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KESTUTIS GUREVICIUS

EEG and Evoked Potentials as Indicators of Interneuron Pathology in Mouse Models of Neurological Diseases

Doctoral dissertation

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

Department of Neurology
University of Kuopio

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Department of Neurobiology
A.I. Virtanen Institute for Molecular Sciences

Author's address: Department of Neurobiology
A.I. Virtanen Institute for Molecular Sciences
University of Kuopio, Bioteknia 1
P.O. Box 1627
FI-70211 KUOPIO
FINLAND
E-mail: Kestutis.Gurevicius@uku.fi

Supervisors: Professor Heikki Tanila, M.D., Ph.D.
Department of Neurobiology
A.I. Virtanen Institute for Molecular Sciences

Ari Pääkkönen, Ph.D.
Department of Clinical Neurophysiology
Kuopio University Hospital

Reviewers: Professor Andreas Draguhn, M.D., Ph.D.
University of Heidelberg
Institute of Physiology and Pathophysiology
Heidelberg, Germany

Docent Tomi Taira, Ph.D., Group Leader
Neuroscience Center and Department of Biosciences
University of Helsinki

Opponent: Docent Aarne Ylinen, Head of the Department
Rehabilitation Research Unit
Tampere University Hospital

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ABSTRACT

Interneurons, which primarily contain the neurotransmitter γ-amino butyric acid (GABA), make up ~20 % of all cortical neurons. They play a key role in the operation of neuronal networks by controlling the number of active pyramidal cells, their firing frequency and discharge timing. Interneurons also play a pivotal role in the generation of network oscillations. In this study, we assessed interneuron function / dysfunction in four genetically modified mouse models. Its main focus is to assess with electrophysiological measures (electroencephalography, event–related potentials) the impact on brain functions of certain pin-pointed mutations which are associated with neurodegenerative diseases and/or interneurons.

Electroencephalography (EEG) was used to test general excitation and inhibition processes in the brain, while event–related potentials (ERPs) were used to test brain activity ranging from sensory reception to cognitive processes (such as learning and memory). The data from electrophysiological recordings was compared to behavioral assays (Morris water maze and automated activity test) and detailed morphological analysis of interneuron pathology.

Pattern of alternation of EEG and ERP was unique for each tested genotype. In line with electrophysiological data, interneuron pathology was different between mutant mouse lines. Developmental or pathological abnormalities caused enhancement or attenuation in various frequency ranges (delta, theta, beta and gamma). Moreover, cortical and hippocampal or even subfield (dentate gyrus vs. CA1) specific EEG alternations were found. Besides intrinsic electrical activity, auditory evoked potentials showed distinctive changes in each genotype as well.

In conclusion, electrophysiological measures (EEG and ERP) proved to be a very sensitive tool to detect neuronal network abnormalities. Specificity of this measurement may be enhanced by increasing the diversity of calculated parameters and the number of recording sites.

National Library of Medicine Classification: WL 102.5, WL 150, WV 270, QU 60, QY 58, QY 60.R6, WL 314

Medical Subject Headings: Interneurons/pathology; Electrophysiology; Electroencephalography; Evoked Potentials, Auditory, Brain Stem; Receptors, GABA; Hippocampus; Neurodegenerative Diseases; Point Mutation; Disease Models, Animal; Mice, Transgenic; Brain Mapping; Sensation; Cognition; Learning; Memory; Behavior, Animal

The peculiar fascination of the brain lies in the fact that there is probably no other object of scientific enquiry about which we know at once so much and yet understand so little.

Gerd Sommerhoff (from *Logic of the Living Brain, 1974*)

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Kuopio, November 2007

Kestutis Gurevicius

ABBREVIATIONS

Aβ	Beta amyloid
ACh	Acetylcholine, neurotransmitter
AD	Alzheimer's disease
AEP	Auditory evoked potentials
AMPA	α-amino-3-hydroxi-5-methylisoxazole-4-propionic acid
APP/PS1	Transgenic mice expressing APPswe and PS1-A264E mutations
BAEP	Brainstem (or short-latency) auditory evoked potentials
CA1	The hippocampal *Cornu Ammonis* subregion 1
CA1Mol	Stratum radiatum/lacunosum moleculare of CA1
CA3	The hippocampal *Cornu Ammonis* subregion 3
CCK	Cholecystokinin, neuropeptide
DG	Dentate gyrus, part of the hippocampal formation
ECM	Extracellular matrix
EEG	Electroencephalography
EPSP	Excitatory postsynaptic potential
ERP	Event–related potential
FFT	Fast Fourier transformation
GABA	γ-aminobutyric acid, neurotransmitter
$GABA_A$	Ionotropic GABA receptor
$GABA_B$	Metabotropic GABA receptor
HIPP	Hilar interneurons with axonal arborization in the PP termination zone
ING	Interneuron network gamma
IPSP	Inhibitory postsynaptic potential
i.p.	Intraperitoneal injection
LAEP	Long-latency auditory evoked potentials
LTP	Long-term potentiation
L-VDCC	L-type voltage dependent Ca^{2+} channel
mAChR	Muscarinic acetylcholine receptors
MAEP	Mid-latency auditory evoked potentials
mGluR	Metabotropic glutamate receptor
mRNA	Messenger Ribonucleic Acid
N/A	Not available
NMDA	*N*-metyl-*D*-aspartate
NPY	Neuropeptide Y
NREM	Non-rapid eye movement, sleep stage
O-LM	Stratum oriens / lacunosum moleculare
PING	Pyramidal-interneuron network gamma
PP	Perforant path, the main input to the hippocampus
PV	Parvalbumin, calcium-binding protein
SOM	Somatostatin, neuropeptide
ST-/-	Mice deficient in the HNK-1 sulfotransferase
TNR-/-	Mice deficient in the extracellular matrix glycoprotein tenascin-R
TNC-/-	Mice deficient in the extracellular matrix glycoprotein tenascin-C

LIST OF ORIGINAL PUBLICATIONS

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

I. Wang J, Ikonen S, **Gurevicius K**, van Groen T, Tanila H (2002). Alteration of cortical EEG in mice carrying mutated human APP transgene. Brain Res 943:181-190.

II. Wang J, Ikonen S, **Gurevicius K**, Van Groen T, Tanila H (2003). Altered auditory-evoked potentials in mice carrying mutated human amyloid precursor protein and presenilin-1 transgenes. Neuroscience 116:511-517.

III. **Gurevicius K**, Gureviciene I, Valjakka A, Schachner M, Tanila H (2004). Enhanced cortical and hippocampal neuronal excitability in mice deficient in the extracellular matrix glycoprotein tenascin-R. Mol Cell Neurosci 25:515-523.

IV. **Gurevicius K**, Gureviciene I, Sivukhina E, Irintchev A, Schachner M, Tanila H (2007). Increased Hippocampal and Cortical Beta oscillations in Mice Deficient for the HNK-1 sulfotransferase. Mol Cell Neurosci. 34(2):189-98.

V. **Gurevicius K**, Kuang F, Irintchev A, Gureviciene I, Iivonen H, Schachner M, Tanila H. Altered brain electrical activity in mice deficient in the extracellular matrix glycoprotein tenascin-C. Manuscript.

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1. INTRODUCTION

Normal cortical function is dependent upon the balanced development of two major neuron types: pyramidal cells and non-pyramidal cells. Non-pyramidal neurons primarily contain the neurotransmitter γ-amino butyric acid (GABA) and make up ~20 % of all cortical neurons. Non-pyramidal neurons also have another name - interneurons. GABAergic synapses make up only about 5% of the synapses on a pyramidal neuron of the CA1 field. However, it is commonly agreed that interneurons play a key role in the operation of neuronal networks. There are number of functions which inhibitory cells provide: i) they control both the number of active pyramidal cells and their firing frequency by feedforward and feedback inhibition; ii) they control the timing of principal cell discharge; iii) they play a pivotal role in the generation of network oscillations.

A balance of interaction between pyramidal neurons and interneurons is very important for the normal brain function. In the intact brain balanced excitation and inhibition give rise to brain rhythms. Despite decades of research, the explicit mechanisms of brain oscillations generation are not fully known. Multiple sources of oscillations are possible in such a complex system as the brain. First of all, the intrinsic properties of neurons themselves contribute towards oscillation. Neurons can have frequency preferences due to passive electrical membrane properties and due to specific expression of voltage-gated channels. This feature enables them to either oscillate spontaneously, or react to input within a narrow frequency range. Second, even a simple connection of two neurons (negative feedback) will create an oscillatory circuit. This simple wiring may be turned to different frequencies by manipulating the $GABA_A$ receptor response. Third, the collective action of neurons with a pivotal role of interneurons is known to generate network oscillations.

To date, electroencephalography (EEG) remains a cost-effective method to measure electrical brain activity. This noninvasive recording technique is still the most widespread method used in clinical and psychological laboratories. Due to its excellent temporal resolution EEG is suitable for monitoring fast, system level events. Signals measured by EEG reflect the coordinated activity of neurons, but also glia cells and even blood vessels can contribute to it. However, in a simplified view extracellular recordings reflects the "average" activity of large numbers of interacting neurons. EEG can be used to test general excitation and inhibition processes in the brain, while event–related potentials (ERPs) can be used to test brain activity ranging from sensory reception to higher cognitive processes (such as learning and memory). Because of ethical limitations, in most cases human EEG or ERP studies are non-invasive (scalp recording), while animal experiment may use deep as well as surface recording. This leads to better understanding of the surface EEG in relation to signals generated in deep brain structures (such as the hippocampus).

Genetically modified mice, which are an invaluable tool for modern neuroscience, give also an opportunity to address questions about the role of interneurons or oscillation in

brain functioning. This PhD project is devoted to four different groups of genetically manipulated mice, and studies changes in balanced excitation and inhibition processes in the living brain. Its main focus is to assess with electrophysiological measures (EEG, ERP) how pin-pointed mutations, which associate with neurodegenerative diseases and/or interneurons, impact brain functions.

2. REVIEW OF THE LITERATURE

2.1. INTERNEURON TYPES AND NETWORKS

Normal cortical function is dependent on the balanced development of two major neuron types: pyramidal cells and non-pyramidal cells. Non-pyramidal neurons primarily contain the neurotransmitter γ-amino butyric acid (GABA) and make up 15–30% of all cortical neurons (Hendry et al., 1987; Meinecke and Peters, 1987; Parnavelas et al., 1977), while the primarily glutamatergic pyramidal neurons constitute the remainder. Non-pyramidal neurons also have another name - interneurons. This name carries an important and descriptive message about the major contribution of these cells in the local networks. The term interneuron was originally used to describe cells at the interface between input and output neurons in invertebrates. However, following the development of the concept of synaptic inhibition (Eccles, 1964), the word 'interneuron' progressively conveyed the unifying principle that inhibitory cells with short axons play an essential role in the regulation of local circuit excitability, in contrast to (excitatory) principal cells with long axons that project information to distant brain regions.

2.1.1. Basic structural elements of Network

Interneurons and principal cells can be combined into a few basic configurations (Fig. 1). In a feedforward inhibitory configuration (Fig. 1A), increased discharge of the interneuron, as the primary event, results in decreased activity of the principal cell. Such pairing of excitation and inhibition can increase temporal precision of firing substantially by narrowing the temporal window of discharge probability. On the other hand, negative (inhibitory) feedback (Fig. 1B) is a self-regulating mechanism. The effect is to dampen activity within the stimulated pathway and prevent it from exceeding a certain critical maximum. In other words, it provides stability for the network. Negative feedback between excitatory and inhibitory neurons opens the possibility of oscillations. An extension of feedback inhibition is lateral inhibition (Fig. 1C). This occurs when principal cell recruits an interneuron to enhance the effect of the active pathway by suppressing the activity of another, parallel pathway. Virtually any kind of network maybe build based on these principles.

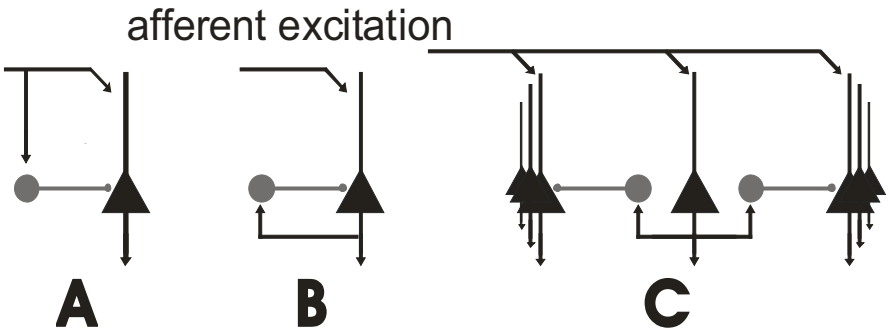

Figure 1. Basic connection principles between interneurons (circles) and principal (triangles) cells. (A) Feedforward loop. (B) Feedback loop. (C) Lateral inhibition.

2.1.2. Cortex vs. Hippocampus

To date, the many aspects of hippocampal interneurons have been documented more extensively than those of neocortical interneurons (Freund and Buzsaki, 1996). However, the data from early Golgi studies, immunocytochemistry and neocortical slices indicate a rich variety of neocortical interneurons and their similarity to hippocampal interneurons (Somogyi et al., 1998). Despite similar constructing elements, network connectivity is diverse in different regions.

The brain is organized hierarchically. Organization at the molecular and cellular levels gives rise to organization at the structural level (different structures of the brain like the cerebellum, amygdala, neocortex, etc.). What distinguishes one brain region from another are the number and types of its neurons and how they are interconnected. It is from the pattern of interconnections that the distinctiveness of function emerges. Paul MacLean (MacLean, 1990), also advocated by György Buzsáki (Buzsáki, 2006), suggested that three gross levels of brain organization is about right. At the bottom of hierarchy is the "reptilian brain". He used the term archipallium as collective name for structures that include the olfactory bulb, brainstem, mesencephalon, cerebellum and the basal ganglia. On the top of the organization lies the neopallium, which is equivalent to the thalamoneocortical system. Sandwiched in between is the paleocortex (comprising the structures of limbic system). From the point of structural organization, the cerebellum or basal ganglia have small neuronal diversity, are dominated by local inhibition and are mainly constructed from feedforward inhibitory loops (Buzsáki, 2006). In contrast, rich variety of neurons and negative feedback connections are common in the neocortex, which holds the key for understanding its dynamics. Besides inhibitory feedback and feedforward loops an important constituent is long-range connections, which provide necessary wire-economy and do not compromise computational needs (Buzsaki et al., 2004). Like the isocortex, most paleocortical structures are constructed from pyramidal cells and GABAergic interneurons, although their layer and wiring organizations vary substantially from the regular isocortical modules.

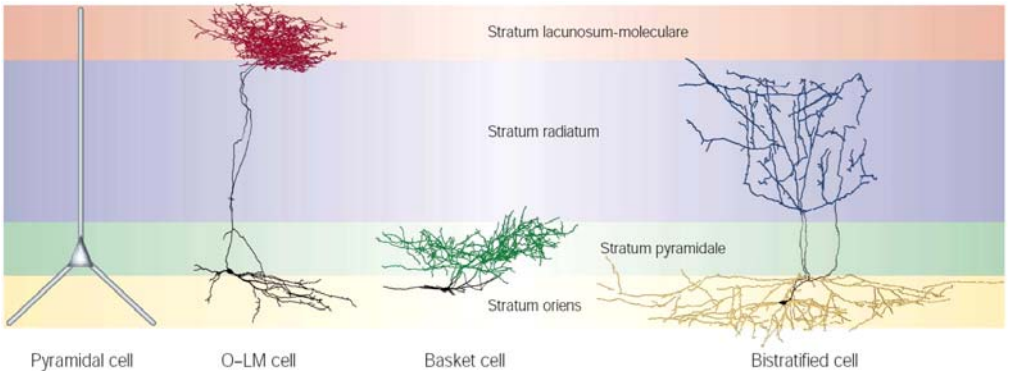

Figure 2. Domain-specific innervation of hippocampal interneurons. Camera lucida reconstructions of three stratum oriens-alveus interneurons showing the domain-specific innervation of pyramidal cells by their axons. A stratum oriens–lacunosum moleculare cell (O–LM cell) projects its axon (red) to pyramidal cell distal dendrites of the stratum lacunosum-moleculare. A basket cell soma, located within stratum oriens, projects its axon (green) to the pyramidal neuron soma and the proximal dendrites. A bistratified cell sends its axon (yellow/blue) to both basal and apical dendrites in stratum oriens and radiatum. Far left, a cartoon of a pyramidal cell showing the approximate location of the basal and apical dendrites, and the cell body. (Figure modified from (Maccaferri et al., 2000)). Adapted by permission from Macmillan Publishers Ltd: [Nature Reviews Neuroscience] (McBain and Fisahn, 2001), copyright 2001.

2.1.3. Classification of interneurons

Interneurons represent a broad class of cells meant to multiply the functional repertoire of principal cells. Multiple interneuron types interact and function within unique circuits that execute complex functions including learning, memory, emotion, motivation, perception, motor behaviors etc. The number of interneurons with different properties is still growing but, to date, there are no commonly agreed classification schemes (Maccaferri and Lacaille, 2003; McBain and Fisahn, 2001; Mott and Dingledine, 2003). Interneurons are so diverse that to date there is no single unifying factor for this class of neurons (e.g. localization, projection, primary neurotransmitter). However, a few descriptors of interneurons are decisive. One of them is their morphological appearance. The anatomy alone can provide intuitive insights into cell-type-specific contributions in an active network, by relating the somatodendritic location to the layer specificity of synaptic input and the axonal projections to the postsynaptic target domain (Fig 2). Based on the aborization of dendritic and axonal processes ~20 cell types have been described (e.g. basket cells, axo-axonic or chandelier cells, oriens-lacunosum moleculare cells etc). Development of new immunohistochemical tools and their combination with morphological data provide new possibilities for interneuron classification. It was found that interneurons contain not only γ-aminobutyric acid (GABA) but a number of other peptides [e.g. somatostatin, cholecystokinin (CCK) and substance P] or Ca^{2+}-binding proteins (e.g. calbindin, parvalbumin and calretinin).

Neurochemical classification adds functional specificity for interneurons as different neurochemical substances are expressed in interneurons of different geometry (Freund and Buzsaki, 1996). However, different types of morphologically defined interneurons could co-exist and overlap in a single neurochemically identified subgroup. Another, and a more useful, characteristic of interneurons is their physiological properties. Usually interneurons have faster kinetics (fast-spiking cells) than principal cells. Interneurons operate with high speed and temporal precision ensured by the expression of specific transmitter receptors and voltage-gated ion channels (Jonas et al., 2004).

Adding further interneuron specific properties will increase heterogeneity in the class to an unlimited number. However, based on their performance in a specific task, interneurons (otherwise with different intrinsic biophysical, morphological and molecular features) may be grouped into a few distinct groups. For example, in terms of their connectivity with the principal cells, three major groups of cortical interneurons are recognized: i) interneurons controlling principal cell output (by perisomatic inhibition), ii) interneurons controlling the principal cell input (by dendritic inhibition), and iii) long-range interneurons coordinating interneuron assemblies (Buzsaki et al., 2004). Furthermore, the division of labor between interneuron classes and proportion in the brain suggest optimization of brain computation power and wiring/metabolic economy.

2.2. FUNCTIONAL ROLE OF INTERNEURONS

Based on most elaborated classification schemes it may be assumed that the interneurons have enormous number of functions (as each subclass of interneurons is likely to have specific function). However, common principles do exist as each feature has an underlying rationale in the evolutionary design of the brain. Despite the fact that GABAergic synapses constitute only about 5% of the synapses in the CA1 field (Megias et al., 2001), it is commonly agreed that interneurons play a key role in the operation of neuronal networks. There are number of functions which inhibitory cells provide: i) to control both the number of active pyramidal cells and their firing frequency by feedforward and feedback inhibition; ii) to control the timing of principal cell discharge; iii) to play a pivotal role in the generation of network oscillations. Many of these functions depend on ability of interneurons to operate with high speed and temporal precision, which in turn depends on the expression of distinct transmitter receptors and voltage-gated ion channels (Jonas et al., 2004).

2.2.1. Control of excitability

Probably one of the most vital functions of interneurons in the brain is to balance neuronal excitability. As early modeling work shows, a network consisting only of principal cells is able to generate avalanches of activity, which would likely exhaust or damage the

brain itself (Buzsáki, 2006). In order to generate harmony in cortical circuits, excitation must be balanced with an equally effective inhibition. There are two $GABA_A$ receptor mediated effects on the postsynaptic membrane which can effectively cancel action potential generation in principal cells. First, the activation of $GABA_A$ receptors usually hyperpolarizes postsynaptic neurons by opening anion channels and allowing an influx of chloride ions. A second event, the importance of which was recognized only recently, is shunting inhibition (Bartos et al., 2007; Mann and Paulsen, 2007). In contrast to hyperpolarization, shunting inhibition drives membrane potential towards more positive value but smaller than action potential firing threshold. However, this depolarization does not drive the principal cell to fire, as increased synaptic conductance (due to the activation of $GABA_A$ receptors) leads to reduced excitability of the cell. The interneurons with negative feedback control (Fig. 1B) may achieve both types of inhibition. In case of the simplest partnership, increased activity of principal cell elevates the interneuron discharge, which in turn decreases or shuts down the principal cell output. By means of feedback inhibition, the activity of an excitatory pathway is dampened and never reaches a certain critical value. An extension of this scheme - or special case of negative feedback - is lateral inhibition (Fig. 1C). Here the principal cell recruits interneurons in order to suppress activity of surrounding principal cells or pathways. Therefore, interneurons serve an important function in the information segregation process, the main mechanism behind David Hubel and Torsten Wiesel observations (Hubel and Wiesel, 1963). Furthermore, a subset of interneurons acts on distinct subcellular compartments, allowing them to selectively control the input, integration and output of the target cells (Gulledge and Stuart, 2003; Miles et al., 1996). For example, a single IPSP initiated by a single perisomatic inhibitory cell could suppress action potential generation in the postsynaptic cell (Miles et al., 1996). In contrast, dendritic inhibition can regulate dendritic integration, back-propagation of sodium spikes and generation of dendritic calcium spikes (Mann and Paulsen, 2007; Miles et al., 1996).

2.2.2. Control of timing

Even though information segregation is an important constituent of brain function, there is plenty of evidence that information integration (coherent processing) is also taking place in the brain. Actually before the 1980s, the main function of a neuron was thought to be to collect information about inputs (integrate), and send this information in the form of action potentials to its downstream peers (see in Buzsáki, 2006). The current view endows a neuron with enormous computational power but the important issue of temporal summation is still relevant. Besides that, temporal relationship between two cells is the substantial matter in Hebb's synaptic plasticity rule. More specifically, when a presynaptic spike and a postsynaptic spike occur within a certain time window, it leads to corresponding plasticity outcomes.

An inhibitory feedforward loop (Fig. 1A) limits the temporal summation of

excitatory postsynaptic potentials (EPSPs) far below the mean interspike interval of principal cells, thus making them precise coincidence detectors (Pouille and Scanziani, 2001). Presumably through somatic inhibition interneurons limit the time window for temporal summation to ~2 ms. A hypothetical scenario of this process is the following. Action potentials in a small number of pyramidal neurons produce a monosynaptic EPSP in neighboring pyramidal neurons, which is rapidly abridged by a disynaptic inhibitory postsynaptic potential (IPSP). In contrast, elimination of inhibitory control by $GABA_A$ antagonists leads to time windows an order of magnitude greater. Therefore, a manipulation of the strength of inhibition will change the principal cell operation mode from precise coincidence detection to integration over a large time window. As a single perisomatic interneuron targets ~1000 principal cells and could suppress their activation by a single IPSP (Miles et al., 1996), basket and axo-axonic cells are in excellent position to regulate timed activity of the hippocampal / cortical network (e.g. synchronize principal cell spiking) (Mann and Paulsen, 2007).

Besides simply providing generalized inhibition, type-specific firing of interneurons during network oscillations are also well characterized (Jinno et al., 2007; Klausberger et al., 2005; Somogyi and Klausberger, 2005; Tukker et al., 2007). The interneurons belonging to different classes fire preferentially at various specific time points during various oscillations (e.g. theta, gamma, high frequency bursts). This would suggest an important role of interneurons in structuring the activity of pyramidal cell discharge. It is also important to consider specific domains of principal cells (proximal or distant dendrites, soma, axon), which different classes of interneurons innervate. Ultimately that would lead to a dynamic spatio-temporal GABAergic control, which is ideally suited to regulate the input integration of individual pyramidal cells and contribute to the formation of cell assemblies and representations in the hippocampus (Somogyi and Klausberger, 2005).

2.3. BRAIN NETWORKS OSCILLATIONS

"Balance of opposing forces, such as excitation and inhibition, often gives rise to rhythmic behavior. Oscillators consisting of only excitatory pyramidal cells also exist, as is the case when GABAergic receptors are blocked pharmacologically. In such case, the frequency of hypersynchronous, epileptic oscillations is determined primarily by the intrinsic biophysical properties of the participating pyramidal cells and the time course of neurotransmitter replenishment after depletion. Under physiological conditions, oscillations critically depend on inhibitory interneurons. In fact, providing rhythm-based timing to the principal cells at multiple time scales is one of most important roles of interneurons." - (Buzsáki, 2006).

2.3.1. Most common oscillations

Since early works of Richard Caton, Adolf Beck and Hans Berger (Berger, 1929; Berger, 1969; Swartz and Goldensohn, 1998), oscillations have been recorded in the brains of numerous mammalian species. Brain rhythms range from ultra slow (with periods of minutes or ~0.01 Hz) to ultra fast (reaching 600 Hz). The first classification was introduced in 1974 by the International Federation of Societies for Electroencephalography and Clinical Neurophysiology (IFSECN, 1974). However, the list is short and incomplete probably because of pragmatic clinical considerations. Nowadays, clinical electroencephalograph (EEG) recording still follows the old tradition and limits the range of recorded frequencies between 0.5 and 70 Hz (or even more narrow). Despite its limitation, that first classification is still widely used: delta corresponds to 0.5-4 Hz, theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), gamma (>30 Hz). However, there is no real frequency border, as the physiology underlying those rhythms maybe influenced by age, species-specific differences, drugs etc. Therefore, the most useful brain oscillation taxonomy would be based on distinct physiological mechanisms involved in particular group of rhythms. Unfortunately, the exact mechanisms of most brain oscillations are not fully understood. As for today, researches are using either the old classification (IFSECN, 1974) or a modernized one, based on an arithmetic progression on the natural logarithmic scale (Penttonen and Buzsáki, 2003). A more recent classification includes also ultra slow and ultra fast frequencies. Either way, exact boundaries between distinct frequency bands may never be drawn.

2.3.2. Mechanisms of network oscillations

As it was mentioned above, the explicit mechanisms of brain oscillation generation are not fully known. The multiple sources of oscillations are possible in such a complex system as the brain. First of all, intrinsic properties of neurons themselves contribute towards oscillation. Neurons can have several oscillatory and resonance properties due to specific expression of voltage-gated channels with opposing properties to depolarize or hyperpolarize the cell. Second, even a simple system of two interconnected neurons (negative feedback, Fig. 1B) will create an oscillatory circuit. This simple wiring may be tuned to different frequencies by manipulating $GABA_A$ receptor responses (for example, prolongation of GABAergic IPSCs will reduce the oscillation frequency). Third, collective action of neurons with a pivotal role of interneurons is known to generate network oscillations. This possibility will be discussed below in detail, mostly when reviewing theta (4-12 Hz) and beta/gamma (20-80 Hz) rhythms, and high frequency bursts (150-250 Hz).

2.3.2.1. Beta/Gamma oscillations

Despite considerable advantages, the mechanisms of gamma oscillations are not fully understood. Data, which have accumulated mostly from hippocampal studies, suggest that GABAergic inhibitory transmission has a major role in the generation of gamma rhythms. A simplified model suggests that two main subtypes of GABA-dependent gamma frequency network activities can be seen in the hippocampus (Whittington et al., 2000). The first, interneuron network gamma (ING), is seen transiently in response to brief periods of direct excitation of populations of interneurons. The second, pyramidal-interneuron network gamma (PING), is seen persistently and does require phasic synaptic excitation of interneurons via α-amino-3-hydroxi-5-methylisoxazole-4-propionic acid (AMPA) receptors. The magnitude of the synaptic inhibition between interneurons governs the frequency of ING. Application of diazepam ($GABA_A$ agonist) to an oscillating brain region increases the amplitude of trains of IPSPs, which generate ING and produce a concentration-dependent decrease in frequency. In contrast, a reduction of IPSP amplitude via the $GABA_A$ receptor antagonist bicuculline, or a reduction of GABA release with morphine, increases the frequency of ING. From this, a prediction can be made that any pharmacological agent or neuromodulator substance that affects the kinetics of the $GABA_A$ response, the amount of GABA released at inhibitory terminals, or the excitability of interneurons themselves, will affect the rhythmicity and frequency of gamma oscillations generated by inhibitory neuronal networks. In addition, the tonic driving force causing the excitation of the interneuron network has to be of sufficient magnitude. As the driving force decreases from optimal, a decrease in the frequency of the population oscillation can be seen until the population oscillation is no longer manifest.

However, both the ING and PING models are an oversimplified view as there are many other factors which influence gamma oscillation in the brain. One of those factors maybe excitatory neurotransmitters. It is known that hippocampal gamma depends on a complex interaction between two oscillatory networks. One is driven by the activation of muscarinic acetylcholine receptors (mAChRs) and the second is driven by the activation of metabotropic glutamate receptors (mGluRs) (Mann and Paulsen, 2005; Palhalmi et al., 2004; Whittington et al., 2000). It has been hypothesized that mAChR activation underlies gamma during theta activity while mGluRs are activated during sharp waves, tetanic stimulation or other large-amplitude events in the hippocampus *in vivo* (Mann and Paulsen, 2005; Whittington et al., 2000).

Another important factor for gamma oscillation generation is the subclass of interneurons involved. Of the large variety of interneuron subtypes particularly important ones for gamma oscillation are the perisomatic inhibitory cells (Freund, 2003; Mann and Paulsen, 2005; Whittington and Traub, 2003). These comprise two types of basket cells: those containing Ca^{2+} -binding protein parvalbumin (PV) and those containing cholecystokinin (CCK). While the assembly of PV-containing cells represents the non-plastic precision

clockwork, the CCK-containing cell assembly is highly modifiable by local neuromodulators (which might allow fine turning of oscillation frequency and amplitude) (Freund, 2003; Klausberger et al., 2005). There are a number of drugs which act differently on PV- and CCK-basket cells. For example, acetylcholine (ACh) excites the CCK-containing cells via nicotinic receptors but inhibit GABA release from the PV-containing cells via presynaptic M_2 receptors (Freund, 2003). As another example, benzodiazepines achieve anxiolysis via potentiating inhibition evoked by CCK-containing ($GABA_A$ receptors mainly with $\alpha 2$ subunit), but not PV-containing ($GABA_A$ receptors mainly with $\alpha 1$ subunit), basket cells (Freund, 2003). *In vitro* studies have found that carbachol induced fast oscillation are enhanced by diazepam (highest affinity to $\alpha 2$ and/or $\alpha 3$ subunit of $GABA_A$) while zolpidem (highest affinity to $\alpha 1$ subunit of $GABA_A$) suppresses oscillations (Palhalmi et al., 2004; Shimono et al., 2000). The importance of parvalbumin-positive interneurons for gamma oscillation has been confirmed by parvalbumin-deficient mice: in hippocampal slice recordings, these mice exhibit increased power of gamma frequency oscillations (Vreugdenhil et al., 2003).

Last but not least important factor in gamma rhythm generation is electrical coupling (gap junction) between neurons (Lamsa and Taira, 2003). Two types of electrical coupling in the hippocampus influence population oscillations. One between distal dendrites of interneuron (at the border between the stratum oriens and the alveus; (Fukuda and Kosaka, 2000; Tamas et al., 2000)) and a second between pyramidal cell axons (suggesting that CA1 pyramidal neurons can be coupled through the contact of processes in the stratum oriens; (Schmitz et al., 2001)). It has been hypothesized that axonal electrical coupling can be used to generate oscillations, and that dendritic gap junctions can be used to sharpen them (Traub et al., 2003). This proposal is based on modeling studies and experimental data from connexin36 knockout mice (Hormuzdi et al., 2001; Pais et al., 2003).

2.3.2.2. Theta oscillations

Similar to gamma oscillations, theta oscillations can be distinguished as atropine-sensitive and atropine-resistant on the basis of pharmacological sensitivity (Kramis et al., 1975). The muscarinic blockers, such as atropine, eliminate theta oscillations in anesthetized animals. In contrast, in the awake rat, the amplitude and frequency of theta oscillation do not change substantially after systemically administered muscarinic blockers. "Classic" theta model in the hippocampus CA1 region assumes two dipoles (current generators) (Buzsaki, 2002). Rhythmic excitation of distal dendrites by entorhinal afferents is assumed to play the most important role in the current generation of extracellular field theta. A second dipole in the CA1 region is assumed to be generated by somatic IPSPs. Cholinergic neurons in the medial septum and diagonal band of Broca (MS-DBB) provide slow depolarization of their targets, pyramidal cells in the CA1 str. lacunusom-moleculare and basket interneuron. At the

same time MS-DBB GABAergic interneurons rhythmically hyperpolarize the basket interneurons.

It is well established that the receptors involved in atropine-resistant type of theta are urethane sensitive (Kramis et al., 1975). This type of theta maybe modeled *in vitro* by co-application of a mGluR agonist and an AMPA receptor antagonist (Gillies et al., 2002). In addition, atropine-resistant component of the hippocampal theta is conveyed by layer II and III entorhinal cortex afferents to the CA1 and CA3/dentate neurons. Although the pharmacological action of urethane is not well understood, it is known to attenuate glutamate release from presynaptic vesicles (Moroni et al., 1981 see in Buzsaki, 2002). However, the theta dipoles mediated by the entorhinal cortex cannot be explained by glutamate activation of pyramidal and granule cells via fast acting AMPA receptors. As Buzsaki review (2002) indicates, *N*-metyl-*D*-aspartate (NMDA) receptors located on the distal apical dendrites are important in spontaneous synaptic events and the maintenance of synaptic function.

One of the possible mechanisms responsible for atropine-sensitive theta is cholinergic modulation of interneurons (Buzsaki, 2002). In this case, tonic cholinergic excitation of interneurons, coupled with their phasic septal GABAergic inhibition, has been suggested to be responsible for the rhythmic discharge of hippocampal interneurons (Freund and Antal, 1988). In support to this view, the remaining theta sinks and sources after a bilateral lesion of the entorhinal cortex are compatible with perisomatic inhibition. This points to an important role of basket inteneurons in theta rhythmogenesis (Reich et al., 2005). As resent findings show, cholecystokinin- and parvalbumin-expressing GABAergic basket cells have different roles *in vivo* in urethane anesthetized rats during theta oscillations (Klausberger et al., 2005). Overall it is agreed that two classes of interneurons are substantial for theta oscillations: i) basket and chandelier cells with perisomatic target, ii) oriens lacunosum-moleculare and hilar interneuron with perforant path axon projection, which specifically innervate the terminal zones of entorhinal afferents (Buzsaki, 2002; Gillies et al., 2002; Klausberger et al., 2003; Traub et al., 2004). In a reduced model, these two interneuron classes alone (interconnected in between) are capable of producing a coherent population theta oscillation (Rotstein et al., 2005). These authors also showed that hyperpolarization-activated h-current is critical for the synchronization mechanism.

Electrical coupling between neurons seems to as important for theta as for gamma oscillations (Whittington and Traub, 2003). As pyramidal cell fire rarely (if at all) during oscillations, the theta oscillation is blocked by NMDA receptor antagonist and manipulation of gap junctions has profound effect on the oscillatory activity, it is reasonable to assume that axonal activity in the pyramidal cell axonal plexus is essential for the those rhythms (Fischer, 2004; Traub et al., 2004). In addition, similar to gamma generators described above, the recurrent network of CA3 pyramidal cells and possibly hilar mossy cells form an intrahippocampal theta oscillator (Buzsaki, 2002).

Altogether, it seems that gamma and theta oscillations mechanisms share many

similarities. Single-cell properties perfectly match circuit features in both principal cells and interneurons. As a result, the multiple theta/gamma oscillation mechanisms can contribute to the computational properties of hippocampal-entorhinal neurons in complex ways (Buzsáki, 2006). However, during normal physiological conditions gamma oscillation are transient (or short lived) while theta is a sustained rhythm (waves occur continuously as long as subject is engaged in the same behavior; see more in Buzsáki, 2006).

2.3.2.3. High frequency (~200 Hz) oscillations

The third major hippocampal pattern includes a ripple complex (fast field oscillation) and its associate "sharp wave". Sharp waves are self-organized endogenous hippocampal events as they occur during waking immobility and sleep. The coordinated discharge of CA3 pyramidal cells depolarizes CA1 pyramidal cells and interneurons, the result of which is a sharp wave in stratum radiatum and a ripple in the pyramidal cell layer (Ylinen et al., 1995). One of the major features of sharp-wave-ripple complex is its widespread effect. In the approximately 100-ms time window of a hippocampal sharp wave, between 50,000 and 100,000 neurons discharge simultaneously in the CA3-CA1–subicular complex–entorhinal axis, qualifying it as the most synchronous network pattern in the brain (Buzsaki and Chrobak, 2005). This number represent 5-15 % of the local population, ten time larger than during theta oscillation (Buzsáki, 2006). Ripple episodes are associated with increased synchrony of pyramidal cells and several classes of interneurons (Klausberger et al., 2003; Ylinen et al., 1995). In addition, axo-axonic interaction also has been show to be important for high-frequency oscillations (Traub et al., 2004). However, the ripple generation is distinct from the mechanisms involved in gamma oscillations, because the power of ripple band in the hippocampal frequency spectrum has a weak if any correlation with power in the gamma frequency band (Buzsaki et al., 2003).

2.3.3. Oscillations and information processing in the brain

The basic unit of information processing in the brain is an action potential. Classical theories viewed brain as a feedforward model, where information in processed in serial steps. The hierarchical organization of the brain was well established by 1950s with basic ideas provided by John Hughlings Jackson already in 1870s (Saper et al., 2000). According to this view, the cortex is organized hierarchically from primary sensory to association areas. This 'bottom-up' connectivity assumes that neurons at the bottom of hierarchy will respond to simples feature of the stimulus while upstream neurons will have 'complex view' by merging representation from 'simple' cells. Ultimately, at the top of hierarchy we would found 'gnostic units' (or 'cardinal neurons') responsible for most complex brain activity. However, a purely feedforward hierarchical model cannot be the whole story. Extensive discussion of this issues

is covered elsewhere (Buzsáki, 2006; Roskies, 1999). Yet in short, the problem outlined by critics are the following. First, the hierarchical model ignores extensive feedback connections in the cortex. Second, there is a 'combinatorial explosion' problem (brain will soon run out of neurons if at least one gnostic cell is required for representation of combination of various object features). Third, anatomical data do not suggest a bottom or a top of the brain, as neuronal connections are organized into infinitive number of loops. Fourth, the feedforward model has limited abilities to compare a newly created representation with knowledge stored about an object or a feature. Fifth, there is a decision-making problem as it is not clear how highly convergent sensory input may lead to extensive divergent output.

An important addition to the hierarchical brain model would be the binding by time solution. The idea of temporal synchrony assumes that functional and anatomical specialization of the brain is brought together by transient synchronization. The temporal binding model assumes that neurons which fire together are bind together, or features which those neurons represent will be bound into a complex representation. Such a temporal integration mechanism would provide an elegant solution to the binding problem, as synchrony would selectively tag the responses of neurons that code for the same object, and demarcate their responses from those neurons activated by other objects (Engel et al., 2001). This highly exclusive temporal structure would allow the system to set up a precise representational pattern (an assembly) for each object. Experimental support for this model was provided almost 20 years ago (Gray and Singer, 1989; Gray et al., 1989). With synchronization oscillatory activity emerges, as neural assemblies have a transient existence that spans the time required to accomplish an elementary cognitive act, but their existence is long enough for neural activity to propagate through the assembly (a propagation that necessarily involves cycles of reciprocal spike exchanges with transmission delays that last tens of milliseconds) (Varela et al., 2001).

From a vast range of oscillations gamma band frequencies are particularly suitable for bringing neuronal population into synchrony. First, a 10-30 ms integration time corresponding to the gamma oscillation appears optimal for discharging a postsynaptic neuron (Harris et al., 2003). This is an important issue as the goal of a synchronized neuronal population is to forward a message to downstream neurons. Second, the same time window is optimal for synaptic modification, such as long-term potentiation (Magee and Johnston, 1997). In a broader context, gamma oscillation may link the 'binding problem' with synaptic plasticity. This is because synchronization by gamma not only does perceptual binding but also stabilizes assemblies representing the current experience.

The complete coverage of information processing in the brain is not possible without inclusion of the 'top-down' processing mode. In its simplest form, this concept means reciprocal or feedback anatomical connections from higher order association areas towards the stimulus perception end (bottom). However, nowadays the 'top-down' concept has much broader meaning than just an idea of a feedback signal flow (Engel et al., 2001; Varela et al.,

2001). An equally important component of 'top-down' processing is endogenous brain activity, which arises from the states of preparation, expectation, emotional context, familiarity with object and attention. Bottom-up and top-down are just concepts for a large-scale network that integrates both incoming and endogenous signaling, and from this interaction emerge synchrony and oscillations. This interaction embraces not only single sensory modalities but also the cross-talk between different brain areas. Although the role of gamma oscillation is well established, the interaction during information processing may occur in different frequency bands, as this brain operation spans on multiple temporal and spatial scales in the nervous system. It seems that high oscillation frequencies (like gamma) are more suitable for local cell population synchronization, while low frequencies (like theta) support long-range coupling (Engel et al., 2001). Moreover, a recent review emphasizes a different role for gamma and theta oscillation in memory formation (Axmacher et al., 2006). However, it is the interaction between theta and gamma that leads to complex learning rules required for realistic formation of declarative memories.

2.4. AUDITORY EVOKED POTENTIALS

Event-related potentials (ERPs) can be obtained by averaging over a large number of EEG epochs that are time locked to a perceptual, cognitive or motor event. This electrical activity of the brain changes rapidly over time and has certain spatial distribution. The magnitude of ERP is typically small in comparison to the amplitude of the ‘background' EEG, especially in human scalp recordings. Since the 1960s, ERPs have provided important insights into perceptual, cognitive and motor functions. Due to high temporal resolution and low cost, ERPs besides EEG remain an essential tool in neuroscience.

2.4.1. Components and latencies

The ERP to auditory (or any other sensory stimulus) may be represented as a series positive-negative waves, "components" (Fig.3). There is no universally accepted definition of what constitutes an ERP component (Otten and Rugg, 2005). Features of the waveform (such as a negative or a positive deflection) can result from summation of several contributing sources. In turn, those sources may not reflect functionally homogeneous neural or cognitive processes. Two extremes of the component definition maybe arbitrary named as "physiological" and "functional" approaches. The first approach (Naatanen and Picton, 1987), assumes that the ERP component should be defined by its anatomical source within the brain. In contrast, the second approach (Donchin, 1981) emphasizes the functional process with which the components are associated. In practice, ERP components are usually defined with respect to both their functional significance and their underlying neuronal source(s) (Otten and Rugg, 2005).

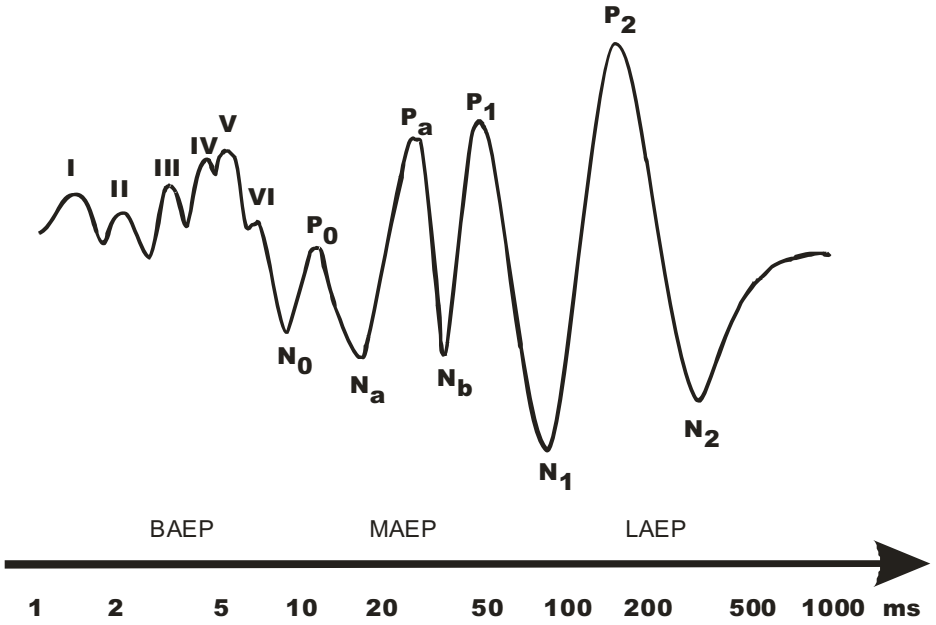

Figure 3. Auditory evoked potentials (AEPs) consist of a sequence of positive and negative peaks which can roughly be divided into three time domains: short or brainstem AEPs (BAEP), mid-latency AEPs (MAEP) and long-latency AEPs (LAEP).

More than thirty years ago, Picton and his colleagues described the principal types of auditory evoked potentials (AEPs) which can be obtained from the human scalp (Picton et al., 1974). This classification scheme groups peaks into three time domains: (1) early or short-latency AEPs which arise within the first 10 ms following the stimulus onset (also now commonly called brainstem AEP (BAEP)); (2) mid-latency AEPs (MAEPs) which are generated between 10 and 50 ms; (3) long-latency AEPs (LAEP). With small modifications the original Picton classification is still widely used nowadays (Shaw, 1995). There is a common agreement on the source of AEPs. According to the classic theory, each wave is generated by the sequential activation of successively higher auditory structures. BAEP is thought to arise principally within the auditory nerve and nuclei of the auditory brainstem (Shaw, 1995). The MAEP is thought to reflect activity mostly in the subcortical structures (such as the colliculus and thalamus) and the auditory cortical areas, while the LAEP is considered to be generated by multiple sources within the auditory cortex and the frontal association areas (Jaaskelainen et al., 2004; Naatanen et al., 2005; Pantev et al., 1995). This classic scheme is widely used in clinical practice as it allows linking ERP waveform abnormalities with particular pathology along the sensory information processing track (Adams and Victor, 1993; Barry et al., 2003; Dorfman, 1983).

2.4.2. Phase resetting of brain oscillation as mechanism of ERP generation

With advancement in computer technology, the averaging method introduced by Dawson introduced in the 1950s became the foundation of a successful experimental program in cognitive and experimental psychology (Hillyard and Kutas, 1983). The assumption behind the averaging procedure is that external stimuli produce a small and constant evoked response, which must be averaged out from much stronger background "noise" EEG. In human scalp recordings, for a reliable extraction of an evoked response hundreds to thousands repetitions are needed, while in animal studies (such as small rodents) this number is an order of magnitude smaller. While the averaging approach is still widely (almost exclusively) used in clinical practice, new mechanisms of ERP generation have been suggested and have gained popularity during the last decade (see references in (Sauseng et al., 2007)). The supporters of the new approach argue that the ERP components are generated by stimulus-induced phase resetting of ongoing oscillatory activity. Despite huge amount of literature advocating for one or the other model of ERP generation neither camp is "winning" (Sauseng et al., 2007), because many of the arguments and methods seem to be unable to dissociate between these two hypotheses.

2.4.3. Auditory gating paradigm

Sensory ERPs have been widely used to examine basic neuronal activity in both normal brain function and disease-related impairments. One of the most widely used stimulation paradigm is so-called Sensory Gating. Normal auditory processing in humans includes a reduced expression in the mid-latency response to the second of two consecutive stimuli. Theoretical considerations of brain function have adverted to such short-term habituation (lasting less than 5 s) as a critical preventive mechanism that protects the limited short-term-memory systems of the brain from overflow by excessive sensory information (Broadbent, 1971). Studies in laboratory animals show a similar strong attenuation in the sensory gating paradigm when recorded from skull surface or in the hippocampus (Bickford-Wimer et al., 1990). Typically, the amplitude of the second response is dramatically reduced, with the maximum reduction being observed around a 500 ms interval between the stimulus pairs (Bickford et al., 1993).

As auditory information reaches hippocampus via two pathways, it is interesting to note that only the non-lemniscal route conveys sensory gating information. In animal studies, recordings from the brainstem reticular nucleus, medial septum and hippocampus show significantly greater gating than the auditory cortex (Bickford et al., 1993; Miller and Freedman, 1993; Moxon et al., 1999; Vinogradova, 1975). Sensory gating is a complex, multisynaptic process and the underlying mechanisms are not fully understood. However, some clues are provided by human as well as animal studies. All those studies point to an

important role of inhibitory interneurons. A series of experiment in rats demonstrated that the inhibition of a response at a 500 ms interval occurs due to presynaptic inhibition (Miller and Freedman, 1995). Furthermore, this inhibitory response must be mediated by $GABA_B$ receptors, because recurrent inhibitory pathways that activate $GABA_A$ receptors on hippocampal pyramidal cells account only for short-term gating of the response to a repeated stimulus (Hershman et al., 1995). The role of interneurons in sensory gating is further supported by human studies. In particular, it has long been known that schizophrenic subjects and some of their relatives demonstrate abnormal sensory gating (Freedman et al., 1996). As a resent review outlined, "some form of dysfunction in the brain's GABAergic system appears to be present in the cortex of schizophrenics" (Benes and Berretta, 2001). Even more straightforward link between abnormal sensory gating and inhibitory interneurons is the genetic factor. The failure to inhibit the AEP in human subjects has been linked to the $\alpha 7$ nicotinic receptor subunit gene (Freedman et al., 1997). This receptor is expressed in interneurons while pyramidal cell rarely show nicotinic responses (Jones and Yakel, 1997; McQuiston and Madison, 1999). Furthermore, the expression of $\alpha 7$ nicotinic receptors is restricted to certain subtypes of hippocampal interneurons, those containing neuropeptide Y (NPY), somatostatin (SOM) or cholecystokinin (CCK) (Freedman et al., 1993). In addition, treatment with nicotine (which is an agonist at the $\alpha 7$ nicotinic receptor) restores auditory sensory gating in schizophrenic patient and fimbria-fornix lesioned rats (Adler et al., 1993; Bickford and Wear, 1995; Stevens and Wear, 1997).

2.5. MOUSE MODELS OF INTERNEURON PATHOLOGY

Genetically modified mice are a primary tool in modern neuroscience to study the specific functional role of certain wild-type and mutated proteins. The possibility to develop transgenic or knockout mouse models for testing a specific hypothesis is very attractive. However, usually it is not straightforward to link variable behavioral observations to pin-pointed changes at the molecular level. Besides behavioral phenotypic characterization of new mouse strains we need methods to directly assess the brain function (sensory or cognitive). One possible such approach is electrophysiological measurements. Electroencephalography (EEG) can be used to test general excitation and inhibition processes in the brain, while event–related potentials (ERPs) can be used to test brain activity ranging from sensory reception to higher cognitive processes (such as learning and memory). Because of ethical limitations, in most cases human EEG or ERP studies are non-invasive (scalp recording), while animal experiments may use deep as well as surface recording. This helps better understand the surface EEG regarding signals generated in deep brain structures (such as the hippocampus).

2.5.1 Transgenic mice expressing APPswe and PS1-A264E mutations (APP/PS1)

Alzheimer's disease (AD) is the most common neurological disorder in elderly individuals. Clinically it is characterized by a progressive impairment in cognitive function along with numerous other symptoms. The pathological hallmarks of AD are beta amyloid (Aβ) deposits, hyperphosphorylation of microtubules associated protein tau and formation of neurofibriallary tangles, degeneration of synapses, and loss of neurons (Selkoe, 2001). Transgenic mice expressing mutated human amyloid precursor protein (APP) and presenilin-1 (PS1) genes mimic certain neuropathological features of AD. These mice have elevated levels of the highly fibrillogenic amyloid beta1-42 peptide and develop amyloid plaques around the age of 9 months.

While loss of cholinergic cells and degeneration of cholinergic projection is hypothesized to play a major role in AD-related cognitive decline (Bartus et al., 1982), dysfunction or loss of interneurons has also been noted. Specifically, neuronal depletion of calcium-dependent protein calbindin in the dentate gyrus has been reported in the brains of AD patients, a mouse model of AD and aging dogs (Palop et al., 2003; Pugliese et al., 2004). Furthermore, loss of SOM and/or NPY in AD patients is a well-reproduced observation (see references in Ramos et al., 2006). Degeneration of the dendritic inhibitory interneurons expressing SOM and NPY has also been reported in a mouse model of AD and aging rats (Ramos et al., 2006; Vela et al., 2003). Considerable attention is focused on nicotinic acetylcholine receptors (nAChRs), which are preferentially expressed on the interneurons rather than the principal cells (Jones and Yakel, 1997; McQuiston and Madison, 1999), and particularly on the α7 subtype. It has been suggested that Aβ peptide may disrupt α7 receptor function in AD due to its high-affinity binding and co-localization with α7 receptor in post-mortem AD tissue (Wang et al., 2000a; Wang et al., 2000b). Whether Aβ binding inhibits or activates the α7 receptor remains controversial (Dineley et al., 2001; Pettit et al., 2001; Spencer et al., 2006) but the balance between excitation and inhibition in the brain will be disturbed.

2.5.2. Tenascins and development of interneuron networks

The architecture of a tissue is determined by recognition mechanisms that involve not only cell-cell interactions but also interactions between cells and the extracellular matrix (ECM). An ECM of collagens, proteoglycans and glycoproteins surrounds the glial cells, neurons and appears in the synaptic terminals. Molecules in the matrix do not only interact with each other - they also activate signal transduction pathways through diverse cell-surface receptors. These pathways coordinate cell functions such as proliferation, migration and differentiation. In the nervous system, they also coordinate synaptogenesis and synaptic activity (Dityatev and Schachner, 2003). The role of ECM constituents has been extensively

studied over the past few decades in knockout animal models. We focus on a few animal models which showed alternation in interneuron network in the brain.

2.5.2.1. Mice deficient in the extracellular matrix glycoprotein tenascin-R

Tenascin-R (TNR, Fig.4a) is an extracellular matrix molecule that has been implicated in axon growth and guidance (Faissner, 1997), neuronal migration, neuritogenesis (Bartsch, 1996; Schachner et al., 1994), and myelination (Bartsch et al., 1993; Wintergerst et al., 1993). It binds to voltage-dependent sodium channels and regulates their conductance (Srinivasan et al., 1998; Xiao et al., 1999). TNR is an important constituent of perineuronal nets surrounding some inhibitory interneurons (Bruckner et al., 2000), most notably parvalbumin-positive interneurons that mediate perisomatic inhibition (Wintergerst et al., 2001). The distribution of extracellular matrix molecules associated with perineuronal nets is altered in TNR deficient (TNR-/-) mice (Bruckner et al., 2000; Weber et al., 1999). Previous *in vitro* studies indicate that TNR and its associated HNK-1 carbohydrate are involved in the modulation of perisomatic inhibition and long-term potentiation (LTP) in the CA1 region of the hippocampus (Saghatelyan et al., 2000; Saghatelyan et al., 2001; Saghatelyan et al., 2003). TNR-/- mice display reduced perisomatic inhibition and increased basal excitatory synaptic transmission in synapses formed on CA1 pyramidal neurons, possibly resulting in an impaired NMDA receptor dependent form of LTP despite normal NMDA receptor-mediated currents. In behavioral studies, TNR-/- mice display deficits in motor coordination, hypoexploration, and increased anxiety (Freitag et al., 2003). The number and density of parvalbumin-positive interneurons (basket and chandelier cells) that account for the perisomatic inhibition are apparently normal in TNR-/- mice (Saghatelyan et al., 2001). However, the number of terminals forming symmetric synapses on the CA1 pyramidal cell somata in TNR-/- mice are reduced by 30–40% compared with their wild-type (WT) controls (Nikonenko et al., 2003). One proposed model for the lack of perisomatic inhibition in TNR-/- mice is the relief of $GABA_B$ receptors from their inhibition by the HNK-1 carbohydrate, the level of which is reduced in TNR-/- mice. Sustained activation of $GABA_B$ receptors may result in elevated levels of extracellular K+, which in turn can inhibit evoked GABA release and $GABA_A$ receptor-mediated inhibition (Fig.4d) (Saghatelyan et al., 2003). However, the mechanisms underlying reduced perisomatic inhibition remain to be elucidated.

2.5.2.2. Mice deficient in the HNK-1 sulfotransferase

The HNK-1 carbohydrate (Fig.4b) (a structure containing a 3′-sulfated glucuronic acid and first discovered on human natural killer cells; hence the name) is carried by many recognition molecules (Kruse et al., 1984), including immunoglobulin (Ig) superfamily members such as the neural cell adhesion molecule (NCAM) (Kruse et al., 1984), P0 (Voshol

et al., 1996), L1 (Faissner, 1987) and F3/F11/contactin (Gennarini et al., 1990), as well as integrins (Pesheva et al., 1987), proteoglycans (Gowda et al., 1989; Xiao et al., 1997) and the ECM glycoproteins tenascin-C and tenascin-R (Kruse et al., 1985). The HNK-1 carbohydrate and its carrier molecules play functionally important roles in neural development, neurite outgrowth, synaptic plasticity, and mediate neuronal cell adhesion (for review, see (Dityatev and Schachner, 2003; Kleene and Schachner, 2004). More recent studies have shown that mice deficient in HNK-1 synthesis due to genetic ablation of the HNK-1 sulfotransferase or a glucuronyltransferase are abnormal in hippocampus-dependent spatial learning and have reduced LTP in area CA1 of the hippocampus (Senn et al., 2002; Yamamoto et al., 2002). Despite the temporally and spatially broad expression of the HNK-1 carbohydrate moiety in many neural and non-neural tissues, mice deficient in HNK-1 sulfotransferase (ST-/- mice; HNK-1 sulfotransferase (ST) is the enzyme that is responsible for transferring the sulfate residue to the terminal glucuronic acid of the HNK-1 core carbohydrate (Schmitz et al., 1994)) appear to develop and behave overall normally (Senn et al., 2002). In particular, analyses of the brain, spinal cord, retina and femoral nerve of ST-/- mice have not revealed abnormalities either at the macroscopic or at the microscopic level. Pertinent to a putative role of HNK-1 in brain electrical activity is the observation that somata and perisomatic synapses of interneurons in wild-type animals are embedded in an HNK-1 carbohydrate-rich extracellular matrix, the perineuronal nets (Ren et al., 1994; Weber et al., 1999; Yamamoto et al., 1988).

Earlier *in vitro* studies have suggested an important role of the HNK-1 carbohydrate and its most prominent carrier, the ECM glycoprotein TNR, in the regulation of inhibitory transmission. Application of monoclonal HNK-1 antibodies to hippocampal slices leads to a decrease in the $GABA_A$ receptor-mediated perisomatic inhibitory postsynaptic currents in CA1 pyramidal neurons of wild-type mice, but has no effect in mice deficient in the HNK-1 carrying glycoprotein tenascin-R (Saghatelyan et al., 2000). Similarly, amplitudes of unitary perisomatic inhibitory currents in CA1 pyramidal neurons are smaller, whereas basal excitatory synaptic transmision is elevated in the CA1 region in tenascin-R deficient mice (TNR-/-) (Saghatelyan et al., 2001). More recently, we have demonstrated alterations in neural network oscillations and increased amplitudes of evoked potentials *in vivo* in both the hippocampus and the cerebral cortex of TNR-/- mice (Gurevicius et al., 2004).

Previous studies showed that the HNK-1 epitope binds to the $GABA_B$ receptor, and tenascin-R is most likely a major carrier of HNK-1 in the mouse hippocampus (Fig.4c) (Dityatev and Schachner, 2003). It has been proposed that reduced perisomatic inhibition in TNR-/- mice results from diminished HNK-1-dependent $GABA_B$ receptor inhibition (Saghatelyan et al., 2003). Sustained activation of $GABA_B$ receptors may result in elevated levels of extracellular K^+, which in turn can inhibit evoked GABA release and $GABA_A$ receptor-mediated inhibition (Fig.4d) (Saghatelyan et al., 2003). In support of the first mechanism, mice deficient in expression of the HNK-1 sulfotransferase (ST) show moderate

increase in basal transmission in the CA1 region of the hippocampus *in vitro* (Senn et al., 2002).

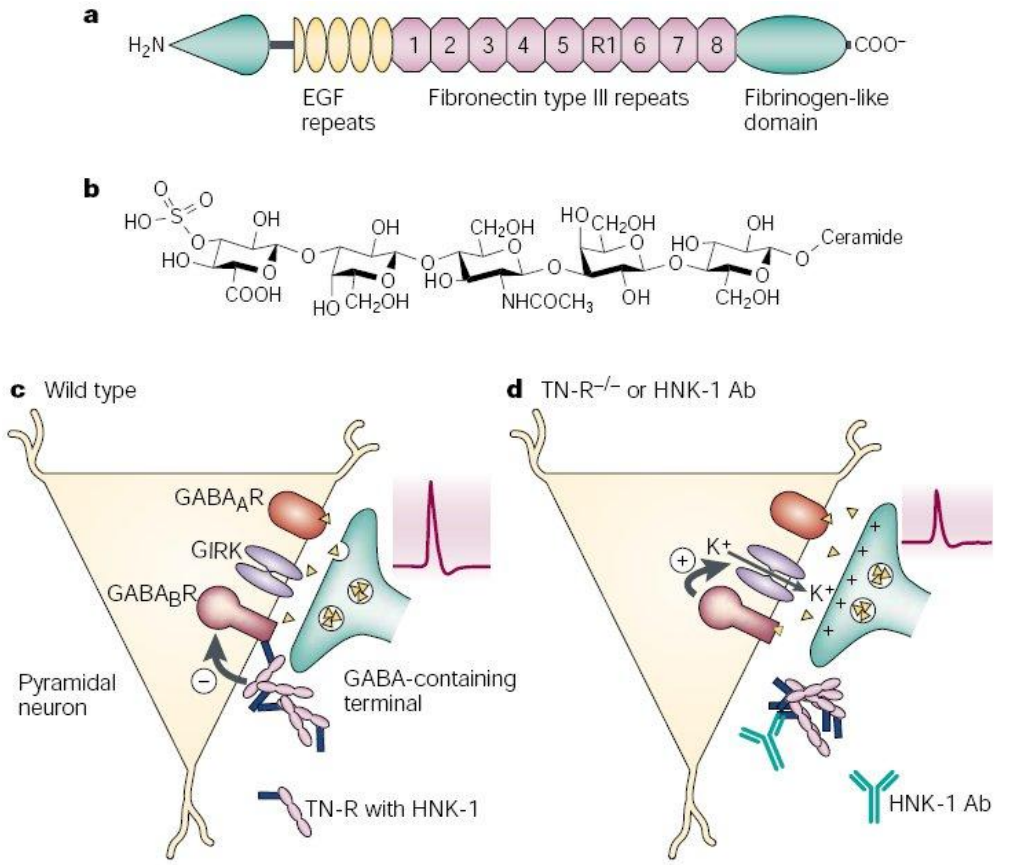

Figure 4. Regulation of perisomatic inhibition in the CA1 region of the hippocampus by glycoprotein tenascin-R and its associated HNK-1 carbohydrate. a | Domain structure of tenascin-R. EGF, epidermal growth factor; R1, alternatively spliced exon. b | Chemical structure of an HNK-1-carbohydrate-carrying glycolipid. c | Hypothetical mechanism by which TN-R and associated HNK-1 regulate perisomatic inhibition. d | Genetic ablation of TN-R or application of monoclonal antibody (Ab) directed against the HNK-1 carbohydrate neutralizes the inhibition of postsynaptic $GABA_BRs$ by the HNK-1 carbohydrate. GIRK, G-protein-coupled inwardly rectifying K+ channel. Adapted by permission from Macmillan Publishers Ltd: [Nature Reviews Neuroscience] (Dityatev and Schachner, 2003), copyright 2003.

2.5.2.3. Mice deficient in the extracellular matrix glycoprotein tenascin-C

Tenascin-C (TNC), an extracellular matrix molecule of the tenascin family of glycoproteins, is abundantly expressed in neural and non-neural tissues during normal development, repair processes in the adult organism and tumorigenesis (Bartsch, 1996; Faissner and Schachner, 1995; Jones and Jones, 2000). Despite the functional importance of TNC, the gross morphology of the central nervous system and the histological appearance of

the neocortex and hippocampus are not detectably affected in TNC deficient (TNC-/-) mice (Evers et al., 2002; Saga et al., 1992). However, stereological analyses have revealed abnormal cellular composition of the neocortex of TNC-/- mice indicating that TNC expression is essential for normal cortical development (Irintchev et al., 2005). It has been suggested that the observed structural aberrations, in particular the reduced ratio of inhibitory to excitatory neurons, may underlie functional deficits such as enhanced cortical responses to somatosensory stimuli in anaesthetized TNC-/- mice.

Nowadays, the connection between TNC expression and synaptic plasticity is well established. The first study of this kind found that TNC was upregulated in the hippocampus, both at the mRNA and protein levels, within hours after stimulation of synaptic activity (Nakic et al., 1998). Later studies in TNC deficient mice showed a reduction of CA1 LTP after stimulation of Schaffer collaterals, whereas CA1 long-term depression was completely abolished (Evers et al., 2002). Furthermore, recording of LTP in the presence of nifedipine, an antagonist of L-type voltage dependent Ca^{2+} channels (L-VDCC), show no effect on TNC deficient mice, but reduced LTP in wild-type mice to the levels seen in the mutant. These findings imply a link between L-VDCCs and TNC in the regulation of synaptic plasticity (for resent review see Dityatev and Schachner, 2006).

3. AIMS OF THE STUDY

The first aim of this project was to develop new tools for the analysis of EEG and event-related potentials (ERP) in mice, which has been studied little so far. The second aim was to validate certain EEG and ERP changes as a measure of the imbalance in the excitation and inhibition processes in the brain. Eventually our aim was to make a direct connection between known manipulation at the molecular level and the electrical signaling of the brain at the systems level. These results will provide the research community with desperately needed new tools to directly assess functional consequences of molecular level changes in the brain.

4. MATERIAL AND METHODS

4.1. ANIMALS

4.1.1. Transgenic mice expressing APPswe and PS1-A264E mutations (APP/PS1)

These experiments were conducted using male APP and PS1 single- and APP/PS1 double-mutant mice. Founders for the mouse lines harboring human PS1 A246E mutation or chimeric mouse/human APP695 mutations (K595N and M596L) were generated by Dr. D. Borchelt at Johns Hopkins University (Baltimore, MD, USA). The mice were of hybrid origin (C57BL/6J×C3H) but were back-crossed to C57BL/6J for six generations. The double-mutant mice were produced through mating between single-mutant transgenic mice of each line. The numbers of animals used in the study were as follows: Experiment 1, APP/PS1 (n=20) and their negative littermates NT (n=21); Experiment 2, APP/PS1 (n=10), NT (n=10), APP (n=8) and PS1 (n=10). The mice were bred in the National Laboratory Animal Center, Kuopio, Finland. The mice were housed individually after the surgery in controlled environmental conditions (21±1°C, humidity at 50±10%, light period 07:00–19:00). Food and water were available *ad libitum*.

4.1.2. Mice deficient in the extracellular matrix glycoprotein tenascin-R (TNR)

Male TNR deficient (Weber et al., 1999) (TNR−/−, n = 8, weight 37.9 ± 1.5 g) and age-matched wild type littermates (WT, n = 7, weight 34.0 ± 1.1 g) from heterozygous breeding pairs (mixed C57BL/6J × 129Ola background, two backcrosses into C57BL/6J) were reared in groups of 4–6 until 4 months of age and individually thereafter in a controlled environment (temperature +21°C, lights on from 7:00 to 19:00 h, water and food available *ad libitum*). The genotypes of mice from the heterozygous breedings were determined by PCR analysis as described earlier (Weber et al., 1999). The mice were bred at the Zentrum für Molekulare Neurobiologie (University of Hamburg, Germany) and shipped to the Department of Neuroscience and Neurology (University of Kuopio, Finland) at the age of 6 months.

4.1.3. Mice deficient in the HNK-1 sulfotransferase (ST)

Male mice deficient in the HNK-1 sulfotransferase (ST) (ST−/−, n = 9, mean weight 35.7 ± 1.0 g SEM) and age-matched wild-type littermates (ST+/+, n = 10, mean weight 35.7 ± 2.0 g) from heterozygous breeding pairs were used in these experiments. The ST−/− mice were originally C57BL/6J × 129SvJ hybrids, but were backcrossed to the C57BL/6J background for 8 generations. The mice were reared in groups of 4–6 until 4 months of age and individually thereafter in a controlled environment (temperature + 21°C, light period 7:00

- 19:00, water and food available *ad libitum*). The genotypes of offspring were identified by multiplex PCR analysis as described earlier (Senn et al., 2002). The mice were bred at the Zentrum für Molekulare Neurobiologie (University of Hamburg, Germany) and shipped to the Department of Neuroscience and Neurology (University of Kuopio, Finland) at the age of 4 months.

4.1.4. Mice deficient in the extracellular matrix glycoprotein tenascin-C (TNC)

Male TNC deficient (Evers et al., 2002; had one backcross) (TNC-/-, n=9, weight mean 34.2 ± 0.8 g) and age-matched wild type littermates (WT, n=9, weight 34.2 ± 0.9 g) from heterozygous breeding pairs (mixed C57BL/6J x 129SvJ background, five backcrosses into C57BL/6J) were reared in groups of 4-6 until 4 months of age and individually thereafter in a controlled environment (temperature +21°C, light period 7:00 - 19:00, water and food available *ad libitum*). The genotypes of mice from the heterozygous breedings were determined by PCR analysis as described earlier (Evers et al., 2002). The mice were bred at the Zentrum für Molekulare Neurobiologie (University of Hamburg, Germany) and shipped to the Department of Neuroscience and Neurology (University of Kuopio, Finland) at the age of 4 months.

4.2. ELECTROPHYSIOLOGICAL RECORDINGS

4.2.1. Surgery

At 5–11 months of age, the mice were chronically implanted with a bundle of two-three recording electrodes (stainless steel wire of 50-µm / 100-µm diameter) in the hippocampus at the following stereotaxic coordinates: AP − 2.2 mm (from the bregma), ML 1.6 mm (from the midline), DV 1.5-1.7 mm (from the dura mater surface) with a vertical separation of the tips of 0.5 / 0.3 mm). In addition, a cortical screw electrode was fixed on the occipital bone at A − 0.8 mm and ML - 1.5 mm (from the lambda), and two frontal screws served as the indifferent and ground electrodes. The mice were anesthetized with a mixture of pentobarbital and chloralhydrate (30-50 mg/kg, i.p.), and, for post-operative analgesia, they received 0.1 mg/kg of buprenorphine (Temgesic©, Reckitt & Colman, Hull, UK) (s.c.) or 5 mg/kg of carprofen (Rimadyl®, Vericore, Dundee, UK) (i.p.) immediately after surgery, and in some cases if needed in the drinking water (carprofen) during two post-operative days. Recordings started after 2-3 weeks of recovery period. The experiments were conducted according to the Council of Europe (Directive 86/609) and approved by the State Provincial Office of Eastern Finland.

4.2.2. EEG / AEP data acquisition

The mouse was placed into a 20 cm x 35 cm wide and 15 cm high plastic box. The EEG was recorded for 3 min during constant movement (free exploratory behavior). Another 3 min time period was recorded during light non-REM (NREM) sleep (not for APP/PS1 mice). This state, which was reached after the animal had remained in the box for 30-100 minutes, was recognized by behavioral immobility, reduced EEG power and/or by 200 Hz ripples in the hippocampal channels. Either four (in three last projects) different channels were recorded (the three hippocampal and the occipital cortical screw electrodes were compared to the reference and ground screws). Or two (in APP/PS1 projects) different channels were recorded (cortical AEP was recorded between the posterior and frontal cortical screw electrodes, and the hippocampal AEP was recorded between the long and short intrahippocampal electrodes; the frontal screw also served as the ground electrode). The signal was analog filtered for frequencies between 1 and 1000 Hz (or 0.1-100 Hz), amplified (x 1000 - 9000), digitized at 2 kHz per channel and processed with fast Fourier transformation (FFT). AEPs were evoked using a pair of clicks (duration 10 ms, 70 dB, 500 ms between the pairs, inter-stimulus interval 10 s). During AEP recordings the mouse stood still in a narrow space between two vertical metal plates. The mouse was continuously observed and all records from the mouse while moving were excluded from further analysis. A total of 30 responses were sampled and averaged. The signal was analog filtered for frequencies between the 1 and 300 (or 500) Hz, amplified (x 1000 - 9000), and digitized at 2 kHz per channel for further processing. Experiment were conducted with the aid of either Experimenter's WorkBench or SciWorks-Experimenter computer program (both DataWave Technologies, Longmont, CO, USA)

At the end of the experiment, the mouse was killed by cervical dislocation, and the sites of the electrode tips were marked by passing a 30 μA anodal current for 5 s through each hippocampal electrode. Subsequently, the brains were immersion fixed overnight with 4% formalin and sectioned at 30 μm with a freezing microtome. The sites of the electrolytic lesions were verified in coverslipped sections stained with cresyl violet.

4.2.3. Electrophysiological data analysis

Offline analysis, including FFT and frequency band filtering, were conducted using MATLAB® (Mathworks, Natick, MA, USA) or Experimenter's WorkBench (DataWave Technologies, Longmont, CO, USA) computer program. Waveform or FFT spectrum averaging and AEP peak detection were conducted by custom made routines in Visual Basic under Microsoft Excel®.

Cortical EEG analysis was based on FFT. The FFT spectrum between 1-20 Hz was averaged to 2 Hz bins and if applicable, the upper frequency ranges (20-100 Hz) were

averaged to 10 Hz bins. The group differences were compared bin by bin using Student's t-test. The hippocampal EEG analysis was based on previously identified main oscillatory components, the theta (4-12 Hz) (Kramis et al., 1975), gamma (30-80 Hz) (Bragin et al., 1995; Wang and Buzsaki, 1996), and high-frequency ripples (180-200 Hz) (Ylinen et al., 1995). The theta peak was defined as the highest power between 5 and 12 Hz. The frequency of the maximum power was also determined. The gamma power was calculated *en block* between 30 Hz and 80 Hz. For the detection of high-frequency ripples, the signal was first filtered with Symlets wavelet (approximately in the range 128 to 256 Hz; adapted from "Uvi_Wave" toolbox for MATLAB). The ripples were defined as events with at least three consecutive cycles crossing the threshold of the mean variance of the baseline amplitude for the original signal. As ripples are never detected during movement, we used their appearance in the EEG recording as an objective measure for time periods of alert immobility or sleep. The theta and gamma rhythms were analyzed from the all hippocampal electrode (fissure/outer molecular layer), while the ripples were analyzed from the electrode closest to the pyramidal cell layer.

The AEP in the mouse typically had three middle-latency components P20, N30, P40 (in the hippocampus) and P50 (in the cortex) (for details see publications III-V). The amplitude of these components was calculated as a deviation from the baseline as follows: first, the baseline was calculated for each mouse from the averaged response between 0 and 100 ms before stimulus onset. Second, the amplitude of each peak was calculated as distance between the baseline and the absolute maximum. When calculating habituation of the AEP (auditory gating), we focused on the middle-latency components only, and further used two parameters as described previously (Bickford-Wimer et al., 1990). One parameter was the amplitude difference between the P20 and N30 peaks, and the other parameter, correspondingly, the amplitude difference between the P40 (or P50) and N30 peaks. Also single peak amplitudes were compared. Paired pulse ratio of the AEPs was calculated by dividing the response amplitude to the second stimulus ($A_{2,}$ test stimulus) by the response amplitude to the first stimulus ($A_{1,}$ conditioning stimulus), and was converted into % by multiplying the values by 100.

4.3. BEHAVIORAL TESTING

4.3.1. Automated activity test

TruScan (Coulbourn Instruments, Allentown, PA, USA) automated activity monitor based on infrared photo detection was used for monitoring exploratory activity. The system consists of a transparent observation cage (26 x 26 x 39 cm) and two rings of photo detectors enabling separate monitoring of horizontal (XY-movement over time) and vertical activity (rearing). Activity was measured for 10 min first day and 10 min after 48 hours.

4.3.2. Water maze

The Morris water maze was used to measure spatial learning and memory. The apparatus was a black plastic pool with a diameter of 120 cm. A black escape platform (square, 14 x 14 cm) was located 1.0 cm below (hidden) the water surface. The temperature of the water was kept constant throughout the experiment (20 ± 0.5°C), and a 10-min recovery period was allowed between the training trials. First, the mice were pre-trained to find and climb onto the platform for 2 days by using an alley (1 m x 14 cm x 25 cm) leading to the platform located 1 cm below the water. The training consisted of 4 consecutive days of testing, with 5 trials per day. If the mouse failed to find the escape platform within the maximum time (60 s), the animal was placed on the platform for 10 s by the experimenter. During the first 3 days of testing, the mice were trained with a hidden platform. The platform location was kept constant, and the starting position varied between four constant locations at the pool rim. The mouse was placed in the water with their nose pointing toward the wall at one of the starting points in a random manner. On the last trial (5^{th}) of the 4^{th} day, the platform was removed, and the mouse was allowed to swim for 60 s to determine its search bias. 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. During the water maze training, we measured swimming speed and latency to find the platform. The wall-swimming tendency (thigmotaxis) was assessed by dividing the pool into three concentric zones of equal surface area (wall zone, platform zone and center) and calculating the time spent in the wall zone. Search bias during the probe trial was measured by dividing the pool area into four quadrants and calculating the time in each quadrant, as well as considering time spent in each zone.

4.4. MORPHOLOGICAL ANALYSES

Morphological analyses were done by collaborators from Hamburg, Germany.

Tissue preparation and immunohistochemistry were performed as described (Irintchev et al., 2005). The mice were anaesthetized with sodium pentobarbital (Narcoren®, Merial, Hallbermoos, Germany, 5 µl g-1 body weight, i.p.) and fixed by transcardial perfusion with 4% formaldehyde and 0.1% CaCl2 in 0.1 M cacodylate buffer, pH 7.3. The brains were post-fixed overnight (4°C) in the same fixative supplemented with 15% sucrose and then immersed in 15% sucrose solution in cacodylate buffer for an additional day at 4°C. The brains were frozen in 2-methyl-butane (isopentane) pre-cooled to -30°C. Serial coronal sections of 25 µm thickness were cut in a caudal-to-rostral direction on a cryostat (Leica CM3050, Leica Instruments, Nußloch, Germany). Sections from 1 mm tissue thickness were collected on a series of 10 SuperFrost®Plus glass slides (Roth, Karlsruhe, Germany) so that 4

sections 250 μm apart were present on each slide. Prior to the immunofluorescence staining, antigen retrieval using 0.01 M sodium citrate solution (pH 9.0) was done in a water bath (80°C, 30 min). Blocking of non-specific binding sites was performed for 1 hour at RT using phosphate-buffered saline (PBS, pH 7.3) containing 0.2% Triton X-100, 0.02% sodium azide and 5% normal goat serum. Incubation with mouse anti-parvalbumin (PV, clone PARV-19, Sigma, Taufkirchen, Germany, 1:1000) or rabbit anti-S-100 (DakoCytomation, Hamburg, Germany, 1:500) in PBS containing 0.5% lambda-carrageenan (Sigma) and 0.02% sodium azide, was carried out at 4°C for 3 days. After wash in PBS (3 x 15 min at RT), goat anti-mouse or anti-rabbit antibody conjugated with Cy3 (Jackson ImmunoResearch Laboratories, Dianova, Hamburg, Germany) diluted 1:200 in PBS-carrageenan solution was applied for 2 hours at RT. Finally, cell nuclei were stained for 10 min at RT with bis-benzimide solution (Hoechst 33258 dye, 5 μg ml-1 in PBS, Sigma) and sections were mounted under coverslips with Fluoromount G (Southern Biotechnology Associates, Biozol, Eching, Germany). For analysis of GABAergic terminals, double immunostaining for PV and vesicular GABA transporter (VGAT) was performed by mixing the primary antibody against PV with a rabbit polyclonal antibody against VGAT (Synaptic Systems, Göttingen, Germany, 1:1000) and using a Cy3-conjugated anti-mouse and a Cy2-labeled anti-rabbit secondary antibody pre-absorbed with rabbit and mouse serum proteins, respectively (multiple-labelling grade antibodies, Jackson ImmunoResearch).

Numerical densities (number per unit volume) of parvalbumin-positive (PV+) cells were estimated using the optical dissector method on an Axioskop microscope (Zeiss, Oberkochen, Germany) equipped with a motorized stage and Neurolucida software-controlled computer system (MicroBrightField Europe, Magdeburg, Germany) as described (Irintchev et al., 2005; Nikonenko et al., 2006). The volume of the dorsal hippocampus and its subdivisions were estimated using spaced-serial sections (250 μm interval) and the Cavalieri principle (Nikonenko et al., 2006). The borders of the hippocampal regions were outlined on the basis of the nuclear staining pattern (Plan-Neofluar® 10x/0.3 objective). Border definitions were those shown in the mouse brain atlas (Franklin and Paxinos, 1997). The small CA2 subfield was added to the CA3 area and the border between CA3 and the dentate gyrus, not shown in the atlas, was defined by straight lines connecting the lateral end of the hippocampal fissure and the lateral tips of the granular layer. The numerical density of PV+ and S-100+ cells was estimated by counting nuclei of labeled cells within systematically randomly spaced optical dissectors. The parameters for this analysis were: guard space depth 2 μm, base and height of the dissector 3600 μm2 and 10 μm, respectively, distance between the optical dissectors 60 μm, objective 40x Plan-Neofluar® 40x/0.75. The same parameters were used for the counting of nuclei in the pyramidal layer except for the base of the dissector which was 625 μm2 and the space between dissectors (25 μm). Nuclei of glial cells in the pyramidal layer were easily recognized and were not counted. Left and right hippocampal areas were evaluated in 4 sections each. Left and right hippocampal areas were evaluated in 4 sections each. Total cell

numbers were calculated by multiplying cell densities by volume estimates. All results shown are averaged bilateral values.

Estimation of perisomatic puncta and area of pyramidal cell bodies was performed as described (Nikonenko et al., 2006). Stacks of images of 1 μm thickness were obtained from sections double-stained for PV and VGAT on a LSM 510 confocal microscope (Zeiss) using 63x oil immersion objective and 1024 x 1024 pixel digital resolution. One merged image (red and green channel) per cell at the level of the largest cell body cross-sectional area was used to measure soma perimeter and area and to count individually discernible perisomatic puncta. Numbers of PV+VGAT+ and PV-VGAT+ puncta, were normalized to the perimeter of the cell profile. These measurements were performed using with UTHSCSA ImageTool 2.0 software (University of Texas, San Antonio, TX, USA, http://ddsdx.uthscsa.edu/dig/). Morphological analyses were performed on coded preparations by one observer. Two-sided t test was used to compare mean group values with a threshold level for acceptance of significance of 5%.

5. RESULTS

5.1. ELECTROPHYSIOLOGICAL FINDINGS

Summary of the results for all studies is provided in Table1. Below are details of findings for each mouse model tested.

Table 1. Electrophysiological finding summary for all mice model.

Parameters to compare	APP/PS1 mice[1,2]	TNR-/- mice[3]	ST -/- mice[4]	TNC-/- mice[5]
Power of cortical EEG θ	↓	↑	≈	↑
Power of cortical EEG β/γ	↑	↑	**only β** ↑	↑
Changes in 200 Hz ripples	**N/A**	**no**	**no**	**no**
Power of hippocampal γ	↑	↑	↓	↑
Power of hippocampal β	↑	≈	↑	↑
Power of hippocampal θ	≈	≈	≈	↑
Frequency of hippocampal θ	≈	↓	≈	≈
Laminar specific hippocampal EEG change	**N/A**	**no**	**no**	**yes**
AEP amplitude	≈	↑	≈	≈
Paired pulse ratio of the AEP	↑	↑	≈	≈
Latency shift of AEP components	↑ **(ctx)**	**no**	↓ **(hipp)**	**no**

[1] data from Wang et al., 2002 (study I)

[2] data from Wang et al., 2003 (study II)

[3] data from Gurevicius et al., 2004 (study III)

[4] data from Gurevicius et al., 2007 (study IV)

[5] data from Gurevicius et al. (study V)

5.1.1. Transgenic APP/PS1 mice

Independent of the age of animals, the cortical EEG in the APP/PS1 mice during waking immobility tended to have a lower power than in NT mice around the theta peak but a increased power over two wide frequency bands ranging from 12 to 23 Hz and 28 to 40 Hz (Fig.1A and Fig.2 in Publication I). The hippocampal EEG during waking immobility showed a different pattern. The total power over 1–40 Hz was significantly higher in APP/PS1 mice (Fig.1B in Publication I). However, neither theta peak amplitude nor frequency was different between groups. An additional experiment with both double and single mutant mice clearly suggests that the observed EEG changes are linked with the APPswe genotype (Fig.5 in Publication I).

In the cortical recording, auditory gating was weaker in APP/PS1 mice than in NT mice (Fig.2A in Publication II). Also the cortical N35 latency to the first (conditioning) stimulus tended to be longer in APP/PS1 than in NT mice ($p = 0.06$) (Fig.2C in Publication II). In contrast, the latencies to the subcortical (most likely inferior collicular) N8 peak did not differ between the genotypes (Fig.3C in Publication II). In addition, the hippocampal recording revealed weaker auditory gating in APP/PS1 mice (Fig.2B in Publication II). In a control experiment with both double- and single-mutant mice, we were able to associate impaired auditory gating with the APP/PS1 genotype, whereas prolonged latency of the N35 response was associated with the presence of the APPswe transgene (Fig.3 in Publication II).

5.1.2. Knockout TNR-/- mice

The characteristic oscillation of hippocampal EEG were tested: high-frequency ripple, gamma/beta and theta. The high-frequency ripple oscillations during immobility were present in both TNR-/- and WT mice, and did not differ in shape or frequency between the genotypes. The mean power of the gamma range (30–80 Hz) was increased by almost 100% in TNR-/- mice compared with their WT controls (Fig.2D and Fig.3 in Publication III). The peak theta power during movement was almost identical in the two groups, but the frequency of the theta peak was lower in TNR-/- mice (8.07 ± 0.18 Hz) than in WT mice (9.04 ± 0.22 Hz) (Fig.2C and Fig.4 in Publication III). The peak looks much sharper in TNR-/- mice, but this is not due to different variability of peak frequency. The theta peak was much more blunted during waking immobility, and neither the maximum theta power nor its frequency differed between the groups. For cortical EEG we focused on waking immobility state to avoid possible contamination by volume conduction from the dominating hippocampal theta rhythm during movement. In general, the cortical EEG power during waking immobility was higher in TNR-/- animals than in their control littermates (Fig.5 and Fig.6 in Publication III).

The amplitudes of all measured cortical AEP components (except for the P50 response to the conditioning tone) were significantly higher in TNR-/- mice than in WT mice

(Fig.7A, B in Publication III). In the hippocampal recordings, only the early components P20 and N30 had higher amplitudes in TNR-/- mice than in WT mice (Fig.8A, B in Publication III). Paired-pulse ratio of the cortical N30–P50 component was clearly increased in TNR-/- mice compared with WT mice, indicating weaker inhibition upon stimulus repetition (Fig.7 in Publication III). A similar genotype difference was also observed in the hippocampal P20–N30 component (Fig.8 in Publication III).

5.1.3. Knockout ST-/- mice

The hippocampal high-frequency ripple oscillations during immobility were present in both ST-/- and ST+/+ mice (wild type littermates of ST -/- mice) and did not differ in their special features between the genotypes. The mean power of the gamma range oscillations (between 30–80 Hz and 50–70 Hz) for the dentate gyrus (DG) was 30–40% lower in ST-/- mice compared with ST+/+ controls, but the difference was not statistically significant because of high between-subject variability (Fig.2 in Publication IV). The same tendency was observed for stratum radiatum/lacunosum moleculare of CA1 (CA1Mol) gamma but the genotype difference was smaller and also non-significant. However, we observed an augmentation of the oscillations within the beta range (16–28 Hz) in the hippocampus during free exploratory movement of ST-/- mice (Fig.2A in Publication IV). Overall, there was a 40–50% increase in the total beta range power in ST-/- mice compared with control animals. A closer examination of the raw EEG data revealed that gamma and beta rhythms alternated on top of theta oscillations during movement (Fig.3 in Publication IV). Brief epochs of beta rhythms were occasionally seen in ST+/+ mice, but these epochs were much more frequent in ST-/- mice. This was evident from a twofold increase in the beta/gamma power ratio in ST-/- mice in comparison to ST+/+ mice. The peak theta frequency during free exploratory movement or immobility (Fig.4 in Publication IV) was lower in ST-/- mice than in ST+/+ mice and the power of theta range was slightly higher but these differences were not statistically significant. Overall, the cortical EEG power was higher in ST-/- than in ST+/+ mice during free movement (Fig.5A in Publication IV) but was lower during immobility (Fig.5B in Publication IV). This difference was significant for the beta range and beta/gamma power ratio but not for the other measured parameters (delta and theta maximum amplitude or maximum frequency, gamma power, delta and theta power).

Overall amplitudes of the cortical AEPs were higher in ST-/- than in ST+/+ mice. However, the genotype difference approached significance only for the early P20 component of the 1st response (Fig.6 in Publication IV). The hippocampal AEP amplitudes were somewhat higher in ST-/- than in ST+/+ mice but none of the differences were significant (Fig.7 in Publication IV). However, the latencies of several components were shorter in ST-/- mice: the P20 latency of the 1st and 2nd responses, and the N30 latency of the 2nd response. Paired-pulse ratio of the single components (P20, N30, P40/P50) was similar between ST-/-

and ST+/+ mice.

Morphological analysis found a lower density of PV+ interneurons in the CA3 subfield of ST-/- compared with ST+/+ mice, but no differences in CA1 or DG (Fig.8A in Publication IV). Subfield volumes (Fig.8B in Publication IV) and total numbers of PV+ interneurons (Fig.8C in Publication IV) were similar in ST-/- and ST+/+ mice. In addition, brain weight, brain volume and soma area of PV+ interneurons were normal in ST-/- mice.

5.1.4. Knockout TNC-/- mice

We found a 20% reduction in the volume the CA1 subfield of TNC-/- mice compared to TNC+/+ mice (Fig.8A in Publication V). This hypoplasia was region-specific, since the DG and the CA3 were normal in size. Further analysis of the CA1 region of TNC-/- mice showed volume reduction of layers containing apical dendrites (strata radiatum and lacunosum-moleculare, -24 % compared with TNC+/+ mice), and basal dendrites (stratum oriens, -17%) of pyramidal cells, but not pyramidal cell bodies (stratum pyramidale) (Fig.8B in Publication V). Despite the volume reduction, the total number of CA1 PV+ cells was similar in TNC-/- mice to TNC+/+ mice, as was the number of pyramidal cells and the ratio of pyramidal cells to PV+ cells (Fig.9A in Publication V). In addition, densities of PV+ and PV- GABAergic terminals on pyramidal cell perikarya as well as pyramidal cell body area were similar between groups (Fig.10 in Publication V). While no abnormalities in neuronal populations were found in the DG of TNC-/- mice (Fig.9B in Publication V), the number of $S\text{-}100^{+}$ astrocytes was increased in both the DG and CA1 (+31 % and +26 % compared to wild-type mice, respectively) (Fig.9A, B in Publication V).

The automated activity test showed no difference between the genotypes. The Morris water maze was used to test spatial learning and memory. The escape latencies during 4 days of task acquisition did not differ between the genotypes (Fig.1A in Publication V), neither did the average swimming speed. However, in a probe test without the platform on the last trial of Day 4 TNC+/+ mice showed a stronger search bias towards the platform quadrant than did TNC-/- mice (Fig.1B in Publication V). In contrast, TNC-/- mice focused their search on the platform zone equally well as TNC+/+ mice.

Overall TNC-/- mice showed a general increase in a wide range of oscillations including theta and gamma (Fig.3-5 in Publication V). While the power of oscillations showed no difference in the DG (Fig.3A in Publication V), the groups differed dramatically when the power was measured in the area close to the CA1 pyramidal cell layer (Fig.3B in Publication V). At the same time, AEPs from both genotypes appeared similar (Fig.6-7 in Publication V). In addition, no difference between genotypes was found in the high-frequency ripple oscillations.

The mean hippocampal power of the gamma range (30-80 Hz) in the DG was similar ($p > 0.5$) in TNC-/- mice compared with TNC+/+ controls (Fig.3A in Publication V).

However, whereas in control mice gamma power decreased almost to a half when the electrode position shifted from the DG to CA1Mol, a slight increase (on average 15 %) was seen in TNC-/- mice (Fig.3B in Publication V). To further assess this laminar specific oscillation change, we calculated the ratio of CA1Mol vs. DG gamma power. We found that for TNC-/- mice this ratio was twice as high as for wild type mice (movement or NREM).

The hippocampal peak theta frequency during movement was almost identical in the two groups, but power the of the theta peak and total power in the range 4-12 Hz were increased by almost 100 % in TNC-/- mice compared to TNC+/+ mice (Fig.4 in Publication V). During NREM sleep the difference in theta power was even higher. Theta peak power and total power in the range 4-12 Hz were 2.5 times higher in TNC-/- mice than in TNC+/+ mice. As with gamma rhythm, the theta oscillation also had a laminar specific pattern. The ratio of CA1Mol vs. DG theta was increased in TNC-/- mice compared to TNC+/+ controls, but the difference did not reach significance due to high variation within groups.

In general, recording of cortical EEG reveal higher power in TNC-/- animals than in their control littermates. This difference reached significance at the number of frequencies ranges (Fig.5 in Publication V). In addition, the delta peak frequency (during NREM sleep) was lower in TNC-/- mice (then only mice with distinguishable delta peak were included).

6. DISCUSSION

6.1. ALTERNATION OF EEG AND ERPS IN TRANSGENIC APP/PS1 MICE

We detected significant differences in the cortical EEG power spectrum, sensory gating and latency of the N35 component between transgenic APP/PS1 mice and their non-transgenic littermates, but these changes were not age dependent as would have been hypothesized on the basis of underlying progressive Aβ accumulation. In a control experiment with both double and single transgenic mice, we were able to associate altered cortical EEG and prolonged N35 latency (corresponding human P50) with the presence of APPswe transgene, whereas impaired auditory gating was associated with the presence of Aβ.

6.1.1. Alternation of EEG

The observed EEG changes were obviously not caused by amyloid deposition in plaques. First, the differences were present at the age of 7 months, before the appearance of the first amyloid deposition, and did not change when plaques started to appear. Second, the number of plaques was small even at the age of 13 months, except in some layers of the hippocampus. Third, the cortical theta activity was found to be different between APP/PS1 and non-transgenic mice, whereas the genotypes did not differ with regard to the hippocampal theta activity, although the hippocampal formation had more amyloid depositions. Similarly, these arguments speak against the role of Aβ42 level as the cause of the EEG changes.

The control experiment with both double and single mutant mice clearly suggests that the observed EEG changes are linked to the APPswe genotype. This association indicates that either elevated levels of Aβ40 or the presence and/or overexpression of mutated human APP protein could be the underlying factor. In our APP/PS1 mice the APP protein levels are about twice as high as endogenous mouse APP levels, and remain relatively constant over the entire age span of the study (Liu et al., 2002a). In contrast, like Aβ42, Aβ40 accumulates in the brain as the mice age. Therefore, the presence of mutated human APP remains the most likely factor underlying the EEG changes. However, the role of soluble Aβ40 cannot be ruled out either.

One specific GABAergic neuronal population may be particularly vulnerable to Aβ deposition. As studies in AD patients and animal models of AD suggest, SOM and/or NPY expressing interneurons selectively degenerate at early stages of AD neuropathology (Ramos et al., 2006). In line with our observation, a recent study in another AD mouse model found an association between selective decrease in SOM and/or NPY expression and APP gene mutation (Ramos et al., 2006). The molecular mechanism underlying the loss SOM / NPY neurons remains largely unknown but high-affinity binding of Aβ to the α7 nicotinic acetylcholine receptor subunit may be one underlying factor (Wang et al., 2000a; Wang et al.,

2000b). The nicotinic α7 receptor is mainly expressed in hippocampus interneurons which contain NPY, SOM or CCK (Freedman et al., 1993). Furthermore, Aβ binds to the α7 receptor and shifts the balance between excitation and inhibition in the brain (Dineley et al., 2001; Pettit et al., 2001; Spencer et al., 2006). This shift is also likely to result in changes of hippocampal gamma and theta oscillations. Theta generation is heavily depend on stratum oriens / lacunosum moleculare (O-LM) and HIPP (interneurons with hilar dendrites and ascending axons) cells (Buzsaki, 2002; Traub et al., 2004) - that is exactly the same neuron population that expresses SOM and/or NPY (Freund and Buzsaki, 1996). On the other hand, both gamma and theta rhythms depend on perisomatic inhibitory cells (Buzsaki, 2002; Freund, 2003; Mann and Paulsen, 2005; Whittington and Traub, 2003), of which CCK but not PV basket cells express the α7 nicotinic-receptor subunit in the hippocampus (Freund, 2003).

6.1.2. Alternation of ERPs

The observed changes in cortical and hippocampal AEPs in APP/PS1 mice were obviously not caused by the amyloid deposition in plaques. Namely, the group differences in auditory gating and N35 latency remained the same between 7 and 11 months of age, although the mouse brains showed dramatic increase in the Aβ load and the formation of first amyloid plaques during this time. Furthermore, the prolongation of N35 latency unlikely relates to Aβ1-42 at all, as it was equally present in APP/PS1 and APP mice despite 20- to 30-fold difference in the levels of soluble Aβ1-42 between these two lines. Interestingly, cortical EEG abnormalities were also similar in magnitude in APP/PS1 and APP mice and contrast with NT or PS1 mice (Publication I).

The most plausible link to prolonged N35 latency is the overexpression of mutated APP. Interestingly, the delayed auditory ERP latency was restricted to mid-latency component, while the subcortical N8 component did not differ between the groups. Consistent with this observation, we did not detect the transgene protein in the subcortical auditory relay nuclei, although it was abundantly present in the auditory cortex and in the hippocampus. It remains open whether this change is specific to the APPswe mutation or whether it is related to increased APP levels in general. Namely, in this transgenic mouse the transgenic APP levels are about twice as high as the endogenous APP protein levels (Liu et al., 2002b).

The association between impaired auditory gating and APP/PS1 genotype is also straightforward, as impaired gating was not observed in either one of the single transgenic mouse lines. Numerous studies show an important linkage between abnormal sensory gating and α7 nicotinic-receptor subunit (Adler et al., 1993; Bickford and Wear, 1995; Freedman et al., 1997; Stevens and Wear, 1997). It is long known, that nicotinic α7 receptor is mainly expressed in hippocampus interneurons which contain NPY, SOM or CCK (Freedman et al., 1993). Thus, in transgenic mice with amyloid accumulation, a selective loss of SOM and NPY interneurons (Ramos et al., 2006) is likely to lead to impaired auditory gating.

6.2. ALTERNATION OF EEG AND ERPS IN KNOCKOUT TNR-/- MICE

The present study provides first in vivo electrophysiological evidence for the importance of tenascin-R (TNR) in the control of certain inhibitory networks in the brain. The TNR-/- mice expressed multiple abnormalities in their EEGs and AEPs not only in the hippocampus, but also in the cortex. In general, TNR-/- mice revealed enhanced amplitudes of either spontaneous or evoked fluctuations in the extracellular field potentials compared to their WT littermates. These findings support the idea of a reduced inhibition in the TNR-/- mice.

Our recordings focused on the hippocampal CA1 layer that is characterized by high-frequency ripple oscillations because we wanted to test the idea that TNR is primarily involved in the perisomatic inhibition of CA1 pyramical cells (Saghatelyan et al., 2000; Saghatelyan et al., 2001; Saghatelyan et al., 2003). Based on these *in vitro* findings, we expected robust changes in the high-frequency ripples, as synchronous discharge of CA1 GABAergic chandelier cells and basket cells and the consequent rhythmic perisomatic inhibition of the pyramidal cells is thought to underlie high-frequency oscillations (Ylinen et al., 1995). However, no difference was found between the TNR-/- mice and their WT controls in the quality or quantity of 200 Hz ripples. It is possible that synchrony of the inhibitory interneurons is more important for the 200 Hz ripples than the number of synaptic contacts between the interneurons and the pyramidal cells, so that an almost 40% loss of perisomatic terminals on CA1 pyramidal cells (Nikonenko et al., 2003) is not enough to remove or even attenuate the ripples. There is evidence for the presence of gap junctions between the dendrites of parvalbumin-containing chandelier and basket cells (Fukuda and Kosaka, 2000; Ylinen et al., 1995), which is a very effective mechanism to synchronize a network of interneurons with minimum number of synaptic contacts. On the other hand, connexin-36 knockout mice, which lack interneuronal gap junctions, also display intact fast ripple oscillations *in vivo* (Buhl et al., 2003). One possible explanation for the presence of ripple oscillations in TNR-/- mice despite weakened mechanisms to maintain synchronization in the interneuronal network is the fact that in vivo ripple oscillations are present only during so-called sharp waves, that is, synchronous discharges of populations of CA3 cells that give a powerful momentary excitation to CA1 pyramidal cells and interneurons (Buzsaki, 1986). This strong discharge in CA1 may be sufficient to synchronize the inhibitory interneurons to such a degree that it overcomes the weakened perisomatic inhibition. Furthermore, a ripple component composed of only excitatory input has been described *in vitro* (Draguhn et al., 1998).

In contrast to the normal high-frequency ripples in TNR-/- mice, we observed clear changes in gamma and theta rhythms that are also dependent on interneuronal networks in the hippocampus. It is noteworthy that the change in theta rhythm was a decreased peak frequency with no change in its amplitude, whereas the amplitude of the gamma oscillations

was robustly increased in TNR-/- mice. Although perisomatic inhibition is assumed to play a role in both gamma and theta oscillations, some treatments have dissociable effects on these two oscillations. For instance, a selective immunotoxin lesion of the cholinergic septo-hippocampal projection cells in the medial septum/diagonal band of Broca results in a robust decrease in theta amplitude with no change in its frequency and no change in the gamma rhythm (Lee et al., 1994). Whereas lesions of the medial septum reduce the theta amplitude without affecting its frequency, lesions of the supramammillary nucleus of the hypothalamus reduce the frequency of the theta rhythm but not its amplitude (McNaughton et al., 1995). In addition, the NMDA-antagonist, phencyclidine, when given systemically to freely moving rats, selectively increases the amplitude of hippocampal gamma oscillations without affecting the amplitude or frequency of hippocampal theta oscillations (Lee et al., 1994). Furthermore, a recent report describes a selective decrease in gamma oscillations with no change in ripples or theta oscillations in connexin-36-deficient mice (that lack gap junctions between interneurons) (Buhl et al., 2003). Another interesting parallel to TNR-/- mice with defects in extracellular matrix around parvalbumin-positive interneurons are parvalbumin-deficient mice. In hippocampal slice recordings, these mice also exhibit increased power of oscillations within gamma frequency (Vreugdenhil et al., 2003). One further mechanism that could account for a selective enhancement of gamma oscillations is an increase in extracellular K^+, which specifically evokes fast oscillations in hippocampal slices (LeBeau et al., 2002). Interestingly, disinhibition of postsynaptic $GABA_B$ receptors and resulting increase in postsynaptic K^+ conductance has been suggested to mediate the lack of perisomatic inhibition in TNR-/- mice (Saghatelyan et al., 2003).

The observed slowing of hippocampal theta oscillations in TNR-/- mice is difficult to attribute to changes in direct perisomatic inhibition of hippocampal CA1 pyramidal cells by PV+ interneurons. On the other hand, a significant and selective loss of calretinin-positive interneurons in the CA1 and CA3 areas of TNR-/- mice has recently been observed (Brenneke et al., 2004), possibly secondary to excitotoxicity. Among other locations, calretinin-positive GABAergic neurons have been described at the border between the medial and lateral septum. These cells receive afferent input from the entrorhinal cortex and terminate in the supramammillary nucleus on calretinin-positive non-GABAergic neurons, which in turn are important regulators of the hippocampal theta oscillations through their projection to the medial septum and hippocampal interneurons (Leranth et al., 1999). Interestingly, lesion of the supramammillary nucleus of the hypothalamus reduces the frequency but not amplitude of theta oscillations (McNaughton et al., 1995).

A comparison of the AEPs of TNR-/- mice and their controls revealed a robust difference in the response amplitudes, such that the response to the first click was about twice as large in amplitude in the TNR-/- mice compared with controls. The effect could be seen in CA1 recordings, but was more pronounced in the cortical recording channel. The enhancement of the first responses to the paired clicks can arise either by enhanced excitatory

drive or reduced feedforward inhibition of pyramidal cells. It needs to be pointed out in this context that TNR has been reported to regulate the activity of voltage dependent sodium channels and would thus directly affect excitability of neurons. However, the lack of TNR would work towards decreased excitation (Srinivasan et al., 1998; Xiao et al., 1999). Therefore, reduced perisomatic inhibition remains a more likely explanation for our observations. In addition, auditory sensory gating (e.g., reduced inhibition upon repetition) was weaker in TNR-/- mice than in control mice, pointing also to a reduced inhibition in TNR-/- mice. However, direct feedback inhibition of pyramidal cell by basket and chandelier cells through $GABA_A$ receptors alone cannot account for the sustained inhibition over a 500-ms inter-stimulus interval (Andersen et al., 1964). In accordance with this notion, involvement of $GABA_B$ receptors has been suggested as the underlying mechanism for auditory gating in the hippocampus (Hershman et al., 1995). In this regard, it is noteworthy that there is evidence that the reduced perisomatic inhibition in TNR-/- mice is actually $GABA_B$ receptor mediated, in that postsynaptic $GABA_B$ receptors may regulate pre-synaptic GABA release via retrograde K^+ signaling (Saghatelyan et al., 2003). The very same mechanism has been suggested to mediate the anxiolytic effect of $GABA_B$ receptor antagonists (Zarrindast et al., 2001).

In conclusion, our study concurs with previous *in vitro* findings that TNR plays an important role in perisomatic inhibition of hippocampal pyramidal cells. Furthermore, the present study extends these findings and shows that the lack of TNR results in a robust decrease of inhibitory control *in vivo*, not only in the hippocampus, but also in the cerebral cortex, manifesting itself in alterations of neural network oscillations and increased amplitude of evoked potentials. The attenuation of inhibitory neurotransmission may explain the behavioral phenotype of these mice, characterized by increased anxiety and impaired motor coordination (Freitag et al., 2003).

6.3. ALTERNATION OF EEG AND ERPS IN KNOCKOUT ST-/- MICE

The present study provides first *in vivo* electrophysiological evidence for the importance of the HNK-1 carbohydrate in the control of inhibitory networks in the brain. The ST-/- mice have abnormalities in EEGs and AEPs not only in the hippocampus, but also in the neocortex.

The most robust EEG abnormality in ST-/- mice was the increase in beta oscillations during free movement with simultaneous decrease in the amount of gamma power. In fact, episodes of beta oscillations alternated with periods of gamma rhythms. This is consistent with *in vitro* recordings in which gamma and beta rhythms often occur in succession (Traub et al., 1999) and with *in vivo* recordings from the rat hippocampus, showing that stimulation-induced beta and gamma oscillations do not coexist (Mikkonen and Penttonen, 2005). Beta frequency oscillations often occurs in epileptic conditions (Amzica and Steriade, 1999; Hirai

et al., 1999). However, we observed no seizure-like behavior of the mice during periods of beta oscillations. Moreover, the episodes of beta oscillations were of short duration (less than 5 s), and were followed by normal EEG. Therefore, it is likely that episodes of beta oscillations are part of a normal EEG of ST-/- mice during movement. To our knowledge, the present data are the first evidence that the gamma-beta shift found in hippocampal slice preparations can occur also in freely moving animals.

The ST-/- mice showed a different pattern of EEG and AEP changes from those reported earlier for TNR-/- mice (study III and Table1 in the result section). The power of hippocampal gamma oscillations, which were increased in TNR-/- mice , tended to be smaller in ST-/- compared with their ST+/+ controls. Although both TNR-/- and ST-/- mice had increased cortical power in the high beta – low gamma range, this difference was seen during different activity states of the animals. Increased low gamma oscillations were observed in TN-R-/- mice during waking immobility, while beta and low gamma rhythms were increased in ST-/- mice only during free movement. Furthermore, TNR-/- mice displayed robust increase in cortical delta power, whereas cortical activity in this frequency range was reduced in ST-/- mice as compared with ST+/+ littermates. The only similarity in EEG abnormalities between TNR-/- and ST-/- mice was the shift of hippocampal theta peak towards lower frequencies during free movements. In addition, AEPs in ST-/- mice bear little similarity to the robustly increased cortical and hippocampal AEP amplitudes recorded from TNR-/- mice. Furthermore, auditory gating was impaired in TNR-/- mice but normal in ST-/- mice. The only significant AEP abnormality in ST-/- mice was a decrease in the latencies of several hippocampal AEP components, a phenomenon not found in TNR-/- mice. Thus, it is unlikely that the EEG and AEP aberrations which we had previously reported for TNR-/- mice are mediated by the sulfate residue to the glucuronic acid of the HNK-1 core carbohydrate. However, this does not rule out the possibility that HNK-1 may influence interneuron function via a different mechanism. In support of this notion, application of monoclonal HNK-1 antibodies to hippocampal slices results in a dramatic decrease of $GABA_A$ receptor-mediated perisomatic inhibitory postsynaptic currents in CA1 pyramidal neurons (Saghatelyan et al., 2000), whereas the corresponding decrease in ST-/- mice is only moderate (Senn et al., 2002). In addition, TNR-/- mice display several developmental abnormalities, not reported for ST-/- mice, such as defective formation of perineuronal nets (Bruckner et al., 2000; Weber et al., 1999) and decreased density of calretinin-immunopositive neurons in the hippocampus (Brenneke et al., 2004), which may also contribute to reduced perisomatic inhibition.

Another question requiring consideration is whether the proposed HNK-1 mediated mechanisms in TNR-/- mice might actually account for the observed EEG abnormalities in ST-/- mice, especially for the increased power of beta oscillations. The generation of beta oscillations has been studied mainly in hippocampal slices, where it can be induced by strong tetanic electrical stimulation or by bath application of the muscarinic acetylcholine receptor

agonist carbachol. The most relevant model of beta rhythm generation to the present observations is the one involving a network of both interneurons and pyramidal cells (Whittington et al., 2000). According to the model, beta oscillations arise when interneurons continue to oscillate at gamma frequencies, while excitatory neurons fire action potentials coherently during every second or third cycle of the gamma rhythm, resulting in net oscillations at beta frequency. Three conditions favor this 'missed beat' beta rhythm: i) sufficient tonic depolarization of the interneuron network, ii) enhanced afterhyperpolarization of pyramidal neurons, and iii) recurrent excitation between pyramidal neurons (Bibbig et al., 2001; Whittington et al., 2000). If in ST-/- mice, similar to TNR-/- mice, inhibition of $GABA_B$ receptors through the HNK-1 carbohydrate is reduced, sustained activation of $GABA_B$ receptors should result in elevated levels of extracellular K^+ (Saghatelyan et al., 2003). This condition could favor tonic depolarization of interneurons, but at the same time it would reduce K^+ currents underlying afterhyperpolarization of pyramidal cells. On the other hand, the decreased PV+ interneuron density in CA3 of ST-/- mice likely increases recurrent excitation among pyramidal neurons. Since recurrent connectivity in CA3 is much higher than in CA1 (Miles and Wong, 1986; Thomson and Radpour, 1991), this subarea is the most plausible generator of synchronous firing of excitatory cells in beta band rhythms in the hippocampus. Beta/gamma oscillations generated in CA3 propagate easily to CA1 (Csicsvari et al., 2003; Fisahn et al., 1998; Shimono et al., 2000) and CA3 may further extend its oscillatory influence into the DG as demonstrated in slice recordings (Arai and Natsume, 2006; Fisahn et al., 1998).

The increase in amplitude and decrease in latency of mid-latency auditory evoked potentials have been shown in many studies on patients after wakening from anesthesia (for example Rundshagen et al., 2000). Higher arousal levels in smokers after smoking a single cigarette is also well known (Pomerleau and Pomerleau, 1984). Interestingly, a recent study on smokers found a decrease in delta EEG rhythms, an increase in beta EEG activity, no changes in theta oscillations, and shortening and increase in amplitude of mid-latency auditory potentials after cigarette smoking (Domino, 2003). All those changes are related to increase in plasma nicotine. The striking similarities of observed differences between ST+/+ vs. ST-/- mice in study-IV and between non-smokers vs. smokers in the above mentioned study (Domino, 2003) suggest an important role of the nicotinic cholinergic system for those findings. The nicotinic $\alpha 7$ receptors are expressed in hippocampal interneurons which contains NPY, SOM or CCK (Freedman et al., 1993). NPY and SOM expressing cells are associated with O-LM and HIPP cells while CCK cells associate with perisomatic inhibitory cells (Freund and Buzsaki, 1996). Nicotinic ACh receptor activation has been shown to excite GABAergic interneurons in the hippocampus, but not the principal excitatory cells in stratum pyramidale and stratum granulosum (for review see Jones et al., 1999). Therefore, alterations in nicotinic ACh neurotransmission may contribute to altered inhibition in ST-/- mice.

In conclusion, lack of the sulfate residue in the glucuronic acid of the HNK-1 core

carbohydrate does not reproduce the robust phenotype of TNR-/- mouse, arguing for the existence of parallel signaling mechanisms through the HNK-1 carbohydrate. However, lack of the sulfate residue is associated with increased cortical and hippocampal beta and corresponding decrease in gamma oscillations. This change in oscillatory pattern in the hippocampus may stem from the decreased density of parvalbumin-positive interneurons in the CA3 subregion, which do not develop or survive normally in ST-/- mice.

6.4. ALTERNATION OF EEG AND ERPS IN KNOCKOUT TNC-/- MICE

The present study provides the first electrophysiological evidence in freely moving animals for the importance of tenascin-C (TNC) in the control of inhibitory networks in the brain. TNC-/- mice express multiple abnormalities in their spontaneous but not evoked brain activity in the cortex and hippocampus. In general, TNC-/- mice express higher EEG amplitude compared to their WT littermates. In addition, laminar specific morphological and oscillatory changes were observed in the hippocampus. These electrophysiological changes were accompanied by impaired spatial memory but unimpaired general learning ability or exploratory activity. As earlier reported for LTP in hippocampal slices (Evers et al., 2002), alterations in L-type voltage sensitive Ca^{2+} channels (L-VDCC) may contribute to EEG changes in TNC-/- mice, since an L-VDCC antagonist or agonist affected differently cortical or hippocampal oscillations in TNC-/- mice compared to WT controls. Morphological analysis in TNC-/- mice revealed CA1-specific hypoplasia (reduced volume of layers containing apical and basal dendrites but not of the pyramidal cell layer). In contrast to earlier findings in the neocortex (Irintchev et al., 2005), numbers of principal cells and PV+ interneurons, and their ratios, were normal in the hippocampus of TNC deficient mice, while increased astrogliosis was observed in both hippocampal subregions. These layer specific structural abnormalities likely account for the altered hippocampal network oscillations and contribute to observed spatial learning deficit.

At a first glance, the finding of impaired spatial memory in TNC-/- mice appears to be in contrast with an earlier report of the same mice (Evers et al., 2002). However, the difference is subtle and most likely attributable to slight differences in the testing procedure. In fact, both studies found normal acquisition in the hidden platform version of Morris water maze in TNC-/- mice, and the only difference between the studies appeared during the probe trial. In the Evers et al. study (Evers et al., 2002), mice were first trained to find a visible platform, and had only three days thereafter to learn the location of the hidden platform, whereas the present study involved four days of acquisition directly with the hidden platform. Thus it is understandable that the wild type mice expressed weaker search bias towards the correct platform location in the study of Evers and colleagues (Evers et al., 2002) than in our study. Another possible contributing factor is a difference in the breeding history. Whereas the earlier study used 129/SsvJ mice with only one generation of backcrossing into C57BL/6J,

mice of the present study had already five generations of backcrossing. Since C57BL/6J mice are better learners in spatial tasks than 129/SvJ mice (Crusio et al., 1987), the background difference may have produced a better spatial learning in general in the present study and revealed a difference in learning strategies between wild type and TNC-/- mice. In fact, both mouse lines learned to locate the escape platform in the same amount of time, but while wild type mice focused on orienting to the correct sector of the pool, TNC-/- mice tried to keep the correct distance from the pool wall. Nevertheless, impaired direction search bias is considered to be the most sensitive water maze parameter indicative of hippocampal dysfunction (Wolfer et al., 1998). Therefore, TNC-/- in this study can be considered to have abnormal hippocampal function.

Earlier *in vitro* electrophysiological studies in TNC-/- mice have revealed a deficit in the induction of LTP in the CA1 subregion of the hippocampus (Evers et al., 2002), but only with stimulation protocols that activate L-VDCCs. No deficit was found in LTP induction in the CA3 or DG, but also a different L-VDCC independent stimulation paradigm was used in these subregions. Interestingly, recording of theta-burst induced LTP in the presence of nifedipine did not affect LTP in TNC-/- mice, but reduced LTP in wild-type mice to the levels seen in mutants (Evers et al., 2002). In the present study, nifedipine and the L-VDCC agonist BayK had opposite effects on theta and gamma oscillation in TNC-/- and control mice, which suggest that impaired function of L-VDCCs may also partially contribute to observed increases in theta and gamma oscillations in TNC-/- mice.

Despite considerable advantages, the neural mechanisms of gamma oscillations are not fully understood. The gamma oscillation in hippocampal slices depends on complex interaction of two oscillatory networks. One is driven by activation of muscarinic acetylcholine receptors (mAChRs) and second driven by activation of metabotropic glutamate receptors (mGluRs) (Mann and Paulsen, 2005; Palhalmi et al., 2004; Whittington et al., 2000). In entraining the network into gamma oscillation, two types of basket cells are particularly important: those containing Ca^{2+}-binding protein PV and CCK. While assembly of PV-containing cells represents the non-plastic precision clockwork, the CCK-containing cell assembly is highly modifiable by local neuromodulators (which might allow fine tuning of oscillation frequency and amplitude) (Freund, 2003; Klausberger et al., 2005). Some pharmacological agents which differently act on PV and CCK basket cells (Freund, 2003) have been shown to enhance hippocampal high frequency oscillations both in vitro and in vivo; these include $GABA_A$ agonist diazepam (Shimono et al., 2000), NMDA-antagonist phencyclidine (Lee et al., 1994; Ma et al., 2004), and cholinesterase inhibitor eserine (Leung, 1985). It is interesting to note the parallel influence on gamma oscillation by the prototype anxiolytic drug diazepam and knockout of TNC, as decreased anxiety has also been reported in TNC-/- mice (Morellini and Schachner, 2006).

Theta oscillations can be divided on the basis of their pharmacological sensitivity into atropine-sensitive (during behavior immobility) and atropine-resistant (during movement)

(Kramis et al., 1975). One way to achieve increased power of theta oscillation in the hippocampus is to influence ACh receptors (Masuoka et al., 2006; Rowntree and Bland, 1986). However, this is an unlikely mechanisms in TNC-/- mice as ACh manipulation also results in a shift in the theta peak frequency, which did not happen in TNC-/- mice (Fellous and Sejnowski, 2000; Keita et al., 2000). Another possibility to increase theta oscillation may arise from facilitated axo-axonic interaction (e.g. with NH4Cl) (Fischer, 2004). Facilitation of axo-axonic interaction (presumable between axons of pyramidal cells) would lead to increased theta oscillation amplitude in hippocampal-cultured slices. Axo-axonic interaction has also been shown to be important for gamma and high-frequency oscillations (approximately 200 Hz) (Traub et al., 2004). Two types of electrical coupling in the hippocampus influence population oscillations: those between distal dendrites of interneurons (at the border between the stratum oriens and alveus) (Fukuda and Kosaka, 2000) and those between pyramidal cells (through the contact of processes in the oriens) (Schmitz et al., 2001). As the volume of apical and basal dendrites is most likely reduced in TNC-/- mice, we hypothesize that the probability of electrical contacts between axons is increased. However, this possibility is difficult to verify in principal neurons as axo-axonic contact number is very low (~1.6 per neuron).

Some of the EEG changes can be attributed to altered morphology in TNC-/- mice. Previous findings in TNC-/- mice demonstrated reduced number / density of PV+ interneurons (Irintchev et al., 2005) in the cortex. This would lead to reduced inhibition or increased excitation and could account for the general increase in cortical EEG power in TNC-/- mice. However, the observed cortical EEG delta peak shift toward lower frequencies during NREM sleep does not fit as a consequence of reduced number of cortical PV+ interneurons. It is more likely a result of alternation in the cortico-thalamic system. Morphological changes in the thalamus were not addressed in the current study but thalamic expression of TNC has been demonstrated in earlier studies (Irintchev et al., 2005; Kusakabe et al., 2001). Morphological changes in the hippocampus were much more subtle than those in the cortex in TNC-/- mice, and no loss of principal cells or PV+ interneurons were observed. However, a volume loss was manifested in a subregion and layer specific manner: only the CA1 subregion was affected and all other layers but the pyramidal cell layer. This pattern of altered morphology is fully compatible with the finding of robust increase in the power of CA1 gamma oscillation in comparison to DG gamma. The layer specificity of the volume loss points to thinning of the dendritic tree of pyramidal cells. As the number of pyramidal cells themselves and that of PV+ interneurons providing somatic inhibition and their perikarya on pyramidal cells remained normal in TNC-/- mice, the basic rhythm generator units thus reside in a smaller tissue volume than in WT mice, resulting in higher power of the oscillation in electrophysiological recordings. Thinning of dendritic trees of CA1 pyramidal cells could partially also account for the impaired LTP induction in the CA1 subregion in TNC-/- mice as compared to the CA3 and DG subregions (Evers et al., 2002).

The possible explanation of shrinkage of CA1 subregion is the specific loss of interneurons that resides in those sub-layers. As a resent study shows, diminished number of O-LM interneurons expressing SOM / NPY will cause shrinkage of the stratum lacunosum-moleculare layer (Ramos et al., 2006).

In conclusion, lack of extracellular matrix glycoprotein TNC in mice produces robust changes in hippocampal and cortical EEG. However, the profile of *in vivo* electrophysiological measures (oscillations power and frequency, structural and laminar specific EEG, AEPs) differs from that in mice deficient in a related glycoprotein TNR (study III) or its associated HNK-1 carbohydrate (study IV). The difference between TNC-/- and TNR-/- mice could be expected because of different localization of these two extracellular matrix glycoproteins. While TNR is an important component of perineuronal nets, TNC is more broadly expressed in the brain. In the hippocampus, the morphological changes in TNR-/- and TNC-/- mice were almost orthogonal. Atrophy of perineuronal nets in TNR-/- mice results in general attenuation of perisomatic inhibition, which manifests as increased power of gamma oscillation and enhancement of AEP amplitudes (study III). In contrast, perisomatic inhibition appears intact in TNC-/- mice, and polysynaptic excitatory drive results in normal AEPs. An increase in gamma oscillation was observed only in the CA1 subregion, where it associates with volume loss of layers with pyramidal cell dendrites. On top of that, TNC-/- mice express dysfunction of L-type voltage sensitive Ca2+ channels, which has not been found in TNR-/- mice.

6.5. GENERAL DISCUSSION

Electrophysiological measures (EEG and ERP) proved to be very sensitive tools for detection of even subtle neuronal network abnormalities. In the light of present knowledge about AD mouse models our data in APP/PS1 mice (study I and II) show EEG and ERP parameter differences after minor (one fifth; (Freedman et al., 1993)) population of interneurons loss or malfunction. It is likely that observed alternations of EEG and/or ERP are due to altered function of interneurons expressing nicotinic $\alpha 7$ receptor (most likely SOM/NPY expressing L-OM and CCK expressing basket interneurons). Similarly, fine physiological changes in the hippocampal circuit may have been left unnoticed by morphological assessment (study IV and V), but were manifest in EEG and/or ERP. Those studies found small alternations in another subpopulation of interneurons (although not yet confirmed in study V) only after a detailed morphological analysis, while beta and/or gamma oscillations alternation were identified by a simple electrophysiological measure. When changes in interneuron are robust (like in study III) EEG and ERP parameters are almost as powerful index of the genotype as genomic analysis with twofold increase in some parameters compared to normal values.

Altogether, findings of this project speak for an underestimated potential of

electrophysiological measurements as simple as EEG and ERP. However, we should admit that lack of specificity of EEG and ERP is a major drawback of these methods. Nevertheless, lack of specificity is not due to limitation of the method itself but due to limited implementation. In order to fully exploit the potential of EEG recording and increase it specificity, we should increase our understanding about the role of each interneuron subpopulation in the complex information processing and/or oscillations in the brain. In the quest of that knowledge not only other neuroscience tools (genetically modified mice, morphological, behavioral analysis etc.) are instrumental but also increasing the diversity of calculated EEG/ERP parameters and number of recording sites is crucial.

7. CONCLUSION

Electrophysiological measurements (EEG and ERP) remain a low-cost but a very sensitive measure of brain interneuron pathology. Studies described here show their potential value in phenotyping various genetically modified mice with interneuron pathology. Diversity of interneurons suggests that each specific group is tuned to a specific task in the brain machinery. Measurement of spontaneous oscillations and evoked potentials helps to discover those functions in which interneuron maybe involved. As our present studies show, specific alternations in a subpopulation of interneurons lead to increase / decrease of unique pattern of electrophysiological parameters. Whether L-OM, perisomatic inhibitory, or nicotine sensitive interneurons, they all play a specific role in information processing in the brain. Notably, no single EEG or ERP parameter (e.g. theta, beta/gamma oscillation, ERP amplitude or paired-pulse ratio) alone is capable of pointing to a specific interneuron population as responsible for the observed alternation. However, specificity of EEG and ERP increase substantially with the number of parameters extracted and with increased number of recorded locations.

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APPENDIX: ORIGINAL PUBLICATIONS (I-V)

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

G 38. Pirttilä, Terhi. Expression and functions of cystatin C in epileptogenesis and epilepsy.
2006. 103 p. Acad. Diss.

G 39. Mikkonen, Jarno Eelis. Short-term dynamics of hippocampal fast brain rhythms and their implications in the formation of functional neuronal networks in vivo.
2006. 68 p. Acad. Diss.

G 40. Wahlfors, Tiina. Enhancement of HSV-TK/GCV suicidegene therapy of cancer.
2006. 65 p. Acad. Diss.

G 41. Keinänen, Riitta et al. (eds.). The eleventh annual post-graduate symposium of the A. I. Virtanen Institute Graduate School: AIVI Winter School
2006. 57 p. Abstracts.

G 42. Nissinen, Jari. Characterization of a rat model of human temporal lobe epilepsy.
2006. 93 p. Acad. Diss.

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.

G 45. Roy, Himadri. Vascular Endothelial Growth (VEGFs) - Role in Perivascular Therapeutic Angiogenesis and Diabetic Macrovascular Disease.
2006. 81 p. Acad. Diss.

G 46. Räty, Jani. Baculovirus surface modifications for enhanced gene delivery and biodistribution imaging.
2006. 86 p. Acad. Diss.

G 47. Tyynelä, Kristiina. Gene therapy of malignant glioma. Experimental and clinical studies.
2006. 114 p. Acad. Diss.

G 48. Malm, Tarja. Glial Cells in Alzheimer's Disease Models.
2006. 118 p. Acad. Diss.

G 49. Tuunanen, Pasi. Sensory Processing by Functional MRI. Correlations with MEG and the Role of Oxygen Availability.
2006. 118 p. Acad. Diss.

G 50. Liimatainen, Timo. Molecular magnetic resonance imaging of gene therapy-induced apoptosis and gene transfer: a role for 1H spectroscopic imaging and iron oxide labelled viral particles.
2007. 81 p. Acad. Diss.

G 51. Keinänen, Riitta et al. (eds.). The first annual post-graduate symposium of the graduate school of molecular medicine: winter school 2007.
2007. 65 p. Abstracts.

G 52. Vartiainen, Suvi. Caenorhabditis elegans as a model for human synucleopathies.
2007. 94 p. Acad. Diss.

G 53. Määttä, Ann-Marie. Development of gene and virotherapy against non-small cell lung cancer.
2007. 75 p. Acad. Diss.