UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Zentrum für Experimentelle Medizin
Institut für Biochemie und Molekulare Zellbiologie

Institusdirektor (komm.): Prof. Dr. rer. nat. Dr. med. habil. Andreas Guse

ApoE Regulates Corticospinal Neuronal Survival Through ApoER2

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
an der Medizinischen Fakultät der Universität Hamburg

vorgelegt von

Farnas Farsian
aus Tehran

Hamburg 2013

Angenommen von der Medizinischen Fakultät am: 28.10.2013

Veröffentlicht mit Genehmigung der medizinischen Fakultät der Universität Hamburg

Prüfungsausschuss, der Vorsitzende: Prof. Dr. Jörg Heeren

Prüfungsausschuss, 2. Gutachter: Prof. Dr. Joachim Herz

Prüfungsausschuss, 3. Gutachter: Prof. Dr. Eckart Förster

TABLE OF CONTENTS

1. INTRODUCTION

Neurodegenerative diseases

The twentieth century witnessed a significant demographic change with a shift to higher life expectancy in the population of the industrialized world. Quality of life of the aging population is to a great extent determined by the normal aging process of neurons in the central nervous system and especially by the occurrence of diseases characterized by accelerated neuronal loss: diseases which are traditionally designated as being neurodegenerative. These disorders of the central nervous system account for major disabilities, and affect patients' families and friends on a personal, emotional, and financial level. On a larger scale, neurodegenerative diseases have already affected most health and social care system in the world.

Alzheimer's disease (AD) is the most prevalent of the neurodegenerative diseases followed by Parkinson's disease. Amyotrophic lateral sclerosis (ALS) and Huntington's disease affect smaller numbers of patients but also have devastating consequences. A large number of other rarer neurodegenerative diseases have similar profound effects on the patients and families who are afflicted by these illnesses.

Research in the last few decades has greatly enhanced our understanding of neurodegenerative disease pathogenesis. This has been fostered by major advances in genetics and molecular biology. We now know that gene mutations account for a large number of neurodegenerative diseases, which include familial forms of AD, Parkinson's disease, ALS, fronto-temporal dementia, Huntington's disease and a variety of ataxias.

Despite differences in pathogenesis, neurodegenerative diseases share loss of neuronal function, structure and ultimately neuron death as common characteristics.

Alzheimer's disease

Historical perspective

In November 1906, the German psychiatrist and neuropathologist Alois Alzheimer gave a lecture to the Meeting of the Psychiatrists of South West Germany on the first case of the disease that Kraepelin some years later named Alzheimer's disease (Moller and Graeber, 1998). In his talk, Alzheimer presented the clinical characteristics with memory disturbances and instrumental signs, as well as the neuropathological features with miliary bodies (plaques) and dense bundles of fibrils (tangles), which became known as the neuropathological hallmarks of the disease.

The patient, Ms Auguste D., who suffered from progressive presenile dementia, was brought to Alzheimer's attention by her worried family. Clinical observation alone made the case of this 51-year-old woman appear so unusual, that Alzheimer could not classify it as one of the recognized illnesses of his time. Over the next 5 years he observed how Auguste D.'s cognitive capacities deteriorated until she finally became bedridden and died. One year later, Alzheimer made his clinical observation and detailed post-mortem neuropathologic findings public (Alzheimer, 1907).

Since then, there has been much scientific interest and effort to understand this neurodegenerative disorder, which has been recognized as the most common cause of dementia.

AD research can be divided into three periods. Initially, clinicians and pathologists defined the disease. Thereafter, discoveries in the neurochemical field led to the identification of the cholinergic lesion in the disease, upon which current therapies are based. In the last three decades molecular, biological, and molecular genetic approaches have helped to further characterize and disentangle the pathogenesis of this debilitating disorder (Hardy, 2006).

In the last century, and especially the last 40 years, since the disease was rediscovered by Blessed, and Tomlinson (Blessed *et al.*, 1968; Tomlinson *et al.*, 1970) there has been an enormous amount of scientific progress in terms of understanding the etiology of this condition. Medical application of this progress has, however, come slower and much remains to be understood and done. If Auguste D. were alive today, her prognosis would be much the same as in 1906, as current pharmacologic treatment can only modulate the course of the disease.

Epidemiology

AD is a neurodegenerative disease that primarily affects older adults. Approximately 10% of all persons over the age of 70 have significant memory loss, and in more than 50 percent of cases the cause is AD. The incidence of AD doubles every five years after 65 years of age, and there are approximately 1,275 new cases of AD per year per 100,000 population aged 65 years or older (Hirtz *et al.*, 2007). It is estimated that more than 35 million people worldwide suffer from this disease (Wimo and Prince, 2010). Women have a slightly higher risk of developing dementia than men with a relative risk of 1.5 (Fratiglioni *et al.*, 1997). This increased risk does not appear to be explained by women's greater longevity.

Autosomal dominant inherited forms of AD, which account for less than five percent of all cases, routinely present earlier, frequently in the fifth decade of life or even earlier. Also, patients with Down syndrome are effected from AD at an

earlier age: virtually all individuals with Down syndrome have neuropathologic changes characteristic of AD beginning at 40 years of age (Schupf *et al.*, 1998).

Pathogenesis

The characteristic lesions of AD are senile plaques in neocortical terminal fields as well as neurofibrillary tangles in the medial temporal lobe structures of the brain together with degeneration of neurons and synapses. Besides, loss of white matter, inflammation, amyloid angiopathy, and oxidative damage are also present (Querfurth and LaFerla, 2010).

AD research has elicited the pathogenesis of several neuropathologic features of the disease, including τ hyperphosphorylation with tangle formation, β amyloid (Aβ) peptide aggregation and deposition with plaque development, and neurovascular dysfunction (Blennow *et al.*, 2006). The findings of a correlation between dementia severity and plaque counts guided research towards understanding the involvement of plaques in the pathogenesis of AD (Blessed *et al.*, 1968). In the mid-1980s researchers were successful in purifying plaque cores and identifying the amino acid sequence of Aβ, which turned out to be the major plaque component (Masters *et al.*, 1985). This discovery paved the path for the cloning of the *APP* gene (Kang *et al.*, 1987).

β-Amyloid

Aβ peptides are natural products of the amyloid precursor protein (APP) metabolism. APP can be processed along two main pathways: the α-secretase pathway (Figure 1) and the β-secretase pathway (Figure 2) (Blennow et al., 2006).

Aβ peptides originate from proteolysis of APP through the β-secretase pathway by the sequential enzymatic actions of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), a β-secretase, and γ-secretase, and a protein complex with presenilin 1 at its catalytic core (Vassar *et al.*, 1999). Monomers of $A\beta_{40}$ are more prevalent than the damaging and aggregation-prone $A\beta_{42}$ species (Tanzi, 2005a; Younkin, 1998).

The central hypothesis for the cause of AD is the "amyloid hypothesis". According to this hypothesis an imbalance between the production and clearance of Aβ leads to Aβ accumulation in the brain, which ultimately leads to neuronal degeneration and dementia (Hardy and Selkoe, 2002; Tanzi and Bertram, 2005).

Aβ spontaneously self-aggregates into soluble oligomers (2 to 6 peptides) and larger insoluble fibrils, which coalesce into intermediate assemblies (Kayed et al., 2003; Klein et al., 2001). Alternatively, Aβ can also form fibrils, which arrange themselves into β-pleated sheets to form amyloid plaques.

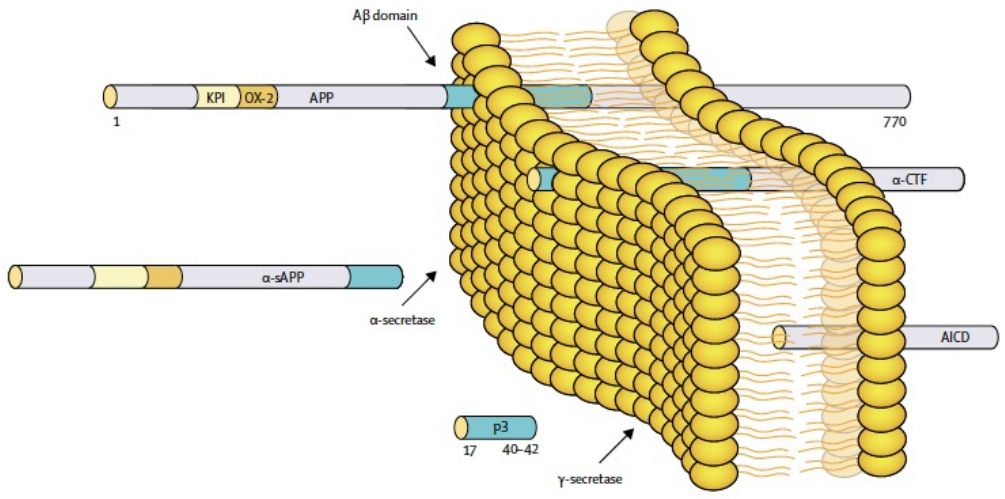

Figure 1
The α-secretase pathway: Non-amyloidogenic metabolism of APP.

APP is a transmembrane protein with a large N-terminal extracellular tail. The Aβ domain is partly embedded in the plasma membrane and includes the 28 residues just outside the membrane and the first 12–14 residues in the transmembrane domain. APP can be processed along two main pathways. In the α-secretase pathway, α-secretase cleaves APP within the Aβ domain, releasing the large soluble APP fragment (α-sAPP). The remaining C-terminal fragment (CTF), α-CTF, or C83, is cleaved by the γ-secretase complex releasing the short p3 peptide. The remaining APP intracellular domain (AICD) is metabolised in the cytoplasm. Since APP cleavage by α-secretase is within the Aβ domain this precludes Aβ generation (Blennow et al., 2006).

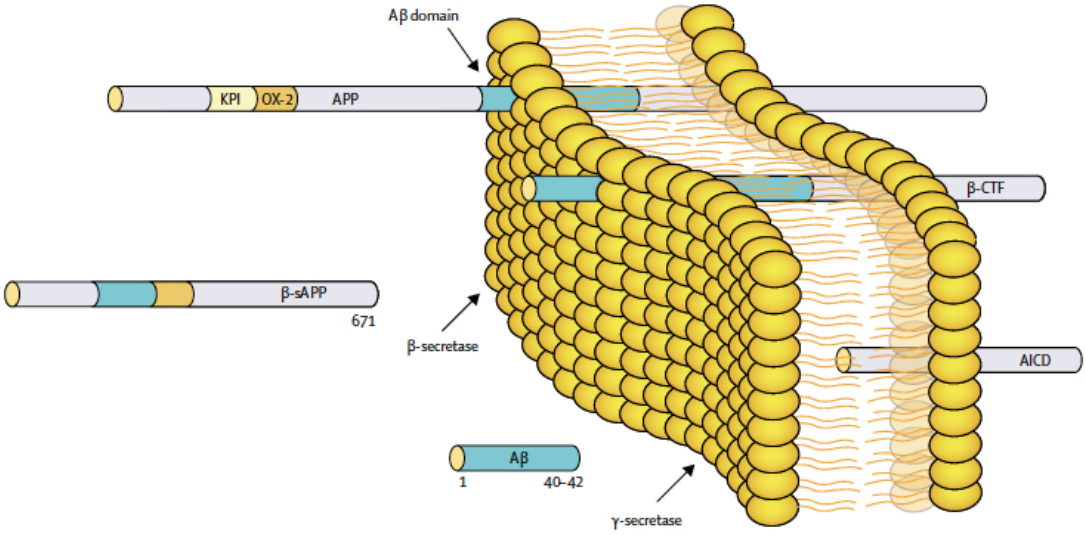

Figure 2
The β-secretase pathway : Metabolism of APP with amyloid β (Aβ) generation.
In the β-secretase pathway, β-secretase cleaves APP just before the Aβ domain, releasing soluble βsAPP. The remaining CTF, β-CTF, or C99 is cleaved by the γ-secretase complex releasing the free 40 or 42 aminoacid Aβ peptide. The remaining AICD is metabolised in the cytoplasm (Blennow et al., 2006).

Given the abundance of senile plaques and neurofibrillary tangles in AD, the issue of whether these brain lesions are neurotoxic, protective, or simply incidental markers of disease was controversial for a long time. In recent years, research has prompted a paradigm shift in which pathogenicity is more strongly correlated with pre-lesion molecules of τ and $A\beta_{42}$ than with neurofibrillary tangles and plaques (Tanzi, 2005b; Walsh and Selkoe, 2007). Experiments in rodents suggest that soluble Aβ oligomers inhibit hippocampal long-term potentiation and to disrupt synaptic plasticity (Walsh and Selkoe, 2004).

Tau

Neurofibrillary tangles are filamentous inclusions in pyramidal neurons. Almost in parallel with the identification of Aβ as the main constituent in plaques, neurofibrillary tangles were shown to be composed of abnormally hyperphosphorylated and aggregated τ protein (Grundke-Iqbal *et al.*, 1986; Nukina and Ihara, 1986). τ, which is normally an abundant soluble protein in axons, binds to microtubules through its microtubule-binding domains and promotes microtubule assembly. τ phosphorylation is regulated by the balance between several kinases (e.g. GSK-3β and CDK5) and phosphatases (e.g. PP-1 and PP-2A) (Iqbal *et al.*, 2005). Hyperphosphorylated τ is insoluble, and lacks affinity for microtubules.

Excessive kinase, reduced phosphatase activities, or both cause hyperphosphorylated τ to detach and self-aggregate, and microtubules to destabilize (Figure 3). τ pathology starts early in the disease process in neurons of the transentorhinal region before it spreads to the hippocampus and amygdala and later involves the neocortial association area (Taylor and Probst, 2008).

According to our current understanding, all of the beforementioned neuroanatomic findings together with neurovascular changes lead to the clinical manifestation of AD.

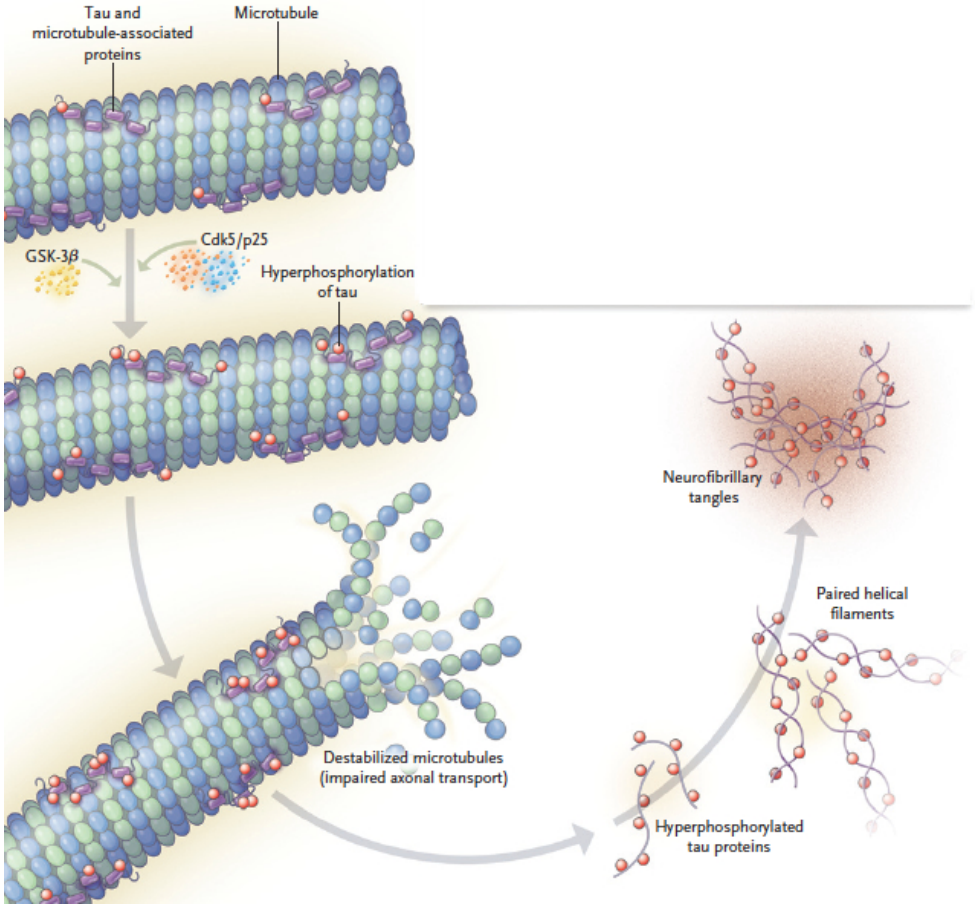

Figure 3. Tau structure and function

Normal phosphorylation of tau occurs on serine and threonine residues. Hyperphosphorylated sites specific to paired helical filament tau in Alzheimer's disease tend to flank the microtubule-binding domain. Tau binding promotes microtubule assembly and stability. Excessive kinase, reduced phosphatase activities, or both cause hyperphosphorylated tau to detach and self-aggregate and microtubules to destabilize (Querfurth and LaFerla, 2010).

Clinical manifestation

AD is a slowly progressive disorder with insidious onset, which leads to progressive memory impairment. The cognitive changes tend to follow a characteristic pattern. Symptoms usually begin with memory deficits. In a minority of AD patients complaints such as organizational, word-finding, or navigational difficulties may precede memory deterioration.

Neurodegeneration in AD is estimated to start two to three decades before clinical onset. During this preclinical phase, tangle and plaque load increase in the brain and at a certain threshold the patient develops first symptoms. Memory loss during this initial phase may be ascribed to benign forgetfulness by the patient or his environment. Yet, once the memory loss begins to affect activities of daily living, such as driving, shopping, housekeeping, or keeping track of finances, or once memory loss falls below 1.5 standard deviations from normal on standardized memory tests, the disease is defined as mild cognitive impairment (Gauthier *et al.*, 2006).

With further progression of disease, the patient is unable to work and is easily lost or confused, and requires daily supervision. Social graces, routine behavior, and superficial conversation may be surprisingly intact. Patients may develop signs and symptoms of apraxia, aphasia, and agnosia, together with general cognitive symptoms, such as impaired decision-making, judgment, and orientation (Blennow *et al.*, 2006).

In the late stage of the disorder, loss of judgment, reason, and cognitive abilities is inevitable. Delusion is common, and patients may develop loss of inhibition, aggression, and change in sleep-wake patterns.

In end stage AD, patients become rigid, mute, incontinent, and bedridden, so that help is needed with the simplest tasks such as eating, dressing and toilet function. Often death results from secondary infections, malnutrition, or heart disease (Fauci, 2008).

ApoE is associated with neurodegenerative diseases

Research over the last couple of years has provided evidence for a genetic link between AD, ALS, and the clinical outcome of neurotrauma and isoforms of apolipoprotein E (ApoE).

ApoE is a ligand for all members of the LDL receptor family and a constituent of lipoprotein particles that transport lipids throughout the circulation and between cells. ApoE is involved in at least three lipid metabolism pathways (Mahley, 1988). First, ApoE plays a key role in the delivery of dietary lipids to the liver, being the ligand for receptor-mediated uptake of chylomicron remnants by the liver. Second, due to their ApoE content, a significant fraction of VLDL remnants and IDL are cleared by the liver, constituting a metabolic cycle for the delivery of free fatty acids to peripheral tissues. Third, ApoE is an essential part of the “reverse cholesterol” transport system, mediating the uptake by the liver of cholesterol-rich HDL. Since the discovery that in humans not only the liver but also the brain produces significant amounts of ApoE mRNA (Mahley, 1988), it became widely appreciated that ApoE may also serve as mediator of local lipid transport in the central nervous system (Weisgraber *et al.*, 1994).

In humans, three alleles of ApoE have been described, which are termed ApoE2, ApoE3, and ApoE4. Among these ApoE3 is considered to be the most common variant (de Knijff *et al.*, 1994). Several studies have shown a relationship

between ApoE4 and different neurodegenerative disorders, among which are AD, ALS, and traumatic brain injury.

Among the different ApoE isoforms, carriers of ApoE4 have been shown to have the highest risk (Corder *et al.*, 1993), and carriers of ApoE2 to have the lowest risk for developing late onset Alzheimer disease (Corder *et al.*, 1994). Based on epidemiologic data, the ApoE4 allele may account for 50% of AD in the United States (Milne and Davis, 1992). Currently, it is not precisely understood how the ApoE isoforms differentially affect an individual's risk for developing AD. Proposed mechanisms include roles for ApoE in cholesterol transport and synapse formation, modulation of neurite outgrowth and synaptic plasticity, destabilization of microtubules, amyloid clearance and fibril formation by direct binding of the Aβ peptide, and impairment of ApoE receptor-dependent protective signals that promote neuronal survival and synaptic plasticity (Beffert *et al.*, 2004).

ALS is a debilitating neurodegenerative disorder, which is characterized by muscle weakness and atrophy throughout the body caused by degeneration of the upper and lower motor neurons. Affected individuals may ultimately lose the ability to initiate and control all voluntary movement, although bladder and bowel sphincters and the muscles responsible for eye movement are usually, but not always, spared. There is evidence that ALS patients, who carry the ApoE4 isoform have a poor prognosis with decreased survival time (Drory *et al.*, 2001), (Lacomblez *et al.*, 2002).

The ApoE4 isoform has also been shown to negatively influence the clinical outcome of patients after traumatic brain injury (Teasdale *et al.*, 1997), (Friedman *et al.*, 1999). These studies were able to demonstrate a strong association between ApoE4 and a poor clinical outcome, implying genetic susceptibility to the effect of brain injury.

In vitro studies of embryonic neuron cultures shows a direct death inducing effect of ApoE4 on neurons. Besides, the different ApoE isoforms display indirect effects on neuron survival by either fostering or reducing the neurotoxicity of Aβ (Hagiwara *et al.*, 2000), (Ji *et al.*, 2002), (Jordan *et al.*, 1998), (Michikawa and Yanagisawa, 1999).

A direct proof for the physiological role of ApoE on neuronal survival regulation is still missing. Animal models, which show a neuroprotective effect after head trauma or cerebral ischemia in ApoE mutant mice, could not show if this is due to a direct effect on the neurons, or whether this effect is a consequence of altered vascular or immunological responses to the injury (Sabo *et al.*, 2000). The current available data suggest that ApoE can affect neuronal survival by LDL family receptor mediated mechanisms, and that the effect on survival include a potential interaction with survival regulating factors in an isoform specific manner.

LDL receptor gene family

The low-density–lipoprotein receptor (LDL) family is an evolutionary ancient gene family of structurally closely related and widely expressed cell-surface receptors. In mammals, the LDL receptor family consists of seven members, namely LDL receptor (LDLR), the VLDL receptor (VLDLR), apoER2, MEGF7, the LDLR-related protein (LRP), LRP1B and Megalin (Figure 4) (Beffert *et al.*, 2004).

The LDLR family falls into two major groups: endocytic receptors that bind their cargo in the form of lipid carrying lipoproteins and mediate their internalization, and a second group that promotes lipid exchange at the plasma membrane without cellular uptake of the protein component of the particle. In addition to their specialized functions as mediators of cellular lipid uptake, lipoprotein receptors have, over the last few years, also been recognized for often unrelated roles as cellular signal transducers or signal modulators (Herz *et al.*, 2009).

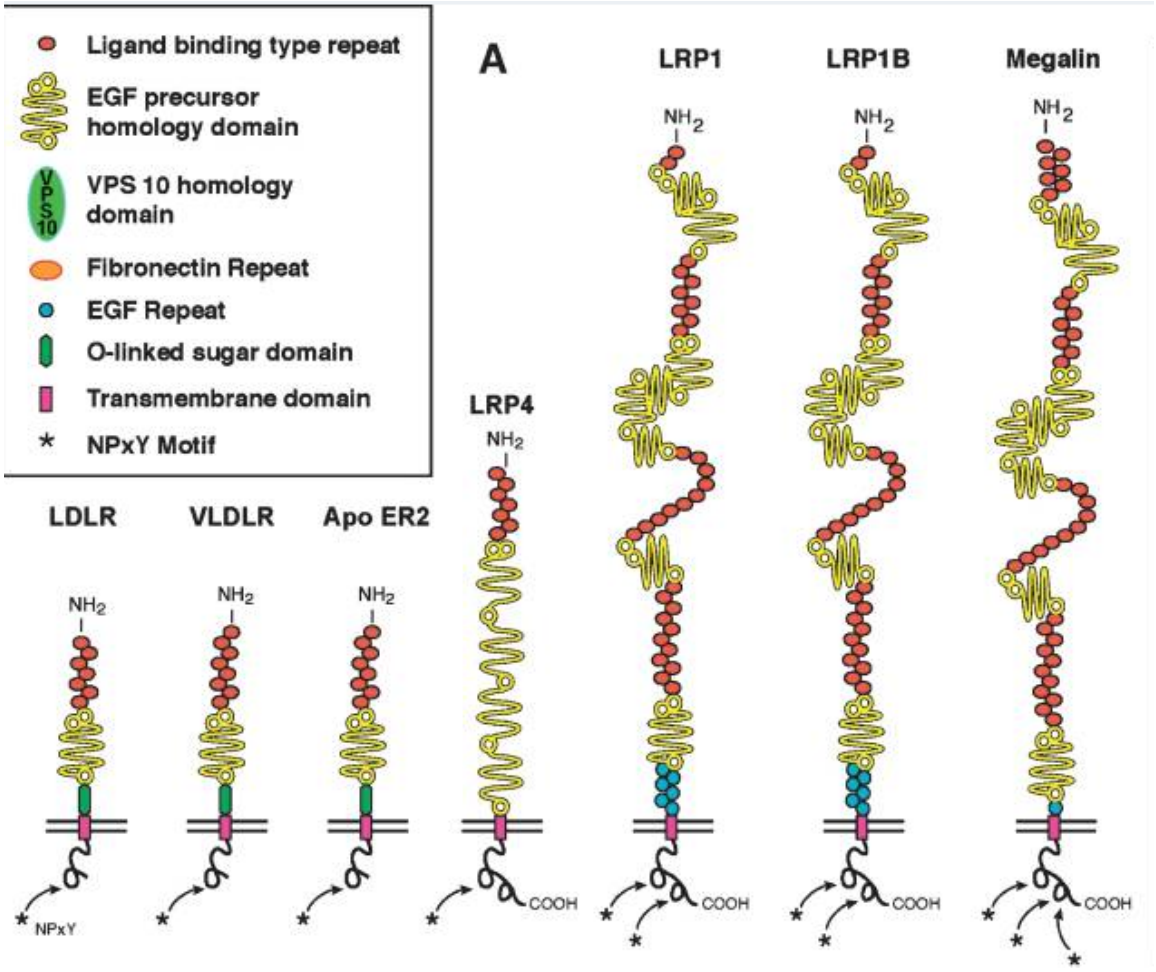

Figure 4. The low-density lipoprotein receptor gene family

(Herz, 2009)

Seven cell surface proteins that bind ApoE make up the core of the LDL receptor gene family. They all contain a single membrane-spanning segment and a short cytoplasmatic tail that harbors various sequence motifs that mediate interaction with cytoplasmatic adaptor and scaffolding proteins (Dieckmann *et al.*, 2010).

ApoER2

ApoE receptors play an essential role during brain development. This became apparent during the analysis of megalin/LRP2 knockout mice and from mice deficient in both ApoER2/LRP8 and VLDL receptor (Trommsdorff *et al.*, 1999). The latter mice strain is phenotypically identical to mice lacking the developmental signaling protein, Reelin, and to another strain of mice carrying mutations in the cytoplasmic adaptor protein, disabled-1 (Dab1). These findings suggested that both these ApoE receptors function together in a linear signaling pathway that is dependent upon the extracellular ligand Reelin and the intracellular adaptor Dab1 for initiating a signal cascade that regulates the migration and positioning of neurons during brain development (Figure 5) (Herz, 2009).

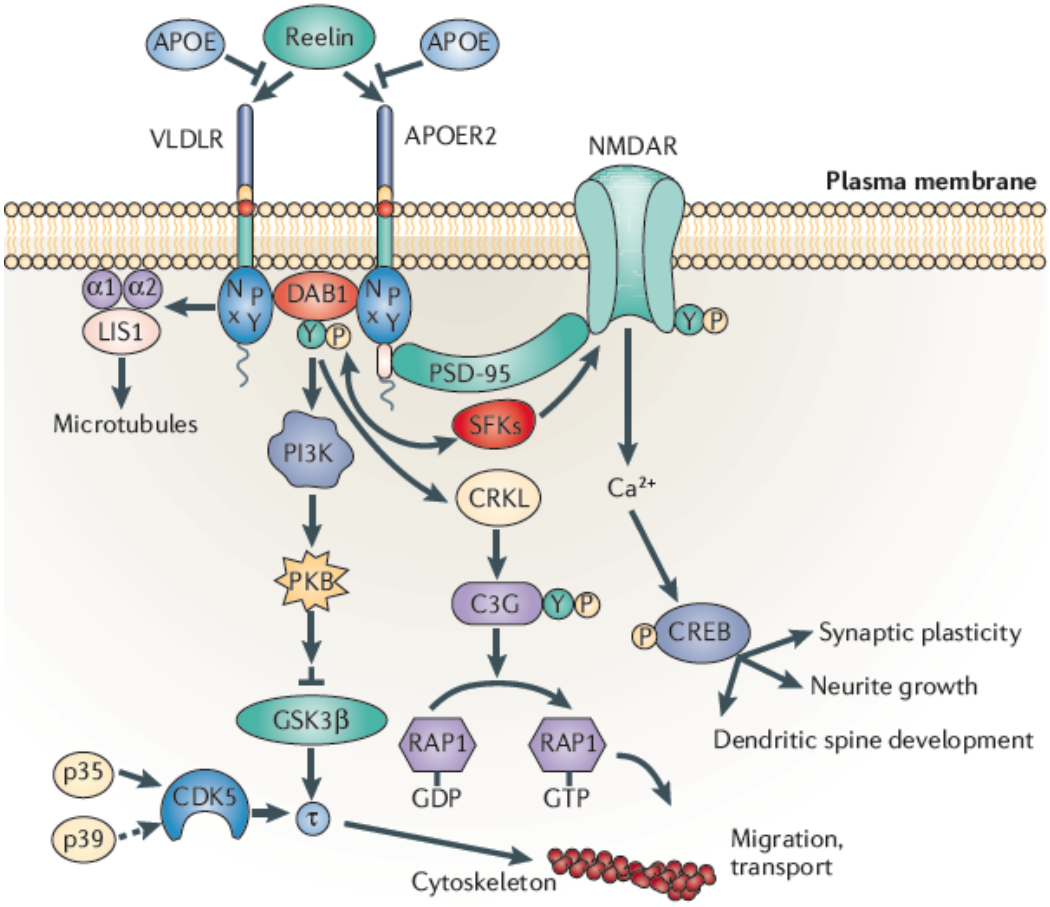

Figure 5. Reelin-initiated intracellular signaling pathway in neurons

The binding of reelin to the lipoprotein receptors VLDLR and APOER2 initiates an intracellular signaling cascade. (Herz and Chen, 2006)

PI3K: phosphatidylinositol-3-kinase, PKB: protein kinase B, GSK3β: glycogen synthase kinase 3β, YP DAB1: tyrosine-phosphorylated DAB1, CRKL: CRK-like, LIS1: Lissencephaly 1, CDK5: Cyclin-dependent kinase 5

Reelin and ApoE are ligands for the cell surface lipoprotein receptors ApoER2 and VLDLR (Figure 5). Binding of reelin to the receptors induces activation of DAB1, an adaptor protein that interacts with NPxY motifs in both receptor tails. The clustering of DAB1 causes activation of the SRC family tyrosine kinases (SFKs), which potentiates tyrosine phosphorylation of DAB1. Phosphorylated DAB1 leads to activation of phosphatidylinositol-3-kinase (PI3K) and subsequently protein kinase B (PKB). PKB activation inhibits the activity of glycogen synthase kinase 3β (GSK3β). As a result, phosphorylation of τ is reduced, promoting microtubule stability. Tyrosine-phosphorylated (YP) DAB1 also recruits CRK-like (CRKL), which induces phosphorylation of a guanine nucleotide exchange factor, C3G. Activated C3G promotes the formation of RAP1-GTP, which controls actin cytoskeleton rearrangement. Lissencephaly 1 (LIS1) is another binding partner of tyrosine-phosphorylated DAB1. It associates with α-subunits to form a Pafah1b complex, which regulates microtubule dynamics. Cyclin-dependent kinase 5 (CDK5) acts in parallel with reelin on numerous substrates, including microtubules. p35 and p39 are activating subunits of CDK5. APOER2 associates with postsynaptic density protein 95 (PSD-95), an abundant scaffolding protein in the PSD, through an alternatively spliced exon. This interaction is crucial for the coupling of the reelin signalling complex to the NMDA (*N*-methyl-D-aspartate) receptor (NMDAR). Reelin-activated SFKs tyrosine phosphorylate the NMDAR on NR2 subunits, resulting in the potentiation of NMDAR-mediated Ca^{2+} influx. Elevated intracellular Ca^{2+} can activate the transcription factor cyclic AMP-response element binding protein (CREB), thereby potentially initiating the expression of genes that are important for synaptic plasticity, neurite growth and dendritic spine development (Herz and Chen, 2006).

Development, and organization of the corticospinal system

The central nervous system (CNS) is one of the most complex tissues of the body, with unparalleled cellular diversity and very complex connectivity. Here, an extraordinary variety of glial and neuronal subtypes are generated during embryogenesis from distinct pools of tissue-specific neural stem and progenitor cells, which are progressively specified to different cellular fates.

The mammalian cerebral cortex is organized into six layers, and neurons in the same layer tend to share similar functional properties and patterns of connectivity. Cells in layers 5 and 6 project to subcortical targets, with thalamic projections arising from layer 6 and projections to the midbrain, hindbrain, and spinal cord originating from layer 5, whereas neurons sending axons to other cortical areas are distributed throughout layers 2-6 (O'Leary and Koester, 1993). After their generation in the ventricular zone, the earliest-generated cortical neurons migrate away to form the preplate, which is split into the marginal zone and subplate during cortical plate formation (Allendoerfer and Shatz, 1994).

Corticospinal neurons (CSN) develop from subplate precursors after embryonic day 13 (Marin-Padilla, 1992). After leaving the cell cycle, they migrate into layer 5 and extend axonal processes to establish connections with spinal cord targets (Jones *et al.*, 1982; O'Leary and Koester, 1993). After leaving the cortex, the axonal projections of CSN travel through the internal capsule, the midbrain cerebral peduncles, the longitudinal fascicles of the pons, the brainstem pyramidal tract to finally reach their projection target in the spinal cord via the proper corticospinal tract.

Unlike peripheral neurons, CSN do not undergo a period of extensive developmental cell death. The fine-tuning of the connectivity between long-distance cortical projections and their subcortical targets is achieved instead by postnatal collateral elimination (Oudega *et al.*, 1994).

The mature corticospinal system plays an essential role in regulating motor function (Porter, 1985). Spinal cord motoneurons are innervated by CSN, either directly or via interneurons.

Mature CSN are located in layer 5 of the cortex in areas, which are associated with motor function. Three major CSN areas have been described in rats and mice: (1) the sensory motor cortex, (2) the medial prefrontal and presumably supplementary motor cortex, and (3) the somatosensory cortex (Bonatz *et al.*, 2000).

Lesions of adult CSN-axons at spinal cord or brainstem levels have been shown to induce atrophy but not death of CSN. However, an axotomy close to the CSN cell body at the internal capsule level induces almost half of the CSN to die within the first 5 days after injury. In our study, we use this axotomy model to identify mechanisms that promote CSN-survival or CSN-death (Bonatz *et al.*, 2000).

Hypothesis

ApoE is a major risk factor for several neurodegenerative disorders, which manifest during midlife or senescence or after neuronal injury. Thus, it can be assumed that ApoE plays either a direct or indirect role in the pathogenesis of neurodegenerative disorders.

Neurodegenerative disorders are characterized by specific neuropathological hallmarks, e.g. amyloid depositions in AD, disturbed neuronal transmission, and neuronal death. ApoE and one of its receptors, ApoER2, are known to influence neuropathology and neuronal transmission in several disease models of neurodegenerative disorders. Whether this receptor-ligand-pair also affects neuronal survival under physiological and pathological conditions in vivo is not known.

We established an experimental model to study survival regulation of intact and damaged adult CSN in vivo. Our recent data indicate that virtually all CSN express ApoER2 mRNA both in the intact state and after pathological damage. The present study was undertaken to show whether ApoER2 and its ligand ApoE regulate survival of normally aging CSN and adult CSN after axonal injury. We hypothesize that both ligand and receptor affect survival of these neurons in normal and pathological conditions in a similar manner.

The aim of my work was to investigate the following hypothesis:
1) Does ApoER2 affect adult CSN survival during normal aging and after axonal injury, and if so, which of the intracellular domains of the receptor known to initiate signal transduction is mediating this effect?
2) Does ApoE affect CSN survival during normal aging and after axonal injury?
To this end, we used the above mentioned experimental model in genetically altered ApoER2 and in $ApoE^{-/-}$ mouse strains using a stereological approach.

2. MATERIALS and METHODS

Mouse models

Using a set of genetic mouse models, we tested in the corticospinal system whether ApoE and ApoER2 have an effect on the survival of neurons because of the following reasons:

(1) CSN express ApoER2.
(2) death of CSN can be induced by axotomy, and the survival of lesioned CSN is regulated by neuronal growth factors.
(3) ApoE is expressed in the glial cells of the cortex.
(4) cortical development is regulated by a reelin / ApoER2 / VLDLR / Dab1 pathway.

All protocols involving the use of animals for the following experiments were in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the UT Southwestern Animal Care and Use Committee.
A total of 52 genetically altered mice were used at the ages of eight, ten to twelve, twelve, and eighteen months for the experiment. The following genetically altered mice strains were used, which have been described previously:

ApoER2[long] (Beffert *et al.*, 2005)
ApoER2[short] (Beffert *et al.*, 2005)
ApoER2[stop] (Beffert *et al.*, 2006b)
ApoER2[Dab-] (Beffert *et al.*, 2006a)
$ApoE^{-/-}$ (Piedrahita *et al.*, 1992)
$ApoER2^{-/-}$ (Trommsdorff *et al.*, 1999)
$ApoER2^{+/-}$ (Trommsdorff *et al.*, 1999)

ApoER2[long] and ApoER2[short] have previously been referred to as ApoER2 ex19 and ApoER2 Δex19, respectively (Beffert *et al.*, 2005).

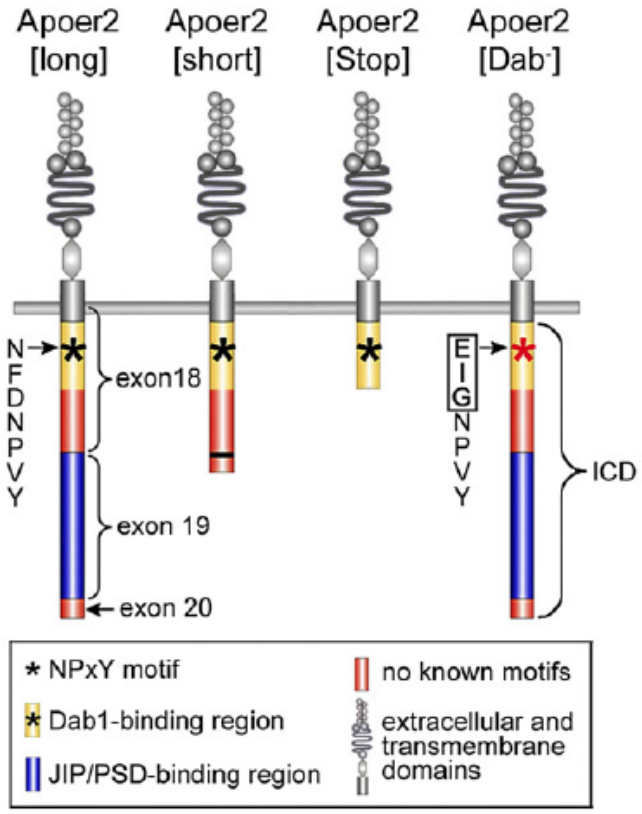

Figure 6. ApoER2 receptor mutations (Beffert *et al.*, 2006b)

Receptor isoforms expressed by Apoer2 knockin mutant mice. PSD95 and JIPs interact with sequences within exon 19 (blue). These sequences are absent in Apoer2[short] and Apoer2[Stop]. The Dab1 binding site is indicated by the asterisk (*). Mutation of this site in Apoer2[Dab2] abrogates binding.

Labeling of corticospinal neurons

Background:

To determine the role of ApoE and ApoER2 in neuronal survival in vivo, we used retrograde fluorescent-dye labeling of CSN to monitor neuronal loss during aging or after injury by deafferation. We chose this method because it allowed us to determine whether reduction of neurons during aging is caused by neuronal loss or by decreased neurogenesis.

Since only 40-50% of all pyramidal neurons in neocortical layer V are CSN, it is necessary to specifically identify them. This is achieved by applying retrograde tracers (e.g. True Blue) to the axons of CSN at the spinal cord levels of the corticospinal tract. These tracers are taken up by the axons from the injection site and are subsequently transported towards the neuronal soma via retrograde axonal transport.

After a transport time of at least 3 days, the tracer is thus far accumulated in the CSN soma that it can be identified by fluorescence microscopy. For assessment of CSN survival, we used only animals that have been labeled at least 5 days before the experimental intervention (for lesion CSN) or before sacrificing the animal (for unlesioned CSN).

Per definition, CSN are the only cortical neurons projecting to the spinal cord. Thus, this procedure is highly specific for the identification of CSN. Cervical spinal cord levels of C4/C5 were chosen for the injection site of the tracer as this yields to a maximum number of labeled CSN-cell bodies as compared to more distal injection sites, e.g. at lumbar levels.

Procedure:

All surgery was done under intraperitoneal anesthesia (single injection) with a combination of chloralhydrate (150 mg/kg body weight) and sodium pentobarbital (64 mg/kg body weight). After reaching deep anesthesia, which was controlled by testing the eye-lid reflex, the animal is placed into a stereotaxic apparatus for rodents (KOPF). Aseptic technique was used for the operation. Only sterile,

previously autoclaved (124 °C for 30 minutes) surgical instruments as well as sterile single use syringes, injection cannulas, and scalpel blades are used. The operation field is shaved and disinfected with 70% Ethanol followed by a desinfection with Betadine solution. The skin is then opened by a longitudinal cut in the dorsal neck region and the subcutaneous fat tissue is pushed laterally to expose the nuchal ligament, which is subsequently cut longitudinally. Then, the superficial and medial layers of the neck muscles of each side of the neck are pulled laterally with a retractor to expose the deep layer of the neck muscle covering the vertebral laminae. Subsequently, the 4^{th} dorsal processus is identified and the neck muscles covering the respective laminae are removed. After clean preparation of the lamina of the 4^{th} vertebrae, its lamina including its dorsal processus are removed using a fine rongeur until the spinal cord segment is visible through the transparent dura mater. Then, both the periostal and the meningeal layer of the dura mater are cut with a fine blade under preservation of the fine vessels covering and supplying the spinal cord. The cerebrospinal fluid leaking through this cut is carefully sucked off and the orientation lines of the spinal cord (dorsal median sulcus, dorsolateral sulcus) are identified, which are necessary for appropriate stereotactic localization of the following tracer injection. We used a maximum of 0.8 µl of the fluorescent tracer True Blue (TB) 2% in 0.2% dimethyl-sulfoxide (DMSO) per injection site. The tracer is injected laterally aside each corticospinal tract. To confirm the completeness of axotomy after the internal capsule lesion (see below), a red tracer (RDX = 15% rhodamine dextran 10000, 10% rhodamine dextran 3000, and 10% rhodamine-b- isothiocyanate in 0.2% DMSO; 1 µl per mouse) was used.

For tracer injections, glass micropipettes (tip diameter 30–40 µm) connected via oil filled teflon tube to a Hamilton 5 µl syringe were used. In total, the spinal cord is maximally exposed to air for 5 minutes. After the injection, the middle and superficial layers of the neck muscles are adapted by a single muscle suture, and the skin wound is closed by clips. The operation field is subsequently cleaned with 70% ethanol.

Axotomy of corticospinal neurons

Background:

To determine the rate of survival of injured neurons, axotomy of tracer labeled CSN is carried out at internal capsule levels and animals were allowed to survive the lesion for 7 days. This time is chosen because axotomy induced death of CSN takes place between 3 and 5 days after the lesion and does not proceed beyond day 7. The axotomy is carried out unilaterally at the internal capsule level in order to induce death of CSN. To achieve complete axotomy, a stereotaxic KOPF lesion knife is placed into the internal capsule and the lesion wire extruded subsequently. This creates a “hook”, which is then rotated twice around the axis of the lesion knife (guidance tube) thereby producing a circular shaped, horizontally oriented cut within the internal capsule (Figure 7B). This procedure completely axotomizes all CSN of the motorcortex on the lesion side (Bonatz *et al.*, 2000).

Procedure:

After reaching deep anesthesia, the operation field is prepared as described above and the animal is subsequently placed into the stereotaxic apparatus. A median longitudinal cut is then carried out over the dorsal skull, which is then exposed in order to visualize the main sutures and Bregma, which are important landmarks for the following stereotaxic procedure. According to the previously determined coordinates, one hole is then drilled into the skull to have access for the lesion knife. Care is taken that the underlying dura mater is left untouched. The dura mater is opened carefully with a microblade under the hole in order to have free access for the lesion knife. The location of these cuts is placed by avoiding injury of blood vessels on the surface of the neocortex. After these procedures, the lesion knife is inserted into the brain parenchyma at a 20° angle to the median with the coordinates br −0.2 mm, lat 3.0 mm, and V 1.7 mm and the internal capsule lesion is performed as described above.

The skin wound is then closed as described above. Surviving neurons were scored one week after injury as described below.

Tissue preparation for survival assays

One week after internal capsule lesion, the animals were sacrificed by an overdose of sodium pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde solution in 0.1 M sodium phosphate, pH 7.2. Brains were postfixed for 2 hours, cryoprotected overnight in 25% sucrose solution in PBS, and subsequently frozen in dry ice cooled isopentane. Brain specimens were stored at -80 °C until further processing.

For cutting, brains have been oriented to yield serial sections that correspond to the frontal planes of mouse brain (Paxinos and Franklin, 2001). Care has been taken to avoid deviations in horizontal/vertical direction and to cut all brains in the same orientation. Serial cryostat sections (20 µm) were obtained throughout the entire cortex, and every second section was collected for cell counts; the remaining sections were used for histological evaluation (Bonatz *et al.*, 2000).

Quantitation of corticospinal neuron numbers

Cell counts were determined with a stereological approach in which the total number of CSN in the entire cortex was assessed. The criterion for a CSN is a TB-filled pyramidal shaped profile larger than 3 µm in diameter. Smaller TB labeled profiles were regarded as dendritic processes from adjacent sections. We verified that CSN size did not affect CSN number by comparing the stereological approach described here to an optical-dissector method. CSN within a specific cortical area were defined by their stereotaxic coordinates. The quantification procedures for intact and lesioned CSN is principally identical. Specific issues are as follows:

Lesioned CSN:

Axotomy as described above is generally complete for the primary motor cortex, whereas some CSN are usually spared in other cortical areas. Thus, only primary motor cortex was scored. The lesion area was determined by the absence of a second tracer (rhodamine dextran) that was applied to the cervical corticospinal tract after axotomy. Within the primary motor cortex a “cell-death area” is defined according to two constant anatomical criteria: (i) The cell-death area begins anteriorly at the level of the anterior commissure and extends 1.6 mm posteriorly; and (ii) in the anterior-posterior direction, the cell-death area is located within the central 50% of the primary motor cortex (Bonatz *et al.*, 2000), (Beffert *et al.*, 2006b). Within the cell-death area percent survival was calculated as (number of TB-labeled CSN on the lesion side / number of TB-labeled CSN contralateral to the lesion side) × 100%.

Intact CSN:

The data of unlesioned CSN are shown as absolute CSN numbers acquired per cortical hemisphere, i.e. each animal gave two values of CSN numbers, one for the right and one for the left hemisphere. The survival assays are based on a total of 800,000 CSN counted.

Statistics

For each animal, sums (absolute number of CSN) and ratios (survival of CSN on the lesion side relative to the control side) were calculated with Microsoft Excel software and analyzed with SigmaStat software by one-way analysis of variance (ANOVA) followed by the post-hoc Newman-Keuls-test (NKT) and Fishers Least Significant Difference Test (Fisher LSD). In addition, single pair-wise comparisons were performed with the Student's t test.

3. RESULTS

ApoER2 protects against neuronal loss during aging

We used retrograde fluorescent-dye labeling of CSN to determine if ApoER2 has any effects on the survival of intact adult CSN during aging. We first determined whether ApoER2 has any effects on the survival of intact adult CSN during aging (Figure 7C-E). Because ApoER2 is required for the control of neuronal migration during the development of the brain, CSN in ApoER2$^{-/-}$ mice are not located in cortical layer 5 as they normally are in the wild-type but are instead aberrantly distributed in layers 4 and 6 (Figure 8A). The total number of CSN is markedly reduced in 4-month-old ApoER2$^{-/-}$ mice (Figure 7C-F) and (Figure 8B), which could either be caused by postnatal loss of CSN or be the result of reduced neurogenesis in these animals. To distinguish between these possibilities, we counted baseline CSN numbers at 1 month after birth, a stage at which development of CSN is essentially complete, and then determined whether the number of cells labeled at this early stage decreases in the ApoER2-deficient animals over a 3 month period (Figure 7E and Figure 8C,D). We found that at 1 month wild-type and ApoER2$^{-/-}$ mice have comparable CSN numbers (Figure 7C-F) and (Figure 8C). However, in 4-month-old mice the number of CSN that had been labeled at 1 month after birth was reduced by approximately 50% in the ApoER2$^{-/-}$ mice, whereas there was no such age-dependent loss in the wild-type animals (Figure 7C-F) and (Figure 8D). Thus, CSN are generated normally in ApoER2$^{-/-}$ mice but suffer accelerated cell death during normal aging.

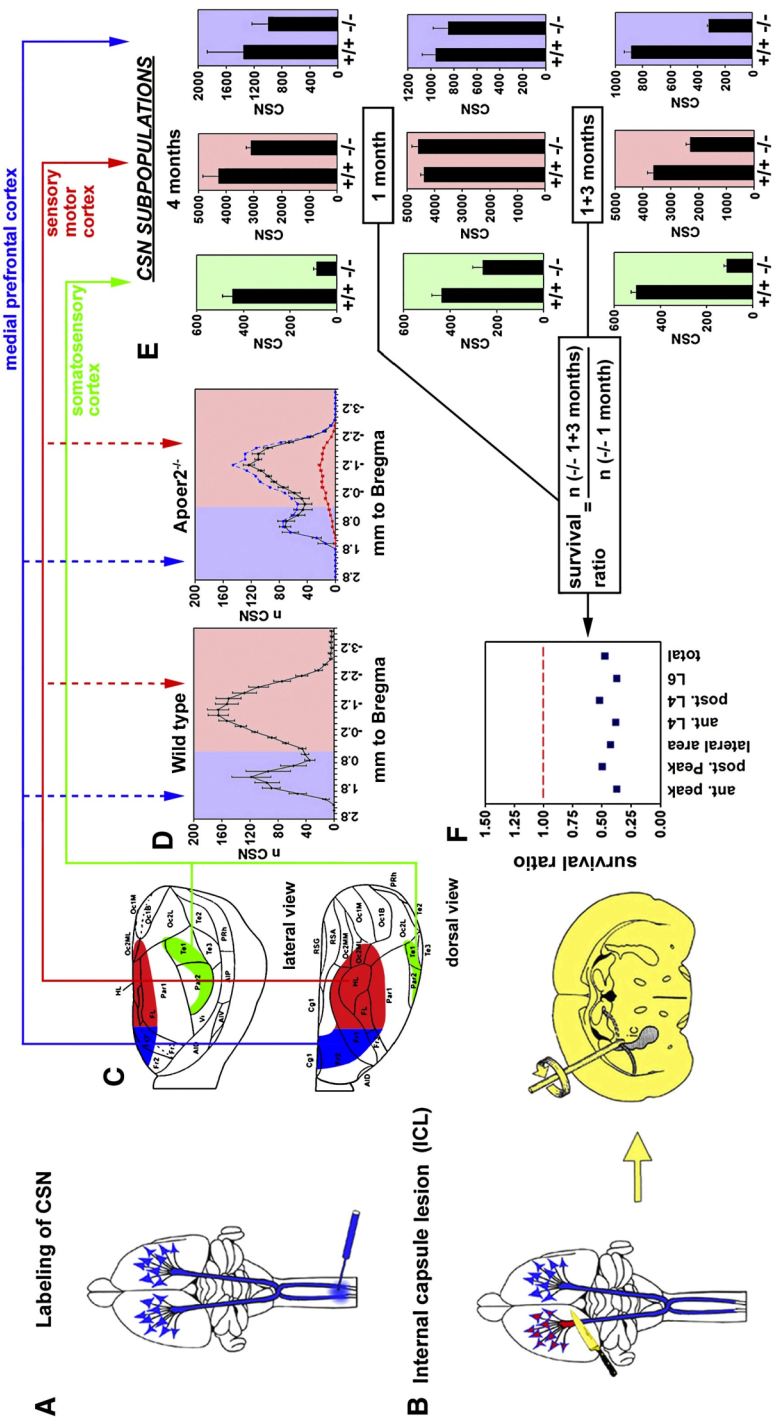

Figure 7. Determination of corticospinal neuron survival (Beffert *et al.*, 2006b)

Figure 7

(A) Schematic dorsal view of the mouse brain and cervical spinal cord showing CSN (blue triangles) labeled with TB injected into the corticospinal tract (blue band) at the cervical spinal cord.

(B) Lesioned animals received an ICL that axotomizes CSN (red) one week after TB labeling. The wire knife used for the ICL is schematically depicted in the yellow frontal plane (right).

(C–F) Scoring of CSN survival in wild-type and ApoER2 knockout mice.

(C) Location of CSN in medial and lateral cortical areas. The medial area (red and blue) consists anteriorly of the medial prefrontal cortex (blue) and posteriorly of the primary motor cortex (red). The lateral CSN area is located in the somatosensory cortex (green). CSN in the medial cortical areas form a contiguous population with regard to their anterior-posterior distribution. However, CSN of the medial prefrontal (blue in [D]) and primary motor cortex (red in [D]) can be separated and are indicated as CSN numbers of each subpopulation peak in the center of the respective areas. CSN of the somatosensory cortex are spatially separated from CSN of the medial areas.

(D) CSN in the medial cortical areas of wild-type and ApoER2$^{-/-}$ animals. In ApoER2 knockouts, CSN split into layer 4 CSN (black line) and layer 6 CSN (red line). Blue dotted line = sum of layer 4 and 6 CSN at the respective frontal levels. The data are from 4-month-old animals.

(E) CSN numbers at different postnatal stages depicted for the individual CSN areas (panel C) of wild-type (+/+; n = 10) and ApoER2 knockout (−/−; n = 8) animals (total CSN numbers in all areas are shown in Figure 8B-D).

For determining whether the reduced CSN number in 4-month-old knockouts ("4 months," see also Figure 8B) reflect death or altered neurogenesis, baseline CSN numbers were determined at one month ("1 month"), and CSN-survival assays were performed on 4-month-old animals that had received CSN tracing at one month ("1+3 months"). One-month-old mice were chosen because CSN connectivity is complete at this age. 1 month: ApoER2 knockouts display slightly increased CSN numbers in the primary motor cortex and reduced numbers in the other areas. The total number of CSN, however, is identical in wild-type and ApoER2$^{-/-}$ animals (see also Figure 8C). 1+3 months: All CSN areas in ApoER2 knockouts have reduced CSN numbers, indicating that CSN die in the absence of ApoER2 (for total CSN numbers, see Figure 8D). Statistical analysis of CSN survival was performed on total CSN because individual subpopulations may be different in ApoER2 knockouts than in wild-type animals as a result of altered migration of CSN in the mutants. Mean ± SEM (all graphs).

(F) The survival ratio of CSN in ApoER2$^{-/-}$ animals illustrates the fraction of CSN that survive in ApoER2 knockouts between the first and fourth postnatal months. This ratio is comparable for all CSN subpopulations, which suggests that similar mechanisms underlie neuronal survival and death.

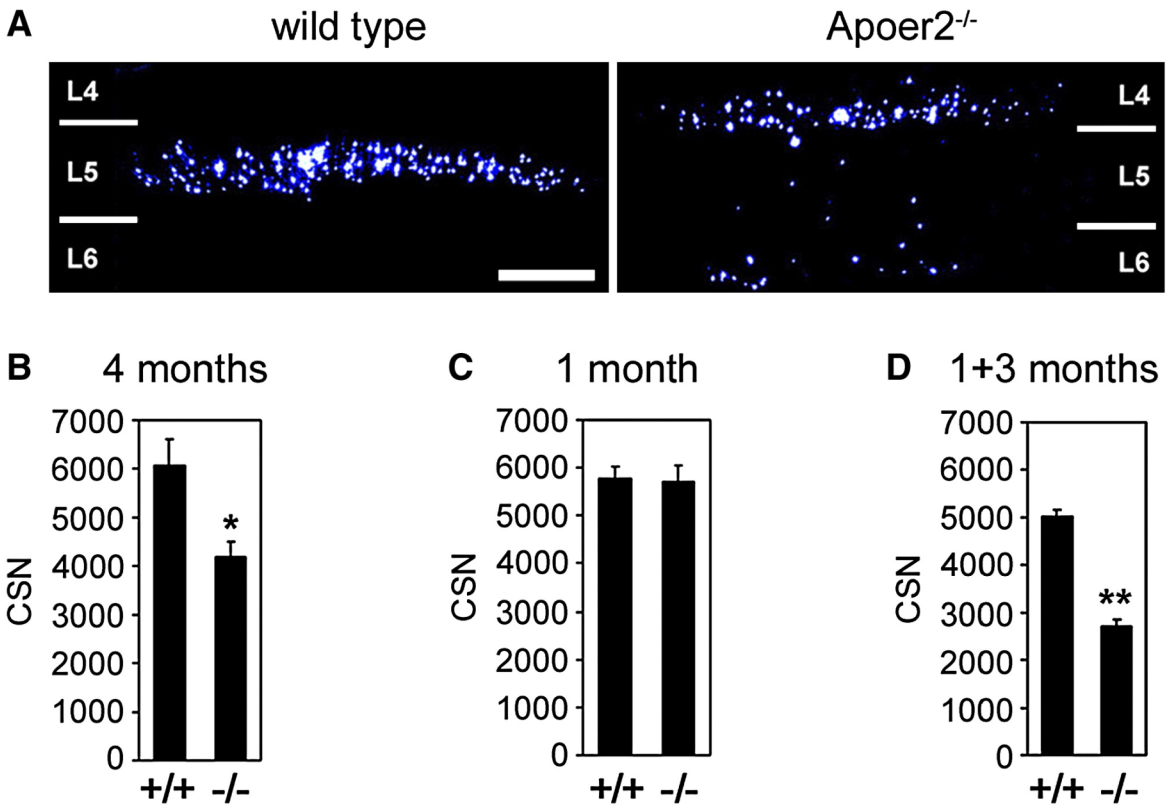

Figure 8. Malpositioning of layer V neurons and age-dependent loss of fluorescently labeled CSN during aging in wild-Type and ApoER2$^{-/-}$ mice (Beffert *et al.*, 2006b)

(A) Fluorescently (True Blue, TB)-labeled CSN in wild-type and ApoER2$^{-/-}$ mice. Location of cortical layers 4 (L4) – 6 (L6) is indicated. The scale bar represents 0.5 mm. (B) and (C) Total CSN number at different ages in wild-type and ApoER2$^{-/-}$ mice. (D) 1+3 months: CSN were TB-labeled at 1 month, and CSN survival was assessed 3 months later. In the other groups, CSN numbers were determined 1 week after tracing. Mean ± SEM (all graphs).

Statistical analysis of total CSN numbers (B-D):

(B) Wild-type (wt): n = 6. ApoER2$^{-/-}$: n = 6.

(C) wt: n = 10. ApoER2$^{-/-}$: n = 8.

(D) wt: n = 8. ApoER2$^{-/-}$: n = 6.

(B) As determined by students t-test, P=0.012 for wild type vs. ApoER2$^{-/-}$

(C,D) As determined by post hoc Newman-Keuls test (NKT), $p<0.01$ for 1 months ApoER2$^{-/-}$ vs. 1+3 months ApoER2$^{-/-}$ and 1+3 months wild type vs. 1+3 months ApoER2$^{-/-}$. As determined by NKT and post hoc Fisher's Least significance test (FLSD), no significant difference between 1 month wild type, 1 month ApoER2$^{-/-}$, and 1+3 months wild types.

Neuronal protection is mediated by the alternatively spliced Exon19

Neuronal death could either reflect an inherent ApoER2 survival-promoting function that might be dependent on the extracellular signals that ApoER2 transmits across the plasma membrane or, in the most trivial case, be simply due to the CSN mislayering that occurs in ApoER2$^{-/-}$ animals (Figure 8A). To distinguish between these possibilities, we made use of knockin strains of mice in which we had selectively altered the intracellular domain of ApoER2 to prevent the receptor from interacting with specific intracellular signaling pathways (Beffert et al., 2006a; Beffert et al., 2005; Herz and Bock, 2002). One of these knockin mouse lines lacks the binding site for Dab1, a cytoplasmic adaptor protein that is required for activation of Src-family tyrosine kinases upon exposure of neurons to the extracellular ligand Reelin. This ApoER2 ligand controls neuronal migration and positioning during embryonic brain development (Trommsdorff *et al.*, 1999), activates anti-apoptotic, and thus potentially survival-promoting signals, and regulates synaptic transmission through NMDA receptors (Chen *et al.*, 2005).

Another strain of knockin mice contains an alternatively spliced exon (exon 19) in the ApoER2 cytoplasmic domain region, that is not present in other LDL-receptor

family members (Beffert et al., 2005; Brandes et al., 2001). Exon 19 mediates the interaction of ApoER2 with Jun N-terminal kinase (JNK)-interacting proteins (JIPs) and with postsynaptic density protein 95 (PSD95) (Stockinger *et al.*, 2000). To determine the function of these regions and sequence motifs for CSN survival, we analyzed knockin mice that exclusively express only one of the following ApoER2 forms (Figure 6):

(1) ApoER2*[long]*, a full-length wild-type receptor containing both functional Dab1- and JIP/PSD-binding regions (Beffert *et al.*, 2005)

(2) ApoER2*[short]*, a truncated wild-type receptor containing the functional Dab1-binding motif but lacking the JIP/PSD-binding region (Beffert *et al.*, 2005)

(3) ApoER2*[Stop]*, a receptor that is truncated downstream of the Dab1-binding motif and thus lacks the JIP/PSD binding region and the rest of the cytoplasmic tail.

(4) ApoER2*[Dab^{-}]*, a full-length receptor that contains the JIP/PSD-binding region but whose Dab1-binding motif carries a mutation that prevents Dab1 interaction and activation (Beffert *et al.*, 2006a).

Analyses were performed on 8-month-old animals. As expected, we found that CSN were located correctly in layer 5 in animals expressing a functional Dab1-binding site (ApoER2*[long]*, ApoER2*[short]*, and ApoER2*[Stop]*) but that they were mislocalized to layers 4 and 6 if this site was inactivated (ApoER2*[Dab^{-}]*) (Figure 9). However, the number of CSN was not affected if only the Dab1-binding site was mutated (ApoER2*[Dab^{-}]*), indicating that mislocalization alone did not impair neuronal survival. In contrast, CSN numbers were reduced by approximately 36% if the JIP/PSD-binding site was missing (ApoER2*[short]*) and even more so, by approximately 64%, when the JIP/PSD-site including the distal cytoplasmic tail was absent (ApoER2*[Stop]*) (Figure 10).

Thus, CSN survival promotion by ApoER2 requires the ApoER2 tail's carboxy-terminal sequences that interact with JIPs and PSD95, but these are not essential for neuronal migration. The apparently normal and indistinguishable neuroanatomy of ApoER2*[long]*, ApoER2*[short]*, and ApoER2*[stop]* animals makes it further unlikely that the striking protection of the animals lacking exon 19 from lesion-induced neuronal death could be caused by differences in axonal pathfinding during development when the axons of exon-19-lacking mice would otherwise have to selectively circumnavigate the site of future lesion induction.

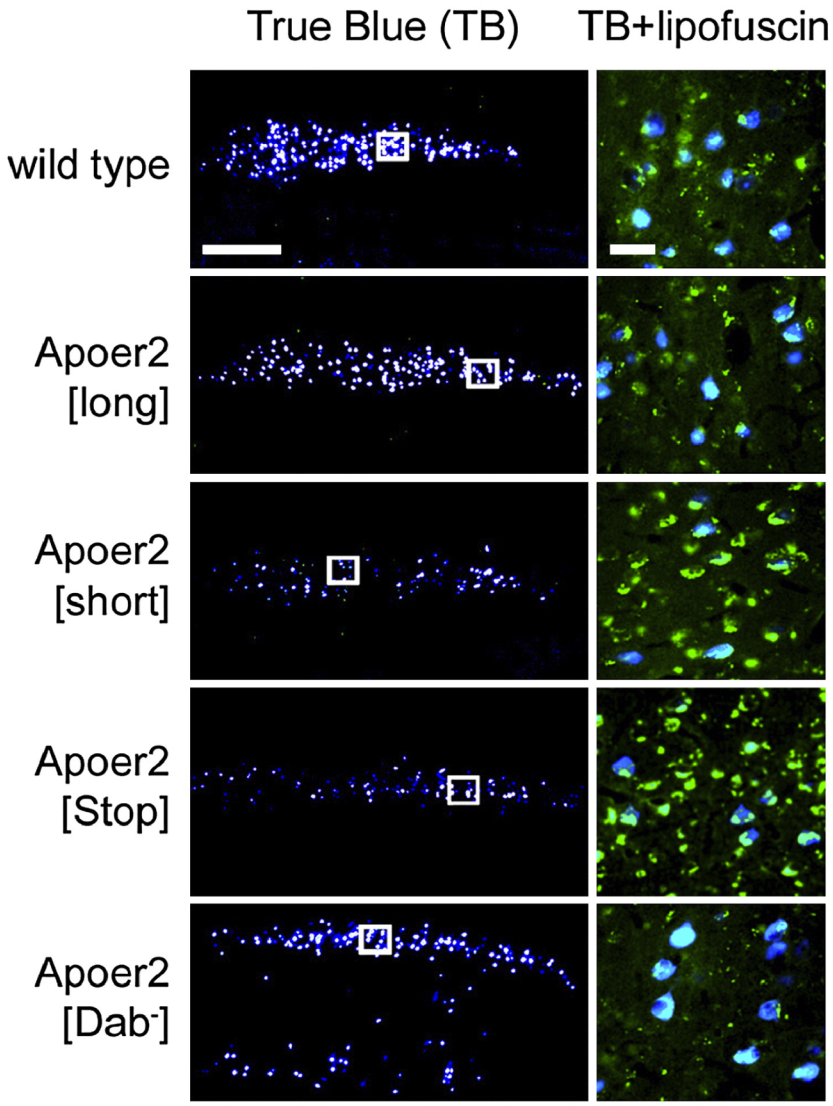

Figure 9. True Blue and lipofuscin labeled CSN (Beffert *et al.*, 2006b)

Left panels: TB-labeled CSN in the indicated mouse strains. The scale bar represents 0.5 mm. Right panels: CSN (DAPI-filter) and lipofuscin deposits (FITC-filter) at higher magnification (from boxed areas at the left side). The scale bar represents 50 µm. Labeled CSN are present in layer 5, except in ApoER2*[Dab^{-}]*, where they are misplaced to layers 4 (top) and 6 (dispersed cell population in bottom part of the image).

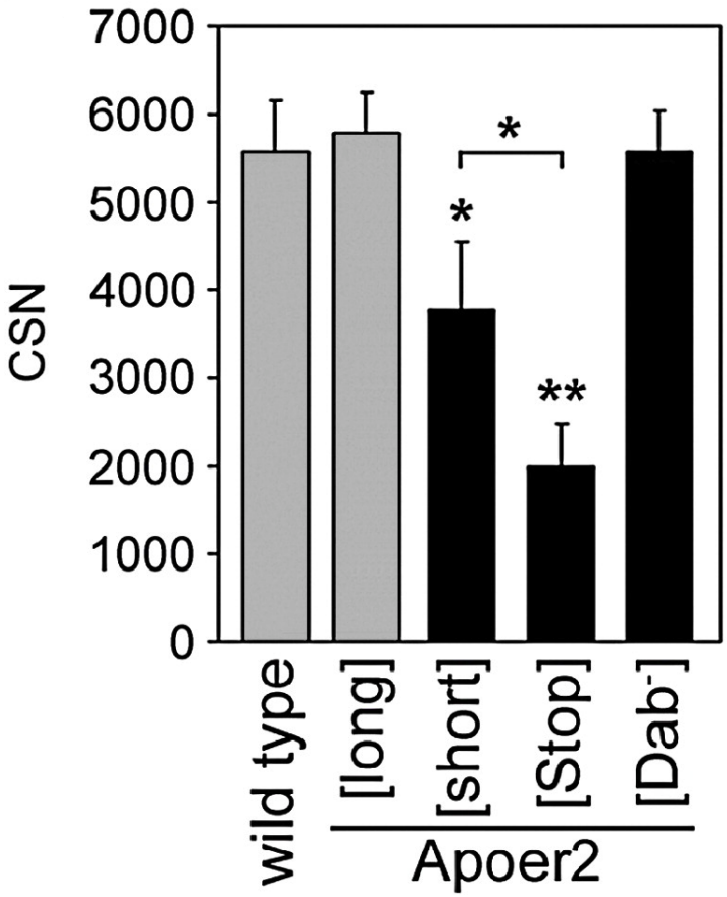

Figure 10. CSN numbers in ApoER2 knockin mutant strains (Beffert *et al.*, 2006b)

CSN numbers in 8 months old ApoER2 knockin mutant strains.

CSN number in wt (n = 8), ApoER2*[long]* (n = 6), ApoER2*[short]* (n = 8), ApoER2*[Stop]* (n = 10), and ApoER2*[Dab^{-}]* (n = 6) animals. The age of animals was 8 months. ApoER2*[short]* and ApoER2*[Stop]* versus others, $p < 0.001$ (ANOVA). Post hoc Newman-Keuls-test: $^{*} = p < 0.05$, $^{**} = p < 0.01$

Error bars represent SEM.

Role of Exon 19 in lesion-induced neuronal loss

The prominent and splice-form-specific role of ApoER2 for the survival of intact CSN led us to investigate whether ApoER2 would also affect the survival of neurons after injury. To investigate this, we chose a model in which CSN are mechanically damaged by stereotaxic deafferation at the level of the internal capsule (Figure 7B). Neurons were retrogradely labeled 7 days before deafferation, and 1-month-old animals were used because CSN numbers are equivalent for all genotypes at this age (Figure 8). Consistent with previous findings, 40% of the injured CSN died in wild-type animals whereas CSN of ApoER2$^{-/-}$ mice were resistant to injury-induced death (Figure 12, left panel). Similar survival ratios (normalized to the unlesioned side) were obtained in 4-month-old ApoER2 knockout animals, indicating that all neurons that have survived to this age are equally protected from lesion-induced death in the absence of ApoER2 (Figure 12, right panel). To determine whether the Dab1- or JIP-binding sites in ApoER2 are involved in injury-induced neuronal death, we assessed CSN survival in 4-month-old ApoER2*[long]*, ApoER2*[short]*, and ApoER2*[Dab^{-}]* mice (Figure 13). At this time point, age-dependent loss of CSN was not yet prominent in *ApoER2[short]* animals, and the total number of CSN in uninjured knockin animals was not significantly different from that in the wild-type. As was the case for survival in the uninjured state, injury-induced death did not depend on the Dab1 site but rather depended on the intracellular region that contains the PSD/JIP binding-sites (Figure 13) and (
Figure 11), with approximately 40% lesion-induced neuronal loss occurring in mice expressing the exon 19 sequences. By contrast, lesion-induced cell death was completely prevented when this exon was absent. These data suggest that the domain that recruits JIP/PSD95 in ApoER2 is critical for regulating survival not only during the aging process but also after traumatic injury to the brain.

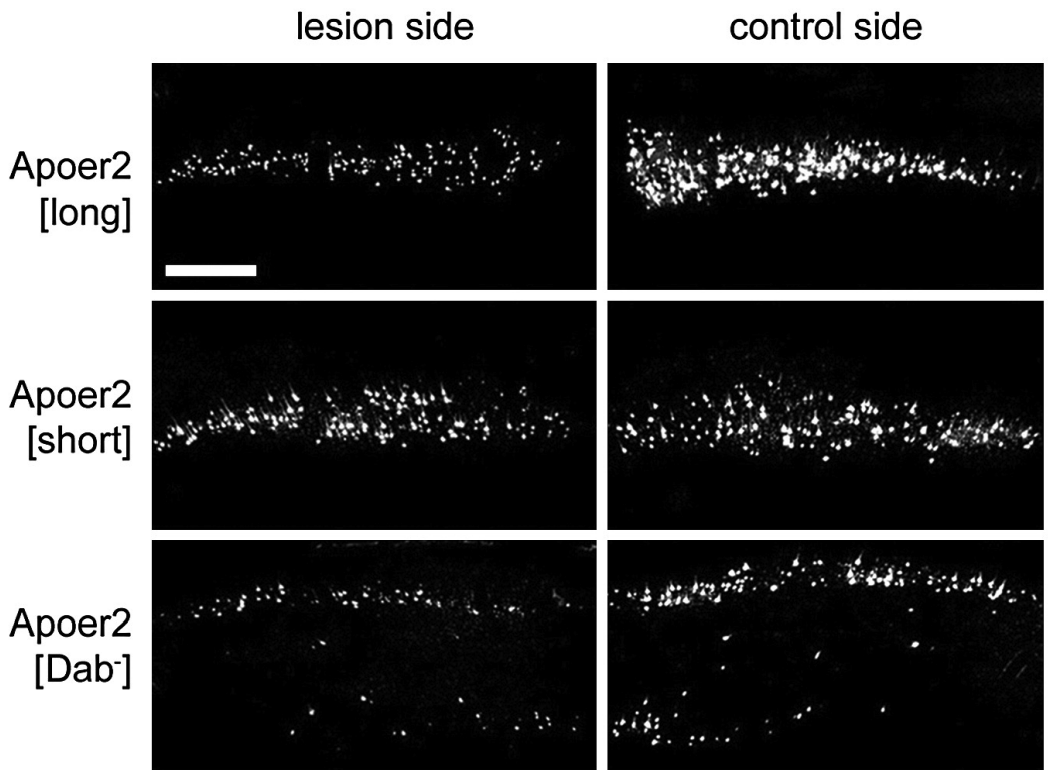

Figure 11. Neuronal degeneration in ApoER2 knockin mutant strains (Beffert *et al.*, 2006b)

Death of CSN by deafferentation is complete within the first 5 days after lesion induction [26]. CSN survival was thus assessed after 7 days. Deafferentation was performed on one side only; the contra- lateral unlesioned side served as a control (Figure 1B). Numbers of surviving CSN on the ipsilateral side were compared to those on the contralateral, unlesioned side. The scale bar represents 0.5 mm.

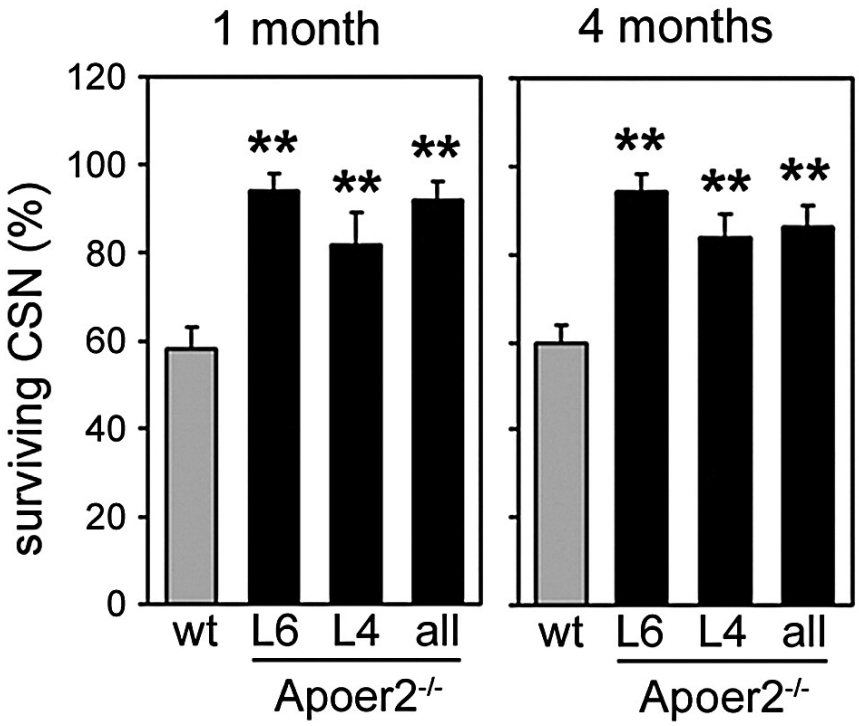

Figure 12. Lesion-induced CSN death is prevented in 1-month-old and 4-month-old ApoER2$^{-/-}$ mice (Beffert *et al.*, 2006b)

L4 = layer 4 CSN, L6 = layer 6 CSN. Four wt and three ApoER2$^{-/-}$ were analyzed. Statistical analysis of survival ratios: [1 month] as determined by NKT, $p<0.01$ for wt (n=5) vs. -/- L6, -/- L4, and -/- all (n=3 each). As determined by NKT and FLSD, non significant between -/- L6, -/- L4, and -/- all. [4 months] As determined by NKT, $p<0.01$ for wt (n=4) vs. -/- L6, -/- L4, and -/- all (n=3 each). As determined by NKT and Fisher LSD, non significant between -/- L6, -/- L4, and -/- all (n=3 each). (**indicates $p < 0.01$)

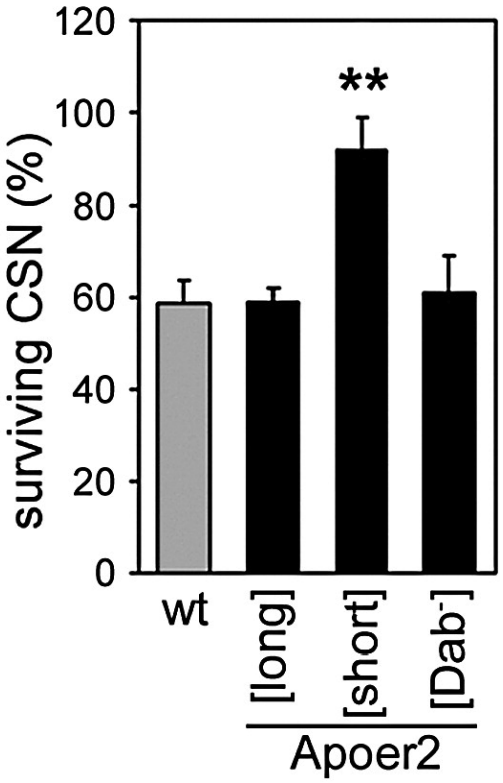

Figure 13. Lesion-induced CSN death in 4-month-old ApoER2[Dab^{-}] and ApoER2[long] animals (Beffert *et al.*, 2006b)

Lesion-induced CSN death in 4-month-old ApoER2*[Dab^{-}]* and ApoER2*[long]* animals is similar to that in wild-type animals. In contrast, lesion-induced death is completely prevented in ApoER2*[short]* animals ($p < 0.05$ as determined by NKT). n values: 4 (wt), 3 (ApoER2*[long]*), 5 (ApoER2*[short]*), and 3 (ApoER2*[Dab^{-}]*). As determined by NKT and Fisher LSD, non significant between wt, [long] and [Dab^{-}]

ApoE protects against neuronal loss during aging

To determine whether ApoE also effects survival of intact adult CSN during aging, we counted and compared CSN numbers in wild-type mice versus ApoE$^{-/-}$ knockout animals. The wild-type mice in the control group consisted of wild type (wt) hybrid as well as wild type B6 animals. The ApoE$^{-/-}$ knockout animals consisted of 3 strains. All animals were 9 months old.

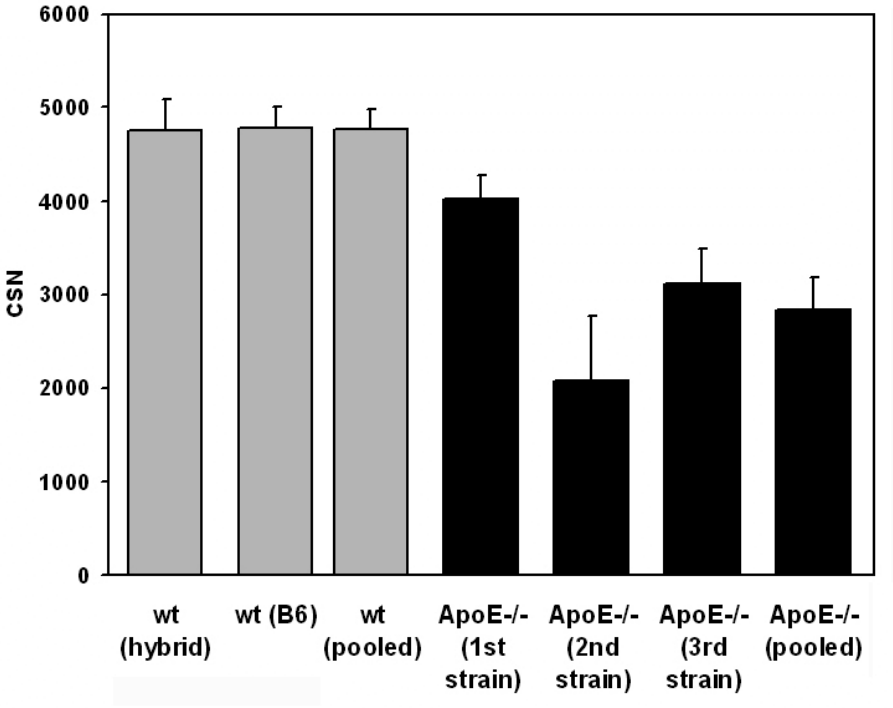

Figure 14. CSN number in wt and ApoE strains

CSN number in wt hybrid (n=8), wt (B6) (n=6), ApoE$^{-/-}$ strains: 1st strain (n=4), 2nd strain (n=10), 3rd strain (n=10). As determined by NKT, wts vs. ApoE$^{-/-}$ (2nd strain) and ApoE$^{-/-}$ (pooled) $p<0.05$ ($p<0.001$ in Fisher LSD).

ApoE induces CSN-death after traumatic brain injury

Lastly, we determined whether ApoE influences the survival of CSN after lesion. We found that CSN are protected from axotomy-induced death in mice deficient for ApoE (Figure 15 A,B). The pattern of survival regulation in ApoE knockout mice resembles that in ApoER2 knockout mice (Figures 8, 11) and ApoER2 [short] mice (Figures 10, 12). In all of these mouse lines, CSN die during aging but are protected from axotomy-induced death. This suggests that ApoE and ApoER2 function as a ligand/receptor pair to promote survival of intact adult CSN and to induce CSN-death after injury.

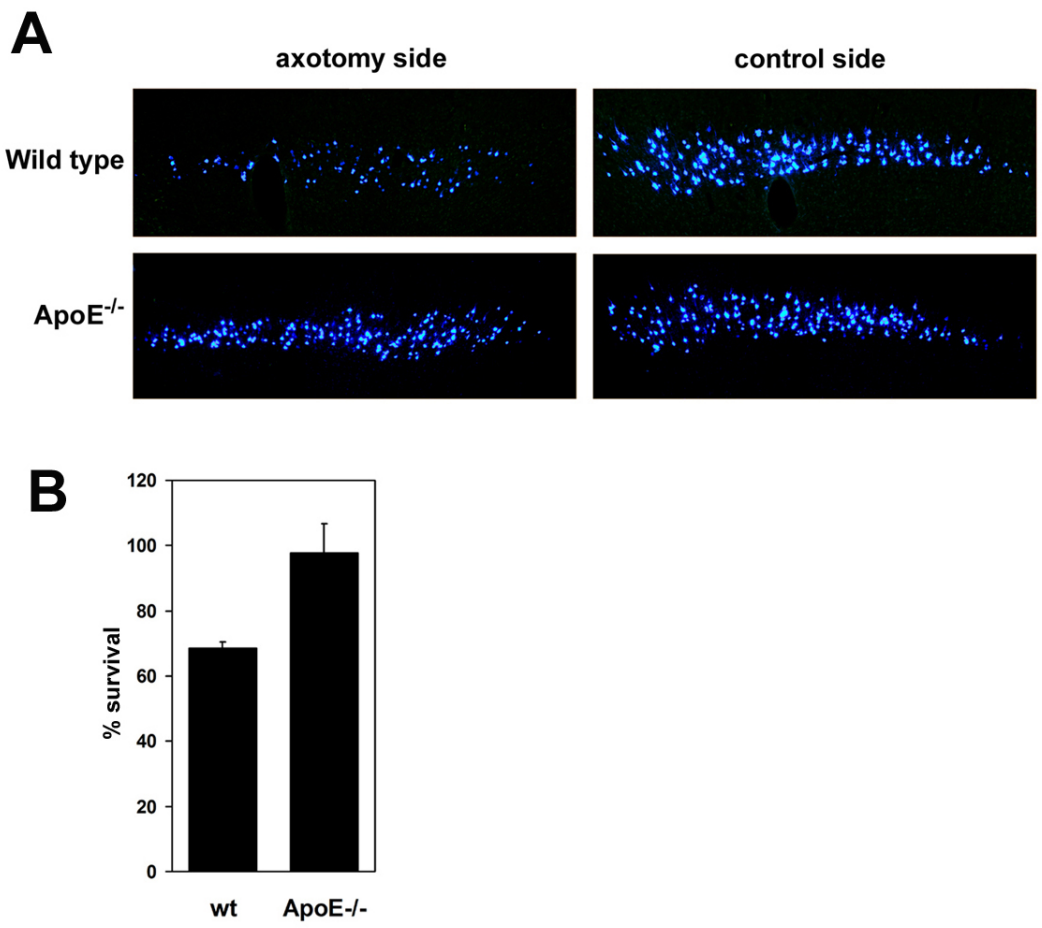

Figure 15. Lesion induced death is prevented in 8 months old ApoE$^{-/-}$ mice

(A) Photomicrographs from wild type (wt) and ApoE knockout mice. Size bar: 0.5 mm. (B) Percent survival of lesioned CSN. N=4 for wt and ApoE$^{-/-}$ respectively. As determined by NKT, wt vs. ApoE$^{-/-}$ $p<0.05$.

4. DISCUSSION

The goal of thesis was to investigate possible roles of ApoE and ApoER2 in the regulation of neuronal survival. The work was based on former data suggesting that ApoE is a major risk factor for several neurodegenerative diseases, including AD (Raber *et al.*, 2004). ApoE and one of its receptors, ApoER2, are known to influence neuropathology and neuronal transmission in several disease models of neurodegenerative disorders. Whether this receptor-ligand-pair also affects neuronal survival under physiological and pathological conditions in vivo was not known.

We established an experimental model to study survival regulation of intact and damaged adult CSN in vivo. Our recent data indicate that virtually all CSN express ApoER2 mRNA both in the intact state and after pathological damage (Giehl, unpublished observations). The present study was undertaken to show whether ApoER2 and its ligand ApoE regulate survival of normally aging CSN and adult CSN after axonal injury.

The fundamental hypotheses of our work where the following:

(i) Does ApoER2 affect adult CSN survival during normal aging and after axonal injury, and if so, which of the intracellular domains of the receptor known to initiate signal transduction is mediating this effect?

(ii) Does ApoE affect CSN survival during normal aging and after axonal injury?
To this end, we used an experimental model in genetically altered ApoER2 and in $ApoE^{-/-}$ mouse strains using a stereological approach.

The LDL receptor family members ApoER2 and VLDLR play an important role in regulation of neuronal migration in the developing brain by mediating reelin-signaling to the adaptor protein disabled-1 (Dab-1) (Trommsdorff *et al.*, 1999). It has been previously shown that normal layering of the cerebral cortex is serverely affected in ApoER2/VLDLR double-knockouts while the single knockouts display a subtle migratory phenotype (Trommsdorff *et al.*, 1999).

In our work we first evaluated the effects of ApoER2 on the migration of CSN. To this end, we retrogradely labeled CSN with fluorescent-dye in 4 months old ApoER2$^{-/-}$ animals. CSN were located in their typical cortical areas, but they were placed in the wrong cortical layers within these areas (Figure 7). Physiologically, CSN migrate to layer 5 during brain development. In our experimental setting CSN of ApoER2$^{-/-}$ mice split into two populations: one was located in layer 4 and the other in layer 6 (Figure 8a). This indicates that CSN are not a uniform cell population regarding migration but are split into two subpopulations. This phenotype has similarities and differences to the CSN layering in yotari and reeler mice. In these two mouse strains, the reeler pathway is affected by either a homozygous depletion of part of the reelin gene in reeler mice (Caviness and Rakic, 1978),(Bar *et al.*, 1995),(D'Arcangelo *et al.*, 1995) or by a mutation in the Dab1 gene in yotari mice (Sheldon *et al.*, 1997). CSN show a splitting in superficially and basally located neurons in the reeler mouse while CSN are predominantly located superficially in the yotari mouse. While the C4-CSN in ApoER2 knockouts form an anatomically well defined layer in a location corresponding to cortical layer 4, none of the CSN subpopulations in reeler or yotari mice form a layer but display unordered and more scattered distribution (Yamamoto *et al.*, 2003). Reelin mediates its developmental effects via both ApoER2 and VLDLR to Dab112 and VLDLR is still present in ApoER2$^{-/-}$ mice. Because reelin is produced in Cajal-Rezius cells at the surface of the developing cortex, this result suggests that ApoER2 mediates a stop signal for L4-CSN and that VLDLR confines this signal to one cortical layer. Thus, the differences in

CSN layering between ApoER2$^{-/-}$ and reelin/yotari mice suggest that ApoER2 and VLDLR convey different migratory signals to CSN in the reelin/Dab1 pathway. Alternatively, the border of layer 4 to 3 may contain an additional, clearly delineated stop signal that is normally not employed by migrating CSN but that is employed if ApoER2 is absent.

We next assessed whether CSN of ApoER2$^{-/-}$ mice display altered area distribution. Physiologically, CSN are located in three major cortical areas, the sensory motor cortex, the medial prefrontal cortex, and the somatosensory cortex (Giehl, 2001). To determine whether CSN in ApoER2 knockouts adhere to this area distribution, we mapped CSN throughout the cortex (Figure 7C). Layer 4 CSN display a normal area distribution pattern. In contrast, layer 6 CSN are absent in the somatosensory cortex and are only found in the posterior two thirds of the medial CSN-areas. This may indicate that layer 6 CSN represent a tangentially migrating CSN population arising from medio-central regions of the ventricular zone that fails to integrate into the very anterior and lateral CSN areas. Alternatively, layer 6 CSN could be a radially migrating population only occurring in the medio-dorsal CSN areas. The analysis of CSN numbers at 35th postnatal day (P35) displayed a relative shift of CSN frequency towards the medio-dorsal CSN areas suggesting that L6-CSN are a tangentially migrating cell population. The answer to this question, however, requires further investigation.

To quantify the size of the CSN population in the different mouse strains, we next plotted their frequencies at the respective frontal brain levels demonstrating that the number of CSN is reduced in the cortex of ApoER2 knockouts compared to the wild-type control group. Three different scenarios could account for the reduction of CSN in ApoER2 knockouts: (i) less CSN were generated in the first place; (ii) CSN die postnatally; (iii) mature CSN do not die but lose their axons. Since CSN are identified by retrograde labeling through their spinal cord axons, those CSN that have already undergone axonal degeneration at the time of tracer application would not be identified.

We initially examined, if CSN are produced at lower numbers in ApoER2 knockout mice during development. The CSN number was determined at the 35th postnatal day (P35), as the CSN development is completed after the first postnatal month. At this stage, CSN numbers are similar in ApoER2 knockout and wild-type mice (Figure 8c). Thus, we can conclude that ApoER2 does not influence CSN generation during brain development. In order to investigate, if CSN-reduction in 4 months old ApoER2 knockouts is due to postnatal axon degeneration, we labeled CSN at P35 and let the animals survive for additional three months. The rational for this experimental setting was that CSN numbers should be identical in wild types and knockouts after these three months if axonal degeneration but not cell death takes place. Our results demonstrate that ApoER2 knockouts have significantly less CSN than their wild type counterparts at four months of age due to CSN death and not because of postnatal axon degeneration (Figure 8d).

The postnatal death of CSN in the ApoER2 knockouts could either reflect a survival promoting function of this receptor, or it could be secondary consequence to the mislayering of CSN, which might not receive their normal survival factors in the wrong layers. To evaluate this, we aimed at determining whether ApoER2 possesses a domain that influences neuronal survival but not migration. Our working hypothesis was that ApoER2 regulates neuronal survival through a domain outside the Dab1-site, which mediates neuron migration. The alternatively spliced ApoER2 exon 19 appeared to be the most likely candidate for such a domain because it harbors motifs that potentially connect via JNK-interacting proteins to JNK pathways, which regulate neuronal survival (Chao, 2003; Herdegen et al., 1997; Herz and Bock, 2002).

This hypothesis was tested in 8 months old knockin mutant strains that exclusively expressed the specifically mutated ApoER2-receptors (Figure 6) ApoER2[long], ApoER2[short], ApoER2[stop], or ApoER2[Dab^{-}]. The gross anatomy (not shown) and cortical layering of CSN in ApoER2[long],

ApoER2[short], and ApoER2[stop] is similar to wild type animals (Figure 9). In contrast, the phenotype of ApoER2[Dab^{-}] mice is different from ApoER2 knockouts both regarding the gross anatomy of the brain (Beffert *et al.*, 2006a) as well as regarding the CSN numbers in the cerebral cortex (Figure 10). Thus, we can conclude that the migratory function of ApoER2 on CSN is mediated by the Dab1-site, but not by exon 19.

The total number of CSN is identical in wild type, ApoER2[long], and ApoER2[Dab^{-}] mice, but it is significantly reduced in ApoER2[short] as well as ApoER2[stop] mice (Figure 10). We can conclude that ApoER2 promotes CSN survival via its alternatively spliced exon 19, and that separate receptor domains of ApoER2 regulate neuronal migration and survival. Most importantly, the results show that mislayering of neurons does not cause postnatal cell death *per se*, and that ApoER2 promotes neuronal survival.

To determine whether ApoER2 also promotes the survival of injured neurons, we examined whether the CSN population surviving axotomy is reduced in ApoER2 knockouts. Axotomy at internal capsule levels causes death of approximately half of the injured neurons (Giehl et al., 2001; Giehl et al., 1998). The lesions were carried out on 4 months old mice. Interestingly, axotomy-induced death of CSN was almost completely prevented in ApoER2 knockouts while approximately 40 % of the CSN died in the wild type mice control group (Figure 12). These findings suggest that the function of ApoER2 switches from survival to death promotion after neuronal lesion. However, the lesions were carried out at the age of 4 months when CSN numbers in the knockouts are already reduced by one third (Figure 8b). If the death of intact CSN in the knockouts would primarily affect those CSN that are sensitive to axotomy-induced death, axotomy would not produce further death. To address this possibility, lesions were performed at 35th postnatal day when wild types and knockouts have identical CSN numbers. Also at this stage, axotomy-induced death was prevented in ApoER2 knockouts.

Thus, it can be concluded that ApoER2 is required for the death of lesioned CSN while it promotes their survival in an intact state.

The protection of lesioned CSN in ApoER2 knockouts shows an important aspect for construing the role of ApoER2 in neuronal survival. In genetic disease models for e.g. AD and ALS, death of adult neurons also occurs without setting a mechanical or ischemic damage. Yet, this death is not caused by the absence of a survival-promoting neurotrophic factor, but by exogenous introduction of a molecular signal into the system that interferes with basic homeostatic functions such as detoxification or metabolism. Similarly, death of unlesioned neurons in ApoER2 knockouts could reflect a crucial function of the receptor in the basic homeostasis of neurons. In this case, however, lesioned CSN would not be protected from axotomy-induced death in the absence of ApoER2. This result indicates that ApoER2 plays a specific role for survival regulation in the sense of a neurotrophic factor receptor.

We also addressed whether ApoE might be a ligand for ApoER2-dependent survival regulation. We focused on ApoE because the alternative ApoER2 ligand reelin mediates its effects via the Dab1-site. Analyses in 9 months old ApoE knockouts revealed that the number of intact CSN is significantly reduced compared to wild-type mice in the control group (Figure 14). Interestingly, axotomy-induced CSN death is prevented in the absence of ApoE.

In conclusion, these data indicate that ApoE, similar to ApoER2, promotes the survival of intact mature neurons while it induces their death after lesion. The similar patterns of ApoE and ApoER2 on survival regulation suggest that ApoE and ApoER2 act in concert, possibly as a ligand/receptor pair, in the regulation of neuronal survival in vivo.

The mechanism of an ApoE and ApoER2 mediated survival regulation of CSN is presently not well understood. It was surprising that activation of Dab1 is not required for the regulation of neuronal survival, despite the fact that the Dab1-binding site is required to activate survival promoting kinases (Chao, 2003), such as PI3-kinase and Akt (Beffert *et al.*, 2002; Beffert *et al.*, 2006b). In contrast, our experiments show that the JIP binding motifs bearing ApoER2 Exon 19 is required for the death induction as well as the survival of CSN. This suggests involvement of a JNK-pathway in this context. Indeed, we show in parallel work (Beffert *et al.*, 2006b) that the PSD95/JIP-binding region of exon 19 recruits the JNK3 activating scaffolding protein JIP1 and that the lesion induced death of CSN requires JNK3 activity. This argues for ApoER2 mediated activation of JNK3 for the massive and rapid death following neuronal injury, but does -at the first glance- not explain exon 19's requirement for ApoER2 dependent long term survival promotion of intact CSN. It is, however, conceivable that ApoER2 in intact CSN sequesters death inducing JNK complexes at the plasma membrane to be held in an inactive state from which they can be released upon activation by neuronal injury. In the absence of ApoE, ApoER2 or exon 19, these JNK complexes would then be constitutively active, resulting in the progressive neuronal loss we have observed in our aging animals. This is consistent with the finding that ApoE genotype has only been associated with neurological disorders that manifest themselves during midlife and senescence, or after neuronal injury.

Alternatively, ApoE/ApoER2 may alter lipid metabolism and/or trafficking in a manner affecting neuronal survival. This possibility is, however, not supported by our results since survival was not affected in animals bearing a mutation in the NPxY motif. This motif is an endocytosis signal for lipoprotein transport across the plasma membrane (Chen *et al.*, 1990) and receptor trafficking (Garcia *et al.*, 2001), (Jones *et al.*, 2003). Thus, its mutation in ApoER2 would be expected to affect CSN survival if lipid metabolism was to play a role in this context.

To our knowledge, ApoE/ApoER2 is so far the only ligand/receptor system described to promote the survival of intact mature CNS neurons *in vivo*. Our results further suggest that this ligand-receptor pair is involved in switching neuronal survival regulation after damage. The mechanisms underlying this switch are unknown. Because both survival-promotion in the intact and death-induction in the lesioned state depend on exon 19, it is unlikely that alternative splicing of this exon causes the switch. One potential mechanism for this switch are alternative splicing events in other parts of the receptor, e.g. the extracellular domain of ApoER2, which potentially affect ligand binding specificity. Alternatively, ApoER2 in intact CSN may associate with functionally different co-receptors or signal transduction molecules than after a lesion. This regulatory principle for neuronal survival regulation is, for example, employed by the neurotrophin receptor p75 (Nykjaer *et al.*, 2005). In an intact state, p75 supports survival by augmenting the affinity of mature neurotrophins to their respective Trk receptors. In contrast, lesion induced sortilin renders p75 to convey apoptosis-inducing signals of proneurotrophins after pathological damage of CSN (Harrington *et al.*, 2004).

Because the human genotype of ApoE predicts the risk for the late-onset form of AD (Corder et al., 1994; Corder et al., 1993), and because the plasma levels of ApoE correlate with disease progression of ALS (Lacomblez *et al.*, 2002), our results might be important for the understanding of neuronal death in the context of neurodegenerative diseases.

5. SUMMARY

Alzheimer's disease is the most prevalent among the neurodegenerative diseases and the most common cause of dementia. The work was based on former data suggesting that ApoE is a major risk factor for several neurodegenerative diseases, including Alzheimer's disease.

ApoE and one of its receptors, ApoER2, are known to influence neuropathology and neuronal transmission in several disease models of neurodegenerative disorders. Whether this receptor-ligand-pair also affects neuronal survival under physiological and pathological conditions in vivo was not known.

This study was designed to characterize the role of ApoER2 and its ligand ApoE in the regulation of neuronal survival of normally aging CSN and after axonal injury. We also investigated, which of the intracellular domain of ApoER2 initiate the signal transduction and mediates survival or neuronal death. To this end, we used an experimental model in genetically altered ApoER2 and in $ApoE^{-/-}$ mouse strains using a stereological approach. To determine the role of ApoE and ApoER2 in neuronal survival in vivo, we used retrograde fluorescent-dye labeling of corticospinal neurons to monitor neuronal loss during aging or after injury by deafferation. Quantitation of corticospinal neuron numbers were determined with a stereological approach in which the total number of CSN in the entire cortex was assessed.

Our data indicate that ApoE, similar to ApoER2, promotes the survival of intact mature neurons while it induces their death after lesion. To our knowledge, ApoE/ApoER2 is so far the only ligand/receptor system described to promote the survival of intact mature CNS neurons *in vivo*. Our work also implies that ApoER2 promotes CSN survival via its alternatively spliced exon 19, and that separate receptor domains of ApoER2 regulate neuronal migration and survival.

The similar patterns of ApoE and ApoER2 on survival regulation suggest that ApoE and ApoER2 act in concert, possibly as a ligand/receptor pair, in the regulation of neuronal survival in vivo. Our results might be important for the understanding of neuronal death in the context of neurodegenerative diseases.

6. ABBREVIATIONS

Aβ	β amyloid peptide
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
ANOVA	analysis of variance
ApoE	apolipoprotein E
APP	amyloid precursor protein
CNS	central nervous system
CSN	corticospinal neurons
Dab-1	adaptor protein disabled-1
FLSD	Fishers Least Significant Difference Test
HDL	high-density lipoprotein
ICL	internal capsule lesion
IDL	intermediate-density lipoprotein
JIPs	JNK-interacting proteins
JNK	Jun N-terminal kinase
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
LRP	low-density lipoprotein receptor-related protein
NKT	Newman-Keuls-test
TB	True Blue (dye)
VLDL	very low-density lipoprotein
VLDLR	very low-density lipoprotein receptor

7. REFERENCES AND BIBLIOGRAPHIE

Allendoerfer KL, Shatz CJ (1994) The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. Annu Rev Neurosci 17:185-218.

Alzheimer A (1907) Ü ber eine eigenartige Erkrankung der Hirnrinde. Allgemeine Zeitschrift fű r Psychiatrie psychisch - gerichtliche Medicin 64:146-148.

Bar I, Lambert De Rouvroit C, Royaux I, Krizman DB, Dernoncourt C, Ruelle D, Beckers MC, Goffinet AM (1995) A YAC contig containing the reeler locus with preliminary characterization of candidate gene fragments. Genomics 26:543-549.

Beffert U, Durudas A, Weeber EJ, Stolt PC, Giehl KM, Sweatt JD, Hammer RE, Herz J (2006a) Functional dissection of Reelin signaling by site-directed disruption of Disabled-1 adaptor binding to apolipoprotein E receptor 2: distinct roles in development and synaptic plasticity. J Neurosci 26:2041-2052.

Beffert U, Morfini G, Bock HH, Reyna H, Brady ST, Herz J (2002) Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. J Biol Chem 277:49958-49964.

Beffert U, Nematollah Farsian F, Masiulis I, Hammer RE, Yoon SO, Giehl KM, Herz J (2006b) ApoE receptor 2 controls neuronal survival in the adult brain. Curr Biol 16:2446-2452.

Beffert U, Stolt PC, Herz J (2004) Functions of lipoprotein receptors in neurons. J Lipid Res 45:403-409.

Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, Li WP, Adelmann G, Frotscher M, Hammer RE, Herz J (2005) Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. Neuron 47:567-579.

Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368:387-403.

Blessed G, Tomlinson BE, Roth M (1968) The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 114:797-811.

Bonatz H, Rohrig S, Mestres P, Meyer M, Giehl KM (2000) An axotomy model for the induction of death of rat and mouse corticospinal neurons in vivo. J Neurosci Methods 100:105-115.

Brandes C, Kahr L, Stockinger W, Hiesberger T, Schneider WJ, Nimpf J (2001) Alternative splicing in the ligand binding domain of mouse ApoE receptor-2 produces receptor variants binding reelin but not alpha 2-macroglobulin. J Biol Chem 276:22160-22169.

Caviness VS, Jr., Rakic P (1978) Mechanisms of cortical development: a view from mutations in mice. Annu Rev Neurosci 1:297-326.

Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 4:299-309.

Chen WJ, Goldstein JL, Brown MS (1990) NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. J Biol Chem 265:3116-3123.

Chen Y, Beffert U, Ertunc M, Tang TS, Kavalali ET, Bezprozvanny I, Herz J (2005) Reelin modulates NMDA receptor activity in cortical neurons. J Neurosci 25:8209-8216.

Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., Rimmler JB, Locke PA, Conneally PM, Schmader KE, et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 7:180-184.

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921-923.

D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 374:719-723.

de Knijff P, van den Maagdenberg AM, Frants RR, Havekes LM (1994) Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. Hum Mutat 4:178-194.

Dieckmann M, Dietrich MF, Herz J (2010) Lipoprotein receptors--an evolutionarily ancient multifunctional receptor family. Biol Chem 391:1341-1363.

Drory VE, Birnbaum M, Korczyn AD, Chapman J (2001) Association of APOE epsilon4 allele with survival in amyotrophic lateral sclerosis. J Neurol Sci 190:17-20.

Fauci AS (2008) Harrison's principles of internal medicine, 17th ed. New York: McGraw-Hill.

Fratiglioni L, Viitanen M, von Strauss E, Tontodonati V, Herlitz A, Winblad B (1997) Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen Project, Stockholm. Neurology 48:132-138.

Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J, Babaey S, Yehuda B, Groswasser Z (1999) Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. Neurology 52:244-248.

Garcia CK, Wilund K, Arca M, Zuliani G, Fellin R, Maioli M, Calandra S, Bertolini S, Cossu F, Grishin N, Barnes R, Cohen JC, Hobbs HH (2001) Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. Science 292:1394-1398.

Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B (2006) Mild cognitive impairment. Lancet 367:1262-1270.

Giehl KM (2001) Trophic dependencies of rodent corticospinal neurons. Rev Neurosci 12:79-94.

Giehl KM, Rohrig S, Bonatz H, Gutjahr M, Leiner B, Bartke I, Yan Q, Reichardt LF, Backus C, Welcher AA, Dethleffsen K, Mestres P, Meyer M (2001) Endogenous brain-derived neurotrophic factor and neurotrophin-3 antagonistically regulate survival of axotomized corticospinal neurons in vivo. J Neurosci 21:3492-3502.

Giehl KM, Schutte A, Mestres P, Yan Q (1998) The survival-promoting effect of glial cell line-derived neurotrophic factor on axotomized corticospinal neurons in vivo is mediated by an endogenous brain-derived neurotrophic factor mechanism. J Neurosci 18:7351-7360.

Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 83:4913-4917.

Hagiwara A, Hashimoto Y, Niikura T, Ito Y, Terashita K, Kita Y, Nishimoto I, Umezawa K (2000) Neuronal cell apoptosis by a receptor-binding domain peptide of ApoE4, not through low-density lipoprotein receptor-related protein. Biochem Biophys Res Commun 278:633-639.

Hardy J (2006) A hundred years of Alzheimer's disease research. Neuron 52:3-13.

Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353-356.

Harrington AW, Leiner B, Blechschmitt C, Arevalo JC, Lee R, Morl K, Meyer M, Hempstead BL, Yoon SO, Giehl KM (2004) Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. Proc Natl Acad Sci U S A 101:6226-6230.

Herdegen T, Skene P, Bahr M (1997) The c-Jun transcription factor--bipotential mediator of neuronal death, survival and regeneration. Trends Neurosci 20:227-231.

Herz J (2009) Apolipoprotein E receptors in the nervous system. Curr Opin Lipidol 20:190-196.

Herz J, Bock HH (2002) Lipoprotein receptors in the nervous system. Annu Rev Biochem 71:405-434.

Herz J, Chen Y (2006) Reelin, lipoprotein receptors and synaptic plasticity. Nat Rev Neurosci 7:850-859.

Herz J, Chen Y, Masiulis I, Zhou L (2009) Expanding functions of lipoprotein receptors. J Lipid Res 50 Suppl:S287-292.

Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R (2007) How common are the "common" neurologic disorders? Neurology 68:326-337.

Iqbal K, Alonso Adel C, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I (2005) Tau pathology in Alzheimer disease and other tauopathies. Biochim Biophys Acta 1739:198-210.

Ji ZS, Miranda RD, Newhouse YM, Weisgraber KH, Huang Y, Mahley RW (2002) Apolipoprotein E4 potentiates amyloid beta peptide-induced lysosomal leakage and apoptosis in neuronal cells. J Biol Chem 277:21821-21828.

Jones C, Hammer RE, Li WP, Cohen JC, Hobbs HH, Herz J (2003) Normal sorting but defective endocytosis of the low density lipoprotein receptor in mice with autosomal recessive hypercholesterolemia. J Biol Chem 278:29024-29030.

Jones EG, Schreyer DJ, Wise SP (1982) Growth and maturation of the rat corticospinal tract. Prog Brain Res 57:361-379.

Jordan J, Galindo MF, Miller RJ, Reardon CA, Getz GS, LaDu MJ (1998) Isoform-specific effect of apolipoprotein E on cell survival and beta-

amyloid-induced toxicity in rat hippocampal pyramidal neuronal cultures. J Neurosci 18:195-204.

Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733-736.

Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486-489.

Klein WL, Krafft GA, Finch CE (2001) Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? Trends Neurosci 24:219-224.

Lacomblez L, Doppler V, Beucler I, Costes G, Salachas F, Raisonnier A, Le Forestier N, Pradat PF, Bruckert E, Meininger V (2002) APOE: a potential marker of disease progression in ALS. Neurology 58:1112-1114.

Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240:622-630.

Marin-Padilla M (1992) Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: a unifying theory. J Comp Neurol 321:223-240.

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci U S A 82:4245-4249.

Michikawa M, Yanagisawa K (1999) Apolipoprotein E4 isoform-specific actions on neuronal cells in culture. Mech Ageing Dev 107:233-243.

Milne RD, Davis JP (1992) The role of the shaft in the golf swing. J Biomech 25:975-983.

Moller HJ, Graeber MB (1998) The case described by Alois Alzheimer in 1911. Historical and conceptual perspectives based on the clinical record and neurohistological sections. Eur Arch Psychiatry Clin Neurosci 248:111-122.

Nukina N, Ihara Y (1986) One of the antigenic determinants of paired helical filaments is related to tau protein. J Biochem 99:1541-1544.

Nykjaer A, Willnow TE, Petersen CM (2005) p75NTR--live or let die. Curr Opin Neurobiol 15:49-57.

O'Leary DD, Koester SE (1993) Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. Neuron 10:991-1006.

Oudega M, Varon S, Hagg T (1994) Distribution of corticospinal motor neurons in the postnatal rat: quantitative evidence for massive collateral elimination and modest cell death. J Comp Neurol 347:115-126.

Paxinos G, Franklin KBJ (2001) The mouse brain in stereotaxic coordinates, 2nd ed. San Diego: Academic Press.

Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N (1992) Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci U S A 89:4471-4475.

Porter R (1985) The corticomotoneuronal component of the pyramidal tract: corticomotoneuronal connections and functions in primates. Brain Res 357:1-26.

Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362:329-344.

Raber J, Huang Y, Ashford JW (2004) ApoE genotype accounts for the vast majority of AD risk and AD pathology. Neurobiol Aging 25:641-650.

Sabo T, Lomnitski L, Nyska A, Beni S, Maronpot RR, Shohami E, Roses AD, Michaelson DM (2000) Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. Neuroscience 101:879-884.

Schupf N, Kapell D, Nightingale B, Rodriguez A, Tycko B, Mayeux R (1998) Earlier onset of Alzheimer's disease in men with Down syndrome. Neurology 50:991-995.

Sheldon M, Rice DS, D'Arcangelo G, Yoneshima H, Nakajima K, Mikoshiba K, Howell BW, Cooper JA, Goldowitz D, Curran T (1997) Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. Nature 389:730-733.

Stockinger W, Brandes C, Fasching D, Hermann M, Gotthardt M, Herz J, Schneider WJ, Nimpf J (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. J Biol Chem 275:25625-25632.

Tanzi RE (2005a) The synaptic Abeta hypothesis of Alzheimer disease. Nat Neurosci 8:977-979.

Tanzi RE (2005b) Tangles and neurodegenerative disease--a surprising twist. N Engl J Med 353:1853-1855.

Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120:545-555.

Taylor KI, Probst A (2008) Anatomic localization of the transentorhinal region of the perirhinal cortex. Neurobiol Aging 29:1591-1596.

Teasdale GM, Nicoll JA, Murray G, Fiddes M (1997) Association of apolipoprotein E polymorphism with outcome after head injury. Lancet 350:1069-1071.

Tomlinson BE, Blessed G, Roth M (1970) Observations on the brains of demented old people. J Neurol Sci 11:205-242.

Trommsdorff M, Gotthardt M, Hiesberger T, Shelton J, Stockinger W, Nimpf J, Hammer RE, Richardson JA, Herz J (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. Cell 97:689-701.

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286:735-741.

Walsh DM, Selkoe DJ (2004) Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 44:181-193.

Walsh DM, Selkoe DJ (2007) A beta oligomers - a decade of discovery. J Neurochem 101:1172-1184.

Weisgraber KH, Roses AD, Strittmatter WJ (1994) The role of apolipoprotein E in the nervous system. Curr Opin Lipidol 5:110-116.

Wimo A, Prince M. 2010. World Alzheimer Report 2010. The Global Economic Impact of Dementia Executive Summary. In: Alzheimer's Disease International.

Yamamoto T, Sakakibara S, Mikoshiba K, Terashima T (2003) Ectopic corticospinal tract and corticothalamic tract neurons in the cerebral cortex of yotari and reeler mice. J Comp Neurol 461:61-75.

Younkin SG (1998) The role of A beta 42 in Alzheimer's disease. J Physiol Paris 92:289-292.

8. ACKNOWLEDGEMENT

Ich bedanke mich herzlich bei Frau Prof. Dr. Ulrike Beisiegel, Herrn Prof. Dr. Joachim Herz und Herrn Dr. Klaus Giehl, die es mir ermöglicht haben, meine Forschungsarbeit in den USA durchzuführen. Zudem gilt mein Dank an Herrn Prof. Dr. Jörg Heeren für die freundliche Weiterbetreuung meiner Doktorarbeit.

Bedanken möchte ich mich ebenfalls bei Frau Christine Runge für Ihre Unterstützung und freundliche Art, die mir immer Kraft gegeben hat.

Ganz besonders danke ich meinen Eltern, meinen Mann, meinen Kindern und meiner Schwester, die immer Verständnis hatten und mir viel Mut und Kraft gaben die Doktorarbeit fertig zu stellen.

9. STATUTORY DECLARATION

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Aussage (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Unterschrift: