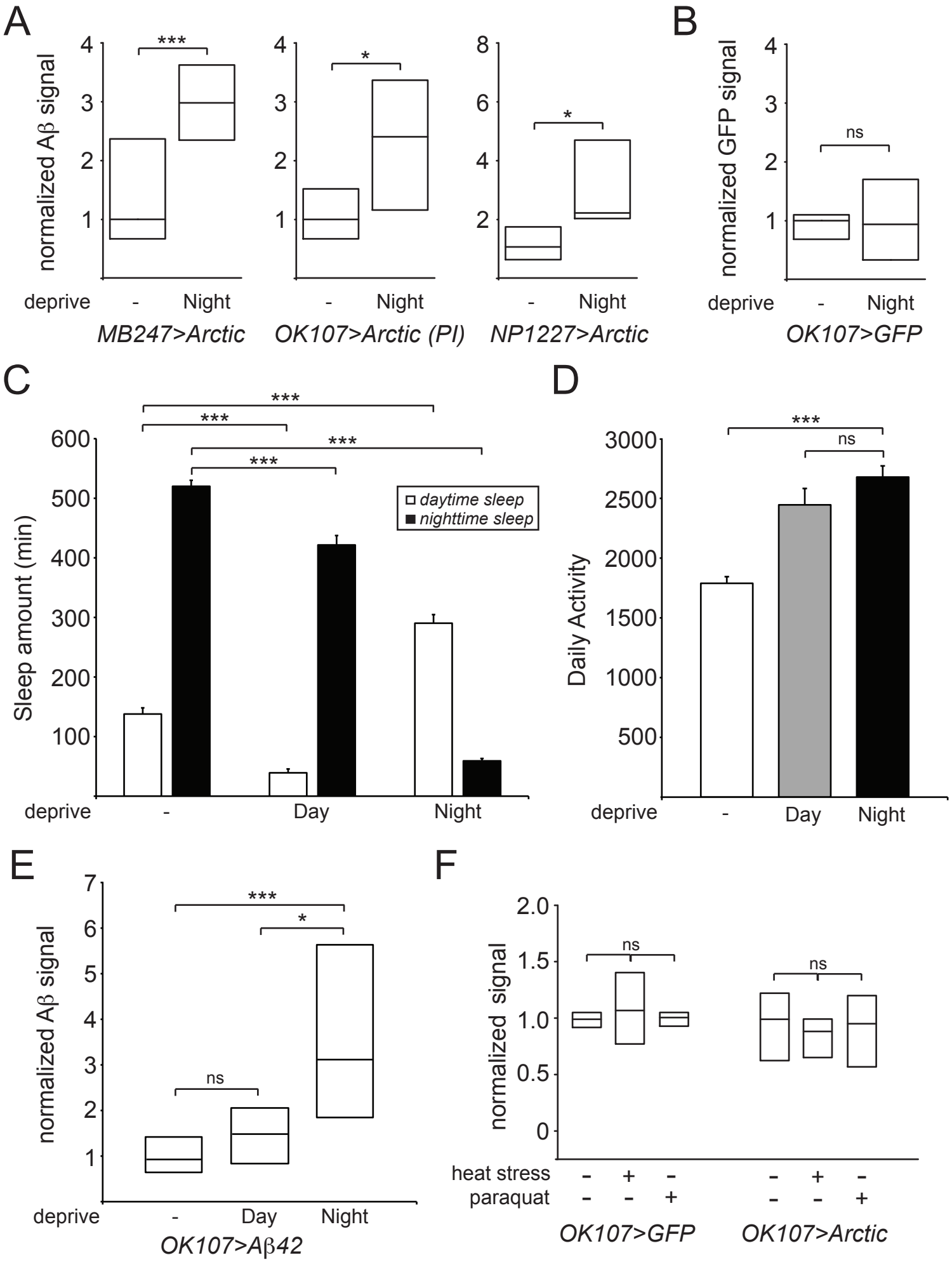
A
normalized Aβ signal
***
*
*
deprive
-
Night
MB247>Arctic
OK107>Arctic (PI)
NP1227>Arctic
B
normalized GFP signal
ns
deprive
-
Night
OK107>GFP
C
Sleep amount (min)
daytime sleep
nighttime sleep
deprive
-
Day
Night
D
Daily Activity
***
ns
deprive
-
Day
Night
E
normalized Aβ signal
***
*
ns
deprive
-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

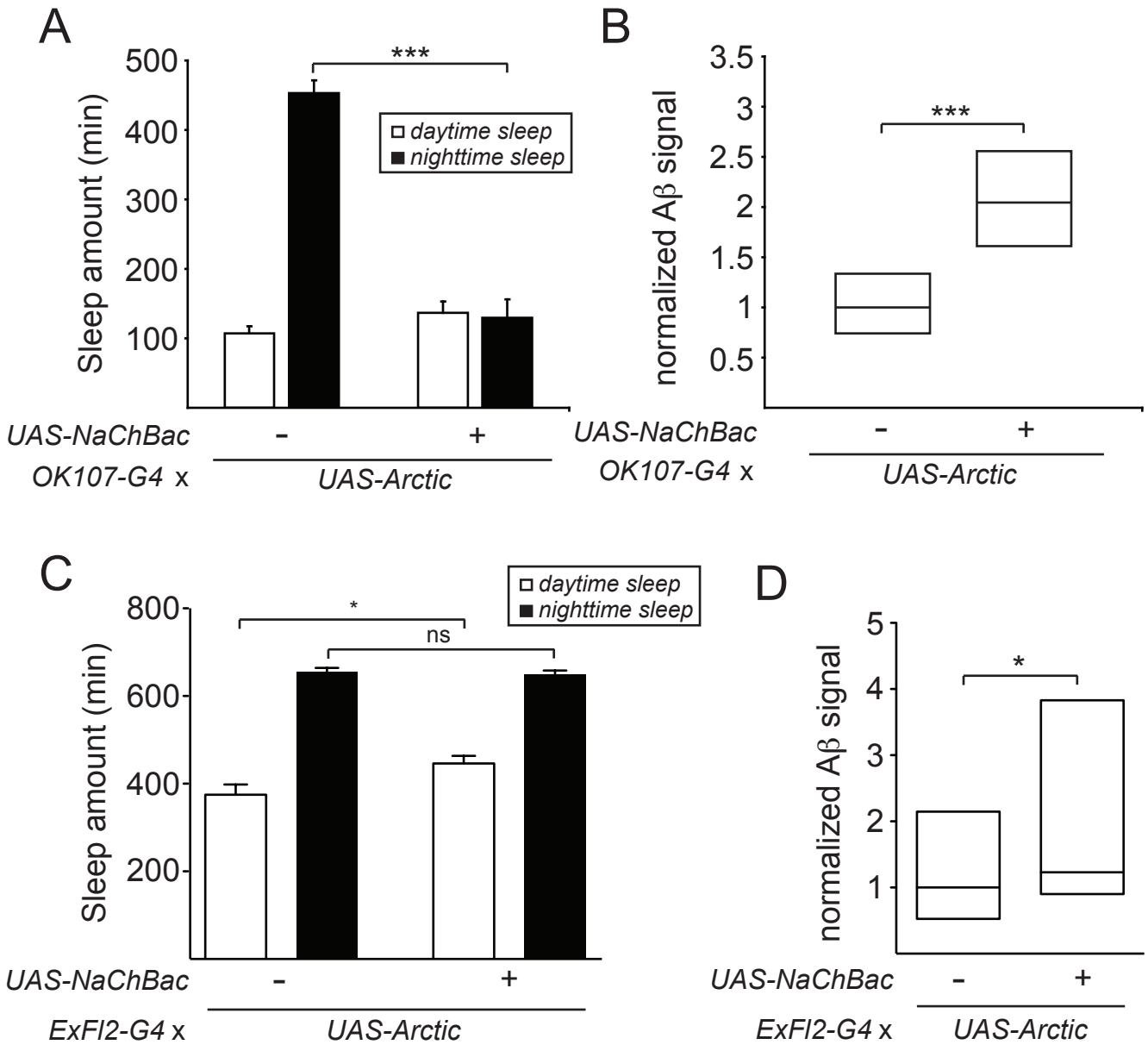

Figure S2. Neuronal Excitation Enhances AβArctic Burden, Related to Figure 4
Daytime and nighttime sleep amount (A) and normalized Aβ signal intensity in the MB KC (B) for *OK107-Gal4>UAS-AβArctic* (n=51) and *OK107>UAS-AβArctic, UAS-NaChBac* (n=53) flies. Daytime and nighttime sleep amount (C) and normalized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

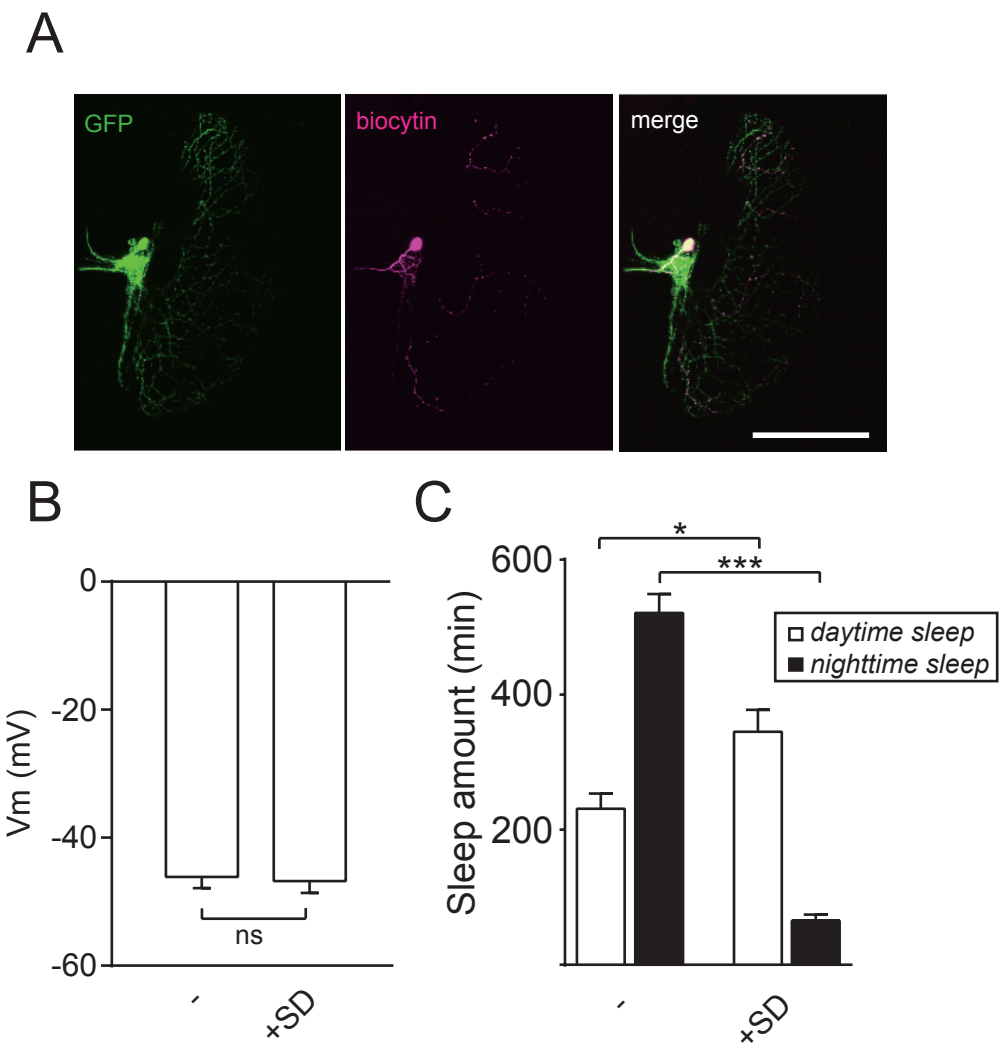

Figure S3. Additional Electrophysiological Parameters, Related to Figure 5

(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.

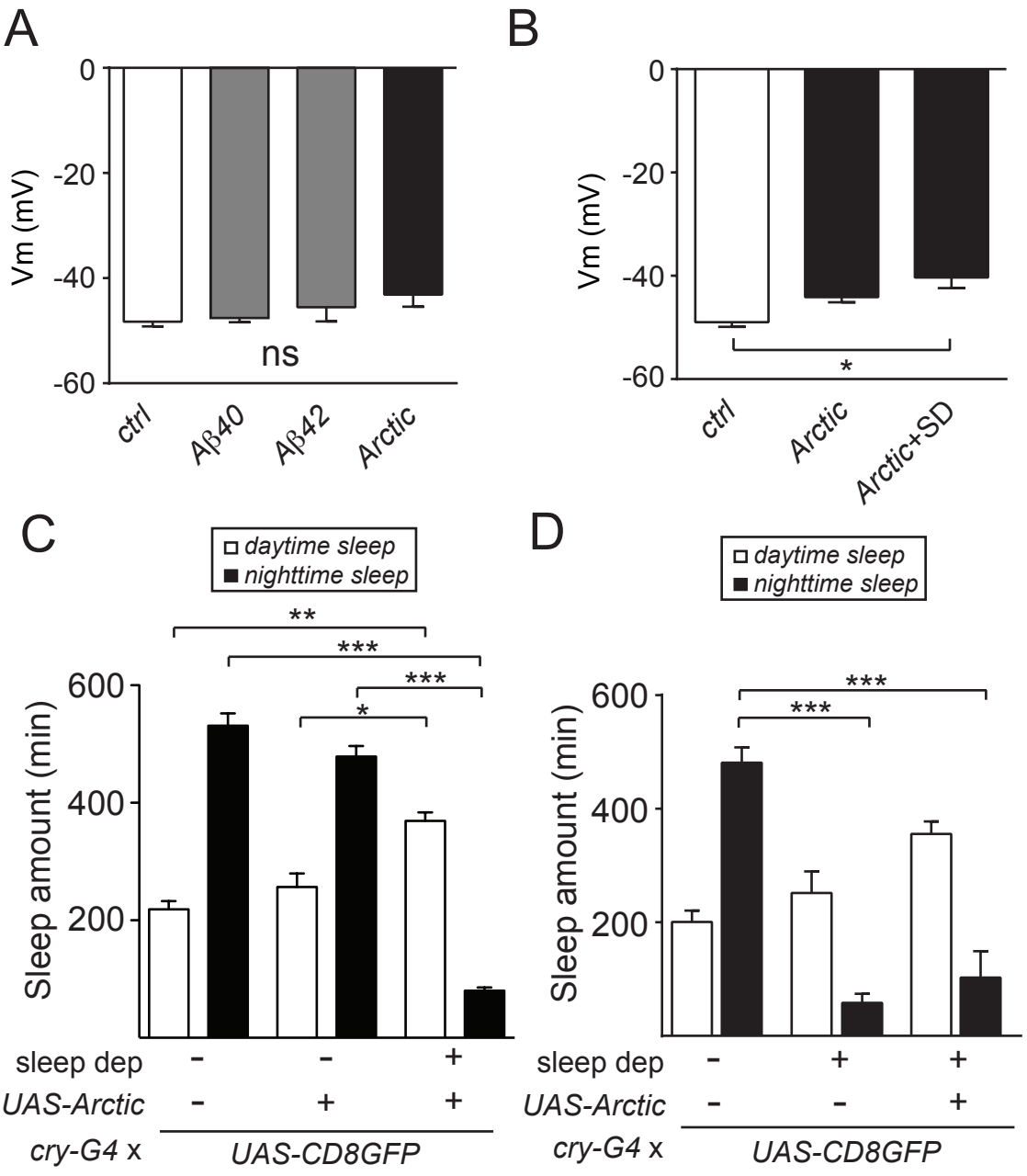

Figure S4. Additional Electrophysiological Parameters, Related to Figure 6
Resting membrane potential of each group during the recording of spontaneous activity, shown in Figures 6A (A) and 6E (B). Daytime and nighttime sleep amounts for groups shown in Figures 6E-6G (C) and Figures 6H-6J (D).

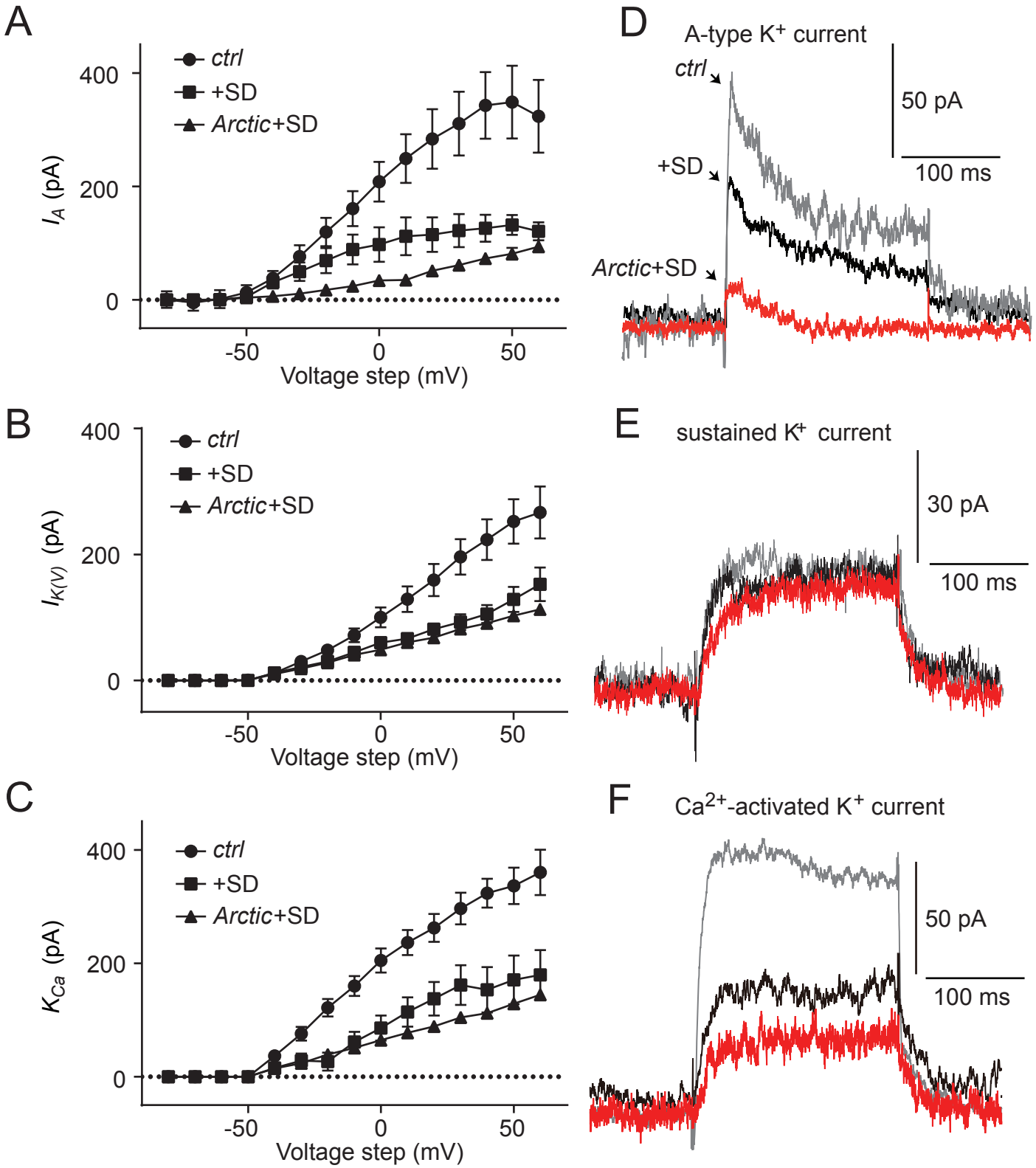
A
ctrl
+SD
Arctic+SD
400
200
0
I_A (pA)
-50
0
50
Voltage step (mV)
B
ctrl
+SD
Arctic+SD
400
200
0
$I_{K(V)}$ (pA)
-50
0
50
Voltage step (mV)
C
ctrl
+SD
Arctic+SD
400
200
0
K_{Ca} (pA)
-50
0
50
Voltage step (mV)
D
A-type K+ current
ctrl
+SD
Arctic+SD
50 pA
100 ms
E
sustained K+ current
30 pA
100 ms
F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

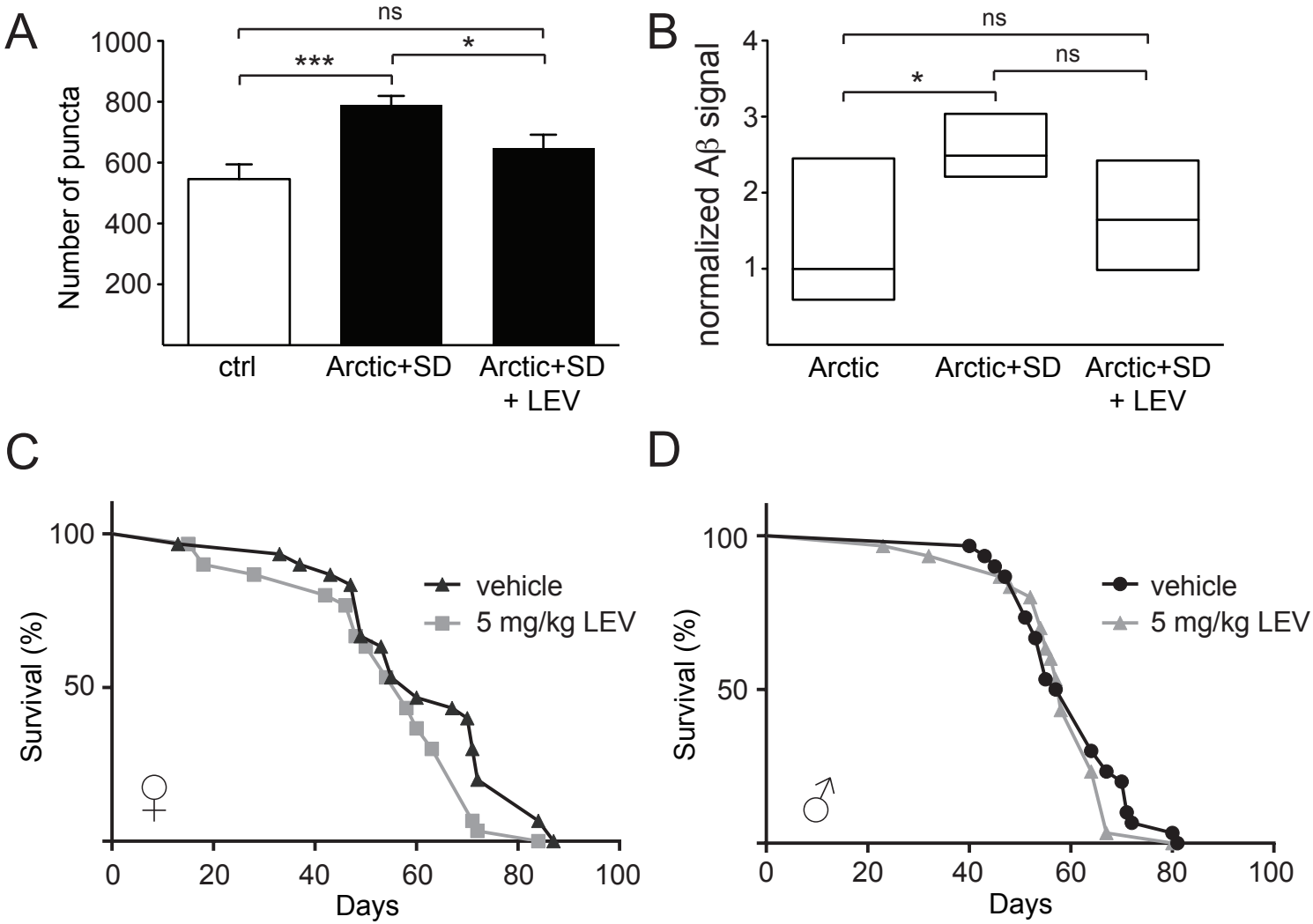

Figure S6. Additional Levetiracetam Data, Related to Figure 7
(A) Number of PDF+ puncta for *cry-Gal4>UAS-CD8::GFP* (n=15, "ctrl") vs *cry-Gal4>UAS-AβArctic* subjected to nighttime sleep deprivation and fed vehicle (n=20) or 5 mg/kg LEV (n=16). (B) Normalized Aβ signal intensity for *cry-Gal4>UAS-AβArctic* (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, 2×10^{-2} TEA, and 5×10^{-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, 4×10^{-3} M 4-AP, and 5×10^{-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 4×10^{-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 5×10^{-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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