicited during NSF-GluA2 blockade was fully reversed within 30 min of stimulus interruption (Duprat et al., 2003). Thus, a ligand-induced AMPAR removal from the synapse results in a reversal of the AMPA signaling even when the rapid AMPAR recycling is impaired. Moreover, while no time course of this reversal was presented, the reversal obviously took place within the same time frame as that presently observed. However, since NMDARs were not blocked the conditions for the reversal observed by Duprat et al. (2003) is not known, i.e., to what extent NMDAR activity was required or not for the AMPAR synaptic reinsertion. Using AMPA application Ehlers (2000) found that the resulting AMPAR internalization resulted in a very slow and incomplete AMPAR surface reinsertion, AMPARs rather becoming targeted for late endosomes and for degradation than for reinsertion. In fact, based on his results Ehlers (2000) suggested that the AMPA induced internalization could be seen as a route for elimination of AMPARs at inappropriately activated synapses. In contrast, Lin et al. (2000) found that the internalized AMPARs were targeted to recycling endosomes with a reinsertion time constant < 10 min. Again, whether this reversal had NMDAR independent and/or dependent components was not examined. Nonetheless, taken together these results suggest that a ligand-induced AMPAR removal can take many different forms which may require different conditions for reversal. In fact, the AMPAR surface reinsertion observed by Ehlers (2000) had its magnitude reduced by ~ half by PKA blockade, indicating a partial dependence on phosphorylation. Plausibly, the intensity and duration of the ligand application can be a deciding factor, the low intensity synaptic activation resulting in more reversible forms of depression than a prolonged AMPA application. With respect to my studies I would therefore predict that during NMDAR/mGluR blockade brief (~ 100 stimuli) low frequency stimulation should result in a shift towards more NMDAR independent and less NMDAR dependent reversal compared to the 900 stimuli used. In fact, as observed in preliminary studies (A. Ermund and B. Gustafsson) the reversal after such brief stimulation is essentially completely NMDAR independent. A plausible explanation for my results is therefore that the low frequency stimulation during NMDAR/mGluR blockade initially results in a ligand-induced AMPAR removal by lateral diffusion only and that these AMPARs are reinserted during stimulus interruption in an NMDAR independent manner. With more prolonged stimulation the AMPARs may become internalized and thus require spontaneous NMDAR activity for synaptic reinsertion. As demonstrated previously (Zhu et al., 2000; Esteban et al., 2003; Kolleker et al., 2003; Qin et

al., 2005), AMPARs containing GluA4 and $GluA2_{long}$ subunits can be inserted synaptically via PKA activity mediated by spontaneous NMDAR activity.

A caveat here is that AMPA application in addition to ligand-induced AMPAR removal also can result in a Ca^{2+}/calcineurin induced AMPAR internalization via depolarization and subsequent VGCC activation (Beattie et al., 2000; Lin et al., 2000). Thus, it can not be excluded that part of the AMPAR internalization described by Ehlers (2000) reflects such Ca^{2+}/calcineurin induced AMPAR removal, and not a ligand-induced AMPAR removal. If so, it remains open to what extent ligand binding per se may cause all of the multiple states of AMPAR removal described by Ehlers (2000).

Reversal of the depression induced in the presence of NMDAR activity

When the low frequency stimulation was evoked in the absence of NMDAR blockade the depression now contained a component that was stable in the face of stimulus interruption. In order to completely reverse the depression Hebbian activity was required (**Paper III**, Fig. 5). Moreover, and interestingly, the depression requiring spontaneous NMDAR activity for its reversal was much reduced (0.2 Hz stimulation) or absent (1 Hz stimulation). It would thus appear that NMDAR activity during the low frequency stimulation has raised the threshold for reversal of one and the same depression component from spontaneous NMDAR activity to Hebbian induced NMDAR activity. A possible interpretation of this finding is that NMDAR activity stabilizes an agonist-induced AMPAR removal by raising the threshold for AMPAR reinsertion. As indicated in the INTRODUCTION, the signaling pathways underlying the depression and the maintenance of this depression may not be the same. Thus, while activation of PP2B (calcineurin) and PP1 may normally be involved in the events leading up to AMPAR removal, the actual maintenance of this depression may be related to other events, such as an autodephosphorylation of another phosphatase (PP2A) (Pi and Lisman, 2008). If, as I have argued above, many AMPARs in the 2nd postnatal week rats already are in a dephosphorylated state, NMDAR activation resulting in PP2B/PP1 activation is therefore not a requirement for this initial AMPAR removal. NMDAR activity during the stimulation will however also result in PP2A activation and to its autodephosphorylation and thereby to a stable depressed state requiring Hebbian activity to reverse this PP2A autodephosphorylation. Spontaneous NMDAR activity will thereby be insufficient to produce such reversal, and this form of depression will no longer be observed.

Hebbian induced plasticity

Prior to P12 Hebbian activity in the form of high frequency tetanization seems merely to result in de-depression of a preceding test pulse induced depression back to the naïve level of AMPA signaling (Abrahamsson et al., 2008). I found that Hebbian activity during this time period resulted in two additional forms of long-lasting (> 2 hours) effects; a potentiation above the naïve level, and an increased threshold for low frequency induced depression.

Following the first description of AMPA silent synapses a number of studies subsequently demonstrated that Hebbian activity resulted in the unsilencing of these synapses (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996) (see also Ward et al. (2006)). The Hebbian induced de-depression of test pulse induced depression was also found to be associated with changes in $1/CV^2$ consistent with unsilencing (Xiao et al., 2004). Furthermore, Abrahamsson et al. (2008) showed that the naïve level at which essentially all the CA3-CA1 synapses should be AMPA signaling acted as a ceiling for the lasting effect of tetanization. These results led to the concept of a developmental LTP explained by AMPA unsilencing, i.e., of an LTP that experimentally is nothing but a de-depression of a prior test pulse induced depression (Abrahamsson et al., 2008). The developmental LTP was found to be the predominant lasting effect of Hebbian activity up to P12 (Abrahamsson et al., 2008).

However, a developmental LTP explained solely by unsilencing contrasts with previous experiments on P6 rats using whole-cell recording and weak stimulation activating only a few synapses at most (Palmer et al., 2004). In these experiments two different forms of LTP were found; a presynaptic LTP explained by an increase in release probability, and a postsynaptic LTP explained by an increased potency. Later studies have identified the presynaptic LTP as a blockade of tonic KAR mediated presynaptic inhibition (Lauri et al., 2006). As described by these authors, this LTP is developmentally down-regulated, and is no longer present at P12. I too failed to find evidence for such KAR mediated inhibition in the P8-P12 rats (**Paper III**), supporting the finding that this presynaptic LTP operates in the 1st postnatal week only. With respect to the postsynaptic LTP described at P6, Abrahamsson et al. (2008) found that Hebbian activity in addition to de-depression resulted in a transient (5-15 min) potentiation above the naïve level, i.e., to an increase in AMPA signaling that should not be explained by AMPA unsilencing. This potentiation was also observed in my experiments, but with somewhat longer duration (~ 30 min) possibly due to the more extensive tetanization

employed compared to the experiments by Abrahamsson et al. (2008) Interestingly, the postsynaptic LTP described by Palmer et al. (2004) (as illustrated in their Fig. 4e) was also not particularly stable but had to a great extent decayed within 20 min. Moreover, in their experiments only synaptic inputs that were stable when exposed to test pulse stimulation (200 baseline stimuli) were used. Taken at face value, this means that their experiments were in fact equivalent to applying tetanization at the naïve level of AMPA signaling, which explains why an LTP explained by unsilencing was not observed. I therefore propose that the LTP described by Palmer et al. (2004) reflects the transient potentiation above the naïve level described by Abrahamsson et al. (2008) and in my thesis (**Paper III**). The potency increase observed by Palmer et al. (2004) was explained by the additional insertion of AMPARs that differed functionally from the pre-existing AMPARs. When examined in P14 rats the transient potentiation, but not LTP, is abolished in GluA1-knock-outs (Jensen et al., 2003). This result would suggest that the transient potentiation above the naïve level as well as the postsynaptic LTP described by Palmer et al. (2004) is explained by the insertion of GluA1-containing AMPARs differing from the GluA4- and $GluA2_{long}$-containing AMPARs normally present at this time period. The experiments by Palmer et al. (2004) further suggested that the inserted AMPARs should have a lower conductance than the pre-existing AMPARs thereby excluding GluA1 homomers. The exact subunit composition of the AMPARs underlying the transient potentiation is thus uncertain.

Importantly, I found this transient potentiation not to be transient in itself but to decay as a consequence of the test pulse stimulation. That Hebbian activity results in a potentiation that lasts for hours unless test pulse stimulation is given is not a new finding. In fact, when examined in the adult rat, Volianskis and Jensen (2003) reported that tetanization of CA3-CA1 synapses results in a potentiation that if test pulse stimulation is discontinued does not decay even during a 6 hour stimulus interruption. When exposed to test pulse stimulation this lasting potentiation in the adult rat could be subdivided into a sustained and a decaying potentiation, respectively, the latter decaying within about an hour using a test stimulus frequency of 1/15 s, and twice as fast using twice that rate. This labile potentiation component could thus explain the initial transient potentiation, commonly referred to as short-term potentiation (STP), which follows tetanization of these synapses (Hanse and Gustafsson, 1994). Volianskis and Jensen (2003) suggested a presynaptic expression for this adult transient potentiation, based on associated changes in paired-pulse plasticity and on the fact

that the potentiation was transient even when test pulse stimulation was applied during a postsynaptic receptor blockade. While paired-pulse plasticity may be an ambiguous tool for differentiating between pre- and postsynaptic expression (Hanse and Gustafsson, 2001b; Poncer and Malinow, 2001), a postsynaptic insertion of AMPARs is not expected to be reversed by stimulation during a postsynaptic receptor blockade. However, a shift in expression during development from a postsynaptic to a presynaptic location, or vice versa, would not be exceptional, e.g. as proposed for mGluR-LTD (Nosyreva and Huber, 2005). In fact, such a shift from postsynaptic unsilencing to a presynaptic modification has also, based on two-photon imaging experiments, been suggested for LTP (Enoki et al., 2009). Based on these developmental considerations, on the results obtained on GluA1-knock-outs, and on the "postsynaptic LTP" described by Palmer et al. (2004), I propose that the presently observed long-lasting potentiation above the naïve level reflects the Hebbian induced addition of AMPARs to AMPA signaling synapses.

Hebbian activity and lability/stability of AMPA signaling

One of the main objectives in my thesis work was to experimentally examine the hypothesis that participation in Hebbian activity stabilizes AMPA signaling. This hypothesis was based on the experimental observation that AMPA signaling appeared to stabilize around the naïve level at least for an hour post-tetanus (Abrahamsson et al., 2008). It was also based on the consideration that if the very low frequency induced depression should reflect a specific synaptic sensitivity in the developing hippocampus to become silenced and eventually eliminated if only participating in sparse synaptic activity, then participation in functional activity should make them resistant towards such depression. The 2nd postnatal week is also a time period during which connectivity between CA3 and CA1 pyramidal cells consists of but a single synapse to retain or to eliminate, and Hebbian activity should thus serve to determine which CA3-CA1 connectivity to retain. The fact that the low frequency induced depression appeared to saturate at a level at which less than half of the field synaptic response was lost (Abrahamsson et al., 2007) suggests that a major part is already stable by the 2nd postnatal week, possibly by Hebbian activity generated by endogenous neural network activity (Hanse et al., 2009).

As noted in the INTRODUCTION, LTP itself should decrease the induction threshold for subsequent LTD by increasing the GluN2A/GluN2B ratio. However, using recordings from

CA3 pyramidal cell pairs in organotypic slice cultures Montgomery and Madison (2002) described that recently unsilenced synapses had became refractory against LTD induction. More recently, using acute slices from 3 week old rats, Peineau et al. (2007) described that tetanization can result in a blockade of NMDAR-LTD through phosphorylation and thereby inactivation of GSK-3β, an enzyme involved in LTD induction. While these effects were only present during the first 30-60 min post-tetanus they nevertheless demonstrate that Hebbian activity may have multiple effects including some form of temporary stabilization of AMPA signaling. Mechanistically, with respect to the very low frequency induced depression, if AMPARs are easily lost during low frequency activation due to deficient PKA phosphorylation, a stable recruitment of PKA to the synapse by Hebbian-induced modulation of PKA binding to AKAP may stabilize the synapses.

To evaluate the possible stabilizing effect of Hebbian activity my experiments were designed so that tetanization was applied just after establishing the naïve level by a few low frequency stimuli. The tetanization was also repeated three times with 5-10 min intervals, which is the optimal protocol to produce stable long-lasting LTP in the more mature rat (Huang and Kandel, 1994). A direct comparison of the time courses of depression from the naïve level of non-tetanized and tetanized synapses, respectively, would reveal any stabilizing action of the tetanization. It should however be noted that when using this procedure synapses are tetanized in a state in which they are AMPA signaling and the only expected effect of Hebbian activity is that of stabilization. This situation may contrast to that in which the synapses have been previously silenced and in which the Hebbian activity should both unsilence and possibly stabilize the AMPA signaling. However, when comparing the effect of tetanization given at the naïve level or given after depression induced by 120 stimuli, respectively, Abrahamsson et al. (2008) did not find any difference in the outcome (see their Figs. 1 and 3, and unpublished data of these authors). This result implies either that the outcome of the tetanization does not depend on whether the tetanized synapses are AMPA signaling or AMPA silent, or that tetanization at the naïve level first depresses and thereafter de-depresses the synapses. Regardless of which interpretation is correct, the actual result obtained by Abrahamsson et al. (2008) suggests that the present results should also be relevant for tetanization of recently unsilenced synapses.

A major complication, however, for the interpretation of the results obtained was that the tetanization also resulted in potentiation above the naïve level. The lability of tetanized synapses to the low frequency stimulation would thus consist of two processes, the lability of the pre-existing, or recently unsilenced, AMPA signaling and the lability of the potentiation above the naïve level. This dual nature of the lability forced me to create two different scenarios for the assessment of the possible stabilizing effect of Hebbian activity. One scenario was based on the observation that even after my extensive tetanization protocol AMPA signaling had practically returned to the naïve level within 30 min post-tetanus, indicating that the potentiation process had to a great extent disappeared at a time when the pre-existing, or recently unsilenced, AMPA signaling has still not begun to depress (Abrahamsson et al., 2008). The depression during the subsequent three hours should therefore solely be due to the pre-existing, or recently unsilenced, AMPA signaling. According to this scenario Hebbian activity resulted in a far from complete stabilization of the pre-existing AMPA signaling, but the depression evoked at tetanized synapses was significantly less than that at non-tetanized synapses (to ~ 40% vs ~ 20% of the naïve AMPA signaling level, respectively after ~ four hours of stimulation) (**Paper III**, Fig. 2A). A similar partial stabilizing action was also found with respect to the 1 Hz stimulation (**Paper III**, Fig. 3). Synapses participating in Hebbian activity may thus not become stabilized but may instead become more resistant towards low frequency stimulation even after 4 hours of stimulation. However, as indicated by the values for depression, the very low frequency induced depression of the tetanized synapses is nonetheless as much as 75% of the depression of non-tetanized synapses, i.e., only a very limited degree of stabilization.

Even so, there are reasons to believe that this scenario assuming a considerably greater lability of the potentiated state than of the pre-existing, or recently unsilenced state results in an exaggerated stabilizing effect of Hebbian activity. Thus, while the postsynaptic LTP described by Palmer et al. (2004) was quite labile, it had still not completely decayed after ~ 1000 stimuli (their Fig. 4e). To the extent that the presently observed potentiation is identical to this postsynaptic LTP, as argued above, it may require several hours of very low frequency stimulation to completely decay. Moreover, the reversal from depression after stimulus interruption was in relative terms not greater at tetanized than at non-tetanized synapses (**Paper III,** Fig. 2B). Therefore a scenario in which no distinction is made between the pre-existing, or recently unsilenced, AMPA signaling and the potentiated state above the naïve

level may be closer to the truth. The low frequency induced depressions of these two processes which formally may represent silencing and depotentiation, respectively, as well as their subsequent reversal, were thus assumed to be parallel, i.e., the lability of these two processes would be the same. In this scenario there were thus two naïve levels, the naïve level of non-stimulated synapses and the naïve level of tetanized synapses, the latter referred to as the post-tetanus-naïve level. According to this scenario my experimental data suggested that the stabilization was to a great extent only temporary and more evident with respect to 0.2 Hz than 1 Hz stimulation (**Paper III**, Figs. 2B and 3C). However, while the amount of depression produced during the 1 Hz stimulation was only marginally affected, the amount of lasting depression produced by this stimulation was substantially impaired by prior tetanization. My results thus suggest a possible gradient of tetanization-induced stabilization where the three different components of depression discussed above are differentially affected. However, further experiments using post-tetanus pharmacological blockade of NMDARs and mGluRs will be required to substantiate this suggestion.

As noted earlier in the DISCUSSION the time-limited stabilizing effect observed by Peineau et al. (2007) was explained by a tetanization-induced phosphorylation and subsequent inactivation of GSK-3β, a kinase involved in LTD induction. The stabilizing effect presently observed was not temporally linked to the tetanization as shown by the fact that the 1 Hz induced depression was equally impaired when applied after two hours of post-tetanus stimulus interruption as applied 15 min post-tetanus. Instead, whatever process that results in a tetanization-induced potentiation of the AMPA signaling that lasts unaltered for hours unless stimulation is given also results in a stabilization of the AMPA signaling with the same property. Thus, if a tetanization-induced phosphorylation would explain this behaviour, the phosphorylated state must remain remain unaltered for hours unless test pulse stimulation is applied.

LTD and long-lasting depression are not the same

In their pioneering study of LTD Dudek and Bear (1992) established the induction protocol that has thereafter with minor variation been used in the CA1 region to produce a long-lasting decrease in synaptic transmission via synaptic activation. In this protocol synapses are initially stimulated at very low frequency, often every 10-30 s, to establish a stable baseline of synaptic transmission. When a stable baseline is established higher frequency stimulation,

usually 1 Hz, is given for 10-15 min in order to induce the long-lasting depression and stimulation is thereafter returned to the initial low rate. The amount of long-lasting depression, i.e., the amount of LTD obtained, is thereafter assessed by comparing the magnitude of the synaptic response 30-60 min after the end of the higher frequency stimulation to the magnitude of the baseline synaptic response. This protocol thus relies on the assumption that long-lasting depression is specifically induced by the higher frequency stimulation only, an assumption that did not appear to have been tested by Dudek and Bear (1992). My thesis work shows that at least in the 2nd postnatal week, stimulation every 10-20 s induces as much depression as 1 Hz stimulation and that most of the depression evoked in this manner is equivalent to NMDAR-LTD induced either in a lasting or a labile form. That is, it is not possible to assess the ability of synaptic activation to induce long-lasting depression using the conventional LTD-inducing protocol. In fact, since stimulation at 0.05-1 Hz results in about the same amount of depression/stimuli and the x-axis is in units of time, the introduction of a higher stimulus rate will in itself result in LTD (**Paper I**, Fig. 1C). This LTD is however only apparent since it does not require that the depression remains after a prolonged stimulus interruption. In my thesis work I thus used the alternative protocol in which the amount of long-lasting depression induced by low frequency stimulation was assessed as the depression still present after 30 min of stimulus interruption. Since I also found that this depression did not significantly differ in magnitude using 30 or 60 min of stimulus interruption it likely represents a fairly stable form of depression. In my thesis I have not used the conventional term LTD for this long-lasting depression. This is because LTD has been assessed in these neonatal CA3-CA1 synapses using the conventional LTD protocol, and I did not want to use the same terminology for depressions evaluated in two such different manners.

That my thesis revealed a very large difference in the amount of NMDAR-LTD and NMDAR dependent long-lasting depression is not difficult to explain. Considering that 1 Hz stimulation did not induce more depression than 0.05 Hz stimulation and since most of this depression is one and the same depression process, most NMDAR dependent depression has in fact already been induced prior to the 1 Hz stimulation. What is observed as an NMDAR-LTD in a conventional LTD experiment in the 2nd postnatal week may thus mostly be an apparent LTD. However, since 0.2 Hz stimulation, in contrast to 1 Hz stimulation, also resulted in a labile version of the long-lasting depression, the synaptic response after 1 Hz

stimulation may contain more synapses that are depressed in a long-lasting manner. Thus, the actual long-lasting depression induced by the 1 Hz stimulation in a conventional LTD experiment will not actually be seen as an LTD.

On the other hand, the contrast between the dominating role of mGluRs in neonatal LTD (Li et al., 2002; Feinmark et al., 2003) (**Paper I**) and the non-existing role of mGluRs in long-lasting depression is more difficult to explain. In their original demonstration of mGluR-LTD in the CA3-CA1 synapse Oliet et al. (1997) noted the more fickle induction requirements for mGluR-LTD compared to NMDAR-LTD in that mGluR-LTD was only induced at certain divalent ion concentrations and synaptic response magnitudes, and required the presence of synaptic inhibition. However, in agreement with my own results (**Paper I**, Fig. 5B), substantial mGluR-LTD is induced in the 2nd postnatal week also when synaptic inhibition is blocked (Feinmark et al., 2003), and under the same experimental circumstances with respect to divalent ions and synaptic magnitude as my present experiments on long-lasting depression. Nonetheless, there are two possible explanations for the discrepancy noted above, both of which however still lack experimental verification. As was noted in my thesis work, the low frequency induced depression was significantly less when only NMDARs were blocked compared to when a combined NMDAR/mGluR blockade was used. Since this mGluR induced impairment of the depression was more pronounced at 0.2 Hz than at 1 Hz stimulation, the introduction of 1 Hz stimulation during a conventional LTD experiment would be expected to result in a decreased impairment and thus a larger depression. To the extent that this reduced impairment is lasting, i.e., continues even after return to test pulse stimulation, an LTD will appear. A second possibility is that the maintenance of the mGluR-LTD is stimulation dependent. As noted in the INTRODUCTION, mGluR-LTD is often induced pharmacologically by a transient application of the mGluR group1 agonist DHPG. For reasons still unexplained, a transient application of an mGluR group I antagonist during the subsequent LTD phase (when DHPG has been washed out) results in a transient de-depression (Palmer et al., 1997). While not explaining the non-existing role of mGluRs in long-lasting depression, the above result indicates that mGluR-LTD is not a lasting depression in itself, as also indicated by my results, but relies on some on-going receptor activity.

CONCLUDING REMARKS

As stated in the first paragraphs of the GENERAL DISCUSSION, my main experimental findings were i) that nearly all AMPAR signaling of neonatal (P8-P12) hippocampal CA3-CA1 synapse are depressed, most likely silenced, by long duration very low frequency stimulation, ii) that this depression completely reverses within 30 min unless NMDARs are active during the stimulation, iii) that NMDAR activation facilitates while mGluR activation impairs the depression, and iv) that preceding Hebbian activity only results in a temporary stabilization of the very low frequency induced depression. The implication of these results is that the neonatal CA3-CA1 synapse has a specific vulnerability to sparse, non-functional, synaptic activity that is only temporarily mitigated by participation in Hebbian activity. It is proposed that this vulnerability is a means to allow only synapses more or less continuously active in cooperation with other synapses during this time period to become part of a functional synaptic network. In this process NMDARs play a complex and dual role, on one hand facilitating the induction of the AMPA silent state during very low frequency synaptic activity and stabilizing it, whilst on the other hand participating in the unsilencing from this silent state both in the absence of evoked synaptic activity and during Hebbian activity.

While not specifically examined in the thesis, comparison with data from the literature suggests that this depression is explained by AMPA silencing due to glutamate binding to dephosphorylated AMPARs, resulting in excessive AMPAR removal and/or impaired recycling. NMDARs and mGluRs may modulate the depression by affecting the phosphorylation state of the AMPARs and/ or the availability of the AMPARs.

ACKNOWLEDGEMENTS

This work has been performed at the Department of Physiology, Institute of Neuroscience and Physiology at the Sahlgrenska academy, University of Gothenburg.
I would not have been able to write this thesis without a lot of support from my surrounding and I would like to express a special thanks to:

Bengt Gustafsson, my supervisor, for putting a great effort into the work of this thesis and being utterly dedicated to science.

Eric Hanse, my co-supervisor, for a lot of support and understanding, for welcoming me to the lab as a "summer student", and for dragging me to the gym early in the morning at conferences. Thank you also for all exciting activities outside of the lab, a retreat to Tjörn every once in a while is always refreshing!

My Jedimasters of electrophysiology, **Pontus Wasling** and **Min-Yi Xiao**, for introducing me to, and teaching me the craftsmanship of electrophysiological recordings and for always being happy and cheerful.

Ingela Hammar for careful and constructive reading of the thesis.

My fellow PhD-students, present and past, **Ilse Riebe**, **Jonny Daborg**, **My Andersson** and **Therese Abrahamsson**, for being great colleagues, for really great times both on and off work and for your support through some rough times.

Dan Magnusson, **Inger Olofsson**, **Karin Göthner**, **Rauni Larsson**, **Staffan Berg** and **Tore Holmström**, for instant and expert help with all technical and administrative matters.

Fredrik Asztély for many good laughs and productive guerilla warfare.

All the people at "neuro", **Andreas**, **Anna**, **Catta**, **Elin**, **Elzbieta**, **Lars-Gunnar**, **Lotta**, **Rick**, **Rong**, **Sergei**, **Simon**, **Ulf**, **Åke** and **Åsa**, for creating a nice atmosphere at our floor.

"The human group", **Helena**, **Håkan**, **Irene**, **Jaquette**, **Johan**, **Karin**, **Linda**, **Line**, **Malin**, **Mikael** and **Simon**, for good times on conferences and various activities outside of the lab.

The nice people at **"Farmen"** and **Emil E** for good company at lunches, great times at skiing conference and super times at parties.

Fredrik Blomstrand and **Malin Stridh** for nice travelling company and lots of good times during these years.

Jonas Faijerson for being a super nice guy and for some refreshing research work on the side. I´ll take a raincheck on the colourful picture…

Jonas Thurdin for helping me with excel magic, it saved me a lot of time!

My dear "kursare" and in most cases fellow researchers, **Denny**, **Fredrik**, **Fredrik**, **Kim**, **Mattias** and **Rasmus**, for great times during the days of our education and research. I am really looking forward to engraving my name yet again on the Bonde-SM trophy…

All the great teachers during my days in school for encouraging me to learn. Foremost I would like to thank **Sture Malmrud**, my chemistry teacher at my high-school **Högbergsskolan**, for great anecdotes, music lectures with Robert Broberg and for getting me interested in the world of research. A special thanks also to **Eva Hogmark**, my "home-room" teacher in high-school.

The people of **MOKIE** for making my time in exile down here much less gray. Especially I would like to thank my great friend, fellow scout and co-founder **Liam**, your time with us was much too short, RIP.

All my friends outside the world of research for taking my mind off the daily struggles.

Everyone worth mentioning that I have forgotten to mention…

Göran, **Maria**, **Richard**, **Henrik**, **Kajsa** and **Kurre** for taking me in to your family.

My mother **Gunbrith**, my father **Lennart** and my sister **Lisa**, for always supporting and loving me. It is a great feeling to know that you are always there for me. I would also like to thank my **grandparents** for always believing in me.

Finally and most importantly, I would like to thank my life-companion **Lotta**, the great love of my life, for your endless support and tremendous patience during the writing of this thesis. You complete me!

The work in this thesis was supported by grants from The Swedish Medical Research Council and Wilhelm och Martina Lundgrens Vetenskapsfond.

REFERENCES

Abbas AK, Dozmorov M, Li R, Huang FS, Hellberg F, Danielson J, Tian Y, Ekstrom J, Sandberg M, Wigstrom H (2009) Persistent LTP without triggered protein synthesis. Neurosci Res 63:59-65.

Abrahamsson T, Gustafsson B, Hanse E (2005) Synaptic fatigue at the naive perforant path-dentate granule cell synapse in the rat. J Physiol 569:737-750.

Abrahamsson T, Gustafsson B, Hanse E (2007) Reversible synaptic depression in developing rat CA3 CA1 synapses explained by a novel cycle of AMPA silencing-unsilencing. J Neurophysiol 98:2604-2611.

Abrahamsson T, Gustafsson B, Hanse E (2008) AMPA silencing is a prerequisite for developmental long-term potentiation in the hippocampal CA1 region. J Neurophysiol 100:2605-2614.

Adesnik H, Nicoll RA (2007) Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. J Neurosci 27:4598-4602.

Adesnik H, Nicoll RA, England PM (2005) Photoinactivation of native AMPA receptors reveals their real-time trafficking. Neuron 48:977-985.

Adesnik H, Li G, During MJ, Pleasure SJ, Nicoll RA (2008) NMDA receptors inhibit synapse unsilencing during brain development. Proc Natl Acad Sci U S A 105:5597-5602.

Ahmed MS, Siegelbaum SA (2009) Recruitment of N-Type Ca(2+) channels during LTP enhances low release efficacy of hippocampal CA1 perforant path synapses. Neuron 63:372-385.

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

Anderson P, Bliss TV, Skrede KK (1971) Lamellar organization of hippocampal pathways. Exp Brain Res 13:222-238.

Arikkath J, Reichardt LF (2008) Cadherins and catenins at synapses: roles in synaptogenesis and synaptic plasticity. Trends Neurosci 31:487-494.

Ayala JE, Niswender CM, Luo Q, Banko JL, Conn PJ (2008) Group III mGluR regulation of synaptic transmission at the SC-CA1 synapse is developmentally regulated. Neuropharmacology 54:804-814.

Bagal AA, Kao JP, Tang CM, Thompson SM (2005) Long-term potentiation of exogenous glutamate responses at single dendritic spines. Proc Natl Acad Sci U S A 102:14434-14439.

Bagri A, Cheng HJ, Yaron A, Pleasure SJ, Tessier-Lavigne M (2003) Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphorin family. Cell 113:285-299.

Banko JL, Hou L, Poulin F, Sonenberg N, Klann E (2006) Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J Neurosci 26:2167-2173.

Barria A, Derkach V, Soderling T (1997a) Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. J Biol Chem 272:32727-32730.

Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997b) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276:2042-2045.

Bartlett TE, Bannister NJ, Collett VJ, Dargan SL, Massey PV, Bortolotto ZA, Fitzjohn SM, Bashir ZI, Collingridge GL, Lodge D (2007) Differential roles of NR2A and NR2B-containing NMDA receptors in LTP and LTD in the CA1 region of two-week old rat hippocampus. Neuropharmacology 52:60-70.

Bastrikova N, Gardner GA, Reece JM, Jeromin A, Dudek SM (2008) Synapse elimination accompanies functional plasticity in hippocampal neurons. Proc Natl Acad Sci U S A 105:3123-3127.

Bats C, Groc L, Choquet D (2007) The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. Neuron 53:719-734.

Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, Somogyi P (1993) The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron 11:771-787.

Bayazitov IT, Richardson RJ, Fricke RG, Zakharenko SS (2007) Slow presynaptic and fast postsynaptic components of compound long-term potentiation. J Neurosci 27:11510-11521.

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. Nat Neurosci 3:1291-1300.

Becker N, Wierenga CJ, Fonseca R, Bonhoeffer T, Nägerl UV (2008) LTD induction causes morphological changes of presynaptic boutons and reduces their contacts with spines. Neuron 60:590-597.

Bellone C, Nicoll RA (2007) Rapid bidirectional switching of synaptic NMDA receptors. Neuron 55:779-785.

Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol Rev 87:1215-1284.

Berberich S, Punnakkal P, Jensen V, Pawlak V, Seeburg PH, Hvalby O, Kohr G (2005) Lack of NMDA receptor subtype selectivity for hippocampal long-term potentiation. J Neurosci 25:6907-6910.

Berkeley JL, Levey AI (2003) Cell-specific extracellular signal-regulated kinase activation by multiple G protein-coupled receptor families in hippocampus. Mol Pharmacol 63:128-135.

Bhattacharyya S, Biou V, Xu W, Schluter O, Malenka RC (2009) A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. Nat Neurosci 12:172-181.

Bjartmar L, Huberman AD, Ullian EM, Renteria RC, Liu X, Xu W, Prezioso J, Susman MW, Stellwagen D, Stokes CC, Cho R, Worley P, Malenka RC, Ball S, Peachey NS, Copenhagen D, Chapman B, Nakamoto M, Barres BA, Perin MS (2006) Neuronal pentraxins mediate synaptic refinement in the developing visual system. J Neurosci 26:6269-6281.

Blanpied TA, Scott DB, Ehlers MD (2002) Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. Neuron 36:435-449.

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

Boehm J, Kang MG, Johnson RC, Esteban J, Huganir RL, Malinow R (2006) Synaptic incorporation of AMPA receptors during LTP is controlled by a PKC phosphorylation site on GluR1. Neuron 51:213-225.

Bolshakov VY, Siegelbaum SA (1994) Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264:1148-1152.
Bolshakov VY, Carboni L, Cobb MH, Siegelbaum SA, Belardetti F (2000) Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. Nat Neurosci 3:1107-1112.
Bourgeron T (2009) A synaptic trek to autism. Curr Opin Neurobiol 19:231-234.
Bourne JN, Harris KM (2008) Balancing structure and function at hippocampal dendritic spines. Annu Rev Neurosci 31:47-67.
Bradley SR, Rees HD, Yi H, Levey AI, Conn PJ (1998) Distribution and developmental regulation of metabotropic glutamate receptor 7a in rat brain. J Neurochem 71:636-645.
Busetto G, Higley MJ, Sabatini BL (2008) Developmental presence and disappearance of postsynaptically silent synapses on dendritic spines of rat layer 2/3 pyramidal neurons. J Physiol 586:1519-1527.
Carr DW, Stofko-Hahn RE, Fraser ID, Cone RD, Scott JD (1992) Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. J Biol Chem 267:16816-16823.
Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci 2:454-460.
Chen J, Martin BL, Brautigan DL (1992) Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. Science 257:1261-1264.
Chen L, Chetkovich DM, Petralia RS, Sweeney NT, Kawasaki Y, Wenthold RJ, Bredt DS, Nicoll RA (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 408:936-943.
Cheng D, Hoogenraad CC, Rush J, Ramm E, Schlager MA, Duong DM, Xu P, Wijayawardana SR, Hanfelt J, Nakagawa T, Sheng M, Peng J (2006) Relative and absolute quantification of postsynaptic density proteome isolated from rat forebrain and cerebellum. Mol Cell Proteomics 5:1158-1170.
Chetkovich DM, Sweatt JD (1993) nMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. J Neurochem 61:1933-1942.
Chevaleyre V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38:461-472.
Choi S, Klingauf J, Tsien RW (2000) Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. Nat Neurosci 3:330-336.
Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N, Kuhl D, Huganir RL, Worley PF (2006) Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52:445-459.
Christopherson KS, Ullian EM, Stokes CC, Mullowney CE, Hell JW, Agah A, Lawler J, Mosher DF, Bornstein P, Barres BA (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell 120:421-433.
Chung HJ, Xia J, Scannevin RH, Zhang X, Huganir RL (2000) Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. J Neurosci 20:7258-7267.
Ciabarra AM, Sullivan JM, Gahn LG, Pecht G, Heinemann S, Sevarino KA (1995) Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. J Neurosci 15:6498-6508.

Coghlan VM, Perrino BA, Howard M, Langeberg LK, Hicks JB, Gallatin WM, Scott JD (1995) Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. Science 267:108-111.

Colledge M, Dean RA, Scott GK, Langeberg LK, Huganir RL, Scott JD (2000) Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. Neuron 27:107-119.

Colledge M, Snyder EM, Crozier RA, Soderling JA, Jin Y, Langeberg LK, Lu H, Bear MF, Scott JD (2003) Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. Neuron 40:595-607.

Collingridge GL, Olsen RW, Peters J, Spedding M (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56:2-5.

Conradi NG, Muntzing K, Sourander P, Hamberger A (1984) Effect of ambient temperature on rectal temperature in normal and malnourished rats during early postnatal development. Acta Physiol Scand 121:147-153.

Corlew R, Brasier DJ, Feldman DE, Philpot BD (2008) Presynaptic NMDA receptors: newly appreciated roles in cortical synaptic function and plasticity. Neuroscientist 14:609-625.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC (1996) Ca2+ signaling requirements for long-term depression in the hippocampus. Neuron 16:825-833.

Das S, Sasaki YF, Rothe T, Premkumar LS, Takasu M, Crandall JE, Dikkes P, Conner DA, Rayudu PV, Cheung W, Chen HS, Lipton SA, Nakanishi N (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 393:377-381.

Daw MI, Chittajallu R, Bortolotto ZA, Dev KK, Duprat F, Henley JM, Collingridge GL, Isaac JT (2000) PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron 28:873-886.

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7-61.

Dobrunz LE, Stevens CF (1997) Heterogeneity of release probability, facilitation, and depletion at central synapses. Neuron 18:995-1008.

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

Duprat F, Daw M, Lim W, Collingridge G, Isaac J (2003) GluR2 protein-protein interactions and the regulation of AMPA receptors during synaptic plasticity. Philos Trans R Soc Lond B Biol Sci 358:715-720.

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

Dzubay JA, Jahr CE (1996) Kinetics of NMDA channel opening. J Neurosci 16:4129-4134.

Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron 28:511-525.

Elias GM, Nicoll RA (2007) Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. Trends Cell Biol 17:343-352.

Enoki R, Hu YL, Hamilton D, Fine A (2009) Expression of long-term plasticity at individual synapses in hippocampus is graded, bidirectional, and mainly presynaptic: optical quantal analysis. Neuron 62:242-253.

Eroglu Ç, Allen NJ, Susman MW, O'Rourke NA, Park CY, Özkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA (2009) Gabapentin

receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. Cell 139:380-392.
Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat Neurosci 6:136-143.
Fabian-Fine R, Volknandt W, Fine A, Stewart MG (2000) Age-dependent pre- and postsynaptic distribution of AMPA receptors at synapses in CA3 stratum radiatum of hippocampal slice cultures compared with intact brain. Eur J Neurosci 12:3687-3700.
Fang X, Yu SX, Lu Y, Bast RC, Jr., Woodgett JR, Mills GB (2000) Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. Proc Natl Acad Sci U S A 97:11960-11965.
Feinmark SJ, Begum R, Tsvetkov E, Goussakov I, Funk CD, Siegelbaum SA, Bolshakov VY (2003) 12-lipoxygenase metabolites of arachidonic acid mediate metabotropic glutamate receptor-dependent long-term depression at hippocampal CA3-CA1 synapses. J Neurosci 23:11427-11435.
Feldman DE, Nicoll RA, Malenka RC, Isaac JT (1998) Long-term depression at thalamocortical synapses in developing rat somatosensory cortex. Neuron 21:347-357.
Feng W, Zhang M (2009) Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. Nat Rev Neurosci 10:87-99.
Ferraguti F, Shigemoto R (2006) Metabotropic glutamate receptors. Cell Tissue Res 326:483-504.
Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci 18:8900-8911.
Fink AE, Sarinana J, Gray EE, O'Dell T J (2007) Activity-dependent depression of local excitatory connections in the CA1 region of mouse hippocampus. J Neurophysiol 97:3926-3936.
Flint AC, Maisch US, Weishaupt JH, Kriegstein AR, Monyer H (1997) NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. J Neurosci 17:2469-2476.
Fonseca R, Vabulas RM, Hartl FU, Bonhoeffer T, Nägerl UV (2006) A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP. Neuron 52:239-245.
Frey U, Krug M, Reymann KG, Matthies H (1988) Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res 452:57-65.
Fu WY, Chen Y, Sahin M, Zhao XS, Shi L, Bikoff JB, Lai KO, Yung WH, Fu AK, Greenberg ME, Ip NY (2007) Cdk5 regulates EphA4-mediated dendritic spine retraction through an ephexin1-dependent mechanism. Nat Neurosci 10:67-76.
Fukunaga K, Stoppini L, Miyamoto E, Muller D (1993) Long-term potentiation is associated with an increased activity of Ca2+/calmodulin-dependent protein kinase II. J Biol Chem 268:7863-7867.
Gallagher SM, Daly CA, Bear MF, Huber KM (2004) Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. J Neurosci 24:4859-4864.
Garaschuk O, Schneggenburger R, Schirra C, Tempia F, Konnerth A (1996) Fractional Ca2+ currents through somatic and dendritic glutamate receptor channels of rat hippocampal CA1 pyramidal neurones. J Physiol 491 (Pt 3):757-772.

Gardner SM, Takamiya K, Xia J, Suh JG, Johnson R, Yu S, Huganir RL (2005) Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. Neuron 45:903-915.

Gasparini S, Saviane C, Voronin LL, Cherubini E (2000) Silent synapses in the developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? Proc Natl Acad Sci U S A 97:9741-9746.

Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15:193-204.

Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci 5:446-451.

Gerges NZ, Backos DS, Rupasinghe CN, Spaller MR, Esteban JA (2006) Dual role of the exocyst in AMPA receptor targeting and insertion into the postsynaptic membrane. Embo J 25:1623-1634.

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ (1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870-873.

Gomperts SN, Rao A, Craig AM, Malenka RC, Nicoll RA (1998) Postsynaptically silent synapses in single neuron cultures. Neuron 21:1443-1451.

Gray EE, Fink AE, Sarinana J, Vissel B, O'Dell TJ (2007) Long-term potentiation in the hippocampal CA1 region does not require insertion and activation of GluR2-lacking AMPA receptors. J Neurophysiol 98:2488-2492.

Greger IH, Khatri L, Ziff EB (2002) RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. Neuron 34:759-772.

Groc L, Choquet D (2006) AMPA and NMDA glutamate receptor trafficking: multiple roads for reaching and leaving the synapse. Cell Tissue Res 326:423-438.

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

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

Groc L, Heine M, Cognet L, Brickley K, Stephenson FA, Lounis B, Choquet D (2004) Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. Nat Neurosci 7:695-696.

Groc L, Heine M, Cousins SL, Stephenson FA, Lounis B, Cognet L, Choquet D (2006b) NMDA receptor surface mobility depends on NR2A-2B subunits. Proc Natl Acad Sci U S A 103:18769-18774.

Grooms SY, Noh KM, Regis R, Bassell GJ, Bryan MK, Carroll RC, Zukin RS (2006) Activity bidirectionally regulates AMPA receptor mRNA abundance in dendrites of hippocampal neurons. J Neurosci 26:8339-8351.

Gustafsson B, Asztely F, Hanse E, Wigström H (1989) Onset Characteristics of Long-Term Potentiation in the Guinea-Pig Hippocampal CA1 Region in Vitro. Eur J Neurosci 1:382-394.

Guzowski JF, Lyford GL, Stevenson GD, Houston FP, McGaugh JL, Worley PF, Barnes CA (2000) Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci 20:3993-4001.

Hanse E, Gustafsson B (1994) Onset and stabilization of NMDA receptor-dependent hippocampal long-term potentiation. Neurosci Res 20:15-25.

Hanse E, Gustafsson B (2001a) Quantal variability at glutamatergic synapses in area CA1 of the rat neonatal hippocampus. J Physiol 531:467-480.
Hanse E, Gustafsson B (2001b) Paired-pulse plasticity at the single release site level: an experimental and computational study. J Neurosci 21:8362-8369.
Hanse E, Gustafsson B (2001c) Vesicle release probability and pre-primed pool at glutamatergic synapses in area CA1 of the rat neonatal hippocampus. J Physiol 531:481-493.
Hanse E, Taira T, Lauri S, Groc L (2009) Glutamate synapse in developing brain: an integrative perspective beyond the silent state. Trends Neurosci 32:532-537.
Hayashi T, Thomas GM, Huganir RL (2009) Dual palmitoylation of NR2 subunits regulates NMDA receptor trafficking. Neuron 64:213-226.
Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262-2267.
Heynen AJ, Quinlan EM, Bae DC, Bear MF (2000) Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. Neuron 28:527-536.
Hohnke CD, Oray S, Sur M (2000) Activity-dependent patterning of retinogeniculate axons proceeds with a constant contribution from AMPA and NMDA receptors. J Neurosci 20:8051-8060.
Holbro N, Grunditz Å, Oertner TG (2009) Differential distribution of endoplasmic reticulum controls metabotropic signaling and plasticity at hippocampal synapses. Proc Natl Acad Sci U S A 106:15055-15060.
Hou L, Antion MD, Hu D, Spencer CM, Paylor R, Klann E (2006) Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. Neuron 51:441-454.
Hsia AY, Malenka RC, Nicoll RA (1998) Development of excitatory circuitry in the hippocampus. J Neurophysiol 79:2013-2024.
Huang CC, Hsu KS (2006) Sustained activation of metabotropic glutamate receptor 5 and protein tyrosine phosphatases mediate the expression of (S)-3,5-dihydroxyphenylglycine-induced long-term depression in the hippocampal CA1 region. J Neurochem 96:179-194.
Huang YY, Kandel ER (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn Mem 1:74-82.
Huber KM, Kayser MS, Bear MF (2000) Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. Science 288:1254-1257.
Huber KM, Roder JC, Bear MF (2001) Chemical induction of mGluR5- and protein synthesis--dependent long-term depression in hippocampal area CA1. J Neurophysiol 86:321-325.
Huber KM, Gallagher SM, Warren ST, Bear MF (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 99:7746-7750.
Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ (2000) Functional requirement for class I MHC in CNS development and plasticity. Science 290:2155-2159.
Ireland DR, Abraham WC (2002) Group I mGluRs increase excitability of hippocampal CA1 pyramidal neurons by a PLC-independent mechanism. J Neurophysiol 88:107-116.

Isa T, Itazawa S, Iino M, Tsuzuki K, Ozawa S (1996) Distribution of neurones expressing inwardly rectifying and Ca(2+)-permeable AMPA receptors in rat hippocampal slices. J Physiol 491 (Pt 3):719-733.
Isaac JT, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: implications for the expression of LTP. Neuron 15:427-434.
Isaac JT, Ashby M, McBain CJ (2007) The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron 54:859-871.
Jane DE, Lodge D, Collingridge GL (2009) Kainate receptors: pharmacology, function and therapeutic potential. Neuropharmacology 56:90-113.
Jensen V, Kaiser KM, Borchardt T, Adelmann G, Rozov A, Burnashev N, Brix C, Frotscher M, Andersen P, Hvalby O, Sakmann B, Seeburg PH, Sprengel R (2003) A juvenile form of postsynaptic hippocampal long-term potentiation in mice deficient for the AMPA receptor subunit GluR-A. J Physiol 553:843-856.
Jin W, Ge WP, Xu J, Cao M, Peng L, Yung W, Liao D, Duan S, Zhang M, Xia J (2006) Lipid binding regulates synaptic targeting of PICK1, AMPA receptor trafficking, and synaptic plasticity. J Neurosci 26:2380-2390.
Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529-531.
Kamal A, Ramakers GM, Urban IJ, De Graan PN, Gispen WH (1999) Chemical LTD in the CA1 field of the hippocampus from young and mature rats. Eur J Neurosci 11:3512-3516.
Kameyama K, Lee HK, Bear MF, Huganir RL (1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. Neuron 21:1163-1175.
Kamikubo Y, Egashira Y, Tanaka T, Shinoda Y, Tominaga-Yoshino K, Ogura A (2006) Long-lasting synaptic loss after repeated induction of LTD: independence to the means of LTD induction. Eur J Neurosci 24:1606-1616.
Kandel ER, O'Dell TJ (1992) Are adult learning mechanisms also used for development? Science 258:243-245.
Kataoka K, Yanase H (1998) Mild hypothermia--a revived countermeasure against ischemic neuronal damages. Neurosci Res 32:103-117.
Kelly MT, Crary JF, Sacktor TC (2007) Regulation of protein kinase Mzeta synthesis by multiple kinases in long-term potentiation. J Neurosci 27:3439-3444.
Kemp N, Bashir ZI (1997) NMDA receptor-dependent and -independent long-term depression in the CA1 region of the adult rat hippocampus in vitro. Neuropharmacology 36:397-399.
Kemp N, McQueen J, Faulkes S, Bashir ZI (2000) Different forms of LTD in the CA1 region of the hippocampus: role of age and stimulus protocol. Eur J Neurosci 12:360-366.
Kennedy MJ, Ehlers MD (2006) Organelles and trafficking machinery for postsynaptic plasticity. Annu Rev Neurosci 29:325-362.
Kerchner GA, Nicoll RA (2008) Silent synapses and the emergence of a postsynaptic mechanism for LTP. Nat Rev Neurosci 9:813-825.
Kidd FL, Isaac JT (1999) Developmental and activity-dependent regulation of kainate receptors at thalamocortical synapses. Nature 400:569-573.
Kim CH, Chung HJ, Lee HK, Huganir RL (2001) Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. Proc Natl Acad Sci U S A 98:11725-11730.
Kim CH, Lee J, Lee JY, Roche KW (2008) Metabotropic glutamate receptors: phosphorylation and receptor signaling. J Neurosci Res 86:1-10.

Kim E, Sheng M (2004) PDZ domain proteins of synapses. Nat Rev Neurosci 5:771-781.
Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, Scott JD (1996) Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science 271:1589-1592.
Kleckner NW, Dingledine R (1988) Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science 241:835-837.
Kolleker A, Zhu JJ, Schupp BJ, Qin Y, Mack V, Borchardt T, Köhr G, Malinow R, Seeburg PH, Osten P (2003) Glutamatergic plasticity by synaptic delivery of GluR-B(long)-containing AMPA receptors. Neuron 40:1199-1212.
Kopec CD, Real E, Kessels HW, Malinow R (2007) GluR1 links structural and functional plasticity at excitatory synapses. J Neurosci 27:13706-13718.
Kornau HC, Schenker LT, Kennedy MB, Seeburg PH (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science 269:1737-1740.
Kullmann DM, Erdemli G, Asztely F (1996) LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. Neuron 17:461-474.
Köhler M, Kornau HC, Seeburg PH (1994) The organization of the gene for the functionally dominant alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunit GluR-B. J Biol Chem 269:17367-17370.
Lai KO, Ip NY (2009) Synapse development and plasticity: roles of ephrin/Eph receptor signaling. Curr Opin Neurobiol 19:275-283.
Lau CG, Zukin RS (2007) NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat Rev Neurosci 8:413-426.
Lauri SE, Vesikansa A, Segerstråle M, Collingridge GL, Isaac JT, Taira T (2006) Functional maturation of CA1 synapses involves activity-dependent loss of tonic kainate receptor-mediated inhibition of glutamate release. Neuron 50:415-429.
Lee HK, Kameyama K, Huganir RL, Bear MF (1998) NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21:1151-1162.
Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955-959.
Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631-643.
Lee SH, Liu L, Wang YT, Sheng M (2002) Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 36:661-674.
Li CL, Mc Ilwain H (1957) Maintenance of resting membrane potentials in slices of mammalian cerebral cortex and other tissues in vitro. J Physiol 139:178-190.
Li R, Huang FS, Abbas AK, Wigström H (2007) Role of NMDA receptor subtypes in different forms of NMDA-dependent synaptic plasticity. BMC Neurosci 8:55.
Li ST, Kato K, Tomizawa K, Matsushita M, Moriwaki A, Matsui H, Mikoshiba K (2002) Calcineurin plays different roles in group II metabotropic glutamate receptor- and NMDA receptor-dependent long-term depression. J Neurosci 22:5034-5041.
Liao D, Hessler NA, Malinow R (1995) Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature 375:400-404.

Liao D, Scannevin RH, Huganir R (2001) Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. J Neurosci 21:6008-6017.
Liao D, Zhang X, O'Brien R, Ehlers MD, Huganir RL (1999) Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. Nat Neurosci 2:37-43.
Lin DT, Huganir RL (2007) PICK1 and phosphorylation of the glutamate receptor 2 (GluR2) AMPA receptor subunit regulates GluR2 recycling after NMDA receptor-induced internalization. J Neurosci 27:13903-13908.
Lin DT, Makino Y, Sharma K, Hayashi T, Neve R, Takamiya K, Huganir RL (2009) Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. Nat Neurosci 12:879-887.
Lin JW, Ju W, Foster K, Lee SH, Ahmadian G, Wyszynski M, Wang YT, Sheng M (2000) Distinct molecular mechanisms and divergent endocytotic pathways of AMPA receptor internalization. Nat Neurosci 3:1282-1290.
Ling DS, Benardo LS, Serrano PA, Blace N, Kelly MT, Crary JF, Sacktor TC (2002) Protein kinase Mzeta is necessary and sufficient for LTP maintenance. Nat Neurosci 5:295-296.
Lissin DV, Gomperts SN, Carroll RC, Christine CW, Kalman D, Kitamura M, Hardy S, Nicoll RA, Malenka RC, von Zastrow M (1998) Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. Proc Natl Acad Sci U S A 95:7097-7102.
Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004) Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304:1021-1024.
Lohmann C, Bonhoeffer T (2008) A role for local calcium signaling in rapid synaptic partner selection by dendritic filopodia. Neuron 59:253-260.
Lopez-Bendito G, Shigemoto R, Fairen A, Lujan R (2002) Differential distribution of group I metabotropic glutamate receptors during rat cortical development. Cereb Cortex 12:625-638.
Lu B, Wang KH, Nose A (2009a) Molecular mechanisms underlying neural circuit formation. Curr Opin Neurobiol 19:162-167.
Lu W, Ziff EB (2005) PICK1 interacts with ABP/GRIP to regulate AMPA receptor trafficking. Neuron 47:407-421.
Lu W, Shi Y, Jackson AC, Bjorgan K, During MJ, Sprengel R, Seeburg PH, Nicoll RA (2009b) Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. Neuron 62:254-268.
Luján R, Nusser Z, Roberts JD, Shigemoto R, Somogyi P (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur J Neurosci 8:1488-1500.
Lüthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, Henley JM, Isaac JT, Collingridge GL (1999) Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. Neuron 24:389-399.
Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.
Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. Annu Rev Neurosci 25:103-126.
Malinow R, Schulman H, Tsien RW (1989) Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245:862-866.

Mameli M, Carta M, Partridge LD, Valenzuela CF (2005) Neurosteroid-induced plasticity of immature synapses via retrograde modulation of presynaptic NMDA receptors. J Neurosci 25:2285-2294.

Man HY, Lin JW, Ju WH, Ahmadian G, Liu L, Becker LE, Sheng M, Wang YT (2000) Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. Neuron 25:649-662.

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ (2001) Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. J Neurosci 21:5925-5934.

Martin LJ, Blackstone CD, Huganir RL, Price DL (1992) Cellular localization of a metabotropic glutamate receptor in rat brain. Neuron 9:259-270.

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

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature 429:761-766.

Megias M, Emri Z, Freund TF, Gulyas AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102:527-540.

Messaoudi E, Kanhema T, Soule J, Tiron A, Dagyte G, da Silva B, Bramham CR (2007) Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo. J Neurosci 27:10445-10455.

Miller SG, Kennedy MB (1986) Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. Cell 44:861-870.

Montgomery JM, Madison DV (2002) State-dependent heterogeneity in synaptic depression between pyramidal cell pairs. Neuron 33:765-777.

Monyer H, Seeburg PH, Wisden W (1991) Glutamate-operated channels: developmentally early and mature forms arise by alternative splicing. Neuron 6:799-810.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529-540.

Morishita W, Lu W, Smith GB, Nicoll RA, Bear MF, Malenka RC (2007) Activation of NR2B-containing NMDA receptors is not required for NMDA receptor-dependent long-term depression. Neuropharmacology 52:71-76.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683.

Moult PR, Gladding CM, Sanderson TM, Fitzjohn SM, Bashir ZI, Molnar E, Collingridge GL (2006) Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. J Neurosci 26:2544-2554.

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

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

Mulkey RM, Endo S, Shenolikar S, Malenka RC (1994) Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486-488.

Mullasseril P, Dosemeci A, Lisman JE, Griffith LC (2007) A structural mechanism for maintaining the 'on-state' of the CaMKII memory switch in the post-synaptic density. J Neurochem 103:357-364.

Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB (2003) Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. Nat Neurosci 6:153-160.

Neki A, Ohishi H, Kaneko T, Shigemoto R, Nakanishi S, Mizuno N (1996) Pre- and postsynaptic localization of a metabotropic glutamate receptor, mGluR2, in the rat brain: an immunohistochemical study with a monoclonal antibody. Neurosci Lett 202:197-200.

Newpher TM, Ehlers MD (2008) Glutamate receptor dynamics in dendritic microdomains. Neuron 58:472-497.

Nicoll RA, Tomita S, Bredt DS (2006) Auxiliary subunits assist AMPA-type glutamate receptors. Science 311:1253-1256.

Niethammer M, Kim E, Sheng M (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci 16:2157-2163.

Nosyreva ED, Huber KM (2005) Developmental switch in synaptic mechanisms of hippocampal metabotropic glutamate receptor-dependent long-term depression. J Neurosci 25:2992-3001.

Nägerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T (2004) Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron 44:759-767.

O'Connor TP, Cockburn K, Wang W, Tapia L, Currie E, Bamji SX (2009) Semaphorin 5B mediates synapse elimination in hippocampal neurons. Neural Dev 4:18.

O'Donnell M, Chance RK, Bashaw GJ (2009) Axon growth and guidance: receptor regulation and signal transduction. Annu Rev Neurosci 32:383-412.

Ohishi H, Neki A, Mizuno N (1998) Distribution of a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat and mouse: an immunohistochemical study with a monoclonal antibody. Neurosci Res 30:65-82.

Okabe S (2007) Molecular anatomy of the postsynaptic density. Mol Cell Neurosci 34:503-518.

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci 7:1104-1112.

Oliet SH, Malenka RC, Nicoll RA (1996) Bidirectional control of quantal size by synaptic activity in the hippocampus. Science 271:1294-1297.

Oliet SH, Malenka RC, Nicoll RA (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. Neuron 18:969-982.

Oliveria SF, Gomez LL, Dell'Acqua ML (2003) Imaging kinase-AKAP79-phosphatase scaffold complexes at the plasma membrane in living cells using FRET microscopy. J Cell Biol 160:101-112.

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

Osten P, Khatri L, Perez JL, Köhr G, Giese G, Daly C, Schulz TW, Wensky A, Lee LM, Ziff EB (2000) Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. Neuron 27:313-325.

Otmakhov N, Griffith LC, Lisman JE (1997) Postsynaptic inhibitors of calcium/calmodulin-dependent protein kinase type II block induction but not maintenance of pairing-induced long-term potentiation. J Neurosci 17:5357-5365.

Otmakhov N, Tao-Cheng JH, Carpenter S, Asrican B, Dosemeci A, Reese TS, Lisman J (2004) Persistent accumulation of calcium/calmodulin-dependent protein kinase II in dendritic spines after induction of NMDA receptor-dependent chemical long-term potentiation. J Neurosci 24:9324-9331.

Palmer CL, Lim W, Hastie PG, Toward M, Korolchuk VI, Burbidge SA, Banting G, Collingridge GL, Isaac JT, Henley JM (2005) Hippocalcin functions as a calcium sensor in hippocampal LTD. Neuron 47:487-494.

Palmer MJ, Isaac JT, Collingridge GL (2004) Multiple, developmentally regulated expression mechanisms of long-term potentiation at CA1 synapses. J Neurosci 24:4903-4911.

Palmer MJ, Irving AJ, Seabrook GR, Jane DE, Collingridge GL (1997) The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. Neuropharmacology 36:1517-1532.

Park S, Park JM, Kim S, Kim JA, Shepherd JD, Smith-Hicks CL, Chowdhury S, Kaufmann W, Kuhl D, Ryazanov AG, Huganir RL, Linden DJ, Worley PF (2008) Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron 59:70-83.

Passafaro M, Piech V, Sheng M (2001) Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 4:917-926.

Patrick GN, Bingol B, Weld HA, Schuman EM (2003) Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. Curr Biol 13:2073-2081.

Pavlov I, Riekki R, Taira T (2004) Synergistic action of GABA-A and NMDA receptors in the induction of long-term depression in glutamatergic synapses in the newborn rat hippocampus. Eur J Neurosci 20:3019-3026.

Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, Lo E, Wu D, Saule E, Bouschet T, Matthews P, Isaac JT, Bortolotto ZA, Wang YT, Collingridge GL (2007) LTP inhibits LTD in the hippocampus via regulation of GSK3beta. Neuron 53:703-717.

Pellmar TC (1995) Use of brain slices in the study of free-radical actions. J Neurosci Methods 59:93-98.

Pérez-Otaño I, Schulteis CT, Contractor A, Lipton SA, Trimmer JS, Sucher NJ, Heinemann SF (2001) Assembly with the NR1 subunit is required for surface expression of NR3A-containing NMDA receptors. J Neurosci 21:1228-1237.

Perez JL, Khatri L, Chang C, Srivastava S, Osten P, Ziff EB (2001) PICK1 targets activated protein kinase Calpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. J Neurosci 21:5417-5428.

Perry VH, O'Connor V (2008) C1q: the perfect complement for a synaptic feast? Nat Rev Neurosci 9:807-811.

Petralia RS, Wang YX, Wenthold RJ (2003) Internalization at glutamatergic synapses during development. Eur J Neurosci 18:3207-3217.

Petralia RS, Wang YX, Niedzielski AS, Wenthold RJ (1996) The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. Neuroscience 71:949-976.

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

Pi HJ, Lisman JE (2008) Coupled phosphatase and kinase switches produce the tristability required for long-term potentiation and long-term depression. J Neurosci 28:13132-13138.

Pickard L, Noel J, Henley JM, Collingridge GL, Molnar E (2000) Developmental changes in synaptic AMPA and NMDA receptor distribution and AMPA receptor subunit composition in living hippocampal neurons. J Neurosci 20:7922-7931.

Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, Isaac JT (2006) Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nat Neurosci 9:602-604.

Plath N, Ohana O, Dammermann B, Errington ML, Schmitz D, Gross C, Mao X, Engelsberg A, Mahlke C, Welzl H, Kobalz U, Stawrakakis A, Fernandez E, Waltereit R, Bick-Sander A, Therstappen E, Cooke SF, Blanquet V, Wurst W, Salmen B, Bosl MR, Lipp HP, Grant SG, Bliss TV, Wolfer DP, Kuhl D (2006) Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron 52:437-444.

Poncer JC, Malinow R (2001) Postsynaptic conversion of silent synapses during LTP affects synaptic gain and transmission dynamics. Nat Neurosci 4:989-996.

Qin Y, Zhu Y, Baumgart JP, Stornetta RL, Seidenman K, Mack V, van Aelst L, Zhu JJ (2005) State-dependent Ras signaling and AMPA receptor trafficking. Genes Dev 19:2000-2015.

Rácz B, Blanpied TA, Ehlers MD, Weinberg RJ (2004) Lateral organization of endocytic machinery in dendritic spines. Nat Neurosci 7:917-918.

Rae MG, Irving AJ (2004) Both mGluR1 and mGluR5 mediate Ca2+ release and inward currents in hippocampal CA1 pyramidal neurons. Neuropharmacology 46:1057-1069.

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

Raymond CR, Thompson VL, Tate WP, Abraham WC (2000) Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong long-term potentiation. J Neurosci 20:969-976.

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

Riebe I, Gustafsson B, Hanse E (2009) Silent synapses onto interneurons in the rat CA1 stratum radiatum. Eur J Neurosci 29:1870-1882.

Roberson ED, Sweatt JD (1996) Transient activation of cyclic AMP-dependent protein kinase during hippocampal long-term potentiation. J Biol Chem 271:30436-30441.

Roberts AC, Diez-Garcia J, Rodriguiz RM, Lopez IP, Lujan R, Martinez-Turrillas R, Pico E, Henson MA, Bernardo DR, Jarrett TM, Clendeninn DJ, Lopez-Mascaraque L, Feng G, Lo DC, Wesseling JF, Wetsel WC, Philpot BD, Perez-Otano I (2009) Downregulation of NR3A-containing NMDARs is required for synapse maturation and memory consolidation. Neuron 63:342-356.

Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16:1179-1188.

Romberg C, Raffel J, Martin L, Sprengel R, Seeburg PH, Rawlins JN, Bannerman DM, Paulsen O (2009) Induction and expression of GluA1 (GluR-A)-independent LTP in the hippocampus. Eur J Neurosci 29:1141-1152.

Ronesi JA, Huber KM (2008) Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. J Neurosci 28:543-547.

Sallert M, Malkki H, Segerstråle M, Taira T, Lauri SE (2007) Effects of the kainate receptor agonist ATPA on glutamatergic synaptic transmission and plasticity during early postnatal development. Neuropharmacology 52:1354-1365.

Sanhueza M, McIntyre CC, Lisman JE (2007) Reversal of synaptic memory by Ca2+/calmodulin-dependent protein kinase II inhibitor. J Neurosci 27:5190-5199.
Sans N, Petralia RS, Wang YX, Blahos J, 2nd, Hell JW, Wenthold RJ (2000) A developmental change in NMDA receptor-associated proteins at hippocampal synapses. J Neurosci 20:1260-1271.
Sans N, Racca C, Petralia RS, Wang YX, McCallum J, Wenthold RJ (2001) Synapse-associated protein 97 selectively associates with a subset of AMPA receptors early in their biosynthetic pathway. J Neurosci 21:7506-7516.
Sans N, Vissel B, Petralia RS, Wang YX, Chang K, Royle GA, Wang CY, O'Gorman S, Heinemann SF, Wenthold RJ (2003) Aberrant formation of glutamate receptor complexes in hippocampal neurons of mice lacking the GluR2 AMPA receptor subunit. J Neurosci 23:9367-9373.
Sasaki YF, Rothe T, Premkumar LS, Das S, Cui J, Talantova MV, Wong HK, Gong X, Chan SF, Zhang D, Nakanishi N, Sucher NJ, Lipton SA (2002) Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. J Neurophysiol 87:2052-2063.
Schenk U, Matteoli M (2004) Presynaptic AMPA receptors: more than just ion channels? Biol Cell 96:257-260.
Schnell E, Sizemore M, Karimzadegan S, Chen L, Bredt DS, Nicoll RA (2002) Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. Proc Natl Acad Sci U S A 99:13902-13907.
Schwenk J, Harmel N, Zolles G, Bildl W, Kulik A, Heimrich B, Chisaka O, Jonas P, Schulte U, Fakler B, Klöcker N (2009) Functional proteomics identify cornichon proteins as auxiliary subunits of AMPA receptors. Science 323:1313-1319.
Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 20:11-21.
Seidenman KJ, Steinberg JP, Huganir R, Malinow R (2003) Glutamate receptor subunit 2 Serine 880 phosphorylation modulates synaptic transmission and mediates plasticity in CA1 pyramidal cells. J Neurosci 23:9220-9228.
Selig DK, Hjelmstad GO, Herron C, Nicoll RA, Malenka RC (1995) Independent mechanisms for long-term depression of AMPA and NMDA responses. Neuron 15:417-426.
Sheng M, Kim E (2000) The Shank family of scaffold proteins. J Cell Sci 113 (Pt 11):1851-1856.
Sheng M, Cummings J, Roldan LA, Jan YN, Jan LY (1994) Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368:144-147.
Shi S, Hayashi Y, Esteban JA, Malinow R (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105:331-343.
Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284:1811-1816.
Shigemoto R, Kinoshita A, Wada E, Nomura S, Ohishi H, Takada M, Flor PJ, Neki A, Abe T, Nakanishi S, Mizuno N (1997) Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. J Neurosci 17:7503-7522.
Shinoda Y, Kamikubo Y, Egashira Y, Tominaga-Yoshino K, Ogura A (2005) Repetition of mGluR-dependent LTD causes slowly developing persistent reduction in synaptic strength accompanied by synapse elimination. Brain Res 1042:99-107.
Silva AJ, Stevens CF, Tonegawa S, Wang Y (1992) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257:201-206.

Skrede KK, Westgaard RH (1971) The transverse hippocampal slice: a well-defined cortical structure maintained in vitro. Brain Res 35:589-593.
Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF (2001) Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat Neurosci 4:1079-1085.
Snyder EM, Colledge M, Crozier RA, Chen WS, Scott JD, Bear MF (2005) Role for A kinase-anchoring proteins (AKAPS) in glutamate receptor trafficking and long term synaptic depression. J Biol Chem 280:16962-16968.
Sobczyk A, Scheuss V, Svoboda K (2005) NMDA receptor subunit-dependent [Ca2+] signaling in individual hippocampal dendritic spines. J Neurosci 25:6037-6046.
Sommer B, Keinänen K, Verdoorn TA, Wisden W, Burnashev N, Herb A, Köhler M, Takagi T, Sakmann B, Seeburg PH (1990) Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS. Science 249:1580-1585.
Stanton PK, Sarvey JM (1984) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. J Neurosci 4:3080-3088.
Steiner P, Higley MJ, Xu W, Czervionke BL, Malenka RC, Sabatini BL (2008) Destabilization of the postsynaptic density by PSD-95 serine 73 phosphorylation inhibits spine growth and synaptic plasticity. Neuron 60:788-802.
Stepien L, Sierpinski S (1964) Impairment of recent memory after temporal lesions in man. Neuropsychologia 2:291-303.
Steward O, Falk PM (1991) Selective localization of polyribosomes beneath developing synapses: a quantitative analysis of the relationships between polyribosomes and developing synapses in the hippocampus and dentate gyrus. J Comp Neurol 314:545-557.
Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131:1164-1178.
Stevens CF, Wesseling JF (1999) Identification of a novel process limiting the rate of synaptic vesicle cycling at hippocampal synapses. Neuron 24:1017-1028.
Strack S, Barban MA, Wadzinski BE, Colbran RJ (1997) Differential inactivation of postsynaptic density-associated and soluble Ca2+/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A. J Neurochem 68:2119-2128.
Suárez LM, Solís JM (2006) Taurine potentiates presynaptic NMDA receptors in hippocampal Schaffer collateral axons. Eur J Neurosci 24:405-418.
Suárez LM, Suárez F, Del Olmo N, Ruiz M, González-Escalada JR, Solís JM (2005) Presynaptic NMDA autoreceptors facilitate axon excitability: a new molecular target for the anticonvulsant gabapentin. Eur J Neurosci 21:197-209.
Sucher NJ, Akbarian S, Chi CL, Leclerc CL, Awobuluyi M, Deitcher DL, Wu MK, Yuan JP, Jones EG, Lipton SA (1995) Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. J Neurosci 15:6509-6520.
Tada T, Sheng M (2006) Molecular mechanisms of dendritic spine morphogenesis. Curr Opin Neurobiol 16:95-101.
Tamaru Y, Nomura S, Mizuno N, Shigemoto R (2001) Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: differential location relative to pre- and postsynaptic sites. Neuroscience 106:481-503.

Thiels E, Norman ED, Barrionuevo G, Klann E (1998) Transient and persistent increases in protein phosphatase activity during long-term depression in the adult hippocampus in vivo. Neuroscience 86:1023-1029.
Triller A, Choquet D (2005) Surface trafficking of receptors between synaptic and extrasynaptic membranes: and yet they do move! Trends Neurosci 28:133-139.
Ullian EM, Christopherson KS, Barres BA (2004) Role for glia in synaptogenesis. Glia 47:209-216.
Waites CL, Craig AM, Garner CC (2005) Mechanisms of vertebrate synaptogenesis. Annu Rev Neurosci 28:251-274.
Waites CL, Specht CG, Hartel K, Leal-Ortiz S, Genoux D, Li D, Drisdel RC, Jeyifous O, Cheyne JE, Green WN, Montgomery JM, Garner CC (2009) Synaptic SAP97 isoforms regulate AMPA receptor dynamics and access to presynaptic glutamate. J Neurosci 29:4332-4345.
Wang XB, Yang Y, Zhou Q (2007) Independent expression of synaptic and morphological plasticity associated with long-term depression. J Neurosci 27:12419-12429.
Wang Y, Small DL, Stanimirovic DB, Morley P, Durkin JP (1997) AMPA receptor-mediated regulation of a Gi-protein in cortical neurons. Nature 389:502-504.
Ward B, McGuinness L, Akerman CJ, Fine A, Bliss TV, Emptage NJ (2006) State-dependent mechanisms of LTP expression revealed by optical quantal analysis. Neuron 52:649-661.
Washbourne P, Bennett JE, McAllister AK (2002) Rapid recruitment of NMDA receptor transport packets to nascent synapses. Nat Neurosci 5:751-759.
Wasling P, Hanse E, Gustafsson B (2004) Developmental changes in release properties of the CA3-CA1 glutamate synapse in rat hippocampus. J Neurophysiol 92:2714-2724.
Waung MW, Pfeiffer BE, Nosyreva ED, Ronesi JA, Huber KM (2008) Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron 59:84-97.
Wenthold RJ, Petralia RS, Blahos J, II, Niedzielski AS (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. J Neurosci 16:1982-1989.
Wenzel A, Fritschy JM, Mohler H, Benke D (1997) NMDA receptor heterogeneity during postnatal development of the rat brain: differential expression of the NR2A, NR2B, and NR2C subunit proteins. J Neurochem 68:469-478.
Vignes M, Clarke VR, Parry MJ, Bleakman D, Lodge D, Ornstein PL, Collingridge GL (1998) The GluR5 subtype of kainate receptor regulates excitatory synaptic transmission in areas CA1 and CA3 of the rat hippocampus. Neuropharmacology 37:1269-1277.
Wigström H, Gustafsson B (1983) Facilitated induction of hippocampal long-lasting potentiation during blockade of inhibition. Nature 301:603-604.
Wigström H, Gustafsson B (1985) Facilitation of hippocampal long-lasting potentiation by GABA antagonists. Acta Physiol Scand 125:159-172.
Wigström H, Gustafsson B (1986) Postsynaptic control of hippocampal long-term potentiation. J Physiol (Paris) 81:228-236.
Voigt T, Opitz T, de Lima AD (2005) Activation of early silent synapses by spontaneous synchronous network activity limits the range of neocortical connections. J Neurosci 25:4605-4615.
Volianskis A, Jensen MS (2003) Transient and sustained types of long-term potentiation in the CA1 area of the rat hippocampus. J Physiol 550:459-492.

Volk LJ, Daly CA, Huber KM (2006) Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. J Neurophysiol 95:2427-2438.

Wong HK, Liu XB, Matos MF, Chan SF, Perez-Otano I, Boysen M, Cui J, Nakanishi N, Trimmer JS, Jones EG, Lipton SA, Sucher NJ (2002) Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. J Comp Neurol 450:303-317.

Wu GY, Deisseroth K, Tsien RW (2001) Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. Nat Neurosci 4:151-158.

Xiao MY, Wasling P, Hanse E, Gustafsson B (2004) Creation of AMPA-silent synapses in the neonatal hippocampus. Nat Neurosci 7:236-243.

Xu J, Xiao N, Xia J (2009a) Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1. Nat Neurosci.

Xu Z, Chen RQ, Gu QH, Yan JZ, Wang SH, Liu SY, Lu W (2009b) Metaplastic regulation of long-term potentiation/long-term depression threshold by activity-dependent changes of NR2A/NR2B ratio. J Neurosci 29:8764-8773.

Yamagata Y, Kobayashi S, Umeda T, Inoue A, Sakagami H, Fukaya M, Watanabe M, Hatanaka N, Totsuka M, Yagi T, Obata K, Imoto K, Yanagawa Y, Manabe T, Okabe S (2009) Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca2+/calmodulin-dependent protein kinase IIalpha in dendritic spine enlargement, long-term potentiation, and learning. J Neurosci 29:7607-7618.

Yamamoto C, McIlwain H (1966) Potentials evoked in vitro in preparations from the mammalian brain. Nature 210:1055-1056.

Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. J Neurophysiol 81:781-787.

Yao Y, Kelly MT, Sajikumar S, Serrano P, Tian D, Bergold PJ, Frey JU, Sacktor TC (2008) PKM zeta maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. J Neurosci 28:7820-7827.

Yoshihara Y, De Roo M, Muller D (2009) Dendritic spine formation and stabilization. Curr Opin Neurobiol 19:146-153.

Yudowski GA, Puthenveedu MA, Leonoudakis D, Panicker S, Thorn KS, Beattie EC, von Zastrow M (2007) Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. J Neurosci 27:11112-11121.

Zakharenko SS, Zablow L, Siegelbaum SA (2001) Visualization of changes in presynaptic function during long-term synaptic plasticity. Nat Neurosci 4:711-717.

Zalfa F, Giorgi M, Primerano B, Moro A, Di Penta A, Reis S, Oostra B, Bagni C (2003) The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell 112:317-327.

Zhang Y, Venkitaramani DV, Gladding CM, Zhang Y, Kurup P, Molnar E, Collingridge GL, Lombroso PJ (2008) The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. J Neurosci 28:10561-10566.

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron 44:749-757.

Zhu JJ, Esteban JA, Hayashi Y, Malinow R (2000) Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nat Neurosci 3:1098-1106.

Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R (2002) Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell 110:443-455.