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Current Biology

# Sleep Interacts with Aβ to Modulate Intrinsic Neuronal Excitability

## Highlights

- Aβ expression leads to reduced and fragmented sleep in *Drosophila*
- Sleep deprivation increases Aβ accumulation via changes in neuronal excitability
- Sleep loss exacerbates Aβ-induced hyperexcitability
- The anti-epileptic drug levetiracetam prolongs lifespan in a fly AD model

## Authors

Masashi Tabuchi, Shahnaz R. Lone, ..., Adam P. Spira, Mark N. Wu

## Correspondence

marknwu@jhmi.edu

## In Brief

Emerging data suggest an important relationship between sleep and Aβ, the peptide implicated in Alzheimer's disease. Using a *Drosophila* model, Tabuchi et al. demonstrate a bidirectional interaction between sleep and Aβ and identify changes in neuronal excitability as a key mechanism mediating the effects of sleep on Aβ.

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Article

# Sleep Interacts with Aβ to Modulate Intrinsic Neuronal Excitability

**Masashi Tabuchi,[1] Shahnaz R. Lone,[1] Sha Liu,[1] Qili Liu,[1] Julia Zhang,[1] Adam P. Spira,[2] and Mark N. Wu[1,3,*]**
[1]Department of Neurology, Johns Hopkins University, Baltimore, MD 21205, USA
[2]Department of Mental Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA
[3]Department of Neuroscience, Johns Hopkins University, Baltimore, MD 21205, USA

## Summary

**Background:** Emerging data suggest an important relationship between sleep and Alzheimer's disease (AD), but how poor sleep promotes the development of AD remains unclear.
**Results:** Here, using a *Drosophila* model of AD, we provide evidence suggesting that changes in neuronal excitability underlie the effects of sleep loss on AD pathogenesis. β-amyloid (Aβ) accumulation leads to reduced and fragmented sleep, while chronic sleep deprivation increases Aβ burden. Moreover, enhancing sleep reduces Aβ deposition. Increasing neuronal excitability phenocopies the effects of reducing sleep on Aβ, and decreasing neuronal activity blocks the elevated Aβ accumulation induced by sleep deprivation. At the single neuron level, we find that chronic sleep deprivation, as well as Aβ expression, enhances intrinsic neuronal excitability. Importantly, these data reveal that sleep loss exacerbates Aβ-induced hyperexcitability and suggest that defects in specific $K^+$ currents underlie the hyperexcitability caused by sleep loss and Aβ expression. Finally, we show that feeding levetiracetam, an anti-epileptic medication, to Aβ-expressing flies suppresses neuronal excitability and significantly prolongs their lifespan.
**Conclusions:** Our findings directly link sleep loss to changes in neuronal excitability and Aβ accumulation and further suggest that neuronal hyperexcitability is an important mediator of Aβ toxicity. Taken together, these data provide a mechanistic framework for a positive feedback loop, whereby sleep loss and neuronal excitation accelerate the accumulation of Aβ, a key pathogenic step in the development of AD.

## Introduction

Alzheimer's disease (AD) is the most common cause of dementia worldwide, whose burden, both in terms of human suffering and health care costs, is expected to rise sharply in the next few decades [1]. β-amyloid (Aβ) peptides, which are generated from sequential cleavage of amyloid precursor protein (APP), have been strongly implicated as having a key role in the pathogenesis of AD by substantial histopathologic, biochemical, and genetic data [2]. Thus, there is intense interest in identifying modifiable factors that modulate Aβ. Emerging evidence suggests potentially important links between sleep and AD [3]. It has long been appreciated that patients with AD have impaired sleep/wake cycles, with fragmented and reduced sleep at night [4–6]. Similarly, mouse models of AD have been shown to exhibit reduced sleep during their consolidated period [7, 8]. In humans, β-amyloid deposition, as inferred by a decrease in Aβ levels in cerebrospinal fluid (CSF), is associated with reduced sleep quality [9], and reduced sleep and poor quality sleep are associated with increased fibrillar Aβ burden in the brain [10].

Intriguingly, recent data also support a bidirectional relationship between sleep and amyloid—i.e., not only may Aβ accumulation impair sleep, but poor sleep may increase Aβ burden [3]. In humans, consolidated sleep attenuates the risk of developing AD conferred by the *E4* allele of *Apolipoprotein E* (*ApoE4*, an important genetic polymorphism for AD) [11, 12]. Moreover, a prospective study revealed that markedly fragmented sleep, as measured by wrist actigraphy, increased the risk of developing AD by ~1.5-fold, compared to those with the least fragmented sleep [13]. Importantly, using mouse models of AD, Kang et al. (2009) demonstrated that chronic sleep deprivation led to an increase in Aβ burden in the brain.

What are the mechanisms by which sleep could modulate Aβ and thus impact AD? A recent study has suggested that the "glymphatic system"—a system of perivascular CSF channels and glial processes that serves to remove metabolic waste from neurons in the brain—is more active during sleep than in wake [14]. In this study, the authors also showed that radioactively labeled Aβ injected into the cortex was cleared more efficiently during sleep, as compared to wakefulness. Another potential mechanism underlying the relationship between sleep and Aβ is an alteration in neuronal activity. Although the function of sleep remains enigmatic, one proposed function of sleep is to downscale synaptic strength following the synaptic potentiation that occurs during wakefulness [15]. For example, in rodents, molecular and electrophysiological markers of synaptic strength are increased following wakefulness, as compared to following sleep [16]. Aβ accumulation also appears to be dependent on neuronal activity. For example, Aβ cleavage from APP is enhanced with increased neural activity [17–19].

The fruit fly *Drosophila melanogaster* has been shown to sleep [20–23] and has also been established as a model for AD [24, 25]. There are several fly AD models, and we focused on a model that uses direct expression of human Aβ42 coupled to a signal peptide. Aβ42-expressing flies have been shown to recapitulate several key features of AD, including Aβ deposition, age-dependent learning impairment, and neurodegeneration [26–28]. Here, using this model, we investigated the functional interactions between sleep, excitability, and Aβ. Our findings support a bidirectional relationship between sleep and Aβ and argue that increased neuronal excitability is a key mechanism underlying the effects of sleep on Aβ.

## Results

### Aβ Expression Leads to Reduced and Fragmented Sleep

To investigate the effects of Aβ expression on sleep behavior in *Drosophila*, we used an established model of AD, whereby Aβ40, Aβ42, or AβArctic peptides are expressed pan-neuronally [28]. Aβ40 is less aggregate prone than Aβ42, whereas AβArctic carries a disease-causing mutation that leads to

*Correspondence: marknwu@jhmi.edu

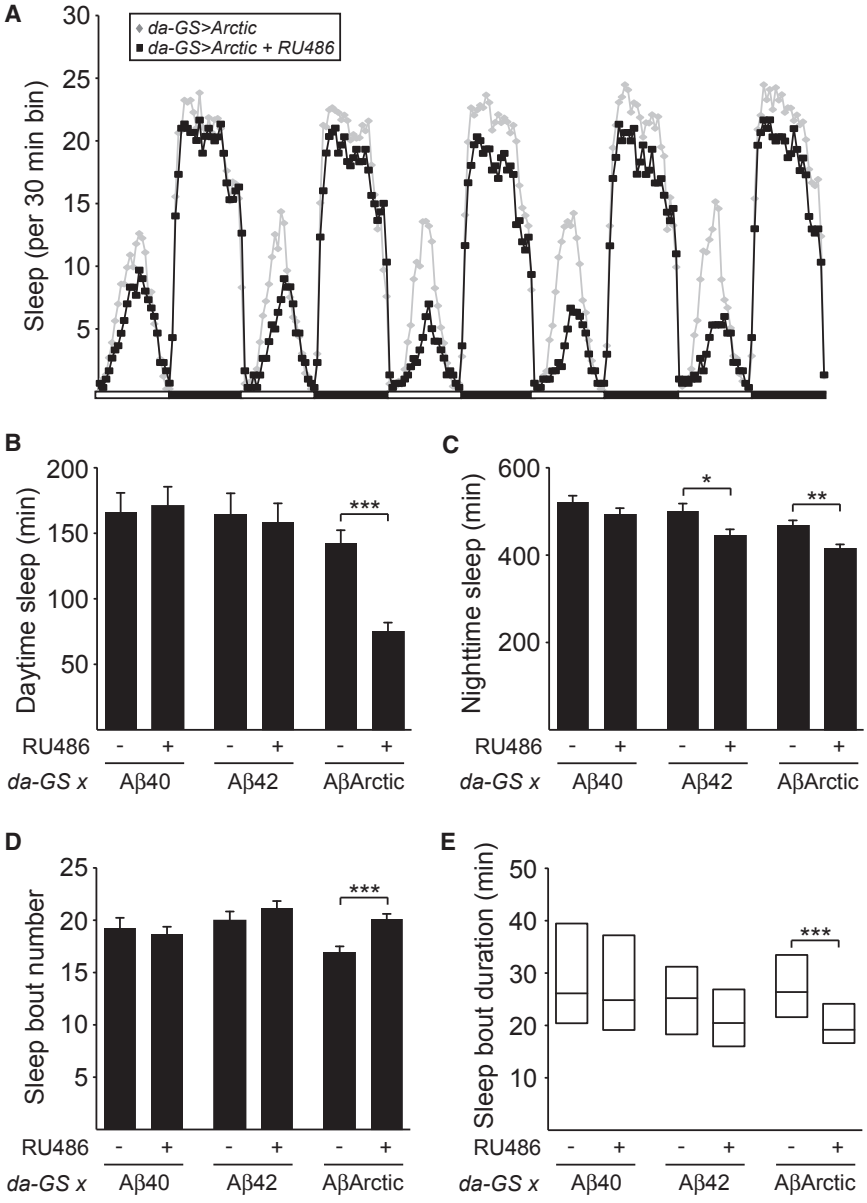


Figure 1. Induction of AβArctic Expression Reduces and Fragments Sleep

(A) Sleep profile for *da-GS>UAS-AβArctic* flies fed 250 μM RU486 (black squares, n = 92) or vehicle control (gray diamonds, n = 77).

(B–E) Daytime sleep (B), nighttime sleep (C), nighttime sleep bout number (D), and nighttime sleep bout duration (E) for *da-GS>Aβ40* fed RU486 (n = 48) or vehicle (n = 38), *da-GS>Aβ42* fed RU486 (n = 70) or vehicle (n = 36), and *da-GS>AβArctic* fed RU486 or vehicle.

Data in (A) are from the same flies as in (B)–(E). Error bars represent SEM; *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

enhanced aggregation [28, 29]. In order to bypass developmental effects, we induced expression of these Aβ peptides in all neurons during adulthood using *daughterless-Geneswitch* (*da-GS*) [30]. We examined daytime and nighttime sleep amount of *da-GS>UAS-Aβ40*, *da-GS>UAS-Aβ42*, and *da-GS>UAS-AβArctic* flies and found that sleep amount was not significantly affected with overexpression of Aβ40 (Figures 1B and 1C). In contrast, nighttime sleep, but not daytime sleep, was significantly reduced with overexpression of Aβ42, while both daytime sleep and nighttime sleep were significantly decreased with overexpression of AβArctic (Figures 1A–1C). These data suggest a "dose-dependent" relationship between Aβ aggregation and sleep amount. We next examined the sleep architecture of these flies and found no significant effect of expression of Aβ40 and Aβ42 on nighttime sleep bout number or duration. However, inducing AβArctic expression resulted in fragmentation of nighttime sleep, as evidenced by an increase in sleep bout number and reduction in sleep bout duration during the night (Figures 1D and 1E). Consistent with these findings, the sleep of AD patients has previously been reported to be fragmented at night [5, 6]. Together, these data suggest that, as is the case in humans and mice, Aβ expression in flies leads to reduced and fragmented sleep.

## Nighttime Sleep Deprivation Increases Amyloid Burden

Previous work in mice has shown that chronic loss of sleep leads to an increase in Aβ burden, raising the intriguing possibility that poor sleep may promote the pathogenesis of AD [31]. We thus addressed whether this phenomenon was conserved in other animals such as fruit flies. To reduce the time needed to visualize Aβ deposits, we mainly focused on *UAS-AβArctic* [28]. In addition, because one of the core features of AD is memory loss [1] and the mushroom bodies (MBs) in *Drosophila* play a critical role in learning and memory [32], we expressed AβArctic peptides in the Kenyon cells (KCs) of the MBs using *OK107-Gal4*. Using mechanical deprivation, we subjected *OK107-Gal4>UAS-AβArctic* flies to nighttime sleep deprivation for 1 week. As the use of chronic mechanical deprivation would increase locomotor activity and potentially result in "physical stress," we also subjected flies to a similar deprivation paradigm during the daytime, to control for this potential confounder. As shown in Figures 2A and 2B, daytime sleep deprivation effectively reduced daytime sleep but left nighttime sleep relatively intact, whereas nighttime sleep deprivation markedly reduced nighttime sleep and led to an increase in daytime sleep, likely reflecting "rebound sleep." As expected, daily activity counts of *OK107-Gal4>UAS-AβArctic* subjected to nighttime sleep deprivation were significantly increased, compared to those of flies not subjected to mechanical deprivation. However, there was no significant increase in locomotor activity in these flies when compared to those undergoing daytime sleep deprivation (Figure 2C). To assess Aβ burden, we immunostained the brains of these flies using 6E10, a monoclonal antibody that detects an N-terminal epitope on

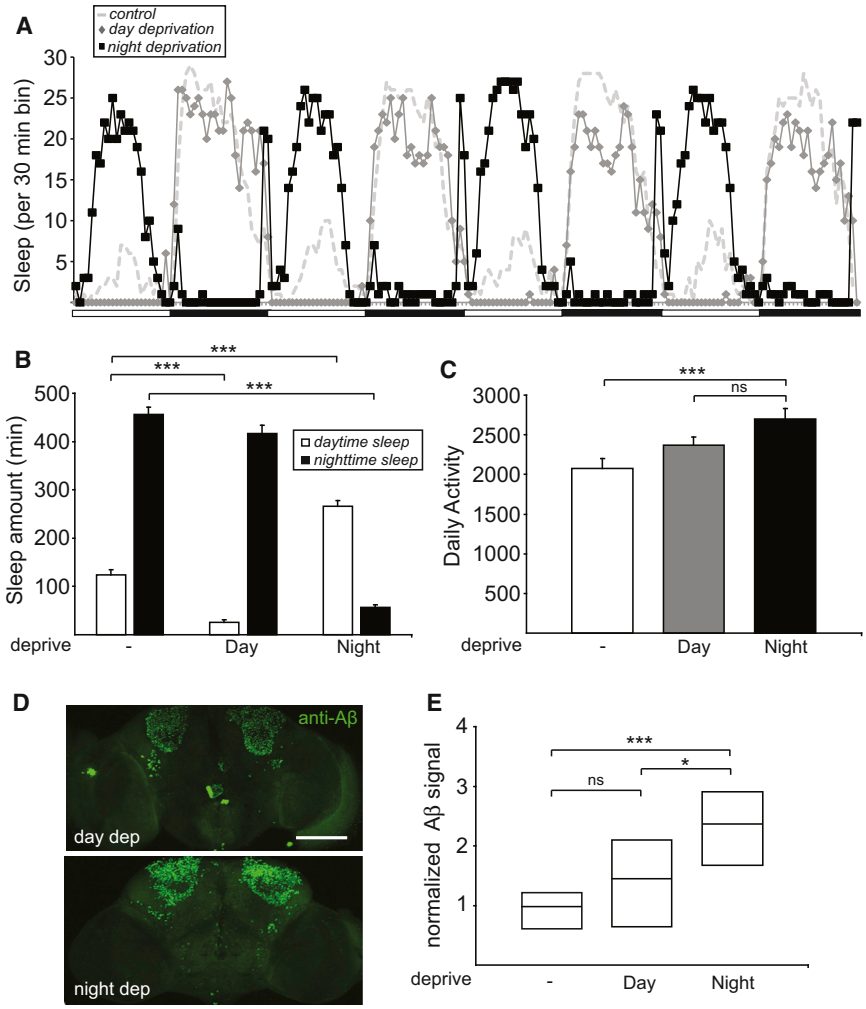


Figure 2. Mechanical Sleep Deprivation Enhances Aβ Burden

(A) Sleep profile for *OK107-Gal4>UAS-AβArctic* flies undergoing no sleep deprivation, daytime sleep deprivation, or nighttime sleep deprivation from a representative experiment. White bars and black bars denote light and dark periods, respectively.

(B and C) Sleep amount (B) and daily activity (C) for *OK107-Gal4>UAS-AβArctic* flies, where "−," "Day," and "Night" denote no sleep deprivation, daytime sleep deprivation, and nighttime sleep deprivation, respectively.

(D) Representative whole-mount brain confocal images for *OK107-Gal4>UAS-AβArctic* flies undergoing daytime ("day dep") or nighttime ("night dep") sleep deprivation, immunostained with anti-Aβ42 antibody (6E10). Maximum projection images are shown.

(E) Normalized Aβ signal intensity in the MB KCs from *OK107-Gal4>UAS-AβArctic* flies undergoing no sleep deprivation (n = 10), daytime sleep deprivation (n = 10), or nighttime sleep deprivation (n = 9).

Aβ signal intensity is not normally distributed and is thus presented here and in subsequent figures as a simplified boxplot, with the median shown as the line inside the box and the 75$^{th}$ and 25$^{th}$ percentiles shown as the top and bottom, respectively. Scale bar represents 100 μm. Error bars represent SEM; *p < 0.05, ***p < 0.001; ns, not significant.

the Aβ42 peptide. Strikingly, confocal imaging of whole-mount brains demonstrated a significant increase in Aβ signal in *OK107-Gal4>UAS-AβArctic* flies undergoing chronic nighttime sleep deprivation, compared to no deprivation or daytime sleep deprivation (Figures 2D and 2E). To reproduce these findings with a different MB-expressing driver, we examined *MB247-LexA>LexAop-AβArctic* flies [33] and obtained similar results (Figure S1A). We asked whether these findings were specific for the MBs and found that nighttime sleep deprivation significantly increased AβArctic accumulation in two other brain regions, the pars intercerebralis (using *OK107-Gal4*) and antennal lobes (using *NP1227-Gal4*) (Figure S1A). To examine whether these effects were specific to using *UAS-AβArctic*, we repeated these experiments using *UAS-Aβ42*. As shown in Figures S1C–S1E, the effects of nighttime and daytime sleep deprivation on sleep amount, daily activity, and Aβ burden were all recapitulated using *OK107-Gal4>UAS-Aβ42* flies. We next assessed whether mechanical sleep deprivation would increase accumulation of an unrelated protein. As shown in Figure S1B, there was no increase in GFP signal when *OK107-Gal4>UAS-GFP* flies were subjected to nighttime sleep deprivation. Finally, we asked whether other manipulations that enhance cellular stress would affect AβArctic or GFP accumulation. Neither chronic exposure to 31°C nor 1 mM paraquat affected AβArctic or GFP burden in the MB KCs (Figure S1F). Together, these data suggest that loss of nighttime sleep specifically leads to an increase in Aβ burden in a fly model of AD.

## Genetic Manipulation of Sleep Modulates Amyloid Burden

To further investigate how changes in sleep affect Aβ accumulation, we used genetic approaches to reduce or increase sleep, instead of mechanical deprivation. To do this, we used two binary expression systems: the Gal4/UAS system to manipulate neurons that regulate sleep and the LexA/LexAop system to express AβArctic in the MBs. We previously demonstrated that activation of a subset of dopaminergic (DAergic) neurons using *TH-D4-Gal4* significantly reduces sleep amount [34]. Thus, we used *TH-D4-Gal4* to drive expression of dTrpA1, a heat-inducible cation channel [35] to activate DA neurons in flies expressing AβArctic in the MBs (*MB247-LexA>LexAop-AβArctic*). As shown in Figure 3C, conditionally activating this subset of DA neurons resulted in a significant decrease in nighttime sleep, compared to controls. Similar to flies undergoing mechanical nighttime sleep deprivation, these flies exhibited a significant increase in Aβ accumulation, compared to controls (Figures 3A and 3D). It was previously shown that the ExFl2 fan-shaped body (FB) neurons promote sleep [36, 37], and we recently identified a restricted Gal4 driver (*R72G06-Gal4*) from the Rubin collection at Janelia Farm that contains these cells (data not shown). Thus, in order to address whether increasing sleep would cause the opposite phenotype, i.e., a decrease in Aβ burden, we generated *R72G06-Gal4>UAS-dTrpA1*, *MB247-LexA>LexAop-AβArctic* flies. As expected, conditional activation of FB neurons in these flies resulted in an increase in daytime and nighttime

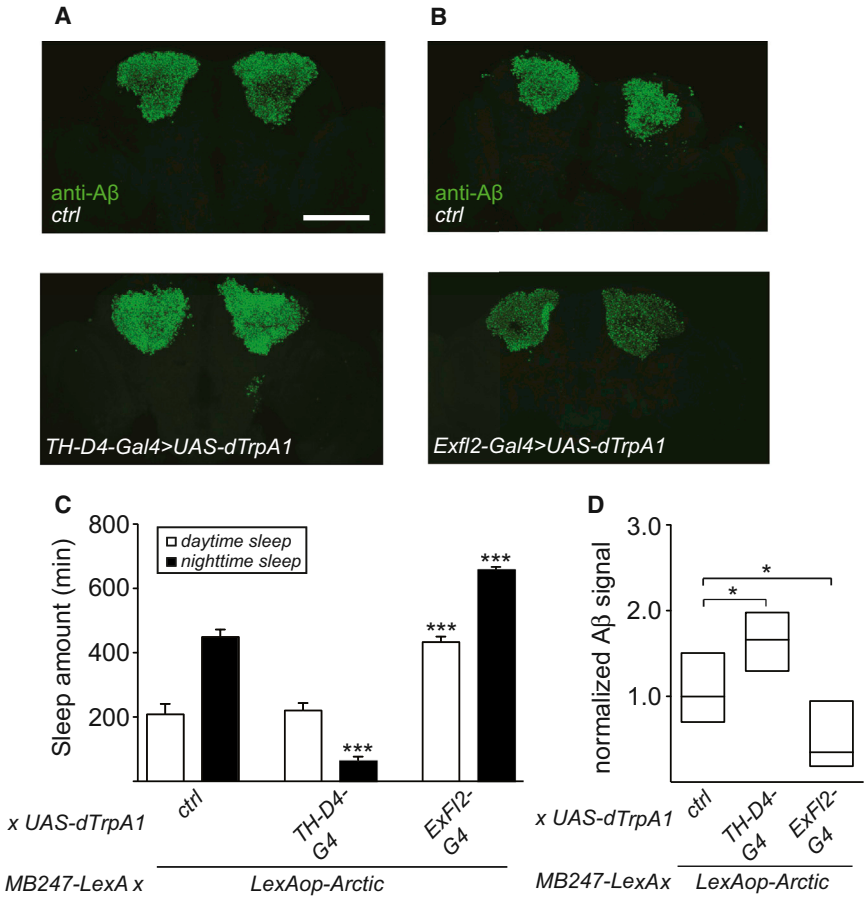


Figure 3. Genetic Manipulation of Sleep Modulates Aβ Levels

(A and B) Representative maximum projection images of whole-mount brains immunostained with 6E10 from *LexAop-AβArctic/+; UAS-dTrpA1/MB247-LexA* ("*ctrl*," top panels) and *LexAop-AβArctic/+; UAS-dTrpA1/MB247-LexA, TH-D4-Gal4* ("*TH-D4-Gal4>UAS-dTrpA1*," bottom panel), and *LexAop-AβArctic/+; UAS-dTrpA1/R72G06-Gal4, MB247-LexA* ("*ExFl2-Gal4>UAS-dTrpA1*," bottom panel) flies.

(C) Daytime and nighttime sleep amounts for *LexAop-AβArctic/+; UAS-dTrpA1/MB247-LexA* (n = 24), *LexAop-AβArctic/+; UAS-dTrpA1/MB247-LexA, TH-D4-Gal4* (n = 24), and *LexAop-AβArctic/+; UAS-dTrpA1/R72G06-Gal4, MB247-LexA* (n = 22).

(D) Normalized Aβ signal intensity in the MB KCs for the flies in (C), shown as a simplified boxplot.

dTrpA1 was chronically activated by subjecting flies to 29°C for 1 week. Scale bar represents 100 μm. Error bars represent SEM; * $p < 0.05$, ***$p < 0.001$.

sleep (Figure 3C). Importantly, genetically increasing sleep in these flies decreased Aβ burden (Figures 3B and 3D). These data thus provide further evidence that sleep can modulate Aβ burden and suggest that enhancing sleep can reduce Aβ pathology.

### Manipulation of Neuronal Excitability Alters Aβ Accumulation

Previous work suggests that sleep deprivation increases neuronal excitability and synaptic transmission [15, 38]. We thus investigated whether alterations in neuronal excitability might underlie the changes in Aβ burden that we observe with manipulations of sleep. We first asked whether increasing neuronal excitability would result in an increase in Aβ accumulation. Expression of the bacterial sodium channel NaChBac [39] in the MBs along with AβArctic (*OK107-Gal4>UAS-NaChBac, UAS-AβArctic*) resulted in a significant increase in Aβ signal in the MBs (Figure S2B). However, this manipulation simultaneously reduced nighttime sleep in these flies (Figure S2A), making it difficult to disentangle whether activating these cells alone leads to an increase in Aβ burden. Therefore, to further assess this issue, we examined Aβ levels in the ExFl2 FB neurons in *R72G06-Gal4>UAS-NaChBac, AβArctic* flies. In flies expressing NaChBac and AβArctic simultaneously in these ExFl2 FB neurons, daytime sleep was increased compared to controls (Figure S2C), and a significant increase in Aβ burden was observed in the ExFl2 cells, compared to controls (Figure S2D). These data thus dissociate changes in sleep from changes in excitability and suggest that enhancing neuronal excitability acts downstream of changes in sleep to increase Aβ burden.

To further address whether changes in excitability act downstream of sleep in modulating Aβ, we asked whether reducing neuronal excitability could suppress the increase in Aβ signal seen with sleep deprivation. We used dORKΔC2, an outward rectifying potassium channel [40], to decrease excitability of MB KC neurons. As a control, we used the non-conducting version of this channel, dORKΔNC. Reducing neuronal excitability in MB KCs led to a trend toward an increase in daytime sleep in animals not undergoing sleep deprivation, while a marked reduction in nighttime sleep was observed in all animals undergoing sleep deprivation (Figure 4A). As shown in Figures 4B and 4C, electrically inhibiting the MB KCs essentially blocked the increase in Aβ burden caused by sleep deprivation. These data suggest that hyperexcitability is necessary for sleep deprivation-dependent increases in Aβ accumulation.

### Sleep Deprivation Increases Intrinsic Neuronal Excitability

In order to investigate the functional relationship between sleep, neuronal excitability, and Aβ, we decided to examine different neuronal cell groups. The circadian network in *Drosophila* consists of ~150 neurons comprised of different cell groups with distinct patterns of neuronal activity [41, 42]. The lateral groups (the ventrolateral and dorsolateral) are particularly suitable for the identification and imaging of individual cells. In addition, the ventrolateral neurons can be readily accessed for whole-cell patch-clamp recordings, and the large ventrolateral neurons (l-LNvs) in particular have been shown to play a role in sleep/wake regulation [43–48]. Thus, the l-LNvs can be used to examine the relationship between sleep, neuronal activity, and Aβ accumulation in a single cell type. We used the *cry-Gal4* driver to manipulate the l-LNvs and the dorsolateral (LNd) groups (Figure 5A). We first expressed AβArctic in these cells and investigated whether chronic sleep deprivation increased Aβ burden. As shown in Figures 5B and 5C, nighttime sleep deprivation

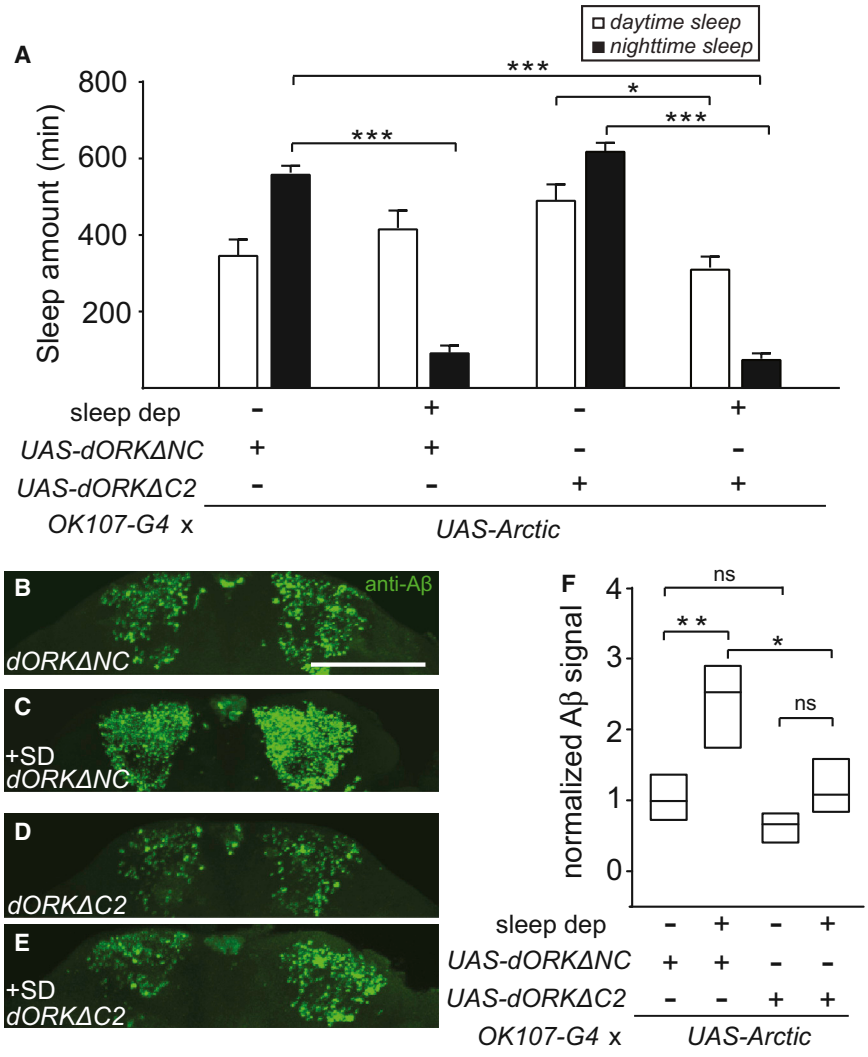


Figure 4. Inhibiting Neuronal Excitability Suppresses Aβ Accumulation Induced by Sleep Loss

(A–F) Daytime and nighttime sleep amounts (A), representative maximum projection images of KCs from whole-mount brains immunostained with 6E10 (B–E), and normalized Aβ signal intensity in the MB KC neurons (F) for *OK107-Gal4>UAS-AβArctic, UAS-dORKΔNC* without sleep deprivation (n = 20) and with sleep deprivation (n = 13) and *OK107-Gal4>UAS-AβArctic, UAS-dORKΔC2* without sleep deprivation (n = 17) and with sleep deprivation (n = 13). Scale bar represents 100 μm. Error bars represent SEM; *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

caused an increase in Aβ signal in l-LNv cells, compared to controls, but not in LNd cells (which may reflect higher baseline Aβ expression in those cells; data not shown).

Our previous data suggest that changes in excitability act downstream of alterations in sleep to modulate Aβ levels. Indeed, previous studies have suggested that sleep "downscales" synaptic strength and that consequently, sleep deprivation leads to increased synaptic transmission [15]. Thus, we sought evidence that sleep deprivation could directly affect neuronal excitability. To address this question, we performed whole-cell patch-clamp recordings of l-LNvs from animals with or without sleep deprivation and examined both the spontaneous action potential (AP) firing rate and evoked firing responses. As expected, nighttime sleep deprivation resulted in a significant decrease in nighttime sleep (Figure S3C). In order to isolate the l-LNv neurons from most excitatory and inhibitory inputs, we performed these recordings in the presence of mecamylamine (50 μM) and picrotoxin (250 μM). As shown in Figures 5D and 5E, the spontaneous AP firing rate of the l-LNv neurons was significantly increased (~1.8-fold) in flies subjected to sleep deprivation versus controls. Similar data were also obtained from measuring evoked responses from these neurons. Evoked AP firing rate was increased at all measured depolarizing currents (Figure 5F), and the frequency-current (*f-I*) slope was significantly increased in sleep-deprived animals, compared with controls (Figure 5G). We did not observe any significant change in resting membrane potential (RMP) in sleep-deprived animals versus controls (Figures S3A and S3B). Together, these data suggest that sleep deprivation enhances both spontaneous and evoked measures of intrinsic neuronal excitability.

### Aβ-Dependent Hyperexcitability Is Exacerbated by Sleep Deprivation

Next, we examined whether Aβ expression itself could also modulate neuronal excitability. Work in mammals has been conflicting, suggesting that Aβ can cause either a decrease or an increase in neuronal excitability [49–51]. To address this question in the *Drosophila* brain, we performed whole-cell patch-clamp recordings on the l-LNv neurons in flies overexpressing Aβ40, Aβ42, or AβArctic using *cry-Gal4*. As shown in Figure 6A, expression of AβArctic, but not Aβ40 or Aβ42, led to a significant increase in spontaneous AP firing rate compared to controls. We next examined evoked parameters of excitability and found that overexpression of AβArctic, but not Aβ40 or Aβ42, resulted in a significant increase in intrinsic neuronal excitability. In particular, the frequency of AP firing was increased in response to a range of injected currents, and the *f-I* slope was similarly increased (Figures 6B and 6C). RMP was not significantly affected in any of these conditions (Figure S4A). Together, these data suggest that AβArctic expression on its own can enhance intrinsic neuronal excitability.

Given our previous finding that sleep deprivation alone could cause neuronal hyperexcitability, we next asked whether chronic sleep deprivation would further exacerbate AβArctic-induced hyperexcitability. We examined *cry-Gal4>UAS-AβArctic* flies subjected to chronic sleep deprivation (Figure S4C) and found that these flies exhibited a further increase in spontaneous and evoked intrinsic neuronal excitability, compared to non-sleep-deprived *cry-Gal4>UAS-AβArctic* as well as controls (Figures 6D–6G). RMP was depolarized, further suggesting that excitability was increased in the presence of AβArctic with sleep deprivation (Figure S4B).

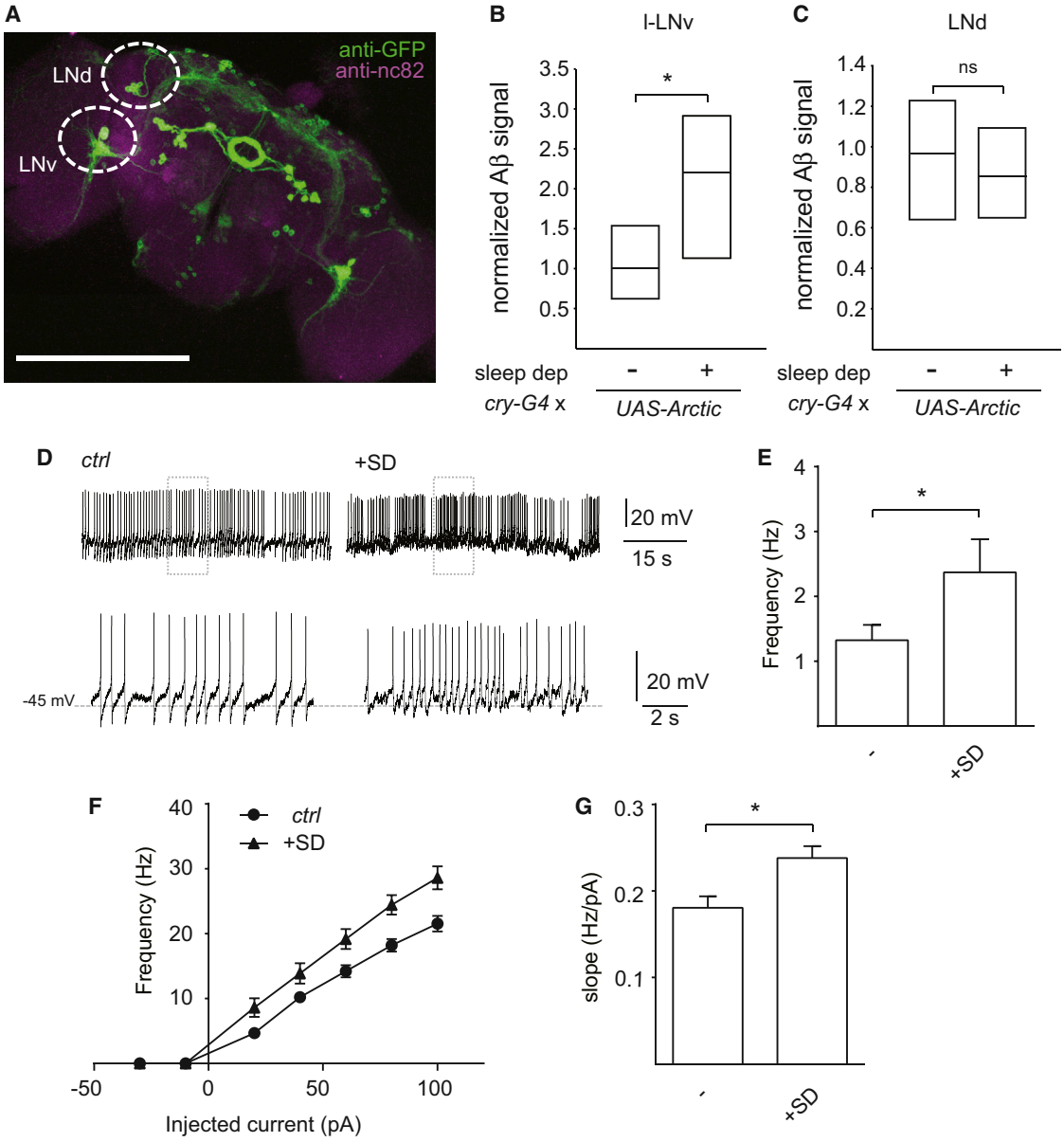


Figure 5. Sleep Deprivation Increases Intrinsic Neuronal Excitability

(A) Maximum projection of a whole-mount brain immunostained with anti-GFP from *cry-Gal4>UAS-CD8::GFP*.

(B and C) Normalized Aβ signal intensity in l-LNv (B) and LNd (C) cells for *cry-Gal4>UAS-AβArctic* with (n = 24) or without (n = 21) sleep deprivation, shown as a simplified boxplot.

(D) Representative traces showing spontaneous AP firing of l-LNvs at ZT0-3 in *cry-Gal4>UAS-CD8::GFP* flies with or without sleep deprivation (SD). The bottom traces in (D) are expanded traces of the boxed regions in the top traces.

(E–G) Mean firing rate of spontaneous activity (E), mean frequency of spikes elicited in response to current injections with 300-ms stepping pulses at 20-pA increments ranging from −30 pA to 100 pA (F), and *f-I* slope (G) of l-LNv neurons in control (*cry-Gal4>UAS-CD8::GFP*) animals with (n = 12) or without (n = 15) sleep deprivation.

Recordings were performed in the presence of mecamylamine (50 μM) and picrotoxin (250 μM) in order to isolate these cells from most excitatory and inhibitory inputs. Scale bar represents 200 μm. Error bars represent SEM; *p < 0.05; ns, not significant.

In summary, these data suggest that sleep deprivation exacerbates the intrinsic neuronal hyperexcitability induced by Aβ expression.

### Sleep Deprivation and Aβ Expression Lead to Impairment of Specific $K^+$ Channel Currents

Changes in excitability could reflect alterations in a variety of ionic currents. Given that $K^+$ channels in particular have been implicated in the regulation of sleep previously, we focused on changes to $K^+$ currents [52–57]. To identify possible mechanisms for the hyperexcitability observed in l-LNv cells following chronic sleep deprivation (Figure S4D), we recorded steady-state activation of three types of $K^+$ currents: A-type $K^+$ current ($I_A$), sustained $K^+$ current ($I_{K(V)}$), and $Ca^{2+}$-activated $K^+$ currents ($K_{Ca}$) under voltage-clamp configuration. We found that over a range of membrane potentials, all three

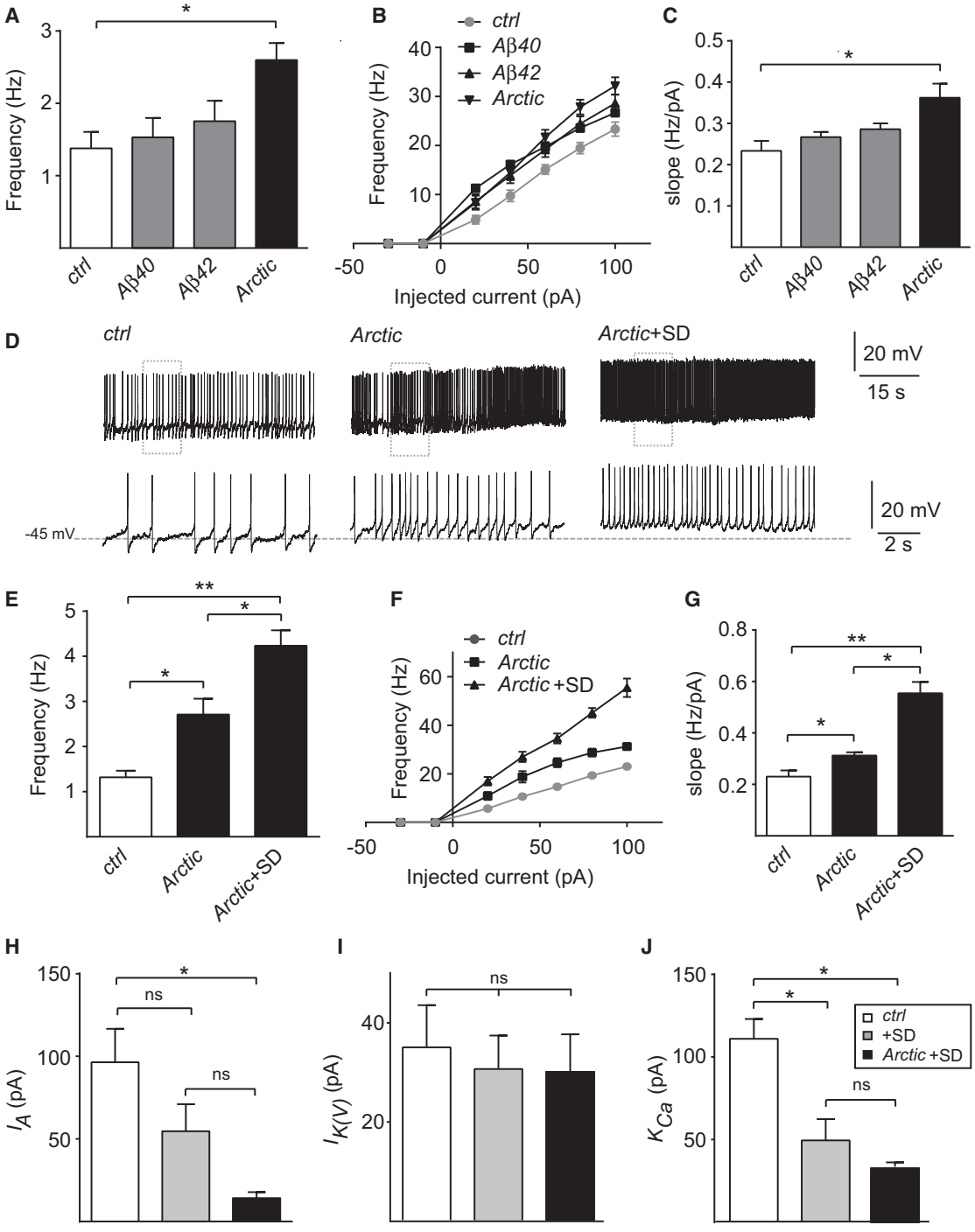


Figure 6. Sleep Deprivation Exacerbates Aβ-Dependent Neuronal Hyperexcitability

(A–C) Mean firing rate of spontaneous activity (A), mean frequency of spikes elicited in response to current injections ranging from −30 pA to 100 pA (B), and *f-I* slope (C) of l-LNv neurons in control *cry-Gal4>UAS-CD8::GFP* (n = 16), *cry-Gal4>UAS-Aβ40, UAS-CD8::GFP* (n = 17), *cry-Gal4>UAS-Aβ42, UAS-CD8::GFP* (n = 15), and *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* (n = 18).

(D) Representative traces showing AP firing of l-LNv neurons in control versus *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* flies ± sleep deprivation (SD). Bottom traces in (D) are expanded traces of the boxed regions in the top traces.

(E–G) Mean firing rate of spontaneous activity (E), mean frequency of spikes elicited in response to current injections ranging from −30 pA to 100 pA (F), and *f-I* slope (G) of l-LNv neurons in control (n = 17) versus *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* with (n = 15) or without (n = 19) sleep deprivation.

(H–J) $I_A$ (H), $I_{K(V)}$ (I), and $K_{Ca}$ (J) current amplitude at the spike threshold (−30 mV) from l-LNvs for *cry-Gal4>UAS-CD8::GFP* with (n = 5, 10, and 11, respectively) or without (n = 5, 7, and 8, respectively) sleep deprivation and *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* with sleep deprivation (n = 5, 6, and 4, respectively).

Recordings were performed in the presence of mecamylamine (50 μM) and picrotoxin (250 μM) in order to isolate these cells from most excitatory and inhibitory inputs. Error bars represent SEM; *p < 0.05, **p < 0.01; ns, not significant.

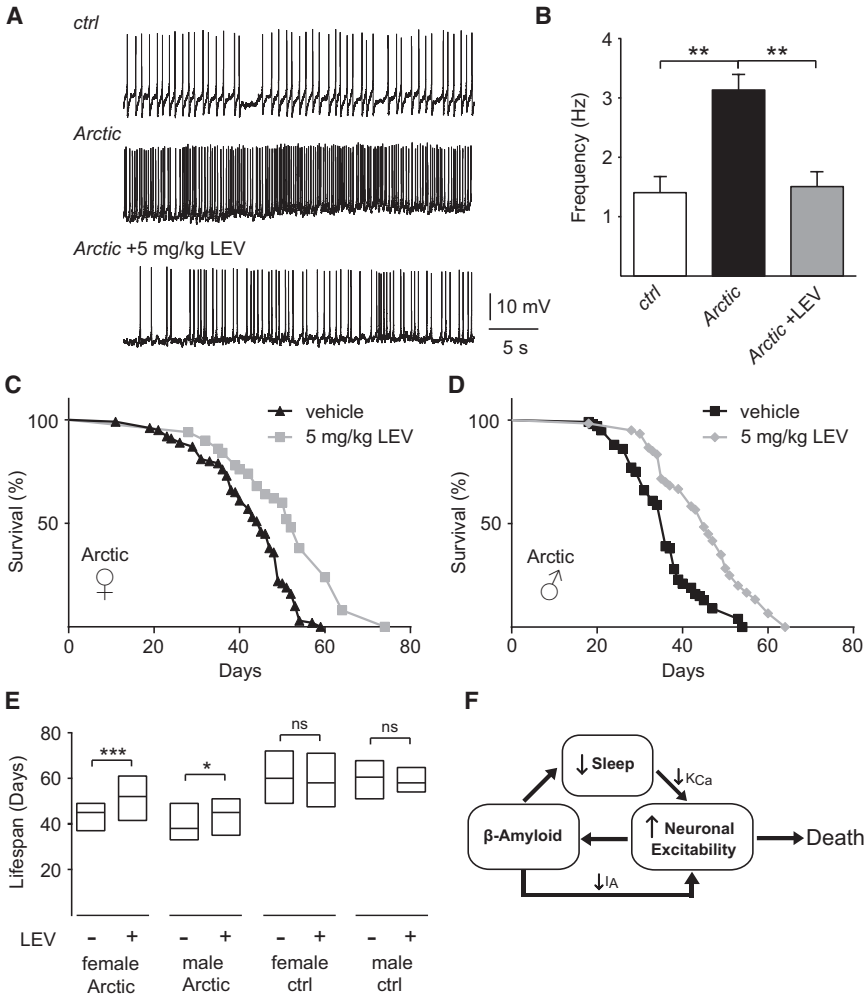


Figure 7. Levetiracetam Suppresses Neuronal Firing and Prolongs Lifespan of AβArctic-Expressing Flies

(A) Representative traces showing spontaneous firing of l-LNv neurons in control (*cry-Gal4>UAS-CD8::GFP*) versus *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* flies fed vehicle or 5 mg/kg levetiracetam (LEV).

(B) Quantification of mean firing rates shown in (A) (n = 4 for control, n = 5 for AβArctic, and n = 8 for AβArctic + LEV).

(C and D) Survivorship curves of *elav-Gal4>UAS-AβArctic* female (C) and male (D) flies fed vehicle or 5 mg/kg LEV.

(E) Lifespan extension of *elav-Gal4>UAS-AβArctic* female and male flies by LEV, where lifespan is displayed as a simplified boxplot. Data for the *elav-Gal4>UAS-AβArctic* ("Arctic") flies shown here are the same as in (C) and (D) (n = 98 for vehicle-fed females and n = 52 for LEV-fed females; and n = 100 for vehicle-fed males and n = 60 for LEV-fed males). For *elav-Gal4/+* ("ctrl") flies, n = 30 for vehicle-fed and LEV-fed females and males.

(F) Model connecting sleep, neuronal excitability, and Aβ. Sleep loss leads to a reduction in $Ca^{2+}$-dependent $K^+$ currents, causing neuronal hyperexcitability. This enhanced excitability, in turn, results in increased Aβ accumulation. Aβ itself reduces sleep and further increases neuronal excitability via a decrease in voltage-gated $K^+$ currents, generating a positive feedback loop whereby sleep loss and Aβ interact to substantially increase neuronal activity and Aβ burden. Increased neuronal excitability then contributes to reduced lifespan.

Error bars represent SEM; *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant.

currents from sleep-deprived flies were significantly reduced compared with non-deprived control flies (Figures S5A–S5C). However, at a potential (−30 mV) near the spike threshold, only $K_{Ca}$ currents showed a significant reduction compared to non-sleep-deprived controls (Figures 6J and S5F). We next subjected *cry-Gal4>UAS-AβArctic* flies to sleep deprivation and found that, in addition to $K_{Ca}$ currents, $I_A$ currents became markedly reduced at potentials near the spike threshold (Figures 6H and S5D). In sleep-deprived animals with or without AβArctic expression, there was no significant effect on $I_{K(V)}$ currents (Figures 6I and S5E). These data suggest that alterations in $K_{Ca}$ and $I_A$ currents may play a role in the changes of excitability observed in l-LNv neurons under conditions of sleep deprivation and AβArctic expression.

## Levetiracetam Prolongs the Lifespan of Aβ-Expressing Animals

Our data point toward changes in neuronal excitability as a key mediator of the effects of sleep loss on Aβ deposition. Furthermore, Aβ expression itself can induce neuronal hyperexcitability. Interestingly, abnormal elevations in neuronal activity can be seen in a variety of "pre-Alzheimer" states, including mild cognitive impairment (MCI) and ApoE4 carrier status [58, 59]. Furthermore, recent studies in humans with MCI and an AD mouse model have suggested that reducing neuronal excitability using the anticonvulsant levetiracetam (LEV) improves performance in learning and memory tests [60, 61]. Therefore, we wished to test the functional relevance of the hyperexcitability observed in AβArctic-expressing flies by using LEV. We first examined whether feeding LEV to AβArctic-expressing flies would suppress the increased AP firing rate seen in their l-LNv neurons. As shown in Figures 7A and 7B, when *cry-Gal4>UAS-AβArctic* flies were chronically fed LEV (5 mg/kg) in their food, the increased AP firing rate of l-LNv neurons was reduced back to control levels. Neuronal activity can be associated with changes in neuronal structure [62], and the LNv neurons have been shown to exhibit experience- and sleep-dependent morphological changes [63, 64]. Therefore, we asked whether AβArctic expression coupled with sleep deprivation affected the synaptic morphology of these neurons and whether LEV might suppress this effect. The LNv neurons express the neuropeptide pigment dispersing factor (PDF) and so we used anti-PDF to label the synaptic terminals of these cells. Sleep-deprived flies expressing AβArctic in their LNv neurons displayed a significant increase in PDF+ puncta in the optic lobes, and LEV treatment significantly inhibited this effect (Figure S6A). However, given that PDF is a releasable neuropeptide, we cannot rule out that these changes reflect alterations in the production or release of PDF. We next asked whether LEV treatment could inhibit the increase in Aβ burden in l-LNv neurons seen with sleep deprivation. As shown in Figure S6B, LEV treatment resulted in a trend toward suppression

of the increased Aβ burden induced by sleep deprivation, although this effect was not statistically significant.

Finally, pan-neuronal expression of AβArctic peptide in flies has previously been shown to reduce lifespan [28], so we asked whether suppressing neuronal hyperactivity by feeding these flies LEV would prolong their lifespan. We examined lifespan in *elav-Gal4>UAS-AβArctic* flies and found, as expected, that these flies exhibited a reduction in their median lifespan, when compared to *elav-Gal4* controls (60 versus 45 days for females and 61 versus 38 days for males) (Figure 7E). Strikingly, when *elav-Gal4>UAS-AβArctic* flies were fed LEV (5 mg/kg), their median lifespan was significantly extended by ~16% and ~18% for females and males, respectively (Figures 7C–7E). In contrast, LEV did not extend the lifespan of control *elav-Gal4/+* female or male flies, suggesting that these effects are specific to AβArctic-expressing flies (Figures S6C and S6D). Together, these data strongly suggest that neuronal hyperexcitability is an important mediator of Aβ-induced toxicity.

## Discussion

Our study supports a bidirectional relationship between sleep and Aβ and points toward an intimate relationship between sleep, neuronal excitability, and Aβ. Loss of sleep leads to neuronal hyperexcitation, which in turn increases Aβ burden. Aβ expression both reduces sleep and further enhances neuronal excitability (Figure 7F). Furthermore, suppression of Aβ-induced neuronal hyperexcitability significantly prolongs lifespan, suggesting that abnormal neuronal activity is an important mediator of Aβ toxicity.

How are changes in sleep related to alterations in neuronal excitability? In animals ranging from flies to humans, sleep is associated with broad changes in patterns of electrical activity [65, 66]. Although the function of sleep remains controversial, one prominent hypothesis is that sleep functions to downscale synaptic strength [15]. Along these lines, prolonged wakefulness has been associated with an increase in evoked cortical local field potential amplitudes in rats [16] and transcranial magnetic stimulation (TMS) measures of cortical excitability in humans [67]. Our data support this hypothesis, as sleep deprivation in flies leads to hyperexcitability of l-LNv neurons. Another recent study found that the ExFl2 neurons in *Drosophila* also exhibited increased excitability with sleep deprivation, although in that case, it was suggested that this phenotype was related to the specific sleep-promoting function of those neurons [37].

There are a number of studies on the effects of APP or Aβ expression on neuronal activity in animal models of AD [49]. These studies, which have largely been conducted in mammalian systems, have been conflicting, possibly because different cell types and neuronal networks may behave differently in response to exposure to Aβ. Here, we have recorded from a single cell type (the l-LNvs) from intact fly brains and found that Aβ expression markedly increases intrinsic neuronal excitability and that this effect is exacerbated by sleep deprivation. Interestingly, patients with epilepsy exhibit elevated amounts of Aβ plaque in their brains [68]. Moreover, seizures are commonly seen in patients with early-onset AD carrying mutations in Presenilin 1 [69], and TMS studies have found increased excitability of primary motor cortex in patients with AD [70].

Our study suggests that neuronal hyperexcitability is an important and early contributor to the pathogenesis of AD. Changes in neuronal excitability in AD likely predate neurodegenerative changes [71]. For example, in young mice overexpressing APP, hippocampal neurons were found to be hyperactive, prior to the formation of Aβ plaques [50]. Furthermore, recent evidence suggests that increases in neuronal excitability may be deleterious for cognitive function. Indeed, patients with amnestic MCI exhibit increased hippocampal activation by high-resolution fMRI, and reduction of this hippocampal activation with LEV improved memory performance in these subjects [60]. Similar observations have been made in a mouse model of AD and in aged rats with cognitive impairment [61, 72]. We now provide evidence that reducing the neuronal activity of Aβ-expressing flies with LEV prolongs their lifespan. Thus, taken together, these findings imply that early treatment of preclinical AD patients with anti-epileptic medications may be beneficial in slowing the course of disease. Our findings reveal an important interaction between Aβ and sleep loss in modulating neuronal excitability and suggest that sleep loss, neuronal hyperexcitability, and Aβ accumulation form a positive feedback loop. As therapeutic interventions exist to manipulate sleep as well as neuronal excitability, these data suggest that targeting these pathways may be a fruitful approach toward slowing the progression or delaying the onset of this incurable disease.

### Experimental Procedures

Details of experimental procedures are available in the online Supplemental Information.

### Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.016.

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**Current Biology**

**Supplemental Information**

# Sleep Interacts with Aβ to Modulate Intrinsic Neuronal Excitability

**Masashi Tabuchi, Shahnaz R. Lone, Sha Liu, Qili Liu, Julia Zhang, Adam P. Spira, and Mark N. Wu**

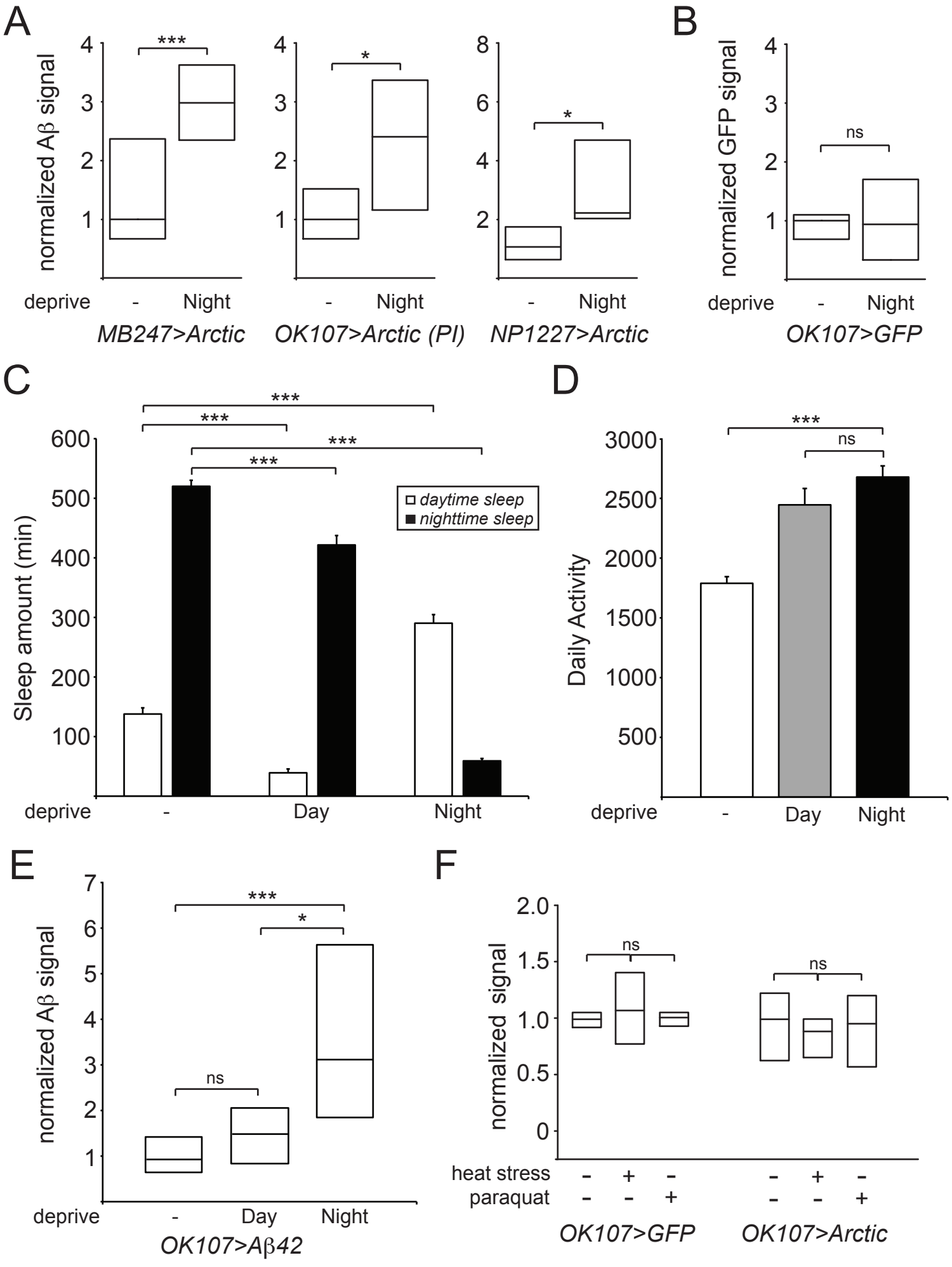
A
normalized Aβ signal
***
*
*
deprive
-
Night
MB247>Arctic
OK107>Arctic (PI)
NP1227>Arctic
B
normalized GFP signal
ns
deprive
-
Night
OK107>GFP
C
Sleep amount (min)
daytime sleep
nighttime sleep
deprive
-
Day
Night
D
Daily Activity
***
ns
deprive
-
Day
Night
E
normalized Aβ signal
***
*
ns
deprive
-
Day
Night
OK107>Aβ42
F
normalized signal
ns
ns
heat stress
paraquat
OK107>GFP
OK107>Arctic

**Figure S1. Sleep Deprivation Specifically Increases Aβ Accumulation, Related to Figure 2**

(A) Normalized Aβ signal intensity for *MB247-LexA>LexAop-AβArctic* with no (n=20) or nighttime (n=24) sleep deprivation, *OK107-Gal4>UAS-AβArctic* with no (n=12) or nighttime (n=10) sleep deprivation, and *NP1227-Gal4>UAS-AβArctic* with no (n=14) or nighttime (n=12) sleep deprivation. (B) Normalized GFP signal intensity in the MB KC from *OK107-Gal4>UAS-GFP* flies undergoing no (n=8) or nighttime (n=7) sleep deprivation. Sleep amount (C), daily activity (D), and normalized Aβ signal intensity in the MB KC (E) from *OK107-Gal4>UAS-Aβ42* flies undergoing no (n=14), daytime (n=16), or nighttime (n=19) sleep deprivation. "-", "Day", and "Night" denote no, daytime, and nighttime sleep deprivation, respectively. (F) Normalized GFP or Aβ signal intensity for *OK107-Gal4>UAS-GFP* subjected to no stress (n=24), 31°C (n=20), and 1 mM paraquat (n=32) for 1 week and *OK107-Gal4>UAS-AβArctic* subjected to no stress (n=24), 31°C (n=20), and 1 mM paraquat (n=32) for 1 week.

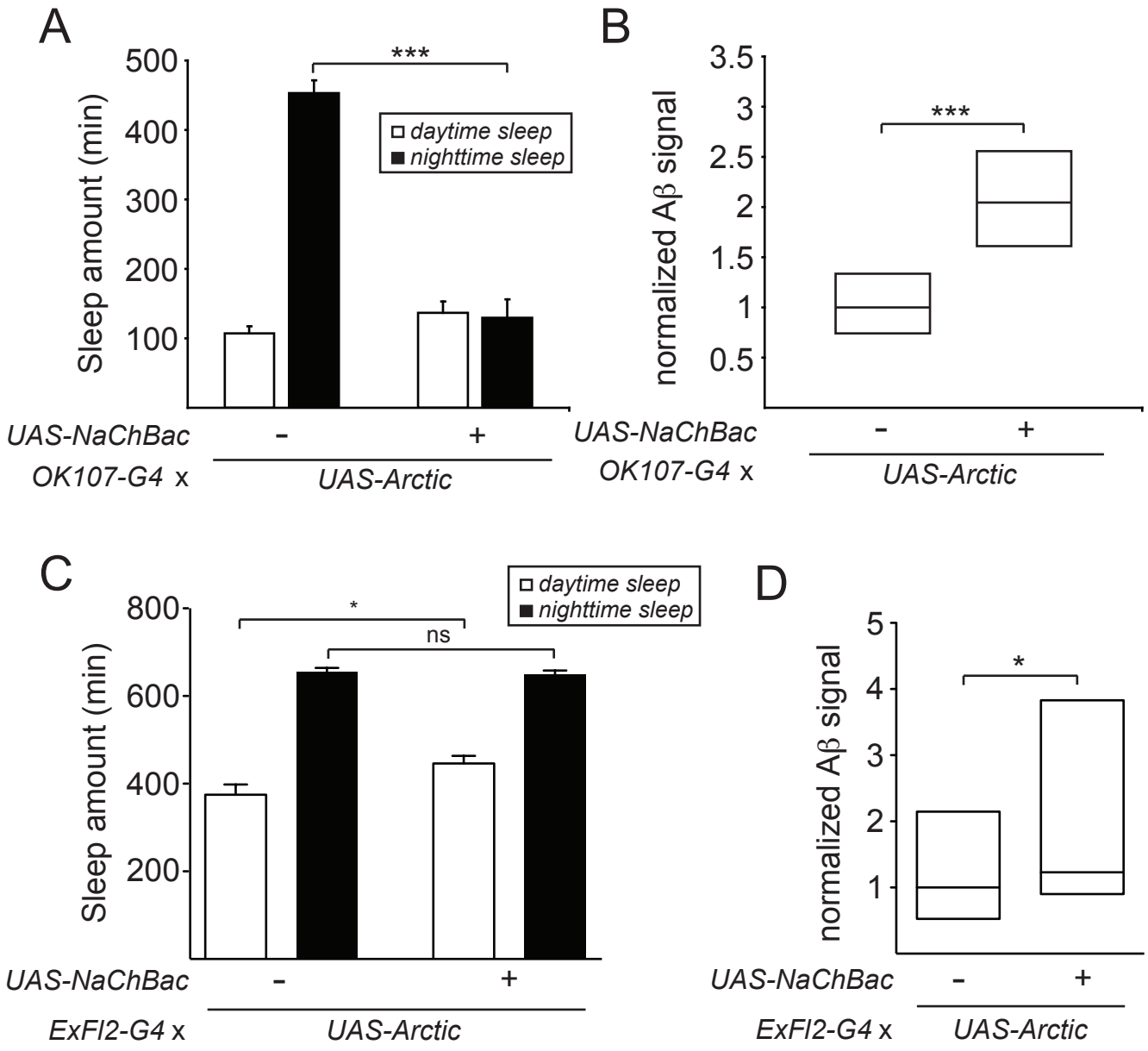


**Figure S2. Neuronal Excitation Enhances AβArctic Burden, Related to Figure 4**
Daytime and nighttime sleep amount (A) and normalized Aβ signal intensity in the MB KC (B) for *OK107-Gal4>UAS-AβArctic* (n=51) and *OK107>UAS-AβArctic, UAS-NaChBac* (n=53) flies. Daytime and nighttime sleep amount (C) and normalized Aβ signal intensity in the ExFl2 neurons (D) for *R72G06-Gal4>UAS-AβArctic, UAS-GFP* (n=25) vs *R72G06-Gal4>UAS-AβArctic, UAS-NaChBac* (n=26) flies

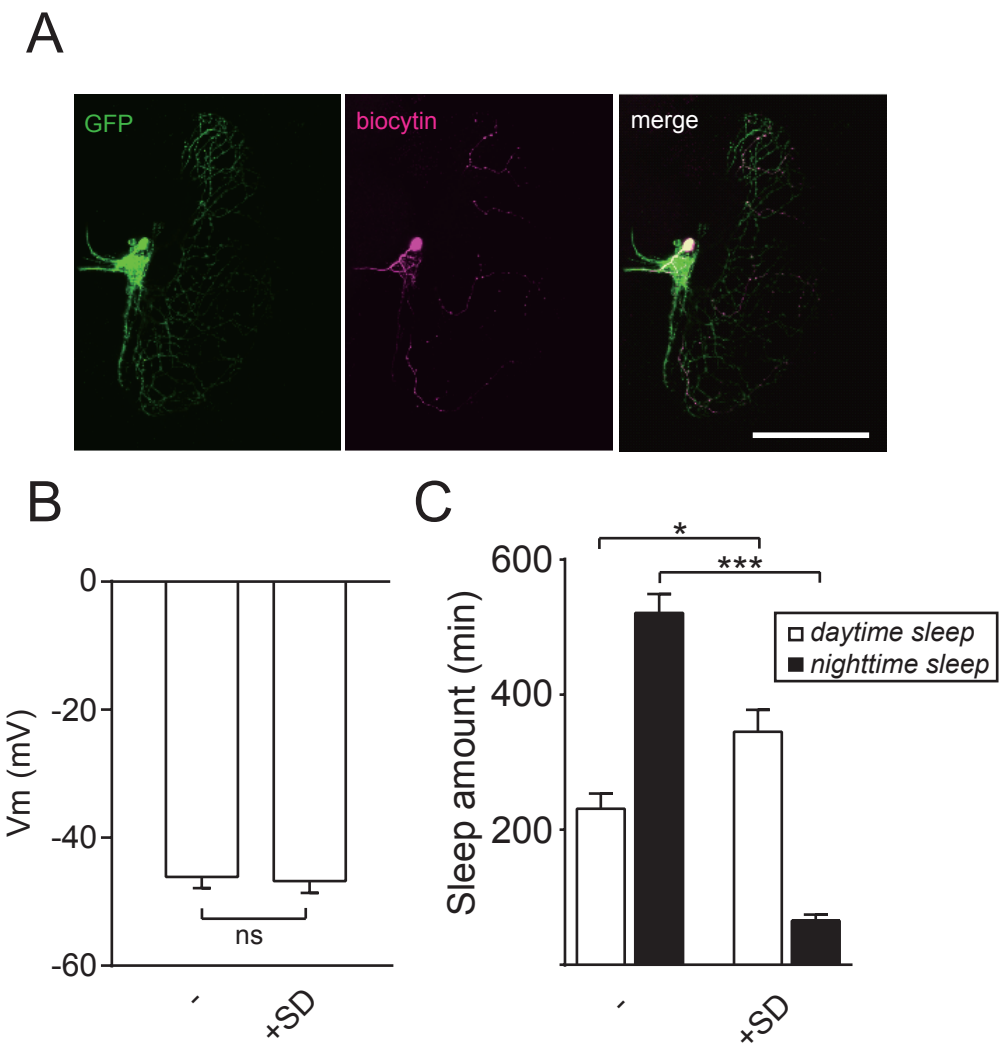


**Figure S3. Additional Electrophysiological Parameters, Related to Figure 5**

(A) Immunostaining of a *cry-Gal4>UAS-CD8::GFP* fly brain with a biocytin-filled l-LNv cell. Scale bar, 100 μm. Resting membrane potential during recording of spontaneous activity (B) and daytime and nighttime sleep amounts (C) of each group shown in Figures 5D-5G.

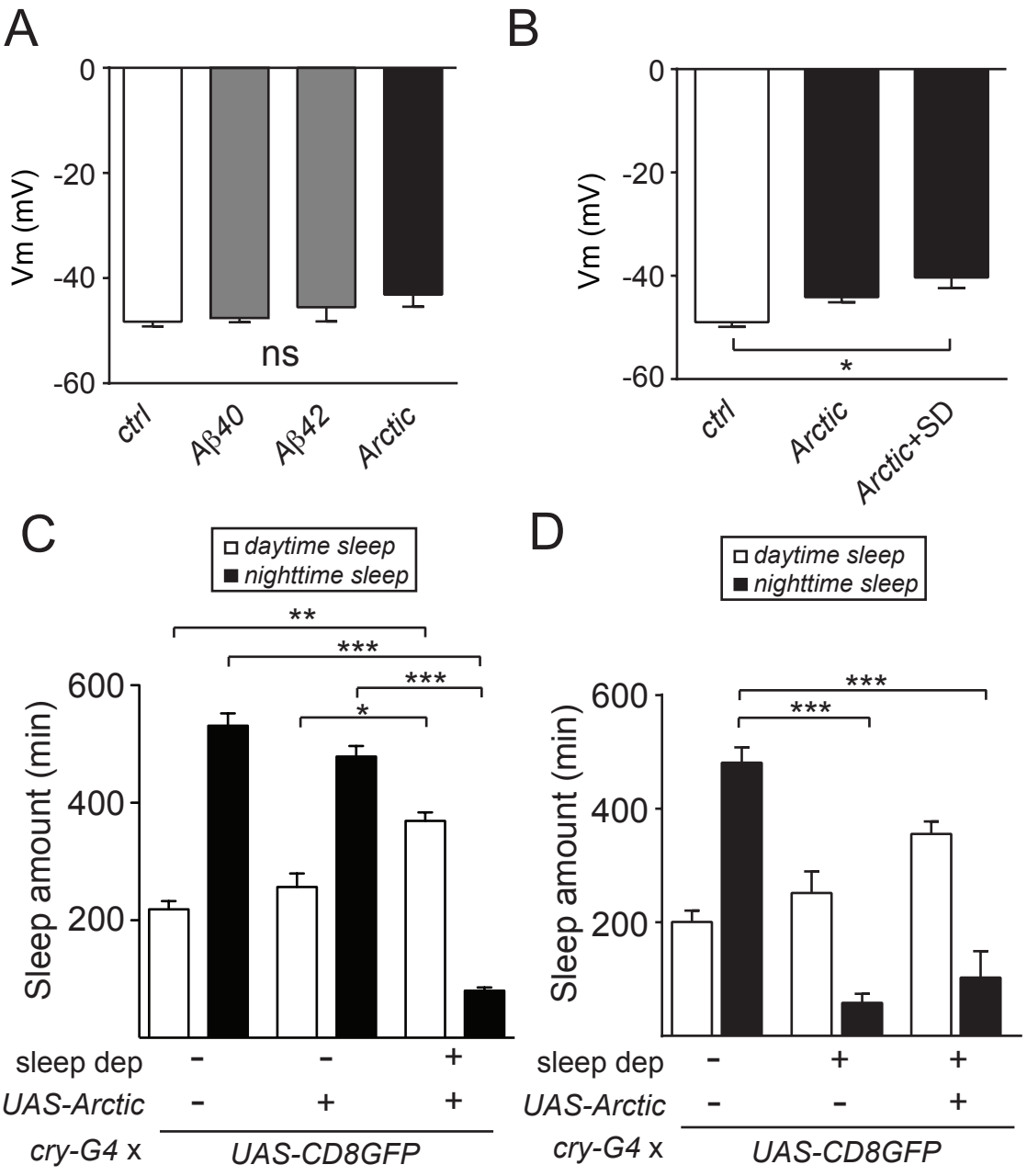


**Figure S4. Additional Electrophysiological Parameters, Related to Figure 6**
Resting membrane potential of each group during the recording of spontaneous activity, shown in Figures 6A (A) and 6E (B). Daytime and nighttime sleep amounts for groups shown in Figures 6E-6G (C) and Figures 6H-6J (D).

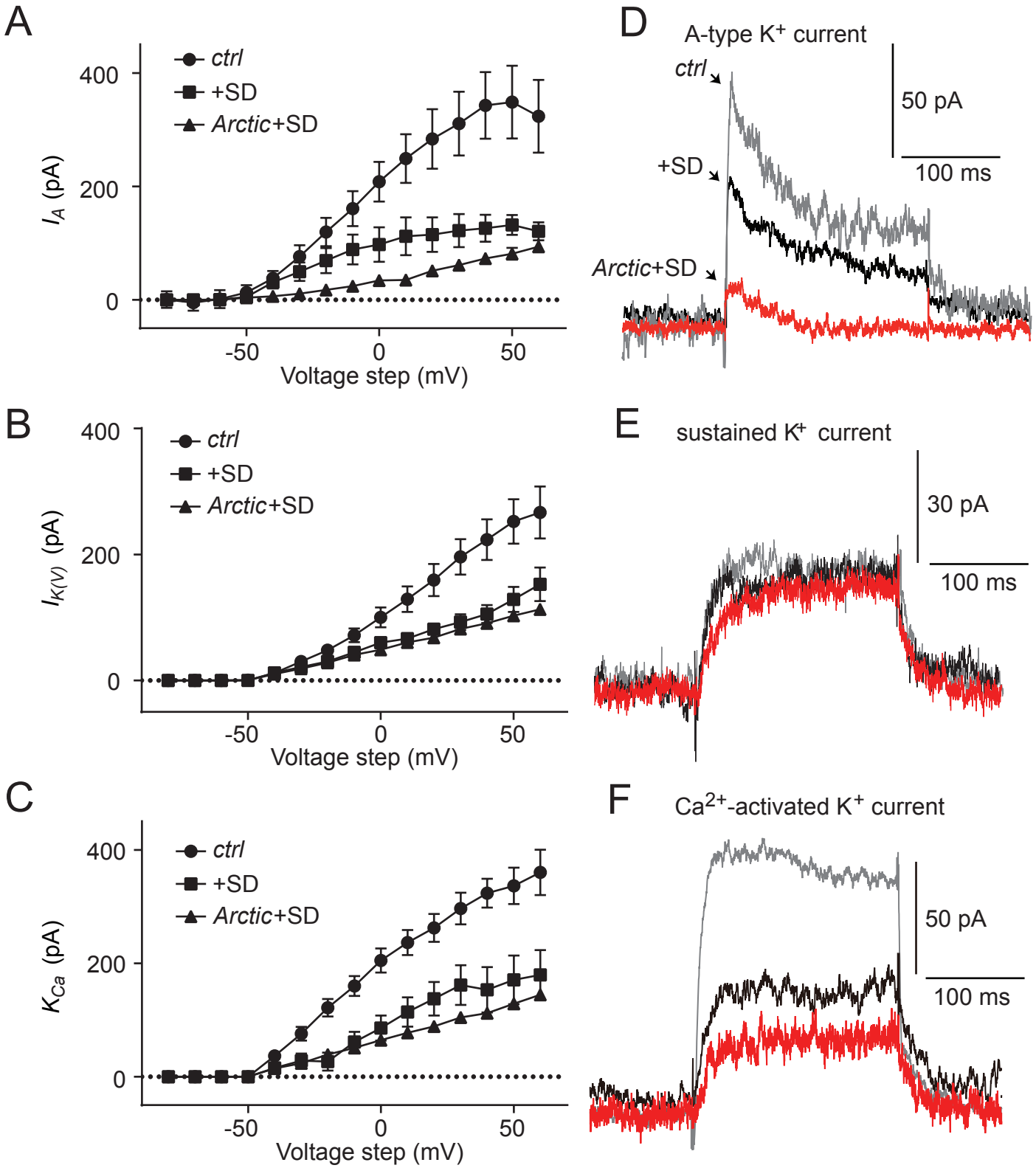
A
ctrl
+SD
Arctic+SD
400
200
0
$I_A$ (pA)
-50
0
50
Voltage step (mV)
B
ctrl
+SD
Arctic+SD
400
200
0
$I_{K(V)}$ (pA)
-50
0
50
Voltage step (mV)
C
ctrl
+SD
Arctic+SD
400
200
0
$K_{Ca}$ (pA)
-50
0
50
Voltage step (mV)
D
A-type K+ current
ctrl
+SD
Arctic+SD
50 pA
100 ms
E
sustained K+ current
30 pA
100 ms
F
Ca2+-activated K+ current
50 pA
100 ms

**Figure S5. Additional Electrophysiological Data, Related to Figure 6**

*I–V* relationships for $I_A$ (A), $I_{K(V)}$ (B), and $K_{Ca}$ (C) current amplitudes measured in l-LNv cells from *cry-Gal4>UAS-CD8::GFP* flies with and without sleep deprivation and *cry-Gal4>UAS-AβArctic, UAS-CD8::GFP* flies with sleep deprivation. Averaged current traces for steady-state activation of $I_A$ (D), $I_{K(V)}$ (E), and $K_{Ca}$ (F) in l-LNvs at the spike threshold (-30 mV). These data are from the same cells as shown in Figures 6H-6J.

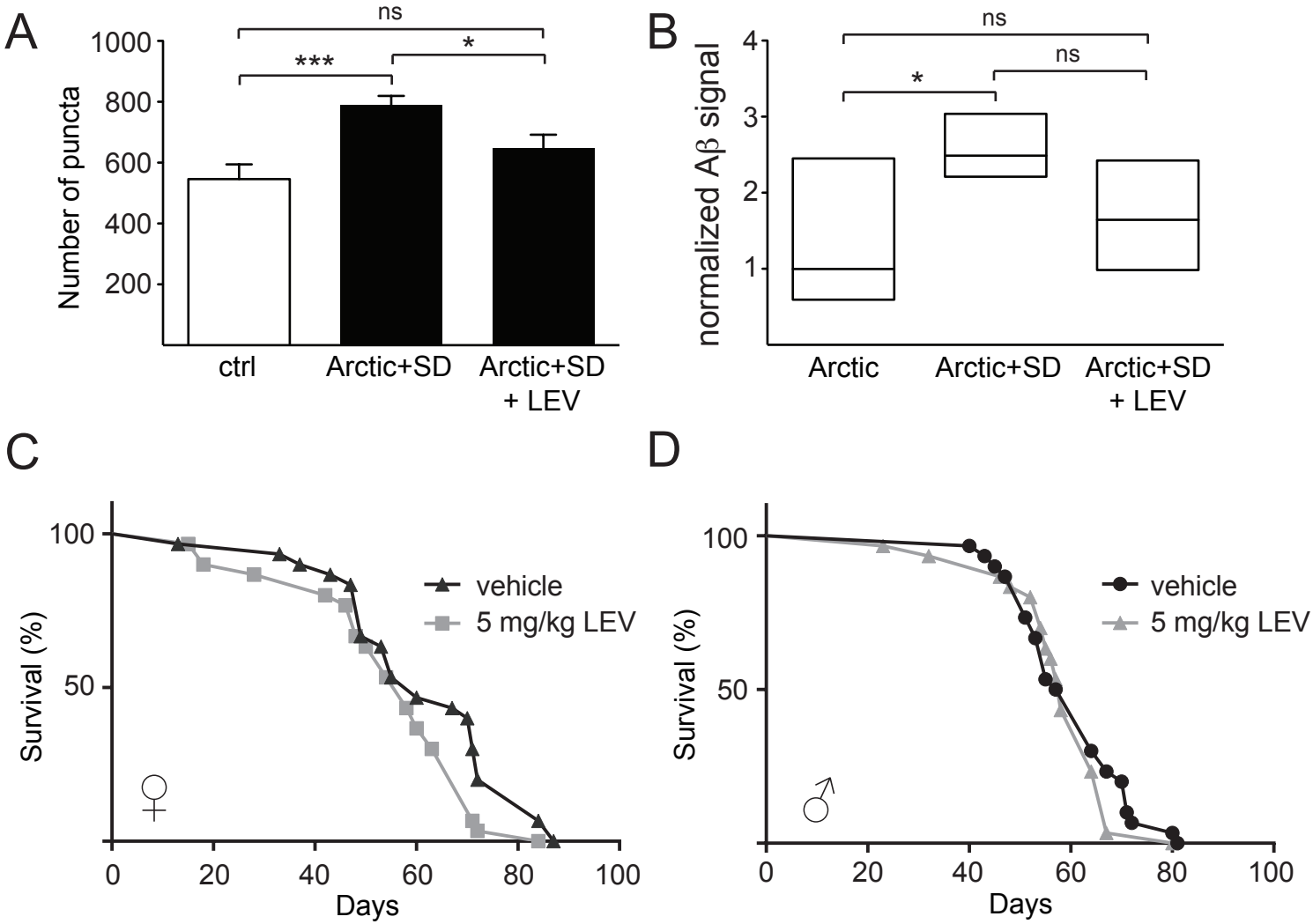


**Figure S6. Additional Levetiracetam Data, Related to Figure 7**
(A) Number of PDF+ puncta for *cry-Gal4>UAS-CD8::GFP* (n=15, "ctrl") vs *cry-Gal4>UAS-AβArctic* subjected to nighttime sleep deprivation and fed vehicle (n=20) or 5 mg/kg LEV (n=16). (B) Normalized Aβ signal intensity for *cry-Gal4>UAS-AβArctic* (n=10) vs *cry-Gal4>UAS-AβArctic* subjected to nighttime sleep deprivation and fed vehicle (n=7) or 5 mg/kg LEV (n=7). (C) Survivorship curves of *elav-Gal4/+* female flies fed vehicle or 5 mg/kg LEV (n=30 for vehicle and n=30 for 5 mg/kg LEV). (D) Survivorship curves of *elav-Gal4/+* male flies fed vehicle or 5 mg/kg LEV in their food (n=30 for vehicle and n=30 for 5 mg/kg LEV).

## Supplemental Experimental Procedures

### Fly strains

Flies were maintained on standard food containing molasses, cornmeal, and yeast at room temperature or a 25°C incubator. All strains, including Gal4 and UAS transgenic lines except *daughterless-Geneswitch* (*da-GS*), were outcrossed into the *iso31* genetic background at least 4 times. *MB247-LexA, NP1227-Gal4,* and *da-GS* were obtained from Drs. Tzumin Lee, Mark Stopfer, and Paul Shaw, respectively. *UAS-Aβ42.1 (Alz3)* and *UAS-Arctic42.1 (Arc2E)* were obtained from Dr. Damian Crowther. *cry16-Gal4, elav-Gal4, UAS-NaChBac, R72G06-Gal4, UAS-dORKΔC2, and UAS-dORKΔNC* were obtained from the Bloomington Stock Center. *LexAop-Arctic* transgenic flies were generated using standard techniques in the *iso31* background (Rainbow Transgenics). Unless otherwise specified, female flies were used for all experiments.

### Behavioral Assays

For experiments measuring sleep behavior, 0-2 day old virgin females were loaded into glass tubes containing standard *Drosophila* medium. Flies were monitored using the *Drosophila* Activity Monitoring System (Trikinetics) in an incubator with a 12 hr:12 hr light:dark (LD) cycle. Activity counts were collected in 1 min bins, and sleep was identified as periods of inactivity lasting at least 5 minutes [S1]. Sleep data were collected for 7 days, after discarding the first day following loading. Sleep parameters were analyzed using custom software. In order to induce expression of Aβ using *da-GS*, flies were fed 250 μM RU486 (Sigma-Aldrich) in standard *Drosophila* medium, and flies were flipped to new glass tubes containing fresh food with or without drug after 3 days. For mechanical sleep deprivation experiments, flies were deprived during the day or during the night for 10 hrs per day (flies were allowed to rest during

the first and the last hour of each 12 hr period); flies were stimulated by shaking using a mechanical vortexer (Trikinetics) for 5s every 5 min at 1000 rpm. Sleep deprivation was conducted for 1 week for AβArctic-expressing animals and 2 weeks for Aβ42-expressing animals. For genetic manipulations of sleep, dTrpA1 was activated by maintaining flies at 29°C for 1 week in order to activate the relevant neurons.

**Immunostaining**

Brains were dissected in PBS, fixed with 4% paraformaldehyde in PBS for 30 min, and then washed in PBS. For AβArctic labelling, brains from 1 week old flies were treated with 70% formic acid for 30 min, and incubated with 6E10 (Covance, 1:500) for 16 hrs for *OK107-Gal4*, *MB247-LexA*, and *NP1227-Gal4* and for 48 hrs for *cry-Gal4* and *R72G06-Gal4* on a shaker at 4°C. After washing in PBS, samples were incubated with Alexa568 anti-mouse (Invitrogen, 1:1000) secondary antibodies for 16 hr for *OK107-Gal4*, *MB247-LexA*, and *NP-1227-Gal4* and for 48 hr for *cry-Gal4* and *R72G06-Gal4* on a shaker at 4°C. To improve penetration of 6E10 when staining *cry-Gal4* and *R72G06-Gal4* brains, the glial sheath enveloping the brain was carefully removed. For *OK107-Gal4>UAS-Aβ42* labelling, brains were treated with 70% formic acid for 30 min and incubated with 6E10 for 48 hrs at 4°C, followed by Alexa 568 anti-mouse (Invitrogen, 1:1000) antibodies for 48 hr. In some preparations, rabbit anti-GFP (Invitrogen, 1:200) was used simultaneously for GFP labelling, followed by incubation with fluorescent Alexa488 anti-rabbit (Invitrogen, 1:1000) secondary antibodies. For quantification of GFP (Figure S1B), mouse anti-GFP (Invitrogen, 1:200) was used, followed by incubation with fluorescent Alexa568 anti-mouse (Invitrogen, 1:1000) secondary antibodies. Images were obtained on a Zeiss LSM-700; Carl Zeiss with 0.7-1.0 μm thick sections. Signal intensities were

quantified on a maximum projection image with ImageJ in different regions of interest (ROIs). Net signal intensity ($signal_{net}$) of the ROI was determined by subtracting the mean background intensity adjacent to the ROI from the mean intensity of the ROI. Total intensity was calculated by multiplying $signal_{net}$ x area of the ROI. For quantification of LNv synaptic terminal number, mouse anti-PDF (Developmental Studies Hybridoma Bank) was used at 1:200, followed by incubation with fluorescent Alexa488 anti-mouse (Invitrogen, 1:1000) secondary antibodies. Number of puncta as a measure for synaptic terminal number in confocal images was quantified using an automated threshold algorithm with ImageJ as previously described [S2].

**Heat and Paraquat Treatment**

To examine whether other treatments that induce cellular stress can also increase Aβ accumulation, we subjected 0-2 day old virgin females to 1 week of elevated temperature (31°C) or 1 week of 1 mM paraquat mixed in standard *Drosophila* media. Aβ immunostaining was performed as described above.

**Molecular Biology**

The Aβ42 Arctic sequence, including the signal peptide sequence from the *Drosophila necrotic* gene [S3], was PCR amplified using primers 5' TCG GAA TTC ATG GCG AGC AAA GTC T 3' and 5' TAT CTC GAG TTA CGC AAT CAC CAC GC 3' from *UAS-AβArctic* genomic DNA. The amplified product was then digested with EcoRI and XhoI and subcloned into pLOT [S4].

**Electrophysiological recordings**

**Preparation** *cry-Gal4>UAS-CD8::GFP, cry-Gal4>UAS-CD8::GFP, UAS-Aβ40, cry-Gal4>UAS-CD8::GFP, UAS-Aβ42,* and *cry-Gal4>UAS-CD8::GFP, UAS-AβArctic* female flies were loaded into 5% sucrose/2% agar tubes within 1 day after eclosion and placed in DAMS monitors (Trikinetics) in an incubator on a 12hr:12hr LD cycle. These flies were then subjected to night-time sleep deprivation vs no sleep deprivation for 7 days, as described above. Brains were removed and dissected in a *Drosophila* physiological saline solution (101 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 4 mM $MgCl_2$, 1.25mM $NaH_2PO_4$, 20.7 mM $NaHCO_3$, and 5 mM glucose; pH 7.2), which was pre-bubbled with 95% $O_2$ and 5% $CO_2$. To better visualize the recording site and to facilitate efficient penetration of drugs, the glial sheath surrounding the brain was removed using ultra-fine forceps (MC40; MORIA). Brains were treated with protease XIV (2 mg/ml; Sigma-Aldrich) at 22°C for 5-8 min, and cleaned with a small stream of saline pressure ejected from a large-diameter pipette. The preparation was immobilized on the bottom of a recording chamber using a custom-made anchor. The recording chamber was placed on an X-Y stage platform (PP-3185-00; Scientifica, UK), and the l-LNvs were visualized with GFP fluorescence on a fixed-stage upright microscope (BX51WI; Olympus, Japan) and viewed with a 40× water-immersion objective lens (LUMPlanFl, NA: 0.8, Olympus). As the recording electrode approached the l-LNvs, visualization was achieved by infrared-differential interference contrast (IR-DIC) optics and a CCD camera (CV-A50IR; JAI, Japan).

**Patch-clamp recordings** Whole-cell recordings were performed at room temperature at Zeitgeber time 0-3, in the presence of 50 μM mecamylamine and 250 μM picrotoxin to isolate the cells from most excitatory and inhibitory inputs. It is worth noting that Cao et al. also observed an increase in spontaneous AP firing at this time in the absence of sleep deprivation

(thus when sleep drive is low), but in those experiments mecamylyamine and picrotoxin were not used [S5]. Patch-pipettes (5-9 MΩ) were fashioned from borosilicate glass capillary with a Flaming-Brown puller (P-1000; Sutter Instrument). The internal solution (102 mM potassium gluconate, 0.085 mM $CaCl_2$, 0.94 mM EGTA, 8.5 mM HEPES, 4 mM Mg-ATP, 0.5mM Na-GTP, 17 mM NaCl; pH7.2) was used for both current-clamp recording and voltage-clamp recording. To label recorded cells, we included 13 mM biocytin hydrazide in the pipette solution. Recordings were acquired with an Axopatch 200B amplifier (Molecular Devices) and sampled with Digidata 1440A interface (Molecular Devices). These devices were controlled on a computer using the pCLAMP 10 software (Molecular Devices). The signals were sampled at 20 kHz and low-pass filtered at 2 kHz. Junction potentials were nullified prior to high-resistance (GΩ) seal formation. After establishing a GΩ seal, the membrane was ruptured with instantaneous suction to establish the whole-cell configuration. Series resistance was compensated and leakage currents were subtracted from all records. Cells were rejected if $R_{access}$>50 MΩ with holding currents ($I_{hold}$) larger than 100 pA (at $V_{hold} = -70$ mV). One or two (from the contralateral cluster) neurons per brain were recorded and stained. To exchange the bath solution, the preparation was perfused with saline by means of a gravity-driven system. Electrophysiological analysis was performed in Igor software (WaveMetrics), MATLAB (MathWorks), and Clampfit (Molecular devices).

**Current isolation** In our pilot experiments, we found that l-LNv cells had at least three types of $K^+$ currents in terms of kinetics and pharmacological sensitivities: an A-type K+ current ($I_A$), a sustained K+ current ($I_{K(V)}$), and a $Ca^{2+}$-activated $K^+$ current ($K_{Ca}$). To isolate $I_A$, the brain preparation was bathed with saline containing (in M) $10^{-7}$ TTX, $2 \times 10^{-2}$ TEA, and $5 \times 10^{-4}$ $CdCl_2$ to substantially reduce non-$I_A$ currents. The neurons were held at −70 mV and two

series of 200-ms voltage pulses were delivered in 10-mV increments between −80 and 60 mV. The first series had a 200-ms prepulse to −90 mV to maximally deinactivate $I_A$. The second series had a 200-ms prepulse to −30 mV, where $I_A$ is almost entirely inactivated, and evoked residual non-$I_A$ currents. These were digitally subtracted from the first series, resulting in "pure" $I_A$. To isolate $I_{K(V)}$, the brain preparation was bathed with saline containing (in M) $10^{-7}$ TTX, $4 \times 10^{-3}$ M 4-AP, and $5 \times 10^{-4}$ $CdCl_2$ to substantially reduce non-$I_{K(V)}$ currents. The neurons were held at −70 mV and then a series of 200-ms voltage pulses were delivered in 10-mV increments between −80 and 60 mV with a 200-ms prepulse to −90 mV. To isolate $K_{Ca}$, the preparation was superfused with saline containing $10^{-7}$ M TTX and $4 \times 10^{-3}$ M 4-AP. The neurons were held at −70 mV and two series of 200-ms voltage pulses were delivered in 10-mV increments between −80 and 60 mV. The second series was recorded with saline containing $5 \times 10^{-4}$ M $CdCl_2$, which abolished voltage-activated $Ca^{2+}$ currents. The difference between the "untreated" and the "$Cd^{2+}$-treated" current series was defined as $K_{Ca}$ current. For levetiracetam-treated flies, flies were fed 5 mg/kg levetiracetam (as described below) for 7-10 days, and then dissected and recorded from as described.

**Single-cell labeling** After the recording, the brain was fixed in 4% paraformaldehyde in PBS for 30 min on ice. After washing for 1 hr in several changes of PBST (0.3% Triton X-100 in PBS) at room temperature, the brain was incubated with mouse anti-GFP antibodies (Invitrogen, 1:200) for 16-40 hrs on a shaker at 4°C, followed by incubation with fluorescent Alexa488 anti-mouse (Invitrogen, 1:1000) secondary antibodies and Alexa-568-conjugated streptavidin (Invitrogen, 1:100) for 24-40 hrs on a shaker at 4°C. After a 1 hr wash, samples were cleared in 70% of glycerol in PBS for 5 min at room temperature and then mounted in Vectashield (Vector Labs). Recorded l-LNvs were imaged using a confocal imaging system (LSM-700; Carl Zeiss).

Serial optical sections were acquired at 0.7-1.0 μm intervals. Only samples where a single cell was both GFP-positive and dye-labeled were included in the analyses.

**Levetiracetam experiments**

Levetiracetam (LEV, Sigma-Aldrich) was dissolved in *Drosophila* physiological saline and added to standard fly food at a concentration of 5 mg/kg. Flies were transferred every 2 days into fresh food vials with or without LEV. For PDF+ puncta and l-LNv Aβ immunostaining experiments, 0-2 day old female flies were collected and maintained on standard food with or without LEV for 10-14 days. For lifespan experiments, newly eclosed adult *elav-Gal4>UAS-AβArctic* and *elav-Gal4/+* females and males were collected and transferred to food vials with or without LEV at a density of ~10 flies/vial and maintained at 25 °C under a 12hr:12hr LD cycle and 50–60% humidity. For lifespan analysis, dead flies were counted during the food exchange.

**Statistical analysis**

For comparisons of 2 groups of normally or non-normally distributed data, t-tests or Mann-Whitney U-tests were performed, respectively. For multiple comparisons, ANOVAs followed by post-hoc Tukey or multiple t-tests with Holm-Bonferroni correction were performed. For multiple comparisons of non-normally distributed data, Kruskal-Wallis tests were performed, with Bonferroni correction for post-hoc comparisons.

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