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IRINA GUREVICIENE

Changes in Hippocampal Synaptic Plasticity in Animal Models of Age-Related Memory Impairment

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium L21, Snellmania building, University of Kuopio, on Wednesday 18th June 2008, at 12 noon

Department of Neurobiology
A.I. Virtanen Institute for Molecular Sciences
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Department of Neurology
University of Kuopio

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ABSTRACT

Dynamic changes in synaptic strength are thought to provide a cellular basis for information storage in the nervous system. Long-term potentiation (LTP) has many features that make it an attractive model of memory formation. It is a long-lasting, activity-dependent form of synaptic plasticity that is expressed by all principal neurons in the hippocampus - a brain structure implicated in certain forms of long-term memory. Studies of the properties of LTP have shown that this form of plasticity contributes to several models of learning and memory.

The aim of the PhD study was to evaluate the hippocampal synaptic functioning in three neurodegenerative conditions that are associated with age-dependent memory impairment, menopause, Alzheimer's disease (AD) and Parkinson's disease (PD). As mice do not go through a rapid decline in estrogen levels as humans, we modeled menopause in young female mice by ovariectomy. The main pathological feature of AD, the accumulation of amyloid β peptide into plaques, was modeled with transgenic mice expressing APP and PS1 mutations linked with familial forms of AD. Finally, we used, both *in vivo* and *in vitro*, a transgenic mouse expressing human A30P

Our results revealed several kinds of alternations in synaptic transmission and plasticity in our animal models, ranging from altered presynaptic mobilization of the neurotransmitter to postsynaptic changes in the number of receptors. We showed that estrogen treatment interacted with pharmacological blockade of NMDA receptors in a way that points to changes in the number of functional NMDA receptors. We also demonstrated that mice carrying mutated human APPswe and PS1 genes had normal induction and maintenance of hippocampal LTP. However, the enhanced fEPSP in the dentate gyrus (DG) declined much faster in these mutant mice than in control littermates, which is compatible with their overnight forgetting of learned spatial information in behavioral studies. Our findings in α-synuclein overexpressing mice lend additional support to the idea that α-synuclein plays an important role in presynaptic mobilization of reserve pool glutamate vesicles. We also revealed that age is an important factor in altering synaptic plasticity *in vivo* of mice carrying mutated α-synuclein and described how model-specific pathological changes of synaptic plasticity interact with general age-related alternations.

Overall, this project revealed that pathological manipulations play an important role in alternations of synaptic strength and plasticity. However, the link between altered synaptic / cellular mechanisms and behavioral manifestations of memory is not straightforward. Behavioral outcome depends on a multitude of neural circuits, while LTP is measured in a limited number of synapses during a single experiment. Also the high-frequency synchronous stimulation of a pathway used for induction of LTP may not fully mimic interplay between neurons during learning. Thus, this PhD project makes a step towards a better understanding of the mechanisms by which molecular alterations lead to impaired memory in neurodegenerative conditions.

National Library of Medicine Classification: QU 450, QU 55.7, QU 60, QY 58, WL 102, WL 102.7-102.8, WL 314, WL 359, WM 173.7, WP 522, WT 155
Medical Subject Headings: alpha-Synuclein; Alzheimer Disease; Amyloid beta-Protein Precursor; Animals, Transgenic; Disease Models, Animal; Estrogens; Hippocampus; Long-Term Potentiation; Membrane Proteins; Memory; Memory Disorders; Neuronal Plasticity; Parkinson Disease; Receptors, N-Methyl-D-Aspartate; Synaptic Transmission

The scientist does not study nature because it is useful to do so.
He studies it because he takes pleasure in it,
and he takes pleasure in it because it is beautiful.

Henri Poincare

ACKNOWLEDGEMENTS

This study was performed in the Department of Neurology and Department of Neurobiology, A. I. Virtanen Institute, at the University of Kuopio during years 1999-2008. I would like to acknowledge all colleagues who contributed directly or indirectly helping me in this quest.

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Kuopio, May 2008

Irina Gureviciene

ABBREVIATIONS

α-syn	α-synuclein, a peptide
Aβ	beta amyloid, a peptide
ACh	acetylcholine, a neurotransmitter
A/C	associational-commissural, a hippocampal path
aCSF	artificial cerebrospinal fluid
ACPD	1-aminocyclopentane-(1S,3R)-dicarboxylic acid, a metabotropic glutamate receptor (mGluR) agonists
AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate
A30P	a mutation in human α-synuclein gene resulting in the substitution of alanine with phenylalanine at position 30
A/P	transgenic mice expressing APPswe and PS1-A264E mutations
AP-V	2-amino-5-phosphonovaleric acid, a N-methyl-D-aspartate (NMDA) receptor antagonist
APP	amyloid beta (A4) precursor protein
CA1	the hippocampal *Cornu Ammonis* subregion 1
CA3	the hippocampal *Cornu Ammonis* subregion 3
CaMKII	Calcium/calmodulin-dependent kinase II
cGK	cGMP-dependent protein kinase
cGMP	cyclic guanosine monophosphate, a cyclic nucleotide derived from guanosine triphosphate
CGP39653	2-amino-4-propyl-5-phosphonopentenoic acid, a competitive N-methyl-D-aspartate (NMDA) receptor antagonist
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione, an α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate (AMPA) receptor antagonist
CNS	central nervous system
CPP	{3-[(+)-carboxypiperazin-4-yl]prop-1-yl}phosphonic acid, a competitive N-methyl-D-aspartate (NMDA) receptor antagonist
D-AP-V	D-2-amino-5-phosphonopentanoic acid, a N-methyl-D-aspartate (NMDA) receptor antagonist

D-AP-VII	D-2-amino-7-phosphonoheptanoic acid, a N-methyl-D-aspartate (NMDA) receptor antagonist
DCG-IV	(2*S*,2*R*,3*R*)-2-(2,3-dicarboxycyclopropyl)-glycine, a group II metabotropic glutamate receptor (mGluR) agonist
DG	dentate gyrus, a part of the hippocampal formation
DLB	dementia with Lewy bodies
DNQX	6,7-dinitroquinoxaline-2,3-dione, a α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate (AMPA) / kainate receptors antagonist
EC	entorhinal cortex, a part of the hippocampal formation
E-LTP	early-long-term potentiation
ER	estrogen receptor
ERα	estrogen receptor family α
ERβ	estrogen receptor family β
ERK	extracellular signal-regulated kinase
ERT	estrogen replacement therapy
fEPSP	field excitatory postsynaptic potential
GABA	γ-aminobutyric acid, a neurotransmitter
$GABA_B$	metabotropic GABA receptor's class B
GAMS	glutamylaminomethylsulfonate, a selective kainate receptor antagonist
HFS	high-frequency stimulation
HIPP	hippocampus, a part of the forebrain
iGlu	ionotropic glutamate receptor
I-LTP	initial-long-term potentiation
I/O	input/output curve
i.p.	intraperitoneally, a type of injection
ISI	inter-stimulus interval
JNK	c-Jun N-terminal kinase
JST	Joro spider (*Nephila clavata*) toxin
L-AP-IV	L-2-amino-4-phosphonobutanoic acid, a groups I/V and II/III metabotropic glutamate receptor (mGluR) agonists
LBs	Lewy bodies
LEC	lateral entorhinal cortex
L-LTP	late-long-term potentiation

LNs	Lewy neurites
LPP	lateral perforant path
LTD	long-term depression
LTP	long-term potentiation
LY382884	3S,4aR,6S,8aR-6-((4-carboxyphenyl)methyl)-1,2,3,4,4a,5,6,7,8,8a-ecahydroisoquinoline-3-carboxylic acid, a kainate receptor antagonist
MAPK	mitogen-activated protein kinase
MEC	medial entorhinal cortex
mGluR	metabotropic glutamate receptor
MK-801	((5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo(a,b)cyclohepten-5,10-imine maleate, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist
MF	mossy fiber, a hippocampal path
MPP	medial perforant path
MWM	Morris water maze, a test to assess spatial memory in animals
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline, a α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate (AMPA) receptor antagonist
NMDA	N-metyl-D-aspartate
NMDA-R	NMDA receptor
NO	nitric oxide
NR	NMDA receptor subunit (i.e. 1, 2A, 2B)
NS-102	5-Nitro-6,7,8,9-tetrahydrobenzo[G]indole-2,3-dione-3-oxime, a competitive kainate receptor antagonist
NT	nontransgenic litters, a control group for the transgenic group
OVX	ovariectomy
OVX+ERT	ovariectomy combined with estrogen replacement therapy
OVX+ERT+CPP	ovariectomy combined with estrogen replacement therapy and CPP treatment
PD	Parkinson's disease
PKC	protein kinase C
PKA	protein kinase A
PP	perforant path
PPD	paired-pulse depression
PPF	paired-pulse facilitation
PPR	paired-pulse ratio

PS	population spike
PS1	presenilin-1, a protein
RRP	readily releasable pool of neuroptransmitter
s.c.	supracutaneously, a type of injection
SC	Schaffer collateral, a hippocampal path
SUB	subiculum, a part of the hippocampal formation
TG	transgenic animals group
TBS	theta bursts stimulation
WT	wild-type animal group

3HPG	(*S*)-3-hydroxyphenylglycine, a competitive metabotropic glutamate receptors antagonist
4C3HPG	(*S*)-4-carboxy-3-hydroxyphenylglycine, a competitive metabotropic glutamate receptors antagonist
4CPG	(*S*)-4-carboxyphenylglycine, a competitive metabotropic glutamate receptors antagonist
7-CK	chlorokynureic acid, a non-competitive N-metyl-D-aspartate (NMDA) receptor antagonist

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals I-IV.

I **GUREVICIENE I.**, PUOLIVÄLI J., PUSSINEN R., WANG J., TANILA H., YLINEN A. **Estrogen Treatment Alleviated NMDA-Antagonist Induced Hippocampal LTP Blockade and Cognitive Deficits in Ovariectomized Mice.** *Neurobiology of Learning and Memory*, 79 (2003) 72 - 80

II **GUREVICIENE I.*,** IKONEN S.*, GUREVICIUS K., SARKAKI A., VAN GROEN TH., PUSSINEN R., YLINEN A., TANILA H. **Normal Induction but Accelerated Decay of LTP in APP+PS1 Transgenic Mice**. *Neurobiology of Disease*, 15 (2004) 188 – 195

III **GUREVICIENE I.**, GUREVICIUS K., TANILA H. **Role of α-Synuclein in Synaptic Glutamate Release.** *Neurobiology of Disease*, 28 (2007) 83 - 89

IV **GUREVICIENE I.**, GUREVICIUS K., TANILA H. **Age-Dependent Effects of α-Synuclein on Synaptic Plasticity in the Dentate Gyrus**. *Manuscript*

* equall contribution

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1. INTRODUCTION

Questions about the exact location of the memory engram in the brain have tickled the human imagination for ages. Now we know that there are different neural systems serving different kinds of learning and memory and the hippocampus together with other medial temporal lobe structures is crucial for forming declarative memories and procedural learning. However the question about how the memories are built remains to be not fully understood. Nowadays we formulate it in the following terms: What is the biological function of long-term potentiation (LTP) in a behaving animal? Is this a physiological phenomenon with the same mechanisms that are responsible for certain forms of learning and memory? Is it reasonable to suggest that LTP is a model for learning and/or memory?

LTP is one of the most widely studied forms of synaptic plasticity, characterized by a long-lasting increase of synaptic strength caused by the pre- and postsynaptic activity (Malenka and Bear, 2004). The great interest is due to LTP properties, making it a useful candidate for cellular processes supporting learning behavior (Andersen, 2003). There are many different types of LTP in the mammalian CNS, varying in their induction paradigm (HFS, TBS), duration of the potentiation (E-LTP, L-LTP) and triggering events at the receptor level (NMDA-R-dependent, NMDA-R-independent). The specific type of LTP exhibited between neurons depends on a number of factors, such as the anatomical location where LTP is observed, the age of the organism when LTP is observed, the differences in signaling pathways expressed by a cell. For instance, LTP in the Schaffer collateral pathway is very different from LTP at the mossy fiber pathway. The molecular mechanisms of LTP in the immature hippocampus differ from those mechanisms that underlie LTP in adults. Some types of hippocampal LTP depend on the NMDA-Rs, while others depend on the metabotropic glutamate receptors or the L-type Ca^{2+} channels.

LTP is experimental phenomena (Malenka and Bear, 2004), which can be used to demonstrate the possible long-lasting modifications of individual synapses. It is still difficult to prove that the same molecular mechanisms underlying certain forms of LTP occur *in vivo* when items and events encountered are encoded into the neural networks. It is even more difficult to prove that these LTP-like modifications play an essential functional role in memory formation. Nevertheless, it is plausible that at least the capacity to form long-lasting activity-dependent synaptic modifications in the brain share some of the same mechanisms by which experience modifies behavior.

The aim of this PhD study was to evaluate hippocampal synaptic functions in three neurodegenerative conditions that are associated with age-dependent memory impairment:

menopause, AD and PD. LTP, basal synaptic transmission, recruitment of cells to fire action potentials and neurotransmitter release were tested in the aforementioned disease models and a number of age groups. Four different studies were conducted on three hippocampal excitatory synaptic pathways: Schaffer collateral pathway – CA1 (**Study I** & **II**), perforant pathway - dentate gyrus (**Study II** & **IV**), and mossy fiber – CA3 pathway (**Study III**). Overall, this project revealed that pathological manipulations play an important role in alternations of synaptic strength and efficacy. However, the link between altered synaptic / cellular mechanisms and behavioral manifestations of memory is not straightforward. Nevertheless, we believe that data we provide extend our knowledge on mechanisms by which molecular changes lead to impaired memory under distinct neurodegenerative conditions. Moreover, understanding the plasticity mechanisms that are responsible for age-related cognitive impairment will help to finding of therapeutic agents that can modify hippocampal neurobiology and slow age-related cognitive decline.

2. LITERATURE REVIEW

2.1. **Anatomy of Hippocampal Formation**

The hippocampal formation is perhaps the most extensively studied structure in the brain. It consists of four subregions: (1) the dentate gyrus (DG); (2) hippocampus proper (HIPP), which is subdivided into three fields (CA1, CA2, and CA3); (3) subicular complex (SUB), which includes 3 fields (subiculum, presubiculum, and parasubiculum); and (4) entorhinal cortex (EC) (**Fig. 1**) (Amaral and Witter, 1994). Most of the knowledge of the organization and functions of the hippocampal formation has been derived from studies in the young adult rat. However, there are differences between the mouse and rat hippocampus (Van Groen and Wyss, 1988) and between animals of different ages (Lopes da Silva et al., 1990).

2.1.1. ***Principle Cells and Layers***

The hippocampus proper (HIPP) and DG consist of three-layered (molecular, pyramidal, and polymorphic) cortex. *Stratum oriens* of CA1 is a relatively cell-free layer located over *alveus*. Pyramidal cells are the principle cells of the hippocampal CA1, CA3 areas, and their bodies form the layer *stratum pyramidale*. CA1 pyramidal cells have basal dendrites extending into *stratum oriens* and apical dendrites into hippocampal fissure. In the area CA3, an additional layer between *stratum pyramidale* and *stratum radiatum* is called *stratum lucidum,* which is formed by the mossy fibers (MF), the DG granule cells axons. The outermost layer of the HIPP is *stratum lacunosum-moleculare*, which is located just under the fissure. Granular cells are the principle cells of DG forming the compact "U" shape layer *stratum granulosum*. The granule cells axons and glia cells form the polymorphic layer (*hilus*). Dendrites of DG granule cells, as well as diverse other cells, occupy the molecular layer (*stratum moleculare*).

2.1.2. ***Connectivity***

The connections within the hippocampus generally follow a laminar pattern and form well-characterized closed loops that originate and end in EC. Principle cells of the hippocampus and DG form a glutamatergic circuit known as the trisynaptic loop. There are three main excitatory pathways in the mammalian hippocampus: perforant path (PP), mossy fibers (MF) and Schaffer collaterals (SC). Thus, there are defined routes for information flow making the hippocampus a very popular target for the study of synaptic transmission and plasticity.

Perforant Path

The perforant path (PP; **Fig. 1, (1)**) is the major input to the hippocampus. The majority of PP axons arise from layers II and III of EC, with a minor contribution from the deeper layers IV and V. Axons from layers II/IV project to the granule cells of the DG and pyramidal cells of the CA3 region. Axons from layers III/V project to pyramidal cells of CA1 and SUB. The PP can be divided into lateral (LPP) and medial (MPP) pathways, depending on whether the fibers arise from the lateral (LEC) or medial (MEC) EC.

Mossy Fiber Path

The mossy fibers (MF; **Fig. 1, (2)**) are 1-µm diameter axons of the DG granule cells. There are about 1 million axons in the rat brain, and 15 million in the human brain. They extend from DG to CA3 pyramidal cells and interneurons. MF synapse forms large aggregations of termini with multiple transmitter release sites and post-synaptic densities on CA3 neurons. Multiple granule cells can innervate a single CA3 pyramidal cell. In rodents, the main MF axons leave the *hilus* and travel through CA3 in an approximately 100 µm in thickness *stratum lucidum*.

Schaffer Collateral/Associational-Commissural Path

Schaffer Collaterals (SCs; **Fig. 1, (3)**) are derived from CA3 axons that project to the CA1 region. The axons either originate from CA3 neurons in the same hippocampus (ipsilateral), forming the Schaffer collateral path (SC); or - from the opposite hemisphere, forming the associational-commissural path (A/C).

Output to Subiculum and Entorhinal Cortex

The pathway from CA1 to SUB and EC forms the principal output from the hippocampus. The connection CA1 - SUB - EC follows a strict anatomical layout, i. e. the distal CA1/proximal SUB projects to the lateral EC while the proximal CA1/distal SUB project to the medial EC. Further connections extend to the perirhinal and postrhinal cortices. The perirhinal cortex projects to the lateral EC and receives returning projections. The postrhinal cortex projects to and receives inputs from the medial EC.

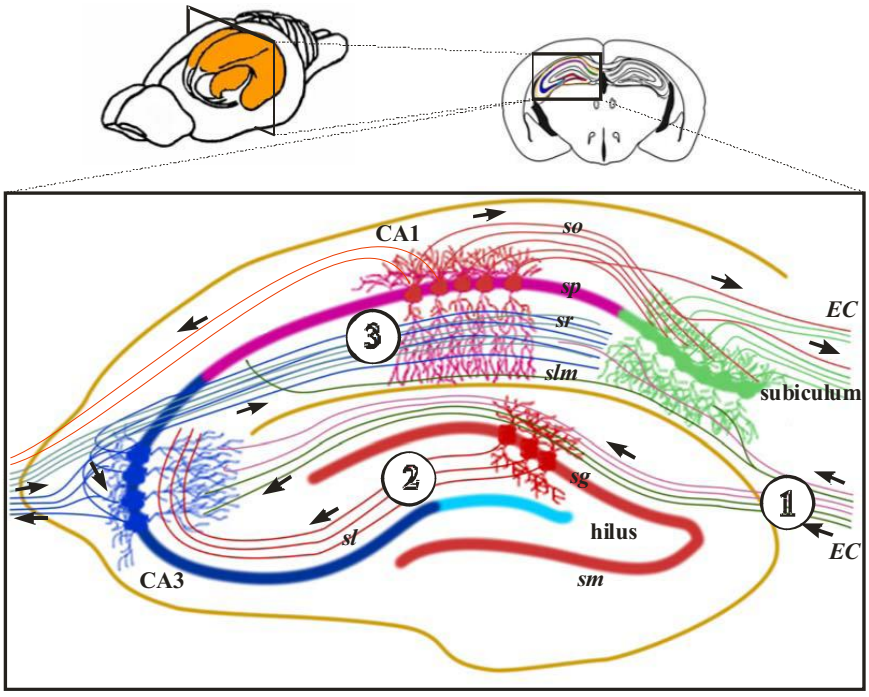


Fig. 1. Major excitatory connections in the rodent hippocampus: the tri-synaptic circuit. The entorhinal cortex (EC) projects through the perforant path (1) on the distal two thirds of granule cell dendrites in *stratum moleculare* (*sm*), and on the distal-most part of the apical dendrites of pyramidal cells in *stratum lacunosum-moleculare* (*slm*). Mossy fibers (2) from granule cells innervate the pyramidal cells of CA3 in *stratum lucidum* (*sl*). The axons of CA3 pyramidal cells (Schaffer collaterals; (3)) then innervate CA1 pyramidal cells, which in turn impinge back upon the subiculum and EC. Abbreviations: *EC*, entorhinal cortex, *sg*, *stratum granulosum*; *slm*, *stratum lacunosum-moleculare*; *sm*, *stratum moleculare*; *so*, *stratum oriens*; *sp*, *stratum pyramidale*; *sr*, *stratum radiatum*. (Figure courtesy provided by Dr. Andrew Doherty, MRC Center for Synaptic Plasticity, School of Medical Sciences, Department of Anatomy, University of Bristol; http://www.bris.ac.uk/Depts/Synaptic/info/pathway/hippocampal.htm).

2.2. **Physiology of Hippocampal Formation. Synaptic Plasticity Mechanisms & Relations to Memory**

Storage of memories in the brain almost certainly involves some forms of synaptic modification that is why LTP might be the experimental analogue of memory formation in the brain (Rosenzweig and Barnes, 2003).

Long-term potentiation (LTP) used to describe the synapse-specific enhancement of postsynaptic responses following the electrical stimulation of presynaptic fibers (Bliss and Lomo, 1973). Extracellularly, postsynaptic responses are recorded as field excitatory postsynaptic potentials (fEPSPs), which describe electric field associated with current flow through the plasma membranes of postsynaptic neurons in response to neurotransmitter release from presynaptic terminals. Increase of fEPSP arises from increasing glutamate concentrations in the presynaptic terminals or from increasing the responsiveness to glutamate by the postsynaptic cell. It is generally accepted that fEPSP is a collective response of a population of neurons in the dendritic regions of area CA1. The typical waveform (**Fig. 2**) consists of a "fiber volley," which is an indication of the presynaptic action potential arriving at the recording site and is an indirect measure of the number of axons activated. The second part of the waveform is the fEPSP itself, which is a manifestation of synaptic activation (depolarization) in CA1 pyramidal neurons. For determination of fEPSPs, the parameter typically measured is the initial slope of the fEPSP waveform. The absolute peak amplitude of fEPSPs can also be measured, but the initial slope is the preferred index. Field EPSPs are downward-deflecting for *stratum radiatum* recordings and upward-deflecting for *stratum pyramidale* recordings. If the cells fire action potentials, the recorded signal has an additional component going in the opposite direction as compared to fEPSP, the population spike (PS), reflecting the sum of action potentials fired by the cells in vicinity to the recording electrode. Increased fEPSP slopes for a given presynaptic fiber volley may reflect either a change in presynaptic glutamate release or postsynaptic receptors.

Field EPSPs are depolarizing postsynaptic potentials, shifting the membrane potential temporarily toward the cell's threshold for producing action potentials. Field EPSPs are relatively long lasting, at least when compared with action potentials. It typically takes 5 to 10 ms before their depolarizing effects are completely disappeared. The size of the fEPSP, produced by a given amount of neurotransmitter, increases with the extent of the change in membrane potential of the postsynaptic cell. Finally, all fEPSPs show a synaptic delay of approximately 1 ms, the time

elapsing between the arrival of an action potential at the presynaptic terminal and appearance of postsynaptic response, which is the time taken to release vesicles with neurotransmitter and for the molecules of neurotransmitter to diffuse across the synaptic cleft and bind postsynaptic receptors. During low-frequency transmission, under standard experimental conditions, the fEPSP is mediated predominantly through AMPA receptors (see paragraph 2.2.1). But during a brief period of synchronized high-frequency synaptic transmission, there is also a significant transient activation of the postsynaptically located NMDA-Rs (see paragraph 2.2.1) (Bashir et al., 1991).

The basal synaptic transmission is usually measured using the fEPSP input/output (I/O) curve, which quantifies the synaptic input to the cells. It determines whether there is a change in presynaptic glutamate release or in postsynaptic receptors. In addition, population spike (PS) I/O relations quantify the size of the population of discharged cells at different stimulus strengths.

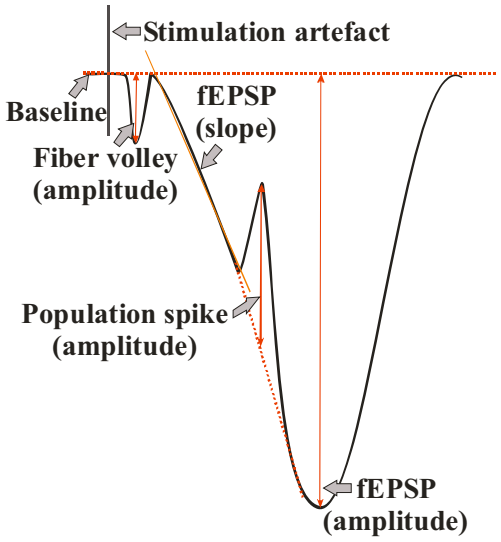


Fig. 2. The typical waveform of the extracellular field response consists of a "fiber volley", the "fEPSP itself" and population spike. For measuring fEPSPs, the parameter typically used is the initial "fEPSP slope" or/and absolute peak amplitude of fEPSPs. For measurement of population spike, the amplitude is calculated using a linear interpolation of fEPSP shape.

2.2.1. ***Glutamate. General Aspects. Receptors and Signal Transduction***

Glutamate (Glu) is the major excitatory neurotransmitter in the mammalian brain. Glutamatergic neurons are particularly prominent in the cerebral cortex from where they project to a

variety of subcortical structures. Various intrinsic glutamatergic pathways have been described also in the hippocampus. Glutamate is involved in fast synaptic transmission, eliciting a postsynaptic depolarization. Besides the fast excitatory effect, which occurs in the millisecond range, glutamate can produce long-lasting activity-dependent changes of neuronal excitability, as is the case in LTP.

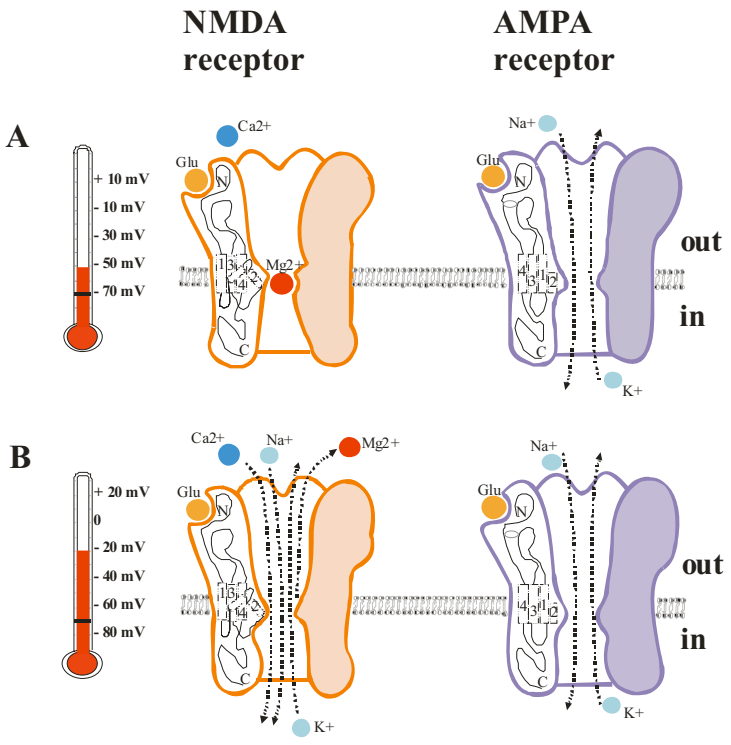


Fig. 3. A schematic diagram illustrating activation mechanisms of NMDA and AMPA receptors at different membrane potentials. (**A)** During normal synaptic transmission, glutamate is released from the presynaptic terminal and binds to both AMPA (right) and NMDA (left) receptors. The degree of membrane depolarization is not large enough to uncouple the Mg^{2+} block on the NMDA-R, while Na^{+} and K^{+} are able to flow through the AMPA-R. (**B)** When the postsynaptic membrane is depolarized more strongly after HFS, depolarization uncouples Mg^{2+} from the NMDA receptor (left). This allows Na^{+}, K^{+} and Ca^{2+} to flow through the receptor. The resulting Ca^{2+} rise in the dendritic spine triggers a cascade of events leading to LTP induction.

Glutamate receptors are widely expressed outside the CNS. NMDA-, AMPA-, kainate and mGlu-Rs have been identified in the heart, spleen, testis, ovary, kidney, bone, bone marrow, pancreas, intestine, oesophagus, hepatocytes, lung and keratinocytes. Moreover, these receptors

have been sequenced and cloned, and their sequences shown to be identical to those of neuronal glutamate receptors (for excellent rev. see (Nedergaard et al., 2002)). Some studies have documented the presence of iGlu and mGlu receptor (**Fig. 3**) subtypes also in astrocytes. They include the NMDA-R subunits NR1, NR2A and NR2B, the AMPA receptor subunits Glu-R1–4, the kainate receptor subunits Glu-R5–7, and the mGlu-Rs (mGluR2, mGluR3 and mGluR5). However, the existence of functional NMDA-Rs in astrocytes is debated, and most reports have failed to identify NMDA-R-mediated responses. AMPA receptor-mediated Ca^{2+} influx in astrocytic processes is required for maintaining the close structural association between neurons and astrocytes (for rev. see (Nedergaard et al., 2002)).

Glutamate receptors (see **Table 1**), which could occur as homomeric or heteromeric structures are classified according either to the binding of the most common agonists or to their functional properties reflecting the pharmacology of the receptors. Based on their general functional properties, two groups can be distinguished: the group of ionotropic receptors (iGlu-Rs) and the group of metabotropic receptors (mGlu-Rs). Ionotropic Glu receptors directly gate on ion channels for sodium (Na^{+}) and calcium (Ca^{2+}) and subsequently elicit fast excitatory responses, measurable in the form of EPSP. Metabotropic Glu receptors indirectly gate on channels through second messengers like inositol triphosphate or cyclic AMP (cAMP), are coupled to G proteins and produce a delayed synaptic response. The iGlu receptors can be divided into two major subtypes: N-metyl-D-aspartate (*NMDA*), which are activated by amino acid analog NMDA and blocked by the drug 2-amino-5-phosphonovaleric acid (AP-V), and *non-NMDA* receptors, which can be activated by the drugs α-amino-3-hydroxy-5-metyl-4-isoxazolepropionate (AMPA), Kainate/Quisqualate, and blocked by CNQX (Kandel and Siegelbaum, 2000). Both iGlu receptors and mGlu receptors are differentially distributed on pre- and postsynaptic sites to contribute to neuronal communication and signal processing, functions that determine learning and memory formation.

NMDA Receptors

NMDA receptors are found in high densities in cerebral cortex, hippocampus, basal ganglia, hypothalamus and olfactory bulb. The NMDA-Rs have received much attention because of their potential involvement in synaptic plasticity and neuronal development (McBain and Mayer, 1994; McDonald and Johnston, 1990), as well as in neurodegenerative disorders (Bradford, 1995; Choi, 1992; Whetsell, 1996).

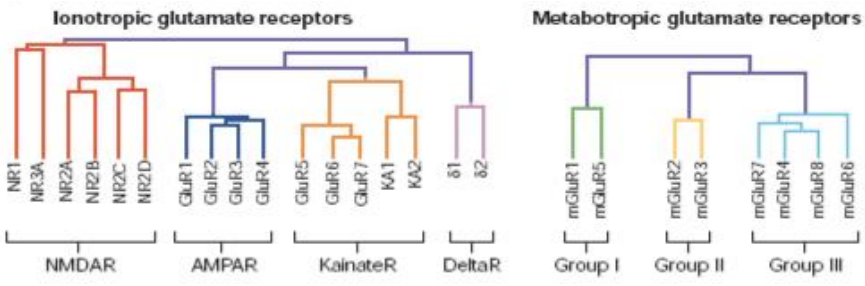
NMDA receptor is heteromultimeric complex consists of four pore-forming subunits (**Table 1**) with different specific binding sites. Within the hippocampus, for example, the

composition of the NMDA-Rs subunits may differ. There are reports that NMDA-R composition of C/A synapses on CA3 pyramidal cell spines includes NR1, NR2A, and NR2B subunits, whereas mossy fiber synapses contain NR1 and NR2A subunits (for more, see (Nusser, 2000)). In addition, it has been reported that NMDA-Rs are present on every synapse in SC terminals and dendritic spines of CA1 pyramidal cells (Takumi et al., 1999), while only 75-85 % of these synapses contain AMPA-Rs.

NMDA ion channel is permeable to Ca^{2+}, Na^{+} and K^{+}. The NMDA-Rs play an important role in the excitatory amino acid induced transmission and in synaptogenesis. Under resting membrane potential, the NMDA-Rs are blocked by Mg^{2+}. NMDA-R requires both its preferred ligand glutamate and a large membrane depolarization in order to open functionally. In addition, presence of glycine is also required for NMDA receptor opening (Parsons et al., 1998). On depolarization, the Mg^{2+} block is released and the channel opens, thereby allowing the exchange of ions through the channel pore. The entry of extracellular Ca^{2+} through the channel activates a variety of processes which alter the properties of the neuron and results in LTP (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). Excess of intracellular Ca^{2+} is also toxic to neurons; and hyperactivation of the NMDA-R is thought to play an important role in several neurodegenerative disorders (see below).

NMDA-Rs play an important role in the summation of synaptic responses and the generation of synaptic plasticity (for rev. see (Ozawa et al., 1998)) in several ways. First, the Ca^{2+} signal mediated by NMDA-Rs may be enhanced at potentiated synapses, and therefore change the stimulus patterns required to induce long-term depression (LTD) or additional LTP. Second, NMDA receptor activation could change the mode of spike generation in DG granule cells. It has been demonstrated that a transient exposure to Mg^{2+}-free aCSF can cause an increase in the NMDA component and as a consequence shift the cell from a single-spiking to a burst-discharging mode of firing (Lynch et al., 2000). Third, changes in the NMDA/non-NMDA ratio may influence neuronal network properties.

Table 1. Glutamate receptor subtypes and their general properties

Receptor type, subtype and subunit	 (Collingridge et al., 2004)			
Functional characteristics	Permeability to Na^+, K^+, Ca^{2+}	Permeability to Na^+, K^+	Permeability to Na^+, K^+, Ca^{2+}	Activation of phospholipase C; inhibition of adenylate cyclase
Selective agonist	Glutamate NMDA Aspartate	Glutamate AMPA Quisqualate Kainate Domoate	Glutamate Kainate Quisqualate Domoate	Glutamate Quisqualate L-AP-IV ACPD L-serine-O-phosphate ibotenic acid
Competitive antagonists	D-AP-V D-AP-VII CGP39653 CPP	CNQX NBQX DNQX	CNQX NBQX GAMS glutamyl-glycine NS-102	Phenylglycine analogs (3HPG, 4CPG)
Inhibitors (Non-Competitive)	MK-801 Ketamine Memantine Dextrophan 7-CK (glycin site)	JST	LY382884	

AMPA Receptors

High densities of AMPA receptors have been identified in the neocortex, hippocampus, lateral septum, basolateral nucleus and lateral nucleus of amygdala, caudate-putamen, nucleus accumbens, olfactory bulb, and in the molecular layers of cerebellum. AMPA-R also consists of a complex (**Table 1**) of four transmembrane proteins with different specific binding sites. AMPA-Rs are permeable for monovalent cations, such as Na^+ and K^+. Na^+ entry through AMPA-Rs is the initial stage of the excitatory synaptic transmission due to the fast membrane depolarization.

The AMPA-Rs are widely distributed in the central nervous system and their pattern is different from that of the NMDA-Rs. The AMPA- and NMDA-Rs display different topologic distributions in the postsynaptic membrane. Electron microscopy of immunogold-labeled synapses has shown that NMDA-Rs tend to cluster near the center of the synapse, while AMPA-Rs are distributed more at the periphery of the synapse (for rev. see (Nusser, 2000)). Also, they are transported to synapses at different times during development, i.e., NMDA-Rs are present on the membrane earlier that AMPA-Rs; and, once installed at the synapse, NMDA-Rs are more firmly attached then AMPA-Rs (for more see (Nusser, 2000)).

As it was already mentioned, only 75-85 % of SC synapses contain AMPA-Rs in juvenile rats. The number of AMPA receptors is positively correlated with the synaptic area, and thus the ratio of AMPA- to NMDA-Rs is linearly related to synaptic diameter (Nusser, 2000). In addition, the mean number of AMPA receptors at mossy synapses has been reported to be more than four-fold compared to C/A synapses. These results suggest that the number and the variability of synaptic AMPA receptors on a given cell depend on the identity of the presynaptic input (Nusser, 2000).

Kainate Receptors

Kainate receptors have been found in the neocortex, piriform cortex and hippocampal formation, as well as in caudate-putamen, reticular nucleus of thalamus and in other brain areas. The distribution pattern of kainate receptors depends on the configuration of the subtype.

Each kainate receptor consists of a tetrameric combination of up to four of the five kainate receptor subunits (**Table 1**). Kainate receptors are restricted to certain sites of the neuron, and depending on their subunit composition are targeted to different sites within a neuron (Mellor, 2006). Within hippocampal CA3 pyramidal neurons, kainate receptors are targeted to the axons and presynaptic terminals, where they regulate neurotransmitter release onto CA1 pyramidal neurons

and interneurons, and to the postsynaptic membrane of the mossy fiber synapse, where they mediate slow EPSPs and regulate the slow after-hyperpolarization (for more see Mellor, 2006). It is possible that these receptors are mainly involved in modulating the release of excitatory amino acids and additional neurotransmitters or neuromodulators. However, they are absent at A/C or PP postsynaptic sites.

Like AMPA-Rs, kainate receptors are activated by the same agonists (**Table 1**) and coexist in the same neurons (Rodriguez-Moreno and Sihra, 2007). And like the NMDA-Rs, the kainate receptors are associated with an ion channel which is permeable for the monovalent cations Na^+ and K^+, and also for Ca^{2+}. There is evidence that kainate receptors are involved in LTP at the mossy fibers (see below in paragraph 2.2.2.).

Metabotropic Glutamate Receptors

The mGlu-Rs are widely expressed throughout the central nervous system, but the different subtypes (**Table 1**) are differentially distributed: they can be localized pre- and/or postsynaptically at the periphery or at a preterminal zone, which predetermines their functions. Metabotropic Glu receptors are localized in all behaviorally relevant brain structures, such as the hippocampus, striatum, amygdala, cerebellum, and cortex.

Metabotropic Glu-Rs regulate the phosphorylation of various kinases, ion channels (for rev. see Ozawa et al., 1998) and receptors, and activate several transcription factors. They contribute to delayed postsynaptic responses and to synaptic plasticity. In some cases, mGlu-Rs stimulation may be sufficient to induce LTP in the hippocampal CA1 (Bortolotto and Collingridge, 1993). On the postsynaptic site at the MF synapse, activation of mGlu-Rs increases cytosolic Ca^{2+} in the CA3 pyramidal cells (Yeckel et al., 1999) and may be necessary for the induction of at least one form of LTP observed at this synapse (Ito and Sugiyama, 1991; Yeckel et al., 1999). However, it should be noted that mGlu-R subtypes have different roles in signal transduction.

Role of Glutamate in Neurological and Neurodegenerative Diseases

In addition to its transmitter function, glutamate is an amino acid and a key molecule in the cell metabolism. On the other hand, high extracellular concentrations of glutamate are toxic to most cells and account for cell loss in conditions such as ischemia, epileptic seizures, schizophrenia, hypoglycemia, AD (Greenamyre et al., 1988; Parsons et al., 1998) and PD (Starr, 1995), amyotrophic lateral sclerosis, anxiety, neuropathic pain and posttraumatic stress disorder.

Furthermore, disruption of glutamatergic neurotransmission may partially account for learning and memory deficits associated with some of these conditions. NMDA-R antagonists have been used to attempt to slow down excitotoxic neurodegeneration in AD. Memantine, an NMDA-R channel blocker, has shown safety and efficacy in slowing the decline in moderate to advanced AD (Danysz and Parsons, 1998; Seow and Gauthier, 2007). The effect of memantine has been attributed to mimicking the voltage-dependent Mg^{2+} blockade of the NMDA-R, which is makes the opening of NMDA-Rs less sensitive to background noise by excessive glutamate release (Parsons et al., 2007).

The excitotoxic effect is related to the massive entry of Ca^{2+} into the cell as a consequence of the sustained activation of glutamate receptors, what leads to multiple cytotoxic neuronal damages. It is generally believed that the most important mechanism mediating the toxic influx of Ca^{2+} into neurons is the NMDA-Rs.

2.2.2. *Modifications of Synaptic Plasticity: Short-Term Modifications. Long-Term Modifications*

There are two main types of stimulation patterns to induce short-term plasticity in hippocampal synapses: paired-pulse paradigms (paired-pulse facilitation vs. inhibition) and stimulus train paradigms (post-tetanic potentiation vs. inhibition and frequency facilitation).

Short-Term Modifications of Synaptic Plasticity

Paired-pulse facilitation (PPF) is a form of short-term synaptic plasticity, which describes the ability of synapses to increase neurotransmitter release on very closely spaced stimuli. When two single stimulus pulses are applied with inter-pulse intervals (IPIs) ranging from 20 to 300 ms, the second fEPSP produced is larger than the first one. Facilitation can be attributed to the transient increase in the concentration of presynaptic intraterminal Ca^{2+} (Zucker and Regehr, 2002) produced by an invading action potential. The concentration declines to basal values over a few hundred milliseconds, but the Ca^{2+} influx at the time of 2nd stimulus adds to the residual Ca^{2+} from the 1st, resulting in an enhanced Ca^{2+} concentration and increased probability of release (Wu and Saggau, 1994). It reflects the fact that at many synapses an individual action potential has a greater chance of evoking release of neurotransmitter when it arrives within a few milliseconds of a preceding action potential. Thus a doublet of action potentials at a short interval has an increased probability to activate the post-synaptic cell. Paired-pulse ratio (PPR; measured as ratio of 2nd response vs. 1st one) is considered a measure of presynaptic release mechanisms (Zucker and Regehr, 2002) and reflects the increase in the probability of neurotransmitter release. The higher the probability of

release, the smaller is the observed PPR (McNaughton, 1982). It represents a means for analysis of the excitation-inhibition balance in neuronal networks (Marder and Buonomano, 2003). Among hippocampal subregions, PPF is very robust at the MF-CA3 synapses. At room temperature, it is about two times larger in amplitude but has a similar time-course as associational/commissural (A/C) synapses (Salin et al., 1996).

If the synaptic transmission is decreased on the 2nd pulse, this phenomenon is called paired-pulse depression (PPD). PPD is elicited with inter-stimulus intervals (ISIs) of 200-2000 ms, and may reflect activation of $GABA_B$ receptors (Rausche et al., 1989).

A typical example of the second form of short-term plasticity is post-tetanic potentiation (PTP), a large enhancement of synaptic efficacy observed after brief periods of high-frequency synaptic activity. The concentration of mitochondrial (Tang and Zucker, 1997) Ca^{2+} in internal presynaptic boutons rises during PTP, suggesting that PTP is a presynaptic process (Tang and Zucker, 1997; Wu and Saggau, 1994). For example, in experiments where LTP is induced with one or two 1-s 100-Hz tetani, a large and transient increase in synaptic efficacy is produced immediately after high frequency stimulation (HFS). PTP decay in SC synapses is less than 1 min, while in MF synapses it decays within 3 min (Zalutsky and Nicoll, 1990). At MF synapses, both *in vitro* (Langdon et al., 1995) and in anaesthetized rats (Derrick and Martinez, 1994), PTP can reach several hundred percent by long tetani.

One special form of short-term synaptic plasticity is frequency-dependent facilitation (FF), which manifests at the hippocampal mossy fibers (MF) synapses and is one of the criteria to identify the MF responses (Wang et al., 2002; Yeckel and Berger, 1998). FF results from Ca^{2+} channel activation and the growing effects of intracellular Ca^{2+} on release (Zucker, 1989). It is mediated, at least in part, by the long-lasting activation of kainate receptors (Schmitz et al., 2001) by synaptically released glutamate (Lerma, 2006). There are indications that FF shows the recruitment of the neurotransmitter storage pool when the readily releasable pool is depleted after the stimulation. While MF synapses show FF at ISIs longer than 40 ms, in A/C synapses it can be seen with ISIs less than 10 ms (Salin et al., 1996). Moreover, the maximal FF for A/C synapses is only 125 % of control, whereas at the MF synapses it can reach up to 600 % (Salin et al., 1996).

Long-Term Modifications of Synaptic Plasticity

LTP is a sustained enhancement of synaptic response to a rapid, brief sequence of excitatory pulses, lasting from hours to several days or even months after the stimulation (Bliss and Lomo, 1973). Within the hippocampal formation, morphological changes, such as modifications in

existing synapses (Desmond and Levy, 1983), addition of newly formed synapses (Chang and Greenough, 1984; Chang et al., 1991) and synaptogenesis (for review see (Wheal et al., 1998)) have been reported after LTP, which support the idea that LTP is relevant for learning and memory formation.

The level of the increase in intracellular Ca^{2+} is critical parameter in determining the direction of changes in synaptic strength. It has been suggested that low elevation of Ca^{2+} activates protein phosphatases to yield LTD, whereas higher concentration activates protein kinases to yield LTP (Lisman, 1989).

The intensity of a stimulus is a combination of the frequency and number of stimulus pulses and the amount of current generated during each stimulus pulse. Higher-frequency stimulation (within the range of frequencies to which the postsynaptic cell can respond) elicits a larger postsynaptic depolarization by increasing temporal summation of postsynaptic potentials. Long-duration stimulation increases the total time when postsynaptic membrane is depolarized, which allows a larger Ca^{2+} influx. Stronger current elicits a larger postsynaptic depolarization by increasing the number of input axons activated simultaneously. All three variables often differ from one experiment to another.

LTP can be induced by several stimulation patterns, which largely determine the properties of resulting LTP. The most common one is a train of 100 Hz for 1 s, called high-frequency stimulation (HFS) protocol. Another widely used protocol is theta-burst stimulation (TBS), in which a common feature is an interval of 200 ms between brief stimulus trains (Rose and Dunwiddie, 1986). It is known that 200 ms is close to the periodicity of the theta rhythm (4-8 Hz) recorded in rats during behavioral activity (Buzsaki, 2002). It is assumed that a prime burst activates GABA interneurons through feedforward connections. Importantly, GABA release activates $GABA_B$ autoreceptors, which produce a transient reduction in GABA release that is maximal at around 100 to 200 ms. Thus the second train consequently enhances the voltage-dependent NMDA receptor-mediated current. A study comparing the TBS and HFS (Hernandez et al., 2005) concluded that the magnitude of LTP is highly dependent on the number of stimuli in a train rather than the pattern of the stimulation itself. Significant differences have been also reported between TBS and HFS in the early phase of LTP, with a high number of pulses (200 and 300) producing greater LTP using TBS (Hernandez et al., 2005). Apparently these two protocols engage different biochemical pathways to produce differences in LTP magnitude and time course kinetics, especially when using stronger induction parameters (Patterson et al., 2001).

In contrast to LTP, long-term depression (LTD) can be achieved using low-frequency stimulation (typically 900 pulses at 1 Hz). There are two distinct types of LTD in CA1: NMDA-R

dependent LTD requires small prolonged rise in postsynaptic Ca^{2+}, while mGlu receptor-dependent LTD is based on both pre- and postsynaptic mechanisms.

The specific type of LTP exhibited between neurons depends on a number of factors, such as the anatomical location where LTP is observed, the age of the organism, and differences in signaling pathways expressed by a cell. For instance, LTP in the SC path is very different from the LTP at the MF path, which is independent of NMDA-R activation and is usually induced by different stimulation protocols. Furthermore, the molecular mechanisms of LTP in the immature hippocampus differ from those mechanisms that underlie LTP in adults (for details see 2.3). Further, some types of hippocampal LTP depend on the NMDA-Rs, while others depend on the mGlu-Rs and voltage-dependent Ca^{2+} channels.

LTP in Schaffer Collateral and Perforant Pathways

NMDA-dependent LTP classically exhibits four main properties: (1) *rapid induction* (by one or more brief tetani to a presynaptic cell), (2) *cooperativity* (induction either by a strong tetanus to a single pathway or by a weaker stimulation to many), (3) *associativity* (when a weak stimulation of a single pathway is insufficient for the induction of LTP, a simultaneous strong stimulation of another pathway will induce LTP at both pathways), and (4) *input-specificity* (once induced at one synapse, LTP does not arbitrarily propagate to an adjacent synapse).

The biochemical model of LTP (Baudry, 1991) consists of four phases: (1) the *induction phase*, which leads to the activation of postsynaptic NMDA-Rs and influx of Ca^{2+} in the postsynaptic cell; (2) the *development phase*, which consists of the stimulation of a complex biochemical cascade involving proteases, lipases, and kinases; (3) the *expression phase*, which is associated with presynaptic membrane modification, changes in configuration of the AMPA-Rs, and changes in the shape of dendritic spines and increase of neurotransmitter release; and (4) the *maintenance* or *anchoring phase*, which represents the stabilization of the new configuration and involves cell-adhesion molecules and integrin proteins.

At the synapses of two major hippocampal pathways, PP-DG and SC-CA1, the induction of LTP obeys the Hebbian rule. In other words, it requires a temporal conjunction of *presynaptic* (release of Glu to activate NMDA-R channel; (Bliss and Lynch, 1988) and *postsynaptic* (depolarization of NMDA-Rs to free the channel from block by extracellular magnesium (Mg^{2+}); (Collingridge and Lester, 1989) activity for subsequent changes in synaptic strength. NMDA-Rs allow Ca^{2+} influx to postsynapse and increase its concentration in postsynaptic spines, and that rise

of Ca^{2+} is necessary for LTP. This association of activity in several afferent axons simultaneously may form a biological basis for memory formation (Henze et al., 2000).

The expression of LTP is often divided into two phases (Squire, 1992): an *early*, protein synthesis-independent phase (*E-LTP*, lasts 2 - 5 h), and a *late*, protein synthesis-dependent phase (*L-LTP*, lasts at least 8 h up to days / months). The very first stage of LTP - initial LTP (*I-LTP*, lasts about 30 minutes), generally referred to as short-term potentiation, is independent of protein kinase activity for its induction. It is a form of NMDA-R dependent synaptic plasticity that is induced by tetanic stimulation, and is a prelude for E-LTP and L-LTP (Roberson et al., 1996). E-LTP depends on the activity of several protein kinases, including CaMKII, PKC, PKA, MAPK, and tyrosine kinases, which provide a *feed-forward mechanism* to increase receptor numbers, receptor trafficking, levels of scaffolding and cytoskeleton proteins that promote surface expression, lateral diffusion and stabilization of AMPA-Rs at potentiated synapses. PKC and CaMKII may also play a role as molecular information storages; autonomously active forms of these kinases subserve the maintenance of E-LTP. In contrast, PKA and MAPK appear to be involved in triggering the induction of E- and L-LTP by increasing of presynaptic Ca^{2+} level, mediating of Ca^{2+} storage, or rising intracellular Ca^{2+} level. L-LTP requires gene transcription and protein synthesis, which leads to prolonged structural changes in the synapse enhancing its strength (Poser and Storm, 2001).

Earlier studies have suggested that in the CA1 region, voltage-dependent Ca^{2+} channels (VDCC) may also be involved in the LTP induction (Grover and Teyler, 1990; Regehr et al., 1989; Regehr and Tank, 1990; Westenbroek et al., 1990), particularly in response to multiple trains of HFS. Furthermore, potassium (K^+) channels are important in the modulation cellular excitability by controlling the repolarization of the membrane after a single or a burst of action potentials. A blockade of repolarizing K^+ channels leads to simultaneous activation of both pre- and postsynapse by increasing the transmitter release. In addition, it increases the amplitude and the duration of the postsynaptic depolarization, facilitates Ca^{2+} entry.

LTP at the Mossy Fibers

Another major hippocampal synapse, the MF-CA3 synapse, expresses LTP which is independent of activation of NMDA-Rs or postsynaptic Ca^{2+} channels (Zalutsky and Nicoll, 1990), but rather depends on presynaptic kainate (Bortolotto et al., 1999; Harris and Cotman, 1986), mGlu-Rs (Conquet et al., 1994), and voltage-dependent calcium channels (Jaffe and Johnston, 1990; Zalutsky and Nicoll, 1990). In many studies, the induction of MF LTP has been shown to be non-associative, non-cooperative, and non-Hebbian (Zalutsky and Nicoll, 1992). However, this form of

LTP may prove to be important in modulating the induction of Hebbian LTP at other synapses (such as DG-A/C synapses; (Derrick and Martinez, 1994; Kobayashi and Poo, 2004).

Studies on MF LTP are complicated by the difficulty of obtaining a pure MF activation by extracellular electrical stimulation. Electrical stimulation of the DG or *stratum lucidum* can lead to activation (and contamination of the responses) of three different synaptic inputs to CA3 pyramidal cells (Claiborne et al., 1993). First, strong stimulation of the DG, hilus, or *stratum lucidum* leads to firing of CA3 pyramidal cells via MF pathway. Second, synaptic responses evoked by DG, hilus or *stratum lucidum* stimulation stems from antidromic activation of associational collaterals of CA3 pyramidal cells projecting to the dentate hilus (Li et al., 1994; Scharfman, 1994). Propagation of action potentials via these CA3 axon collaterals may then evoke a monosynaptic non-MF synaptic response in CA3 pyramidal cells (Henze et al., 2000). This possible contamination of DG-evoked MF responses has been suggested and been functionally demonstrated several times before (Claiborne et al., 1993). Third, bulk stimulation of the DG or hilus often results in activation of the hilar collaterals of MF axons. When an action potential then travels antidromically to the main MF axon, it is conducted orthodromically into CA3 in a so-called anti-orthodromic sequence (Claiborne et al., 1993; Henze et al., 2000; Nicoll and Schmitz, 2005). Several special measures have been suggested that should allow one to separate these inputs and record a 'pure' MF response in are CA3.

Depending on the specific pattern of high-frequency stimulation (HFS), LTP at the MF synapse can be both, non-Hebbian or Hebbian (Urban and Barrionuevo, 1996). Specifically, long-lasting tetanus (L-HFS, three 1-s, 100-Hz trains presented at 0.1 Hz) induces LTP at the MF depending on the level of postsynaptic hyperpolarization (Katsuki et al., 1991; Langdon et al., 1995), and requires postsynaptic increase of Ca^{2+} (Yeckel et al., 1999) through VDCC (Jaffe and Johnston, 1990; Kapur et al., 1998; Yeckel et al., 1999). Specifically, the activation of mGlu-Rs during L-HFS is sufficient to cause release of Ca^{2+} from intracellular stores. In contrast, a brief tetanus (B-HFS, eight 0.1-s, 100-Hz trains presented at 0.2 Hz) induces LTP at the MF depending on depolarization of the postsynaptic CA3 cell and the activity of the presynapse. The difference between Hebbian and non-Hebbian induction protocols arises from differences in the source of Ca^{2+} elevation in the postsynaptic cell. Both L-HFS and B-HFS induced LTP at the MF depends on cAMP-dependent signaling cascades. It has been reported that L-HFS induced MF LTP is caused by presynaptic Ca^{2+} influx which triggers a cAMP cascade leading to long-term changes in neurotransmitter release (Huang et al., 1994; Weisskopf et al., 1994). In contrast, the B-HFS induced MF LTP depends on postsynaptic cAMP cascade activated by increase of postsynaptic Ca^{2+} (Kapur et al., 1998; Yeckel et al., 1999). This leads to the generation of a retrograde messenger

arachidonic acid which, in turn, activates a presynaptic PKC and cAMP cascade (Collingridge and Lester, 1989; Son et al., 1996; Son et al., 1997). In addition, the maintenance of E-LTP at the MF depends on the integrity of communication between DG granule cell somata and the MF buttons (Calixto et al., 2003; Lonart, 2002). E-LTP involves an enhancement of transmitter release which may be under control of a synaptic active zone protein, RIM1 (Lonart and Sudhof, 1998; Villacres et al., 1998).

There are controversial opinions as to whether LTP at the MF depends on protein synthesis in the postsynapse. Some previous studies have reported that the E-LTP in the MF (Huang et al., 1994; Huang and Kandel, 1996) and the in the SC (Frey et al., 1993; Huang et al., 1996) is independent of protein synthesis. In contrast, other studies indicate that the E-LTP in area CA1 (Scharf et al., 2002), in DG (Otani and Abraham, 1989; Otani et al., 1989), and at the MF synapse (Barea-Rodriguez et al., 2000) is protein synthesis and RNA synthesis dependent (Calixto et al., 2003), because the blockade of protein (by protein synthesis inhibitors emetine or cycloheximide) or of RNA synthesis selectively inhibits the early maintenance phase of MF LTP without affecting the induction process (Calixto et al., 2003).

Relevance of LTP to Spatial Learning and Memory

It is well established that the integrity hippocampal formation is crucial for certain forms of memory (Jarrard, 1993; Squire, 1992). Animal studies reveal that hippocampal lesions (surgical or pharmacological) result in learning difficulties or impaired spatial memory (Morris et al., 1986; Pastalkova et al., 2006). Collective data from human and animal studies provide correlative evidence that episodic-like learning and memory involves hippocampal activity (Neves et al., 2008). However, despite accumulating knowledge from intensive research over the past 30 years the *precise* neural mechanisms of such hippocampal involvement are still unclear. Synaptic plasticity in the hippocampus remains a major experimental system for those studies. It is widely believed that learning induces long-lasting changes in the synaptic connections of central glutamatergic synapses (Dragoi et al., 2003; Gruart et al., 2006; Whitlock et al., 2006). These alterations include strengthening and weakening of synapses, changes in postsynaptic transduction processes, changes in the distribution of receptor proteins, and changes in the morphology of synapses. A common prediction is that memory can be encoded by these changes. Even today, LTP is still the most attractive electrophysiological cellular correlate of memory formation. However, a causal link between these two is still far from being proven (Neves et al., 2008).

The probable link between synaptic plasticity and memory has been formulated as the synaptic plasticity and memory hypothesis: *Activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed* (Martin et al., 2000). The necessity and sufficiency of synaptic plasticity for memory is also discussed in a recent review by Neves and colleagues (Neves et al., 2008). There are number of experimental strategies that have been used to assess the hypothesis (Martin et al., 2000). First, the behavioral parameters of learning should correlate with some properties of synaptic plasticity (Morris et al., 1986; Pastalkova et al., 2006; Shimizu et al., 2000). However, despite compelling evidence it is difficult to exclude the possibility that effects on processes unrelated to the maintenance of LTP cause the learning impairment (Neves et al., 2008). Second, learning should be associated with the induction of measurable changes in synaptic efficiency at synapses (Whitlock et al., 2006). Third, saturation of synaptic plasticity in a network should destroy the pattern of trace strengths corresponding to established memories and occlude new memory encoding. Despite positive findings, scepticism remains about the additional changes in hippocampal and extrahippocampal circuitry that disrupt learning rather than LTP saturation per se (Martin et al., 2000; Moser and Moser, 1999). Fourth, blockade or enhancement of synaptic plasticity, achieved by pharmacological, genetic or other manipulations, should have commensurate effects on learning or memory. For example, the NMDA-R antagonist AP-V blocks hippocampal LTP *in vivo* and impairs spatial learning. In addition, APV impairs spatial memory and hippocampal LTP *in vivo* at similar concentrations which also block the induction of LTP *in vitro* (Davis et al., 1992). Other hippocampal-dependent tasks are also impaired by NMDA antagonists (for rev. see Shapiro and O'Connor, 1992). However, NMDA antagonists do not block memory consolidation, i. e. do not affect performance once learning has occurred (Bannerman et al., 1995; Watanabe et al., 1992) or if LTP has been established (Collingridge et al., 1983). Fifth, erasure of synaptic plasticity should, at least shortly after learning, induce forgetting. However, protocols for inducing depotentiation (as in case of saturation) remain elusive (Martin et al., 2000). In conclusion, despite the abundance of supporting data, definitive evidence that LTP is necessary and sufficient for hippocampal-dependent learning is still lacking (Martin et al., 2000; Neves et al., 2008).

2.2.3. *Hippocampal Plasticity and Normal Aging*

Morphological Age-Related Changes

Previous studies have reported that the medial temporal lobe, which includes the hippocampus, a critical area for memory formation, is especially sensitive to the effects of aging. In general, the total number of DG granule cells and pyramidal neurons in CA3 and CA1 remains stable in aged mice (Calhoun et al., 1998), rats (Rapp and Gallagher, 1996; Rasmussen et al., 1996), monkeys (Peters et al., 1996), and humans (West, 1993). Animal studies have confirmed also that there is no regression of dendrites with age (for excellent review see Burke and Barnes, 2006). In particular, in rats there is no significant change in dendritic length in hippocampal granule cells and branching extent in CA1 between young (3 months), middle-aged (12–20 months) and aged (27–30 months) rats. In addition, there is no significant reduction in spine density in DG or CA1 in aged rats when compared with young. Similar to the investigations on dendritic branching during aging, the data on spine density suggest that age-associated alterations are also region-specific (for review see (Burke and Barnes, 2006)). In contract, studies of synaptic density suggest that hippocampal connectivity is more susceptible to aging (Geinisman et al., 1995). However, synapse loss is highly specific, affecting only the cortical inputs to the hippocampus, in particular perforant path inputs from layer II of the EC to DG granule cells and CA3 pyramidal cells. Furthermore, loss of perforant path input correlates with spatial memory impairment of aged rats (Smith et al., 2000). In contrast, layer III EC inputs to CA1 pyramidal cells and synapses onto CA3 cells from their own recurrent collaterals are not reduced in number during aging (Smith et al., 2000). Also, the total number of SC–CA1 synapses remains the same across different age groups (Geinisman et al., 2004).

The morphological changes are accompanied by changes in neurotransmission. Neurochemical studies have shown age-related changes in cholinergic, catecholaminergic and other neurotransmitters systems, including the glutamatergic system. The latter include loss of high affinity glutamate transporters in glutamatergic terminals and decreased density of glutamatergic receptors (for excellent rev. see Segovia et al., 2001). In particular, decreased NMDA-R density has been described in most of the cortical areas, striatum and hippocampus in rats and mice. Changes in the number of AMPA-Rs are more controversial: a reduced number of cortical AMPA-Rs have been reported in the mouse but not in the rat (for rev. see Segovia et al., 2001). In the hippocampus, different age-dependent changes may also be region-specific. For example, decreased AMPA receptor density has been described in area CA1 (for rev. see Segovia et al., 2001). In some studies, the decrease of NMDA and AMPA receptors in the hippocampus also significantly correlates with

age-related declines in learning (Magnusson, 1998). Regarding the kainate receptor, there have been reports of both, decreases or no changes in the density of kainate binding with age in the cerebral cortex and hippocampus of the rat. In contrast, the number of mGlu-Rs seems to be constant during aging (Simonyi et al., 2005). In addition, there are reports on increased density of L-type Ca^{2+} channels in the aged hippocampus that might lead to disruptions in Ca^{2+} homeostasis, contributing to the plasticity deficits that occur during aging (Toescu et al., 2004).

Electrophysiological Findings of Age-Related Changes

Most physical properties of hippocampal neurons do not change with age, including resting membrane potential, input resistance, amplitude of the action potential, membrane time constant, and fEPSP rise time and half-width (for rev. see (Rosenzweig and Barnes, 2003)). There is evidence though that aged hippocampal CA1 pyramidal cells are less excitable, i.e, they are further from action potential threshold than are young neurons (*in vitro*; Moyer et al., 1992). However, when pyramidal neurons are recorded *in vivo* in behaving rats, there is no difference in the firing rates of CA1 pyramidal neurons between young and aged animals (Tanila et al., 1997; Wilson et al., 2005), and the firing rates of CA3 pyramidal neurons are actually slightly higher in aged than in young rats (Wilson et al., 2005).

Loss of functional synapses in CA1 correlates with reduced amplitude of the fEPSP recorded in the same area in aged rats compared with young ones (Deupree et al., 1993). However, there is neither a decrease in the fEPSP amplitude in SC (Barnes, 2001) nor a reduction in the number of functional synapses in the SC fibers in aged animals. In accordance with the morphological finding of loss of perforant path synapses in aged DG, there is evidence for reduced amplitude of fEPSP (Barnes, 1979) and presynaptic fiber potential (Barnes, 2001) in response to perforant path stimulation.

The effects of changed neuronal morphology, biophysical properties, synaptic connections and plasticity can be assessed by measuring age-associated alterations in LTP. In general, aged rats have some deficits in LTP induction and maintenance which are complex and depend on the pathway under investigation and experimental protocol (for rev. see (Burke and Barnes, 2006))). In particular, when chronically implanted animals have been used to examine the effects of aging on LTP, recordings in DG have revealed that aged (10-16 months) rats reach the maximal potentiation of fEPSPs more gradually but to eventually to the same extent as young ones. However, the potentiation is lost more rapidly in the aged animals (Barnes, 1979). Changes in the molecular mechanisms of LTP are unlike to change radically during normal aging: LTP induction, for

example, still requires the activation of NMDA-Rs (Barnes et al., 1996). However, age-related alterations in LTP could contribute to a decline in cognitive function. In aged rats LTP decays faster in PP-granular cell (Barnes, 1979) and in PP-CA3 synapses (Dieguez and Barea-Rodriguez, 2004), and this faster decay correlates with the rate of forgetting. At CA1 synapses, there is an age-related reduction in the magnitude of LTP, which may derive from lower depolarization during induction, lower activation of NMDA-Rs (Deupree et al., 1991; Moore et al., 1993) and age-related alterations in Ca^{2+} signaling.

Similarly to the PP-DG synapses, potentiation in the SC-CA1 fEPSP is weaker in aged (15-25 months) rats compared to young (2-3 months) ones for the same stimulus intensity (Barnes et al., 1992; Deupree et al., 1993). However, the presynaptic fiber volley and the ratio of the fEPSP amplitude to fiber volley amplitude do not differ between the age groups. Although the development of maximal LTP in CA1 is delayed in aged rats (older than 1 year) compared to young ones (3-4 months), the extent of potentiation level remains the same in both age groups (Landfield, 1988). In one *in vitro* study on aged (26-27 months) rats, CA1 LTP was elicited by HFS of associational fibers (Chang et al., 1991), and the slices were examined morphologically 30 min after LTP induction. Control slices taken from the same animal were stimulated at a low frequency that does not evoke LTP. The general pattern of LTP-induced structural synaptic changes and the enhancement of the PS amplitude in CA1 pyramidal cells were similar in young adult (3-4 months old) and aged animal.

Although age-related reduction of fEPSP has been reported in both DG and in CA1, aged animals can show intact LTP induction in PP–granule cell synapses, in CA3–CA1 SC synapses and in PP–CA3 pyramidal cell synapses when robust HFS is used (for rev see Burke and Barnes, 2006). However, when supra-threshold stimulation parameters are used, aged rats have a deficit in the maintenance of LTP in both DG and CA3. When peri-threshold stimulation parameters are used, LTP induction deficits can be observed in both DG and CA1. When weak presynaptic stimulation is combined with direct depolarization of the granule cell in DG, a larger current injection is required to elicit LTP in PP–granule cell synapse of aged rats than in young ones. This indicates that aged DG granule cells have an increased threshold for LTP induction (for rev see Burke and Barnes, 2006).

In summary, plasticity changes during the normal aging process result from region-specific changes in dendritic morphology, cellular connectivity, Ca^{2+} dysregulation and other factors, that may alter the network dynamics that supports cognition.

2.2.4. *Synaptic Plasticity in Age-Related Neurodegenerative Conditions. Alzheimer's Disease. Parkinson's Disease*

The hippocampus is among the most vulnerable brain structures for age-related degeneration. Below is a brief summary of observed functional and morphological changes in the hippocampus associated with most common neurodegenerative conditions.

Alzheimer's Disease

Alzheimer's disease (AD) is a major cause of disability and mortality in developed countries, where the percentage of elderly individuals in the population grows all the time. Memory loss, the most prominent symptom of Alzheimer disease (AD), is linked with changes in synaptic plasticity. The etiology of AD is still not fully understood. Deposition of neurotoxic β-amyloid (Aβ) protein aggregates (Selkoe, 2001) or plaques and the formation of neurofibrillary tangles are typical neuropathological hallmarks of the disease.

The most widely accepted theory about AD pathophysiology is "amyloid cascade hypothesis", which is based on the findings that all known mutations linked with familial, early-onset AD, including amyloid precursor protein (APP) and presenilin 1 or 2 (PS1, PS2) mutations, lead to increased production and aggregation of Aβ (Selkoe, 1997). However, the mechanism by which Aβ accumulation leads to memory impairment is still unknown, although accumulating evidence indicates that abnormal signaling via p38 MAPK and JNK underlies Aβ induced deficits in synaptic plasticity and memory (Rowan et al., 2004). Transgenic (TG) mice have provided a powerful model to study the molecular and synaptic pathology of AD and its relationship to memory loss. TG mice carrying APP mutation alone or combined with PS1 mutation show an age-dependent increase in Aβ levels (Kawarabayashi et al., 2001) and in many cases also age-dependent memory impairment (Chen et al., 2000; Koistinaho et al., 2001; Puolivali et al., 2002; Westerman et al., 2002).

Two strategies are used to assess mechanisms underlying cognitive decline in mouse models of AD. Clinically, AD is characterized by an early loss of declarative memory. So it is not surprising that all APP TG models have been screened in the water maze task (hippocampal-dependent measurement of spatial learning and memory). Deficits in hidden-platform-MWM performance in animals may be related to memory loss in human AD patients. The majority of these studies have reported impaired memory in this navigation task (Duyckaerts et al., 2008). Impairment in spatial learning tasks is consistent with the fact that amyloid pathology is most

severe in the hippocampus in all these mouse lines (Arendash et al., 2001; Borchelt et al., 1997; Dodart et al., 2000; Hsiao et al., 1996). However, some reports have failed to demonstrate significant learning and retention deficits in the water maze task (Arendash et al., 2001; Holcomb et al., 1999). The reasons for such discrepancies are still unclear.

Memories are thought to be kept in the brain as a change in the strength of synapses. Therefore, the electrophysiological studies in AD animal models have focused on the measurement of synaptic strength among hippocampal cells. Both basal synaptic transmission and LTP have been studied, however, the results have been controversial. While some studies reported a reduction of LTP in TG mice with Aβ accumulation (Chapman et al., 1999; Moechars et al., 1999; Nalbantoglu et al., 1997; Trinchese et al., 2004), others were not able to report similar findings (Fitzjohn et al., 2001; Hsia et al., 1999; Jolas et al., 2002; Larson et al., 1999). A possible explanation for these conflicting results could be that handling, trafficking, and signaling properties of APP are likely different between full-length APP and its natural cleavage products, which would impair different aspects of synaptic function. Also different background strains are likely to contribute to these observed differences among APPswe mice.

Estrogen in Neuronal Plasticity, Learning and Memory

Estrogen plays a critical role in the development, maintenance, and physiology of male and female reproductive tissues, as well as of nonreproductive systems, including the cardiovascular, skeletal, and CNS (Gruber et al., 2002). Epidemiological studies indicate that estrogen deprivation due to menopause is a risk factor in both the initiation and progression of AD, and that estrogen replacement therapy (ERT) may be protective. However, the recent Women's Health Initiative Memory Study found that hormone therapy (HT) increased rather than decreased the risk of dementia in women after the age of 64 (Shumaker et al., 2003). Many observational studies, however, report numerous beneficial actions in brain (Behl et al., 2000; Petanceska et al., 2000; Wise, 2002; Woolley and McEwen, 1994). In order to resolve these contradictory observations few explanations have been suggested (Rosario et al., 2006). Composition of HT and delivery of hormone components are the most problematic factors. However, skepticism remains as evidence on hormone therapy is insufficient to guide practice decisions (Henderson, 2007).

The classic mechanism of estrogen action is through one of two estrogen receptors (ERs). Estrogen receptor alpha (ERα) and beta (ERβ) are members of the steroid nuclear receptor family that modulate gene transcription in an estrogen-dependent manner. ER mRNA and protein have been detected both peripherally and in the CNS in both genders (Kuiper et al., 1998; Mitra et al.,

2003). Distribution of ER receptors was studied in a great detail in the mouse brain (Mitra et al., 2003). ERβ is primarily localized to cell nuclei within multiple regions of the brain, including the olfactory bulb, cerebral cortex, septum, preoptic area, bed nucleus of the stria terminalis, amygdala, paraventricular hypothalamic nucleus, thalamus, ventral tegmental area, substantia nigra, dorsal raphe, locus coeruleus, and cerebellum. ERβ extranuclear localization has been observed in the olfactory bulb, CA3 stratum lucidum, and CA1 stratum radiatum of the hippocampus and cerebellum. Although nuclear ERα is generally expressed in a similar manner throughout the brain, it is the predominant subtype in the hippocampus, preoptic area, and most of the hypothalamus, whereas it is sparse or absent in the cerebral cortex and cerebellum (Mitra et al., 2003).

Estrogen has been shown to acutely modify the intrinsic excitability of neurons in diverse brain regions, including the hypothalamus/preoptic area, amygdala, striatum, cerebellum, and hippocampus (for rev. see Woolley, 2007). When estradiol is applied either *in vivo* or *in vitro* it induces rapid alterations in neuronal firing rates and/or modulation of K^+ currents that control the resting membrane potential and limit action potentials (Woolley, 2007). It has been suggested that one of the proximal effects of estradiol is to suppress Ca^{2+} influx through L-type Ca^{2+} channels (Kumar and Foster, 2002). Besides its effects on membrane excitability, 17β-estradiol also modulates synaptic physiology in the hippocampus. This was first suggested almost three decades ago (Teyler et al., 1980). Subsequent studies confirmed 17β-estradiol effect on neuronal excitability both *in vitro* (Bi et al., 2000; Foy et al., 1999) and *in vivo* (Cordoba Montoya and Carrer, 1997; Wong and Moss, 1992). Namely, it increases amplitude of PS and fEPSP in all major subfields of the hippocampus (CA1, CA3 and DG; Woolley, 2007). Interestingly, only a subset of hippocampal cells is responsive to estradiol and the proportion of responsive cells increases when animals are pretreated with estradiol for several days (Rudick and Woolley, 2003; Woolley, 2007). In addition to its effects on baseline synaptic responses, estradiol can acutely increase the capacity for synaptic plasticity in CA1 (Barraclough et al., 1999; Cordoba Montoya and Carrer, 1997; Good et al., 1999). Also, other forms of hippocampal neuronal plasticity are positively affected by estrogen: filopodial outgrowth (Brinton, 1994), dendritic spines maturation (Harris, 1999), increase of synaptic density (Gould et al., 1990; Woolley and McEwen, 1992; Woolley and McEwen, 1993). Together, these data illustrate that estradiol activation of intracellular signaling pathways in hippocampal neurons can enhance neuronal excitability, glutamatergic synaptic transmission and synaptic plasticity.

It is now well established that 17β-estradiol effect on excitatory synaptic responses in CA1 is mediated by non-NMDA-Rs (Rudick and Woolley, 2003; Wong and Moss, 1992). Furthermore, it was suggested that estradiol increases non-NMDA-R-dependent postsynaptic

responses through cAMP/PKA-mediated phosphorylation in the postsynaptic cell (Gu and Moss, 1998). However, another group reported a similar effect of 17β-estradiol on both non-NMDAR- and NMDAR-mediated EPSPs *in vitro* (Foy et al., 1999). Therefore, it is likely that estradiol potentiates both types of glutamate receptor-mediated synaptic responses through PKA-dependent mechanism Woolley, 2007), because both non-NMDA- and NMDA-Rs can be positively modulated by PKA-dependent phosphorylation (Greengard et al., 1991). Similar to basal synaptic transmission, LTP enhancement by 17β-estradiol application is NMDA- and/or AMPA-R-dependent (Foy et al., 1999). Nevertheless, accumulating evidence suggests that estrogen-induced synaptic strengthening in the hippocampus is dependent on NMDA-R activation (Bi et al., 2000; Pozzo-Miller et al., 1999; Rudick and Woolley, 2001; Woolley et al., 1997).

Reports on estrogen-induced electrophysiological and morphological changes in the rat hippocampus have been accompanied by several reports on improved learning in hippocampal-dependent tasks after estrogen treatment (Daniel et al., 1997; Fader et al., 1998; Gibbs, 1999; Luine et al., 1998). However, there are studies showing that short-term estrogen treatment has no effect on spatial memory in OVX rats (Chesler and Juraska, 2000; Luine et al., 1998) or that estrogen might impair spatial learning and memory in OVX and gonadally intact female rats (Daniel et al., 1999). On the other hand, a number of studies report improved performance in the same tasks after longer (10 days or more) estrogen treatment (Daniel et al., 1997; Gibbs, 1999; Luine et al., 1998). While benefit of prolonged estrogen treatment is far from proven, positive short-term effects on memory were reported recently (Woolley, 2007). Series of studies showed that intrahippocampally injected estradiol immediately following training in the water maze improved memory for the platform location tested 24 h later, while it was ineffective when injected after 2 h (Packard et al., 1996; Packard and Teather, 1997). In similar studies, estrogen given systemically 30 min before training or immediately post-training, but not 2 h later, improved memory in rats (Luine et al., 2003; Rhodes and Frye, 2006).

Parkinson's Disease

Parkinson's disease (PD) is another devastating age-related neurodegenerative disorder which is accompanied by cognitive and movement impairment. Its etiology remains unknown, even though human genetic studies as well as TG animal models suggest that multiple events, both genetic and environmental, interactions trigger its initiation and progression. Data from human post-mortem studies indicate that alternations in the glutamatergic system may contribute to PD development. Glutamate excitotoxicity, which changes the permeability of cells to Ca^{2+} by acting

through NMDA-Rs, might play an important role in the development of PD. Massive activation of glutamate receptors can result in excessive nitric oxide (NO) formation (Jenner, 2003), mitochondrial dysfunction (Schinder et al., 1996), and neuronal death (Mody and MacDonald, 1995). Mitochondrial dysfunction also can be the reason of the ubiquitin-proteasome system (UPS) deficit, another feature of PD. Furthermore, aggregation of β-amyloid and α-synuclein (α-syn) (Ding et al., 2006; Widmer et al., 2006) are clear signs of proteolysis in neurons because of impaired proteasome and mitochondria function (Dawson and Dawson, 2003; Song et al., 2004; Wood-Kaczmar et al., 2006). It leads to neuroinflammation, DNA and RNA brakeage, and disturbance of other signaling processes, such as dopamine misfolding and degeneration, aggregation of misfolded protein parkin, which triggers the oxidative stress response and promotes neuronal death (Imai et al., 2002; Takahashi and Imai, 2003). It has also been suggested that PD might be triggered by disorganization of neuronal cytoskeleton structures (intrafilaments, synaptic vesicles and their ultrastructures; (Iseki et al., 1998)), which results in formation of Lewy bodies (LBs) in living neurons in certain neuronal populations (Gibb and Lees, 1988; Spillantini et al., 1997).

Dementia with Lewy Bodies

Another common form of dementia in older people is dementia with Lewy bodies (DLB). Clinically DLB is characterized by the presence of progressive cognitive decline resulting in significant social and occupational functional impairment, visual hallucinations and parkinsonism (McKeith et al., 1996). However, relatively preserved memory functions are also frequently reported in DLB (for rev. see (Mukaetova-Ladinska and McKeith, 2006)). Neuropathologically, DLB is characterized by the presence of intraneuronal inclusions, Lewy bodies (LBs), found in the brain stem and in various cortical areas, and Lewy neurites (LNs), predominantly present in the CA2/3 hippocampal region (Klucken et al., 2003; Mukaetova-Ladinska and McKeith, 2006). In this context it is worth noticing that CA2/3 regions receive afferents mainly from brain stem (locus coeruleus, raphe complex), septal cholinergic nuclei and diagonal band of Broca, layer III of EC and amygdala. α-Syn is a principal component of the intraneuronal LB aggregates/filaments, glial inclusions and LNs (Mukaetova-Ladinska and McKeith, 2006). Hippocampal pathology is important in LBD as well as in PD and AD, because memory disturbance, a leading symptom of these neurodegenerative disorders, is closely related to the degeneration of PP (Hyman et al., 1986; Klucken et al., 2003).

In addition, cytoplasmic LBs also occur in sporadic and familial forms of AD, Down syndrome, and in elderly with no cognitive impairment. One to two thirds of patients with a definite diagnosis of AD has LBs in their allo- and/or neocortical areas, defining the so-called Lewy body variant of AD (Mukaetova-Ladinska and McKeith, 2006).

α-Synuclein: General Aspects

Both human and rodent synuclein family consists of four (α-, β-, γ-synucleins and synoretin) small 15-20 kDa proteins with a relatively similar amino acid sequence but encoded by different genes (Goedert, 2001). This 140 amino acid protein is abundant in presynaptic terminals in close association with different membranes, including synaptic vesicles (Gitler and Shorter, 2007; Lotharius and Brundin, 2002), which implies its role in synaptic transmission (Liu et al., 2004; Liu et al., 2007) as well as regulation of synaptic vesicle cycle. However, normal function of α-syn is still unknown. The mouse α-syn gene was mapped to the chromosome 6 (Touchman et al., 2001). The intron/exon structure of both mouse and human α-syn genes is highly conserved (Touchman et al., 2001).

It has been reported that α-syn can inhibit protein kinase C (PKC) activity (Ostrerova et al., 1999) and down-regulate extracellular signal-regulated kinase (ERK) activity (Hashimoto et al., 2003). Since ERK plays a central role in various neuronal functions such as synapse formation and neuronal growth, survival (Subramaniam et al., 2003), synaptic plasticity, and LTP (Impey et al., 1999), modulation of ERK might be an important mechanism for normal α-syn functions. Additionally, there are the reports on the important role of α-syn in neurotransmitter release through NO and cGMP-dependent protein kinase (cGK) activation (Arancio et al., 2001). In addition, CaMKII substrates play a vital role in the process of synaptic strengthening within the terminal and acts as a functional switch for many presynaptic proteins (one of them is α-syn) involved in the neurotransmitter release process (for review see Liu et al., 2007).

The Role α-Synuclein in Hippocampal Synaptic Plasticity

α-Syn became a focus of intensive research when three point mutations (Ala53Thr, Ala30Pro and E46K) in its gene became associated with rare forms of familial PD (for rev. see Liu et al., 2007). Additionally, a triplication of the α-syn gene has been shown to cause severe PD (for a review see Dauer and Przedborski, 2003).

When α-syn is expressed at physiological levels, it functions as a negative regulator of vesicle fusion and neurotransmitter release at the synapse. However, its accumulation beyond a certain threshold might lead to dysregulation of this function at the synapse or perhaps promiscuous inhibition of additional trafficking steps (Gitler and Shorter, 2007). Overexpression of α-syn might decrease the number of dopamine vesicles docked at the plasma membrane and available to release their contents, or might inhibit "priming", a reaction that transfers morphologically docked vesicles to a fusion competent state (Gitler and Shorter, 2007).

α-Syn accumulation in disease may temporarily inhibit synapses from releasing neurotransmitter in response to repetitive action potentials within a certain frequency range and could, therefore, alter the normal pattern of synaptic activity (Steidl et al., 2003). Also, synaptic release mechanisms downstream of Ca^{2+} influx may be affected by the overexpression of α-syn. Mice with inactivated α-syn gene develop normally and show no gross morphological or behavioral changes (Abeliovich et al., 2000; Cabin et al., 2002; Chen et al., 2002). Nevertheless, recordings in striatal (Abeliovich et al., 2000) and hippocampal (Cabin et al., 2002) slices have revealed slightly abnormal kinetics of neurotransmitter release in α-syn knockout mice. These mice did not show any altered LTP in CA1 by using tetanic stimulation (Abeliovich et al., 2000). These findings were conflicting in that replenishment of readily releasable pools of dopamine was enhanced in striatal recordings (Abeliovich et al., 2000), whereas it was attenuated in hippocampal recordings (Cabin et al., 2002). However, we showed that α-syn knockout and A30P mutation similarly affect release of the neurotransmitter at the glutamatergic synapses, manifested as reduced PPF and altered LTP in MF synapses (Gureviciene et al., 2007) and PPD (Steidl et al., 2003). It supports the idea that the function of α-syn is not restricted to dopaminergic synapses only.

3. AIMS

The aim of this PhD study was to evaluate the hippocampal synaptic functioning in different age-related pathological conditions mimicked by experimental mouse models. To this end, we have recorded electrophysiological parameters in these mice both *in vitro* and *in vivo*.

The specific aims were as following:

Study I

To test whether the effect of estrogen treatment on hippocampal synaptic plasticity in OVX mice is NMDA-R mediated.

Study II

To search for a correlation between hippocampal synaptic plasticity and age-related spatial memory impairment in A/P mice.

Study III

First, to investigate the functional implications of α-syn accumulation in MFs in tg mice expressing mutated A30P α-syn, and second, to assess whether α-syn changes the dynamics of synaptic glutamate release as was shown for dopaminergic neurons.

Study IV

To extend findings of **Study III** to the input side of DG granule cells and investigate the interaction between α-syn and aging, which was not possible with MF recordings.

4. MATERIAL AND METHODS

4.1. **Animals**

The strain, gender and age of the mice used in the experiments are summarized in **Table 2**.

The mice were individually housed in a controlled environment (temperature 21°C, humidity 50-60 %, lights: 7:00 –19:00). Food and water was available *ad libitum*. The studies were conducted according to guidelines set by the Council of Europe (Directive 86/609) and Finnish guidelines, and approved by the State Provincial Office of Eastern Finland.

In **Study II** we used mice carrying both human APP695swe mutation (K595N and M596L) and PS1 A246E mutation (**A/P**) or their nontransgenic littermates (**NT**). The mice were of hybrid origin (C57BL/6J x C3H) but were back-crossed to C57BL/6J for 10 generations. In **Study III** we used **KO** (a subpopulation of C57BL/6J originating from Harlan Olac® (Bicester, UK), which carries a spontaneous chromosomal deletion of the α-syn locus; **WT1** mice, another subpopulation of C57BL/6J originating from Charles River Wiga® (Sulzfeld, Germany) with no reported deletion of the α-syn locus. This line was used as the wild-type control for the α-syn knockout mice. Our **TG1** is a transgenic mouse line carrying human α-syn with A30P mutation, which was overexpressed under prion-protein promoter; and **TG2** mice are expressing human wild-type α-syn under α-syn promoter, and **WT2** are negative littermates of TG2. In **Study IV** we used mouse line carrying human α-syn with A30P mutation, which was overexpressed under prion-protein promoter, **TG,** and their negative littermates, **NT**.

Table 2. The strain, gender and age of the mice

			Age, months	Total number	Sex & Genetic background
Study I	OVX		5	110	female C57BL/6J
	OVX+ERT		12	90	
	rota-rod & open field		13	**20***	
	OVX			10	
	OVX+ERT			10	
	OVX+ERT+CPP	3.0 mg/kg		10	
	MWM		13	**90**	
	OVX			12	
	OVX+CPP	2.0 mg/kg		12	
		0.5mg/kg		12	
		5.0 mg/kg		11	
	OVX+ ERT			11	
	OVX+ERT+CPP	2.0 mg/kg		12	
		0.5 mg/kg		12	
		5.0 mg/kg		8	
	in vitro LTP		14	**44**	
	OVX			8	
	OVX+CPP	5 μM		4	
		10 μM		4	
	OVX+ERT			14	
	OVX+ERT+CPP	5 μM		9	
		10 μM		5	
				132	
Study II	***in vitro LTP***		3–4	12	male A/P
				7	male NT
	in vivo LTP		17–18	12	male A/P
				11	male NT
				42	
Study III	***in vitro LTP***		4–5	13	male KO
				16	male WT1
				15	male TG1
				16	male WT2
				13	male TG2
				73	
Study IV	***MWM & activity test***		9	9	male TG
			7	9	male NT
			25	10	male TG
			22	8	male NT
	in vivo LTP		10	7	male TG
			10	10	male NT
			24	8	male TG
			25	5	male NT
				56**	

Notes.

* In the rota-rod and open field tests (**Study I**), the mice were tested in a counterbalanced order, i. e., on the first test day, half of the mice got CPP while other half got saline; on the second test day, the treatment groups were exchanged.

** In **Study IV** 10 animals which were included in behavioral testing, were later used in electrophysiological *in vivo* studies.

4.2. **Surgical Procedures**

4.2.1. ***OVX and ERT***

The procedures were conducted under general anesthesia (pentobarbital + chloral hydrate (50/50; 40 mg/kg, i.p.). For OVX, an incision was made in the back and the ovaries were removed and the muscles and skin were stitched. The sham animals were given only the incision on the skin under anesthesia, but the ovaries were not touched.

The estrogen treatment was conducted using mini-pellets (0.18 mg of 17β-estradiol; Innovative Research of America®, Sarasota, FL, USA), releasing estradiol for 90 days. The pellets were implanted s.c. in the upper neck. The non-treated control animals were given only an incision in the skin under anesthesia. After the experiments mice were sacrificed by cervical dislocation and the uterine weights were measured.

4.2.2. ***Electrode Implantation***

Under general anesthesia (bentobarbiturate-chloralhydrate (50/50, 70 mg/kg, i.p.; **Study II**) or urethane (1.2 g/kg, i.p.; **Study IV**) the animal was placed on a stereotaxic frame (David Kopf, Tujunga, CA, USA), a recording electrode (a pair of tungsten wires, 50 µm in diameter, tip separation 1 mm; **Study II**) was implanted into the left hemisphere at A: -2.0 (from bregma), M: +1.4 (from midline). The longer electrode was aimed at the dentate hilus and the shorter (reference electrode) at the cortex. The depth coordinate was adjusted during the operation based on perforant path (PP) stimulation and monitoring of the fEPSP. The stimulating electrodes (a pair of stainless steel wires, 100 µm in diameter, tip separation 500 µm; **Study II**) were implanted into the left hemisphere at A: -3.8 (from bregma), M: -3.0 (from midline), D: -1.5 (from the dura mater surface). The electrode tips were aimed to straddle the angular bundle. The implant was fixed to the skull

with acrylic cement and three anchor screws. The mice received 0.15 mg/kg of buprenorphine postoperatively and were allowed to recover for 2 weeks before the experiment.

In **Study IV** fEPSPs from the dentate gyrus (upper blade of granular cell layer or polymorphic layer of hilus) were evoked by a bipolar stimulation electrode (a pair of silver wires, 100 µm in diameter) inserted into the right hemisphere at AP -3.8 (from bregma), ML -3.0 (from midline), DV -1.7 (from dura mater surface). It was aimed to straddle the angular bundle.

During surgery (**Study II and IV**) and *in vivo* recordings (**Study IV**) body temperature of the animal was kept stable (34^{o} C ± 0.5) using custom made heating pad and monitored throughout the experiment using the YSI Precision 4000A rectal thermometer (YSI Yellow Springs Instruments Co., Inc. Yellow Springs, Ohio, USA). The animal was given a saline injection (1 ml, s.c.) every 2 h after the onset of anesthesia.

4.2.3. ***Slice Preparation for in vitro Recordings***

Under halothane anesthesia, the mouse was decapitated and the hippocampus was dissected for LTP experiments. Acute hippocampal slices (450 µm) were prepared from the rostral hippocampus using a McIlwain tissue chopper (The Mickle Laboratory Engineering, Gomshall, UK; **Study I** & **II**), or the brain was dissected using Vibratome 1000 from "Pelco101" (Redding, CA, USA; **Study III**). Slices were moved into the chamber and were maintained at the interface between an oxygen-rich atmosphere and artificial cerebrospinal fluid (aCSF), which was perfused at a rate of 0.8 ml/min. The aCSF contained (in mM): NaCl 124, KCl 3:0, KH_2PO_4 1.25, $CaCl_2$ 3.4, $MgSO_4$ 2.5, $NaHCO_3$ 26, D-glucose 10, and L-ascorbate 2. Calcium precipitation was prevented by equilibrating the slice with 95% O_2 and 5% CO_2 and keeping it at 35 ± 1^{o} C. The slices were let incubate for at least 1 h before placing the electrode and starting the stimulation.

4.3. **Histology**

After the *in vivo* recordings (**Study II** and **IV**), the mice were deeply anesthetized, and an anodal current of 30 µA was passed through the electrodes for 3 s. Brains were fixed with 4 % paraformaldehyde, cut in coronal sections (35 µm) using a freezing, sliding microtome. In all studies, one series of sections was stained for cresyl violet to confirm the location of the electrodes.

In **Study II**, one series was stained for Aβ with human specific WO-2 antibody (Dr. T. Hartmann, Heidelberg, Germany, Mouse antihuman Aβ1–16; (Ida et al., 1996)), and one for Aβ with G 2-10 (Dr. T. Hartmann, Heidelberg, Mouse anti-Aβ40; (Ida et al., 1996)) following a 30-min

of pretreatment with a Nacitrate solution at 85° C, and the fourth series was processed for amyloid β-sheets with thioflavine S histochemistry using a standard protocol (Guntern et al., 1992).

In **Studies III-IV**, for immunohistochemistry, a separate groups of 12-month-old transgenic mice expressing human mutated A30P α-syn and 7-month-old mice expressing human wild-type α-syn, α-syn knockout (C57BL/6J-Harlan) and C57BL/6J wild-type mice were anaesthetized and transcardially perfused with 50 ml of buffered saline, followed by 100 ml of a 4 % phosphate buffered (pH 7.4) paraformaldehyde solution to which 0.5 % picric acid was added. The brains were removed from the skull and stored in the fixative for 4 h, thereafter they were transferred to a 30 % sucrose solution. Three series of coronal sections (35 m) were cut. One series of sections was immunohistochemically stained for human α-syn using the mouse anti-human α-syn antibody (clone 4B12, Signet, Dedham, MA, USA), another series was stained for species non-specific α-syn antibody (Syn-1) (Transduction Laboratories, Lexington, KY, USA). The sections were pre-treated for 30 min with hot (85° C) citrate buffer. The series of sections were transferred to a solution containing the primary antibody (mouse antihuman α-syn at 1:4000) in TBS with addition of 0.5 % Triton X-100 (TBS-T). Following incubation in this solution for 18 h on a shaker table at room temperature (20° C) in the dark, the sections were rinsed three times in TBS-T and transferred to the solution containing the secondary antibody (goat anti-mouse*biotin, Sigma-Aldrich, St. Louis, MO, USA). After 2 h, the sections were rinsed three times with TBS-T and transferred to a solution containing mouse ExtrAvidin® (Sigma-Aldrich). Following rinsing, the sections were incubated for approximately 3 min with Ni-enhanced DAB.

All stained sections were mounted on slides and coverslipped.

4.4. **Behavioral Tests**

4.4.1. ***Rota-rod*** (**Study I**)

Motor coordination and balance of A30P vs. WT mice was tested in accelerating rota-rod test (Stoelting, IL, USA; Jones and Roberts, 1968). The mice were allowed to walk on accelerating rotating beam, and time that mice could stay on the beam was recorded. The mice were tested on 2 days, 48 h apart.

4.4.2. ***Activity Test*** **(Study I & IV)**

Locomotor and exploratory activity of the mice was tested in an automated activity monitor employing infrared beam detection (TruScan®, Coulbourn Instruments, Allentown, PA, USA). The system consisted of four 26 x 26 x 39 cm transparent plastic cages with two photobeam sensor rings enabling separate monitoring of horizontal (XY-movement over time) and vertical activity (rearing). All observation cages were connected to a computer for recording and data analysis. The mice were gently placed at the center of the arenas and the recording was started at once. The mice were tested in two 10-min sessions, 48 h apart.

4.4.3**.** ***Morris Water Maze*** **(Study I & IV)**

Morris water maze was used to assess spatial memory. Performance in the test is highly sensitive to dysfunction of the hippocampus (Morris et al., 1982).

Before the actual water maze testing took place, the mice were given 2 days of pre-training in a 1m x 14cm alley with high walls leading to a black rubber-coated platform (14 x 14 cm), located 1 cm below the water surface. This gave them experience in climbing onto the platform from the water. The mice were allowed to swim until they found the platform or for a maximum of 20 s, after which they were placed on the platform for 10 s. This was repeated four times in a row on both pre-training days.

The actual water maze test employed a black plastic circular pool (diameter 120 cm) and the same platform as in pre-training. The testing took place in a room rich with visual cues on the walls. The starting locations, labeled as North, South, East, and West were located arbitrarily on the pool rim. The timing of the latency to find the submerged platform was started and ended by the experimenter. A computer connected to an image analyzer (HVS Image, Hampton, UK) monitored the swim pattern. Mice were placed in the water with their nose pointing towards the wall at one of the starting points in a random manner. If the mouse failed to find the platform in the maximum time (50 s), it was placed on the platform by the experimenter. Mice were allowed to stay on the platform for 10 s. A recovery period of 30 s was allowed between the training trials. The temperature of the water was kept constant throughout the experiment (20 ± 1^{o} C).

The training schedule consisted of 5 consecutive days of testing. Four platform trials of 50 s were run per day. The platform location was kept constant (the South-West quadrant) during training. After the fourth trial on the 5^{th} day, the platform was removed, and the mice were allowed

to swim for 40 s without the platform. The spatial probe trial was run for all mice that were trained during initial acquisition phase. During the platform training trials, path length, latency to find the platform, percentage of trials when mice found the platform and swimming speed were recorded. Furthermore, the pool surface was divided into three annuli of equal surface area, and the time spent in each annulus was counted separately. The data from platform finding were normalized using arcsin correction before the statistical analyses. In the spatial probe trial, the time that the mouse spent in the vicinity (within a radius of 12 cm from the former platform center) of previous platform position was measured.

4.5. **Electrophysiological Tests (Study I-IV)**

4.5.1. ***In vitro Recordings***

Preparation of slices for *in vitro* recordings is explained in chapter 4.2.3. For electrophysiological recordings a slice was used 4-5 h after cutting. Responses were evoked by stimulation of the SC projection in *stratum radiatum* of the CA1 field (**Study I** and **II**) or by stimulation of the mossy fiber pathway in the dentate hilus (**Study III**) through a pair of twisted nichrome bipolar electrodes and recorded using a glass microelectrode filled with 2M NaCl. In **Study I** and **II**, the recording electrode was positioned in the CA1b subfield between two stimulating electrodes placed in CA1a and CA1c subfields; this allowed us to activate separate inputs in the targeted pyramidal cells (**Study I** and **II**). The stimulation intensity (20–70 μA) was adjusted so as to obtain approximately 50 % of the maximum slope of the population spike free fEPSPs. The inputs were stimulated (0.1 ms pulse duration) every 30 s. In **Study III** the recording electrode was placed in *stratum lucidum* of the hippocampal CA3 field. For baseline recording the stimulation intensity (80 – 140 μA) was adjusted so as to obtain 40 % of the maximum slope as the response, and inputs were stimulated (0.1 ms pulse duration) every 30 s. The slope of the fEPSPs was measured between 30 % and 70 % of maximum. A pair of stimuli with inter-stimulus interval (ISI) of 75 ms was delivered to simultaneously record paired-pulse facilitation (PPF) in **Study II**. In **Study III** input/output (I/O) curves were constructed by taking the slopes of fEPSPs obtained at different stimulation intensities. The slope of the I/O curve was calculated in MS Excel® using Method of Least Squares for all points between minimum and maximum.

In addition, in **Study III** paired stimuli with ISIs of 20, 40, and 80 ms were delivered to record PPF twice during the recording - on baseline and 40 min after HFS. PPF was expressed as a ratio of the slope of the second fEPSP to that of the first. Frequency facilitation (FF) was also tested

in **Study III** before and 45 min after HFS. FF was induced by 1 Hz stimulation for 30 s. FF was expressed as a ratio of last fEPSP to the first out of 30 responses. After establishing 30 min of stable baseline, LTP was elicited by delivering eight trains of theta burst stimulation (TBS; 100 Hz trains of 4 pulses, duration 0.2 ms, separated by 200 ms, repeated twice at 30-s interval) to one of inputs in **Study I** and **II**. Stimulation with eight bursts was used to produce a sub-maximal LTP to see clear effect. For induction of LTP, the stimulation intensity was increased by setting the pulse duration to 0.2 ms (Arai and Lynch, 1992). In **Study III** HFS (100 pulses at 100 Hz, repeated four times at 10-s interval) was delivered to empty the readily releasable pool of glutamate and to assess mobilization of the storage pool. If the baseline did not stabilize in 30 min, the slice was discarded.

In **Study I**, 70 min after TBS, CPP (RBI, USA), a competitive NMDA-Rs antagonist at 5 or 10 μM was infused into perfusion line with a syringe pump. During drug infusion (25 min), TBS (as previously described) was administered to the 2nd, unstimulated input. Monosynaptic responses (sweep time 50 ms) for both inputs were recorded for 70 min (wash-out of the drug). As in **Study III**, MK-801, a non-competitive NMDA-Rs antagonist (Tocris Bioscience, Bristol, UK) at 20 μM was added to aCSF to avoid contamination with CA3 commissural NMDA-dependent responses (Weisskopf and Nicoll, 1995). In addition to the inclusion criteria for the mossy fiber (MF) recording (see Result section), for the second set of animals (TG2 and WT2) the MF origin of fEPSPs was verified at the end of each experiment by application of the mGlu-R II agonist DCG-IV (Ascent Scientific Ltd., Weston-Super-Mare, UK) at 1 μM (Kamiya et al., 1996; Yoshino et al., 1996). The experiments were discarded if the inhibition produced by DCG-IV was ≤ 20 % of pre-DCG-IV level.

Field EPSPs were amplified (x 1000), filtered (0.1 Hz – 1 kHz), and digitized (10 - 20 kHz), acquired using Clampex 8.0 software and analyzed using Clampfit 8.0 software (both Axon Instruments, Foster City, CA, USA). We measured the fEPSP amplitude and slope. Both parameters can be used interchangeably in a situation where fEPSP is population spike-free (the sweep to sweep correlation between slope and amplitude for individual slices in this material was 0.99).

4.5.2. *In vivo Recordings*

The implantation of the electrodes for chronic recordings (**Study II**) is explained in chapter 4.2.3. Single monopolar pulses (duration 50 μs) were delivered at 30-s intervals and an I/O curve was established as a ratio between the fEPSP amplitude vs. stimulus intensity at eight different current intensities (50, 100, 150, 200, 250, 300, 350, and 400 μA). Based on this curve, the baseline intensity was selected that yielded a fEPSP that was 40 % of its maximum amplitude. The signal

was amplified (x 100–900), filtered (0.1 Hz–3 kHz), digitized at 2 kHz, and stored on the computer using Experimenter's WorkBench® (DataWave Technologies, CO, USA) software. HFS to induce LTP consisted of six trains of 6 pulses (50 μs) at 400 Hz, 100 ms between each train, repeated six times at a 20-s interval. During the HFS, the stimulation intensity was raised to produce a fEPSP amplitude that was 80% of its maximum. The animals were habituated for 10 min to the recording chamber (plastic cage). On Day 1, an I/O curve was generated to ascertain the test and tetanus intensities. Following the I/O curve, a 20-min baseline period was recorded. HFS was delivered and fEPSP were recorded for a further 60-min period at the test intensity. On the ensuing two days, a 30-min period was recorded at the test intensity. The LTP induction was analyzed as percentage of increase in the fEPSP or PS amplitude as compared with the baseline amplitude. Furthermore, the fEPSP enhancement was analyzed only in those mice in which the PS did not mask the fEPSP maximum, while the PS enhancement was analyzed only in those mice that demonstrated a clear PS during the baseline recordings with the selected stimulus current.

The implantation of the electrodes for chronic recordings (**Study IV**) is explained in chapter 4.2.3. For baseline recording the stimulation intensity (about 90 μA) was adjusted to obtain 40 % of the maximum slope of the response and inputs were stimulated (0.1 ms pulse duration) every 30 s. The slope of the fEPSPs was measured between 30 % and 70 % of maximum. I/O curves were constructed by taking the amplitude of fEPSPs obtained at different stimulation intensities (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 μA). The slope of the I/O curve was calculated by custom made routines in Visual Basic under MS Excel® (version 2002) using Method of Least Squares for points between 10 and 90 % of maximum of absolute value. In addition, paired stimuli with ISIs of 25, 50, 75, 100, 600 and 900 ms were delivered to record paired pulses on baseline. It was expressed as a ratio (PPR) of the slope of the 2nd fEPSP to that of the 1st. HFS to induce LTP consisted of 6 trains of 6 pulses (50 μs) at 400 Hz, 100 ms between each train, repeated 6 times at 20 s interval. During the tetanus the stimulation intensity was raised to produce a fEPSP height that was 80 % of its maximum. After 120 min of follow-up after HFS, the second I/O curve was established at the same 14 different current intensities as it was described earlier. The signal was amplified (x 500), filtered (0.5 - 3 kHz), digitized (at 20 kHz) using Clampex® software (Axon Instruments, Foster City, CA, USA), and stored in the computer.

4.6. **Statistical Analyses**

All statistical analyses were carried out using SPSS for Windows software (versions 9.0 - 14, SPSS Inc., USA).

The uterus and body weights were analyzed using t-test. The effects of CPP and estrogen and their interaction on water maze performance (platform finding percentage, swimming speed, and probe trial success), rota-rod and open field performance were evaluated by General Linear Model for repeated measures (GLM-RM) followed by contrast analyses, and by t-test.

Amyloid burden (surface area covered with amyloid deposits) was analyzed using t-test.
In electrophysiological studies input/output (I/O) curves were constructed by taking the slopes of fEPSPs obtained at different stimulation intensities. The mean slope of fEPSP was also plotted against FV amplitudes (if recorded) to establish I/O relationships. The slope of the I/O curve was calculated in MS Excel® using the Method of Least Squares for all points between minimum and maximum. The basal synaptic transmission was determined from I/O curves was evaluated by using GLM-RM followed by t-test or Dunnett's test post-hoc.

The electrophysiological parameters (enhancement and / or decay of fEPSP slope or amplitude, PS amplitude, paired-pulse ratio) were evaluated by GLM-RM followed by t-test or Dunnett's test post-hoc, or by univariate analysis of variance (ANOVA).

5. RESULTS

5.1. **Behavioral Tests**

5.1.1. ***Effects of Estrogen and CPP on Spatial Navigation and Motor Activity*** (**Publication I**)

Morris Water Maze

The swimming speed was dramatically affected by the treatment, such that both estrogen and CPP decreased swimming speed (**Publication I**, **Fig. 2C and D**). When we analyzed the averaged number of successful platform findings over 5 training days, we found a significant overall CPP effect, but no estrogen effect (**Publication I**, **Fig. 1A and B**). In the probe trial, used to assess search bias when the platform was no longer present in the pool, CPP impaired but estrogen treatment improved the performance (**Publication I**, **Fig. 3**).

Motor Tests

CPP decreased the time spent on the rotating rod. However, the performance did not differ between OVX and OVX+ERT mice. In the automated activity monitoring, OVX+ERT mice spent more time in the center of the arena than OVX mice. There were no differences in ambulatory distance, stereotypic movement time or number of rearings between OVX and OVX+ERT mice. CPP decreased stereotypic movement time and number of rearings, but had no effect on ambulatory distance or time spent in the center of the arena. No interaction has been found (**Publication I**, **Table 1**).

Overall, the protecting effect of estrogen against NMDA-receptor blockade was associated with milder cognitive impairment in a hippocampal-dependent test. On the other hand, estrogen did not alleviate motor side effect induced by an NMDA-receptor antagonist.

5.1.2. ***Age-Dependent Effect of Mutated α-Synuclein on Motor Activity*** (**Publication IV**)

Activity Test

We found a significant difference between A30P (TG) and wild-type (WT) mice in ambulatory distance (horizontal gross movement; **Publication IV**, **Fig. 2**), such that old TG mice moved almost 50 % less than old WT mice or young mice of either genotype. In contrast, the number of rearings did not differ between the genotypes.

Morris Water Maze

The escape latencies during 4 days of task acquisition did not differ between the genotypes, but got progressively shorter indicative of task acquisition. Old mice swam considerably slower than young ones during all testing days (**Publication IV**, **Fig. 3B**). However, swimming distance did not differ between groups, and only decreased across successive days. When spatial memory in terms of search bias was assessed in a probe trial (last trial on Day 4) without the platform, we observed that old animals were as good as young ones in locating the platform. There was no difference between the group in time spent in platform quadrant or time in the near vicinity of the former platform location. Furthermore, when memory retention was assessed in a second probe trial on Day 5, no group difference was found in the search bias towards the platform quadrant. However, search strategies were significantly different between age groups. In particular, young mice spend more time in the zone closest to pool wall than the old mice.

To sum up, we did not observe impaired spatial learning and memory as a consequence of aging and expression of A30P mutated α-synuclein. The only significant difference in behavioral tests was decreased locomotion in aged mice, especially, in aged transgenics.

5.2. **Electrophysiological Tests**

5.2.1. ***Effect of Estrogen on Synaptic Plasticity in the Hippocampus*** (**Publication I**)

In the absence of CPP, the fEPSP slope right after the tetanus tended to be higher in the OVX+ERT mice compared to OVX mice. But no group differences were found in the fEPSP slope at 15, 30, and 60 min after the tetanus (**Publication I, Fig. 1**).

CPP at the concentration of 5.0 μM completely blocked LTP in OVX mice, while OVX+ERT mice still expressed robust LTP. OVX+ERT mice had a significant elevation of the fEPSP slope compared to baseline at 15 min, 30 min, 45 min, but no longer at 60 min after the tetanus. On the other hand, the OVX group showed no elevation of fEPSP slope at any time point. Furthermore, the OVX+ERT group differed from the OVX group at 15, 30, and marginally at 45 min after the tetanus, but no longer at 60 min. No significant difference was found between the groups in the fEPSP right after the tetanus (**Publication I**, **Fig. 1**).

In contrast, 10 μM CPP blocked the induction of LTP in both OVX and OVX+ERT groups. Neither group had elevated fEPSP slope compared to baseline at any time point. No group difference was found between OVX and OVX+ERT mice in the EPSP slope at any time between 0 to 60 min after the tetanus (**Publication I**, **Fig. 1**).

In agreement with behavior findings, LTP induction hippocampal slices taken from estrogen-treated OVX mice was more resistant to the effect of a the lower dose of CPP than slices from non-treated OVX mice. However, the higher CPP dose overran the effect of estrogen. Our results are consistent with the idea that estrogen increases the number of NMDA-receptors on the cell membrane, thereby rendering the neurons more resistant to the action of the competitive channel blocker CPP (**Publication I**, **Fig. 4**).

5.2.2. *Effect of Amyloid β Peptide on Synaptic Plasticity* (**Publication II**)

Recordings in Schaffer Collateral – CA1 Path in vitro

The baseline fEPSP slope tended to be smaller in APP/PS1 transgenics (A/P) mice than in nontransgenic (NT) mice, but the age x genotype interaction was not significant. Input-output (I/O) curve was determined by plotting the stimulus intensities needed to elicit maximum fEPSP slope and 50 % of the maximum. The resulting slopes did not differ between A/P and NT mice, and no genotype x age interaction was found (**Publication 2, Table 1**). Paired-pulse facilitation did not differ between the genotypes, and no genotype x age interaction was found (**Publication 2, Table 2**). The theta burst stimulation (TBS) paradigm used resulted in about 50 % increases in the fEPSP slope at 15 min after TBS. We compared the extent of LTP and its short-term decay between the genotypes and ages at four times points (15, 30, 45, and 60 min after TBS), and neither a genotype effect nor a genotype x age interaction was found in this comparison (**Publication 2, Fig. 2**).

Despite robust amyloid pathology, aged A/P mice had normal basal synaptic transmission, LTP induction and maintenance when measured *in vitro* in CA1.

Recording of Perforant Path - Dentate Gyrus Synapses in vivo

The groups did not differ in their basal synaptic transmission as assessed by I/O curves. Although fEPSP at low stimulus currents and population spikes (PS) at all stimulus currents were somewhat smaller in A/ P mice than in the NT controls, neither fEPSP nor PS amplitude displayed a significant current x genotype interaction. The latency to fEPSP maximum was slightly but not significantly longer in A/P mice than in NT mice. A clear LTP induction was observed in both genotypes. The LTP decay over 3 days was faster in A/P mice compared with NT mice (**Publication II**, **Fig. 5A** and **B**), as a significant day x genotype interaction was found for the fEPSP amplitude. By contrast, the LTP decay for the PS was similar in both groups.

To further evaluate whether the more rapid decay of fEPSP over 24 h in A/P mice was due to impaired long-term maintenance of LTP or just a continuation of initially steeper decay curve, we also analyzed the LTP decay during the first 60 min after its induction. The slope of the decay curve did not differ between the genotypes.

Aged A/P mice with robust amyloid pathology had normal basal synaptic transmission, LTP induction and maintenance when measured in DG *in vivo* 60 min after the tetanic stimulation. However, the enhanced fEPSP in the DG declined much faster in the A/P mice than in their nontransgenic controls over 24 h.

5.2.3. ***Role of α-Synuclein on Glutamate Release in vitro*** (**Publication III**)

Our results indicate that overexpression of mutated or wild-type human α-syn, or lack of α-syn does not increase excitability of MF-CA3 pyramidal cell synapses (**Publication III**, **Fig. 2**).

As for paired-pulse facilitation (PPF), paired-pulse ratio (PPR) before HFS tended to be smaller in mice deficient in α-syn and in mice overexpressing wild-type or mutated human α-syn compared to their wild-type controls, but the effect approached significance only for mice overexpressing human wild-type α-syn. Although the HFS did not induce any overall change in the PPR, it enhanced the group differences (**Publication III**, **Fig. 3A and B**). When the PPR was measured again after HFS, α-syn deficient mice and both transgenic groups had significantly smaller PPR than their controls.

HFS induced somewhat weaker post-tetanic potentiation in α-syn deficient mice than in their wild-type control and in mice overexpressing wild-type human α-syn, but the group difference was not significant. Similarly, post-tetanic potentiation did not differ between mice overexpressing wild-type human α-syn and their wild-type controls. However, only both transgenic groups displayed enhancement at 25 min (**Publication III**, **Fig. 4A** and **B**).

Frequency facilitation was found in wild-type mice and A30P transgenic mice, but was totally absent in α-syn deficient mice before and after HFS.

In summary, basal synaptic transmission was normal but FF and PPF after HFS was impaired in α-syn deficient mice. Similarly, transgenic animals overexpressing wild-type or mutated human α-syn showed normal basal synaptic transmission and attenuated PPF. However, they had normal FF and enhanced LTP.

5.2.4. ***Combined Effect of α-Synuclein and Aging on Synaptic Plasticity*** (**Manuscript IV**)

In terms of fEPSP, the basal synaptic transmission in PP – DG synapses before HFS was significantly higher in A30P TG animals (**Manuscript IV**, **Fig. 4 A** and **B**), while young animals tended to have higher excitability compared to old ones. However, after HFS young animals had significantly stronger synaptic transmission than the old ones, while the genotype effect was nonsignificant. To assess the overall genotype effect before and after HFS, we included both age groups in the same GLM-RT model. In this analysis, TG animals did not differ in basal synaptic transmission when compared to NT. However, the effect of age became significant. Thus it appears that in general young animals had increased basal synaptic transmission and that transgenic α-syn further enhanced basal synaptic transmission before HFS.

As for PS, none of analyzed parameters (PS maximal amplitude, minimal current to elicit PS and I/O curve tangential line) was significantly different between the groups before HFS. However, after HFS, PS decreased significantly in all groups.

The PPR for fEPSP slope on baseline was significantly smaller in old animals compared to young ones. In addition, young animals had a modest facilitation with maximum at 25 and 50 ms, while in old animals no such peak was observed and PPR dropped bellow 1 for ISIs longer than 50 ms. Similarly to PPR of fEPSP slope, PPRs of PS tended to be higher in young animals, but at a different ISI.

Activity-dependent plasticity was tested with a HFS protocol. In our recordings, the only consistent finding was the expression of LTD in old TG group 75-120 min after HFS (**Manuscript IV**, **Fig. 6B**). Both fEPSP slope and amplitude in all other groups did not differ significantly from baseline starting from 45 min after HFS. In addition, only young animals displayed a short-term (first 15 min) increase of fEPSP amplitude, which later decayed to baseline, however.

In summary, we found decreased basal synaptic transmission and PPF in the perforant path - dentate gyrus granule cell synapses of old mice independent of their genotype. In addition, α-syn accumulation in old A30P mice led to LTD expression after a stimulation protocol that normally induces LTP. These findings suggest that α-syn exacerbates the aging process and leads to impaired synaptic plasticity.

6. DISCUSSION

6.1. The Effect of Estrogen on Synaptic Plasticity in Hippocampus

Our results indicate that the potency of the competitive NMDA-antagonist CPP to block LTP induction is dependent on the estrogen status of the animal: slices taken from estrogen-treated OVX mice were more resistant to the effect of CPP than slices from non-treated OVX mice. Also, our results suggest that estrogen treatment might increase the number of functional NMDA-receptors in hippocampus in OVX mice, and in that way antagonize a blockade of hippocampal NMDA-receptors by CPP. The protecting effect of estrogen against NMDA-receptor blockade was also associated with milder behavioral impairment in a hippocampal-dependent cognitive test. However, estrogen treatment did not reduce the effects of CPP to decrease locomotor activity and impair balance and motor-coordination in OVX mice.

Previous *in vivo* studies have shown positive estrogen effect on LTP. *In vivo* recordings during anesthesia have revealed an augmented LTP in female rats during the proestrus (Good et al., 1999; Warren et al., 1995). LTP enhancement has also been found after a single injection of estradiol in awake rats (Cordoba Montoya and Carrer, 1997; Smith and McMahon, 2005). The effect of chronic estrogen treatment (14 days) on LTP *in vitro* (Barraclough et al., 1999) is consistent with our study in mice, i.e. no any effect in the CA1 area was found.

Estrogen has been reported to increase the number of dendritic spines and synaptic density of CA1 pyramidal neurons in OVX rats (Gould et al., 1990; Woolley and McEwen, 1993), sensitivity of CA1 pyramidal cells to NMDA-R mediated synaptic input (Woolley et al., 1997), and enhanced NMDA-dependent Ca^{2+} signals (Pozzo-Miller et al., 1999), suggesting that the new spines and synapses induced by estrogen are enriched in NMDA-receptors. Thus, an increase in the number of NMDA-Rs would be one of possible explanation for different dose-response curves to CPP application in the present study. A 20–30 % increase in the receptor number would not necessary result in significant enhancement of LTP. Also, if the competitive antagonist is present in excess, such a small increase in the number of binding sites should not affect the outcome. However, if the concentration of the antagonist is large enough to block all binding sites in the OVX slices, but leaves 20–30 % of NMDA-Rs free in the OVX+ERT slices, only OVX+ERT slices should express LTP, as indeed was the case with CPP at 5 μM.

A study that was published two years later than ours supports our hypothesis by confirming that after estrogen treatment the number of NMDA-Rs increases through insertion into newly formed or pre-existing synapses, which increases NMDA-Rs-mediated neurotransmission (Smith

and McMahon, 2005). Importantly, this study pointed out that hippocampal synaptic plasticity is enhanced only when the spine density is increased simultaneously with an increase in NMDA-R transmission relative to AMPA-R transmission. With ongoing estrogen treatment, also AMPA-R transmission gradually increases, while increased spine density and NMDA-R transmission are maintained. Eventually, the balance between NMDA-R and AMPA-R transmission is re-established, and the magnitude of attained LTP is similar to that in the baseline condition. This finding indicates that the hormone-induced increase in functional synapse density alone is not sufficient to support heightened plasticity (Smith and McMahon, 2005). A further complexity in this scenario is that only about 30 % of the synapses containing estrogen receptors co-express NMDA-R (Adams et al., 2004). Future studies also need to examine the relationships between estrogen receptors and NMDA receptor subunits.

In our study, we found that estrogen treatment initiated 2 weeks before water maze testing did not improve the initial acquisition of mice being OVX for 7 months. The effect cannot be a general slowing of locomotion or impaired coordination because estrogen did not affect movement parameters in the open field or rota-rod tests. Slowing of swimming speed may partially explain why estrogen did not improve initial task acquisition. It may also account for the reported impairing effect of repeated estrogen injections on water maze performance in OVX mice (Fugger et al., 1998), because that study reported the impairment as longer escape latency but did not measure the swimming speed. Consistent with frequently reported spatial learning impairment with NMDA-antagonists (Morris et al., 1986; Whishaw and Auer, 1989), CPP treatment dose-dependently impaired spatial navigation of mice as indicated by decreased platform finding percentage during acquisition and poorer probe trial success. Consistent with previous reports (Millan and Seguin, 1994; Parada-Turska and Turski, 1990), CPP also dose-dependently reduced locomotor activity and impaired motor coordination of mice. Therefore, impaired water maze acquisition cannot be ascribed to impaired hippocampal function alone. However, the main finding of the water maze test was that the estrogen treatment alleviated the CPP-induced deficit in a dose-dependent manner. This was most clearly seen as an improved performance during the probe trial, which is considered the best measure for hippocampal-dependent spatial navigation in this task (Lipp and Wolfer, 1998; Morris et al., 1982). Importantly, the performance of CPP-treated OVX+ERT mice on the probe trial was superior to that of OVX mice despite their slower swimming speed that should impair the acquisition. A specific effect of ERT on hippocampal-dependent navigation was further indicated by similar effects of CPP in OVX+ERT and OVX mice on the non-cognitive functions in the open field and rota-rod tests.

Based on literature CPP almost completely blocks LTP induction in a freely moving rat at the systemic dose of 5 mg/kg (Morimoto et al., 1991), and a dose of 10 mg/kg completely blocks LTP induction in a freely moving mouse (Davis et al., 1997). Such a large dose could not be studied in the water maze because of the motor side effects. Assuming that 10 mg/kg of CPP leads to a complete blockade of NMDA-receptors as did the 10 μM concentration *in vitro*, we sketched the following dose-response curves for CPP in OVX and OVX+ERT mice. The relative difference in NMDA-receptor occupancy between OVX and OVX+ERT mice with the CPP dose of 0.5 mg/kg would be about 15 %, which is not large enough to affect their behavior differentially. However, the dose 5 mg/kg should block all NMDA-R in the OVX mice while leaving about 20 % of the receptors free in the OVX+ERT mice, thus revealing a significant group difference also at the behavioral level. The dose-response curves indicate a 30 % rightward shift of the curve in the ERT group, which in consistent with the about 30 % increase in the number of NMDA-receptors of estrogen-treated OVX rats (Gould et al., 1990).

A later study confirmed that following estradiol treatment NMDA-Rs density was restored in aged animals back to the control level in all hippocampal subregions, especially at a high dose (El-Bakri et al., 2004), and thus ERT might help maintain cognitive functions as well in old animals as in young ones (Adams et al., 2004). It is believed that the memory enhancing effect of estrogen involves interaction of estrogen with different neurotransmitters system such as the cholinergic (El-Bakri et al., 2004; Packard and Teather, 1997) and the monoaminergic systems (Luine et al., 1998). Therefore, it is possible that estrogen affects the glutamatergic system indirectly through an interaction with other neurotransmitter systems.

Our study provided first indirect evidence that estrogen may affect the number or properties of hippocampal NMDA-receptors in the mouse. Taken together with later studies it shows that NMDA receptor functions in the hippocampus are strongly regulated by estradiol. These findings provide insight into the interaction of estradiol and the glutamatergic system, which may be relevant in the treatment of memory problems in postmenopausal women and in AD patients.

6.2. **The Effect of Accumulation of Amyloid β Peptide on Synaptic Plasticity**

At the age of 17–18 months and with robust amyloid pathology, transgenic mice carrying mutated human APPswe and PS1(A246E) genes (A/P mice) had normal induction and 60 min maintenance of hippocampal LTP both when measured *in vitro* in CA1 and *in vivo* in DG. However, the enhanced fEPSP in the DG declined much faster in the A/P mice than in their NT controls over 24 h. This finding is fully compatible with the behavioral findings in these mice.

Namely, in the water maze, the A/P mice show similar learning within a session as control mice, but show markedly less improvement across days than the controls (Liu et al., 2002).

Different mouse strains have been shown to vary in evoked fEPSPs and population spikes and their potentiation, but not in the presynaptically determined PPF (Bampton et al., 1999). The frequent presence of two population spikes in A/P mice may indicate increased excitability of dentate granule cells in these mice. However, the I/O plot for the PS did not differ among the genotypes, speaking against any genotype difference in excitatory synaptic transmission. Also, no genotype difference was observed in PPF. As the focus of this study was on LTP maintenance, we did not systematically test paired-pulse inhibition with varying intervals, which may have revealed genotypes differences in the dynamics of inhibitory neurotransmission. However, in a previous study, we found that A/P mice display reduced habituation of auditory evoked responses to paired click stimuli separated by 500 ms (Wang et al., 2002). Reduced inhibition of dentate granule cells in A/P mice may explain why the decay of population spike enhancement was similar in A/P mice and their controls despite much faster decay of fEPSP enhancement in A/P mice. Interestingly, however, a similar dissociation between fEPSP and PS enhancement in the DG has also been reported elsewhere (Chapman et al., 1999), but they never observed multiple PSs in the APPswe mice. The observation that the dentate fEPSP enhancement is more vulnerable to Aβ accumulation than the PS enhancement is consistent with the current understanding of the underlying pathology. Namely, a study (Lazarov et al., 2002) showing that Aβ accumulation in the dentate molecular layer is dependent on axonal transport of APP from the entorhinal cortex through the perforant path indicates that Aβ peptides are released and subsequently deposited close to the nerve terminals. Therefore, Aβ should have a stronger influence on the fEPSP generated in the dendritic tree of dentate granule cells than on the PS initiated in the soma and proximal axon further away from the terminal zone.

Earlier we suggested that mainly APP mutation is responsible for the majority of effects in our A/P double mutant mice (Wang et al., 2002; Wang et al., 2003). A more recent report on triple 3xTg-AD mice expressing APP, PS1 and tau mutations (3xTg-AD mice) strongly supports this hypothesis. In particular, 3xTg-AD mice manifest impaired synaptic functions measured as basal synaptic transmission, LTP maintenance and PPF, already at the age of 6 months or older (Oddo et al., 2003). In contrast, double mutants without APP mutation did not show significant synaptic alterations in area CA1. Importantly, synaptic dysfunction, including LTP deficits, appeared before plaque and tangle pathology, manifested in an age-related manner and correlated with the accumulation of intracellular Aβ at later time points (Oddo et al., 2003). In fact, a number of

previous studies have reported that young APP transgenics undergo synaptic, electrophysiological and behavioral changes even months before any amyloid plaque formation (Hsia et al., 1999; Mucke et al., 2000). These findings indicate that some soluble form of Aβ is a more likely candidate for the perturbed synaptic plasticity than amyloid plaques themselves. Recent evidence point to a special importance of small soluble Aβ aggregates (oligomers and protofibrils) in this respect (Clearly et al., 2005; Wang et al., 2004). Naturally secreted Aβ oligomers from transfected human cell lines can interact with neurons *in vivo*, altering their normal physiology. In particular, Aβ oligomers, in the absence of monomers and amyloid fibrils, markedly inhibited hippocampal LTP in rats *in vivo* at concentrations found in human brain and cerebrospinal fluid (Walsh et al., 2002). Whereas in the presence of Aβ monomers or dimers, oligomers do not express such an effect, as high-frequency stimulation produced a robust LTP which was fully maintained for over 180 min. These observations strongly support the hypothesis that soluble Aβ oligomers are the principal effectors of the synaptic dysfunction and synaptic loss that characterize AD (Walsh et al., 2002; Clearly et al., 2005).

A similar parallel between electrophysiological findings (**Study II**) and MWM performance (Liu et al., 2002) was later observed in another AD mouse model, the 3xTg-AD mice (Billings et al., 2005; Oddo et al., 2003). At a young age, these mice displayed equal performance in hippocampal dependent memory tasks such as the spatial reference version of MWM when compared to age-matched controls (Billings et al., 2005). However, their memory problems appeared at the age of 6 months, at the same age when amyloid plaques were detected in the hippocampus, cortex and some other regions. The initial cognitive impairments manifested as retention, but not learning, deficit, as the 3xTg-AD mice effectively learn the task within a day but fail to retain essential information from day to day, exactly as was the case with our APP/PS1 mice (Liu et al., 2002). These findings parallel observations in patients with mild cognitive impairment or early AD who show normal immediate recall of a word list but have impaired recall after another intervening task (Grundman et al., 2004). The striking Aβ-dependent behavioral and LTP maintenance deficits suggest that Aβ interferences with intracellular signaling cascades supporting memory consolidation.

6.3. **The Effect of α-Synuclein Deletion on Glutamate Release**

Mossy fibers (MFs) display the most robust overexpression of A30P mutated α-syn in our transgenic mice, and therefore our electrophysiological recordings focused on the MF-CA3

synapses. Notably, the literature on MF slice recordings is based on results in juvenile animals, whereas we wanted to study the effect of A30P mutation in the adult brain. The age difference likely explains some atypical features of our recordings. First, paired-pulse facilitation (PPF) was only about 1.5-fold in our mice while it is typically over 2 in the literature. Second, clear frequency facilitation (FF) is another characteristic feature of MF conducted responses while it was only marginal in our animals. Third, high-frequency stimulation at 100 Hz with the currently used protocol is reported to induce robust LTP in wild-type rats and mice, whereas only transgenic mice expressing human α-syn showed typical LTP in our study. Another factor that may explain some of the unconventional results is the fact that we used extracellular recordings, while the majority of earlier reports are based on intracellular or patch-clamp recordings. Despite all precaution measures taken to avoid "contamination" of recorded responses by CA3-CA3 fEPSPs, it is difficult to fully exclude them from consideration using extracellular recordings (Claiborne et al., 1993; Dityatev A, personal communication).

The I/O curve for fEPSP slope as a response to increased stimulation current or presynaptic fiber volley did not differ between α-syn KO mice and their wild-type controls. This is consistent with a previous study on SC–CA1 synapses in α-syn KO mice, reporting no change in basal synaptic transmission (Cabin et al., 2002). Also in line with the earlier study, our α-syn KO mice displayed attenuated responses after prolonged stimulation. Both 30 pulses at 1 Hz and repeated 100 Hz stimulation resulted in less facilitation in α-syn KO than in control mice. Additionally, PPF was impaired in α-syn KO mice compared to wild-type mice after the repeated 100 Hz stimulation. All these findings are compatible with the idea that lack of α-syn results in a larger capacity or faster refilling of the readily releasable transmitter pool, while reducing the capacity of the reserve pool (Yavich et al., 2004).

Despite similar I/O curves and attenuated PPF after repeated 100 Hz stimulation, the A30P transgenic mice differed from the α-syn KO mice in two important aspects. First, their FF did not differ significantly from the wild-type control mice, except for a modest decline in FF after the repeated 100 Hz stimulation. Second, and more importantly, A30P mice and mice with transgenic expression of the wild-type human α-syn were the only groups showing true LTP. Their LTP was typical of MF synapses with prolonged post-tetanic potentiation followed by a slowly emerging LTP that reached its peak at 50 min after the end of high frequency stimulation (Derrick and Martinez, 1994). The stimulation protocol used induces a pure presynaptic synaptic enhancement according to previous literature (Kapur et al., 1998; Zalutsky and Nicoll, 1990). The presynaptic nature of LTP in human α-syn transgenic mice is further supported by the use of MK-801 during all

recordings to block NMDA-receptors and the observed attenuation of PPF. In fact, despite ostensibly similar to PPF attenuation in α-syn KO mice, the underlying mechanism of this attenuation in A30P mice is likely different, and can be simply attributed to enhanced glutamate release upon the first stimulation. It is worth noting that the A30P mice carry both the endogenous mouse α-syn and the transgenic mutant one, so that their total α-syn levels are about 1.6-fold compared to the wild-type (Yavich et al., 2005). Their enhanced LTP compared with wild-type mouse could derive from increased amount of available α-syn protein in MF terminals. In favor of this notion, an LTP was also induced with the present HFS protocol in mice with transgenic overexpression of human wild-type α-syn protein, which also have about double levels of α-syn (Kallunki P., personal communication). If the role of α-syn is to augment transfer of transmitter vesicles from the reserve pool to the readily releasable pool (RRP), it makes sense that additional amount of α-syn would be beneficial after HFS which empties the RRP. One could have expected similar enhanced facilitation in both TG groups in the FF paradigm. However, maybe because of the low frequency used in this paradigm, additional α-syn may not provide extra capacity to replenish the RRP, even though lack of α-syn did result in the absence of FF.

In conclusion, our findings lend additional support to the idea that α-syn plays an important role in presynaptic mobilization of reserve pool neurotransmitter vesicles, not only for dopamine but also for glutamate. On the other hand, the presence of A30P mutated (or wild-type) human α-syn does not appear to perturb the functions of endogenous mouse α-syn in glutamate mobilization in MF terminals, although it did so for striatal dopamine release (Yavich et al., 2004). This difference may explain the link of α-syn with PD in particular.

6.4. **The Age Effect of α-Synuclein Mutation on Glutamate Release**

The focus of the present study was the age-depended effect of mutated human α-syn on synaptic plasticity in the dentate gyrus (DG). An earlier study from our laboratory showed age-depended (between 11 and 21 months of age) increase in total α-syn protein levels in A30P mice, while their life span, body size and physical condition did not differ from congenic control mice (Yavich et al., 2005). The characterization of DG response in this model was of special interest because α-syn-positive dystrophic neurites have been described in this structure in patients with diffuse Lewy body disease (LBD) (Iseki et al., 1998). In addition, perforant pathway is degenerated in LBD patients. To our knowledge, this is the first *in vivo* electrophysiological investigation of

perforant pathway-dentate granule cell (PP-DG) synapses in transgenic mice overexpressing A30P α-syn, in which age-depended effect of α-syn accumulation also was assessed.

Behavioral analysis of A30P mice revealed decreased locomotion in aged animals. This data is consistent with previous study in the same mice and in parallel with α-syn accumulation in old but not in young TG mice (Yavich et al., 2005). However, when spatial learning and memory was tested in Morris water maze, we did not observe any genotype or age effects. This may suggest that α-syn accumulation does not affect hippocampal dependent learning and memory at system level.

The basal synaptic transmission of PP-DG synapses was tested on two occasions, before high frequency stimulation (HFS) and 120 min after it. Our data indicates reduced excitability in old animals. In addition, the genotype had some additional effect, as TG animals tend to have higher basal synaptic transmission compare to controls. The trend of increased basal synaptic transmission in TG animals was also found in our previous study on the same mice in mossy fibers *in vitro* (**Study III**). This is in line with reports of reduced fEPSP in DG of aged rats (Barnes and McNaughton, 1980), probably due to a reduction in axon collaterals from the entorhinal cortex to the granule cells (Burke and Barnes, 2006).

When presynaptic release mechanism in PP-DG synapses was probed with measurement of paired-pulse ratio (PPR), for fEPSP we observed typical facilitation at interstimulus intervals of less than 200 ms and depression at longer intervals in young mice (McNaughton, 1982). However, old animals showed no sign of paired-pulse facilitation at shorter intervals and overall the facilitation was significantly lower than in young animals. This is consistent with previous *in vitro* findings of age-dependent decrease of fEPSP facilitation in lateral PP input to DG (Froc et al., 2003; Zheng et al., 2005). However, this age-dependent decrease is unlikely of presynaptic origin as usually considered (Zucker and Regehr, 2002), but may stem from compensatory mechanisms that increases postsynaptic sensitivity in response to the reduced medial PP input (Barnes and McNaughton, 1980; Burke and Barnes, 2006). One such mechanism may be alterations in postsynaptic AMPA receptors, which has been reported to contribute to paired-pulse facilitation (PPF) in CA1 synapses (Wang and Kelly, 1997).

Results in our study show that PS PPR in young mice resembles triphasic pattern typical of PP-DG synapses: early depression (10-40 ms), facilitation (70-100 ms), and late depression (150-2000 ms) (Gilbert and Burdette, 1996). PPF and depression of the PS can give useful information about feedforward and feedback inhibitory circuits in the hippocampus (Bliss et al., 2007). If the first stimulus in a pair is able to evoke PS, $GABA_A$-mediated feedback inhibition lasting 10-20 ms

prevents the second stimulus from evoking a population spike. The spike facilitation at longer intervals may be explained by suppression of feedforward inhibition mediated by presynaptic $GABA_B$ autoreceptors (the peak effect at 100-200 ms). Thus, it can be suggested that decreased PPF in old animals is due to stronger feedforward inhibition, which maybe a result of partial loss of feedback interneurons. In this regard, it should be noted that hilar cells in DG represent a population of feedback interneurons (Freund and Buzsaki, 1996) which are among the most vulnerable cell types to aging process (Gavilan et al., 2007).

High frequency stimulation (HFS) induced LTD in old TG mice, short term potentiation in young NT mice, and neither potentiation nor depression in old NT and young TG mice. Deficits in both LTP induction and maintenance in aging animals is not surprising as numerous studies have shown this before (for review see (Burke and Barnes, 2006)). However, LTD is considerable more difficult to obtain in the intact animals, especially in the DG (Errington et al., 1995). It is well establish that postsynaptic intracellular Ca^{2+} is a central messenger in NMDA-R-dependent forms of LTP and LTD. The current hypothesis states that a rapid rise in Ca^{2+} produces potentiation, whereas a small, prolonged increase induces depression (Bi and Rubin, 2005; Bliss et al., 2007). In support of this hypothesis, lowering of external Ca^{2+} transforms a protocol that normally induces LTP into one that produces LTD (Mulkey and Malenka, 1992). Interestingly, a recent study revealed that α-syn is responsible for acceleration of Ca^{2+} release in cultured cells upon treatment with a wide range of agonists (Narayanan et al., 2005). Therefore α-syn accumulation in old animals may alter postsynaptic Ca^{2+} signaling in a way which favors manifestations of activity-dependent depression. Alternatively, α-syn accumulation may alter another distinct form of LTD, mGlu-R-dependent, in which signaling mechanisms are very different from those involved in NMDA-R-dependent LTD (Bliss et al., 2007).

At a first glance, the finding of intact spatial memory in old TG mice appears to be in contrast with a consistent expression of LTD in the same animals. However, it should be recognized that water maze is a systems level test assessing general cognitive function (or integrity of large neural networks involving the hippocampus), while LTP/LTD is a measurement of a single synaptic pathway. As PP-DG synapses are an integral part of the hippocampal circuitry, other synapses may compensate for the observed alternations. Our previous study (**Study III**) may provide one example of such compensation by showing that human α-syn expressing mice were the only ones which exhibited LTP in MF-CA3 synapses.

A previous *in vitro* study on medial PP-DG granule cell synapses showed no changes in basal synaptic transmission and enhanced PPD (Steidl et al., 2003) in mice carrying similar A30P

mutation as our mice. In addition, no age and genotype interaction was found for measured parameters. However, this study is difficult to compare with our study due to a number of methodological differences. First, Steidl and colleagues (Steidl et al., 2003) employed a mouse with much higher overexpression level of the transgene induced by the hamster prion protein (PrP) promoter (15-fold) as compared to the mouse PrP promoter in our mice (1.5-fold). Second, one study was conducted *in vitro* and the other one *in vivo*.

In conclusion, our findings lend additional support to the contention of altered synaptic plasticity in aged mice. We found decreased basal synaptic transmission and PPR in the PP-DG granule cell synapses of old mice. In addition to normal aging processes, α-syn accumulation may impose additional stress on the tested synapses. Likely by altering postsynaptic Ca^{2+} signaling, α-syn accumulation in old TG animals leads to LTD expression after stimulation protocol that normally induces LTP.

6.5. GENERAL DISCUSSION

The mouse models used in these studies may be instrumental for future investigation of the pathogenesis of neurodegeneration in both AD and PD. We hope that investigations on how AD and PD affect excitatory synaptic transmission and plasticity in the hippocampus will eventual reveal the underlying cause for early memory deficits in AD and PD/Lewy Body Dementia.

Despite all difficulties to record from hippocampal slices *in vitro*, they are the most popular preparations to study short- and long-term synaptic plasticity in mammals. However, even though physiological properties of synapses in slice preparations are close to *in vivo* condition, the percentage of synaptically connected neurons in slices is very low. Therefore, combination of *in vitro* and *in vivo* methods provides a more powerful way to test or at least to formulate plausible hypotheses on the mechanisms of transmitter release and to link intracellular changes and memory formation in normal aging and in disease.

7. CONCLUSION

1. The present study provided first indirect evidence that estrogen may affect the number or properties of hippocampal NMDA-receptors in the mouse. The complexity of estrogen effect is emphasized by number of factors (such as a dose and duration of estrogen treatment and a length of deprivation), which may be relevant in determining its neural and cognitive efficacy. The observed effects of estrogen on NMDA-R-mediated synaptic plasticity in mice will open up this field of research to modern gene targeting techniques.

2. The present study provides some additional data on how AD pathology underlies functional abnormalities involving the mechanisms of synaptic transmission. These deficits could involve changes in one or more enzymes, second messengers, transcription factors etc., which could affect plasticity of neurotransmitter receptors without modifying basic synaptic plasticity. Defining the relationships among APP, PS1, Aβ and mechanisms contributing to the induction, expression or maintenance of LTP will therefore be critical for our understanding of the pathogenesis of AD.

3. Our findings lend additional support to the idea that α-syn plays an important role in presynaptic mobilization of reserve pool neurotransmitter vesicles, not only for dopamine but also for glutamate. On the other hand, the presence of A30P mutated (or wild-type) human α-syn does not appear to perturb the functions of endogenous mouse α-syn in glutamate mobilization in MF terminals, although it does so for striatal dopamine release. This difference may explain the link of α-syn with PD in particular.

4. Our findings lend additional support to the idea of altered synaptic plasticity in aged mice. In addition to normal aging processes, α-syn accumulation can disturb synaptic plasticity. For instance by altering postsynaptic Ca^{2+} signaling, α-syn accumulation in old TG animals leads to LTD expression after a stimulation protocol that normally induces LTP.

All in all, a comparison of the behavioral and electrophysiological findings in these experiments showed a close relationship between spatial memory function and the rates of synaptic transmission and plasticity depending on age and the pathological status.

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APPENDIX:

ORIGINAL PUBLICATIONS (I-IV)

Kuopio University Publications G. - A.I.Virtanen Institute

G 43. Nairismägi, Jaak. Magnetic resonance imaging study of induced epileptogenesis in animal models of epilepsy.
2006. 77 p. Acad. Diss.

G 44. Niiranen, Kirsi. Consequences of spermine synthase or spermidine/spermine N1-acetyltransferase deficiency in polyamine metabolism - Studies with gene-disrupted embryonic stem cells and mice.
2006. 72 p. Acad. Diss.

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