Prion-Like Aspects of β-Amyloid Aggregation: Seeded Strain-Like Propagation of β-Amyloid Morphotypes and Peripheral Transmission of Cerebral β-Amyloidosis in APP Transgenic Mice

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Erklärung

Hiermit erkläre ich, dass ich die zur Promotion eingereichte Arbeit mit dem Titel:

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Seeded strain-like propagation of β-amyloid morphotypes and peripheral transmission of cerebral β-amyloidosis in APP transgenic mice"

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Für Hanne und Helmut

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Contents

Deutsche Zusammenfassung

Bei vielen neurodegenerativen Erkrankungen wie z.B. Morbus Alzheimer, Morbus Parkinson, Morbus Huntington und bei Prionerkrankungen tritt die Anhäufung krankheitsspezifischer Proteine auf. Folglich wurde der Begriff "Protheopathien" für diese Erkrankungen eingeführt. Die Alzheimer Erkrankung ist die häufigste dieser Proteopathien. Die Anzahl betroffener Patienten steigt aufgrund zunehmender Überalterung unserer Gesellschaft stetig an.

Die Anhäufung von Amyloid-Beta (Aβ) im Gehirn von Alzheimerpatienten wird als initialer Prozeß in der Pathogenese dieser Krankheit betrachtet, gefolgt von dem Auftreten neurofibrillärer Bündel ("neurofibrillary tangles"), dem Absterben von Nervenzellen und zunehmendem Verlust kognitiver Fähigkeiten. Zur Identifizierung von Therapiezielen ist daher einerseits das Verständnis der Mechanismen, die der Aggregation von Aβ zugrunde liegen, sowie andrerseits auch deren Auswirkungen von großer Bedeutung. Das Ziel dieser Doktorarbeit war die Untersuchung mechanistischer Gemeinsamkeiten zwischen der Induktion der Aβ-Aggregation und Prionen, den infektiösen Proteinablagerungen, die bei Prionenerkrankungen auftreten.

Im ersten Projekt dieser Doktorarbeit wurde die Übertragbarkeit der β-Amyloidose an einem Mausmodell, welches ein Transgen für mutiertes, humanes β-Amyloid–Vorläufer Protein (Amyloid Precursor Protein, APP) trägt, untersucht. Hierbei war das Ziel, eine potentielle Induktion zerebraler Ablagerung von Aβ im Gehirn von jungen, APP-transgenen Mäusen nach peripherer Exposition gegenüber Hirnextrakt, welches aggregiertes Aβ (Aβ Nukleationskeime) beinhaltet, zu eruieren. Bisherige Mausstudien haben gezeigt, dass die intrazerebrale Applikation von Hirnextrakt mit aggregiertem Aβ die zeit- und konzentrationsabhängige Aggregation und Ablagerung im Gehirn induziert (Meyer-Luehmann et al., 2006). Die in dieser Doktorarbeit präsentierten Ergebnisse demonstrieren, dass die intraperitoneale Injektion von Hirnextrakt von gealterten APP-trangenen Mäusen ausreicht, um die Ablagerung von Aβ im Gehirn junger APP-transgener Mäuse zu initiieren. Die intraperitoneale Injektion benötigt jedoch eine längere Inkubationszeit im Vergleich zur intrazerebralen Applikation. Diese Studie wurde in *Science* publiziert, aufgrund meines Beitrags bin ich Co-Autor dieser Studie (Eisele et al., 2010).

Im zweiten Teil dieser Doktorarbeit wurde die Übertragung unterschiedlicher Konformationsvarianten von β-Amyloid Ablagerungen mit unterschiedlichen Verhältnissen von Aβ40 zu Aβ42 untersucht. Zu diesem Zweck wurden junge, transgene APP23 und APPPS1 Mäuse intrazerebral mit Hirnextrakt mit aggregiertem Aβ von gealterten, transgenen APP23 oder APPPS1 Mäusen inokuliert. Die Aβ Ablagerungen von Spender- und Empfängertieren wurde sowohl anhand histologischer Färbung und darauf folgender Spektralanalyse mit Hilfe von lumineszenten konjugierten Polythiophenen (LCP) als auch durch biochemische Analyse von lasermikrodissektiertem Gewebe aus Hirnschnitten charakterisiert. Die Resultate zeigen, dass in den induzierten Ablagerungen sowohl das Verhältnis von Aβ40 zu Aβ42 als auch die Konformation der induzierten Aβ-Morphotypen von den Eigenschaften des injizierten Gehirnextrakts sowie der Empfängertiere abhängig ist. Diese Resultate weisen Parallelen zu Studien auf, die zur Identifikation von Prionenstämmen führten. Diese Studie wurde in Kollaboration mit K. P. R. Nilsson von der Universität Linköping in Schweden durchgeführt. Diese Studie, bei welcher ich Erstautor bin, wurde in *EMBO Reports* publiziert (Heilbronner et al., 2013).

In einem dritten Teil dieser Doktorarbeit wurde der Einfluss von induzierten Aβ-Ablagerungen im Hippocampus von APP-transgenen Mäusen auf deren kognitive Fähigkeiten untersucht. Um den Einfluss von Hirnextrakt-induzierter hippocampaler Ablagerung von Aβ auf Lernen und Gedächtnis zu untersuchen wurde ein Verhaltenslabor mit einem Morris-Wasserlabyrinth eingerichtet. Die Interpretation der Resultate dieses Experiments war jedoch diffizil und zum Teil uneindeutig. Es wurde jedoch keine schwerwiegende oder offensichtliche kognitive Beeinträchtigung im Zusammenhang mit induzierten Amyloid-Ablagerungen festgestellt.

Zusammenfassend läßt sich festhalten, dass die Ergebnisse dieser Doktorarbeit Ähnlichkeiten zwischen der experimentellen Übertragung zerebraler β-Amyloidose und der Übertragung von Prionenerkrankungen nachweisen. Diese Ähnlichkeiten basieren einerseits auf der Induktion von Aβ-Ablagerung durch periphere Exposition gegenüber dem induzierenden Agens, andererseits auf der Übertragung und Verbreitung intrinsischer Eigenschaften des induzierenden Agens. Die Ergebnisse der Verhaltensexperimente deuten nicht auf einen schwerwiegenden Einfluss der induzierten hippocampalen Aβ-Ablagerungen auf das räumliche Gedächtnis APP-transgener Mäuse hin. Obwohl die Resultate dieser Experimente einen Beitrag zum

umfassenderen Verständnis der Übertragung amyloidogener Erkrankungen leistet, ist es wichtig darauf hinzuweisen, dass es keine Beweise für eine horizontale Übertragung zerebraler β-Amyloidose von Mensch zu Mensch gibt. Dennoch bietet die Identifizierung und Charakterisierung von Aβ-Subtypen oder Aβ-Varianten das Potenzial neue Ziele für präventive oder kurative Therapien der Alzheimer Krankheit zu finden.

Summary

Aggregation of disease-specific proteins occurs in a variety of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and the prionoses. Hence, the term "proteopathies" has been used to refer to these disorders. Among them, the most prevalent is Alzheimer's diseases with an increasing number of people affected due to the aging of our society.

The aggregation of amyloid beta (Aβ) in the brain of patients with Alzheimer's disease is considered an initial and early event in disease pathogenesis, followed by neurofibrillary tangle formation, neuronal loss, and progressive cognitive decline. Therefore, it is important to understand the underlying mechanisms and impact of early Aβ aggregation that in turn, is a prime target for therapy. The aim of this thesis was to study mechanistic similarities between the induction of Aβ-aggregation and prions, the infectious protein aggregates in prion diseases.

In the first project, the transmissibility of β-amyloidosis in an amyloid precursor protein (APP) transgenic (tg) mouse model was studied. The aim was to investigate the possible induction of cerebral Aβ deposition in young APP tg mice after peripheral inoculations with brain extract containing aggregated Aβ ("Aβ seeds"). Previously, it had been shown that intracerebral administration of brain extract containing aggregated Aβ induces the aggregation and deposition of Aβ in brain in a time and concentration dependent manner (Meyer-Luehmann et al., 2006). Results presented in this thesis demonstrate that the intraperitoneal injection of brain extract from aged APP tg mice is sufficient to trigger Aβ deposition in brain in young APP tg mice. However, intraperitoneal injection requires a prolonged incubation time when compared to intracerebral inoculation. This study, to which I contributed and consequently became a co-author, has been published in *Science* (Eisele et al., 2010).

In the second part of the thesis, transmission of conformational variants of β-amyloid deposits with different Aβ42 to Aβ40 ratios was studied. To this end young APP23 or APPPS1 tg mice were intracerebrally inoculated with brain extract containing aggregated Aβ either from aged APP23 or aged APPPS1 tg mice. Biochemical analysis of laser-dissected tissue pieces and histological staining with spectral analysis with luminescent conjugated polythiophenes (LCP) was used to characterise the Aβ aggregates in the donor tissue and in the host. Results revealed that the Aβ40/42 ratio

of the induced Aβ deposits and the conformation of the induced Aβ-morphotypes was dependent on the properties of both the brain extract and the inoculated host. This 'strain-like' aspect of Aβ-amyloid is reminiscent to findings from studies on prion diseases. This study was done in collaboration with K. P. R. Nilsson from the University of Linköping in Sweden. This study was published in *EMBO Reports* (Heilbronner et al., 2013).

In a third project, the functional impact of induced Aβ deposits in the hippocampus of APP tg mice was investigated. To study the impact of brain extract-induced hippocampal deposition of Aβ on learning and memory, a behavioural laboratory using the Morris water maze was established. Interpretation of the data of this experiment was however difficult and somewhat inconclusive. Overall, however, no severe or obvious cognitive impairment associated with the induced amyloid deposits was found.

In summary, the results of this thesis indicate similarities between the experimental transmission of cerebral β-amyloidosis and the transmission of prion diseases regarding (a) the observed induced Aβ aggregation after peripheral application of the Aβ seeds and (b) the transmission and propagation of intrinsic properties of the Aβ seeds. In behavioural experiments we did not find a severe impact of induced hippocampal Aβ deposition on spatial memory in APP tg mice. While our experimental findings contribute to the understanding of the principles underlying the transmission of amyloidogenic proteopathies, it is important to note that evidence for horizontal transmission of cerebral β-amyloidosis in humans is lacking. Nevertheless, identification and further characterisation of subtypes or variants of Aβ aggregates might potentially lead to the identification of new targets for preventive or curative therapies of Alzheimer's disease.

1. Synopsis

1.1. Alzheimer's Disease

In 1906 Alois Alzheimer reported the case of Auguste Deter, one of his patients deceased at 51 years of age after suffering from rapidly progressing memory impairment. By post mortem examination of her brain he found global atrophy and distinct lesions by silver staining. He described extracellular aggregates and intracellular fibrils, later named Alzheimer plaques or senile plaques and neurofibrillary tangles, in the cerebral cortex (Alzheimer, 1907). In the following years, articles reporting cases with similar pathological lesions were published (Bonfiglio, 1908; Perusini, 1909; Alzheimer, 1911) and Emil Kraepelin introduced the name Alzheimer's disease (AD) in 1910 (Kraepelin, 1910; Maurer et al., 1997).

The typical course of AD follows different stages of cognitive decline. Early symptoms include inability to focus attention, social withdrawal and decrease of vitality (Bidzan et al., 2008). In later stages memory impairment is accompanied by mood swings, hallucinations, language difficulties and further cognitive decline leading to severe dementia (Förstl and Kurz, 1999). Average survival time after diagnosis is six years and most frequent causes of death are septicaemia and pneumonia followed by myocardial infarction (Förstl and Kurz, 1999; Helzner et al., 2008).

1.2. Future Economical Impact of AD

At the time of its discovery AD was considered as a rare neurological disorder and did not receive much attention in medical science. Today, old age is considered as the major risk factor for AD (Yoshitake et al., 1995). Constantly prolonging life spans during the last century due to progression of medical care and improved nutritional status in industrialised countries led to increasing the number of people suffering from age-related diseases including AD.

In 2010, estimated 35 million people are living with dementia. Beside vascular dementia, dementia with Lewy bodies and frontotemporal dementia, AD is the most common form of dementia. Estimated worldwide costs of dementia are US$604 billion and account to approximately 1% of the world's gross domestic product. The number of people affected by dementia is expected to rise to 66 million by 2030 and 115 million by 2050 mainly due to increasing life expectancy in low and middle-income countries (Wimo and Prince, 2010). The aging population and increasing healthcare costs in first

world countries are also major contributing economic factors. Finding a cure for his fatal disease remains the ultimate goal. However, delaying the onset and progression can have a significant impact on AD related healthcare costs. Delaying the onset and progression by one year would yield a reduction of AD cases by about nine million in 2050 (Brookmeyer et al., 2007). Facing a global epidemic of AD within the next decades, there is strong need for therapeutic and preventive strategies to delay onset and progression of the disease.

1.3. Pathological Hallmarks of AD and Procession of the Amyloid Precursor Protein

To distinguish AD from other neurodegenerative diseases two major hallmarks have been defined. Extracellular lesions described by Alzheimer have been identified as fibrillar aggregates of amyloid beta (Aβ), a protein generated by cleavage of amyloid precursor protein (APP). APP is a cellular ubiquitous type 1-transmembrane protein involved in cell adhesion (Small et al., 1994), neuronal outgrowth (Qiu et al., 1995) and synaptic transmission (Kamenetz et al., 2003; Hsieh et al., 2006). Alternative splicing leads to three major isoforms of APP, APP_{695}, APP_{751} and APP_{770} (Kitaguchi et al., 1988; Ponte et al., 1988; Tanaka et al., 1988). APP is cleaved by secretases into amyloidogenic and non-amyloidogenic fragments. In the non-amyloidogenic pathway APP is cleaved within the Aβ sequence by α-secretase resulting in soluble sAPPα and C83, the c-terminal fragment of APP (Esch et al., 1990; Sisodia et al., 1990; Golde et al., 1992; Pasternack et al., 1992; Sisodia, 1992). Subsequent cleavage of C83 by γ-secretase leads to production of p3 and APP intracellular C-terminal Domain (AICD). The amyloidogenic pathway plays a central role in AD. Here, APP is first cleaved by β-secretase generating C99 and sAPPβ. The latter fragment is further cleaved by γ-secretase generating Aβ peptides ranging from 37 to 42 amino acids in length with Aβ40 and Aβ42 being the most prominent species (De Strooper, 2010).

The second hallmark, neurofibrillary tangles (NFT), is intracellular accumulation of hyperphosphorylated tau, a microtubule-associated protein involved in axonal transport by binding to microtubules (Grundke-Iqbal et al., 1986; Kosik et al., 1986). Alternative mRNA splicing leads to various isoforms of the protein (Goedert et al., 1989). Progression of the disease is associated with brain atrophy and neuronal loss in certain brain areas including the hippocampus (Jobst et al., 1994; West et al., 1994) and cerebral

inflammatory processes and gliosis (Akiyama et al., 2000; Wyss-Coray, 2006; Venneti et al., 2009).

1.4. The Amyloid Hypothesis and Genetic Background of Early Onset AD

Identification of the Aβ peptide by isolation and sequencing of vascular amyloid aggregates and senile plaques from AD patients initiated what is today known as the "amyloid hypothesis" (Glenner and Wong, 1984; Masters et al., 1985). A small fraction of AD patients with an early onset of the disease (before 60-65 years of age) and similar findings for patients suffering from Down syndrome suggested a gene locus on chromosome 21 being involved in onset and progression of the disease. Further linkage studies with DNA markers derived from individuals suffering from familiar Alzheimer's Disease (FAD) identified the gene encoding APP (St George-Hyslop et al., 1987; Mann and Esiri, 1989; Rumble et al., 1989). By cloning the cDNA of this particular gene the role of APP as the precursor of Aβ was confirmed (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987).

According to the amyloid hypothesis aggregation of Aβ is an initial event in AD pathology causing tau aggregation, neuron loss and the clinical manifestation of the disease (Hardy and Allsop, 1991; Hardy and Higgins, 1992; Hardy and Selkoe, 2002). In 1990 the first mutation in APP associated with cerebral amyloid angiopathy (CAA) was reported (Levy et al., 1990; Van Broeckhoven et al., 1990) followed by publication of point mutations in APP linked to EOAD (Goate et al., 1991).

To identify additional loci associated with FAD, further research was broadened on secretases involved in APP processing. Since γ-secretase is responsible for the final cleavage of APP after β-secretase mediated proteolysis it was a promising candidate to unravel the genetic background of AD. In 1995, a gene loci later associated with γ-secretase, a proteolytic complex of proteins including presenilin 1 (PSEN1) and presenilin 2 (PSEN2) encoded on chromosome 14 and 1, respectively, was suggested (Levy-Lahad et al., 1995; Rogaev et al., 1995). Most mutations in PSEN1 and PSEN2 related to EOAD are associated with a shift the ratio between Aβ40 and Aβ42 peptides (De Strooper, 2007). In addition to APP processing, γ-secretase cleaves a broad range of type 1 membrane proteins and is essential for notch signalling pathway and is therefore is necessary for development and differentiation (Selkoe and Wolfe, 2007). Meanwhile

more than 25 mutations for APP and over 170 for presenilins associated with EOAD have been identified (Bertram and Tanzi, 2008).

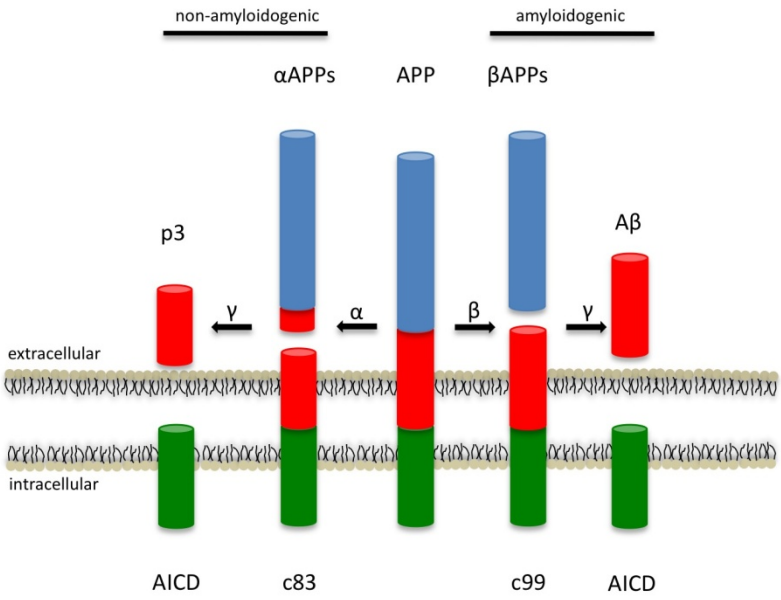

Figure 1: APP processing

The integral membrane protein APP can be cleaved following the non-amyloidogenic or amyloidogenic pathway. Initiated by α-secretase activity the non-amyloidogenic pathway results in release of the soluble peptide αAPPs. Subsequent cleavage of the residual fragment c83 by membrane-associated γ-secretase produces p3 and the APP-intracellular domaine (AICD). Proteolysis of APP by β-site APP cleavage enzyme 1 (BACE) generates the soluble fragment βAPPs and c99 including the Aβ sequence. Further cleavage of c99 by γ-secretase releases Aβ prone to aggregate and cytosolic AICD.

1.5. Late Onset AD

The vast majority of AD cases are not associated with mutations in the above mentioned gene loci and are characterised by an onset of pathology after 65 years of age. To uncover further AD genes genome wide association studies (GWAS) have been conducted. Testing up to ~500,000 single-nucleotide polymorphisms (SNPs) in over 1,000 AD patients and healthy controls revealed only SNPs located in the proximity of the apolipoprotein E (APOE) gene associated with an increased risk for AD (Coon et al., 2007; Grupe et al., 2007; Li et al., 2008). APOE is involved in the regulation of cholesterol

and trigliceride metabolism and is associated with senile plaques and vascular Aβ (Breslow et al., 1982; Saunders et al., 1993). The difference of the three APOE alleles (ε2, ε3 and ε4) is based on two SNPs at position 112 and 158 (Saunders et al., 1993). For the influence of APOE on onset and risk a gene dosage effect of the ε4 allele was found. Individuals being homozygous for ε3 or carrying the ε2 and ε3 allele had a risk of 20%, subjects being heterozygous for ε4 had a risk of 50% and carriers of both ε4 alleles had a risk of 90% to develop late onset AD (Corder et al., 1993). The mechanistic relation between APOE and AD is still under discussion but an influence on generation, clearance and toxicity of Aβ has been shown (Bu, 2009). Due to decreasing costs for genomic sequencing, GWAS with increased sample size are still conducted. To further analyse the bulk of data generated by these studies in order to identify genetic polymorphisms associated with an increased risk of AD a publicly available database (http://www.alzgene.org) was established (Bertram et al., 2007). Taken together, studies on late onset AD imply a crucial role of Aβ in the initiation of the disease strongly supporting the hypothesis of aggregated Aβ as a central upstream event followed by tauopathy and neuronal loss.

1.6. Transgenic animal models of AD

Most mammals do not develop age-related pathologies resembling AD. Beside humans and non-human primates only few species including bears and dogs spontaneously develop neuropathological features as observed in AD patients (Jucker, 2010). Therefore, organisms suitable for experimental research are essential to gain insights of the pathogenesis of this disease.

Approaches using popular invertebrate model organisms as nematode worms C. elegans and fruit fly D. melanogaster are suitable to study some aspects of AD on a cellular and molecular level and are cost and time effective research tools. For presenilins and tau clear homologous genes in worms and flies have been identified (Levitan and Greenwald, 1995; Goedert et al., 1996; Boulianne et al., 1997; Heidary and Fortini, 2001). The Aβ bearing sequence of APP is well conserved among vertebrates but in invertebrates homologous genes only for two paralogs of APP in humans (APLP1 and APLP2) both lacking the Aβ sequence have been found (Rosen et al., 1989; Daigle and Li, 1993). While invertebrate models offering the possibility to investigate aspects of neurodegeneration (Teschendorf and Link, 2009) and to screen potential therapeutic

compounds (Götz and Ittner, 2008) but being phylogenetically distant to humans and the simplicity of those organisms are limiting factors to study complex mechanisms of AD present in the human brain.

Identification of mutations in FAD cases provides an important tool to gain information about onset and progression of pathology by generation of transgenic (tg) mouse and invertebrate models. To model amyloid pathology in mice human APP bearing mutations associated with EOAD were generated. Depending on the mutations these APP tg models display broad range of age-dependent Aβ aggregation including vascular and/or parenchymal deposition (Games et al., 1995; Hsiao et al., 1996; Sturchler-Pierrat et al., 1997). Double transgenic mice additionally carrying mutated presenilins are associated with a shift in Aβ-species in favour for Aβ42, early onset and fast progression of pathology (Duff et al., 1996; Borchelt et al., 1997; McGowan et al., 2005; Radde et al., 2006). Despite exhibiting a broad range of Aβ-related phenotypes APP tg mouse models are lacking tauopathy and substantial neuron loss and therefore do not simulate all aspects of AD. Today, over 50 different APP tg mouse models have been reported, an overview can be found at www.alzforum.org/res/com/tra/app/default.asp.

Since rats are considered as better suiting laboratory rodents for behavioural phenotyping and experimental manipulation (Tesson et al., 2005) several tg rat models for AD have been engineered. While the initially developed rat models failed to exhibit robust age-related extracellular Aβ aggregation (Echeverria et al., 2004; Ruiz-Opazo et al., 2004; Clarke et al., 2007; Folkesson et al., 2007) a recently established model with amyloid deposition starting at 6 months of age carrying human APP with a double mutation has been reported (Leon et al., 2010). According to findings in tg mouse models co-expression of mutated human PS1 facilitates amyloid deposition and accelerates progression of this pathology (Liu et al., 2008; Flood et al., 2009).

Aggregation of hyperphosphorylated tau followed by neuronal loss is associated with neuronal disorders including Pick's disease, progressive supranuclear palsy, Parkinson's disease (PD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) among others (Lee and Trojanowski, 1999). Based on mutations in the tau encoding gene Mtapt (Microtubuli-associated protein tau) transgenic animal models developing features of tauopathy including hyperphosphorylation, intracellular filament formation and extensive neuronal loss (Lewis et al., 2000; Allen et al., 2002). Interestingly, double transgenic progeny from crossings of mouse lines carrying

mutated APP and mutant tau results in enhanced tau pathology (Lewis et al., 2001) arguing in favour for Aβ aggregation as an upstream event in the proposed amyloid hypothesis leading to NFTs and neurodegeneration.

While to date none of the mouse models generated is able to recapitulate the entirety of AD pathology in humans the investigation of single aspects offers the advantage to differentiate causes and effects of this complex disorder (Radde et al., 2008; Jucker, 2010).

1.7. Exogenous Induction of Cerebral Amyloidosis

The first evidence for transmission of cerebral β-amyloidosis came from studies of transmissible spongiform encephalopathies (TSE). TSEs are caused by prions, infectious proteins, giving rise to a range of fatal diseases in a variety of mammals. Among these, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) are the most prominent due to a BSE epidemic in UK in the 1980s. For CJD three subtypes have been identified and based on epidemiological evidence and biochemical similarities it is believed that one of these (vCJD, variant of CJD) is associated with BSE (Collinge et al., 1996; Will et al., 1996). Experimental prion infection of non-human primates with brain tissue from a deceased patient with prion disease and concomitant cerebral β-amyloidosis resulted in cerebral amyloid angiopathy (CAA) and parenchymal Aβ aggregation (Baker et al., 1994). These results initiated a long-term study with marmoset monkeys but incubation times up to 10 years after inoculation strengthened the need for a more time efficient experimental design (Ridley et al., 2005). For prion diseases it was shown that overexpression of prion protein (PrP) in tg mouse models facilitates prion disease transmission (Prusiner et al., 1990). Similar studies in APP overexpressing mouse models followed and yielded a substantial Aβ deposition after infusion of Aβ-containing brain extracts from AD patients or aged APP tg mice (Kane et al., 2000; Walker et al., 2002; Meyer-Luehmann et al., 2006). This locally induced amyloid pathology is accompanied with tau hyperphosphorylation (Walker et al. 2002) and spreads to functionally connected brain areas (Eisele et al., 2009) implying a diffusible mediator underlying this mechanism. Recent studies trying to identify the nature of the pathology inducing agent reported soluble Aβ-species resistant to protease degradation efficiently initiating this reaction (Langer et al., 2011).

While for the mentioned studies APP tg mice with age related amyloid deposition was a necessity new approaches using tg rodent models lacking pronounced endogenous Aβ aggregation have been reported. By inoculation of APP tg mice only developing deposition after 15 months of age with Aβ-laden brain extract it was shown that induction of amyloid formation is independent from age and prolonged incubation times are associated with extensive spreading of deposition throughout the brain (Hamaguchi et al., 2012). Similar findings have been reported using APP tg rats lacking amyloid deposition until 30 months of age. Nine months post inoculation of three-month-old recipient rats with brain extract from deceased AD patients induced amyloid in vasculature and pyrenchyma was detected (Rosen et al., 2012). Using mice expressing human APP without any mutation injection of brain material from a AD patient induced Aβ deposits though it has to be mentioned that only very long incubation times (>19 months) resulted in substantial deposition (Morales et al., 2012).

1.8. Functional impact of Aβ aggregation

While Aβ deposition in cerebral parenchyma is considered as the primary event prior to tau aggregation neuronal loss, the relation between senile plaques and their impact on memory and cognition is still controversial. Post mortem examination of non-demented elderly reveals significant amyloid pathology and were not clinically distinguishable from AD patients (Crystal et al., 1988; Katzman et al., 1988; Arriagada et al., 1992; Dickson et al., 1992). Rather than amyloid deposition and NFT pathology cognitive decline assessed by neuropsychological tests correlates with synapse loss (Terry et al., 1991).

Studies on APP tg mouse models could not reveal a clear relationship between Aβ, senile plaques and cognitive impairment. For PDAPP mice, the first transgenic mouse model with substantial Aβ deposition starting at 10-13 months of age (Games et al., 1995), deficits in various behavioural tasks have been reported starting at three months of age (Dodart et al., 1999; Chen et al., 2000). Behavioural assessment of the subsequently published Tg2576 mice (Hsiao et al., 1996) with similar onset of plaque-like deposition gave ambiguous results. Initially, impairment in memory-related behavioural tests beginning at 10 months of age was reported (Hsiao et al., 1996) but further analysis revealed a deficit starting at seven months (Pompl et al., 1999) and gender-specific, progressing impairment at three months of age (King et al., 1999). In a sophisticated approach by measuring detergent soluble and insoluble Aβ in Tg2576 mouse brains a

link between progressive spatial memory decline and levels of insoluble Aβ was detected (Westerman et al., 2002). For APP23 mice (Sturchler-Pierrat et al., 1997) age related cognitive deficits were found in several studies. Amyloid formation in this mouse model starts at six months in cortex, reaching the hippocampus at twelve months progressing with age resulting in global Aβ deposition (excluding cerebellum) at 24 months, CAA and hippocampal neuron loss (Sturchler-Pierrat et al., 1997; Calhoun et al., 1998; Calhoun et al., 1999). Progressively worsening cognitive deficits in hippocampus-depending tests have been reported starting at three months of age (Lalonde et al., 2002; Kelly et al., 2003; Van Dam et al., 2003; Van Dijck et al., 2008).

It has been demonstrated that Aβ dimers isolated from deceased AD patients interfere with the memory of a learned behaviour and inhibit long-term potentiation (LTP) in normal rats while insoluble plaque cores were unable to impair LTP unless being solubilised to release Aβ dimers (Shankar et al., 2008). Similar findings have been reported for PDAPP mice. By passive immunisation impaired cognitive behaviour could be reversed by clearance of soluble Aβ without altering total Aβ burden (Dodart et al., 2002). For other immunisation studies a reduction of deposited Aβ was associated with restored behavioural deficits (Janus et al., 2000; Morgan et al., 2000).

Taken together, the relationship between soluble Aβ, plaque load and cognitive impairment in APP tg mice is uncertain but local induction of deposition in the hippocampus may help to clarify the impact of Aβ aggregation on behavioural deficits.

Behavioural phenotyping of APP tg mice additionally carrying human mutated tau transgene is not applicable due to severe motor phenotypes associated with tau transgenic mice (Lewis et al., 2001).

1.9. Toxicity of Amyloid β

The identification of Aβ as the major component of vascular and parenchymal deposits in AD brains was initiating research about a potential toxicity of Aβ. In cerebral amyloid angiopathy (CAA), the amount of amyloid deposited in the vasculature of the brain correlates with vessel rupture and haemorrhages (Dierksen et al., 2010). This vascular toxicity is shared by Aβ and other CAA–associated amyloidogenic proteins (ADan in familial Danish Dementia, ABri in familial British Dementia, Cystatin C). Vascular amyloid deposition promotes thickening of the basal lamina of vessels, loss of smooth

muscle cells and perivascular inflammation, leading to cognitive decline as a consequence of microhaemorrhages and fatal strokes (Revesz et al., 2009).

However, it was found that the initially expected direct correlation between cognitive decline and fibrillar Aβ deposits in the brain parenchyma in elderly humans is weak (Crystal et al., 1988; Katzman et al., 1988; Dickson et al., 1992). Instead, post mortem analysis of AD brains revealed elevated levels of soluble oligomeric Aβ, but not fibrillar deposits, correlating with severity of dementia (Haass and Selkoe, 2007). Also, isolated oligomeric assemblies from APP tg mice interfere with cognitive tasks when administered intracerebrally to young rats (Lesné et al., 2006). Furthermore, extracellular administered Aβ oligomers, isolated from autopsy material of AD patients, disrupt synaptic function and structure in hippocampal brain slices of wild-type mice and rats. Initially ineffective insoluble plaque cores can be rendered into neurotoxic oligomers, demonstrating that plaques can serve as a reservoir for neurotoxic Aβ assemblies (Shankar et al., 2008) and sequestration of this assemblies may contribute to observed synaptic abnormalities in the vicinity of fibrillar Aβ deposits (Tsai et al., 2004). It is assumed that the ability to infiltrate synaptic clefts and other compartments by diffusion is associated with the synaptotoxicity of those soluble aggregates (Haass and Selkoe, 2007; Shankar and Walsh, 2009; Eisenberg and Jucker, 2012). Regarding the specific toxicity of the two most dominant peptides Aβ40 and Aβ42, no direct relation between the absolute quantities of the two species and the degree of neurodegeneration is known. Despite the finding that early and aggressive forms of the disease are associated with mutations of APP and/or presenilins promoting the generation of Aβ42 (Suzuki et al., 1994; Duff et al., 1996; Scheuner et al., 1996), cell culture studies have shown that even subtle changes of the relative ratio of the two peptides stabilises toxic oligomers with intermediate conformations (Kuperstein et al., 2010). Taken together, recent findings are pointing to soluble, oligomeric Aβ assemblies as the neurotoxic species instead of fibrillar Aβ as found in senile plaques. However, these deposits can serve as a reservoir sequestering the bioactive, soluble aggregates.

2. Transmission of Cerebral β-Amyloidosis

In Reference to

Eisele YS, Obermüller U, **Heilbronner G**, Baumann F, Kaeser SA, Wolburg H, Walker LC, Staufenbiel M, Heikenwalder M, Jucker M (2010)
Peripherally Applied Aβ-Containing Inoculates Induce Cerebral β-Amyloidosis. Science

2.1. Amyloids

The term amyloid is derived from the Latin word for starch (amylum). It was introduced by the German botanist Mattias Schleiden to describe a constituent in plants. Based on the finding to stain corpora amylacea of the nervous system with iodine, similar to starch, German physician Rudolph Virchow postulated in 1854 a starch-like nature of these structures (Kyle, 2001). Today the term amyloid refers to proteins with the following characteristics: the ability to polymerize into non-branching fibrils approximately 10nm in diameter; polypeptide conformation rich in β-structure in a cross-β arrangement, i.e. β-strands are perpendicular to the fibril axis forming β-sheets running parallel to the fibril axis. Due to the high content of β-sheet structure amyloids bind the *bis*-diazo dye congo red and exhibit green birefringence when viewed under polarized light (Sipe et al., 2010). This secondary structure of proteins forming an amyloid is either natively rich in β-strands (e.g. transthyretin) or gain these structures by conformational changes (e.g. PrP, Aβ) (Sunde and Blake, 1998). For a selection of amyloidogenic proteins and associated pathologies see Table 1. In former times proteins like α-synuclein (involved in Parkinson's disease) and tau (involved in AD and frontotemporal Dementia) were terminologically not considered as amyloids while resembling most necessary characteristics (β-sheet structure, fibrillar aggregation, congo red binding and green birefringence for tau). This is due to the fact that amyloids by definition of pathologists are necessarily extracellular aggregates (Westermark et al., 2005). By definition of biophysicists these lesions are considered as amyloids since disease related proteins are able to form amyloids in vitro, other proteins are able to fibrillize depending on their state of denaturation (Fändrich et al., 2003) and biologically functional amyloidogenic proteins have been identified in various species.

PROTEIN	PATHOLOGY	LOCAL/SYSTEMIC
Prion Protein (PrP)	Transmissible spongiforme Encephalopathies	local
Amyloid β (Aβ)	Alzheimer's Disease	local
Islet Amyloid Polypeptide (IAPP)	Type-2 Diabetes	local
Transthyretin (TTR)	Familial amyloid polyneuropathies	systemic
Serum Amyloid A	AA Amyloidosis	systemic

Table 1: Selected amyloidogenic proteins and associated pathologies
For a detailed overview see Sipe et al., 2010.

A special feature of amyloid fibrils is the property of nucleated polymerization. The formation of an amyloid fibril starts with a slow reaction leading to a nucleus. By recruiting additional subunits the fibril grows at both ends. Due to its non-branching architecture, the growth rate can be accelerated by fragmentation of fibrils into seeds to provide more growing ends. Thereby, structural properties of the seed replicate in a self-propagating manner (Petkova et al., 2005; Baxa, 2008).

2.2. Protein Misfolding Diseases and Transmissibility

As mentioned, the key characteristic of amyloid forming proteins is the abundance of β-strands. Intense research on transmissible neurodegenerative diseases revealed a new pathogenic mechanism based solely on proteins (without any nucleic acid sequence involved as for viruses or microorganisms) as the infectious agent (Prusiner, 1982). For his discovery Stanley Prusiner received the Nobel Prize in Physiology or Medicine in 1997. Later, the prion hypothesis of infectivity was formulated. According to the hypothesis cellular prion protein (PrP^{c}), undergoes a helix-to-sheet conversion to PrP^{Sc} (for Scrapie, a prion disease in sheep) and replicates in vivo by a mechanism called permissive templating (Prusiner, 2001; Weissmann, 2004; Hardy, 2005). This mechanism could be applied for a wide range of diseases and gave rise to the idea of protein misfolding disorders or proteopathies. Today proteopathies in humans include Parkinson's disease, AD, Huntington's disease, FTD, prion diseases like BSE, CJD, Kuru

among many others. Adapting the paradigms studying the transmissibility of prions to enlighten the seeding-like mechanism in β-amyloidosis depending on properties of the inducing agent and host (Kane et al., 2000; Walker et al., 2002; Meyer-Luehmann et al., 2006) initiated a discussion about the contagiousness of AD. Since induction of Aβ aggregation in these studies was achieved via intracerebral (i.c.) inoculation into the hippocampus and cerebral cortex, the induction in other brain regions and the possibility of transmission via peripheral routes or contaminated surgical instruments was investigated (Eisele et al., 2009). In this study it was shown that the susceptibility of the brain regions to develop parenchymal and vascular amyloid deposits differs resembling the pattern of age-dependent β-amyloidosis of the particular APP tg mouse model, starting in cerebral cortex and hippocampus and enthorinal cortex and later affecting other regions. Addressing the question of transmission by contaminated surgical instruments, steel wires were immersed in Aβ-containing brain extract and implanted into predepositing APP tg mice. Analysis after four months showed robust induction in the vicinity of the implanted wires that could not be abolished by heating the wires in PBS for ten minutes at 95°C. Only plasma sterilization prevented induction of Aβ-positive deposits completely. To investigate similarities in transmission between prion mediated diseases and induced aggregation of Aβ in APP tg mice, protocols sufficient for prion transmission have been applied. Therefore Aβ-laden brain extract was applied by intravenous (i.v), intraocular and intranasal inoculation. For prions a raised threshold has been shown using extra-cerebral routes for transmission of the related disease. Accounting for this expected reduced efficiency the amount of inoculated Aβ was up to 2E4-fold higher than required for prominent Aβ deposition after i.c. injection. Analysis after four to eight months post inoculation revealed no induction in any of the peripheral approaches. Since expression of APP in the mouse model used in this study (APP23) is restricted to neuronal tissue the lack of substrate was speculated to hinder the spread of the domino-like reaction of Aβ-misfolding and subsequent aggregation. But non-existing induction in brain regions receiving neuronal input from the site of inoculation (olfactory bulb for intranasal and visual cortex for intraocular inoculation) do not support this hypothesis.

Despite the partly negative results from this study the importance of a possible contagiousness of cerebral β-amyloidosis its impact regarding the history of prion infections following the BSE epidemic in the late 20th century urged to intensify research

on this particular topic. To rule out a transmission of cerebral β-amyloidosis, we initiated a study with the same mouse model susceptible for age-dependent Aβ deposition in cerebral parenchyma and vasculature. A relatively high amount of Aβ-laden brain extract from aged APP23 mice was intraperitoneal (i.p.) injected into young predepositing recipient mice of the same APP tg line. The animals were sacrificed 6-7 months post injection and histological brain analysis revealed significant Aβ-positive deposits mainly associated with vasculature but also spreading into the adjacent parenchyma. Again, the pattern of affected brain regions resembled the age-related deposition in this mouse model. We confirmed induced vascular Aβ deposition by electron microscopy and immunoblotting, being associated with gliosis, tau hyperphosporylation and other pathologies. To further investigate the infectious principle of peripheral induction of cerebral Aβ deposition, additional mice were i.p. injected and analysed one hour, one day or one week post inoculation. Considering a systemic deposition and amplification of amyloid in peripheral organs (lung, heart, kidney, liver and spleen) were analysed at all time points but no manifestation of amyloidosis could be observed. Blood plasma analysis revealed an increase of cytokines and chemokines (interleukins, monocyte chemotactic protein-1, tumor necrosis factor-α and macrophage inflammatory protein-1β) at one hour post-injection. This also occurred in control animals injected with wild-type (wt) brain extract. We found a slight increase for interleukine-6 at one week post injection for the tg extract by ELISA. Replication of prions in peripheral tissue involves the immune system, particularly follicular dendritic cells (FDC), B cells and less characterised bone-marrow derived populations. The significance of parasympathic and sympathic innervation for prion migration into the CNS was demonstrated by extended incubation times of i.p. inoculated prions after sympathectomy (surgical removal of sympathetic ganglia and nerves) in tg mice susceptible for prion diseases (Aguzzi et al., 2008). Further, inflammatory processes and activation of the immune system enhances prion susceptibility after peripheral inoculation in animal models of prion diseases (Bremer et al., 2009). But above-mentioned lack of expression of human APP in APP23 mice replication of Aβ with aggregation inducing properties is unlikely. Since the required amount of peripherally applied Aβ-containing brain extract to induce cerebral aggregation and the far less efficiency of induction indicated by prolonged incubation times post inoculation and reduced extent of Aβ burden in the brain compared to

cerebral inoculation, we speculate rather a migration of the originally injected seeds from the peritoneal cavity to the brain than a preceding replication of misfolded Aβ in peripheral tissue. Still, a route for seeds capable of inducing pathology-related protein aggregation from peripheral tissue to the central nervous system can be postulated and strongly promotes further research to identify mechanisms underlying this transport to clarify the infectious nature of the still growing family of protein misfolding diseases.

3. β-Amyloid Strains

In reference to

Heilbronnner G, Eisele YS, Langer F, Kaeser SA, Novotny R, Nagarathinam A, Åslund A, Hammarström P, Nilsson KPR, Jucker M (2013)

Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice. EMBO Reports.

3.1. β-Amyloid Morphotypes

For Aβ deposits heterogeneity in various aspects has been observed. Seeded growth of Aβ40 fibrils in vitro differ structurally depending on growth conditions and structural properties of the seeds initiating the polymerization reaction (Petkova et al., 2005; Meinhardt et al., 2009). Aβ40 and Aβ42 and alteration of amino acid position 22 and 35 differ in aggregation properties, fibre morphology and cellular toxicity (Seilheimer et al., 1997; Yoshiike et al., 2003). While Aβ deposition in senile plaques is a prerequisite for AD diagnosis, brains of non-demented elderly can exhibit substantial Aβ burden (Crystal et al., 1988) and differences concerning toxicity and aggregation between normal aging and AD have been found (Piccini, 2005). Further, comparing patients with independent EOAD mutations in presenilins revealed variations in relative Aβ40 and Aβ42 levels, distribution and morphology of deposits and tau pathology (Maarouf et al., 2008). In vitro and cell culture studies showed altered toxicity and changes of synaptic activity based on subtle changes of the ratio of Aβ40 and Aβ42.

Amyloid probes suitable for positron emission tomography like Pittsburgh compound B (PiB), a fluorescent analogue of Thioflavin T, have been developed for ante mortem diagnosis of AD (Klunk et al., 2004a). Based on PiB binding mild cognitive impaired individuals (MCI) and AD patients can be distinguished from the healthy elderly. However, PiB signal for deposits derived from mouse models of AD is much weaker due to reduced numbers of high affinity binding sites per mol Aβ. Recently, a case lacking PiB binding despite neuropsychological and histopathological confirmed AD was reported (Rosen et al., 2009). An even lower binding capacity for PiB was observed studying fibril preparations from synthetic Aβ40 or Aβ42 (Klunk et al., 2004b). Diagnostic dyes like PiB, Congo red or Thioflavins are sterically rigid molecules and their binding is solely based on β-sheet rich conformation of the substrate.

3.2. Structural Phenotyping of Amyloids with Luminescent Conjugated Polythiophenes

A new approach to assess the conformational state of amyloidogenic proteins was made by developing new luminescent conjugated thiophene polymers (LCP) able to bind to amyloids. The key feature of these polymers is the flexibility of conformation due to the thiophene (C_4H_4S) backbone of the molecule. This enables it to twist under specific conditions. The repetitive order of this heterocycle within the polymer attaches to the symmetric repetitive amyloidogenic molecules. Subtle conformational changes of this substrate alters the conformation of the polymer and thereby its optical properties such as absorption and fluorescence (Åslund et al., 2007). Accuracy of LCP typing of amyloids was shown by blinded analysis of deposits derived from systemic amyloidoses (Nilsson et al., 2010). Concerning Aβ, it has been shown that different conformational states of Aβ42 fibrils can be characterised using LCPs in vitro and in vivo (Nilsson et al., 2007).

3.3. Prion Strains

Based on a pattern of brain regions affected after scrapie infection of mice the existence of prion strains was proposed by Fraser and Dickinson in 1973. A surprising finding studying prion infectivity was the phenomenon of a species barrier. Hence, transmission between mammalian species is less efficient than within species (Fraser and Dickinson, 1973). Primary structure (amino acid sequence) of the proteins could only partially explain this finding since transmission of prions with identical primary structure is depending on intrinsic properties of the host (Collinge et al., 1996; Hill et al., 1997). Further studies implied variants of secondary, tertiary and quaternary protein structure (e.g. fibril conformation) contribute significantly to the species-dependent limitations of transmission (Jones and Surewicz, 2005). Beside distinct incubation times, distinguishable neuropathological phenotypes, variation of glycosylation patterns, altered fragmentation by proteolysis and propagation of conformational properties supported the strain hypothesis for prions (Kocisko et al., 1994; Bessen et al., 1995; Collinge et al., 1996; Collinge and Clarke, 2007). To characterise prion strains and their interaction within the same host LCPs have been proven to be a sensitive and powerful tool and are underlining the role of tertiary and quaternary structures in prion strains (Sigurdson et al., 2007; Nilsson et al., 2010).

3.4. Seeded Induction of β-Amyloidosis

It was shown that exogenous (seeded) induction of cerebral β-amyloidosis in APP tg mice from different sources results in morphological heterogeneous deposits of Aβ. Variations seem to depend on the nature of the amyloid-inducing agent and endogenous properties of the recipient (Meyer-Luehmann et al., 2006). In order to further analyse the principle of potential propagation of intrinsic properties of the seed in a different host we initiated a new study. Here we used two different APP tg mouse models (APP23 and APPPS1) and characterised the biochemical composition and conformation of deposits after cross-induction. APP23 mice expressing mutated human APP develop age-dependent large plaques and diffuse amyloid with Aβ40 dominating over Aβ42 (Sturchler-Pierrat et al., 1997). In contrast APPPS1 mice show a faster progression of neuropathology associated with smaller, compact plaques with a majority of Aβ42 due to the additional expression of mutated human presenilin 1 (Radde et al., 2006). Aβ-containing brain extracts from aged mice of both lines were separately injected bilateral into hippocampi of predepositing APP23 and APPPS1 mice. The induced deposits were analysed morphologically, concerning the ratio of Aβ40 to Aβ42 and by LCP typing to assess the conformation of the amyloid and compared to untreated Aβ-laden brains of the two tg mouse lines.

3.5. Propagation of Conformation and Composition in Induced Aβ Deposits

In this study we are able to show that the APP23 phenotype can be altered by intracerebrally introduced APPPS1 seeds. It was demonstrated that the conformation of APPPS1 seeds is propagated in the APP23 host. Regarding the ratio of Aβ40 to Aβ42, a significant shift in favour of Aβ42 in the APP23 host after APPPS1 inoculation compared to either untreated APP23 mice or deposits induced by APP23 extract was detected. It should be mentioned that the higher variation of Aβ ratios may be at least partially due to the method of sample generation. While the amyloid conformation was assessed by measuring fluorescence of LCPs bound to fibrils of single plaques, patches of tissue from hippocampal sections were biochemically analysed to determine the Aβ ratio. Therefore, these patches may contain diffuse and compact amyloid, possibly contributing to the variance of the ratio and its intermediate value compared to APP23 and APPPS1 mice. Analysis revealed a ratio of approximately 5/1 for the native APP23, 0.5/1 for native APPPS1 and 1.5/1 for the APP23 mice inoculated with APPPS1 seeds). Changes in morphology of induced deposits were also observed. APP23 mice inoculated with APP23

seeds exhibited a morphotype reminiscent to endogenous deposits of this mouse line compared to amyloid induced by APPPS1 extract. APP23 are associated with large plaques with diffuse coronas of amyloid. While induction with APPPS1 seeds resulted in a granular pattern of small plaques, APP23 induced deposits were larger and surrounded by diffuse, reticular extensions. For the vice versa experiment, APP23 or APPPS1 brain extract introduced in the APPPS1 host, a propagation of the conformation of the Aβ seeds was also observed but no statistical significant difference of the ratio of Aβ40 to Aβ42 of the induced deposits was detected (Aβ40/42 ratio of 0.35/1 for the APP23 extract vs. 0.25/1 for APPPS1 extract).

Taken together, we demonstrated selective recruitment of truncated Aβ-species based on the composition of the amyloid-inducing seeds. The tertiary and/or quaternary structure of the incorporated peptides is imposed by the conformation of the seeds as shown by LCP probing. In our experiments, intrinsic properties of the seeds alter the morphology of induced aggregates. These findings imply the existence of Aβ variants with distinct conformations and biochemical compositions capable of self-replication in vivo in a susceptible host similar to the proposed model of prion strains.

4. Functional Impact of Amyloid Induction

4.1. The Morris Water Maze – A Paradigm for Hippocampus-mediated Learning

In a third work package we were interested in the impact of induction of senile plaques in the hippocampus of APP tg mice on learning and memory. To evaluate hippocampal function in laboratory rodents the Morris water maze is the most frequently used behavioural paradigm. Invented by Richard G. M. Morris the test was used to investigate neuronal substrates involved in spatial learning and memory in rats (Morris et al., 1982; Morris, 1984) and mice (Logue et al., 1997). In general, this test is based on the principle of a rodents urge to escape from water. The subject is placed in a circular pool with a hidden underwater escape platform. Provided with distal visual cues the animal learns in consecutive trials to find the location of the platform and escape from the water. After several days of training with the platform remaining at the same position within the pool, the platform is removed to run a test trial. Being able to navigate to the former position of the platform the animal exhibits a preference for this area of the pool. For APP tg mouse models a relation between Aβ and memory loss was found (Hsiao et al., 1996; Chen et al., 2000; Janus et al., 2000; Morgan et al., 2000; Gordon et al., 2001; Westerman et al., 2002; Kelly et al., 2003), but the relation between soluble and insoluble Aβ species and memory decline is still under discussion. Our approach to induce Aβ aggregation promoting the deposition of soluble Aβ in senile plaques in APP tg mice offers a tool to investigate the impact of aggregated, insoluble Aβ on hippocampus-mediated learning and memory.

4.2. Design of the Study

To assess the behavioural impact of amyloid formation in the hippocampus male tg APP23 mice underwent surgery at three months of age and were tested 8.5 months after surgery. One group received bilateral intrahippocampal injections of seeding extract of brains from aged APP23 mice (experimental group) while the other group consisted of male APP23 mice injected with brain extract of an aged non-transgenic littermate (control group). Mice received surgery and were tested in two different cohorts. 14 animals treated with APP23 brain extract (six for the first cohort, eight for the second) and 21 animals treated with WT brain extract (eight for the first and 13 for the second

cohort) were tested in the Morris Water Maze. After behavioural testing mice were sacrificed and the brains analysed to confirm Aβ-deposition in the hippocampus.

4.3. Methods

Transgenic Mice used for Morris Water Maze

As mentioned above, male APP23 mice were used. APP23 mice carry cDNA for human APP with the Swedish double mutation at positions 670/671 (KM→NL), driven by a murine neuron-specific promoter (Sturchler-Pierrat et al., 1997). APP23 mice have been backcrossed with B6 mice for more than ten generations (C57BL/6J-Tg (Thy1-APPK670N;M671L)23).

Preparation of Tissue Extracts for Intracerebral Infusion

Mouse brain extracts were prepared from the whole brain without cerebellum of aged (27 to 28 month-old) APP23 transgenic mice and age-matched, non-transgenic control mice.

Tissue samples were fresh-frozen and stored at -80°C until use. Tissue was then homogenized at 10% (w/v) in sterile, phosphate-buffered saline (PBS), vortexed, sonicated 3 x 5 s and centrifuged at 3000 x g at 4 °C for five minutes. The supernatant was aliquoted and immediately frozen.

Aβ levels of the extracts were estimated by electrochemiluminescence-linked immunoessay using the MSD 96-well MULTI-SPOT Human (6E10) Aβ Triplex Assay (Meso Scale Discovery). Extracts were treated with formic acid (95%), sonicated for 30 s on ice and centrifuged at 25,000x g for 1 h at 4 °C. Supernatants were neutralized (1 M tris base, 0.5 M Na_2HPO_4, 0.05% NaN_3) and Aβ detection was performed according to the manufacturer's instructions. The plates were analysed using a Sector Imager 6000, extracts revealed Aβ concentrations of 10-20 ng/μl.

Stereotaxic surgery

Host mice were anaesthetized with a mixture of ketamine (110mg/kg body weight) and xylazine (20 mg/kg body weight) in saline. Bilateral stereotaxic injections of 3 μl brain extract were placed with a Hamilton syringe into the hippocampus (AP -2.5 mm, L +/- 2.0 mm, DV -1.8 mm). Injection speed was 1 μl/min and the needle was kept in place for an additional five minutes before it was slowly withdrawn. The surgical area was cleaned with sterile saline, the incision was sutured, and the mice were monitored until

recovery from anaesthesia. Surgery was performed with assistance of Yvonne S. Eisele 8.5 months prior behavioural testing. After stereotaxic surgery mice were housed individually in standard lab cages (1284L-116, Tecniplast, Milan, Italy) on a 12 h light cycle (lights on at 06.00 am) with ad libitum access to food and water. All animal experiments were in compliance with protocols approved by the local Animal Care and Use Committee.

Preparation of brain sections

At 8.5 months post surgery mice were sacrificed by decapitation under deep inhalation anaesthesia. Brains were removed, fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose, and sectioned (25 μm-thick) on a Microtome (SM200R, Leica Microsystems, Wetzlar, Germany) in ice-cold PBS. Until use, sections were stored in cryoprotectant solution (25% (v/v) glycerine; 30% (v/v) ethylene glycol in PBS) at -20°C.

Aβ-immunohistochemistry of brain sections

For immunohistochemical staining sections were washed in tris-buffered saline (TBS) and incubated with 0.3% H_2O_2 in TBS for 30 minutes. Subsequently, sections were washed in TBS, incubated 10 minutes with 0.3% Triton X-100 (Sigma, St. Louis, MO, USA) in TBS and blocked for 30 minutes with 5% goat serum. Sections were then probed overnight with polyclonal antibody NT12 or CN3 raised against human Aβ40 (dilution 1:2000 in TBS). Sections were washed in TBS and incubated with biotinylated goat anti-rabbit IgG (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) for 90 minutes. Sections were again washed in TBS and incubated in 0.8% avidin and 0.8% biotin (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA) in TBS for 90 minutes. Sections were again washed and reacted with 3% peroxidase substrate (SG Blue, Vector Laboratories, Burlingame, CA, USA) and 3% H_2O_2 in 0.1M PBS for approx. 2 minutes until desired intensity of staining was achieved. Sections were washed in TBS and mounted on gelatine coated glass slides (Super Frost+, R. Langenbrinck, Emmending, Germany) and dried for 2 hours at room temperature. Through a series of graded ethanols, sections were dehydrated and coverslipped with xylene-based mounting medium (Pertex, Cellpath, Newtown, UK)

Apparatus and procedure for behavioural testing

A circular pool (Noldus, Wageningen Netherlands) with 120 cm diameter and a depth of 70 cm was filled with 24 ±1 °C warm water. The water level was set 20 cm below the rim and prominent visual cues (black triangle, cross, circle and square on white background) were placed around the maze and stayed at the same position during the whole experiment. The pool was divided into four imaginary quadrants named North-East (NE), South-East (SE), South-West (SW), North-West (NW) and a round escape-platform (Noldus, Wageningen Netherlands) was placed in the centre of the target quadrant (NW) at 1-2 cm below the water level. To hide the platform 20 ml of white finger paint (Giotto, Pero, Italy) was added to the water. The setup was placed in a 2 x 2 x 3.2 m tent self-made from pond groundsheets to avoid distraction of the animals and to ensure a controlled environment during the experiment. 40 W lights in the top corners illuminated the inside of the tent. A black and white video camera (ICD-49E, Ikegami Tsushinki CO., LTD, Tokyo, Japan) connected to a PC was placed 250 cm above the centre of the maze to record the experiment. Videos were captured and analysed using Noldus Ethovision 7.0 software (Noldus, Wageningen Netherlands). To minimize the influence of handling, the mice were always gently released into the water using a 500 ml plastic beaker attached to a 100 cm metal stick. With aid of a large metal scoop the mice were transferred back to their home cage. To avoid hypothermia the mice were dried with paper tissue after each swim trial and the home cage was placed under a red light (Sanitas, Uttenweiler, Germany)

Habituation to the experimental task

All water maze experiments were conducted between 08.00 am and 05.00 pm. Before the experiment started, mice were habituated to the task of swimming in water, finding and climbing on the escape platform and being handled by the experimenter. Three days prior to the experiment mice were habituated for two consecutive days. Therefore the animals were one after another placed in a standard rat cage filled with 24±1 °C warm water with a submerged escape platform. Each mouse had the opportunity to find and climb on the platform within 60 s and had to stay there for ten seconds to before being transferred to their home cage. If it could not find the platform within 60 s or jumped back into the water the mouse was gently guided to the platform and transferred to their home cage after staying on the platform for ten seconds. Every day, each mouse had a minimum of ten trials or was trained until it completed the task for three

consecutive times. Mice were tested consecutively and had an inter trial interval (ITI) of three to five minutes. Habituation took place in a different environment than the rest of the water maze experiment.

Training phase

During the second phase of the experiment the mice were trained to find the hidden platform in the target quadrant North-West (NW). The animals were released facing the wall of the maze at the centre of one of the remaining quadrants South-East, North-East and South-West (SE, NE and SW) in a pseudo-randomized order. Each trial lasted 60 s or until the mouse reached and stayed on the platform for a minimum of three seconds. The time to reach the platform (latency) was calculated by analysing the videos with Noldus Ethovision 7.0 software. If the mouse failed to find the platform within the 60 s it was gently guided to the platform and after staying on the platform for ten seconds transferred to its home cage and a latency of 60 s was recorded. Animals were trained for seven consecutive days with five trials per day. Performance of each mouse per day was averaged over trials from which the average performance of the respective group and the standard error of the mean (SEM) were calculated for statistical analysis.

Probe Trial/Test phase

After seven days of training to learn the position of the hidden platform using distal visual cues the animals had an intermission of two days before they were subjected to the probe trial. For the probe trial the platform was removed from the maze and the mice were released from the opposite quadrant as the target quadrant. The mice were able to explore the maze for 60 s and the time spend in each quadrant, the time spend at the former location of the platform and the number crossings of this area was analysed from the videos using Noldus Ethovision 7.0 software. Again, the average of the groups and SEM were calculated for statistical analysis.

4.4. Results

Induction of Aβ deposition in APP23 mice

Immunohistochemical analysis of hippocampal brain sections of mice tested in this behavioural paradigm revealed very prominent Aβ deposition in animals injected with tg brain extract (See Fig. 2). Induced aggregates are focused on the upper and lower blades of the dentate gyri, spreading throughout the hippocampus to adjacent brain areas and the overlaying cerebral cortex. Surprisingly, seven out of 21 mice injected

with wt brain extract exhibited some Aβ-positive deposition which was less pronounced compared to mice receiving tg extract. Interestingly, all mice with unexpected hippocampal deposition belonged to the first cohort of mice receiving surgery. Previous conducted studies in our department with highly diluted tg brain extract demonstrated that even minute amounts are able to trigger amyloid aggregation (Meyer-Luehmann et al., 2006) within 4 months post surgery. Therefore, we assume a contamination of the wt brain extract or surgical instruments. This would also explain the lower extend of amyloid deposition in these animals. For mice receiving tg brain extract in both cohorts the amyloid formation was exceeding the hippocampal tissue spreading into adjacent brain areas and overlying cortex.

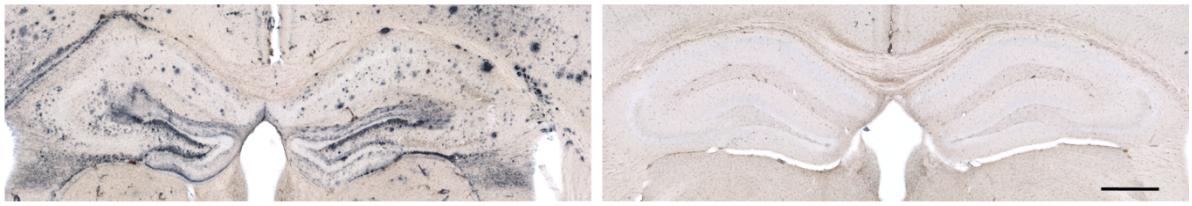

Figure 2: Immunohistochemical analysis of Aß deposits induced by infusion of tg or wt brain extract in the hippocampus of APP23 mice

The picture on the left displays induced Aβ deposition 8.5 months post surgery in a hippocampal section of a mouse injected with tg brain extract. On the right, a corresponding section of a mouse treated with wt brain is shown. Note the abundance of induced deposits in the upper and lower blades of the dentate gyri spreading from the hippocampus to adjacent brain regions in the left picture. Scale bar corresponds to 500 µm.

Behavioural Testing

Training phase

The two consecutive cohorts of mice tested in the water maze task were analysed separately in order to exclude a distortion of the data due to unnoticed changes of the environment of behavioural testing. Thereby, we observed a discrepancy of the behaviour between the 2 cohorts. While the first cohort of mice reduced the latency to find the hidden platform from day one (36.7±3.3 s for the experimental group versus 34.9±3.1 s for the control group) to day seven (20.5±3.0 s in the experimental group versus 15.9±2.2 s in the control group) this reduction for the second cohort was less prominent. Here, latency to find the platform was reduced from day one (39.9±3.4 s for

the experimental group versus 40.5±2.3 s) to day seven (27.0±3.2 s for the experimental group versus 22.3±1.9 s for the control group). Data analysis of the probe trial on day 10 without platform also confirmed differences in the performance of these two cohorts of animals. Usually, since the animals are trained to find the hidden platform located in the target quadrant (NW) it is expected that the animals focus on this area while exploring the maze during the probe trial. This was the case for both experimental and control group of the first cohort (experimental group: yielding 21.3±4.3 s spent in the target quadrant versus 9.2±1.7 s and 15.5±2.3 s for the neighbouring quadrants and 13.0±2.6 s for the opposite quadrant of the maze. Control group: 22.2±3.0 s spent in the target quadrant versus 10.6±1.4 s and 13.0±1.1 s for the neighbouring quadrants and 13.0±1.9 s for the opposite quadrant). However, data of the probe trial of the second cohort could not confirm these expected findings. Surprisingly, both groups of the second cohort explored mainly quadrant SE located at the opposite of the target quadrant. The experimental group spent 13.0±2.0 s in the target quadrant NW, 14.5±1.1 s and 13.2±0.9 s in the neighbouring quadrants but 19.5±2.1 s in the opposite quadrant. Data of the control group of the second cohort could confirm this finding. This group spent 13.2±1.0 s in the target quadrant, 12.6±0.7 s and 12.9±0.6 s in the neighbouring quadrant but 19.4 ±1.1 s in the opposite quadrant of the maze. Since there was no ascertainable change of the experimental set up between the two cohorts a reason for this fundamental change of behaviour could not be identified. Due to these findings, the results of the behavioural test are presented for both cohorts separately.

Learning Curve

Both groups of the first cohort exhibited a reduction in latency to find the hidden platform during the seven days of training (See Fig. 3). On the first day the experimental group showed a latency of 36.7±3.3 s for the experimental group versus 34.9±3.1 s for the control group going down to 20.5±3.0 s versus 15.9±2.2 s, respectively. A mixed 2-way ANOVA with repeated measurements for time (factor 1) and between wt or tg extract treated group (factor 2) of the data only confirmed a highly significant effect of time on the results ($F(6, 476)=7.28$, $p<0.0001$) meaning both groups improved their performance to find the platform during the seven days of training. While an effect of treatment was found to be significant ($F(1, 476)=7.63$, $p=0.006$) Bonferroni post-hoc test revealed a significant effect of treatment only for day four on which the performance of the experimental group dropped due to unknown reasons.

Also for the second cohort of mice, a reduction of latency during the seven days of training was observed (see Fig. 3). Latency to find the platform was reduced from day one (39.9±3.4 s for the experimental group versus 40.5±2.3 s) to day seven (27.0±3.2 s for the experimental group versus 22.3±1.9 s for the control group). A mixed 2-way ANOVA with repeated measurements for time (factor 1) and between wt or tg extract treated group (factor 2) revealed a highly significant difference for time ($F(6, 721)=7.82$, $p<0.001$) meaning that both groups improved their performance to find the platform during seven days of training. Statistical analysis of the data 2-way ANOVA with repeated measurements yielded no significant effect of treatment between the two groups.

Probe Trial

During the probe trial on day ten, both groups of the first cohort showed a preference for the target quadrant (see Fig. 2). Mice of the experimental group spent 21.3±4.3 s in the target quadrant and 9.2±1.7 s and 15.5±2.3 s for the neighbouring quadrants and 13.0±2.6 s for the opposite quadrant of the maze compared to 22.2±3.0 s spent in the target quadrant versus 10.6±1.4 s and 13.0±1.1 s for the neighbouring quadrants and 13.0±1.9 s for the opposite quadrant for mice of the control group. A 2-way ANOVA (factor 1: Quadrant; factor 2: Treatment) and Bonferroni post hoc test revealed a significant effect on the quadrant ($F(3, 48)=8.814$, $p<0.0001$) but not for the treatment ($F(1, 72)=0.02$ $p=0.8801$) indicating that both groups preferred exploring the target quadrant without significant difference between the experimental and the control group. Time spent in the former area of the platform (platform duration) was 1.1±0.1 s for the experimental group and 1.4±0.4 s for the control group. Statistical analysis using unpaired Student's t-test revealed no significant difference between the two groups. Statistical analysis of the number of crossing the former area of the platform (platform crossings) showed the same result as for the platform duration. Mice of the experimental group had a frequency of 3.3±0.2 crossings compared to 4.4±1.0 crossings in the control group. Again, unpaired Student's t-test did not reveal any significant differences between the two groups.

During the probe trial the experimental group of the second cohort spent 13.0±2.0 s in the target quadrant, 14.5±1.1 s and 13.2±0.9 s in the quadrants enclosing the target quadrant (NE and SW) but 19.5±2.1 s in quadrant SE located at the opposite of the target quadrant. A similar result could be observed for the control group. Here, time spent in

the target quadrant was 13.2±1.0 s, for the neighbouring quadrants 12.6±0.7 s and 12.9±0.6 s were recorded. Also for this group a preference of the quadrant SE was observed (19.4±1.1 s). Therefore, a 2-way ANOVA (factor 1: Quadrant; factor 2: Treatment) and Bonferroni post hoc test revealed no preference for the target quadrant (see Fig. 3) but instead a statistical significant preference for quadrant SE located at the opposite of the target quadrant NW ($F(3, 76)=13.66$, $p<0.0001$). A reason for this change in behaviour of the second cohort compared to the first cohort could not be determined. Avoiding the target quadrant lead also to lower times spent in the former area of the platform for both groups (0.6±0.2 s in experimental group, 0.6±0.1 s in the control group) compared to the first cohort tested (1.1±0.1 s and 1.4±0.4 s, respectively). Statistical analysis using unpaired, two-tailed t-test revealed significant differences for the time spent in the former location of the platform between the two cohorts independent from treatment ($t(33)= 2.645$; $p=0.012$).The number of platform crossings was also reduced for both groups of the second cohort compared to the first cohort (2.1±0.6 and 1.7±0.3 for the experimental and control group of the first cohort versus 3.3±0.2 and 4.4±1 for the experimental and control group). Statistical significant differences using unpaired, two-tailed t-test analysing the frequency of platform crossing between the two cohorts independent from treatment could be detected ($t(33)=3.358$; $p=0.002$).

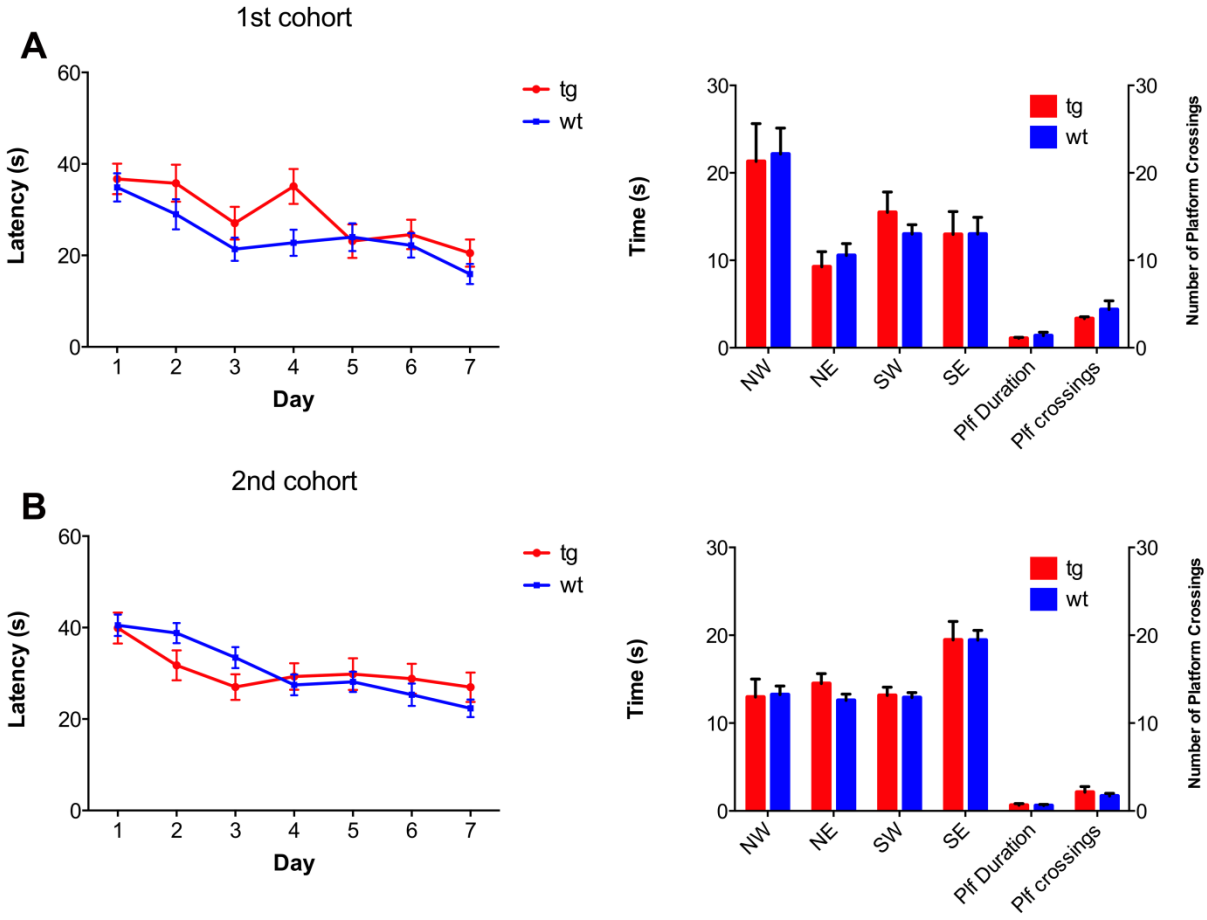

Figure 3: Behavioural Data of the first (A) and second cohort (B) of APP23 mice 8.5 months post surgery

On the left the average latency to find the hidden platform for mice injected with tg and wt brain extract is plotted. The bar diagrams on the right indicate the average time spent in the four quadrants, at the former position of the platform and the average number of crossing this area. Note the altered search pattern of the second cohort focussing on the SE quadrant. For the first cohort n=8 mice injected with wt extract and n=6 mice treated with tg brain extract, for the second cohort n=13 and 8, respectively.

Behavioural Difference Between the First and Second Cohort of Mice tested in the Morris Water Maze

Despite all efforts to provide an identical experimental set-up and environment for both cohorts tested in the Morris Water Maze, a severe alternation of behaviour was observed. Animals of the second cohort tend to either avoid the target quadrant (NW) or prefer the quadrant SE located at the opposite of the maze. Since no observable modification of the set-up could be observed one cannot exclude subtle changes of the experimental environment detectable for mice. This observed systematic error prohibits pooling both cohorts for analysis, as originally intended. Importantly, discussion with

another researcher using this particulate water maze set-up in our institute showed that this alternated behaviour occurred for all mice tested at that particular time being independent from strain, department, experimenter, treatment, housing conditions and any other capable factor (Marco Benevento, personal communication).

4.5. Discussion

Induction of β-Amyloidosis in APP23 Mice and Behavioural Testing in the Morris Water Maze

Infusion of brain extract from aged APP23 mice induced local deposition of Aβ in the hippocampus. In order to test whether massive Aβ deposition is affecting learning and memory a long incubation time of 8.5 month was chosen. Histological analysis, however, revealed discrepancy between the two control groups of this experiment. In seven out of eight animals of the first cohort receiving wt brain extract a weak, unexpected induction of Aβ deposition occurred. Since seeded Aβ deposition is known to be time and concentration dependent (Meyer-Luehmann et al., 2006) and the agent responsible for induction seems to be heat- and protease-resistant (Eisele et al., 2009), a contamination of the used extract during procession of the brains or during surgery can not be excluded. Absence of amyloid induction in the control group of the second cohort of animals support this hypothesis and indicates that even after prolonged incubation after surgery wt brain extract is not inducing or accelerating amyloid pathology in APP23 mice. Nevertheless, in the first cohort severity of amyloid induction in mice treated with wt brain extract was strongly reduced compared to mice infused with tg brain extract. Still, discussion of the results of this study is limited due to the following reasons: Results of the first cohort may be biased due to some induction of amyloid formation in mice treated with wt brain extract. For the second cohort of mice a systemic error for both, the experimental and control group, was detected, limiting the discussion of the results of this cohort of mice. Beside these restrictions, the data of the experiment do not indicate impaired spatial memory in APP23 mice with substantial Aβ deposition in the hippocampus since for both groups of the two cohorts no significant difference of latency or search behaviour during the probe trial was detected.

To discuss the functional impact of amyloid deposition recent studies to determine the role of soluble oligomeric forms of Aβ versus insoluble, deposited Aβ have been conducted. These studies have shown that low-oligomeric species, especially dimers of Aβ, potently and consistently induce Alzheimer's disease phenotypes in laboratory

rodents including synaptic plasticity (Klyubin et al., 2005) inhibition of long term potentiation (LTP) in the hippocampus and alterations of spine density (Shankar et al., 2008; Tackenberg and Brandt, 2009), tau hyperphosphorylation and neuritic degeneration in vitro (Jin et al., 2011) and are able to initiate amyloid deposition (Langer et al., 2011). Despite the shift of research from insoluble Aβ (in senile plaques) to soluble species of the peptide, prevention of aggregation in early stages remains the final goal since aggregated Aβ is a reservoir sequestering soluble Aβ. It has been shown that neuronal activity of hippocampal neurons in APP tg mice is altered including silencing and hyperactivation in presence of soluble and deposited Aβ (Busche et al., 2012).

5. Conclusions and Outlook

The aim of this PhD thesis was to study prion-like aspects and the functional impact of seeded cerebral β-amyloidosis in APP tg mouse models. This was achieved by the demonstration of induction of cerebral Aβ deposition by peripheral application of small amounts of brain extract containing aggregated Aβ and by the finding that characteristics of the induced β-amyloid deposits are dependent on the nature of Aβ in the brain extract and host. Behavioural testing using a classic behavioural paradigm of spatial learning and memory did not detect any severe impact of seeded Aβ deposition in the hippocampus of APP tg mice.

In neurodegenerative diseases, differences and similarities of pathomechanisms between prion diseases and other proteopathies such as AD, Parkinson's disease, Huntington disease, and systemic amyloidoses are under intensive discussion (Prusiner, 1998; Kane et al., 2000; Lundmark et al., 2002; Meyer-Luehmann et al., 2006; Clavaguera et al., 2009; Desplats et al., 2009; Ren et al., 2009; Soto, 2012; Fritschi et al., 2013). A special feature of prion diseases is the infectivity of the disease-inducing agent. As demonstrated by the BSE crisis, even ingestion of contaminated food is sufficient to cause spongiform encephalopathy. In previous studies only direct, intracerebral inoculation of the β-amyloidosis-inducing agent was shown to trigger amyloid formation in brain of APP tg mice. Data presented in this dissertation demonstrate the induction of cerebral β-amyloidosis after peripheral exposure to Aβ-laden brain extract. While the potency to spread from one organism to another is much higher for prions than for Aβ, the presented findings have sparked the discussion about transmission of cerebral β-amyloidosis or even AD. Due to the experimental approach (high amount of inoculated brain extract, recipients prone to amyloid deposition by expression of mutated APP), our data do not implicate a transmission of β-amyloidosis between individuals but do indicate a prion-like aspect of Aβ. Just recently, Irwin et al. have published a study addressing the possible transmission of proteins associated with neurodegenerative diseases. In this study, recipients of cadaver-derived human growth hormone (hGH) from pituitary glands have been examined for a human-to-human transmission of AD, PD and other proteins associated with neurodegenerative diseases. No cases of AD or PD but three cases of amyotrophic lateral sclerosis could be confirmed in at total of 796 recipients of hGH (Irwin et al., 2013). Nevertheless, the transmission of cerebral β-

amyloidosis after peripheral exposure of Aβ-seeds strengthens the need for further research to clarify the fundamental principles determining the infectivity of proteopathies.

Another characteristic feature of prions is the existence of distinct prion strains. Initially, the existence of prion strains was concluded due to differences in incubation times, histopathological lesions and affected brain areas after transmission to identical hosts (Telling et al., 1996). Propagation of differences in susceptibility to proteolytic fragmentation and posttranslational modifications e.g. glycosylation patterns imply variations of conformation of PrP as an underlying principle of prion strains (Collinge and Clarke, 2007). The propagation of intrinsic conformation and the alteration of Aβ ratio after inoculation of Aβ-seeds from different sources as presented are implying similar strain-like properties for Aβ. Since these are only initial findings, further research to strengthen the hypothesis of Aβ-strains is needed. This would include for example serial passage experiments of different Aβ-strains (using induced amyloid for inoculation) in various hosts and biochemical characterisation of amyloids from other APP tg animal models, familiar and sporadic AD patients and non-demented elderly. This may lead to identification of subtypes of Aβ aggregates that characterise disease stage or differ between in AD and normal aging. In turn specific Aβ aggregates may become targets for novel therapeutic interventions e.g. inhibiting the formation of specific disease-associated amyloid. Along this line further research concerning the functionality of Aβ aggregation and subtypes of β-amyloid is needed. This would include behavioural experiments as presented in this thesis to assess the impact of variants of β-amyloid on cognitive function, and assays to study differences in neurotoxicity of Aβ strains. Studying the relation between variants of β-amyloid and AD is crucial to evaluate the implications of insights gained from research on cerebral β-amyloidosis and to understand the proceeding from Aβ deposition to neuronal degeneration and finally dementia in patients suffering from AD.

The term prion was introduced by Prusiner in 1982 and is defined as a “small, proteinaceous infectious particle which is resistant to inactivation by most procedures that modify nucleic acids” in order to distinguish prions from viruses, plasmids and viroids (Prusiner, 1982). While the term prion was originally coined after scientific study of the PrP protein and its ability to be converted from PrP^{C} (rich in α-helical

content) to β-sheet rich amyloidogenic PrP^{SC}, today this conformational change is viewed as the central event in prion formation. Most notably our understanding of prion biology has been greatly enhanced as additional proteins with prion-like properties have been identified, including Aβ. The discovery that additional proteins involved in neurodegenerative disease also undergo conformational changes followed by propagation of these infectious states has initiated the hypothesis that most, if not all, amyloidogenic proteopathies might share conformational properties previously attributed to prions (Colby and Prusiner, 2011). If this hypothesis proves to be true, then therapeutics can potentially be designed to target general conformational properties common to various amyloidogenic proteopathies, and not one amyloidogenic protein (Jucker and Walker, 2011). Since most of these diseases are associated with the later stages of life, their impact on our aging society will be tremendous and urges to pool forces to mitigate their socioeconomic effects.

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7. Publications

7.1 Description of Personal Contribution

1) Peripherally applied Aβ-containing inoculates induce cerebral β-amyloidosis

   Eisele YS, Obermüller U, **Heilbronner G**, Baumann F, Kaeser SA, Wolburg H, Walker LC, Staufenbiel M, Heikenwalder M, Jucker M

   Own contribution:
   Assistance in experimental design of the study together with MJ and YSE; Analysis of blood samples; Preparation and immunoblot analysis of tissue extracts; Help with figure generation.

2) Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice.

   Heilbronnner G, Eisele YS, Langer F, Kaeser SA, Novotny R, Nagarathinam A, Åslund A, Hammarström P, Nilsson KPR, Jucker M

   Own contribution:
   Experimental design together with MJ and YSE; Immunoblot analysis; Statistical Analysis of data; Design and figure preparation with the help of YSE, AN, SAK, MJ and KPRN. Assistance in manuscript preparation by MJ and KPRN.

7.2. Peripherally Applied Aβ-Containing Inoculates Induce Cerebral β-Amyloidosis

Yvonne S. Eisele, Ulrike Obermüller, **Götz Heilbronner**, Frank Baumann, Stephan A. Kaeser, Hartwig Wolburg, Lary C. Walker, Matthias Staufenbiel, Matthias Heikenwalder, Mathias Jucker

Published in:

Science (2010): 330: 980-982

7.3. Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice

Götz Heilbronnner, Yvonne S. Eisele, Franziska Langer, Stephan A. Kaeser, Renata Novotny, Amudha Nagarathinam, Andreas Åslund, Per Hammarström, K. Peter R. Nilsson, Mathias Jucker

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8. Appendix

8.1. Abbrevations

AD	Alzheimer's disease
Aβ	amyloid β
AICD	APP intracellular domain
am	ante meridiem
APP	amyloid precurser protein
APP tg	APP transgenic
ANOVA	analysis of variance
ApoE	apolipoprotein E
BACE	β-site APP cleavage enzyme 1
BSE	bovine spongiform encephalopathy
C	Celsius
CAA	cerebral amyloid angiopathy
CJD	Creutzfelldt-Jakob disease
cm	centimetre
CNS	central nervous system
CTF	C-terminal fragment
DNA	Deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EOFAD	early onset FAD
FAD	familial Alzheimer's disease
FDC	follicular dendritic cells
FTD	fronto-temporal dementia
h	hour
i.c.	intracerebral
i.p.	intraperitoneal
ITI	inter-trial interval
LCP	luminescent conjugated polythiophene
LOAD	late onset Alzheimer's disease
m	meter
ml	millilitre
µl	microliter
MCI	mild cognitive impairment
min	minute
NE	North-East
NFT	neurofibrillary tangles
NW	North-West
PBS	phosphate-buffered saline
PD	Parkinson's disease
PET	positron emission tomography
PiB	Pittsburgh compound B
pm	post meridiem
PRP^{C}	cellular prion protein
PRP^{SC}	prion protein scrapie
PSEN	presenilin

s	second
SEM	standard deviation of the mean
SE	South-East
SNP	single-nucleotide polymorphism
SW	South-West
TBS	tris-buffered saline
tg	transgene
TSE	transmissible spongiform encephalopathy
TTR	Transthyretin
W	watt
wt	wild-type

9. Bibliography

Publications
Eisele YS, Obermüller U, **Heilbronner G**, Baumann F, Kaeser SA, Wolburg H, Walker LC, Staufenbiel M, Heikenwalder M, Jucker M (2010)
Peripherally applied Aβ-containing inoculates induce cerebral β-amyloidosis. Science

Heilbronnner G, Eisele YS, Langer F, Kaeser SA, Novotny R, Nagarathinam AÅslund A, Hammarström P, Nilsson KPR, Jucker M (2013)
Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice. EMBO Reports.