

Supplementary Online Material

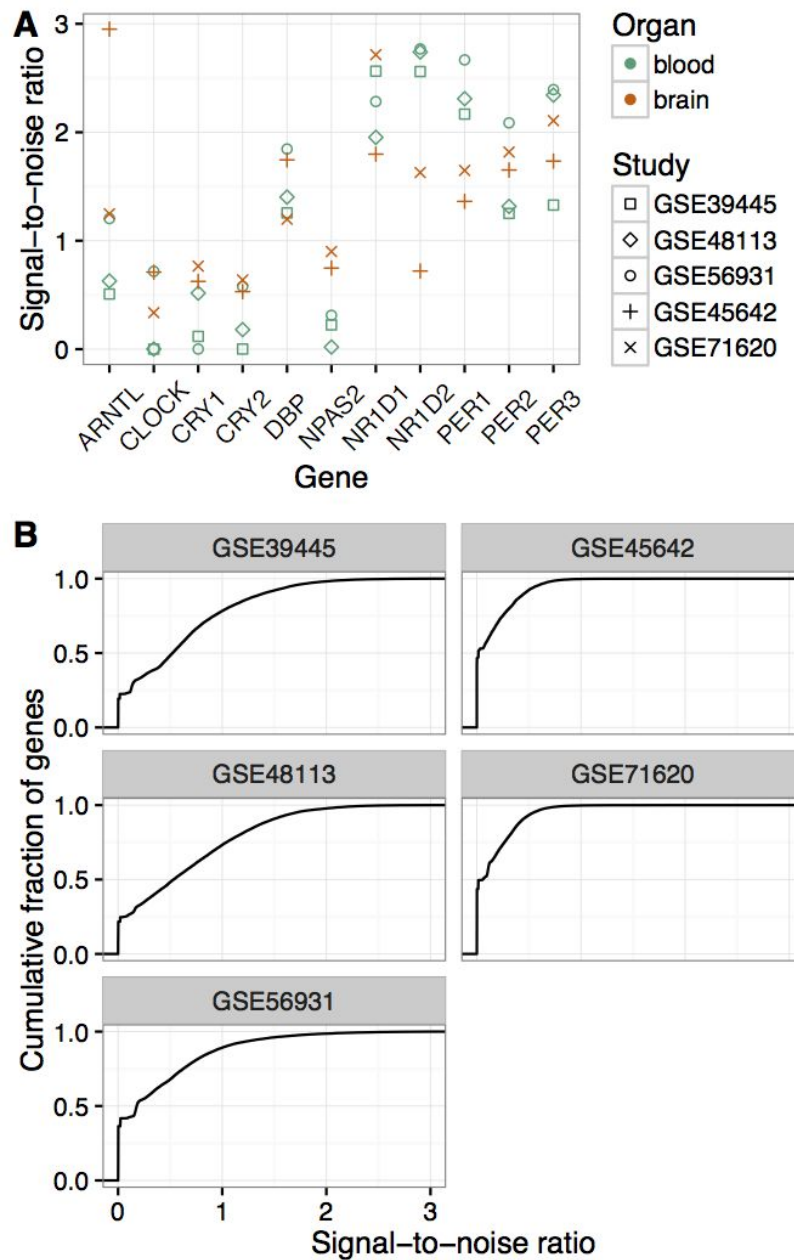
Differential phasing between circadian clocks in the brain and peripheral organs in humans

Jacob J. Hughey^{*,1} and Atul J. Butte[†]

^{}Department of Biomedical Informatics, Vanderbilt University School of Medicine, Nashville, Tennessee, [†]Institute for Computational Health Sciences, University of California, San Francisco, California*

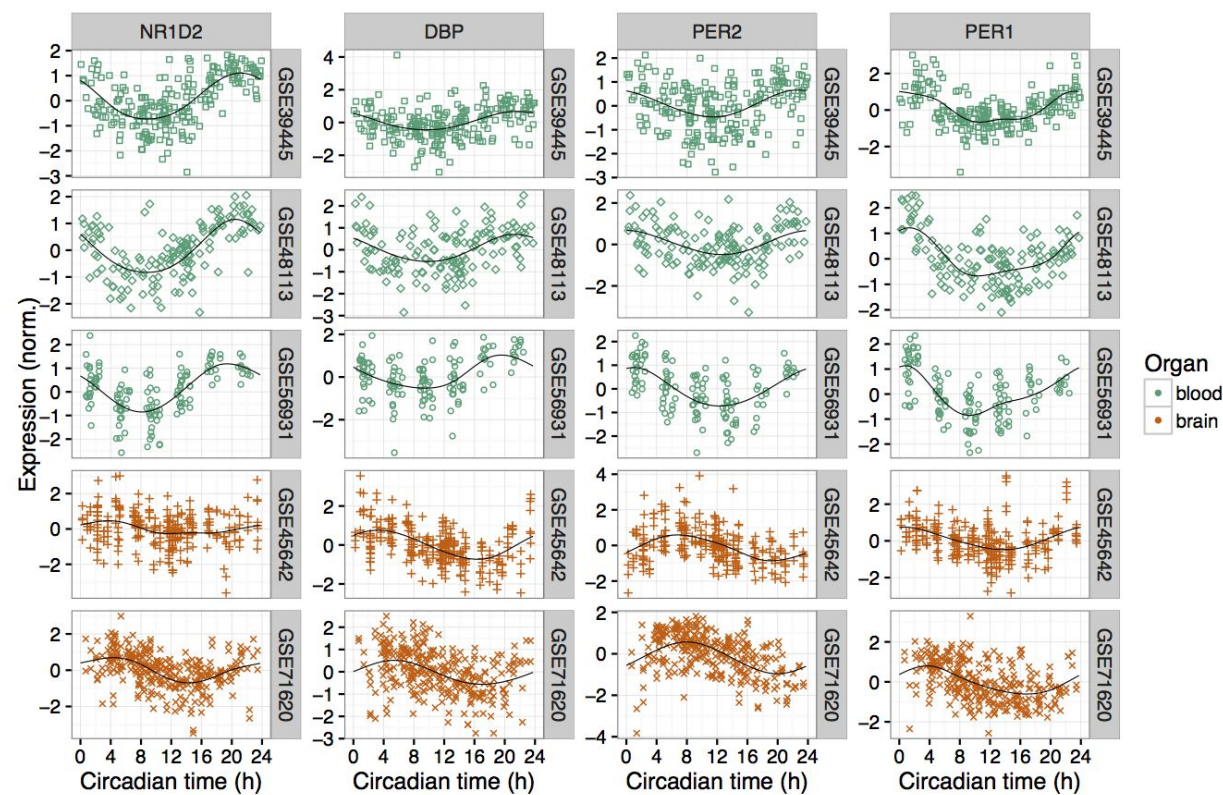
1. To whom all correspondence should be addressed: Jacob J. Hughey, Department of Biomedical Informatics, Vanderbilt University School of Medicine, 2525 West End Ave, Suite 1475, Nashville, TN 37203, USA; e-mail: jakejhughey@gmail.com

Suppl. Fig. S1



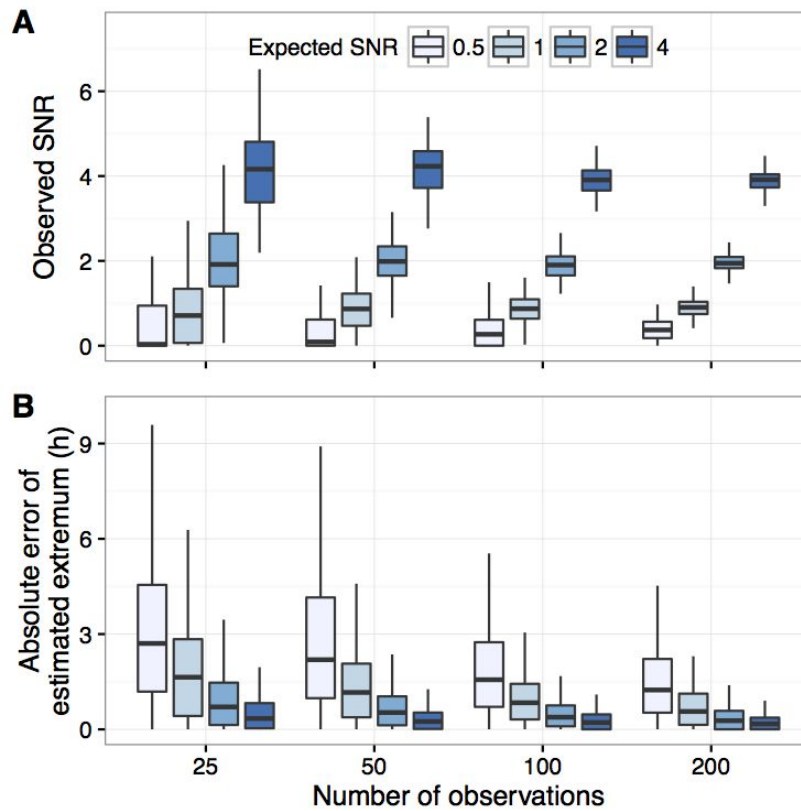
(A) Signal-to-noise ratio (SNR) of circadian rhythmicity for clock genes in each dataset. The SNR is based on the fit of the periodic smoothing spline, and is calculated as the maximum fitted value minus the minimum fitted value, divided by the square root of the mean of squared residuals. **(B)** Cumulative distribution function of SNR over all genes in each dataset. Many genes in each dataset have an SNR of zero, indicating no circadian rhythmicity.

Suppl. Fig. S2



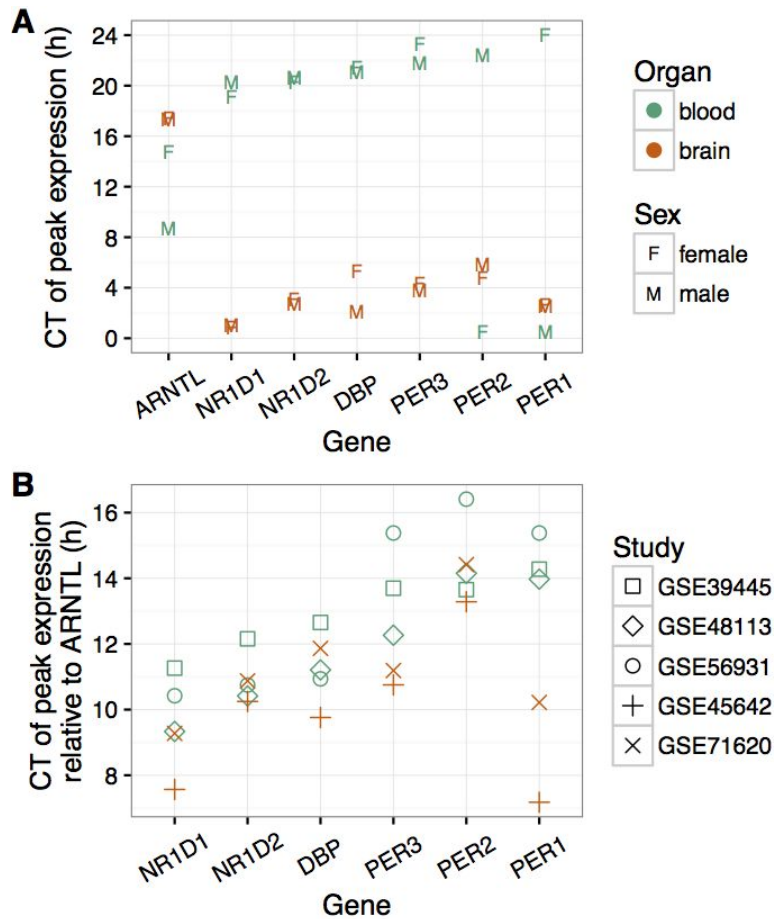
Circadian expression of the four other clock genes across the five datasets.

Suppl. Fig. S3



Simulations to determine accuracy of peak and trough time detection, as described in the Materials and Methods. Boxplots of **(A)** Observed signal-to-noise ratio and **(B)** absolute error of estimated peak or trough time for various numbers of observations and values of the expected signal-to-noise ratio. Because the number of outliers depends on the somewhat arbitrarily chosen number of simulations, we have omitted outliers from both plots. For ease of comparison with the rest of the analysis, absolute error is expressed in hours (24 hours per day, so maximum absolute error is 12 h).

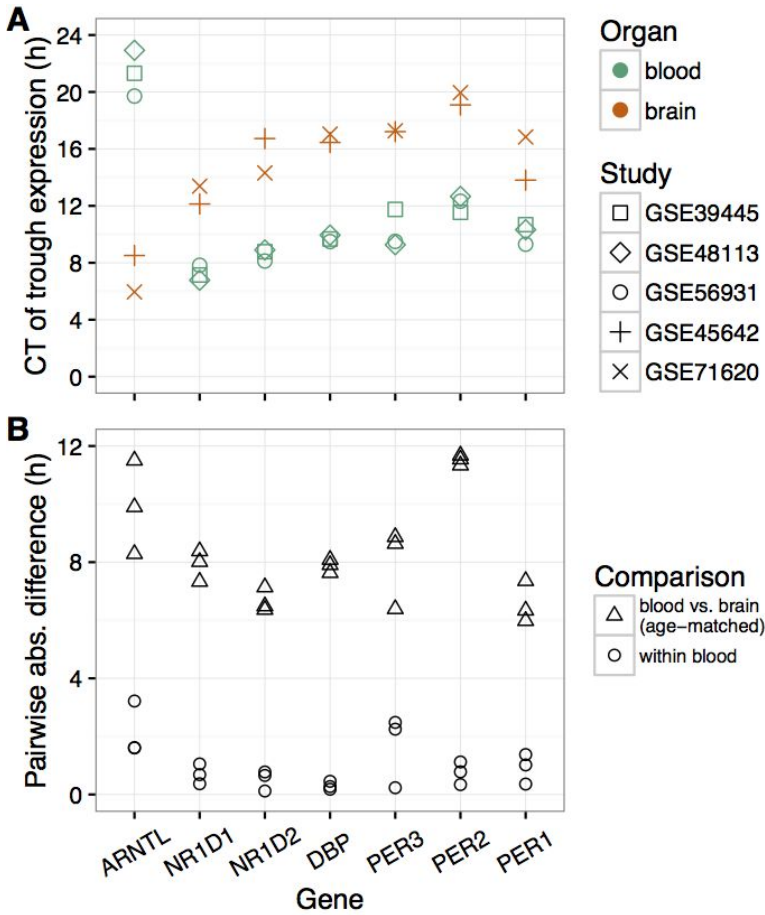
Suppl. Fig. S4



(A) Time of peak expression for each clock gene in each organ, stratified by biological sex. For each organ, we first merged the respective datasets as described in the Materials and Methods, then calculated the time of peak expression in the merged data for each clock gene. To avoid confounding with the effect of age and because the brain datasets had few samples from young female donors, only samples from donors at least 40 years old were included. For the brain datasets, subject sex was provided by the original researchers. For the blood datasets, subject sex was inferred based on expression of *RPS4Y1*, as described in the Materials and Methods.

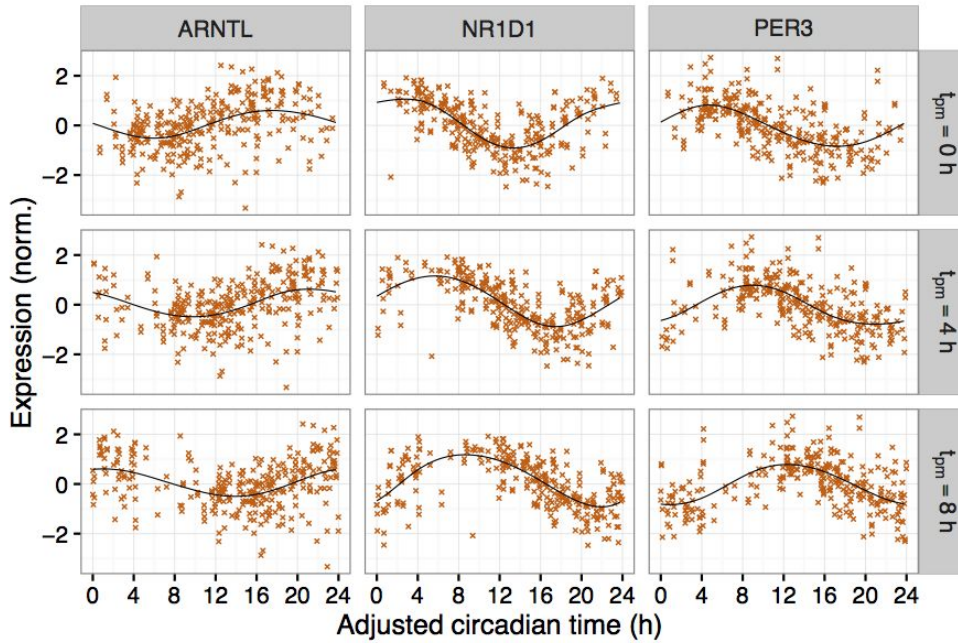
(B) Difference between the peak time for each clock gene and the peak time for *ARNTL* in each dataset (based on Figure 2A). For ease of visualization, values less than zero were shifted by 24 h.

Suppl. Fig. S5



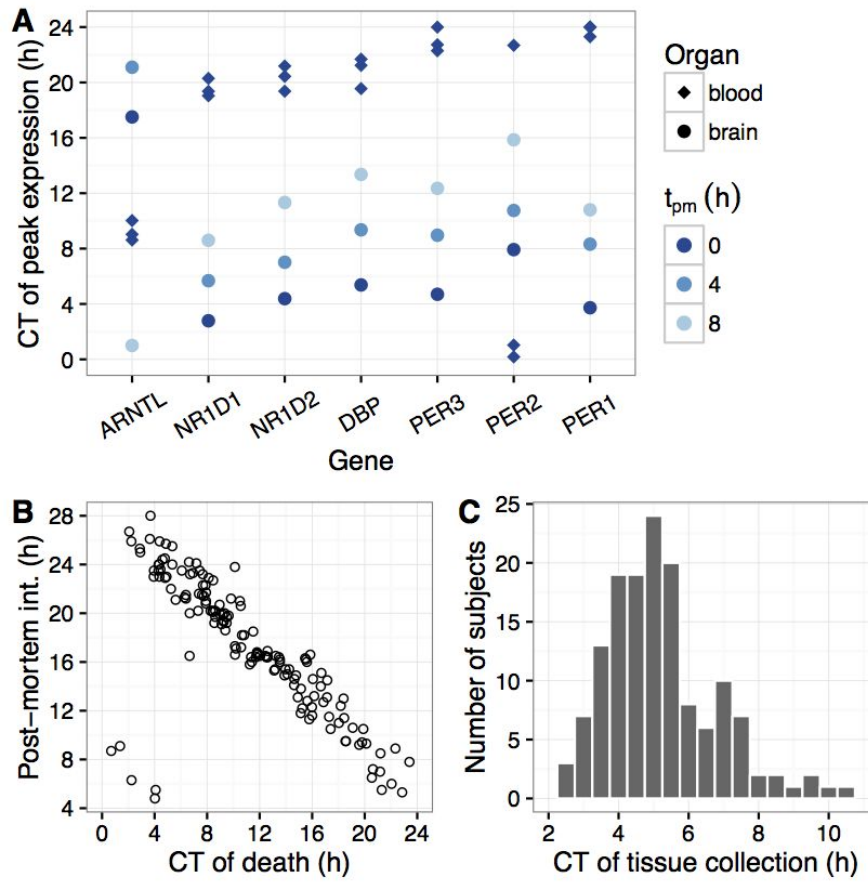
(A) Time of trough expression for each clock gene in each dataset, analogous to Figure 2A. **(B)** Pairwise absolute differences in trough time within and across organs, analogous to Figure 2C.

Suppl. Fig. S6



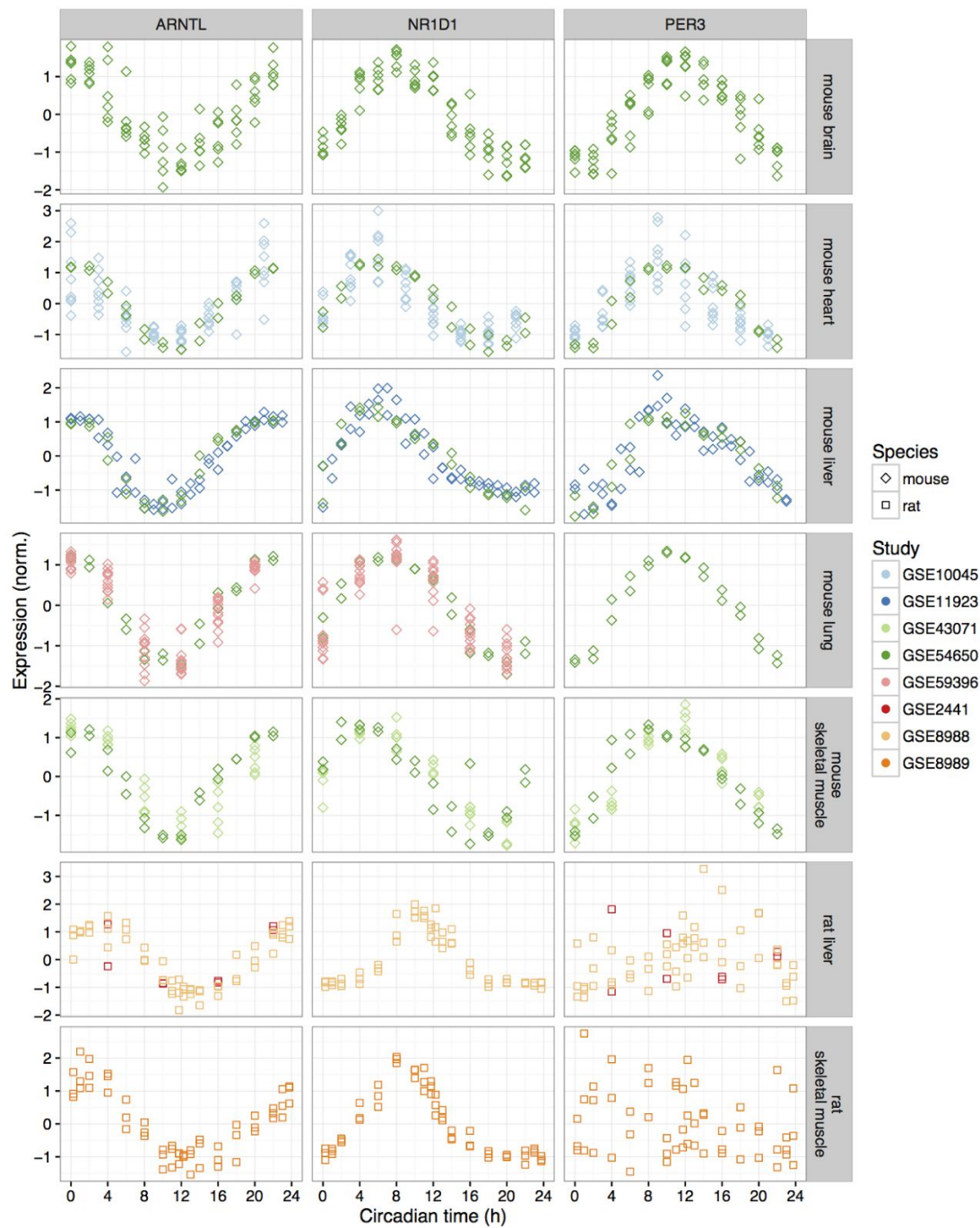
Normalized expression of three clock genes in samples from GSE71620 for different values of t_{pm} , which corresponds to the maximum amount of time the circadian clock continues to progress after the official time of death. Adjusted circadian time is then circadian time of death plus either the postmortem interval or t_{pm} , whichever is less.

Suppl. Fig. S7



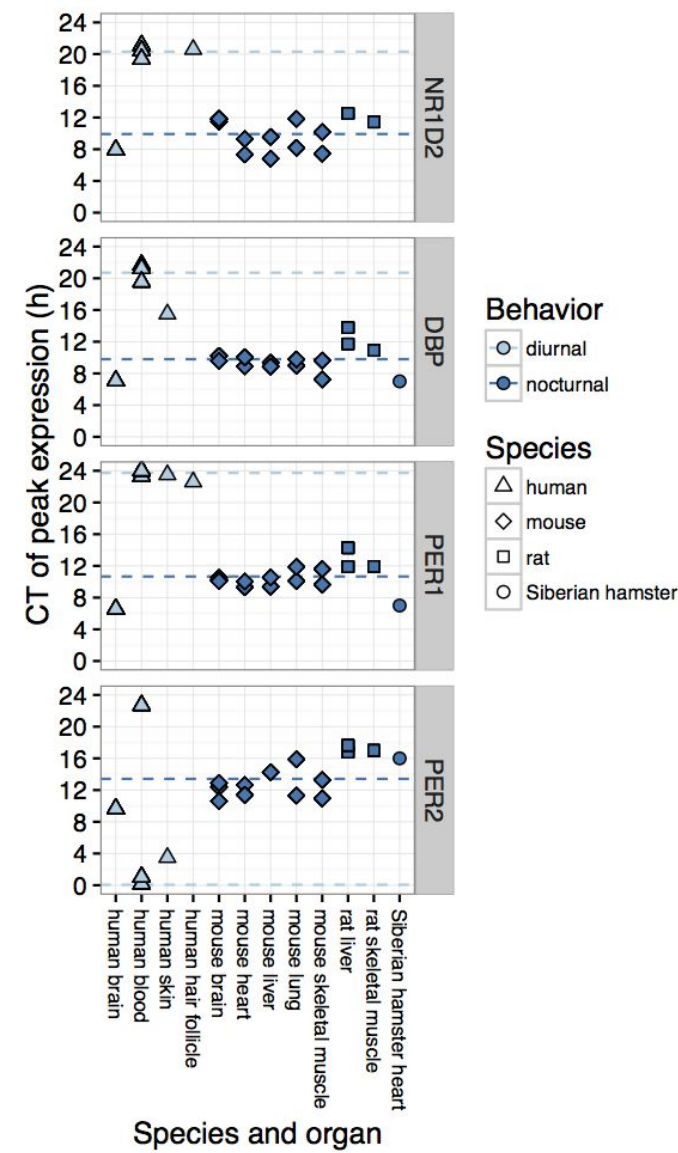
(A) Times of peak expression for clock genes in blood and in samples from GSE71620, given different values of t_{pm} . **(B)** Confounding of postmortem interval and time of death in GSE71620. Each point is a sample. **(C)** Histogram of circadian time of tissue collection in GSE71620 (CT of death plus postmortem interval). For ease of visualization, one sample for which tissue was collected at CT23 has been omitted.

Suppl. Fig. S8



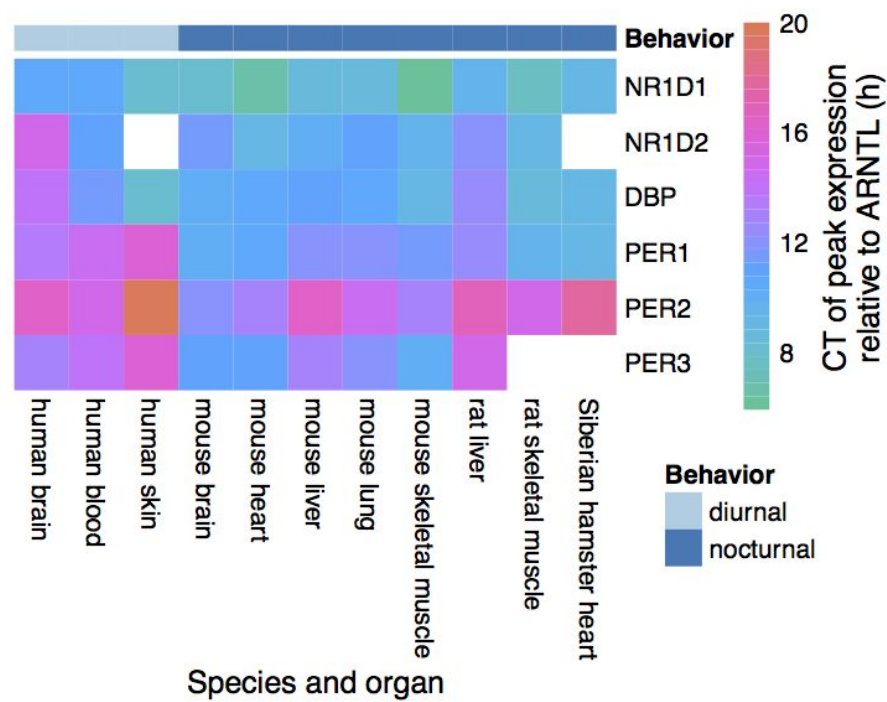
Expression vs. circadian time for three clock genes across multiple species and organs. Only datasets with publicly available microarray data are shown here, although Figure 3 also includes published results for which raw data was not available.

Suppl. Fig. S9



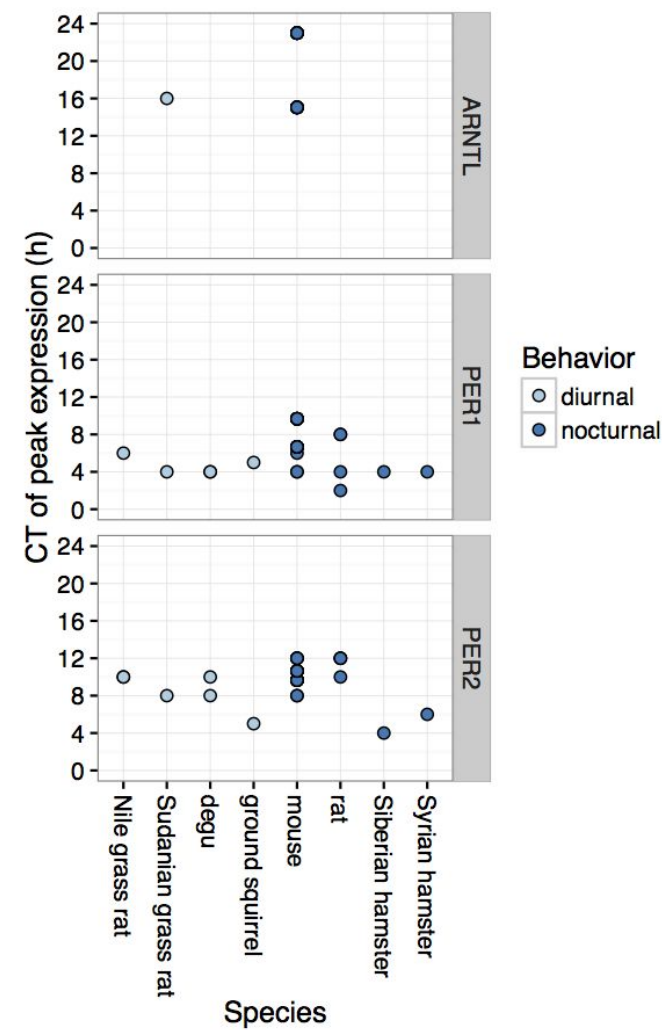
Time of peak expression for the clock genes not shown in Figure 3A.

Suppl. Fig. S10



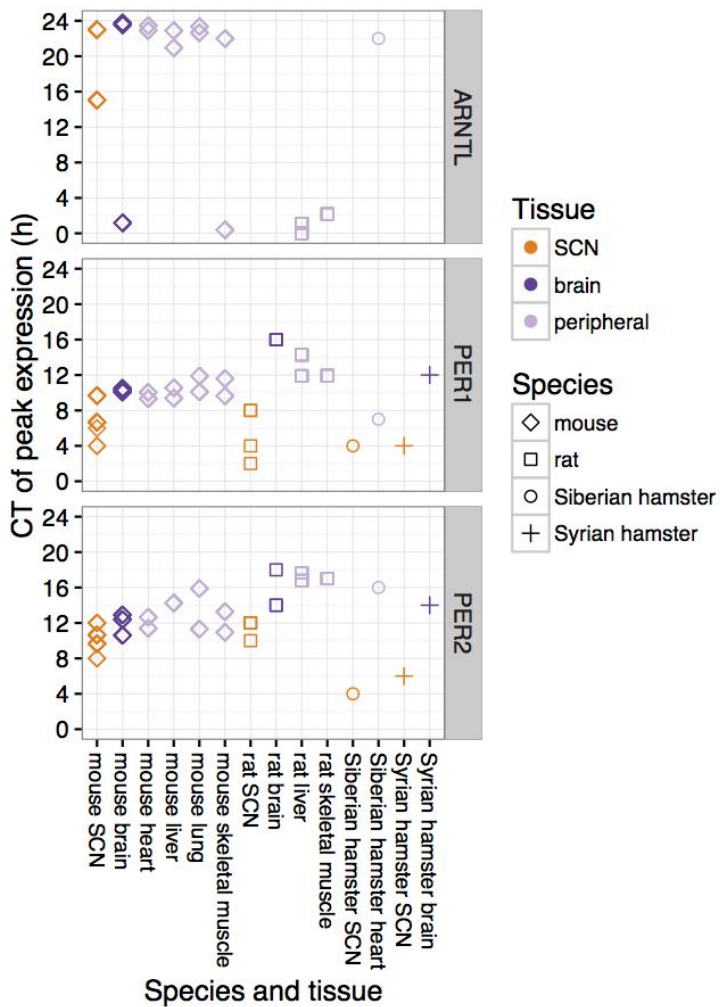
Differences between the peak time for each clock gene and the peak time for the *ARNTL* ortholog in each species and organ (analogous to Suppl. Fig. S4B).

Suppl. Fig. S11



Circadian phasing of clock gene expression in the SCN of various diurnal and nocturnal rodents.

Suppl. Fig. S12



Circadian phasing of clock gene expression across nocturnal mammalian species and including the SCN. Most of the SCN data comes from published studies of in situ hybridization, although the mouse SCN data includes one RNA-seq dataset and one microarray dataset.