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Supplemental Information

Antagonistic Regulation of Circadian Output and Synaptic Development by JETLAG and the DYSCHRONIC-SLOWPOKE Complex

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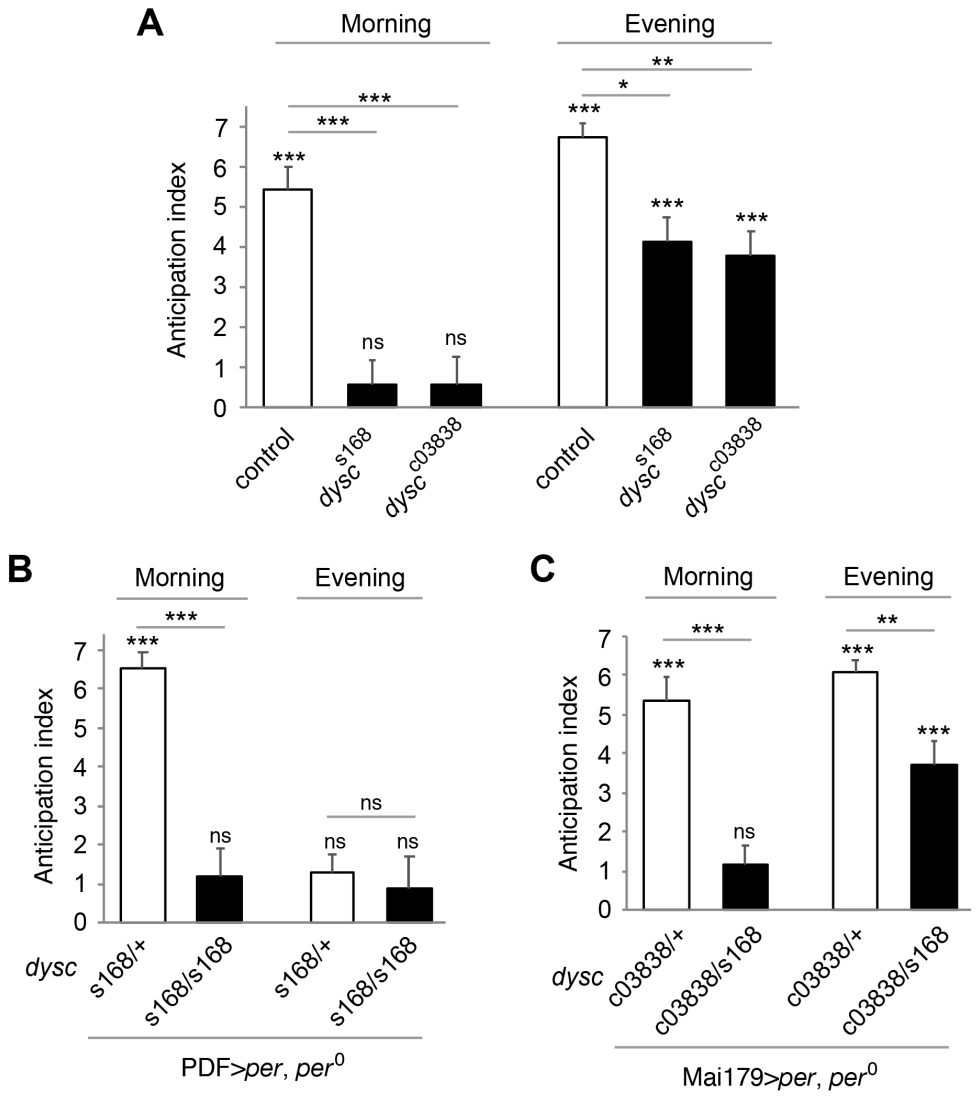

Figure S1. Loss of DYSC differentially affects morning and evening oscillators, Related to Figure 1.

(A-C) Anticipation index for the data presented in Figures 1A and 1C. FaasX was used to calculate anticipation index, defined as the slope of the best-fitting regression line for the activity counts over a period of 6 h prior to a light-dark transition. Bars represent anticipation index ± standard error. Statistics above the lines indicate whether the two genotypes are different, while those above the bars indicate whether anticipation index of each genotype is different from 0. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, ns: not significant; two-way ANOVA (genotype and morning vs evening as the two main factors) followed by Tukey post-hoc tests for pairwise comparisons; *t* test with Bonferroni correction for comparing each anticipation index to 0.

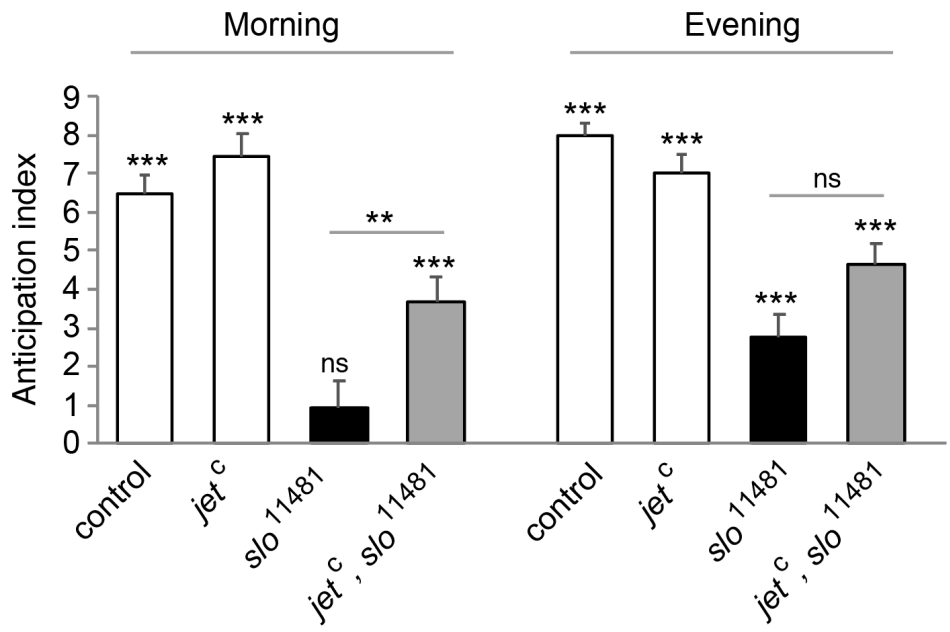

Figure S2. Reduced JET rescues morning anticipation in *slo* mutants, Related to Figure 4.
(A) Anticipation index for the data presented in Figure 4C. Anticipation index is calculated over a period of 6 h prior to a light-dark transition. Bars represent anticipation index ± standard error. Statistics above the lines indicate whether the two genotypes are different from each other, while those above the bars indicate whether anticipation index of each genotype is significantly different from 0. ** $p < 0.005$, *** $p < 0.0005$, ns: not significant, two-way ANOVA (genotype and morning vs evening as the two main factors) followed by Tukey post-hoc tests for pairwise comparisons; *t* test with Bonferroni correction for comparing each AI to 0.

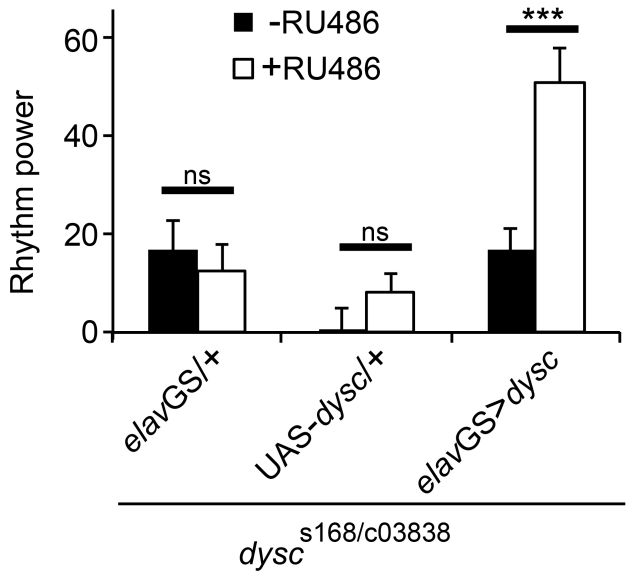

Figure S3. Adult-specific restoration of *dysc* partially rescues the arrhythmic phenotype of *dysc* mutants, Related to Figure 5. Mean power of rhythmicity of *dysc*$^{s168/c03838}$ transheterozygous males carrying *elav*-GS, UAS-*dysc*, or both. Flies raised on standard food without RU486 were tested in the presence or absence of 500 μM RU486. N = 22-34. Bars represent mean ± s.e.m. *** $p < 0.0005$, ns: not significant, two-way ANOVA with genotype and RU486 treatment as main factors, followed by Tukey post-hoc tests, only 3 of which are shown for clarity. The interaction between genotype and RU486 treatment was significant at $p < 0.005$.

Transparent Methods

Fly strains

Flies were raised on standard food containing cornmeal, yeast, and molasses. Isolation of *jet*^{c}, *jet*^{r}, and *dysc*^{s168} was previously described (Jepson et al., 2012); *cry*^{02} was obtained from Charlotte Helfrich-Förster (Dolezelova et al., 2007; Gegear et al., 2008); *per*^{0} and UAS-*per16* from François Rouyer (Blanchardon et al., 2001); *dysc*^{c03838} from the Exelixis collection at the Harvard Medical School; and *slo*^{11481} from the Bloomington Stock Center (BL29918). PDF-Gal4, Mai179-Gal4 (Grima et al., 2004; Siegmund and Korge, 2001), and *elav*-GS (Osterwalder et al., 2001) were obtained from Drs. Amita Seghal, François Rouyer, and Haig Keshishian, respectively. All mutant flies were outcrossed to the iso31 (*white*-) background at least 5 times, except for *per*^{0} flies. In the *per* rescue experiments, all flies had the same mixed genetic background between *per*^{0} and iso31.

Locomotor assays

To assay locomotor behavior, 2- to 5-day old male flies were entrained to a 12 h:12 h LD cycle for at least 3 days and placed individually in glass tubes containing 5% sucrose and 2% agar. Locomotor activity was monitored using the *Drosophila* Activity Monitoring System (Trikinetics) at 25^{o}C under indicated light conditions. For conditional expression of *dysc* using *elav*-GS, stock solution of RU486 dissolved in ethanol was added to food containing 5% sucrose and 2% agar to a final concentration of 500 μM. Food with 1% ethanol without RU486 was used as a vehicle control. For quantification of circadian behavior in constant conditions (DD, RR or LL), activity counts (beam breaks) were collected in 30-min bins over a 6-day period, and power of rhythmicity (rhythm strength) was determined using Fly Activity Analysis Suite for Mac OSX (FaasX, M. Boudinot). The power of rhythmicity is defined as the difference between the χ^2

value and the significance value at p=0.05. Average rhythm power was determined for all flies including arrhythmic ones. Individual actograms were generated using ClockLab (Actimetrics), and 24 h activity plots showing the mean beam breaks per 30-min bin over a 1-3 day period were generated using FaasX, which was also used to calculate anticipation index. Anticipation index is defined as the slope of the best-fitting linear regression line over a period of 6 h prior to the light-dark transition. The slope was normalized so that the maximum activity count for a given period was set to 100.

Measurement of synaptic bouton number and size at the larval NMJ

The morphology of synapses from muscle 6/7, segment 3, from wandering 3rd instar larvae was assessed using an Olympus Fluoview confocal microscope. Larvae from both sexes were pooled. Larvae were dissected in a low Ca^{2+} HL3.1 solution of composition (mM): 70 NaCl, 5 KCl, 0.2 $CaCl_2$, 20 $MgCl_2$, 10 $NaHCO_3$, 5 Trehalose, 115 Sucrose, and 5 HEPES, pH 7.2. Dissected larval fillets were fixed in 4% PFA (in PBS) for 10-20 mins at room temperature. For immuno-staining, antibodies were diluted in PBS with 0.15% Triton-X and 5% goat serum. Fluorescently conjugated (Alexa-488) anti-HRP (Jackson ImmunoResearch Labs Cat# 123-065-021, RRID:AB_2314646, 1:200) was used to label neuronal membranes. The number of boutons was quantified for each synapse using an antibody against cysteine string protein (CSP; Developmental Studies Hybridoma Bank Cat# DCSP-2 (6D6), RRID:AB_528183, 1:1000), which specifically labels the presynaptic bouton (Zinsmaier et al., 1990). Goat anti-mouse Alexa-fluor 555 (Molecular Probes, 1:1000) was used to label the anti-CSP primary antibody. Quantification of synaptic morphological parameters was performed and analyzed blind to genotype. Type 1b and Type 1s boutons were counted simultaneously. Synaptic bouton size was measured using ImageJ, with a minimum cut-off size of 5 μm^2 (Jepson et al., 2014).

Statistical Analysis

GraphPad Prism was used for statistical tests. Either *t* tests for pairs of groups or one-way ANOVAs for multiple groups were performed. If the groups had unequal variances, *t* tests for unequal variances or the Brown-Forsythe and Welsh version of ANOVA was used. ANOVAs were followed by Dunnett, Tukey, or Sidak post-hoc tests depending on the number and type of pairwise comparisons performed. For multiple *t* tests for the same dataset, Bonferroni correction for multiple comparisons was applied. For comparisons of non-normally distributed data, Kruskal-Wallis tests were performed, followed by Dunn's post-hoc tests.

Supplemental References

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