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The Nexus of A β , Aging, and Sleep

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Abstract

Roh *et al.* report a positive feedback loop between sleep-wake irregularities and aggregation of β -amyloid (A β) peptide, suggesting that sleep alterations could be an early event in Alzheimer's disease (AD).

Alzheimer's disease (AD), the most common cause of dementia, is characterized by aggregation of β -amyloid (A β) peptide in the brain. The most prevalent genetic risk factor for the common sporadic form of AD is a variant of apolipoprotein E (apoE). In the brain, apoE is made by glial cells, most notably astrocytes, and aids in the degradation and clearance of A β (1). The rare early-onset autosomal dominant familial form of AD (FAD) is caused by mutations in amyloid precursor protein (APP) or presenilin proteins (PS1, PS2) that result in the production of toxic forms of A β . Although very rare, FAD has provided scientists with useful mouse models of AD (e.g., the APP^{swe}/PS1 transgenic mouse) to elucidate pathogenesis of this disease and to test potential therapies. Although sporadic and familial forms of AD show similar pathology, namely aggregates of A β in the brain called amyloid plaques, we still do not understand the relationship between changes in A β concentrations, A β aggregation, sleep-wake behavior, and metabolism. A new study by Roh *et al.* (2) in this issue now tries to connect these different factors. The authors describe an association between diurnal cycling of A β and A β aggregation in transgenic FAD mouse models and in humans with a PS1 mutation, who will ultimately develop AD but are currently asymptomatic. They also examine changes in the diurnal cycling of lactate associated with the sleep-wake cycle and A β aggregation, providing evidence for metabolic coupling of neuronal activity and A β metabolism. Given that A β aggregation is associated with poor sleep-wake patterns and occurs years prior to clinical manifestations of AD, alterations in sleep may be an early event in AD (Fig. 1A).

These new observations build on the earlier studies from the same group (3). Kang and colleagues showed, using microdialysis, that brain interstitial fluid (ISF) A β concentrations cycle in young AD mice (APP^{swe}/PS1) and wild-type mice, with an increase in ISF A β concentrations during the hours of wakefulness and a decrease during sleep. Diurnal changes in total APP, APP fragments, or A β were not observed during the light-dark cycle in mice; only ISF A β concentrations oscillated in response to wakefulness and sleep (3). This

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suggested that the pool of extracellular ISF A β is somehow differentially regulated from total intracellular and membrane-bound A β . A similar oscillation in A β was observed in the cerebrospinal fluid (CSF) of young, healthy humans, with peak concentrations occurring in the evening and lower concentrations overnight (3). Although ISF exists around the primary brain parenchyma, and CSF in the subarachnoid space, this suggests that similar mechanisms regulate diurnal cycling of A β concentrations in the extracellular space of the mammalian central nervous system (CNS). Given the correlation of A β concentrations with wakefulness in mice and humans, Kang *et al.* investigated changes in A β concentrations during altered sleep homeostasis. Six hours of forced wakefulness during a normal sleeping period increased ISF A β concentrations in the APPswe/PS1 AD mice, but an immediate reduction occurred upon sleep recovery. In addition, chronic sleep restriction (4 hours of sleep daily for 21 days) resulted in greater A β deposition in the brains of APPswe/PS1 mice compared to non-restricted control mice (3). When a 6-hour intracerebroventricular (ICV) infusion of the wake-promoting peptide orexin-A was given during the light period, mice showed a concomitant increase in both wakefulness and ISF A β concentrations. Conversely, ICV infusion of Almorexant, an orexin receptor 1 and 2 antagonist, lowered ISF A β concentrations and reduced the amount of time spent awake (3). Kang *et al.* concluded that wakefulness regulates ISF A β concentrations and that endogenous orexin signaling is necessary for diurnal oscillations in ISF A β .

Although these results show that behavioral state regulates A β , the effect of age and amyloid plaque burden on this process is unknown. Now, in their follow-up study, Roh *et al.* (2) examine the relationship between diurnal A β oscillations, sleep-wake behavior, and A β aggregation in wild-type and AD mice of different ages. They investigated two brain regions: the hippocampus, which is more susceptible to A β plaque formation, and the striatum, a region less susceptible to A β plaque formation. Prior to A β aggregation, young APPswe/PS1 AD mice have normal diurnal ISF A β oscillations and sleep-wake cycles (2, 3). However, in aged APPswe/PS1 mice in which A β had aggregated into amyloid plaques, the diurnal ISF A β oscillations stopped and mice show increased wakefulness (2) (Fig. 1B). Both rapid eye movement (REM) and non-REM sleep were decreased, particularly during the “lights on” inactive period (mice are nocturnal). Interestingly, the authors observed that local ISF A β oscillated depending on the presence of plaques in the different brain regions. Hippocampal A β showed diurnal oscillations in mice aged 3 months, but not those aged 6 or 9 months, coinciding with plaque formation in the brain confirmed with immunohistochemistry. However, in the striatum, ISF A β oscillated in mice aged 3 and 6 months but stopped oscillating in 9-month-old mice. Disruption in the diurnal ISF A β oscillation correlates with a progressive increase in wakefulness during the normal sleep phase, an effect that follows A β aggregation. This suggests that the diurnal ISF oscillation is associated with the sleep-wake cycle and that both of these events are disrupted in aged mice with A β plaque formation.

To test the clinical relevance of this finding, Roh *et al.* examined A β concentrations in the CSF of humans with mutations in PS1 or control subjects without the mutation. Positron Emission Tomography imaging of patients with mutant PS1 only showed a loss of CSF A β oscillation when positive for the imaging agent Pittsburgh Compound B (PIB), a marker of A β aggregation. Subjects without the PS1 mutation or those who were PIB-negative PS1-

mutant carriers still showed oscillation of A β (2). These data support the murine findings and suggest that the diurnal oscillation of A β negatively correlates with A β aggregation. Therefore, the authors concluded that the oscillation in ISF/CSF A β continues until A β aggregation and is associated with progressive sleep loss (Fig. 1B). More work is needed to confirm if the diurnal CSF A β association with plaque formation correlates with increased wakefulness in humans.

Roh *et al.* next asked whether removal of A β would rescue the diurnal ISF A β oscillation and reduce A β aggregation in older animals. They immunized mice with A β starting at 1.5 months old and observed restoration of diurnal ISF A β oscillation and prevention of A β plaque formation in the hippocampus of 9-month-old vaccinated APPswe/PS1 AD mice. Roh *et al.* used lactate to study the relationship between neuronal activity and ISF A β cycling in vaccinated animals. They observed diurnal changes in lactate concentrations, which correlated with an increase in wakefulness in immunized APPswe/PS1 mice, confirming recent studies with similar observations in normal mice (4). Given that the correlation between ISF A β and ISF lactate is lost at 9 months when amyloid plaques have formed (Fig. 1C), the authors tested this relationship in immunized APPswe/PS1 mice. Indeed, they observed a restoration of lactate-metabolic coupling with ISF A β concentrations in the hippocampus of 9-month-old APPswe/PS1 mice that had been vaccinated.

The authors attribute the loss of A β oscillation in the ISF/CSF to incorporation of soluble A β into insoluble aggregates (2). In light of these new findings describing a relationship between A β concentrations, lactate metabolism, sleep-wake rhythms, and A β aggregation, it will be important to identify the mechanisms that are responsible for the time-of-day clearance of A β . Binding of apoE to A β is thought to be one mechanism for clearing A β and preventing its aggregation in the brain (1). Recently, apoE-directed pharmacological strategies were reported to clear ISF A β , reduce A β aggregation, and restore memory and cognitive function in APPswe/PS1 mice (5). This study used agonists directed at transcriptional regulators of apoE, including peroxisome proliferator-activated receptor gamma (PPAR γ) and retinoid X receptors (RXRs) (5). When associated with lipids, apoE binds to soluble A β , thereby influencing its transport, uptake, metabolism, and plaque formation. A drug that enhances apoE expression via PPAR γ activation cleared A β , suppressed relocation of astrocytes around amyloid plaques, and rescued cognitive deficits in AD mice (6). This supports the role of astrocytes and other types of glial cells in A β clearance by apoE (Fig. 1C). In addition, it supports the hypothesis that localized glial cell responses around A β aggregation inhibit normal glial cell function, namely clearance of soluble A β and metabolic support of proximate neurons. The astrocyte-neuron-lactate shuttle hypothesis posits that astrocytes take up glutamate released by neurons and, in turn, supply lactate as fuel (Fig. 1C). Localized glial cell responses around A β aggregates could inhibit normal glial cell function, including clearance of soluble A β and metabolic support of proximate neurons. These effects would have implications for the neurometabolic coupling of diurnal ISF A β and the sleep-wake cycle. Improperly cleared A β due to glial cell dysfunction would contribute to further plaque formation, and increased newly aggregated A β would attract more astroglia, instituting a positive feedback loop. Glutamatergic synapses would show increased excitotoxicity and hyperexcitability due to

impaired glutamate clearance by the relocated astroglia (Fig. 1D). This increased excitatory neurotransmission could explain increased wakefulness and the progressive sleep loss observed with augmented plaque formation (Fig. 1D). Whether or not drugs that restore uptake of A β by glia would also affect the sleep-wake cycle remains to be tested.

These concepts lead to the question: What are the mechanisms that contribute to diurnal changes in glial cell regulation of A β clearance coupled to sleep-wake activity? Astrocyte metabolism is thought to be closely tied to the sleep-wake cycle and is affected by A β concentrations (7). A β increases glucose uptake by astrocytes, lactate release, and hydrogen peroxide production and impairs neuronal viability in coculture experiments (8). Recently, it was shown that extracellular lactate and glutamate concentrations increase upon wakefulness in the brain, whereas glucose acutely decreases (4). This association is reversible upon changes in behavioral state from wake to sleep. The relationship of neurometabolic coupling of glutamatergic excitatory neurotransmission with extracellular release of lactate and intracellular incorporation of glucose by astrocytes supports the astrocyte-neuron-lactate shuttle model proposed 18 years ago [for full review, see (9)]. The extracellular increases in lactate concentrations associated with enhanced wakefulness have been attributed to the increase in energy demand by neurons needed to facilitate glutamatergic excitatory neurotransmission. Roh *et al.* (2) also observed increases in lactate associated with wakefulness. However, the association of diurnal lactate concentrations with oscillating ISF A β is disrupted upon A β aggregation, suggesting that neuronal energy demand associated with sleep-wake states and normal A β metabolism may become decoupled as A β aggregation increases.

Recently, it was shown that glial cells respond to A β deposition by clustering around A β plaques (6). Retraction of glial cells and their processes away from neurons due to their attraction toward amyloid plaques could contribute to decoupling of the astrocyte-neuron-lactate shuttle, causing the abandonment of glutamate and A β -uptake mechanisms normally facilitated by astrocytes (Fig. 1, C and D). This model provides a potential mechanism underlying the positive feedback loop relating sleep-wake irregularities and A β aggregation with neuro-glial metabolic coupling but as yet is still hypothetical and needs to be robustly tested.

Much effort has gone into slowing the progression of AD after A β plaques have already formed and cognition is impaired. Unfortunately, at this point, it is likely too late to reverse or slow cognitive decline in these patients. In fact, bapineuzumab, which helps patients build immunity to A β to facilitate plaque clearance, recently failed to show efficacy in phase III clinical trials in patients with mild-to-moderate AD (10). We would predict, in accordance with the Roh *et al.* findings, that neuro-glial coupling in these patients has already been, and continues to be, disrupted. Therefore, removing the cause of the uncoupling does not ameliorate the effects of the uncoupling. Further, as astrocytes may have already migrated away from their original microdomain, neuronal function and viability would continue to be negatively affected. There has been very little progress in identifying early, testable markers of AD. The results of the Roh *et al.* study suggest that monitoring sleep-wake disturbances and metabolomics could provide a noninvasive indicator of early AD. Of course, much more work in rodents and humans must be conducted to validate this hypothesis. Nonetheless, the

Roh *et al.* study adds support to an emerging area of investigation in neurodegeneration—the role of sleep-wake disturbances in altering the progression of these disorders.

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Fig. 1. A β and the sleep-wake cycle.

(A) Sleep amount and quality decline with age. This effect is even more pronounced in AD patients. A β aggregation increases during AD and is associated with a decline in sleep. (B) In the absence of A β aggregation, ISF A β in mice and CSF A β in humans show diurnal oscillations associated with sleep (S) and awake (W) states (2). Following A β aggregation, the diurnal oscillation of A β is lost in mice and the animals spend more time awake ($\uparrow$ W). Whether the loss of diurnal CSF A β associated with A β aggregation contributes to increased wakefulness in humans with early AD remains to be determined. (C) ApoE derived from astrocytes and other glial cells is believed to facilitate A β clearance in the extracellular space of the brain (1). In the absence of A β aggregation, there is coupling of lactate with diurnal oscillations in ISF A β , and a normal sleep-wake rhythm. The astrocyte-neuron-lactate shuttle hypothesis (9) posits metabolic coupling of the sleep-wake cycle to astrocyte function (4). (D) In this model, lactate metabolic coupling with diurnal oscillation of A β

would be lost after A β aggregation, and wakefulness would increase. One way that coupling could be lost is that astrocytes responsible for clearing A β and also glutamate become mobilized by A β plaques (6), moving toward the plaques and away from neurons. This could initiate a positive feedback loop in which astrocytes would no longer metabolize A β or glutamate from nearby neurons, leading to further aggregation of A β and increased excitotoxicity and neuronal injury due to glutamate accumulation.

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