Don't Let Amyloid Keep You Awake at Night

The accumulation in the brain of the neurotoxic β-amyloid (Aβ) peptide is a key event in the pathogenesis of Alzheimer’s disease (AD). Aβ accumulation in amyloid plaques begins about 10 to 15 years before cognitive decline and is already very substantial by the time memory and thinking problems begin. It is critical to determine whether there are functional and biochemical changes in the brain that are present when Aβ is accumulating but before the appearance of dementia to initiate therapy earlier as well as to assess the therapeutic effects of new drugs. Previous work has shown that soluble forms of Aβ fluctuate in the brain with the sleep-wake cycle. Now, Roh and colleagues show that diurnal fluctuation of Aβ occurs in different brain regions in young adult mice that develop accumulation of Aβ. However, Aβ fluctuation disappeared with the onset of amyloid plaque deposition, most likely due to insoluble Aβ plaques sequestering soluble forms of Aβ. Similar findings were seen in the cerebrospinal fluid of humans with genetic mutations that cause early-onset, autosomal dominant AD. Coincident with increasing Aβ accumulation, the researchers found that the amount of time mice were awake when they were supposed to be asleep increased by 50%. Actively immunizing mice with Aβ prevented amyloid plaque formation, as well as maintaining normal circadian Aβ fluctuation and normal sleep patterns. These findings suggest that changes in the sleep-wake cycle may be caused by Aβ accumulation. If analogous abnormalities in the sleep-wake cycle are present in cognitively normal and very mildly impaired humans who are developing AD pathology, the sleep-wake cycle may be a useful indicator of early brain dysfunction that could be assessed as an outcome measure in response to therapeutic interventions.
Disruption of the Sleep-Wake Cycle and Diurnal Fluctuation of Amyloid-β in Mice with Alzheimer’s Disease Pathology

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Aggregation of amyloid-β (Aβ) in the brain begins to occur years before the clinical onset of Alzheimer’s disease (AD). Before Aβ aggregation, concentrations of extracellular soluble Aβ in the interstitial fluid (ISF) space of the brain, which are regulated by neuronal activity and the sleep-wake cycle, correlate with the amount of Aβ deposition in the brain seen later. The amount and quality of sleep decline with normal aging and to a greater extent in AD patients. How sleep quality as well as the diurnal fluctuation in Aβ change with age and Aβ aggregation is not well understood. We report a normal sleep-wake cycle and diurnal fluctuation in ISF Aβ in the brain of the APPswe/PS1ΔE9 mouse model of AD before Aβ plaque formation. After plaque formation, the sleep-wake cycle markedly deteriorated and diurnal fluctuation of ISF Aβ dissipated. As in mice, diurnal fluctuation of cerebrospinal fluid Aβ in young adult humans with presenilin mutations was also markedly attenuated after Aβ plaque formation. Virtual elimination of Aβ deposits in the mouse brain by active immunization with Aβ42 normalized the sleep-wake cycle and the diurnal fluctuation of ISF Aβ. These data suggest that Aβ aggregation disrupts the sleep-wake cycle and diurnal fluctuation of Aβ. Sleep-wake behavior and diurnal fluctuation of Aβ in the central nervous system may be functional and biochemical indicators, respectively, of Aβ-associated pathology.

INTRODUCTION

Aggregation of the amyloid-β (Aβ) peptide in the extracellular space of the brain is one of the pathological hallmarks of Alzheimer’s disease (AD). Aβ is produced from amyloid precursor protein (APP) by sequential cleavage by the β- and γ-secretases (1–3) and exists as a soluble, monomeric form throughout life (4). Monomeric Aβ begins to aggregate in the human brain ~10 to 15 years before the clinical symptoms and signs of AD become apparent, by which time a substantial amount of neuronal and synaptic loss in several brain regions is present (5, 6). In humans with Aβ aggregation in the brain who are clinically asymptomatic (preclinical AD), there is evidence of decreased functional connectivity in brain networks affected by amyloid deposition (7, 8). There may be other functional changes associated with Aβ aggregation during preclinical AD (9). Identification of such factors that might be cognitive, behavioral, or physiological measures will be important so as to better assess functional impairment during this period of time as well as to assess responses to new disease-modifying therapies as they become available.

APP transgenic mice that develop Aβ aggregation in the brain are neuropathological and functional models of preclinical AD in that they develop Aβ aggregation, inflammation, neuritic dystrophy, as well as functional disconnection between brain areas but do not develop marked neurodegeneration including tauopathy (10–13). Therefore, understanding Aβ metabolism and functional deficits induced by Aβ aggregation in such mice may provide useful clues to aid in development of early diagnostic markers in humans as well as provide insights into functional abnormalities induced by Aβ aggregation before significant cognitive decline.

Aβ is secreted by neurons into the extracellular space of the brain in the interstitial fluid (ISF). Using in vivo microdialysis, ISF Aβ concentrations in APP transgenic mice have been found to be closely associated with brain synaptic and neuronal activity before Aβ plaque formation (14, 15) and also to be related to subsequent amyloid plaque formation and growth in vivo (16). We previously reported in young APPswe transgenic and wild-type mice without Aβ plaques in the brain that ISF Aβ increases during wakefulness and decreases during sleep (17), similar to the diurnal fluctuation of cerebrospinal fluid (CSF) Aβ observed in humans (17–19). However, whether the sleep-wake cycle and Aβ fluctuation become disrupted after Aβ aggregation in the brain is not clear. Further, the causal relationship between changes in the sleep-wake cycle and changes in Aβ metabolism is not understood. Here, we characterized the amount and quality of sleep and the degree of diurnal Aβ fluctuation across two brain regions with different vulnerability to Aβ deposition before and after the onset of Aβ aggregation in a mouse model of β-amyloidosis. We also assessed CSF Aβ concentrations over 36 hours in humans carrying mutations that cause autosomal dominant forms of AD. Moreover, we examined whether preventing Aβ aggregation was sufficient to normalize the sleep-wake cycle and biochemical abnormalities in the mouse model.

RESULTS

Changes in the sleep-wake cycle and Aβ diurnal fluctuation with Aβ deposition

APPswe/PS1ΔE9 mice (20) at 3 months of age, before Aβ deposition begins, displayed a diurnal fluctuation of ISF Aβ in the hippocampus...
and in the striatum (Fig. 1, A, D, G, and J) and a normal sleep-wake cycle (Fig. 2A), similar to that seen in wild-type littermates (Fig. S1, A to F). APPswe/PS1ΔE9 mice begin to deposit substantial amounts of Aβ plaques in the hippocampus by 6 months of age, whereas striatal deposition is not detectable until 9 months (Fig. S2). At 6 months, diurnal fluctuation of ISF Aβ was disrupted in the hippocampus (Fig. 1, B and E) but maintained in the striatum (Fig. 1, H and K). There was also a trend for an increase in wakefulness and a decrease in sleep during the light phase (Fig. 2, B and G to I) at 6 months. At 9 months, when Aβ plaques were increased to a greater extent in hippocampus and now were present in striatum (Fig. S2, E and F), loss in diurnal fluctuation of ISF Aβ was observed in both brain regions (Fig. 1, C, F, I, and L). There was also a marked disruption of the sleep-wake cycle with significantly increased wakefulness and decreased rapid eye movement (REM) and non-REM (NREM) sleep (Fig. 2). Absolute concentrations of ISF Aβ were decreased in the hippocampus and remained unchanged in the striatum (Fig. 1, M and N).

**Attenuated diurnal fluctuation of CSF Aβ in human subjects with presenilin mutations**

In addition to a loss of diurnal fluctuation of ISF Aβ seen in APPswe/PS1ΔE9 mice, we also observed attenuation of the diurnal pattern of Aβ in the CSF of humans with mutations in the presenilin (PS) gene (Fig. 3). These individuals also had Aβ deposition as detected by amyloid imaging with Pittsburgh Compound B (PiB). Cosinor analysis is a statistical method to fit a cosine wave to data sets obtained over time to capture a circadian oscillation pattern. Cosinor analysis was used to assess the diurnal patterns of CSF Aβ dynamics in mutation∗.

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**Fig. 1.** Chronological changes in sleep-wake patterns and diurnal fluctuations of ISF Aβ in APPswe/PS1ΔE9 mice. (A to C and G to I) Diurnal changes of ISF Aβ<sub>1-40</sub> in APPswe/PS1ΔE9 mice at 3, 6, and 9 months across 2 days shown as percent average of 2 days of absolute values of ISF Aβ<sub>1-40</sub> in the hippocampus (A to C) and striatum (G to I). (D to F and J to L) Comparison of percent average of 2 days of ISF Aβ<sub>1-40</sub> between dark and light periods in the hippocampus (D to F) and striatum (J to L) for each age group (n = 6 to 8 per group; two-tailed t test). (M and N) Absolute concentrations of ISF Aβ<sub>1-40</sub> in the hippocampus (M) and striatum (N) of 3-, 6-, and 9-month-old APPswe/PS1ΔE9 mice (n = 6 to 8 per group; one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test). ∗P < 0.05; **P < 0.01; ***P < 0.001. Values represent means ± SEM.
**Normalization of sleep-wake cycle and Aβ diurnal fluctuation by active immunization**

To investigate whether Aβ aggregation is responsible for the changes in sleep amount and quality as well as the attenuation of the diurnal fluctuation of ISF Aβ, we actively immunized APPswe/PS1E9 mice starting at 1.5 months and then monthly with subcutaneous injections of synthetic Aβ1-42 or phosphate-buffered saline (PBS) and compared the patterns of ISF Aβ and the sleep-wake cycle at 9 months. PBS-treated APPswe/PS1E9 mice showed a pattern of Aβ plaque deposition similar to untreated 9-month-old APPswe/PS1E9 mice (Fig. 4, A and B). Diurnal fluctuation of ISF Aβ was absent in the hippocampus of PBS-treated animals, and the mice had strongly disrupted sleep-wake patterns (Fig. 5, A, B, D, and E). In contrast, APPswe/PS1E9 mice actively immunized with Aβ1-42 showed markedly decreased Aβ deposits in the brain (Fig. 4, C and D) and exhibited both a normal sleep-wake cycle and a diurnal fluctuation of ISF Aβ at 9 months (Fig. 5, G to L). During the light period, PBS-treated mice were awake 29.6 ± 4.1 min/hour, whereas Aβ1-42-vaccinated mice were awake 17.2 ± 2.4 min/hour (*P = 0.0256) (Fig. 5, A, D, G, and J). Wild-type littermates had a normal sleep-wake cycle and diurnal fluctuation of endogenous Aβ through 9 months (fig. S1, G to L).

**Changes in neuronal activity and the sleep-wake cycle after Aβ accumulation**

Lactate is an indicator of neuronal activity both in vitro and in vivo that is increased during wakefulness and decreased during sleep (15, 21, 22). We measured ISF lactate in the hippocampus and striatum of APPswe/PS1E9 mice and found differences across the dark and light phases. To investigate whether lactate concentrations could be a biological indicator of wakefulness irrespective of Aβ pathology in the brain, we compared ISF lactate concentrations and the amount of wakefulness in APPswe/PS1E9 mice at different ages. ISF lactate concentrations showed a significant correlation with the amount of wakefulness at 3, 6, and 9 months (fig. S3). Additional analysis of the amplitude of diurnal fluctuation of lactate as assessed by cosinor analysis (23) showed a decrease in amplitude by 9 months (Fig. 6) corresponding to an increase in wakefulness. To further investigate a potential causal relationship between changes in Aβ metabolism and changes in brain neuronal activity, we compared the chronological changes in correlation between ISF Aβ and ISF lactate in relation to Aβ accumulation. Hippocampal concentrations of ISF lactate and ISF Aβ correlated at 3 and 6 months, but the correlation was lost by 9 months (fig. S4, A to C). This was validated in PBS-treated APPswe/PS1E9 mice, where there was no correlation between ISF lactate and ISF Aβ at 9 months (fig. S5A). In contrast, APPswe/PS1E9 mice actively immunized with Aβ1-42 maintained a significant correlation between concentrations of ISF lactate and ISF Aβ in the hippocampus and striatum at 9 months (fig. S5, B and D). These data demonstrate a strong relationship between the sleep-wake state and the neuronal activity even after the acquisition of Aβ pathology and suggest that the cause of ISF Aβ fluctuation disruption is more likely due to the biochemical effect of the formation of Aβ plaques resulting in sequestration of ISF.
Aβ rather than a change in neuronal activity associated with the sleep-wake cycle.

Loss of Aβ diurnal fluctuation associated with absolute decrease in ISF and CSF Aβ42

We also investigated whether ISF Aβ42 behaved similarly to Aβ1-40 and Aβ40 across 36 hours in no mutation carriers (mutation ; n = 4) (A and D), mutation carriers who are PiB− (mutation− PiB− ; n = 4) (B and E), and mutation carriers who are PiB+ (mutation+ PiB+ ; n = 4) (C and F) as shown by cosinor curves. Cosinor analysis was used to assess diurnal patterns of CSF Aβ dynamics in each group, and diurnal patterns were considered significant when amplitudes were different from zero (P < 0.05).

Aβ plaque deposition in the hippocampus and striatum of 9-month-old APPswe/PS1ΔE9 mice treated with PBS or immunized with Aβ42.

(A to D) Representative brain sections of the hippocampus (A and C) and striatum (B and D) of mice from each group stained with HJ3.4 antibody to visualize Aβ-immunoreactive plaques (Aβ-IR). (E and F) Amount of Aβ deposition in the PBS-treated mice and Aβ42-vaccinated mice are shown with amount of Aβ deposition in 6- and 9-month-old APPswe/PS1ΔE9 mice in the hippocampus (E) and striatum (F) (n = 5 to 6 in each group; two-tailed t test). ***P < 0.001. n.s., not statistically significant. Values represent means ± SEM. Scale bar in (A), 500 μm.
Fig. 5. Sleep-wake patterns and diurnal fluctuation of ISF Aβ in 9-month-old PBS-treated and Aβ42-immunized APPswe/PS1ΔE9 mice. (A and G) Sleep-wake patterns in 9-month-old PBS-treated (A) and Aβ42-immunized (G) APPswe/PS1ΔE9 mice across 2 days (two light-dark periods) shown as minutes awake per hour. (D and J) Comparison of minutes awake per hour between the dark and the light periods in each group (n = 5 to 6 per group; two-tailed t test). (B and H) Diurnal fluctuation of ISF Aβ1–42 in the hippocampus of 9-month-old PBS-treated (B) and Aβ42-immunized (H) APPswe/PS1ΔE9 mice across 2 days presented as percent average of absolute values of ISF Aβ1–42. (E and K) Comparison of percent average of absolute values of ISF Aβ1–42 in the hippocampus between the dark and the light periods (n = 5 to 6 per group; two-tailed t test). (C and I) Diurnal fluctuation of ISF Aβ1–42 in the striatum of 9-month-old PBS-vaccinated (C) and Aβ42-immunized (I) APPswe/PS1ΔE9 mice across 2 days. (F and L) Comparison of percent average of absolute values of ISF Aβ1–42 in the striatum between the dark and the light periods (n = 5 to 6 per group; two-tailed t test). *P < 0.05; ***P < 0.001. Values represent means ± SEM.

APPswe/PS1ΔE9 mice (Fig. 7A). In contrast to Aβ, concentrations of ISF lactate, which does not aggregate in the brain, continue fluctuating at 6 months with no change in absolute concentrations through 9 months (Fig. 7, B and E). Absolute concentrations of CSF Aβ42 in humans also decreased in those with Aβ plaque formation. CSF Aβ42 was highest in nonmutation carriers and lowest in PiB+ mutation carriers, and the amount of CSF Aβ42 inversely correlated with the amount of fibrillar Aβ in the brain as measured by amyloid imaging (Fig. 7, C and F).

DISCUSSION

Here, we found an Aβ accumulation–associated disruption of the sleep-wake cycle and loss of diurnal fluctuation of ISF Aβ in a mouse model of AD amyloidosis. Similar findings, namely, loss of diurnal fluctuation of CSF Aβ associated with amyloid deposition, were also seen in humans with mutations that cause autosomal dominant AD. These changes were not seen in age-matched wild-type mice in ISF nor in age-matched humans lacking PS mutations in CSF, suggesting that overexpression of APP/PS1 transgenes associated with autosomal dominant AD in mice or some form of Aβ aggregation was responsible. The findings that active immunization with Aβ42 prevented the changes in sleep disruption as well as diurnal fluctuation of Aβ in mice and that aged littermates maintained both sleep-wake cycle and diurnal fluctuation of Aβ strongly suggest that Aβ accumulation rather than overexpression of transgenes is responsible for these changes.

The sleep-wake cycle is a fundamental property of the brain. Diurnal fluctuation of Aβ is a physiologic finding observed in brains of mice and humans that occurs before the development of Aβ plaque deposition. We found that the diurnal fluctuation of ISF Aβ was disrupted sequentially in line with a hierarchical deposition of Aβ plagues in different brain regions. Because changes in Aβ fluctuation occurred before the changes in sleep quality and perturbation of neuronal activity, the results suggest that the changes in ISF Aβ fluctuation are likely due to a biochemical change in Aβ metabolism induced by plaque formation rather than changes in the sleep-wake cycle itself. Using mouse models of β-amyloidosis, we previously showed that sleep deprivation and administration of the neuropeptide orexin, which regulates arousal and wakefulness, acutely increased ISF Aβ, and chronic sleep deprivation strongly increased Aβ plaque formation (17). Blocking orexin receptors acutely and chronically decreased ISF Aβ and plaque formation (17). These results suggested the possibility that sleep disruption and disorders might be a risk factor for the development of Aβ deposition and possibly AD. Emerging evidence in humans suggests that this may be the case (24, 25), although additional longitudinal studies with biomarkers are required because studies with elderly participants could not assess a cause-effect relationship between AD
pathology and changes in sleep. Although disrupted sleep has the potential to lead to Aβ aggregation, our current results suggest that once Aβ aggregates, some of the damage it induces in the central nervous system leads to dysregulation of the sleep-wake cycle. Previous reports on AD patients and mouse models of Aβ amyloidosis support the possibility that the presence of Aβ-related pathology in the brain is associated with a disrupted sleep-wake cycle or circadian rhythm by affecting molecules including orexin, melatonin, and associated brain regions (26–32). Thus, there could be a positive feedback loop between the sleep-wake cycle and the Aβ metabolism. The early increase in wakefulness possibly initiated by the aggregation of Aβ may accelerate Aβ accumulation, which may lead to further neuronal dysregulation and increase sleep-wake cycle abnormalities.

Observations from this study also demonstrate that at least the initial changes in sleep and Aβ fluctuation are likely due to Aβ aggregation and not the effect of aging. Recent human studies assessing CSF noted that diurnal fluctuation of CSF Aβ in young adults was attenuated in older adults who had a mean age of 73.4 years. Whether this attenuation was due to aging or Aβ aggregation was not clear (19). The oldest APPswe/PS1dE9 mice used in this study only reached the equivalent of middle age, and wild-type littersmates at the same age did not have sleep-associated impairments. Because the mouse model we are using expresses mutations found in humans with dominantly inherited AD who begin to have AD pathology as young adults, we further investigated presymptomatic individuals within autosomal dominant AD families. In those young subjects (mean age of 42.4 years) with and without Aβ pathology in the brain, we demonstrated that the aggregation of Aβ in the brain is associated with the attenuation of diurnal fluctuation of Aβ in human CSF. This attenuation was not present in age-matched siblings that lacked these mutations. This suggests that

**Fig. 6.** Chronological changes in the amplitude of diurnal fluctuation of ISF lactate in 3-, 6-, and 9-month-old APPswe/PS1dE9 mice (A to F) Diurnal fluctuation of ISF lactate in the hippocampus (A to C) and striatum (D to F). (G and H) Chronological changes in the amplitude of diurnal fluctuation in the hippocampus (G) and striatum (H) as measured by amplitude of cosinor analysis (n = 6 per group; one-way ANOVA after cosinor analysis for measurement of amplitude, Tukey’s post hoc test for multiple comparisons). *P < 0.05. Values represent means ± SEM.
changes in brain Aβ metabolism associated with amyloid plaque formation induce attenuation in the fluctuation of CSF Aβ independent of an effect of aging. Although the change in diurnal fluctuation in CSF Aβ in presenilin carriers with versus without amyloid deposition is significant, it is small, suggesting that it may be difficult to be used as a biomarker. However, if the changes in sleep quality and amount seen in APPswe/PS1E9 mice are also present in humans, this may provide a useful quantitative and functional endophenotype during the period of preclinical AD that can be assessed in response to therapeutic intervention.

Aβ aggregation was also associated with disruption of homeostatic fluctuation of neuronal activity in the brain. Before Aβ plaque deposition, there was a strong correlation between concentrations of ISF Aβ and ISF lactate, suggesting neuronal activity-associated release of Aβ within the brain (14, 15, 33). The correlation between ISF Aβ and ISF lactate, however, was lost after substantial accumulation of Aβ in the brain. Sequential loss of correlation between ISF lactate and ISF Aβ with a decrease in absolute amount of ISF Aβ indicates that changes in the equilibrium between ISF Aβ and Aβ plaques may cause dissociation between ISF Aβ concentrations and neuronal activity. The decrease in absolute concentrations of ISF Aβ and CSF Aβ in humans with Aβ plaque formation suggests that soluble ISF and CSF Aβ is being sequestered by amyloid plaques, consistent with other studies (4, 34). On the other hand, lactate, which does not aggregate within the brain, did not show changes in absolute concentration in ISF. The significant decrease in the fluctuation of lactate in both hippocampus and striatum by 9 months in APPswe/PS1E9 mice occurred in concert with the changes in the sleep-wake cycle, the most prominent change of which was a marked increase in wakefulness during the light phase by 50%, a time when the animals would otherwise be sleeping most of the time. This increase in wakefulness may be very damaging to the brain in an additive fashion to other Aβ-linked pathways of damage, because many studies have shown the important function of sleep to learning, memory, synaptic plasticity, and risk for other medical disorders (35–38). It is possible that the Aβ-induced changes to sleep are due to local cortical and hippocampal Aβ-induced changes to synaptic activity and excitability occurring throughout affected brain regions (39–41). Notably, at all ages assessed in our studies, APPswe/PS1E9 mice had no phenotypic or electroencephalogram evidence of seizures. Thus, the disruption of the sleep-wake cycle, which may be due to synaptic alterations, was not secondary to seizures.

Sleep-wake patterns of the human subjects who participated in this study were not investigated. Sleep changes in young adults with autosomal dominant AD before or after Aβ pathology in the brain will be important in future studies to determine whether similar changes in the sleep-wake cycle are present because Aβ pathology develops in the preclinical stages of disease. Because changes in the sleep-wake cycle and Aβ fluctuation in both mice and humans were all in the presence of presenilin mutations, it will be important in the future to assess whether the same changes occur in the absence of such mutations. Thus, it will also be interesting to determine whether changes in the sleep-wake cycle are present in the preclinical stages of late-onset AD, which would have important implications both diagnostically and for therapeutic assessment.

**MATERIALS AND METHODS**

**Mice**

All studies were approved by the Animal Studies Committee at Washington University. Female APPswe/PS1E9 on a B6C3 background (The Jackson Laboratory) mice (20) and their wild-type littermates (B6C3) were used at 3, 6, and 9 months for sleep-wake analysis and for microdialysis for ISF Aβ and lactate measurement. Aβ immunohistochemistry and X-34 staining were performed at the completion of experiments. Animals were given ad libitum access to food and water.

**In vivo microdialysis**

In vivo microdialysis to assess Aβ and lactate in the brain ISF of awake, freely behaving mice was performed as described (4, 15). Briefly, guide cannulae (BR style, Bioanalytical Systems) were stereotaxically implanted into hippocampus (bregma –3.1 mm, 2.5 mm lateral to midline, 1.2 mm below the dura at a 12° angle) and striatum (bregma +0.5 mm, 2.5 mm lateral to midline, 1.6 mm below the dura at a
14.5° angle) simultaneously. Probe placement in the regions of interest was confirmed by cresyl violet staining. Microdialysis probes (2 mm; 38-kD molecular size cutoff; BR style, Bioanalytical Systems) were connected to a syringe pump (Stoelting Co.), and artificial CSF (pH 7.35) containing 1.3 mM CaCl$_2$, 1.2 mM MgSO$_4$, 3 mM KCl, 0.4 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, and 122 mM NaCl was continuously perfused through the microdialysis probe. For measurement of $\Delta\beta_{40-42}$ and lactate in APPswe/PS1E9 mice, a flow rate of 1.0 μl/min was used. For measurement of $\Delta\beta_{40-42}$ in wild-type littermates and for measurement of $\Delta\beta_{42}$ in APPswe/PS1E9 and wild-type littermates, a flow rate of 0.5 μl/min was used. Guide cannulae were implanted 2 weeks before the beginning of microdialysis. After insertion of the microdialysis probe, mice were habituated to a 12-hour light/dark cycle for three more days. On the fourth day, samples were collected and stored for analyses.

**Immunization**

We actively immunized APPswe/PS1E9 mice beginning at 1.5 months with synthetic $\Delta\beta_{1-42}$ or PBS as described (42). Briefly, $\Delta\beta_{1-42}$ peptide was freshly prepared from lyophilized powder. Then, 2 mg of $\Delta\beta_{42}$ (human $\beta_{21-42}$, US Peptides) was added to 0.9 ml of deionized water, and the mixture was vortexed to generate a relatively uniform suspension. A 100-μl aliquot of 10X PBS was added to make a final 1X PBS (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.5). The suspension was vortexed again and incubated overnight at 37°C. $\Delta\beta_{42}$ was 1:1 (v/v) emulsified with complete Freund’s adjuvant for the first immunization, followed by boost injections with incomplete Freund’s adjuvant at 2 weeks, 4 weeks, and monthly thereafter until 9 months. For PBS treatment, the exact same methods were used, except using PBS instead of $\Delta\beta_{1-42}$. Titters were determined by serial dilutions of sera against $\Delta\beta_{42}$ protein, which had been coated on enzyme-linked immunosorbent assay (ELISA) plates. Detection used goat anti-mouse immunoglobulin conjugated to horseradish peroxidase (HRP) and slow-TMB (3,3’,5,5’-tetramethylbenzidine; Sigma-Aldrich) substrate. Titters were defined as the dilution yielding 50% of the maximal signal.

**Enzyme-linked immunosorbent assay**

Microdialysis samples were analyzed for $\Delta\beta_{k-40}$, $\Delta\beta_{k-42}$, or $\Delta\beta_{1-13}$ with sandwich ELISAs. Briefly, $\Delta\beta_{k-40}$, $\Delta\beta_{j-42}$, and $\Delta\beta_{1-13}$ were captured with monoclonal antibodies targeted against amino acids 35 to 40 (HJ2), 37 to 42 (HJ7.4), and 13 to 28 (m266) of $\beta\theta$, respectively. For $\Delta\beta_{k-40}$ and $\Delta\beta_{k-42}$ assays, a biotinylated N-terminal domain monoclonal antibody (HJ5.1) followed by streptavidin–poly–HRP40 (Fitzgerald) was used for detection. For $\Delta\beta_{1-13}$ assays, a biotinylated N-terminal domain monoclonal antibody (3D6) followed by streptavidin–poly–HRP20 (Fitzgerald) was used. The antibodies m266 and 3D6 were gifts from Eli Lilly. All assays were developed with Super Slow ELISA TMB (Sigma-Aldrich) and read on a Bio-Tek Synergy 2 plate reader at 650 nm.

**Lactate assay**

An enzymatic lactate assay kit (BioVision) was used to measure ISF lactate according to the manufacturer’s instructions. Assays were read on a Bio-Tek Synergy 2 plate reader at 570 nm. To calculate the absolute values of steady-state concentration of lactate being dialyzed, we used the zero-flow extrapolation method by varying flow rates from 0.4 to 1.2 μl/min (43). Zero-flow data for each mouse were fit with an exponential decay regression, and the maximum concentration at the point at which there is no flow of the perfusion buffer was calculated with GraphPad Prism 5.0 software, as described (44).

**Sleep-wake monitoring**

Polysomnographic sleep-wake cycle analysis of mice was performed as described previously (15, 17). Briefly, electroencephalograph (EEG) and electromyogram (EMG) electrodes were implanted simultaneously with the microdialysis guide cannula. For EEG recording, two stainless-steel screws attached to wire electrodes were placed over the right frontal bone (bregma +1.0 mm, 1.5 mm lateral to midline) and the right parietal bone (bregma −3.0 mm, 2.5 mm lateral to midline). Two wire electrodes were directly inserted into the neck musculature for EMG recording. The ground electrode was placed on the skull over the cerebellum. Insulated leads from the EEG and EMG electrodes were soldered to a mini-connector. After surgery, mice were housed in 12-hour light/12-hour dark for 2 weeks before recording began. To monitor the sleep-wake cycle, we transferred the mice to recording cages maintained in 12-hour light/12-hour dark conditions (light phase began at 6 a.m.), and we connected the mini-connector to flexible recording cables. Mice were habituated to the recording cages for 3 days. At the end of the habituation period, EEG and EMG recording began simultaneously with collection of microdialysis samples. EEG and EMG signals were displayed on a monitor and stored in a computer for analysis of sleep states. EEG and EMG recordings were assessed with a PS11K AC pre-amplifier (Grass-Telefactor Instruments), digitized with a DigiData 1440A Data Acquisition System (Molecular Devices), and recorded digitally with pClamp 10.2 (Molecular Devices). EEG and EMG signals were filtered (EEG: high pass 1 Hz, low pass 30 Hz; EMG: high pass 10 Hz, low pass 100 Hz) and used to identify vigilance states. EEG and EMG recordings were scored semiautomatically with sleep scoring software (SleepSign, Kissei Comtec Co. Ltd.) and binned into 10-s epochs as wakefulness, REM sleep, and NREM sleep on the basis of standard criteria of rodent sleep. Semiautomatic sleep scoring was visually inspected and corrected when appropriate. The automatic analysis and visual inspection was performed in a blinded state to the genotype and age of mice.

**Plaque deposition analyses**

After mice were perfused with PBS transcardially, brains were removed, fixed in 4% paraformaldehyde for 24 hours (4°C), cryoprotected with 30% sucrose in PBS (4°C), frozen in powdered dry ice, and cut on a freezing sliding microtome. Serial coronal sections (50 μm thick) were collected from the genu of the corpus callosum to caudal hippocampus. Sections (each separated by 300 μm) were stained with biotinylated HJ3.4 ($\beta_{11-13}$) antibody to visualize $\beta\theta$-immunopositive plaques or X-34 dye to visualize fibrillar amyloid plaques. Immunostained sections and X-34–stained sections were imaged with a NanoZoomer slide scanner (Hamamatsu Photonics). Quantitative analysis of percent area covered by immun- or X-34–positive staining was performed as described previously (43). Briefly, images of immunostained sections were exported with NDP viewer software (Hamamatsu Photonics), converted to 8-bit grayscale with ImageJ software (National Institutes of Health), thresholded to highlight $\beta\theta$–specific staining, and analyzed with ACDSee Pro 2 software (ACD Systems). Images of X-34–stained sections were converted to 16-bit grayscale, thresholded to highlight X-34–specific staining, and analyzed with ImageJ software. A mouse brain atlas (45) was used to identify hippocampus (−1.7, −2.0, −2.3) and striatum (0.8, 0.5, 0.2) for quantitative analysis of immun- and X-34–positive staining.
Human CSF analysis

Human participants were enrolled for the Familial Adult Children Study conducted by the Knight Alzheimer Disease Research Center (ADRC) at Washington University. Mutations in PS1 and PS2 were genotyped for each participant at the ADRC Genetics Core. For participants with PS mutations, we determined the status of their brain amyloid deposition by PiB positron emission tomography scan. An MCBD of 0.2 or greater was considered amyloid plaque–positive (PiB⁺) (46). Serial CSF samples were collected from participants with mutations that are known to cause autosomal dominant AD and PiB⁺ (mutation 1 PiB⁺, n = 4), those with mutations and PiB⁺ (mutation 2 PiB⁺, n = 4), and age-matched nonmutation carriers (mutation−, n = 4). The samples were collected at the same time of day, starting at ~8 a.m. for each participant. Samples were analyzed for concentrations of Aβ40 and Aβ42 with ELISA (19). The average ages for the mutation−, mutation 1 PiB⁺, and mutation 2 PiB⁺ group were 38.0 ± 1.4, 46.3 ± 16.9, and 43.0 ± 10.3 years, respectively. Participants in mutation 1 PiB⁺ group had two types of PS1 mutations (His141Leu or Ala272Val) and one type of PS2 mutation (Asn19Arg, His163Arg, or Met226Leu). Three types of PS1 mutation (Leu226Arg, His163Arg, or Met226Leu) and one type of PS2 mutation (Asn141Ile) were present in subjects in the mutation− PiB⁺ group. There was no statistical difference in age between groups (P = 0.602). Circadian patterns of Aβ concentrations were investigated for the three groups with cosinor analysis using the mean-adjusted group average data as described before (19). The mean value of CSF Aβ42 obtained over 36 hours was used for comparison of absolute values.

Statistical analysis

Statistical significance was determined by two-tailed Student’s t test, if the data sets fulfilled the normality test (Kolmogorov-Smirnov test). When the data set did not meet the assumptions of a parametric test, Mann-Whitney rank sum test was performed. One-way ANOVA followed by Tukey’s post hoc test for multiple comparisons was performed, if the data sets fulfilled the equal variance test (Levene’s test, if normality test (Kolmogorov-Smirnov test). If data sets did not fulfill both tests, Kruskal-Wallis test was performed. Pearson test was used for correlation analysis. Single cosinor analysis was used to analyze the 24-hour circadian patterns of ISF lactate fluctuation in each animal with Prism (GraphPad Software) (19). A cosine transformation was applied to the time variable with 24 hours as the default circadian cycle, and amplitude [distance between the peak and the midline of the Aβ oscillation (mesor)] was calculated for each mouse (19, 23). In the human study, mean-adjusted group average data were used for analysis. Cosinor analysis was applied to data after linear trend was subtracted from the group-averaged Aβ values. Cosinor analysis is a statistical method that uses the least squares method to fit a sinusoidal wave to data sets obtained over time. If the wave fits to a linear line of zero, the P value is higher than 0.05, which means a lack of circadian oscillation. All statistical analyses were performed with Prism version 4.0 for Windows (GraphPad Software) and SPSS 15.0 for Windows (SPSS Inc.). Values were accepted as significant if P < 0.05.

SUPPLEMENTARY MATERIALS

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Fig. S1. Sleep-wake pattern and diurnal fluctuation of ISF Aβ in the hippocampus and striatum of 3- and 9-month-old wild-type littermate mice.

Fig. S2. Aβ plaque deposition in female APPswe/PS1E9 (APP/PS1) mice at 3, 6, and 9 months and in wild-type littermates at 9 months.

Fig. S3. Correlation between wakefulness and concentrations of ISF lactate.

Fig. S4. Correlation between concentrations of ISF lactate and ISF Aβ in APPswe/PS1E9 mice.

Fig. S5. Correlation between concentrations of ISF lactate and ISF Aβi in Aβ1- and PBS-immunized APPswe/PS1E9 mice.

Fig. S6. Changes in diurnal fluctuation of ISF Aβ40 in the hippocampus and striatum of 3- and 9-month-old APPswe/PS1E9 mice.

Fig. S7. Changes in diurnal fluctuation of ISF Aβ42 in the hippocampus and striatum of 9-month-old PBS-treated and Aβ42-vaccinated APPswe/PS1E9 mice.

Fig. S8. X-34-positive amyloid plaque deposition in the hippocampus and striatum of 6- and 9-month-old APPswe/PS1E9 mice and 9-month-old PBS- and Aβ42-vaccinated APPswe/PS1E9 mice.

REFERENCES AND NOTES


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