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Amyloid- β Dynamics are Regulated by Orexin and the Sleep-Wake Cycle

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Abstract

Amyloid- β (A β) accumulation in the brain extracellular space is a hallmark of Alzheimer's disease (AD). The factors regulating this process are only partly understood. A β aggregation is a concentration-dependent process that is likely to be dependent on changes in brain interstitial fluid (ISF) levels of A β . Using *in vivo* microdialysis, we found that ISF A β levels correlated with wakefulness. ISF A β levels also significantly increased during acute sleep deprivation and during orexin infusion, whereas they decreased with infusion of a dual orexin receptor antagonist. Importantly, chronic sleep restriction significantly increased and a dual orexin receptor antagonist decreased A β plaque formation in amyloid precursor protein transgenic mice. Thus, the sleep-wake cycle and orexin may play a role in the pathogenesis of AD.

Alzheimer's disease (AD) is the most common cause of dementia. The accumulation of the amyloid- β (A β) peptide in the brain extracellular space is a critical event in the pathogenesis of AD. A β is produced by neurons and secreted into the brain interstitial fluid (ISF). An initiating factor in AD pathogenesis occurs when soluble, monomeric A β undergoes a conformational change and converts into forms such as oligomers, protofibrils, and fibrils. The accumulation of these forms of A β is concentration-dependent and confers toxicity (1). Elucidating factors that regulate soluble A β levels is important for understanding AD pathogenesis. Synaptic activity regulates the release of A β from neurons into the ISF (2,3). How ISF A β is regulated by normal physiology is poorly understood.

To investigate ISF A β metabolism, we monitored hippocampal A β levels using *in vivo* microdialysis in both wild-type mice and human *APP* transgenic (Tg2576) mice, which express a mutated form of human amyloid precursor protein (APP) (4). ISF A β was assessed in Tg2576 mice at 3 months of age, several months earlier than A β deposition begins. We found diurnal variation of ISF A β levels. A β levels were significantly increased during the dark period compared to the light period (Fig. 1A). ISF A β levels fluctuated over a 24-hour period with mean levels during the light period being ~75% of mean A β levels during the dark period (Fig. 1B). ISF A β levels were significantly correlated with the amount of time spent awake (Fig. 1, C–D). Conversely, ISF A β levels were negatively correlated with the amount of time spent

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asleep. This negative correlation was even stronger with non-REM sleep (Fig. S1). Despite fluctuations in ISF A β levels, full-length APP, APP C-terminal fragments, and A β ₁₋₄₀ and A β ₁₋₄₂ were not significantly different in total tissue homogenates of hippocampus between dark and light periods (Fig. S2). Thus, the pool of ISF A β is likely to be independently regulated from total intracellular and membrane-associated A β .

Next, we asked if diurnal A β fluctuation was also present in C57BL6, wild-type mice. Similar to Tg2576 mice, C57BL6 mice also showed a significant difference in ISF A β levels between dark and light phases, when samples were pooled over longer periods of time (Fig. 1, E and F). Thus, the diurnal variation in A β is intrinsic to normal cellular physiology.

To determine the underlying mechanism of the diurnal variation in ISF A β levels, we tested whether the light stimulus itself could affect ISF A β levels. Using C57BL6 mice, we measured ISF A β levels over 2 days under constant dim light conditions. Diurnal fluctuations of ISF A β still occurred, as did normal sleep-wake behavior (Fig. 1, G and H). Thus, ISF A β fluctuations are linked to the sleep-wake cycle and not to light or dark exposure.

To see whether the diurnal fluctuation of ISF A β is present in humans, we assessed cerebrospinal fluid (CSF) levels of A β in N=10 young healthy male volunteers via lumbar catheters over a 33 hour period and found clear evidence of diurnal fluctuation of A β in the CSF. A β levels increased throughout the first day with a peak in the evening, then decreased overnight, and again increased throughout the second day (Fig. 1I).

Because A β levels correlated with wakefulness, we asked whether manipulating sleep behavior would alter ISF A β levels. Mice were forced into wakefulness for 6 hours at the beginning of the second 12 hour light period when they would naturally be asleep. During sleep deprivation (SD), ISF A β levels were significantly higher compared to ISF A β levels during the normal light period 24 hours previously (Fig. 2, A-C). Following SD, mice spent more time sleeping and had an immediate reduction in ISF A β levels. Thus, the state of wakefulness, and not time of day, is associated with increased ISF A β levels.

Restraint stress in Tg2576 mice can acutely increase ISF A β mediated by corticotropin releasing factor (CRF) (5). α CRF₉₋₄₁, an antagonist of CRF receptors, was administered by reverse microdialysis at the beginning of SD. In the presence of the CRF receptor antagonist, ISF A β levels were still significantly higher compared to ISF A β levels during the normal light period 24 hours previously (Fig. 2, D-F). The SD-induced increase in ISF A β did not significantly differ in the presence or absence of the CRF antagonist, thereby excluding the CRF stress pathway as mechanism of action for SD to increase A β levels.

We next asked what molecular mechanism might mediate the diurnal fluctuation of A β levels. Orexin is a molecule that regulates wakefulness and other physiological functions, and is strongly implicated in narcolepsy/cataplexy and disorders of sleep and arousal (6). Orexin release from hypothalamic neurons shows a diurnal fluctuation similar to that of ISF A β (7). Orexin neurons project to the hippocampus where orexin receptors are expressed and is the location where we monitored ISF A β (8). We asked if orexin administration would modulate ISF A β levels. Intracerebroventricular (icv) infusion of orexin-A (1.5 pmole/hr) was given for 6 hours at the beginning of the light period. This dose induces wakefulness in rodents (9). During orexin infusion, ISF A β levels were significantly increased compared to ISF A β levels measured during the light period of the preceding day (Fig. 3, A-B). Infusion of vehicle did not significantly affect ISF A β (Fig. S3, A-B).

The orexin family (orexin-A and orexin-B) has two receptor subtypes: orexin receptor 1 (OXR1) and orexin receptor 2 (OXR2). We asked whether endogenous orexin signaling via orexin receptors is involved in the diurnal variation of A β levels. We infused a dual orexin

receptor antagonist, almorexant, during in vivo microdialysis for ISF A β . Icv administration of almorexant for 24 hours suppressed ISF A β levels and abolished the natural diurnal variation of A β (Fig. 3, D–E). Removal of almorexant immediately restored the diurnal rhythm in ISF A β levels during the next 24 h period. Control icv infusions of vehicle did not affect ISF A β levels (Fig. S3, C–D). Almorexant decreased the total amount of time spent awake by approximately 10% (Fig. 3F). Thus, endogenous orexin signaling via orexin receptors is required for the diurnal rhythm of ISF A β levels.

Because sleep-wake behavior modulates ISF A β levels, we asked whether chronic sleep deprivation could ultimately affect A β plaque deposition in the brain. APP transgenic mice of the APP^{swe}/PS1^{dE9} genotype were subjected to chronic sleep restriction for 20 hours daily for 21 days. Sleep-restricted animals showed markedly greater A β plaque deposition compared to their age-matched littermate controls (Fig. 4A–G). We also observed significantly greater A β plaque burden using Tg2576 mice (Fig. S4). We next asked whether chronic orexin receptor blockade could decrease A β plaque deposition in APP^{swe}/PS1^{dE9} mice at an age when plaques are just forming. Systemic treatment with almorexant once daily for 8 weeks significantly decreased A β plaque formation in several brain regions compared to vehicle-treated age-matched control mice (Fig. 4H).

Herein, we demonstrated diurnal variation in A β levels in the brain of awake and behaving animals. Perturbations in both orexin signaling and the sleep-wake cycle had acute effects upon A β dynamics. Furthermore, chronic sleep restriction accelerates A β plaque burden, while enhancing sleep via orexin receptor blockade markedly inhibits A β plaque accumulation.

One factor that influences A β levels is synaptic activity. Periods of wakefulness are associated with a net increase in synaptic strength, and periods of sleep are associated with a net decrease in synaptic strength (10–12). Differences in synaptic activity between sleep and wake states, specifically via orexin signaling, may underlie the dynamic fluctuations in ISF A β levels.

How might changes in hourly ISF A β levels contribute to eventual A β plaque deposition? Recent work with a gamma secretase inhibitor has shown that changes in ISF A β levels as little as 20% blocks plaque formation and growth over weeks (13). Thus, behavioral and pharmacological manipulations of wakefulness that resulted in changes in ISF A β of 20–25% likely caused the observed changes in A β accumulation.

Sleep is a complex behavioral state whose ultimate functions remain poorly understood. Sleep disturbances, in addition to being prominent in neurodegenerative diseases (14), could exacerbate a fundamental process leading to neurodegeneration, and optimization of sleep time could potentially inhibit aggregation of toxic proteins and slow the progression of AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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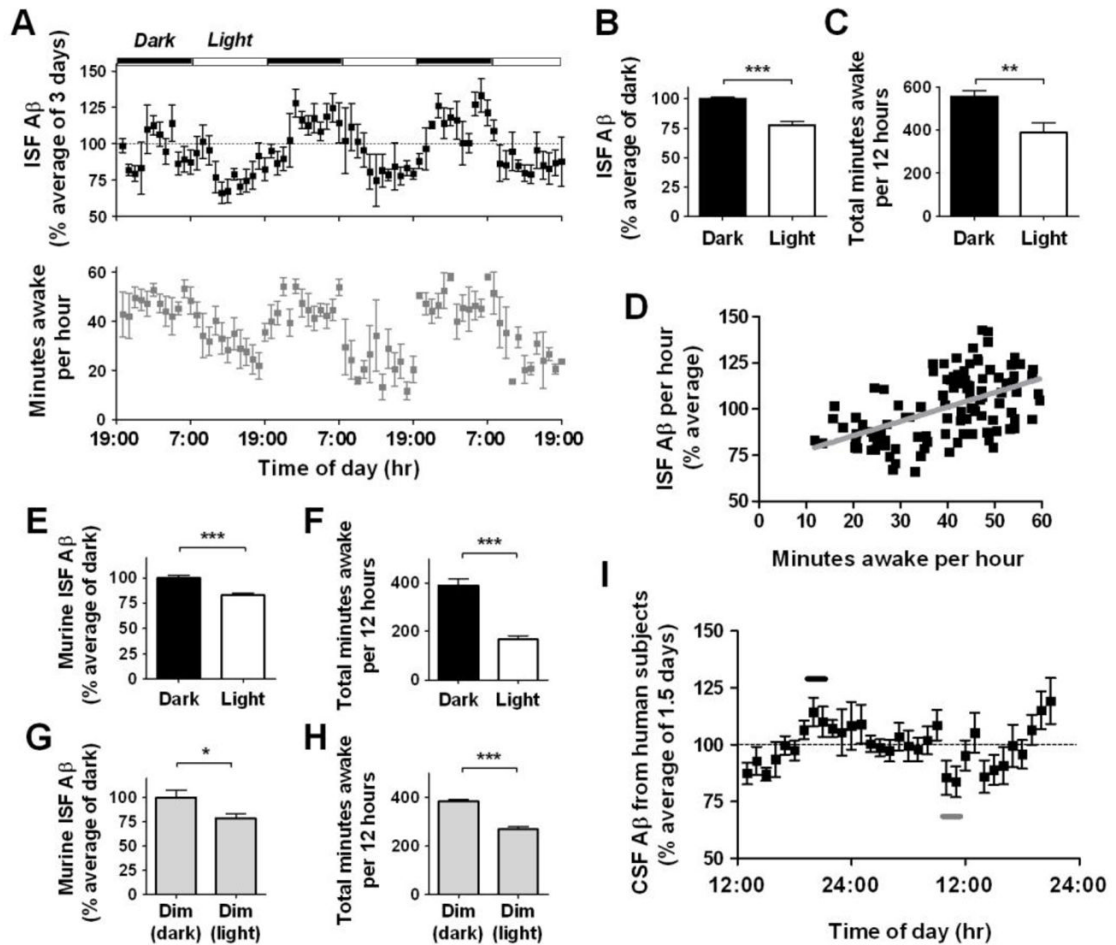


Figure 1.

Diurnal rhythm of ISF A β levels in the hippocampus of mice and CSF A β levels in human subjects. **(A)** ISF human A β levels expressed as a percentage of basal ISF A β levels over 6 light-dark periods in Tg2576 mice ($n = 8$). Total number of minutes spent awake per hour in the same mice. **(B, C)** Mean ISF A β levels were 24.4% higher ($***P < 0.0001$, $n = 8$) and the number of minutes awake were 167 minutes higher ($**P = 0.007$, $n = 7$) during dark vs. light periods. **(D)** ISF A β levels correlate with the number of minutes awake per hour ($r = 0.53$, $***P < 0.0001$, $n = 7$). **(E, F)** Mean ISF murine A β levels and minutes awake over 2 days in C57BL6 mice. Under 12 hr dark/12 hr light conditions, ISF murine A β levels were 18.5% higher ($***P < 0.0001$, $n = 10$) and the number of minutes awake was 223 minutes higher ($***P = 0.0001$, $n = 5$) during the dark periods. **(G, H)** Under constant dim light conditions, ISF A β levels were 22.7% higher ($*P = 0.05$, $n = 10$) and the number of minutes awake was 114 minutes higher ($***P = 0.0003$, $n = 5$) during the typical hours for the dark phase. **(I)** CSF A β_{1-40} levels from human subjects expressed as a percentage of basal CSF A β_{1-40} levels over 33 hours ($n = 10$). Mean peak CSF A β_{1-40} levels (black bar) at 8–10 PM were 27.6% higher than mean trough CSF A β_{1-40} levels (gray bar) at 9–10 AM ($112.3 \pm 6\%$ vs. $84.7 \pm 6\%$ respectively, $P = 0.004$). Data shown are mean \pm SEM.

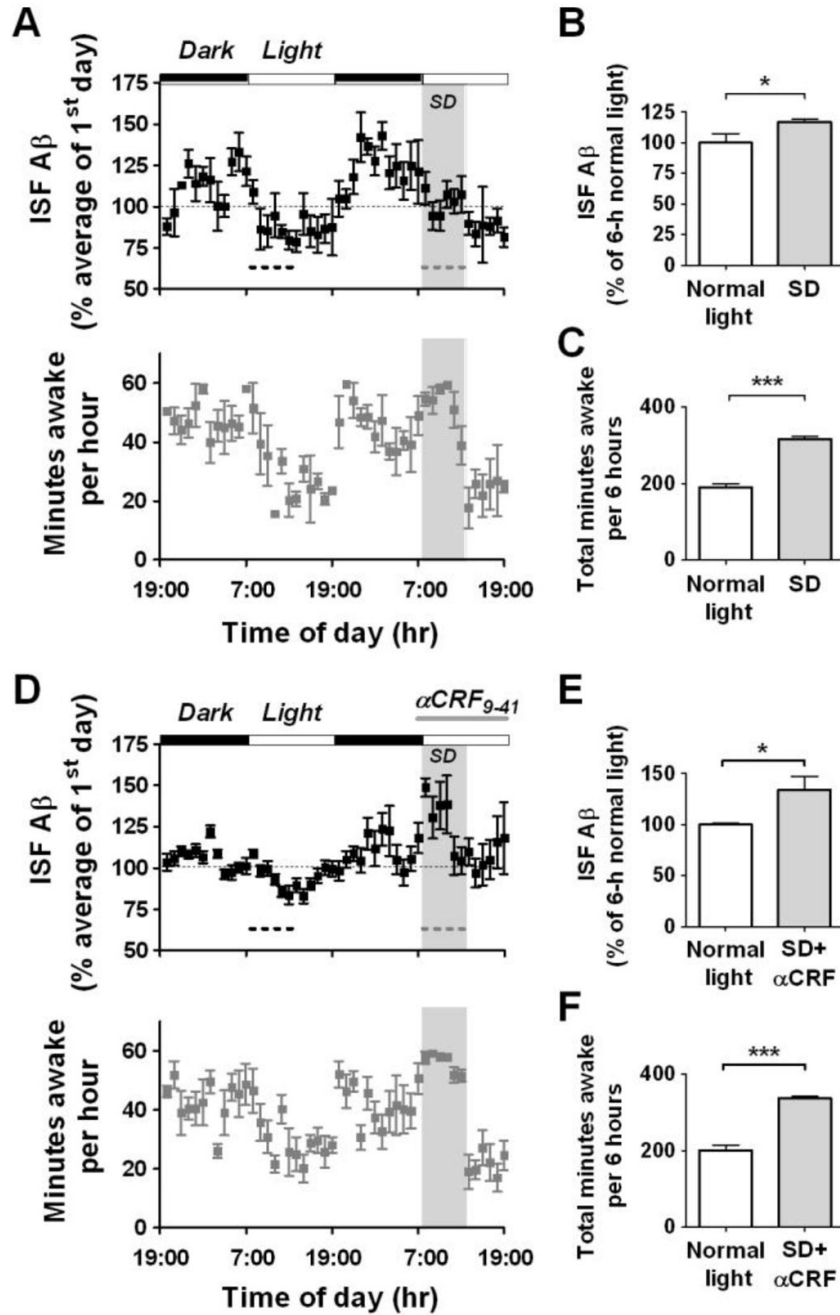


Figure 2.

Acute sleep deprivation alters ISF A β diurnal rhythm independently of CRF receptor signaling in Tg2576 mice. **(A)** Mice underwent acute sleep deprivation (SD – grey dashed line) for 6 hours at the beginning of the light period. This prevented the normal decrease in ISF A β levels that occurs during this period ($n = 8$). **(B)** Mean ISF A β levels during SD were 16.8% higher compared to those during the light period 24 hours earlier (black dashed line, $P = 0.05$, $n = 8$). **(C)** Animals spent 126 more minutes awake during SD ($***P < 0.0001$, $n = 5$). **(D)** Mice underwent acute SD (grey dashed line) at the beginning of the light period. 860 pmoles of α CRF₉₋₄₁ was infused into the hippocampus from 30 min before SD until the end of the light period ($n = 8$). **(E)** Mean ISF A β levels during SD with α CRF₉₋₄₁ infusion were 33.7% higher

compared to those during the light period 24 hours earlier (black dashed line, $P = 0.01$, $n = 8$). (F) Mice spent 136 more minutes awake during SD with α CRF₉₋₄₁ infusion ($***P < 0.0001$, $n = 5$). Data represent mean \pm SEM. SD = sleep deprivation.

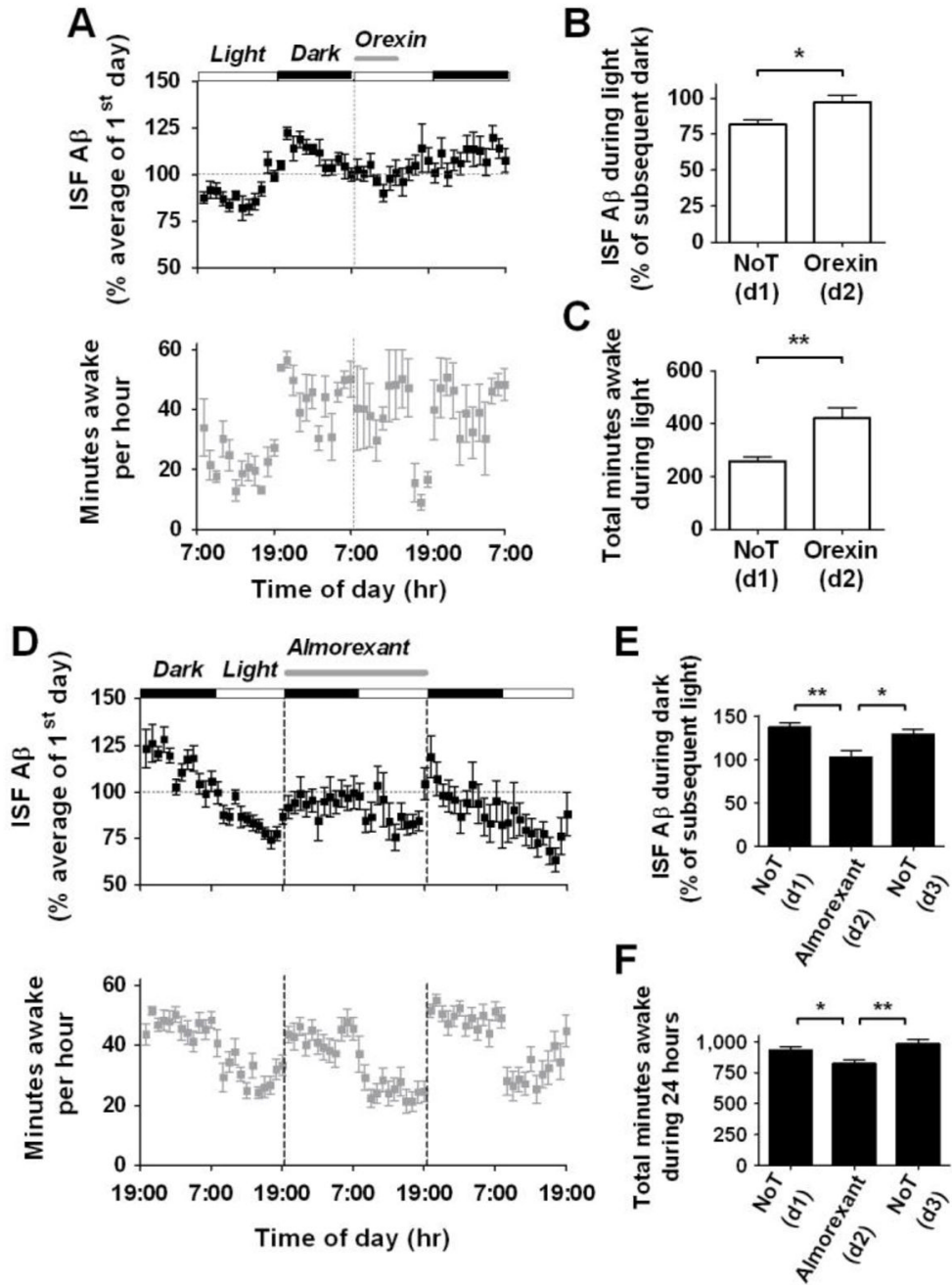


Figure 3. Effects of orexin and a dual orexin receptor antagonist on ISF A β levels in Tg2576 mice. (A) After 24 hr of baseline measurement, 1.5 pmole/hr of orexin was infused icv for 6 hours at the beginning of the light period. This sustained ISF A β levels from the dark period, and kept the mice awake longer. (B, C) Infusion with orexin increased A β levels during the light period and thereby abolished the normal 20% difference between the dark and light period (* $P = 0.01$, $n = 7$). Orexin increased the amount of minutes awake by 163 minutes (** $P = 0.009$, $n = 5$), compared to that during the light period 24 hours previously. (D) After 24 hr of baseline measurement, 13.9 nmole/hr of almorexant was infused icv for 24 hr from the beginning of the dark period ($n = 8$). This continued to suppress ISF A β levels from the light

period. **(E)** Mean ISF A β levels differed by 29% between the dark and light period during the control days, whereas there was no difference between the dark and light period during the 24 hour infusion of almorexant (** $P = 0.001$, $n = 8$). **(F)** During almorexant treatment, the number of minutes spent awake was decreased by 108 minutes over the 24 hour period compared to control days (** $P = 0.005$, $n = 13$). Data represent mean \pm SEM. NoT = no treatment; d = day.

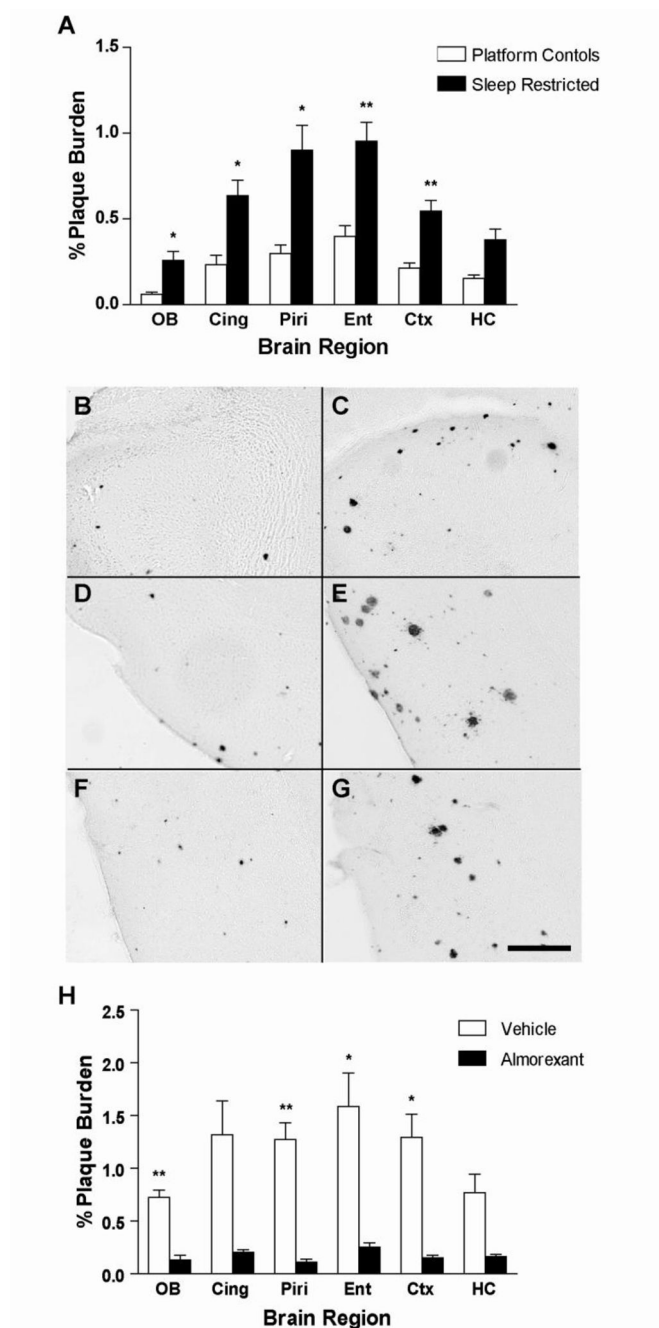


Figure 4.

A β plaque deposition after chronic sleep restriction and chronic orexin receptor blockade in APPsw/PS1dE9 transgenic mice (**A**) Mice that underwent chronic sleep restriction for 21 days showed significantly greater A β plaque deposition in multiple subregions of the cortex compared to age-matched control mice (** $P < 0.0008$, * $P < 0.008$, $n = 9-11$ per group, using Bonferroni-adjusted $P < 0.0083$ for multiple t-tests. For hippocampus, $P < 0.009$).

Representative photomicrographs of A β plaques are shown in (**B**) control and (**C**) sleep restricted olfactory bulb (**D**) control and (**E**) sleep restricted piriform cortex, (**F**) control and (**G**) sleep restricted entorhinal cortex. (**H**) Mice treated with daily i.p. injections of almorexant for 8 weeks showed significantly less A β plaque deposition in multiple subregions of the cortex

compared to age-matched vehicle controls (** $P < 0.0008$, * $P < 0.008$ $n = 5$ per group, using Bonferroni-adjusted $P < 0.0083$ for multiple t-tests. For cingulate cortex and hippocampus, $P < 0.009$). Scale bar = 200 μm . OB = olfactory bulb, Cing = cingulate cortex, Piri = piriform cortex, Ent = entorhinal cortex, Ctx = cortex (immediately dorsal to dorsal hippocampus), and HC = hippocampus.