Alzheimer's disease: sleep, neuronal activation, and the default mode network

A.M. Krajnc, BoD master student, Utrecht; J.A.Claassen, MD, PhD

Abstract

More than 100 years after the first publication on Alzheimer's disease (AD), this neurodegenerative disease now affects a significant and increasing part of the elderly population. The cause of Alzheimer's disease is however still unclear and effective treatment is lacking. Understanding the role of amyloid-β in AD pathology, but also in health, is of great significance for the development of an eventual treatment. In the past decade it has become clear that, in health, neuronal activation increases amyloid-β levels and that sleep decreases neuronal activity and amyloid-β. We speculate that by increasing amyloid-β burden in the structures involved in the default mode network – the major affected areas in AD – sleep disorders can increase AD risk. Treating sleep disorders early in life could therefore have a major impact on worldwide AD burden.

Key words: Alzheimer's disease, amyloid-β, neuronal activation, default mode network, sleep disorder

1. Introduction

In 1907 Alois Alzheimer reported the first case of a peculiar disease (1). He examined his patient's brain and described fibrils and small miliary foci, currently known as tangles and amyloid-ß plaques, the main characteristics of Alzheimer's disease (AD) (2). Often AD begins with disruption of brain cell function in brain areas involved in memory, leading to gradually increasing difficulty remembering new information (3). To diagnose Alzheimer's disease the diagnostic and statistical manual of mental disorders (DSM) IV-TR and the NINCDS-ADRDA criteria, or the new NIA-AA revised criteria, are used (4-6).

1.1 Prevalence and economic burden of AD

More than 100 years after the first publication AD has developed into a disease affecting a significant and increasing part of the population of 65 years and older (3). The prevalence of AD in 2001 was estimated at 14.6 to 19.5 million cases worldwide, in 2006 at 26.6 million, and by 2050 the prevalence will be quadrupled (7, 8). With this major and increasing prevalence, AD exerts a great and increasing economic burden with each case costing approximately £27,647 per year, or €33,021 (exchange rate 12-2011) (9). In comparison, the costs of patients with cancer, stroke and heart disease are less than a quarter of this amount per year; oddly enough more is spent for research into these diseases. The sum of costs of dementia in terms of health and social care, informal care and productivity losses in 2008 were estimated at €20 billion per year in the UK (9), and at €177 billion per year in all of Europe (10). Besides the high prevalence and increasing incidence the costs of AD are high due to the failure of current treatment to alter disease progression.

1.2 Available pharmacological treatment for AD

Currently there are five Food & Drug Administration (FDA) approved drugs for Alzheimer's disease treatment (11). Following the finding that degeneration of cholinergic nerves in the basal forebrain is closely linked to cognitive decline, in 1993, the first acetylcholinesterase inhibitor (AChEI) tacrine was approved by the FDA for the treatment of AD. Tacrine was soon replaced by AChEIs with less side effects. Currently under investigation are AChEIs that are also claimed to inhibit amyloid-ß (Aß) production and aggregation (11). The excessive glutamate stimulation in the brain of AD patients is targeted by the fifth approved drug, memantine, a moderate NMDA receptor antagonist. These current treatments, developed with lack of knowledge of AD etiology, however, solely alleviate the symptoms of AD and are mildly effective for only approximately one year (7, 11).

1.3 Etiology of AD: a brief summary of current knowledge and introduction to the position of sleep.

The main factor that predicts the onset of Alzheimer's disease in a given individual is aging (3). The cause of Alzheimer's disease however remains unclear in these older patients. The amyloid cascade hypothesis (see 2.2 for more details), based on gene mutations found in AD, suggests Aß deposition and tau protein hyperphosphorylation lead to neuronal cell death, thereby causing the impaired memory formation in AD (12, 13). Aβ oligomers and Aβ induced tau protein hyperphosphorylation (which impairs normal microtubule association) could impair memory by decreasing synaptic transmission, increasing long-term depression, and decreasing long-term potentiation. The Aβ hypothesis was developed based on gene mutations found in hereditary AD patients and Down's syndrome patients with AD. These gene mutations involve enzymes that affect the processing of Aβ and may thus explain the initiation of the amyloid cascade. The amyloid hypothesis however does not elucidate the initial cause in non-genetic AD.

In the absence of a known genetic cause to explain the initiation of the amyloid cascade in the majority of patients, a number of factors have been investigated that may directly or indirectly trigger this amyloid cascade. Over the years, a number of such risk factors have been proposed, and their putative contribution to the world-wide prevalence of AD have recently been reviewed (14). Barnes *et al*. concluded that diabetes mellitus, midlife hypertension, midlife obesity, depression, physical inactivity, smoking, and cognitive inactivity each account for 2 to 19% of AD cases worldwide. They estimated that half of the worldwide AD prevalence is attributable to these seven modifiable risk factors together and pleaded that lifestyle factors thus play a major role in AD development (14). Besides these lifestyle risk factors, sleep disorders have also been associated with AD (15).

The past decade the concept of a default-mode network (DMN) of brain areas activated during inwardly directed mental activities and sleep has gained interest (16-18). DMN activity has also been linked to mental disorders; among others to Aß deposition in AD (19). First, the topography of amyloid-ß deposition is correlated to DMN brain

areas (20). Moreover, DMN activity appears disrupted in AD and the areas affected in AD, even before symptoms emerge, involve parts of the DMN (16). Recently it was demonstrated that neuronal activity affects both the amount of Aß in the interstitial fluid (ISF) as well as the development of amyloid plaques and that sleep attenuates the increased ISF Aß levels in mice after sleep deprivation (21, 22). The role of DMN activity increases and sleep disorders, which are common in AD patients, should be elucidated. It is tempting to speculate that by increasing Aß burden in the structures involved in the DMN – the major affected areas in AD – sleep disorders can increase AD risk. To comprehend possible AD risks, an overview of the physiological and pathological role of Aβ is given first and this review will then focus on the relation of Aβ with neuronal activation, the DMN, and sleep in AD.

2. Pathology in Alzheimer's disease

The hippocampus, key player in memory formation, is one of the major areas affected in AD; other areas include layer II of the entorhinal cortex, and some parts of the temporal, parietal and frontal neocortex (23, 24). The two main pathologic hallmarks of Alzheimer's disease are amyloid-β plaques and neurofibrillary tangles of hyperphosphorylated tau protein, both associated with the neuronal loss (12, 25). Aβ is however also present in the healthy population, therefore, before discussing AD pathology, an overview of the possible physiological roles of Aβ is given in section 2.1 first. Understanding the physiological role of AD is of great significance to appreciate the effect of the sleep/wake cycle in AD.

2.1 Physiological function of Aβ

Extracellular Aβ peptides of 39 to 43 amino acids result from the cleavage of the precursor protein of Aß (AβPP), a transmembrane protein found in neurons (25). Aβ could have one or more biological functions in the CNS as it is also expressed in the healthy population (26). Some studies indicate that the biological functions of Aß could be participation in synaptic function and facilitation of neuronal growth and survival, others indicate Aß could protect against oxidative stress, bind neuroactive compounds, toxins, and pathogens (26).

Increases in neuronal activity induce increases in Aβ and Aβ could be involved in a physiological feedback loop decreasing neuronal activity (see 3.2 for Aβ role in neuronal activity). The possible participation in synaptic function is based on effects of Aβ on NMDA receptors, also seen as the major detrimental effect of Aβ in AD, and is supported by the presence of Aβ throughout life. AβPP null mice (lacking the Aβ precursor protein) have reduced synapse numbers, and impaired LTP and memory. However, the majority of studies demonstrated Aβ to inhibit long-term potentiation, essential for memory formation (26). Besides Aß effects on synaptic function, i*n vitro* studies demonstrated that Aβ has neurotrophic effects and that neuronal growth increases in response to Aβ (26).

The role of Aβ in binding of oxidative metal ions, neuroactive compounds, toxins and pathogens could be mediated through the aggregating properties of Aβ. Aß has a high binding affinity for iron, copper, zinc and aluminum (26). Moreover, the injection of both iron and human $Aß_{42}$ in rat cortices was

demonstrated to be significantly less toxic than injection of iron solely, supporting the possible neuroprotective role of Aß (27). However, Aß oligomers are also thought to induce oxidative stress instead of protecting against it (28). The binding of Aß to neuroactive compounds, like apolipoproteins and many others, was demonstrated in human CSF and rat brain homogenates (26). Moreover, Bishop and Robinson suggested that Aß could bind to pathogens, however, only serum amyloid component P, a component of senile plaques, has been demonstrated to bind lipopolysaccharide, a component of gram negative bacteria, and the binding of Aß itself to pathogens has not been demonstrated (26, 29).

We should however be careful at drawing conclusions from studies investigating the physiological function of Aß that are performed in non-physiological mouse, rat, and *in vitro* models of AD. Overall Aβ could have several physiological effects at low concentrations, leading to the opposite when levels are increased, for example in AD patients.

2.2 Aβ as the key player in AD, insights from early and late onset AD

Besides the possible physiological roles of Aβ, Aß is generally accepted as the key player in AD. Gene mutations in enzymes processing Aβ have been associated with the rare early onset AD (<65 years of age), supporting the Aβ cascade hypothesis. These mutations are present in the Aβ precursor protein (AβPP) or presenilin genes (PS1 & PS2). AβPP cleavage by β- and γ-secretase results in the production of extracellular Aβ peptides of 39 to 43 amino acids, and this route is known as the amyloidogenic pathway. AβPP can also be cleaved first by α-secretase, a pathway which does not lead to Aβ production. PS1 and PS2 mutations alter γ-secretase mediated AβPP cleavage. Mutations in AβPP or in the presenilin genes therefore result in increased Aβ production. In theory, any cause of β- or γ-secretase stimulation or α-secretase inhibition may stimulate the amyloidogenic pathway, as can any cause of increased AβPP production. Of the Aβ peptides, $Aβ_{42}$ aggregates more easily than $Aβ_{40}$ and is therefore seen as more neurotoxic (25). In a recent study, however, overproduction of $Aß_{43}$ in mice induced impairment of short-term memory and AD-like Aß plaque formation, indicating $Aß_{43}$ might be an important player in AD development as well (30).

The more common sporadic, late onset AD has been associated with the ApoE4 (ε4/ε4) genotype. Biomarkers of human brain Aβ were found to be present in accordance with ApoE genotype, with the highest levels present in subjects expressing ApoE4 (E4>E3>E2) (31). Moreover, using *in vivo* microdialysis, Castellano *et al*. demonstrated that the concentration of ISF Aβ was greatest in the hippocampus of mice expressing human ApoE4 compared with mice expressing human ApoE3 or ApoE2. The increased Aβ levels in late onset AD patients seem to be partly caused by impaired Aβ metabolism, specifically clearance, leading to increased Aβ levels (31, 32).

Aβ could impair memory by interfering with NMDA receptors needed for long-term potentiation,

leading to disrupted synaptic plasticity (see 2.3 for Aβ effects on NMDA receptors) (24, 33, 34). The mutations found in AD patients indicate that other characteristics of AD, like hyperphosphorylated tau protein, neurofibrillary tangles, vascular damage and inflammation are more likely consequences of Aβ deregulations rather than causes of AD. Transgenic mice with a mutation in AβPP, a combination of AβPP and tau protein, or presenilin mutation develop neuronal loss, Aβ accumulation and memory deficits and are, although not ideal, used as AD models (35).

Some remarks have however to be made regarding the Aβ cascade hypothesis. First, Aβ plaques are also present in the cognitive healthy population and, second, the amount of Aβ plaques does not seem to correlate with disease progression in every study (34, 36). However, it is possible that Aβ is increased long before disease onset. Stern proposed a cognitive reserve enabling some individuals to cope better with challenges that come with aging, like increased Aβ. A more efficient recruitment of alternate, not affected, brain networks or more efficient utilization of the networks may be better developed in some individuals (37). Second, soluble forms of Aβ, oligomers, have been demonstrated to be more toxic than Aβ fibrils and plaques, increases in Aβ oligomers could therefore be more meaningful than plaque amounts (24, 38). Indeed, since the use of ELISA, soluble Aβ levels were demonstrated to better correlate with cognitive decline than previous plaque counts in post mortem brain sections (34, 39). Moreover, there is evidence that quantitative changes might not be as important as changes in Aß type ratios. Investigating ratios of $Aß_{40}$, $Aß_{42}$ and $Aß_{43}$ might be more meaningful in predicting disease progression (30). All these factors may thus confound the relationship between Aβ plaques and clinical AD symptomatology.

2.3 Aβ effects on tau hyperphosphorylation and NMDA receptor signaling

Besides Aβ plaque formation, neurofibrillary tangles (NFTs) are characteristic for AD. These NFTs are composed of the hyperphosphorylated, aggregated form of tau protein. Tau protein normally binds tubulin in microtubules for stabilization, however, the hyperphosphorylated tau protein dissociates from microtubules and forms NFTs by self-aggregation (23). Several AD mouse models have demonstrated that a direct or indirect interaction of Aβ with tau protein accelerates NFT formation (35). An interplay of Aß and tau protein is likely to be more detrimental in lieu of solely Aβ or tau protein pathology, as was previously suggested by supporters of either the Aβ or tau hypotheses. Ittner & Götz proposed that tau protein indirectly sensitizes NMDA receptors through FYN re-localization to the postsynaptic compartment. FYN, a tyrosine protein kinase, could then link NMDA receptors to downstream signaling pathways, which sensitizes NMDA receptors, and increases Aβ toxicity. Continued exposure to Aβ exerts toxic effects and hyperphosphorylates tau protein, leading to impaired tau protein interaction with microtubules and even more sensitization of NMDA receptors (13). Recent *in vitro* research indicates that Aβ could induce tau protein phosphorylation through upregulation of RCAN1 (regulator of calcineurin gene). Calcineurin, which dephosphorylates tau

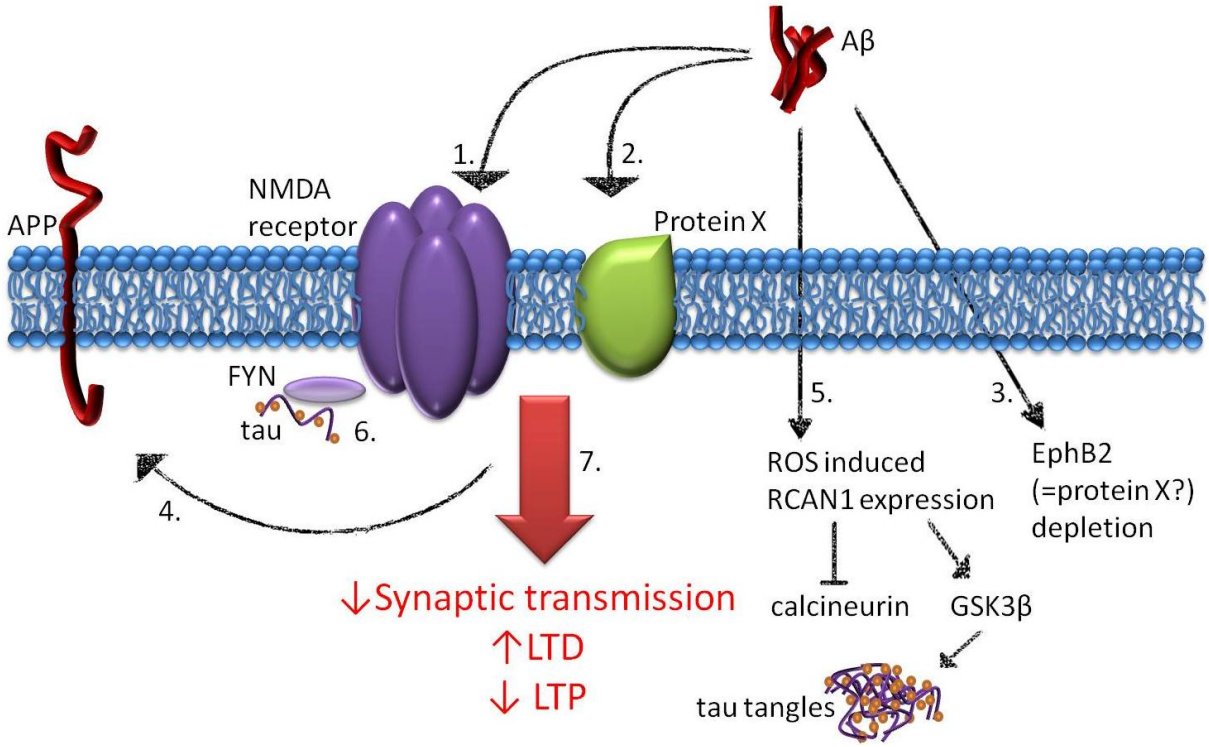

Figure 1. Possible direct and indirect effects of Aβ on NMDA receptor signaling. *1. Aβ binding to NMDARs/affecting NMDAR activity. 2. Aβ binding protein X, affecting NMDAR. 3. Aβ induced EphB2 depletion, candidate protein X, leading to impaired NMDAR activity. 4. NMDAR/NMDAR-protein X affecting Aβ formation. 5. Aβ induced RCAN1 gene expression through ROS, inhibiting tau dephosphorylation through calcineurin inhibition and inducing tau phosphorylation via GSK3β increases. 6. Hyperphosphorylated tau sensitizes NMDARs through FYN re-localization. 7. Overall Aβ oligomers impair memory by decreasing synaptic transmission, increasing LTD, and decreasing LTP. NMDAR: NMDA receptor, ROS: reactive oxygen species, GSK3β: glycogen synthase kinase-3β, LDP: long-term depression, LTP: long-term potentiation. Adapted from Malinow & extended (40).*

protein, is inhibited by RCAN1 proteins. RCAN1 proteins also induce upregulation of glycogen synthase kinase-3β (GSK3β), a tau kinase. Aβ induced upregulation of RCAN1 therefore leads to both decreased dephosphorylation and increased phosphorylation of tau protein, impairing normal microtubule association, and causing toxicity (Fig.1). Antioxidants were able to counteract the *in vitro* Aß induced upregulation of RCAN1, which indicates upregulation is likely mediated through oxidative stress (28).

Besides the effects of Aβ on tau protein, Aβ oligomers were recently proved to deplete EphB2 by binding to the fibronectin repeats of EphB2, inducing EphB2 degradation in the proteasome (33). EphB2 is a tyrosine kinase that regulates NMDA-receptor trafficking and function. Cissé *et al*. demonstrated that lentiviral EphB2 depletion causes functional deficits in NMDA receptor synaptic transmission strength and gene expression, and leads to impaired LTP and memory. By lentiviral increase of EphB2 in transgenic mice expressing human AβPP, the Aβ induced deficits were reversed. Thus, EphB2 increases can reverse Aβ effects on NMDA receptors, LTP, and memory (33). These direct and indirect effects of Aβ on NMDA receptors and tau leading to neuronal loss are induced by increased levels of Aβ, therefore, elucidation of the cause of increased Aβ in AD is essential and will be discussed in the next sections.

3. Neuronal activity and amyloid-β deposition

3.1 Neuronal activity affects Aβ *in vitro* and *in vivo*

In the past decade evidence for increased Aβ deposition during neuronal activity has accumulated. In 2003 Kamenetz *et al*. demonstrated that *in vitro* neuronal activity induces endocytosis of AβPP, which promotes the β-secretase accessibility in the endosomal compartments, and thereby enhances the chances for AβPP to be processed into Aβ (41). In mouse models increases in neuronal activity were also demonstrated to increase Aβ, while decreased activity led to decreased Aβ levels. Electrical stimulation in Tg2576 mice, recorded by EEG and microdialysis, induced a 30% increase in ISF Aβ 1h after stimulation (42). The transgenic Tg2576 mice express human AβPP and develop Aβ deposition in amyloid plaques at approximately 9 months of age. Moreover, Tg2576 mice have similar Aβ deposition patterns in the DMN as Alzheimer's patient do (21). Besides these effects of electrical stimulation, decreases in neuronal activity were demonstrated to decrease Aβ. First, in Tg2576 mice, LY354740 treatment, which decreases glutamate release and therefore neuronal activity, resulted in a 18.2% ± 4.9% decrease of Aβ. Second, tetrodotoxin treatment, which blocks sodium channels and thereby neuronal activity, displayed a high correlation with decreases in Aβ. Third, tetanus toxin, which inhibits synaptic vesicle release, decreased Aβ levels in absence of significant EEG amplitude change (42). More recently vibrissal stimulation, which increases neuronal activity, induced an increase in ISF Aβ levels, and vibrissal removal, which decreases neuronal activity, induced a decrease ISF Aβ levels in mice. Moreover, vibrissal deprivation reduced plaque growth by 78% and new plaque formation by 65% (21). Furthermore, Bero *et al*. revealed that reduced clearance of Aβ, or differential AβPP processing, are unlikely to be a sufficient explanation for Aβ deposition in older mice. Neither levels of clearance, nor AβPP processing significantly differed among regions with different levels of Aβ deposition. Besides inducing modifications in vibrissal activity, they demonstrated that endogenous regional ISF $Aβ_{42}$ levels in young Tg2576 mice could predict subsequent regional amyloid deposition in old mice. ISF lactate levels, a marker of neuronal activity, in young mice are closely associated with regional steady-state ISF Aβ levels and Aβ deposition later in life (21). These studies proved a significant correlation of neuronal activity with Aβ levels *in vitro* and *in vivo* and a significant correlation of early in life ISF Aβ levels and late life Aβ deposition.

3.2 Deregulation of Aβ and neuronal activity in AD

Neuronal activity increases Aβ levels; neuronal activity is however essential for synaptic plasticity and memory formation and, therefore, activity would seem important to prevent clinical AD progression. Chronic reduction in synaptic activity resulted in impaired memory in AD transgenic mice while Aβ plaques were reduced, and Aß intraneuronal immunoreactivity was increased (43). This increase in intraneuronal Aβ levels while synaptic activity is decreased is however in contradiction with other studies (21, 41, 42). Naturally a chronic reduction in neuronal activity compared to baseline activity is not likely to be beneficial for memory formation.

Overexpressed AβPP, cleaved into Aß, led to neuronal depression, which is dependent on NMDA receptor activity. This implies a negative feedback loop in which increased neuronal activity leads to Aß increases, which then binds to NMDA receptors and thereby depresses neuronal activity. In AD, Aß desensitization of NMDA receptors could lead to constant high neuronal activity. Highly increased neuronal activity and high levels of Aß could then lead to excitotoxicity. Another possibility is a consistent Aß production regardless of synaptic activity leading to neuronal depression and toxicity (41). However, neuronal activity appears disrupted in the default mode network (DMN) of AD patients and therefore Aβ desensitization and increased neuronal activity are more likely in those brain areas than solely consistent Aβ overproduction (44-46).

4. DMN activity and Aβ deposition

4.1 DMN and AD

In 2001 Raichle *et al*. first introduced the default mode network (DMN); a network of brain areas activated during inwardly directed mental activities (17). The core regions of the DMN are the ventral medial prefrontal cortex, the posterior cingulate cortex, the inferior parietal lobule, the lateral temporal cortex, the dorsal medial prefrontal cortex and the hippocampal formation (16).

DMN activity has been correlated to mental disorders including autism and schizophrenia. The DMN also appears disrupted in AD, moreover, the areas affected in AD, even before symptoms emerge, involve parts of the DMN (16). The functional connectivity of the DMN decreases with normal aging, however, decreases are more severe in AD and subjects at risk for AD development (47). Moreover, there is a correlation between DMN patterns in cortical regions in young adults and the topography of amyloid deposition in elderly AD patients (20). Aß deposition was also correlated with the DMN brain areas in cognitive healthy elderly; these elderly could be in an early stage of AD or could be able to compensate the impairments induced by the Aβ deposition (37, 48).

4.2 Increased DMN neuronal activity prior to AD onset

Both Aβ plaque deposition and brain aerobic glycolysis seem to be concentrated in the DMN. PET imaging (^{11}C-PiB imaging) revealed that the areas of aerobic glycolysis in normal young adults correlate spatially with the Aβ deposition seen in AD patients and subjects with elevated Aβ levels (46). Therefore, increased neuronal activity in the DMN of (young) adults could lead to increased plaque deposition in AD. Indeed, young healthy carriers of the ApoE4 genotype, a risk factor for late onset AD, have increased DMN activity compared to age matched non-carriers. Increases in hippocampal activity were found in ApoE4 carriers in both resting (DMN) state and during memory task (44). On the contrary, the older ApoE4 carriers expressed decreased brain activity while non-carriers expressed increased brain activity with aging (45). These results indicate that increased DMN activity in young subjects could result in regionally increased Aβ deposition and DMN disruption later in life and causes of increased DMN activity are thus essential to elucidate.

4.3 DMN activity and sleep disorders

Since increased neuronal activity increases Aβ levels, and DMN areas are correlated to AD pathology, it is tempting to speculate that prolonged neuronal activity in the DMN can lead to an increased AD risk. DMN activity correlates with posterior networks involved in memory retrieval, thus memory in AD patients is possibly affected due to DMN activation (20). The brain's resting state during introspection, memory retrieval, daydreaming and imagination is associated with increased DMN activity (17). However, BOLD fMRI indicated that the correlations among brain regions of the DMN also persisted during light sleep (49). One year later Horovitz *et al*. demonstrated that DMN activity persists during deep sleep as well, however, with a dynamical modulation in strength of correlations of the DMN components. During deep sleep, the posterior areas strengthen their connectivity but connectivity with the frontal areas is lost. Deep sleep induces a DMN with compromised integrity, however, the activity levels in the individual network components are preserved (18). Sleep disorders have been associated with AD, and induce increased neuronal activity and Aβ deposition, both characteristics seen in the DMN of AD patients (15, 16, 21). Recently, a study proved that sleep deprivation results in a reduction of DMN functional connectivity (50). Moreover, sleep deprivation resulted in less task-related deactivation in the dorsal anterior cingulate cortex and increased deactivation in the precuneus of the DMN compared to controls with a longer period of prior sleep (51). The correlation of sleep disorders and DMN activity is of great interest regarding AD, and still needs to be further investigated.

5. Sleep

5.1 Sleep and cognition

Sleep has been proven to be essential for healthy cognition. Sleep deprivation is associated with deficits in attention, learning and memory, emotional reactivity, and higher order processes, such as executive decision making. Moreover, sleep deprivation inhibits long-term synaptic plasticity in the hippocampus (52). Wakefulness is associated with a net increase in cortical synaptic strength and periods of sleep with a net decrease and it has become clear that sleep probably is necessary for recovery of the homeostatic balance of neuronal activity (53, 54). Sleep disorders thus have a major effect on cognition.

5.2 Sleep disturbances in AD

Sleep disturbances are common in AD patients (55). In AD the rhythmic expression of clock genes is no longer found, possibly due to functional disconnection between the suprachiasmatic nucleus and the pineal gland. Neuronal loss in brain areas involved in wakefulness like the basal nucleus of Meynert, locus coeruleus, upper raphe nuclei, and tegmentopontine reticular nuclei may contribute to insomnia and sleep fragmentation (55). Studies have both reported that increased and decreased sleep are associated with a cognitive decline. Benito-León *et al*. reported a significant association between long self-reported sleep duration and incident dementia (56). Depression was however not assessed which

could be associated with the increased sleep. Excessive daytime sleepiness has also been reported as an important risk factor for cognitive decline (57). Daytime sleepiness could reflect decreased nighttime sleep and impair homeostatic recovery due to sleep fragmentation. Moreover, late-life chronic insomnia in men was associated with an increased risk of cognitive decline (58). Studies report both an association of increases and decreases in sleep with cognitive decline; this could be caused by the different AD stages of the investigated subjects. Early in life decreases in sleep could impair the recovery of the homeostatic balance of neuronal activity and therefore affect Aß deposition, while late life increased sleep duration could reflect progression into AD.

5.3 Sleep affects Aβ levels

The past years, sleep and wakefulness have been correlated to Aβ levels in mice and humans (21, 22, 59). In mice that express human AβPP, ISF Aβ levels correlate with the duration of wakefulness (22). Aβ levels were significantly increased during the dark period (when the mice are awake) compared to the light period and correlated significantly with the time spent awake. A negative correlation with sleep, especially with non-REM sleep, was found (22). Diurnal fluctuation of mouse-Aβ was also present in wild type B6SJL mice and is therefore assumed as physiological. ISF Aβ levels significantly increased during acute sleep deprivation and during orexin (a natural peptide that induces wakefulness) infusion (22). Moreover, Bero *et al*. demonstrated that increased neuronal activation leads to increased ISF Aβ levels and that lactate, a marker for neuronal activity, fluctuates diurnally. ISF Aβ levels correlate with lactate levels and both decrease during sleep (21). Factors, like sleep disorders, that elevate neuronal activity over prolonged periods may accelerate progression of Aβ deposition and therefore of AD. These data indicate that physiological neuronal activity regulates ISF Aβ levels in animal models and that neuronal activity and Aβ levels are decreased during sleep.

Recently, CSF Aβ patterns were assessed in young normal human controls, aged subjects with normal amyloid levels and an aged group with increased amyloid levels. In healthy controls a circadian pattern of Aβ CSF was found; this pattern was disrupted by increasing age (aged amyloid negative and positive groups). In young controls, both $A\beta_{40}$ & $A\beta_{42}$ levels were inversely correlated with sleep after a 6-hour delay, while no significant total protein circadian pattern was present. In young controls, wakefulness thus causes increased levels of CSF Aβ, while sleep decreases CSF Aβ, indicating that sleep disorders early in life could be an important risk factor for Alzheimer´s disease (Fig.2) (59).

6. Discussion

6.1 Summary

AD affects a major and increasing part of the elderly population and exerts a great economic burden worldwide. Lack of knowledge of AD pathology impairs effective treatment development and research into the mechanisms of AD is therefore essential. Aβ is thought to affect NMDA receptors directly and indirectly (13, 28, 33). Overall Aβ

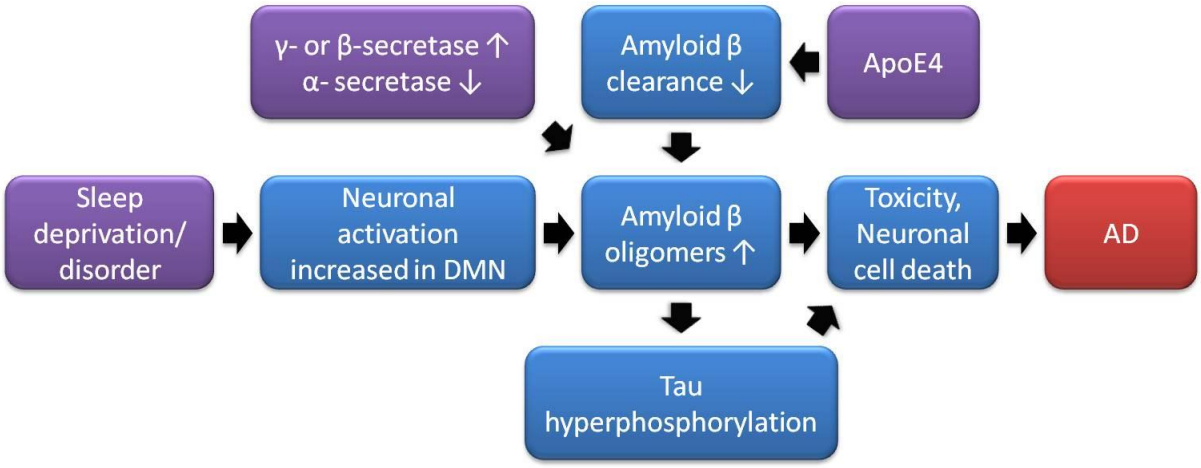

***Figure 2. Simplified scheme indicating the effects of sleep disturbances, secretase activity, and ApoE4 on AD development*.**

oligomers could impair memory by decreasing synaptic transmission, increasing LTD, and decreasing LTP. These possible effects of Aβ have however not yet been indisputably proven and more research is needed.

Aβ deposition is influenced by neuronal activity, and more specifically by activity in the DMN, and is influenced by sleep, but the exact correlations remain to be elucidated. Neuronal activity was proved to increase Aβ deposition *in vitro* and *in vivo* (21, 41, 42). The default mode network appears disrupted in AD, with increased DMN neuronal activity prior to AD onset and decreased DMN activity in elderly with AD (16, 20, 44-47). Moreover, areas with Aβ deposition are correlated with brain areas of the DMN, however, Aβ is distributed more widely than solely the DMN (60). Brain areas of the DMN remain activated during light and deep sleep (18, 49), however, sleep reduces overall cortical synaptic strength and could therefore be essential for recovery of the homeostatic balance of neuronal activity in the DMN (53, 54). If sleep is needed for recovery of homeostatic balance, a decrease of activity in DMN associated brain areas would be expected since both increased DMN activity and disturbed sleep patterns are present in the brain of AD patients. Sleep does indeed affect the DMN; sleep deprivation resulted in a reduction of DMN functional connectivity and resulted in less task-related deactivation in areas of the DMN compared to controls with a longer period of prior sleep (50, 51). Early in life sleep disorders could thus increase AD risk due to increased neuronal activity, impaired neuronal recovery, and by directly affecting DMN connectivity, all inducing increased Aß levels.

6.2 Future directions

The exact mechanisms of AD onset and disease progression remain unknown. The role of Aβ on tau protein or tau protein itself in AD has not yet been explained. In transgenic mice models, tau protein's role in AD, also independent of Aβ, should be further assessed. Moreover, using transgenic mice models, a possible disrupted feedback loop of Aß and neuronal activation should be investigated as it could give more insight into the presence of NMDA

receptor desensitization or consistent Aß overproduction regardless of neuronal activity in AD.

Sleep disorders are common in AD patients, however, prior to AD sleep disorders early in life, when Aβ levels fluctuate significantly in correlation to sleep, need to be investigated. By increasing Aβ burden in structures involved in the DMN, sleep disorders could increase AD risk. Research in human subjects that investigates Aβ diurnal fluctuations and both sleep deprivation and Aβ deposition will give more insight into the possibility of sleep disturbances early in life as a risk factor for AD. Ideally, sleep behavior, DMN activity, Aβ CSF levels, Aβ plaques and tau protein should each be assessed in a longitudinal study in young subjects. However, from a practical point of view a parallel study with young subjects (with and without predisposition to AD) and old subjects (with and without AD) is more realistic. Effects of sleep disorders in the young population on later dementia could be of great interest and the DMN and Aβ levels should be investigated by following young subjects in time using fMRI and PET imaging (^{11}C-PiB imaging). Naturally, these long lasting studies come with a price but the economic impact of AD is significant and these studies would give important insights into this neurodegenerative disease affecting a major part of the elderly. In the young population Aβ decreases are significant during sleep and a possible approach against AD would therefore be to treat sleep disorders early in life effectively. If sleep is proven to significantly affect AD onset, early treatment of sleep disorders could postpone AD onset with a few years, which is thought to significantly decrease AD prevalence and have a major impact on global AD burden (7, 14).

Abbreviations

^{11}C-PiB	^{11}C-Pittsburgh compound B
Aß	amyloid-β protein
AChEI	acetylcholinesterase inhibitors
AD	Alzheimer's disease
ApoE	apolipoprotein E
AβPP	amyloid-β precursor protein
BOLD	blood oxygenation level dependent
CNS	central nervous system
CSF	cerebrospinal fluid
DMN	default-mode network
DSM IV-TR	diagnostic and statistical manual for mental disorders IV text revision
EEG	electroencephalography
ELISA	enzyme-linked immuno sorbent assay
EphB2	ephrin type B2
FDA	food & drug administration
fMRI	functional magnetic resonance imaging
GSK3ß	glycogen synthase kinase 3ß
ISF	interstitial fluid
LTD	long-term depression
LTP	long-term potentiation
NFT	neurofibrillary tangles
NIA-AA	National Institute on Aging and the Alzheimer's Association
NINCDS	National Institute of Neurological and Communicative Disorders and Stroke
ADRDA	Alzheimer's Disease and Related Disorders Association
NMDA	N-Methyl-D-Aspartic acid
NMDAR	N-Methyl-D-Aspartic acid receptor
NREM	non-rapid-eye-movement
PET	positron emission tomography
PS1/PS2	presenilin 1/2
ROS	reactive oxygen species
TTX	tetrodotoxin
RCAN1	regulator of calcineurin 1
UK	United Kingdom

Acknowledgements

I thank my supervisor, J.A. Claassen, MD, PhD, for giving me the opportunity to do a literature research at the UMC Nijmegen - Alzheimer Centre Nijmegen. The feedback was clear, leading to a pleasant collaboration and made it possible to write this master thesis.

References

1. Alzheimer A, Stelzmann RA, Schnitzlein HN, Murtagh FR (1995) An english translation of alzheimer's 1907 paper, "uber eine eigenartige erkankung der hirnrinde". *Clin Anat*. **8**, 429-31.

2. Cipriani G, Dolciotti C, Picchi L, Bonuccelli U (2011) Alzheimer and his disease: A brief history. *Neurol Sci*. **32**, 275-279.

3. Thies W, Bleiler L (2011) Alzheimer's disease facts and figures. *Alzheimers Dement*. **7**, 208-244.

4. American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders, fourth edition, text revision. Washington, DC, 1-955.

5. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on alzheimer's disease. *Neurology*. **34**, 939-944.

6. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR,Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to alzheimer's disease: Recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimers Dement*. **7**, 263-269.

7. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of alzheimer's disease. *Alzheimers Dement*. **3**, 186-191.

8. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M (2005) Global prevalence of dementia: A delphi consensus study. *Lance*t. **366**, 2112-2117.

9. Luengo-Fernandez E, Leal J, Gray A (2010) Dementia 2010, executive summary. *Alzheimer's research trust*. 1-12.

10. Wimo A, Jonsson L, Gustavsson A, McDaid D, Ersek K, Georges J, Gulácsi L, Karpati K, Kenigsberg P, Valtonen H (2011) The economic impact of dementia in europe in 2008-cost estimates from the eurocode project. *Int J Geriatr Psychiatry*. **26**, 825-832.

11. Haas C (2011) Strategies, development, and pitfalls of therapeutic options for alzheimer's disease. *J Alzheimers Dis*. **28**, 1-41.

12. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (2011) Alzheimer's disease. *Lancet*. **377**, 1019-1031.

13. Ittner LM, Gotz J (2011) Amyloid-beta and tau--a toxic pas de deux in alzheimer's disease. *Nat Rev Neurosci*. **12**, 65-72.

14. Barnes DE, Yaffe K (2011) The projected effect of risk factor reduction on alzheimer's disease prevalence. *Lancet Neurol*. **10**, 819-828.

15. Osorio RS, Pirraglia E, Aguera-Ortiz LF, During EH, Sacks H, Ayappa I, Walsleben J, Mooney A, Hussain A, Glodzik L, Frangione B, Martínez-Martín P, de Leon MJ (2011) Greater risk of alzheimer's disease in older adults with insomnia. *J Am Geriatr Soc*. **59**, 559-562.

16. Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: Anatomy, function, and relevance to disease. *Ann N Y Acad Sci*. **1124**, 1-38.

17. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. *Proc Natl Acad Sci U S A*. **98**, 676-682.

18. Horovitz SG, Braun AR, Carr WS, Picchioni D, Balkin TJ, Fukunaga M, Duyn JH (2009) Decoupling of the brain's default mode network during deep sleep. *Proc Natl Acad Sci U S A*. **106**, 11376-11381.

19. Walker LC, Jucker M (2011) Amyloid by default. *Nat Neurosci*. **14**, 669-670.

20. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005) Molecular, structural, and functional characterization of alzheimer's disease: Evidence for a relationship between default activity, amyloid, and memory. *J Neurosci*. **25**, 7709-7717.

21. Bero AW, Yan P, Roh JH, Cirrito JR, Stewart FR, Raichle ME, Lee JM, Holtzman DM (2011) Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. *Nat Neurosci*. **14**, 750-756.

22. Kang JE, Lim MM, Bateman RJ, Lee JJ, Smyth LP, Cirrito JR, Fujiki N, Nishino S, Holtzman DM (2009) Amyloid-beta dynamics are regulated by orexin and the sleep-wake cycle. *Science*. **326**, 1005-1007.

23. Holtzman DM, Morris JC, Goate AM (2011) Alzheimer's disease: The challenge of the second century. *Sci Transl Med*. **3**, 77sr1.

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

25. Hendriksen JV, Nottet HS, Smits HA (2002) Secretases as targets for drug design in alzheimer's disease. *Eur J Clin Invest*. **32**, 60-68.

26. Bishop GM, Robinson SR (2004) Physiological roles of amyloid-beta and implications for its removal in alzheimer's disease. *Drugs Aging*. **21**, 621-630.

27. Bishop GM, Robinson SR (2003) Human Abeta1-42 reduces iron-induced toxicity in rat cerebral cortex. *J Neurosci Res*. **73** 316-323.

28. Lloret A, Badia MC, Giraldo E, Ermak G, Alonso MD, Pallardo FV, Davies KJ, Viña J (2011) Amyloid-beta toxicity and tau hyperphosphorylation are linked via RCAN1 in alzheimer's disease. *J Alzheimers Dis*. **26**, 1-9.

29. de Haas CJ (1999) New insights into the role of serum amyloid P component, a novel lipopolysaccharide-binding protein. *FEMS Immunol Med Microbiol* **26** 197-202.

30. Saito T, Suemoto T, Brouwers N, Sleegers K, Funamoto S, Mihira N, Matsuba Y, Yamada K, Nilsson P, Takano J, Nishimura M, Iwata N, Van Broeckhoven C, Ihara Y, Saido TC (2011) Potent amyloidogenicity and pathogenicity of Abeta43. *Nat Neurosci*. **14**, 1023-1032.

31. Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, Fagan AM, Morris JC, Mawuenyega KG, Cruchaga C, Goate AM, Bales KR, Paul SM, Bateman RJ, Holtzman DM (2011) Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med*. **3**, 89ra57.

32. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS beta-amyloid in alzheimer's disease. *Science*. **330**, 1774.

33. Cisse M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu GQ, Mucke L (2011) Reversing EphB2 depletion rescues cognitive functions in alzheimer model. *Nature*. **469**, 47-52.

34. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol*. **8**, 101-112.

35. Schaeffer EL, Figueiro M, Gattaz WF (2011) Insights into alzheimer disease pathogenesis from studies in transgenic animal models. *Clinics* **66**, S1 45-54.

36. Rodrigue KM, Kennedy KM, Park DC (2009) Beta-amyloid deposition and the aging brain. *Neuropsychol Rev*. **19**, 436-450.

37. Stern Y (2002) What is cognitive reserve? theory and research application of the reserve concept. *J Int Neuropsychol Soc*. **8**, 448-460.

38. Dahlgren KN, Manelli AM, Stine WB,Jr, Baker LK, Krafft GA, LaDu MJ (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem*. **277**, 32046-32053.

39. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA*. **283**, 1571-1577.

40. Malinow R (2011) New developments on the role of NMDA receptors in alzheimer's disease. *Curr Opin Neurobiol.* doi:10.1016/j.conb.2011.09.001.

41. Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S, Malinow R (2003) APP processing and synaptic function. *Neuron*. **37**, 925-937.

42. Cirrito JR, Yamada KA, Finn MB, Sloviter RS, Bales KR, May PC, Schoepp DD, Paul SM, Mennerick S, Holtzman DM (2005) Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron*. **48**, 913-922.

43. Tampellini D, Capetillo-Zarate E, Dumont M, Huang Z, Yu F, Lin MT, Gouras GK (2010) Effects of synaptic modulation on beta-amyloid, synaptophysin, and memory performance in alzheimer's disease transgenic mice. *J Neurosci*. **30**, 14299-14304.

44. Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A.* **106**, 7209-7214.

45. Filippini N, Ebmeier KP, MacIntosh BJ, Trachtenberg AJ, Frisoni GB, Wilcock GK, Beckmann CF, Smith SM, Matthews PM, Mackay CE (2011) Differential effects of the APOE genotype on brain function across the lifespan. *Neuroimage*. **54**, 602-610.

46. Vlassenko AG, Vaishnavi SN, Couture L, Sacco D, Shannon BJ, Mach RH, Morris JC, Raichle ME, Mintun MA (2010) Spatial correlation between brain aerobic glycolysis and amyloid-beta (abeta) deposition. *Proc Natl Acad Sci U S A*. **107**, 17763-17767.

47. Hafkemeijer A, van der Grond J, Rombouts SA (2011) Imaging the default mode network in aging and dementia. *Biochim Biophys Acta*. doi:10.1016/j.bbadis.2011.07.008

48. Sperling RA, Laviolette PS, O'Keefe K, O'Brien J, Rentz DM, Pihlajamaki M, Marshall G, Hyman BT, Selkoe DJ, Hedden T, Buckner RL, Becker JA, Johnson KA (2009) Amyloid deposition is associated with impaired default network function in older persons without dementia. *Neuron*. **63**, 178-188.

49. Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2008) Low frequency BOLD fluctuations during resting wakefulness and light sleep: A simultaneous EEG-fMRI study. *Hum Brain Mapp*. **29**, 671-682.

50. De Havas JA, Parimal S, Soon CS, Chee MW (2011) Sleep deprivation reduces default mode network connectivity and anti-correlation during rest and task performance. *Neuroimage*. **59**, 1745-1751.

51. Gujar N, Yoo SS, Hu P, Walker MP (2010) The unrested resting brain: Sleep deprivation alters activity within the default-mode network. *J Cogn Neurosci*. **22**, 1637-1648.

52. McCoy JG, Strecker RE (2011) The cognitive cost of sleep lost. *Neurobiol Learn Mem*. **96**, 564-582.

53. Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. *Sleep Med Rev*. **10**, 49-62.

54. Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat Neurosci.* **11**, 200-208.

55. Hauw JJ, Hausser-Hauw C, De Girolami U, Hasboun D, Seilhean D (2011) Neuropathology of sleep disorders: A review. *J Neuropathol Exp Neurol*. **70**, 243-252.

56. Benito-Leon J, Bermejo-Pareja F, Vega S, Louis ED (2009) Total daily sleep duration and the risk of dementia: A prospective population-based study. *Eur J Neurol*. **16**, 990-997.

57. Ohayon MM, Vecchierini MF (2002) Daytime sleepiness and cognitive impairment in the elderly population. *Arch Intern Med*. **162**, 201-208.

58. Cricco M, Simonsick EM, Foley DJ (2001) The impact of insomnia on cognitive functioning in older adults. *J Am Geriatr Soc*. **49**, 1185-1189.

59. Huang Y, Potter R, Sigurdson W, Santacruz A, Shih S, Ju YE, Kasten T, Morris JC, Mintun M, Duntley S, Bateman RJ (2011) Effects of age and amyloid deposition on A{beta} dynamics in the human central nervous system. *Arch Neurol*. 2011 doi:10.1001/archneurol.2011.235.

60. Jagust WJ, Mormino EC (2011) Lifespan brain activity, beta-amyloid, and alzheimer's disease. *Trends Cogn Sci*. **15**, 520-526.