Orexin and Alzheimer's Disease

Claudio Liguori

Abstract Alzheimer's disease (AD) is the most frequent age-related dementia. It prevalently causes cognitive decline, although it is frequently associated with secondary behavioral disturbances. AD neurodegeneration characteristically produces a remarkable destruction of the sleep–wake cycle, with diurnal napping, nighttime arousals, sleep fragmentation, and REM sleep impairment. It was recently hypothesized that the orexinergic system was involved in AD pathology. Accordingly, recent papers showed the association between orexinergic neurotransmission dysfunction, sleep impairment, and cognitive decline in AD. Orexin is a hypothalamic neurotransmitter which physiologically produces wakefulness and reduces REM sleep and may alter the sleep–wake cycle in AD patients. Furthermore, the orexinergic system seems to interact with CSF AD biomarkers, such as beta-amyloid and tau proteins. Beta-amyloid accumulation is the main hallmark of AD pathology, while tau proteins mark brain neuronal injury due to AD pathology. Investigations so far suggest that orexinergic signaling overexpression alters the sleep–wake cycle and secondarily induces beta-amyloid accumulation and tau-mediated neurodegeneration. Therefore, considering that orexinergic system dysregulation impairs sleep–wake rhythms and may influence AD pathology, it is hypothesized that orexin receptor antagonists are likely potential preventive/therapeutic options in AD patients.

Keywords Alzheimer's disease • Beta-amyloid • Orexin • Polysomnography • REM • Sleep disturbances • Sleep–wake cycle • Tau

C. Liguori (✉)
Sleep Medicine Centre, Neurophysiopathology Unit, Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy
e-mail: dott.claudioliguori@yahoo.it

© Springer International Publishing AG 2016
Curr Topics Behav Neurosci
DOI 10.1007/7854_2016_50

Uncorrected Proof

Contents

1 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which has been identified as the main cause of cognitive decline in the elderly [1, 2]. The neuropathological hallmarks of AD are the accumulation of both amyloid-containing neuritic plaques and neurofibrillary tangles (NFTs) of tau proteins [3]. These neuropathological brain changes have been suggested to occur 15–20 years before the onset of AD symptoms [4]. Accordingly, the preclinical AD concept has been recently established as the presence of AD markers in cognitively normal individuals. In this regard, the expectations for disease-modifying therapeutic strategies are highly relevant for preclinical AD patients, since this condition may last years before the appearance of clinical AD. Therefore, the identification of risk factors for developing AD pathology is a current significant biological issue. Accordingly, researchers are presently highly focused at recognizing causes of AD, in order to counterbalance the pathological mechanisms triggering AD pathology. Therefore, substantial efforts have been made in the last decade to understand the pathophysiological processes underpinning beta-amyloid aggregation and deposition in order to delay or possibly prevent the AD neurodegeneration. In particular, biomarkers, expression of AD neuropathology, are actually invoked, thus requiring extensive investigations. In keeping with this observation, many studies have been performed in order to identify and possibly establish early biomarkers of AD pathology.

Sleep disruption is considered a core component of AD, and also a preclinical biomarker, since sleep impairment may emerge before the clinical onset of AD. Moreover, insufficient sleep facilitates the accumulation of β-amyloid, potentially triggering earlier AD neuropathological changes [5]. Therefore, sleep dysfunction has been hypothesized as an AD biomarker, since it may promote and/or accelerate the neurodegenerative AD processes, and thus the cognitive decline [5–8]. In agreement with this supposition, sleep impairment has been demonstrated as altering the physiological homeostatic brain processes essential to ensure the clearance of toxic substrates which accumulate during wakefulness, such as beta-amyloid [8]. In fact, sleep reduction and dysregulation significantly lessen the demonstrated homeostatic and restorative effects of sleep against neurodegenerative processes, ensured by the glymphatic system [8]. This recently discovered that

Uncorrected Proof

the macroscopic waste clearance system is active during sleep and alleviates brain from deposition of toxic substrates [8]. Importantly, glymphatic failure may precede β-amyloid deposits, thus representing an early biomarker of AD. However, an animal model study documented that restoring glymphatic inflow and brain interstitial-fluid (ISF) clearance potentially act as therapeutic targets slowing the onset and progression of AD [9]. Hence, restoring sleep in AD may recover the glymphatic system function and thus reduce the progression or possibly stop the ongoing chain of events started with the reduction of β-amyloid cerebrospinal-fluid (CSF) levels and leading to β-amyloid neurotic plaques deposition.

In keeping with this supposition, it has been recently hypothesized that poor sleep quality may promote cognitive decline and AD neurodegeneration [10–13].

Clinically, AD is characterized by the deterioration of memory, language, and intellect. Although cognitive decline is the main feature of AD, sleep disturbances are a common highly disruptive behavioral symptom associated with AD pathology. Indeed, epidemiological studies have reported that sleep disturbances occur in up to 45% of AD patients [14–16]. The main sleep disorder in AD is obstructive sleep apnea syndrome, although also insomnia is a frequent and disabling disorder affecting AD patients. What is certain is that sleep disorders accelerate AD pathology [17, 18].

The origin of sleep disturbances in AD is thought to be multifactorial. In fact, several hypotheses have been established and tested. Accordingly, degeneration of suprachiasmatic nucleus, pineal gland, hypothalamus, and brain nuclei containing circadian clock-regulating neurons in basal forebrain and brainstem is one of the possible pathological mechanisms proposed of sleep dysregulation in patients with AD [19]. However, neurodegeneration in these regions does not totally explain sleep impairment occurring in AD patients. In keeping with this need of better identifying the key regions related to sleep dysregulation in AD, investigations have been carried out in order to better explain and possibly treat sleep impairment in AD. Therefore, the possible pathological changes in the hypothalamic regulation of the circadian rhythm have been recently investigated in AD pathology. In fact, hypothalamus, and specifically the lateral hypothalamus containing the orexinergic system, is considered essential in controlling the sleep–wake cycle, since it projects to several crucial brain nodes of the sleep–wake cycle controlling system [20]. Such areas include: locus coeruleus, dorsal raphe, substantia nigra, ventral tegmental area, hypothalamic tuberomammillary nucleus, melanin-concentrating hormone neurons, and basal forebrain.

On these bases, since the orexinergic system could have a significant impact on sleep in AD neurodegeneration, several reports investigated the CSF orexin levels in AD patients from the preclinical to the advanced stages of the disease. Moreover, literature proposed the interesting mutual relationship among orexinergic system dysregulation, sleep impairment, and CSF AD biomarkers (tau proteins and beta-amyloid).

Uncorrected Proof

2 Orexin and Cognition

The hippocampal formation is principally involved in learning and memory. Alterations in hippocampal structure and function are usually contributors to cognitive dysfunction [21]. Hippocampus receives many inputs from several brain regions; also orexinergic system sends projections to this cognitive-fundamental region. In fact, the orexinergic system has influences on a vast number of homeostatic and physiological behaviors, such as attention, arousal, and cognition [22–24]. Vigilance and daytime activity are necessary components for cognitive performances. It has been also demonstrated that orexin promotes both wakefulness and energy expenditure by interconnecting with the ventrolateral preoptic area, and thus stimulates spontaneous physical and mental activity [25]. Therefore, orexin may play a significant role in hippocampal-dependent cognitive tasks. Accordingly, orexin controls hippocampal neurotransmission through direct as well as transsynaptic modulation of various pathways [26]. In particular, orexin-mediated modulation of GABA and glutamate tone in the hippocampus could be a potential contributor to disruption of the sleep–wake cycle as well as cognitive performances [26]. However, not only deficient synaptic activity, but also aberrant networks activities, which can be caused by orexinergic system upregulation, may cause cognitive deficits, as already demonstrated in AD animal model studies [27]. In fact, orexin has excitatory properties in both animals and humans by interacting with the mesolimbic pathway and amplifying dopamine release [28]. Therefore, the dysregulation of orexinergic signaling may interfere with cognition, in particular causing hippocampal-related cognitive deficits. Nevertheless, these suppositions need to be better addresses also considering that a single animal model study documented that orexin receptor antagonists did not have effects on cognitive processes in rats [29].

3 Orexin and Alzheimer's Disease

The activity of the orexinergic system can be evaluated by measuring the CSF levels of orexin. Orexin-A (Hypocretin-1) is a neuropeptide produced by the lateral hypothalamic neurons, which regulates the sleep–wake cycle by increasing arousal levels and maintaining wakefulness [20]. Several reports have evaluated CSF orexin levels in AD patients using different techniques, such as radioimmunoassay (RIA) [4], fluorescence immunoassay (FIA) [30], enzyme immunoassay (EIA) [6, 7], and mass spectrometry [31].

Orexin levels in the brain are under a complex regulation. In particular, recent animal studies indicate that the orexinergic system is under the influence of light and present diurnal variation and thus a circadian pattern of release and activity [32]. In humans, it has been also demonstrated that CSF orexin levels vary with season, principally correlating with day length and duration of the light period

Uncorrected Proof

[33]. Therefore, the orexinergic system seems to be, like other neurotransmitter systems, subjected to long-term modulation. Moreover, the orexinergic system is affected by the physiological aging, since an overall decrease (averaging 23–25%) in the proportion and density of orexinergic neurons from infancy to older age (0–60 years) has been demonstrated in the human hypothalamus [34]. Finally, the circadian rhythmicity of CSF orexin levels in AD patients and aged controls has been depicted by the well-designed paper from Slats and colleagues examining CSF orexin levels in AD patients and controls at eight individual time points chosen during a 24-h period. Authors demonstrated that in AD pathology the orexinergic system is significantly affected since both the decrease of mean orexin CSF levels and the increase of the orexin circadian rhythm amplitude were observed in the examined AD population compared to the elderly controls. Nevertheless, this study was limited by the lack of polysomnographic recordings, thus not allowing the correlation between orexinergic system dysregulation and the sleep–wake rhythm.

In the last decade, RIA analysis was validated for the quantification of CSF orexin levels in narcoleptic patients [35]. In 2006, for the first time Baumann and coauthors investigated CSF orexin levels in small populations of patients affected by dementia processes documenting normal CSF levels of this biomarker in AD. In 2007, Friedman and colleagues confirmed this finding in a larger group of AD patients. Few years later, Wennstrom and coauthors compared CSF orexin levels among AD patients, Lewy-Body dementia (LBD) patients, and non-demented controls. They detected lower CSF orexin levels in LBD patients compared to both AD patients and controls, whereas CSF orexin level did not differ between AD patients and non-demented controls. However, when dividing AD patients by gender, higher CSF orexin levels were found in females with respect to males. Using FIA analysis, this finding was replicated by Schmidt and colleagues, who analyzed CSF orexin levels in AD patients. Although both groups supposed that female AD patients secrete abnormal orexin levels with a possible higher production rate in respect to males, their discussions did not provide substantial explanations. In fact, the following investigations did not confirm this supposition thus revealing comparable CSF orexin levels between male and female patients [6, 7, 31, 36–38].

Despite no differences found between AD patients and controls [37], a significant increase in CSF orexin concentrations was documented in moderate–severe with respect to mild AD patients [6, 7]. Furthermore, also in mild cognitive impairment (MCI) due to AD patients higher CSF orexin concentrations has been found with respect to controls [38] or patients affected by other dementing processes [36].

The finding that moderate–severe AD patients as well as MCI due to AD patients present increased CSF orexin levels suggests that the orexinergic neurotransmission system may be dysregulated in the early as well as in the advanced stages of the AD neurodegenerative processes. However, this observation could be somewhat paradoxical and has been drawn from few studies. Hence, further evidence that the orexinergic system impairment persists from the onset throughout the progression of AD is needed. Nevertheless, it is possible to speculate that orexin may play a significant role along the entire progression of AD pathology.

Uncorrected Proof

Even though the orexin levels were extensively examined in in vivo CSF samples, only one report interrogated *postmortem* AD brains in order to assess orexinergic neurons and ventricular CSF orexin concentrations. Fronczek et al. documented a 40% decrease of orexin immunoreactive neurons in *postmortem* brain hypothalamic tissues and a modest reduction in orexin-A ventricular CSF levels of AD patients compared to aged controls [39]. Nevertheless, how CSF orexin levels correspond to the number of intact orexinergic neurons in the human brain is difficult to quantify. In rodent models, it was reported that a substantial loss of orexinergic neurons (50–70%) is required before a significant decrease in CSF concentrations of orexin appears [40]. A possible explanation for the finding by Fronczek and colleagues could be achieved from the recent paper by Zhu et al. [41] demonstrating that intermittent short sleep (ISS) produces premature senescence of orexinergic neurons in mice. In fact, chronic ISS, a condition easily observed in AD patients, causes a significant reduction of orexinergic neurons, which also showed an altered morphology. Moreover, ISS induces the reduction of projections from orexinergic neurons, thus possibly causing the increased release of orexin neurotransmitters to ensure the interconnections between orexin and its output terminals. However, this supposition needs to be completely demonstrated.

On this basis, it could be plausible that the moderate reduction of orexinergic neurons (40%) found in AD patients does not significantly modify CSF orexin levels; conversely, the increase in CSF orexin levels found in MCI and moderate–severe AD patients suggests that the dysregulation of the orexin system in AD pathology could be functional and not structural. In fact, it could be hypothesized that the high CSF orexin levels found in patients with AD at the MCI and moderate–severe stages could be the result of increased orexin release, as a compensatory mechanism involving the lateral hypothalamus in the context of the AD neurodegenerative processes [42]. Indeed, the wakefulness-promoting neurons, particularly the basal forebrain cholinergic ones, are principally affected during AD neurodegeneration [43]. This cholinergic neurodegeneration could lead to the upregulation of the other arousal systems, including orexin-producing neurons, not only in the advanced stages but even at early stages, thus contributing to the sleep alteration frequently reported in these patients.

4 Orexin, Alzheimer's Disease, and the Sleep–Wake Cycle

Once established that CSF orexin levels are normal or slightly increased in both MCI and AD patients, researchers investigated the relationship between the activity of the orexinergic system and the sleep–wake cycle in AD neurodegeneration.

Circadian disruption in AD has been well established. In fact, AD patients show reduced amplitude and period length of circadian rhythm, increased intradaily variability, and a decreased interdaily stability of a rhythm [44]. Pathophysiologic mechanisms underlying dysregulation of the circadian rhythmicity in AD have been identified in suprachiasmatic nucleus impairment and loss of pineal gland function

Uncorrected Proof

[45, 46]. However, also orexinergic signaling dysregulation has been invoked in as a possible cause of circadian disruption in AD. Accordingly, a single previous work using actigraphic recordings investigated the sleep–wake cycle and the circadian rest activity of AD patients in relation to CSF orexin concentrations [47]. Since lower CSF orexin concentrations are documented in narcoleptic patients who present diurnal fragmentation with several naps, authors correlated CSF orexin levels with daytime wakefulness in AD patients. Consistently, lower CSF orexin levels were correlated with the higher number and duration of daytime naps in AD patients, thus suggesting that orexin neurotransmission deficiency could be responsible for the daytime napping of AD patients. However, taking into account that CSF levels were in a normal range in all the AD patients evaluated, this supposition remained unconfirmed in the following studies investigating CSF orexin levels exclusively in respect to nighttime sleep.

It is well known that the role of the orexinergic system is not only limited to control the diurnal wake, but also to influence the nocturnal sleep. In fact, orexin seems to primarily reduce REM and slow wave sleep (SWS) and increase wakefulness. Therefore, the orexinergic system shows a wake-on and REM-off pattern of firing, since it physiologically promotes arousal through activation of the wake-active monoaminergic populations and the deactivation of the REM-on cholinergic network [48, 49]. However, the correct orexinergic signaling is considered essential in ensuring the physiological rhythmicity of the entire sleep–wake cycle.

It is well known that Alzheimer's pathology interferes with sleep physiology; in fact, MCI and AD patients suffer from sleep disturbances, such as reduced REM and SWS duration and decreased sleep efficiency, coupled with increased wakefulness after sleep onset (WASO) [14–16, 50]. In detail, increase in fragmented daytime naps, earlier times of sleep onset, and alterations in the timing and frequency of nighttime REM and SWS are usual sleep–wake characteristics of AD patients. However, the most distinct change of sleep architecture in AD neurodegeneration is the reduction of REM sleep, which is featured by longer latency and severe fragmentation [51, 52]. This significant change in REM sleep quality and quantity is already evident in the MCI stage of AD, possibly representing the first sign of sleep impairment in AD pathology [38, 53, 54]. Consistently, sleep disturbances are very common in the AD process and are likely related to the cholinergic depletion. In fact, it has been already reported that the impairment of the cholinergic networks in AD neurodegeneration could be responsible for sleep disruption and SWS/REM sleep alteration [55–59].

However, the relationship between sleep impairment and AD neurodegeneration has not yet been fully elucidated. Therefore, taking into account that AD patients show a dramatic impairment of sleep with frequent arousals coupled with the reduction of REM and SWS, recent reports investigated the relationship between CSF orexin levels and sleep macrostructure in AD patients ranging from the mild to the advanced disease stages [6, 7].

In the last decades, actigraphy emerged as a noninvasive tool for determining sleep patterns; in fact, it can measure sleep in individuals going about their usual activities, thus representing an appropriate method of sleep measurement also for

Uncorrected Proof

the research questions. However, polysomnography (PSG) remains the gold standard for measuring sleep, since it is a well-validated approach to study and define the sleep architecture.

Two reports investigated and correlated the polysomnographic sleep with CSF orexin concentrations in MCI and AD patients [6, 7, 38]. The first investigation correlated the PSGs performed in mild to severe AD patients with CSF orexin concentrations and documented that higher CSF orexin levels correlated with longer sleep latency (SL), higher WASO, and decreased sleep efficiency and SWS. Significantly, the main finding consisted in the correlation between CSF orexin levels and the reduction of REM sleep. In this study, it emerged that, beyond the simple correlation between SL/REM and orexin, the additional multivariate regression analysis revealed the significant mutual interplay between CSF orexin levels and both SL and REM sleep. Therefore, it appeared evident that the orexinergic system overexpression may result in longer SL and REM sleep impairment in AD patients ranging from mild to severe cognitive decline [6, 7]. The second study demonstrated that orexin system dysregulation is already evident in the MCI stage of AD pathology. In fact, it documented that the orexinergic system overexpression is related to REM sleep impairment and sleep fragmentation in MCI due to AD patients [38]. Notably, by dividing the MCI population into two subgroups on the basis of subjective sleep concerns, authors found that MCI patients with subjective sleep complaints presented higher CSF orexin levels compared to MCI patients without sleep disturbances. Moreover, the further analysis between MCI patients complaining of sleep disturbances and controls affected by similar sleep impairment documented that MCI patients showed higher CSF orexin levels than controls. These findings propose the suggestion that in MCI due to AD patients sleep impairment may be related to the orexinergic system dysregulation, which seems to cause insomnia, prolonged SL, and nocturnal awakenings. Surprisingly, in the results section authors reported that the highest CSF orexin concentrations were found in two patients who presented a remarkable impairment of nocturnal sleep, with REM sleep suppression. Hence, the already proposed association between orexinergic system dysregulation and REM sleep impairment was early evident in MCI patients [38].

Although the association between orexinergic system overexpression and sleep impairment in the AD pathology is well documented, the mechanisms linking orexin system dysregulation to REM sleep impairment and sleep fragmentation have not yet been investigated. Up to now, it has been largely supposed that the failure of the cholinergic network may represent the main candidate in provoking the derangement of sleep in AD pathology. However, considering the abovementioned studies, the dysregulation of the orexinergic system may also be a factor that induces sleep alteration in the AD pathology. Therefore, the sleep impairment in MCI/AD patients may be caused by the dysregulation of both the cholinergic and the orexinergic systems. In particular, overexpression of the orexinergic neurotransmission system has been suggested owing to the malfunctioning of the damaged cholinergic network, thus resulting in an unbalance between these two systems [60]. Moreover, in vitro intracellular recordings identified that orexinergic neurons

Uncorrected Proof

present a depolarized resting membrane potential, with spontaneous firing in the absence of stimuli (Lee et al. 2002; [61]). This considering the absent feedback of the cholinergic network on the orexinergic terminals may produce the spontaneous firing of the orexinergic neurons. Moreover, in animal models it has been demonstrated that REM sleep deprivation increases CSF orexin levels [62]. These findings suggest that the raised CSF orexin levels found in MCI/AD patients could be also linked to REM sleep impairment, which is related to the cholinergic system failure. Hence, on the basis of the recent evidence linking AD pathology, REM sleep suppression, and orexinergic system dysregulation, it is conceivable to speculate that the upregulation of the orexinergic system present in the AD neurodegeneration could be likely mediated by the lacking deactivation of the wake-on orexinergic neurons due to the derangement of the cholinergic neurotransmission. In particular, this evidence is drawn from studies investigating polysomnographic nocturnal sleep in AD patients and CSF orexin levels. However, based on the observations previously described by [47], further studies evaluating the circadian activity of AD patients (thus investigating both sleep and wake periods) related to CSF orexin levels changes are needed.

5 Orexin and Alzheimer's Disease Biomarkers: Beta-Amyloid

Beta-amyloid deposition is the main hallmark of AD pathology. It is widely accepted that beta-amyloid dynamics are altered many years before the onset of clinical symptoms [4]. In fact, the proposed amyloid cascade hypothesis suggests that AD neurodegeneration starts with aggregation of non-soluble monomeric beta-amyloid peptides. In keeping with this biomarker view, it has been demonstrated that low CSF β-amyloid$_{42}$ levels represent a very strong predictor of AD pathology since the preclinical stage. On these bases, in order to target the possible pathological processes promoting AD preclinical neurodegenerative processes, researchers focused their work in understanding the possible mechanisms that early alter beta-amyloid dynamics.

In 2009, a seminal scientific report by Kang and coauthors described that cerebral beta-amyloid dynamics are regulated by orexin, which in turn influences the sleep–wake cycle. By using an animal mouse model, authors documented that intracerebroventricular infusion of orexin, inducing wakefulness in mice, produces the significant increase of β-amyloid concentrations in the brain ISF. To confirm these findings, in a second phase, authors infused for 24 h a dual orexin receptor antagonist, thus detecting that ISF beta-amyloid levels reduced significantly with the abolishment of beta-amyloid diurnal fluctuations and the inhibition of beta-amyloid plaque formation. Based on these findings, authors proved that perturbations in orexin signaling not only alter the sleep–wake cycle by promoting wakefulness and reducing REM sleep, but also have acute effects on cerebral

Uncorrected Proof

beta-amyloid dynamics. In fact, the high orexinergic tone increases the diurnal fluctuation of beta-amyloid ISF levels and promotes the cerebral beta-amyloid plaque formation.

The impact of sleep deprivation and prolonged wakefulness has been also tested in humans. In fact, it has been documented that sleep deprivation increases CSF β-amyloid$_{42}$ levels, whereas a night of unrestricted sleep leads to decrease of β-amyloid$_{42}$ levels [12]. This finding confirms a previous study documenting that sleep impairment is associated with the diagnosis of preclinical AD [63]. Reading these papers, it is interesting to note that β-amyloid deposition, as assessed by CSF β-amyloid$_{42}$ levels, is described in patients presenting with worse sleep quality and lower sleep efficiency [63]. On the other hand, Ooms and coauthors described a difference of 75.8 pg/mL of β-amyloid$_{42}$ CSF levels between the unrestricted sleep and sleep deprivation groups. Therefore, it appeared evident that sleep impairment and in particular WASO are the main candidates in altering brain β-amyloid dynamics, thus possibly representing risk factors for preclinical AD. However, animal model studies that were subsequently performed tried to determine whether sleep impairment or orexin-mediated nocturnal wakefulness is related to the dysregulation of: (1) β-amyloid metabolism; (2) the increase of ISF β-amyloid levels, and (3) the induction of β-amyloid cerebral deposition. In fact, modulation of sleep, rather than orexin per se, seems to be important in causing AD neuropathological changes. In keeping with this hypothesis, the paper from Roh et al. [64] documented that stereotaxic injection of orexin into the hippocampus of amyloid precursor protein/presenilin 1 transgenic mice did no change β-amyloid deposition, also nor changing sleep time. Nevertheless, the injection of orexin in the hypothalamus of orexin knockout mice increased the amount of wakefulness as well as increased the amount of β-amyloid deposition. Considering that sleep deprivation induced β-amyloid pathology also in the absence of orexin, Roh et al. [64] concluded that wakefulness and sleep deprivation concurrently affect β-amyloid clearance and deposition more than orexinergic hyperactivation. Therefore, this animal model study totally agrees with clinical evidence of lower CSF β-amyloid$_{42}$ levels in sleep deprived humans. Hence, it appears plausible that the complex interaction between orexin signaling and sleep regulation could alter β-amyloid dynamics. After all, orexin fluctuations are related to the sleep–wake cycle and the diurnal fluctuations of β-amyloid. In agreement with this supposition, Kang and colleagues observed in a small group of healthy volunteers that fluctuations of CSF beta-amyloid levels are present during the day, with reduced levels overnight and increased levels during the wake period with a peak in the evening. Later, Slats et al. replicated this study in six AD patients compared to six elderly controls documenting the circadian rhythm of CSF orexin and β-amyloid levels in AD patients, obtained thanks to a longitudinal CSF collection throughout a 36-h intrathecal catheter. From this experiment, several observations were achieved. Although no differences in CSF orexin levels were observed between AD patients and controls, authors showed that CSF orexin levels were increased during the night and with a higher mean amplitude in AD patients with respect to controls. Significantly, orexin CSF levels changed in relation to β-amyloid levels in both AD patients and controls. Consistently, lower mean CSF

Uncorrected Proof

beta-amyloid concentrations (consistent with a higher cerebral beta-amyloid burden) were related to both lower orexin levels and higher amplitude of orexin circadian rhythm.

Different from the aforementioned study, the previously documented association between CSF orexin and β-amyloid levels described either in an animal model study or in a small sample of AD patients was not evident in the following reports investigating the orexinergic systems in MCI and AD patients [6, 7, 30, 37, 38, 65]. This lack of correlation has been described as the possible effect of the plateau of low (pathological) CSF β-amyloid levels reached by both MCI and AD patients, which cannot allow correlations with other CSF biomarkers such as orexin [6, 7]. Moreover, in MCI and AD patients, CSF β-amyloid fluctuations disappeared since levels in the CSF were lower due to the β-amyloid deposition in amyloid plaques. Therefore, the significant reduction of CSF β-amyloid levels influences possible interplays between this biomarker and other molecules present in the CSF. In keeping with this supposition, in cognitively normal elderly subjects (not showing β-amyloid pathology) it was demonstrated the significant relationship between CSF β-amyloid and orexin levels, although this correlation seemed to be driven by phosphorylated tau CSF levels [66]. Moreover, if considering patients affected by narcolepsy in which CSF orexin levels are dramatically reduced, it was evident the lack of correlation between CSF orexin and β-amyloid levels [7]. Therefore, it is possible to speculate that disease-specific alterations (orexinergic system damage in narcolepsy and β-amyloid pathology in AD) cause the loss of the reciprocal modulation between orexin and β-amyloid$_{42}$ CSF levels. However, it could be very interesting to further investigate the possible in vivo relationship between CSF orexin and β-amyloid levels in larger groups of preclinical AD patients, when it is hypothesized that brain could still be salvageable from AD pathology. In fact, the evaluation of this correlation could be important in preclinical stages of AD in order to better investigate how orexin may modify in vivo β-amyloid dynamics, thus representing a novel therapeutic target.

6 Orexin and Alzheimer's Disease Biomarkers: Tau Proteins

Although the correlation between CSF orexin and beta-amyloid levels appeared controversial in animal model and human studies, in human studies the significant relationship between CSF orexin and tau protein levels has been widely documented either in AD patients or in depressed and cognitively normal healthy subjects [6, 7, 37, 66].

It has been hypothesized that in the AD process the hyperphosphorylation and accumulation of tau proteins appear in a temporal ordering after the accumulation of beta-amyloid plaques [67, 68]. Consistently, tau proteins mark the neuronal injury occurring in AD pathology. In fact, increased CSF tau protein levels

Uncorrected Proof

correspond to higher NFT pathology [69]. Moreover, higher tau protein levels in the CSF are considered a marker of rapid cognitive decline, since they have been associated with faster and more pronounced neuronal degeneration, significantly supporting the transition from early to more advanced disease stages [70].

Reports from the recent literature suggested that the dysregulation of the orexinergic system, as expressed by the increased CSF orexin levels found in AD patients, was related to a faster and more marked tau-mediated neurodegeneration. This observation was carried out in studies investigating CSF orexin levels in mild and moderate–severe AD patients [6, 7, 37]. In fact, it has been demonstrated that CSF orexin levels directly correlate with CSF tau levels in AD patients, with particular evidence in moderate–severe AD subjects [6, 7]. Explanations have been only suggested. One of them is that the higher neuronal activity mediated by the increased orexinergic function may be responsible for the higher CSF tau protein levels found in AD patients. This supposition is based on the recent report documenting that neuronal activity could be a regulator of extracellular tau levels [71]. Further alternative explanations are related to the effects of sleep–wake cycle alterations on both orexinergic signaling and tau pathology [66]. In fact, age-related increases in orexin may promote wakefulness and sleep fragmentation, which in turn may promote accumulation of tau proteins. An alternative model of this correlation could be achieved from the results by Davies et al. [72] documenting that application of β-amyloid in cell cultures induced both amyloid plaques formation and tau phosphorylation coupled with the downregulation of orexin receptors thus inducing the possible increase in orexin neurotransmission due to the reduced available receptors. Therefore, AD neuropathology may influence orexinergic function by reducing orexin receptors.

Hence, the mutual relationship between the orexinergic system and tau pathology emerged in human as well as in animal model studies and these findings open new frontiers for better understanding and possibly counterbalancing the tau-mediated neurodegeneration in AD patients by reducing the overexpression of the orexinergic system. In keeping with this supposition, dual orexin receptor antagonists, recently approved for the treatment of insomnia, should be investigated as a potential preventive or therapeutic measure against AD pathology.

7 Conclusion: Orexin and Alzheimer's Disease Pathogenesis

Researches so far conducted highlighted that the overactivation of the orexinergic system is associated with the dysregulation of the sleep–wake cycle in patients suffering from AD neurodegeneration. Moreover, the orexinergic system dysfunction is also related to β-amyloid and tau protein brain dynamics, either in aging or in AD pathology. However, the associations found among the orexinergic system dysfunction, the biomarkers consistent with AD pathology, and the sleep–wake

Uncorrected Proof

cycle alteration suggest a mutual relationship of these three factors. The more accredited model, emerged from the recent studies investigating orexin, sleep, and AD, explains these interplays focusing on the effect of the orexinergic system dysfunction on the sleep–wake cycle impairment, which in turn has detrimental effects on β-amyloid and tau proteins deposition.

Therefore, these findings suggest that orexin may be considered as a novel biomarker of sleep impairment in AD pathology, secondarily influencing both beta-amyloid and tau pathologies.

Evidence proposed by animal model and in vivo human studies enforced this hypothesis and inaugurated new potential preventive/therapeutic strategies. Therefore, considering that sleep disruption has been proposed to exacerbate neurodegeneration in AD, results from the recent studies investigating the orexinergic system, sleep disruption, and cognitive decline in MCI and AD patients could propose novel therapeutic approaches to improve sleep by reducing the orexinergic tone. Additionally, taking into account that the dysregulation of the orexinergic system seems to influence AD pathology by acting on the sleep–wake rhythm, it could be hypothesized the use of orexin receptor antagonists as potential preventive, therapeutic, or neuroprotective ways targeting the AD neurodegenerative process in order to improve sleep, slow the cognitive impairment, and thereby hamper the pathological processes at the basis of AD pathology. However, these observations require solid confirmations in AD patients, preferably in the preclinical stage of the disease, in order to test if sleep improvement mediated by orexin receptor antagonisms may stop the ongoing chain of events leading to AD neurodegeneration.

Uncorrected Proof

References

1. Alzheimer (1907) Über eine eigenartige Erkan kung der Hirnrinde. Psych Genchtl Med 64:146–148
2. Blessed G, Tomilson B-E, Roth M (1968) The association between quantitative measures of dementia, and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 114:797–811
3. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259
4. Sperling RA, Jack CR Jr, Black SE, Frosch MP, Greenberg SM, Hyman BT, Scheltens P, Carrillo MC, Thies W, Bednar MM, Black RS, Brashear HR, Grundman M, Siemers ER, Feldman HH, Schindler RJ (2011) Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. Alzheimers Dement 7(4):367–385. doi:10.1016/j.jalz.2011.05.2351 (Review)
5. Mander BA, Winer JR, Jagust WJ, Walker MP (2016) Sleep: a novel mechanistic pathway, biomarker, and treatment target in the pathology of Alzheimer's disease? Trends Neurosci 39 (8):552–566
6. Liguori C, Romigi A, Nuccetelli M, Zannino S, Sancesario G, Martorana A, Albanese M, Mercuri NB, Izzi F, Bernardini S, Nitti A, Sancesario GM, Sica F, Marciani MG, Placidi F

(2014) Orexinergic system dysregulation, sleep impairment, and cognitive decline in Alzheimer disease. JAMA Neurol 71(12):1498–1505. doi:10.1001/jamaneurol.2014.2510
7. Liguori C, Placidi F, Albanese M, Nuccetelli M, Izzi F, Marciani MG, Mercuri NB, Bernardini S, Romigi A (2014) CSF beta-amyloid levels are altered in narcolepsy: a link with the inflammatory hypothesis? J Sleep Res 23(4):420–424
8. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. Science 342(6156):373–377. doi:10.1126/science.1241224
9. Peng W, Achariyar TM, Li B, Liao Y, Mestre H, Hitomi E, Regan S, Kasper T, Peng S, Ding F, Benveniste H, Nedergaard M, Deane R (2016) Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease. Neurobiol Dis 93:215–225
10. Cricco M, Simonsick EM, Foley DJ (2001) The impact of insomnia on cognitive functioning in older adults. J Am Geriatr Soc 49(9):1185–1189
11. Nebes RD, Buysse DJ, Halligan EM, Houck PR, Monk TH (2009) Self-reported sleep quality predicts poor cognitive performance in healthy older adults. J Gerontol B Psychol Sci Soc Sci 64(2):180–187. doi:10.1093/geronb/gbn037 (Epub 9 Feb 2009)
12. Ooms S, Overeem S, Besse K, Rikkert MO, Verbeek M, Claassen JA (2014) Effect of 1 night of total sleep deprivation on cerebrospinal fluid β-amyloid 42 in healthy middle-aged men: a randomized clinical trial. JAMA Neurol 71(8):971–977
13. Peter-Derex L, Magnin M, Bastuji H (2015) Heterogeneity of arousals in human sleep: a stereo-electroencephalographic study. Neuroimage 123:229–244. doi:10.1016/j.neuroimage.2015.07.057 (Epub 26 July 2015)
14. McCurry SM, Logsdon RG, Vitiello MV, Teri L (2004) Treatment of sleep and nighttime disturbances in Alzheimer's disease: a behavior management approach. Sleep Med 5(4):373–377
15. Moran M, Lynch CA, Walsh C, Coen R, Coakley D, Lawlor BA (2005) Sleep disturbance in mild to moderate Alzheimer's disease. Sleep Med 6(4):347–352 (Epub 31 Mar 2005)
16. Vitiello MV, Prinz PN (1989) Alzheimer's disease. Sleep and sleep/wake patterns. Clin Geriatr Med 5(2):289–299
17. Osorio RS, Gumb T, Pirraglia E, Varga AW, Lu SE, Lim J, Wohlleber ME, Ducca EL, Koushyk V, Glodzik L, Mosconi L, Ayappa I, Rapoport DM, de Leon MJ (2015) Alzheimer's disease neuroimaging initiative. Sleep-disordered breathing advances cognitive decline in the elderly. Neurology 84(19):1964–1971
18. Troussière AC, Charley CM, Salleron J, Richard F, Delbeuck X, Derambure P, Pasquier F, Bombois S (2014) Treatment of sleep apnoea syndrome decreases cognitive decline in patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry 85(12):1405–1408
19. Kondratova AA, Kondratov RV (2012) The circadian clock and pathology of the ageing brain. Nat Rev Neurosci 13(5):325–335. doi:10.1038/nrn3208 (Review)
20. Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437(7063):1257–1263
21. Rosenzweig ES, Barnes CA (2003) Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. Prog Neurobiol 69(3):143–179
22. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci U S A 95(1):322–327
23. Deadwyler SA, Porrino L, Siegel JM, Hampson RE (2007) Systemic and nasal delivery of orexin-A (Hypocretin-1) reduces the effects of sleep deprivation on cognitive performance in nonhuman primates. J Neurosci 27(52):14239–14247
24. Jaeger LB, Farr SA, Banks WA, Morley JE (2002) Effects of orexin-A on memory processing. Peptides 23(9):1683–1688
25. Mavanji V, Perez-Leighton CE, Kotz CM, Billington CJ, Parthasarathy S, Sinton CM, Teske JA (2015) Promotion of wakefulness and energy expenditure by orexin-A in the ventrolateral preoptic area. Sleep 38(9):1361–1370

Uncorrected Proof

26. Stanley EM, Fadel JR (2011) Aging-related alterations in orexin/hypocretin modulation of septo-hippocampal amino acid neurotransmission. Neuroscience 195:70–79
27. Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A 109(42): E2895–E2903
28. Scheurink AJ, Boersma GJ, Nergårdh R, Södersten P (2010) Neurobiology of hyperactivity and reward: agreeable restlessness in anorexia nervosa. Physiol Behav 100(5):490–495
29. Dietrich H, Jenck F (2010) Intact learning and memory in rats following treatment with the dual orexin receptor antagonist almorexant. Psychopharmacology (Berl) 212(2):145–154
30. Schmidt FM, Kratzsch J, Gertz HJ, Tittmann M, Jahn I, Pietsch UC, Kaisers UX, Thiery J, Hegerl U, Schönknecht P (2013) Cerebrospinal fluid melanin-concentrating hormone (MCH) and hypocretin-1 (HCRT-1, orexin-A) in Alzheimer's disease. PLoS One 8(5):e63136. doi:10.1371/journal.pone.0063136 (Print 2013)
31. Hirtz C, Vialaret J, Gabelle A, Nowak N, Dauvilliers Y, Lehmann S (2016) From radioimmunoassay to mass spectrometry: a new method to quantify orexin-A (hypocretin-1) in cerebrospinal fluid. Sci Rep 6:25162
32. McGregor R, Wu MF, Barber G, Ramanathan L, Siegel JM (2011) Highly specific role of hypocretin (orexin) neurons: differential activation as a function of diurnal phase, operant reinforcement versus operant avoidance and light level. J Neurosci 31(43):15455–15467
33. Boddum K, Hansen MH, Jennum PJ, Kornum BR (2016) Cerebrospinal fluid hypocretin-1 (orexin-A) level fluctuates with season and correlates with day length. PLoS One 11(3): e0151288
34. Hunt NJ, Rodriguez ML, Waters KA, Machaalani R (2015) Changes in orexin (hypocretin) neuronal expression with normal aging in the human hypothalamus. Neurobiol Aging 36 (1):292–300
35. Ripley B, Overeem S, Fujiki N, Nevsimalova S, Uchino M, Yesavage J, Di Monte D, Dohi K, Melberg A, Lammers GJ, Nishida Y, Roelandse FW, Hungs M, Mignot E, Nishino S (2001) CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 57 (12):2253–2258
36. Dauvilliers YA, Lehmann S, Jaussent I, Gabelle A (2014) Hypocretin and brain β-amyloid peptide interactions in cognitive disorders and narcolepsy. Front Aging Neurosci 6:119. doi:10.3389/fnagi.2014.00119 (eCollection 2014)
37. Deuschle M, Schilling C, Leweke FM, Enning F, Pollmächer T, Esselmann H, Wiltfang J, Frölich L, Heuser I (2014) Hypocretin in cerebrospinal fluid is positively correlated with Tau and pTau. Neurosci Lett 561:41–45. doi:10.1016/j.neulet.2013.12.036 (Epub 25 Dec 2013)
38. Liguori C, Nuccetelli M, Izzi F, Sancesario G, Romigi A, Martorana A, Amoros C, Bernardini S, Marciani MG, Mercuri NB, Placidi F (2016) Rapid eye movement sleep disruption and sleep fragmentation are associated with increased orexin-A cerebrospinal-fluid levels in mild cognitive impairment due to Alzheimer's disease. Neurol Aging 40:120–126
39. Fronczek R, van Geest S, Frölich M, Overeem S, Roelandse FW, Lammers GJ, Swaab DF (2012) Hypocretin (orexin) loss in Alzheimer's disease. Neurobiol Aging 33(8):1642–1650. doi:10.1016/j.neurobiolaging.2011.03.014 (Epub 5 May 2011)
40. Gerashchenko D, Murillo-Rodriguez E, Lin L, Xu M, Hallett L, Nishino S, Mignot E, Shiromani PJ (2003) Relationship between CSF hypocretin levels and hypocretin neuronal loss. Exp Neurol 184(2):1010–1016
41. Zhu Y, Fenik P, Zhan G, Somach R, Veasey RX (2016) Intermittent short sleep results in lasting sleep wake disturbances and degeneration of locus coeruleus and orexinergic neurons. Sleep 39(8):1601–1611. pii: sp-00094-16 (Epub ahead of print) AU90
42. Jones BE (2004) Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. Prog Brain Res 145:157–169
43. Francis PT, Palmer AM, Snape M, Wilcock GK (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry 66(2):137–147

Uncorrected Proof

44. Videnovic A, Zee PC (2015) Consequences of circadian disruption on neurologic health. Sleep Med Clin 10(4):469–480
45. Stopa EG, Volicer L, Kuo-Leblanc V, Harper D, Lathi D, Tate B, Satlin A (1999) Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J Neuropathol Exp Neurol 58(1):29–39
46. Wu YH, Feenstra MG, Zhou JN, Liu RY, Toranõ JS, Van Kan HJ, Fischer DF, Ravid R, Swaab DF (2003) Molecular changes underlying reduced pineal melatonin levels in Alzheimer disease: alterations in preclinical and clinical stages. J Clin Endocrinol Metab 88(12):5898–5906
47. Friedman LF, Zeitzer JM, Lin L, Hoff D, Mignot E, Peskind ER, Yesavage JA (2007) Alzheimer disease, increased wake fragmentation found in those with lower hypocretin-1. Neurology 68 (10):793–794 (No abstract available)
48. Arrigoni E, Mochizuki T, Scammell TE (2010) Activation of the basal forebrain by the orexin/hypocretin neurones. Acta Physiol (Oxf) 198(3):223–235
49. Lee MG, Hassani OK, Jones BE (2005) Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 25(28):6716–6720
50. Beaulieu-Bonneau S, Hudon C (2009) Sleep disturbances in older adults with mild cognitive impairment. Int Psychogeriatr 21(4):654–666
51. Bonanni E, Maestri M, Tognoni G et al (2005) Daytime sleepiness in mild and moderate Alzheimer's disease and its relationship with cognitive impairment. J Sleep Res 14(3):311–317
52. Montplaisir J, Petit D, Lorrain D et al (1995) Sleep in Alzheimer's disease: further considerations on the role of brainstem and forebrain cholinergic populations in sleep-wake mechanisms. Sleep 18(3):145–148
53. Maestri M, Carnicelli L, Tognoni G, Di Coscio E, Giorgi FS, Volpi L, Economou NT, Ktonas P, Ferri R, Bonuccelli U, Bonanni E (2015) Non-rapid eye movement sleep instability in mild cognitive impairment: a pilot study. Sleep Med 16(9):1139–1145
54. Naismith SL, Hickie IB, Terpening Z, Rajaratnam SM, Hodges JR, Bolitho S, Rogers NL, Lewis SJ (2014) Circadian misalignment and sleep disruption in mild cognitive impairment. J Alzheimers Dis 38(4):857–866
55. Grothe M, Zaborszky L, Atienza M et al (2010) Reduction of basal forebrain cholinergic system parallels cognitive impairment in patients at high risk of developing Alzheimer's disease. Cereb Cortex 20(7):1685–1695
56. Kundermann B, Thum A, Rocamora R, Haag A, Krieg JC, Hemmeter U (2011) Comparison of polysomnographic variables and their relationship to cognitive impairment in patients with Alzheimer's disease and frontotemporal dementia. J Psychiatr Res 45(12):1585–1592
57. Mallick BN, Joseph MM (1997) Role of cholinergic inputs to the medial preoptic area in regulation of sleep-wakefulness and body temperature in freely moving rats. Brain Res 750:311–317
58. Perry E, Walker M, Grace J, Perry R (1999) Acetylcholine in mind: a neurotransmitter correlate of consciousness? Trends Neurosci 22:273–280
59. Power AE (2004) Slow-wave sleep, acetylcholine, and memory consolidation. Proc Natl Acad Sci U S A 101:1795–1796
60. Eggermann E, Bayer L, Serafin M et al (2003) The wake-promoting hypocretin-orexin neurons are in an intrinsic state of membrane depolarization. J Neurosci 23(5):1557–1562
61. Pedrazzoli M, D'Almeida V, Martins PJ et al (2004) Increased hypocretin-1 levels in cerebrospinal fluid after REM sleep deprivation. Brain Res 995(1):1–6
62. Roh JH, Huang Y, Bero AW et al (2012) Disruption of the sleep-wake cycle and diurnal fluctuation of β-amyloid in mice with Alzheimer's disease pathology. Sci Transl Med 4 (150):150ra122
63. Ju YE, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, Duntley SP, Morris JC, Holtzman DM (2013) Sleep quality and preclinical Alzheimer disease. JAMA Neurol 70(5):587–593
64. Roh JH, Jiang H, Finn MB, Stewart FR, Mahan TE, Cirrito JR, Heda A, Snider BJ, Li M, Yanagisawa M, de Lecea L, Holtzman DM (2014) Potential role of orexin and sleep

Uncorrected Proof

modulation in the pathogenesis of Alzheimer's disease. J Exp Med 211(13):2487–2496. doi:10.1084/jem.20141788
65. Wennström M, Londos E, Minthon L, Nielsen HM (2012) Altered CSF orexin and α-synuclein levels in dementia patients. J Alzheimers Dis 29(1):125–132. doi:10.3233/JAD-2012-111655
66. Osorio RS, Ducca EL, Wohlleber ME, Tanzi EB, Gumb T, Twumasi A, Tweardy S, Lewis C, Fischer E, Koushyk V, Cuartero-Toledo M, Sheikh MO, Pirraglia E, Zetterberg H, Blennow K, Lu SE, Mosconi L, Glodzik L, Schuetz S, Varga AW, Ayappa I, Rapoport DM, de Leon MJ (2016) Orexin-A is associated with increases in cerebrospinal fluid phosphorylated-tau in cognitively normal elderly subjects. Sleep 39(6):1253–1260
67. Hardy J (2009) The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem 110:1129–1134
68. Jack CR Jr, Knopman DS, Jagust WJ et al (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9:119–128
69. Blennow K, Hampel H (2003) CSF markers for incipient Alzheimer's disease. Lancet Neurol 2 (10):605–613
70. Kester MI, van der Vlies AE, Blankenstein MA et al (2009) CSF biomarkers predict rate of cognitive decline in Alzheimer disease. Neurology 73(17):1353–1358
71. Yamada K, Holth JK, Liao F et al (2014) Neuronal activity regulates extracellular tau in vivo. J Exp Med 211(3):387–393. doi:10.1084/jem.20131685 (Epub 17 Feb 2014)
72. Davies J, Chen J, Pink R, Carter D, Saunders N, Sotiriadis G, Bai B, Pan Y, Howlett D, Payne A, Randeva H, Karteris E (2015) Orexin receptors exert a neuroprotective effect in Alzheimer's disease (AD) via heterodimerization with GPR103. Sci Rep 5:12584
73. Baumann CR, Hersberger M, Bassetti CL (2006) Hypocretin-1 (orexin A) levels are normal in Huntington's disease. J Neurol 253(9):1232–1233 (Epub 5 Apr 2006, No abstract available)
74. 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(5955):1005–1007. doi:10.1126/science.1180962 (Epub 24 Sep 2009)
75. Slats D, Claassen JA, Lammers GJ, Melis RJ, Verbeek MM, Overeem S (2012) Association between hypocretin-1 and amyloid-β42 cerebrospinal fluid levels in Alzheimer's disease and healthy controls. Curr Alzheimer Res 9(10):1119–1125

Uncorrected Proof

Author Queries

Chapter No.: 50

Query Refs.	Details Required	Author's response
AU1	The references should be sequentially cited in the text, hence the references have been renumbered both in the text and in the reference list. Please check, and correct if necessary.	
AU2	Please note that the reviewer comments given in the manuscript of this chapter have been retained. Kindly check them and amend if required.	
AU3	Needs a reference: suggest Villemagne VL et al Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. Lancet Neurol. 2013 Apr;12 (4):357-67	
AU4	Please check the sentence "In particular, biomarkers, expression of …" for clarity.	
AU5	This section starts to bring in items that are repeated in later sections. i.e this para is about sleep disruption in AD, while there is a section entitled "Orexin Alzheimer's Disease and the Sleep-Wake Cycle" on page 9. Suggest structuring so as to minimise repetition.	
AU6	Biomarkers have very specific criteria; being part of the pathological cascade is not neccesarily one of them.	
AU7	Please check if the edit made to the sentence "In agreement with this supposition, …" conveys the intended meaning and amend if required.	
AU8	This whole para describing Xie's findings could be much more tightly written.	
AU9	Repetition vs 1 page previously	
AU10	Repetition vs 1 page ago	

Uncorrected Proof

AU11	The citation "[18] [Troussiere et al. 2014 (originally)]" has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.	
AU12	Again, need to either do the section on sleep and AD here or on page 9; not both. IF here, this section needs a more full description of the sleep disruptions in AD (i.e. circadian rythym, NREM, Rem etc)	
AU13	Please check if the sentence "Accordingly, degeneration of suprachiasmatic nucleus, . . ." conveys the intended meaning and amend if required.	
AU14	English (2 x "better", has said in preceding sentence that regions does not fully explain impariments, then wants tpo go looking for more regions)	
AU15	This is not established in the review yet	
AU16	I probably would have put this section later, after putting together the orexin AD CSF/biomarker section, to suggest that indeed, dysregulated orexin may potentially have a direct influence on cognitive processes in AD as well.	
AU17	Not necesarily (only) hippo dependent. This conclusion is not well established by the preceding sentences. This comes in the next few sentences...!	
AU18	Reference?	
AU19	Repetition	
AU20	Please check the sentence "Nevertheless, these suppositions need to be better addresses. . ." for clarity.	
AU21	Not ture – they showed almorexant did not impair memory in the MWM or the passive aoidance task, but also, that almorexant appeared to enhance performance in the MWM, since they learned the task more quickly than the vehicle-treated rats.	
AU22	Repetition	

Uncorrected Proof

AU23	The citation "[4] [Sperlin et al. 2011 (originally)]" has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.	
AU24	Might want to add what the primary collection method was (i.e intrathecal port?)	
AU25	Delete, replace with "patients" or the like	
AU26	of the orexin system	
AU27	But also extended the finding to Associate lower orexin CSF with increased daytime napping in the AD group (as Ligouri describes later in the chapter)	
AU28	Please check the sentence "Furthermore, also in mild cognitive impairment . . ." for clarity.	
AU29	"MCI due to AD" is a bit of an unusual terminnology (used throughout the chapter) – it implies the MCI patients were later confimed as AD.. was this really thecase or are they just normally diagnosed MCI (i.e. by cognitive testing) ???	
AU30	Why do some studies show no influences on AD on orexin levels in the CSF and Ligouri's studies do?	
AU31	Not much difference between 40% reported above and 50% here...	
AU32	commonly	
AU33	Reference?	
AU34	...demonstrated experimentally....?	
AU35	This is a thin line of argumentation	
AU36	...may suggest	
AU37	Mander et al., 2016 thinks this too, but as far as I know there is not really experimental evidence to support it. . .yet.	
AU38	See earlier comment about conglomerating the earlier sentences about sleeop in AD in this section or visa versa	
AU39	What sort of impairment?	

Uncorrected Proof

AU40	Please check if the edit made to the sentence “It is well known that the role of the . . .” is fine and amend if required.	
AU41	Chicken or egg? Correlation does not equal causality	
AU42	Really? What about sun-downer behaviour in advanced AD?	
AU43	Repetition	
AU44	Ore orexin?	
AU45	Please check the sentence “In the last decades, actigraphy emerged as . . .” for clarity.	
AU46	Actigraphy cannot measure sleep architeture at all	
AU47	When? In the day time? Night time? Overall?	
AU48	Please check if the sentence “In this study, it emerged that, beyond . . .” for clarity.	
AU49	predict?	
AU50	Again – when was CSF orexin measured, or is this an average?	
AU51	support	
AU52	hypothesis	
AU53	may	
AU54	Really?	
AU55	What – normally, or in an AD cell model or ...?	
AU56	Ref. “Lee et al. 2002” is cited in the text but not provided in the reference list. Please provide it in the reference list or delete the citation from the text.	
AU57	Wrong reference – this one shows increased ox in CSF after REM sleep dep	
AU58	Please check the sentence “This considering the absent feedback of the . . .” for clarity.	
AU59	Wrong reference – Roh didn’t do sleep dep/ox/REM – probably mixed up with Pedrazzoli above	
AU60	Support (not really evidence, is it?)	

Uncorrected Proof

AU61	The citation "[47] [Friednam et al. 2007 (originally)]" has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.	
AU62	No – Abeta pathology also occurs in non-AD brain. Tau and Abeta pathology (aggregation) are the two hallmarks of AD brain	
AU63	Please check if the edit made to the sentence "It is widely accepted that . . ." is fine and amend if required.	
AU64	Reference would be nice (maybe Blennow et al., Mov Disord. 2016 Jun;31(6):836-47; Cerebrospinal fluid biomarkers in Alzheimer's and Parkinson's diseases-From pathophysiology to clinical practice.), and really Abeta 42:40 is thought by some to be a stronger biomarker than 42 alone. This plus CSF tau & pTau are the real triad of AD CSF biomarkers	
AU65	Need to be careful with causality here; one could say from the work that Abeta dynamics are regulated by sleep/wake, which is also regulated by orexin (which is a theme followed in the subsequent papers by Roh also from the Holtzman lab). Main issue is one cannot use orexin agonists or antagonists without altering sleep/wake!From the Kang paper itself:"-Perturbations in both orexin signalingand the sleep-wake cycle had acute effects upon Ab dynamics. Furthermore, chronic sleep restriction accelerates Ab plaque burden, whereas enhancing sleep via orexin receptor blockade markedly inhibits Ab plaque accumulation."	
AU66	Please check if the sentence "By using an animal mouse model, . . ." conveys the intended meaning and amend if required.	
AU67	Plaque formation was not assessed with the 24 hr infusion, but with 8 weeks of chronic dosing for ALM in an amyloidogenesis mouse model (APP/PS1 transgenic)	
AU68	Yes – OxA injection increased ISF Abeta	

Uncorrected Proof

AU69	Actually Kang did not do this expt – they did sleep deprive APP/PS1 Tgs for 20 hr a day for 21 days (wow!), which increased plaques, but they did not measure orexin in the CSF or brains of these mice.	
AU70	Please check the sentence "Reading these papers, . . ." for clarity.	
AU71	Please check the latter part of the sentence "In keeping with this hypothesis, the paper from Roh . . ." for clarity.	
AU72	No – it was a lentivirus for orexin overexpression injected into thie hippocampus, and it did not influence sleep or Abeta depositionin the brain. Then they injected the orexin lentivirus vector into the hypothal into APP-PS1/orexin KO mice, which restored Ox expression there and reduced sleep – this increased abeta depostion in the hippoc & ctx. Hence the inseparability of sleep and orexin.	
AU73	No – see above	
AU74	OXKOs crossed with APP-PS1 Tgs	
AU75	Please check the latter part of the sentence "Later, Slats et al. replicated this study in . . ." for clarity.	
AU76	40 or 42? I assume it must be 42, but it should be stated clearly	
AU77	As someone who has worked on BACE inhibition (where Abeta 40 is used as a pharmacodynamic biomarker), I'd really like to see 42 specified where ever CSF amyloid is refered to (that is what he means, since 42 goes down as AD progresses, presumably due to deposition, whereas 40 is not greately affected by diseaes state; but the isoform in question should be clearly stated each time)	
AU78	observation?	
AU79	Please check the sentence "In keeping with this supposition. . ." for clarity.	
AU80	eh??? First mention of tau	
AU81	Please check the sentence "Moreover, if considering patients . . ." for clarity.	

Uncorrected Proof

AU82	Weird – this paper says CSFAbeta42 is significantly LOWER in narcolepsy patients	
AU83	This does not make sense with regard to the preceding sentence	
AU84	Please check if the edit made to the sentence "This observation was carried out ..." is fine and amend if required.	
AU85	State that this info is from animal models	
AU86	Reference	
AU87	What is the eveidence for overexpression? (of orexin, or it's receptors? Does he mean overactivationby more orexin peptide? If so, he did not build a very substantive case for this.	
AU88	Please check the sentence "Additionally, taking into account that ..." for clarity.	
AU89	References "73–75" were not cited anywhere in the text. Please provide a citation. Alternatively, delete the items from the list.	
AU90	Please check the edits made to the Ref. [41] and amend if required.	

Uncorrected Proof