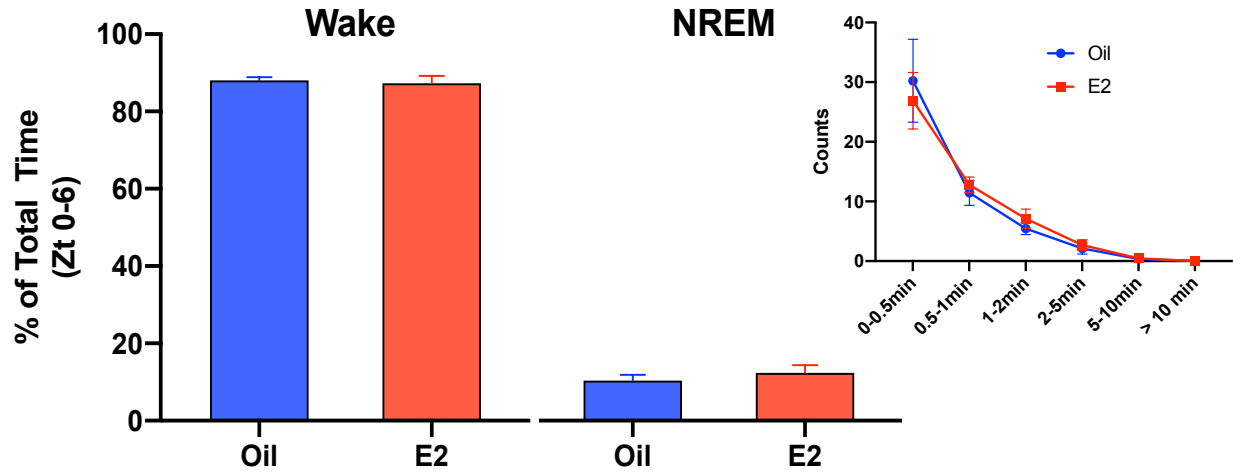
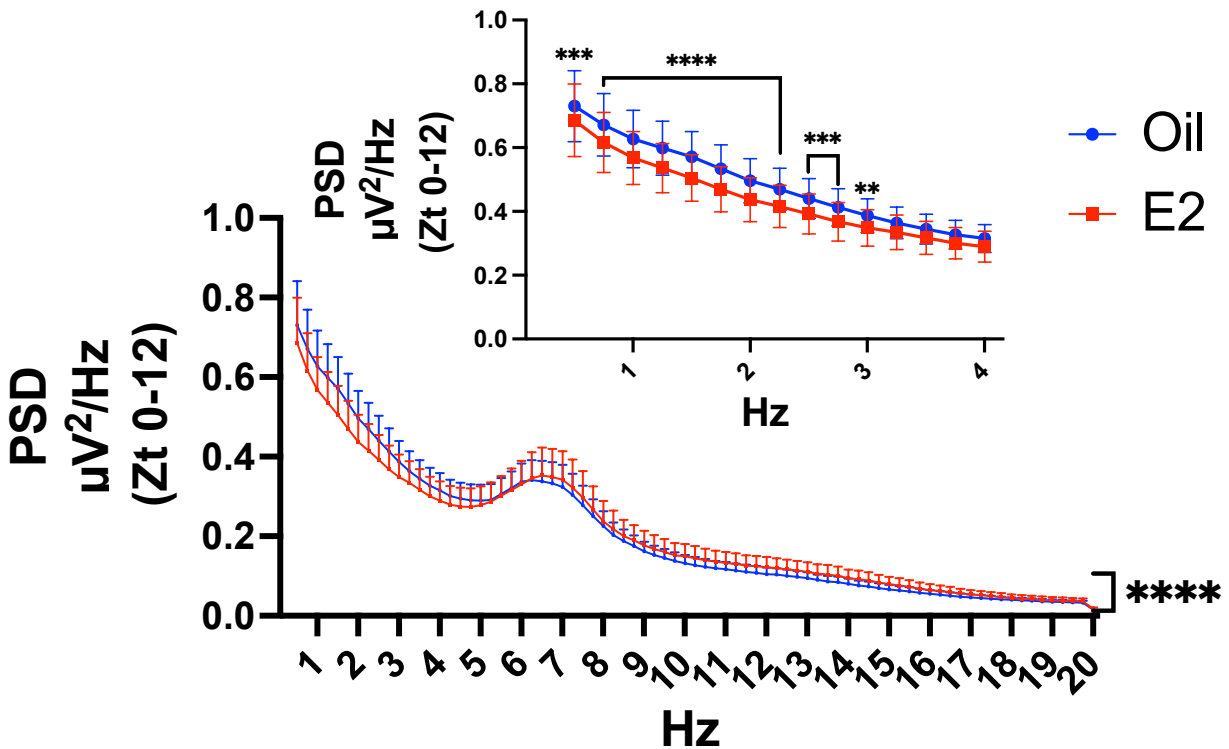


Supplementary Figure S1



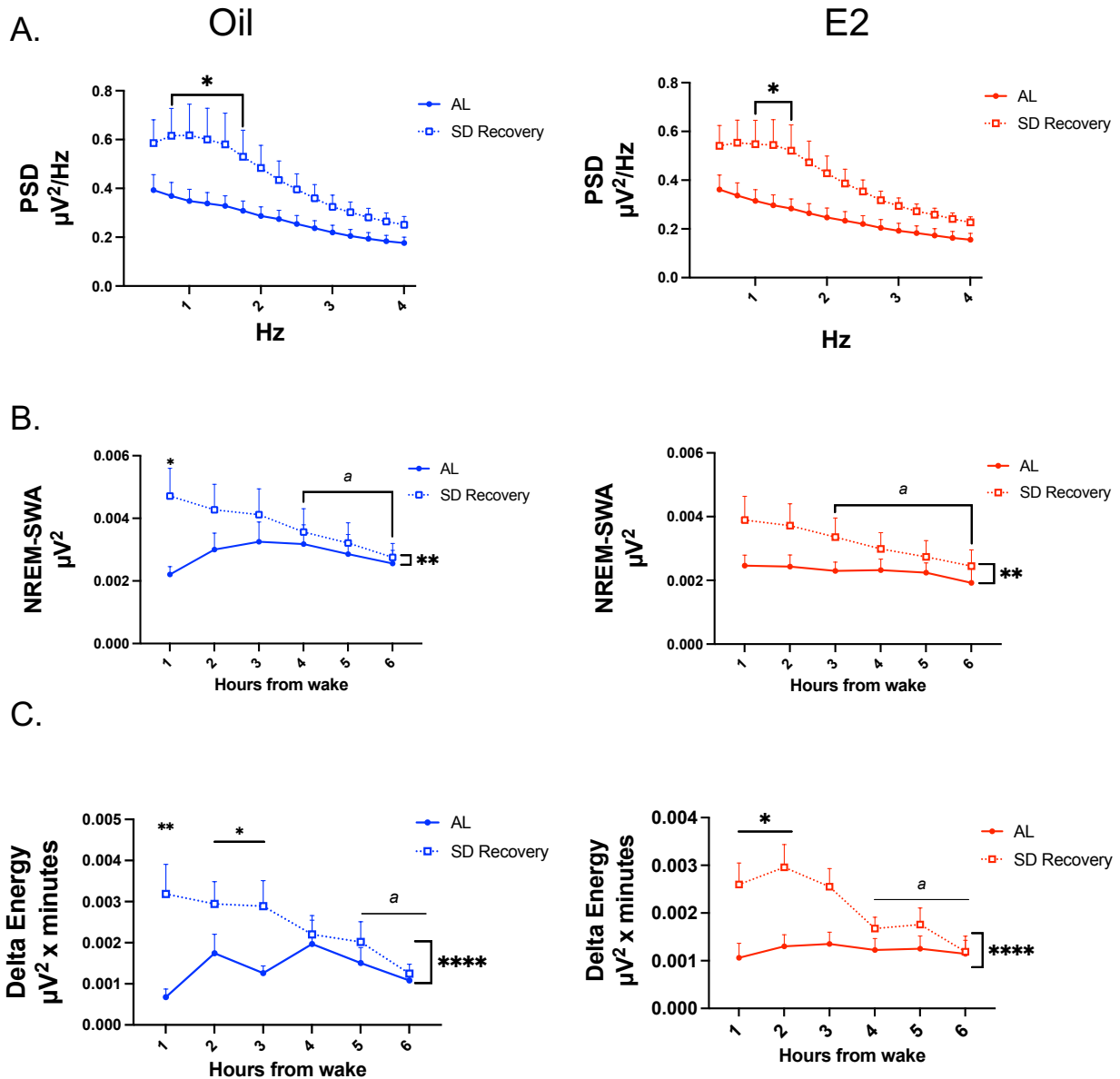
Supplementary Figure S1: Sleep Deprivation Efficiency. Sleep deprivation was induced in the animal's home cage between Zt 0-6. Analysis of the percent of total time spent in wake, NREM sleep and REM sleep over the 6-hour deprivation period was quantified for each animal following E2 or oil treatment from the associated EEG/EMG traces. The mean percent for each state was compared between the E2 and oil treatment via a paired t-test. The amount of accumulated Wake, NREM sleep and REM sleep was similar for both Oil and E2 treatment days (two-tailed paired t-test: Wake: $p=0.67$, $t_{(8)}=0.444$; NREM: $p=0.34$, $t_{(8)}=1.026$; REM: $p=0.26$, $t_{(8)}=1.23$). The majority of accumulated NREM sleep occurred in bouts that were less than 1 minute and most often less than 30 seconds (inset). There was less than 0.1% of accumulated REM in either group (Data not shown). Data are the mean \pm SEM.

Supplementary Figure S2



Supplementary Figure S2: E2 decreased Power Spectral Density (PSD) in AL sleep across the 12-hour light phase (A) PSD of the 12 hours of AL-NREM sleep. In AL sleep, analysis of the spectral power distribution from Zt 1-12 for NREM sleep revealed that E2 treatment significantly decreased the spectral distribution compared to oil treatment (Repeated Measure Two-Way ANOVA; main effect of E2: $F_{(1,553)}=57.02$, **** $p < 0.0001$). (Inset) A multiple comparison post-hoc test further revealed that the significant differences were limited to the lower delta frequency range of 0.5-3Hz (Šídák correction for multiple comparisons; *** $p < 0.005$, **** $p < 0.0001$). Data are the mean \pm SEM.

Supplementary Figure S3

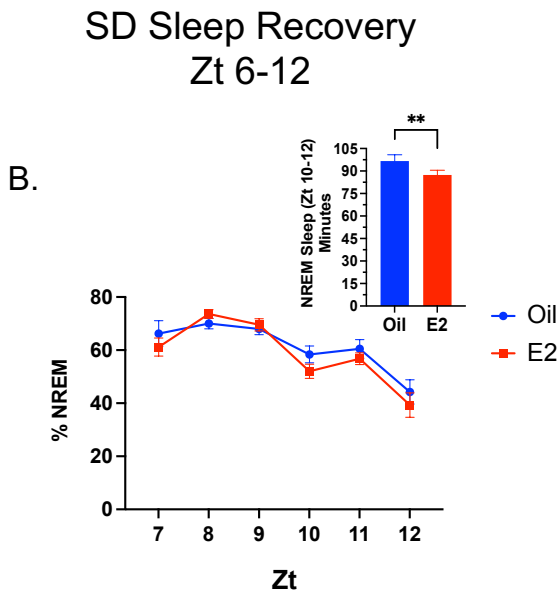
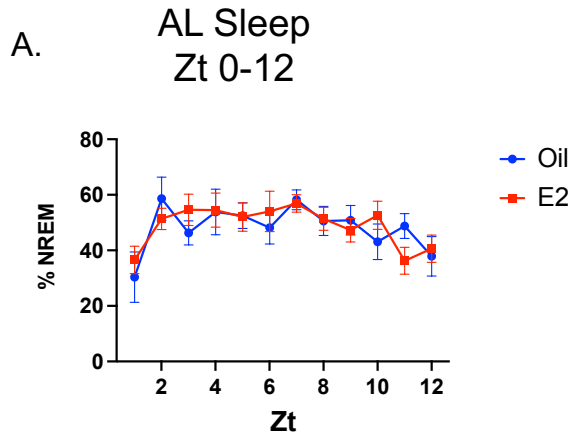


Supplementary Figure S3: Sleep deprivation increased measures of homeostatic pressure in NREM sleep compared to AL sleep following oil and E2 treatments. (A) Comparison of NREM-PSD (0.5-4 Hz) between AL sleep (Zt 0-6) and SD recovery sleep (Zt 6-12) following oil and E2 treatment. Sleep deprivation increased homeostatic sleep pressure following oil and E2 treatment as represented by the significant increase in the

PSD within the delta frequency band (0.5-4 Hz) in SD recovery sleep compared to AL sleep (Repeated Measure Two-Way ANOVA; main effect of sleep condition: Oil, $F_{(1,105)}=62.14$, **** $p<0.0001$ and E2, $F_{(1,105)}=65.85$, **** $p<0.0001$). A multiple comparison post-hoc test further revealed that the significant differences in the Oil treatment were between the 0.75 to 1.5 Hz range (Šídák correction for multiple comparisons; * $p<0.05$); whereas the significant differences in the E2 treatment were between the 1.0 to 1.5 Hz range (Šídák correction for multiple comparisons; * $p<0.05$). Data are the mean \pm SEM. (B) *Comparison of NREM-SWA between AL sleep (Zt 0-6) and SD recovery sleep (Zt 6-12) following oil and E2 treatment.* Sleep deprivation significantly increased NREM-SWA during recovery sleep compared to the first 6 hours of AL sleep (Repeated measure two-way ANOVA; main effect of sleep condition: Oil, $F_{(1,48)}=7.788$, ** $p=0.0075$ and E2, $F_{(1,48)}=9.17$, ** $p=0.0042$). NREM-SWA declined across the hours in the SD recovery sleep condition but not in the first 6 hours of AL sleep for both E2 and oil treatment (Repeated measure two-way ANOVA; main effect of hour, Oil: $F_{(5,40)}=4.750$, ** $p=0.0017$ and E2: $F_{(5,45)}=19.22$, **** $p<0.0001$; a denotes hours that are significantly different from hour 1). Specifically, in the oil control condition, NREM-SWA was significantly decreased starting at hour 4 of SD recovery sleep (Dunnett's multiple comparison test; hour 1 vs hour 4, * $p=0.0294$; Zt 1 vs Zt 5, ** $p=0.0029$ and Zt 1 vs Zt 6, **** $p<0.0001$). Following E2 treatment, NREM-SWA was significantly decreased starting at hour 3 of SD recovery sleep (Dunnett's multiple comparison test; hour 1 vs hour 3, ** $p=0.0058$; Zt 1 vs Zt 4-6, **** $p<0.0001$). (C) *Comparison of NREM-delta energy between AL sleep (Zt 0-6) and SD recovery sleep (Zt 6-12) following oil and E2 treatment.* Sleep deprivation significantly increased delta energy during recovery sleep compared to the first 6 hours of AL sleep

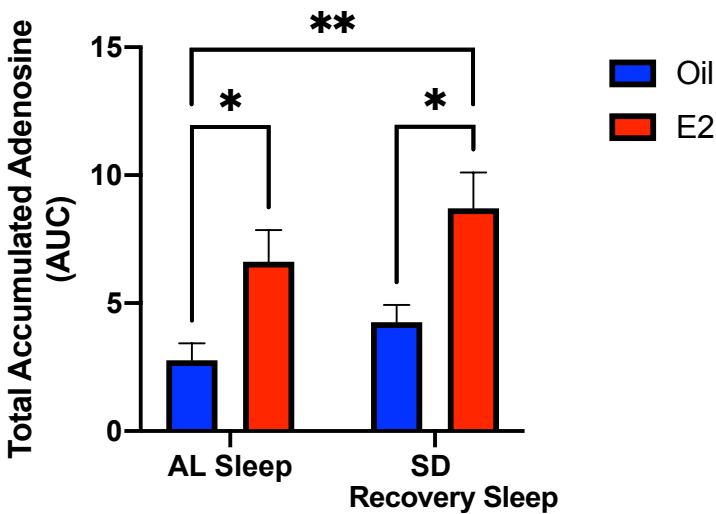
for both oil and E2 treatments (Repeated measure two-way ANOVA; main effect of sleep condition: Oil, $F_{(1,48)}=18.81$, **** $p<0.0001$ and E2, $F_{(1,48)}=21.63$, **** $p<0.0001$). Delta energy declined across the hours in the SD recovery sleep condition but not in the first 6 hours of AL sleep for both E2 and oil treatment (Repeated measure two-way ANOVA; main effect of hour, Oil: $F_{(5,76)}=4.175$, ** $p=0.0021$ and E2: $F_{(5,76)}=9.072$, **** $p<0.0001$; a denotes hours that are significantly different from hour 1). Specifically, in the oil control condition, delta energy was significantly decreased starting at hour 5 of SD recovery sleep (Dunnett's multiple comparison test; hour 1 vs hour 5, * $p=0.0499$; Zt 1 vs Zt 6, and *** $p=0.0001$). Following E2 treatment, delta energy was significantly decreased starting at hour 4 of SD recovery sleep (Dunnett's multiple comparison test; hour 1 vs hour 4, *** $p=0.0007$; Zt 1 vs Zt 5, ** $p<0.0025$, and Zt 1 vs Zt 6, **** $p<0.0001$).

Supplementary Figure S4



Supplementary Figure S4: The hourly duration of time spent in NREM was not significantly different between oil and E2 treatments for both the AL sleep and SD recovery sleep periods (Repeated Measure Two-Way ANOVA). However, comparison of the cumulative duration for the last 3 hours of SD recovery sleep did reveal E2 induced a significant decrease in NREM sleep (paired two-tailed t-test; $t_{(8)}=3.699$; $**p=0.0061$) which likely contributed to the observed differences in Figure 2B.

Supplementary Figure S5



Supplementary Figure S5: Area under the curve comparison of accumulated extracellular adenosine levels between the AL and SD sleep experiments.

Comparing the AUC calculations across the four treatment conditions demonstrated that SD did increase total adenosine concentration in both the oil and E2 treated groups compared to AL sleep; however, the increase did not reach statistical significance (Two way ANOVA; main effect of E2; $F_{1,29} = 15.96$, $p = 0.0004$; no main effect of sleep condition $F_{1,29} = 2.961$, $p = 0.0960$). This could be due to the experimental variability between the AL sleep and SD sleep recovery cohorts which were run as separate experiments. Post hoc analysis revealed that significant differences between E2 vs oil in AL sleep and SD (Šídák correction for multiple comparisons, $p = 0.0381$ and $p = 0.0402$, respectively) as well as E2 in SD vs Oil in AL sleep ($p = 0.0017$). Data are the mean \pm SEM.

Supplementary Table 1

A.

Epoch Score	EMG Threshold	Delta Threshold	Theta Threshold	Theta/Delta Ratio
Wake				
	>	<	<	N/A
	>	>	>	N/A
	>	>	<	N/A
	>	<	>	N/A
	<	<	>	<1
NREM				
	<	>	<	N/A
	<	>	>	N/A
REM				
	<	<	>	>1

B.

Treatment	Mean Artifact Duration (minutes)	SEM	Minimum	Maximum
AL/Oil (12h)	0.19	0.08	0	0.9
AL/E2 (12h)	0.09	0.07	0	0.5
SD/Oil (6h)	0.09	0.09	0	0.4
SD/E2 (6h)	0.17	0.07	0	0.9

Supplementary Table 1: MATLAB Automated Vigilant State Scoring. (A) *Automated scoring decisions.* Wake, NREM sleep and REM sleep were assigned to each 10 second epoch based on threshold decisions of muscle tone (EMG) and Delta/Theta power. Determinations of the low or high thresholds were made relative to each animal's median value of the 10 second epoch for the given parameter. >, signifies greater than the threshold values and <, signifies less than the threshold value. (B) *The mean duration of*

artifacts and range for each treatment paradigm. Overall, the mean duration of artifacts was less than a minute for the entire 12 hours of recordings for each treatment /sleep condition. Under the *ad libitum* baseline sleep condition, a two-way ANOVA (steroid x hour) revealed no main effects of treatment ($F_{1,9} = 0.7949$; $p=0.3958$) or time ($F_{11,99} = 0.7976$; $p=0.7976$) nor an interaction ($F_{11,76} = 1.042$; $p=0.4192$) between the groups. Similarly, in the sleep deprivation recovery condition, a two-way ANOVA (steroid x hour) revealed no main effects of treatment ($F_{1,9}=0.1422$; $p=0.7148$) or time ($F_{5,45} = 0.7651$; $p=0.5798$) nor an interaction ($F_{5,39}=1.068$; $p=0.3930$) between the two.

Supplementary Table 2

	Wake		NREM		REM	
	Oil	E2	Oil	E2	Oil	E2
AL Sleep (12 hours)	81.30 ± 6.7	82.67 ± 5.1	155.4 ± 8.3	167.70 ± 6.3	37.90 ± 2.2	48.00 ± 3.5
SD Recovery Sleep	75.11 ± 10.5	72.44 ± 2.4	81.78 ± 10.58	75.67 ± 2.7	33.33 ± 5.2	29.22 ± 2.4

Supplementary Table 2: Total number of bouts in AL sleep and SD Recovery Sleep during (Zt 6-12). In either AL sleep or SD Recovery Sleep, E2 treatment did not significantly affect the total number of Wake, NREM or REM bouts when compared to the oil treatment under the same sleep condition. (Two-Way Repeated Measure ANOVA). Data are the mean ± SEM.

Supplementary Table 3

Comparison	Difference Between Treatments	95% CI	Adjusted P Value
AL Sleep: PSD post hoc comparison Oil vs E2 (Fig. 3 A)			
0.5 Hz	$\frac{\mu\text{V}^2}{\text{Hz}}$ 0.03160	0.006371 to 0.05683	0.0016
0.75Hz	0.03240	0.007170 to 0.05763	0.0010
1.0 Hz	0.03352	0.008294 to 0.05876	0.0005
1.25Hz	0.04109	0.01586 to 0.06632	<0.0001
1.50 Hz	0.04472	0.01949 to 0.06995	<0.0001
1.75Hz	0.04401	0.01878 to 0.06924	<0.0001
2.0 Hz	0.03960	0.01437 to 0.06483	<0.0001
2.25 Hz	0.04019	0.01495 to 0.06542	<0.0001
2.50 Hz	0.03449	0.009259 to 0.05972	0.0003
2.75Hz	0.03224	0.007005 to 0.05747	0.0011
3.0 Hz	0.02778	0.002548 to 0.05301	0.0138
SD Sleep: PSD post hoc comparison Oil vs E2 (E2, Fig. 3B)			
0.75Hz	$\frac{\mu\text{V}^2}{\text{Hz}}$ 0.06191	0.01067 to 0.1132	0.0031
1.0 Hz	0.06958	0.01834 to 0.1208	0.0003
1.25Hz	0.05585	0.004608 to 0.1071	0.0160
1.50 Hz	0.05894	0.007696 to 0.1102	0.0071
1.75Hz	0.05685	0.005615 to 0.1081	0.0123
2.0 Hz	0.05523	0.003996 to 0.1065	0.0188
AL sleep: NREM-SWA post hoc comparison Oil vs E2 (Fig. 4 A)			
Zt 3	$\frac{\mu\text{V}^2}{\text{Hz}}$ 0.001227	0.0002745 to 0.002179	0.0034
Zt 4	0.001116	0.0001640 to 0.002069	0.0107

Supplementary Table 3: Šídák correction for multiple comparisons test Adjusted P values and confidence intervals for Figure 3. List of the mean difference, confidence intervals (CI) and adjusted P values for the comparisons in Figure 3 and 4.