1. **Table S1.** Coeffecient and R2 values of regressions between interpolated τ values and
2. τ value calculated

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **A** | Interpolated Ultradian Periods Modulated by Circadian Phase | | | |
| Analysis window | τ'm | | | |
| Method of Calculating Ridge  Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |
| Post-Parsed Dark Phase Data | 0.732 | 0.217 | 0.815 | 0.497 |
| Post-Parsed Light Phase  Data | 0.76 | 0.279 | 0.907 | 0.631 |
| Pre-Parsed Dark Phase Data | 0.659 | 0.228 | 0.822 | 0.541 |
| Pre-Parsed Light Phase Data | 0.692 | 0.317 | 0.901 | 0.694 |
| Un-Parsed Data | 0.34 | 0.119 | 0.41 | 0.283 |
| Analysis window | τ'l | | | |
| Method of Calculating Ridge  Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |
| Post-Parsed Dark Phase Data | 0.598 | 0.198 | 0.803 | 0.535 |
| Post-Parsed Light Phase  Data | 0.608 | 0.238 | 0.774 | 0.577 |
| Pre-Parsed Dark Phase Data | 0.426 | 0.233 | 0.809 | 0.705 |
| Pre-Parsed Light Phase Data | 0.251 | 0.186 | 0.76 | 0.753 |
| Un-Parsed Data | 0.365 | 0.134 | 0.441 | 0.33 |
| **B** | Interpolated Ultradian Periods Not Modulated by Circadian Phase | | | |
| Analysis window | τ'm | | | |
| Method of Calculating Ridge  Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |
| Post-Parsed Dark Phase Data | 0.752 | 0.266 | 0.825 | 0.56 |
| Post-Parsed Light Phase  Data | 0.773 | 0.322 | 0.887 | 0.719 |
| Pre-Parsed Dark Phase Data | 0.659 | 0.228 | 0.822 | 0.541 |
| Pre-Parsed Light Phase Data | 0.706 | 0.301 | 0.887 | 0.715 |
| Un-Parsed Data | 0.792 | 0.294 | 0.879 | 0.638 |
| Analysis window | τ'l | | | |
| Method of Calculating Ridge  Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Post-Parsed Dark Phase Data | 0.76 | 0.347 | 0.874 | 0.684 |
| Post-Parsed Light Phase Data | 0.781 | 0.389 | 0.901 | 0.783 |
| Pre-Parsed Dark Phase Data | 0.426 | 0.233 | 0.809 | 0.705 |
| Pre-Parsed Light Phase Data | 0.446 | 0.281 | 0.831 | 0.833 |
| Un-Parsed Data | 0.793 | 0.368 | 0.903 | 0.73 |
| **C** | Ultradian Structure Randomized and Circadian Structure  Intact | | | |
| Analysis window | τ'm | | | |
| Method of Calculating Ridge Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |
| Post-Parsed Dark Phase Data | 0.001 | 0.003 | 0.00013 | 0.002 |
| Post-Parsed Light Phase Data | 0.000063 | -0.001 | 0.002 | 0.009 |
| Pre-Parsed Dark Phase Data | 0.004B | 0.007B | 0.000023 | 0.001 |
| Pre-Parsed Light Phase Data | 0.00019 | -0.001 | 0.00013 | 0.002 |
| Un-Parsed Data | 0.0000092 | -0.0002 | 0.00031 | 0.004 |
| Analysis window | τ'l | | | |
| Method of Calculating Ridge Period | Ridgewalk Function | | Ridge Maximum | |
| Regression Component | R2 | Slope | R2 | Slope |
| Post-Parsed Dark Phase Data | 0.001 | 0.003 | 0.001 | 0.009 |
| Post-Parsed Light Phase Data | 0.000021 | 0.001 | 0.001 | -0.008 |
| Pre-Parsed Dark Phase Data | 0.00023 | 0.002 | 0.00045 | 0.006 |
| Pre-Parsed Light Phase Data | 0.003 | -0.008 | 0.007A | 0.023A |
| Un-Parsed Data | 0.001 | 0.003 | 0.00003 | 0.001 |

3

1. Note: (A & B). All are significant (p<0.0001). (C). Not significant unless denoted. AP =
2. 0.0073. BP = 0.0409
3. Generation of Artificial Activity Records for Validation of Modified Wavelet Transform
4. Workflow. Time series data were evaluated using a modified wavelet transform
5. analyses. Experiments 1.1 - 1.3 evaluated the precision of the CWT workflow and
6. verified that the analysis procedure itself did not generate systematic artifacts in period
7. or power estimates, especially because we concatenated dark and light periods
8. separately for some analyses. To accomplish this we use simulated activity data
9. (artificial activity record) which possessed defined rhythmic features in the UR domain.
10. Artificial activity records mimicked the amplitude and variability inherent in actual activity
11. data generated by mice. Each artificial activity record contained a robust CR in activity,
12. and 2 or 4 unique signals in the UR domain (1 - 6.5 h). Artificial activity records also
13. contained random noise. Procedures for generating artificial activity records were as
14. follows:
15. 1. Each of the simulation experiments (Experiments 1.1 - 1.3) contained 10
16. treatment groups, and each treatment group (‘Group’) contained 10 individual
17. simulated ‘animals’ (i.e., 10 artificial activity records). Each artificial activity record
18. was composed of an ‘entrained’ CR with a period of 24 h, 2 or 4 URs, each with
19. unique periods.1
20. 2. To generate known periodicities in the CR and UR domains, but against a
21. background of naturalistic variance/noise, artificial activity record were generated
22. with constraints that defined the limits of CR and UR period and amplitude but
23. allowed for UR period to vary between simulated Groups and among individuals
24. within each Groups, as well as for random variability to be inserted (inter-
25. individual variability, see Step #4, below).
26. 3. Each Group was assigned a fundamental CR (fCR) period of 24.0 and two
27. different fundamental UR (fUR) periods, which were randomly selected from
28. values bounded by defined period ranges: faster fURs (fUR1) had periods
29. between 1.07 - 2.13 h, and slower fURs (fUR2) had periods between 2.13 and
30. 4.26 h). This resulted in the generation of 10 different Groups each defined by a
31. fCR of 24.0 h, and two fURs (UR1 [faster] and UR2 [slower]) unique to each
32. treatment Group.
33. 4. Next, artificial activity records for 10 simulated individual ‘animals’ were
34. generated to populate each Group. To generate individual differences among
35. these subjects (without affecting the mean fUR value of the Group), inter-
36. individual variation was added to the fURs within each artificial activity record.
37. The range of the inter-individual variation was limited by margins that were based
38. on the natural range in variability exhibited in free-running CRs of C57BL6/J mice
39. (mean+sem = 23.8 +0.02, n=10; SD=0.063; 3SD = 0.19, or ±0.8% of the period
40. value; [1] scaled down into the UR domain. Thus, within a treatment Group, each
41. of the 10 individuals each had a different unique UR (qUR), which was randomly
42. selected, but was bounded in its range to ±0.8% of the defined fUR for that
43. Group. The introduction of inter-individual variation was performed separately for
44. UR1 and UR2 of each simulated animal within each treatment Group.[1](#_bookmark0)
45. 5. Steps 1-4 were repeated to define fUR periods for each Group and to generate
46. unique qUR periods for each constituent artificial activity record. URs, and the 24
47. h CR common to all artificial activity records, were generated as square wave
48. functions. artificial activity records were 14,400 min (10 days) in duration.[2](#_bookmark1)

1 Intra-individual (i.e., cycle-to-cycle) variability in the qUR of each member of the treatment Group was added in a later step, but any such experimentally-introduced within-animal period variability from cycle to cycle adhered to the period defined by the qUR.

2 For purposes of clarity, this description considers procedures for artificial activity records adulterated with 2 fURs. In some simulations, (e.g., Expt. 1.1), 4 fURs were interpolated into some artificial activity record records. This was necessary to generate artificial activity records with URs that changed period during the L and D phases (‘CR modulated’ rhythms; see *Methods*). In these instances, procedures identical to those described here for 2 fURs (and their constituent qURs) were followed, with the only difference being the number of fURs that were randomly generated (4 vs. 2). artificial activity records with 4 fURs were comprised of

1. 6. Square wave CRs and URs were generated according to the above schema with
2. a length of 14400 minutes (10 days). All records began with CRs and URs in the
3. ON state. CR and UR phases interacted to generate absolute levels of activity at
4. any point in time. In a given 1 min bin, the CR could be in the ON (active) state,
5. or in the OFF (rest) state; simultaneously, either of the 2 URs could likewise be in
6. an ON or OFF state.

58

1. 7. To introduce intra-individual variability and random noise, CR and UR state did
2. not obligately code for a fixed amount of activity, rather, the ON or OFF state
3. coded for a probability of activity. Because activity at each point in the artificial
4. activity record resulted from information derived from 3 interacting rhythms (one
5. CR and two URs)[3](#_bookmark2), a total of 6 permutations of rhythm-states existed at each
6. point in time. The following rules and probabilistic functions were used to
7. determine the absolute activity level (bin value) for each permutation (CR-UR-UR
8. permutation indicated [in brackets]). Probability-driven mean activity levels for a
9. bin with each unique permutation are summarized in Table S.2.
10. a. [OFF-OFF-OFF] If the CR was in the OFF state, and both URs were also
11. in OFF states, then: 99% of the time the bin value = 0, and 1% of the time
12. it was set to a random integer ranging from 0 - 30. This chance was also
13. added to all of the below conditions.

2 different fUR1s, and 2 different fUR2s, modulated by circadian phase. For the two fUR1s, one was randomly chosen to be inserted into the active phase of the CR and the other was inserted into the rest phase of the CR; similarly, the two fUR2s were each assigned to a specific circadian phase (see *Methods*). All unique qURs still behaved the same with respect to range of variability around their respective fUR periods. See #7 below for details of quantitative generation of intra-individual variability in records that contained 2 vs. 4 fURs.

3 Although artificial activity records in the CR modulated group in Experiment 1.1 possessed 4 fURs, note that at any point in time only 2 of the fURs were being expressed, thus even in these records, a maximum of 3 rhythms would be interacting.

72

73 ∑30 . 01 ∗ 1/31 ∗ 𝑛 = .15

𝑛=0

74

1. b. [OFF-ON-OFF] If the CR was in the OFF state, and one of the two URs
2. was in the ON state but the other UR was in the OFF state, then: 79% of
3. the time the bin value = 0, and 20% of the time it was set to a random
4. integer ranging from 0 – 10. 1% of the time it was set to a random integer
5. ranging from 0 - 30.

80

81 ∑20

.2 ∗ 1/21 ∗ 𝑛 + ∑30

. 01 ∗ 1/31 ∗ 𝑛 = 2.15

𝑛=0

82

𝑛=0

1. c. [OFF-ON-ON] If the CR was in the OFF state, but both URs were in the
2. ON states, then: 59% of the time the bin value = 0, 20% of the time it was
3. set to a random integer ranging from 0-30 (additive), and 13.34% of the
4. time it was set to a random integer ranging from 0-20 (interference)[4](#_bookmark3) 1% of
5. the time it was set to a random integer ranging from 0 - 30.

88

89 ∑20

.2 ∗ 1/21 ∗ 𝑛 + ∑30

.1334 ∗ 1 ∗ 𝑛 + ∑30

. 01 ∗ 1/31 ∗ 𝑛 = 4.15

𝑛=0

90

𝑛=0

31 𝑛=0

1. d. [ON-OFF-OFF] If the CR was in the ON state, but both URs were in OFF
2. states, then: 49% of the time the bin value = 0, and 50% of the time it was

4 These two settings were selected in an effort to remain agnostic about the nature of UR-UR interactions when both are in the ON state, The probabilistic range of 0-25 accounts for URs interacting in an additive manner, whereas the range from 0-15 accounts for URs interfering with one another.

1. set to a random integer ranging from 0 – 20. 1% of the time it was set to a
2. random integer ranging from 0 - 30.

95

96 ∑20

.5 ∗ 1/21 ∗ 𝑛 + ∑30

. 01 ∗ 1/31 ∗ 𝑛

= 5.15

𝑛=0

97

𝑛=0

1. e. [ON-ON-OFF] If the CR was in the ON state, and one of the two URs was
2. in the ON state but the other UR was in the OFF state, then: 29% of the
3. time the bin value = 0, and 70% of the time it was set to a random ranging
4. from 0 – 20. 1% of the time it was set to a random integer ranging from 0 -
5. 30.

103

104

∑20 .7 ∗ 1

∗ 𝑛 + ∑30

. 01 ∗ 1/31 ∗ 𝑛

= 7.15

105

𝑛=0 21

𝑛=0

1. f. ON-ON-ON] If the CR was in the ON state, and both URs were in the ON
2. states, then: 9% of the time the bin value = 0[5](#_bookmark4), and 18% of the time it was
3. set to a random integer ranging from 0 - 10 [low activity], 54% of the time it
4. was set to a random integer ranging from 0 - 20 [moderate activity], and
5. 18% of the time it was set to a random integer ranging from 0 – 30 [high
6. activity][6](#_bookmark5). 1% of the time it was set to a random integer ranging from 0 -
7. 30.

113

5 Bouts of LMA inactivity (rest, grooming, sleep) occur abundantly during the active phase, thus, the CR ON cannot obligately code for a non-zero integer activity value.

6 To generate 3 different levels of higher-probability activity during the ON-ON-ON permutation.

114

10

𝑛=0

∑

.18 ∗ 1/11 ∗ 𝑛 + ∑20

.54 ∗ 1/21 ∗ 𝑛

30

𝑛=0

+ ∑

.18 ∗ 1

31

∗ 𝑛 +

115

∑30

. 01 ∗ 1

∗ 𝑛

= 9.15

𝑛=0 31

𝑛=0

116

117

1. **Table S.2 Mean activity value for each of the 6 unique permutation bins (UP mean**
2. **E.V.) activity value being inserted**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| CR | UR | UR | UP E.V. | Range | Probability (P0) of 0 value |
| - | - | - | 0.15 | 0 – 30 | P = 0.99 |
| - | + | - | 2.15 | 0 – 30 | P = 0.79 |
| - | + | + | 4.15 | 0 – 30 | P = 0.59 |
| + | - | - | 5.15 | 0 – 30 | P = 0.49 |
| + | + | - | 7.15 | 0 – 30 | P = 0.29 |
| + | + | + | 9.15 | 0 – 30 | P = 0.09 |

120

1. Note: Table indicates mean value for each unique bin, but probabilistic function ensures
2. bin-to-bin variability, even if they possess the same permutation.

123

1. 8. For each artificial activity record, a low-amplitude time series of random noise
2. equal in length to each artificial activity record was generated and the random
3. values were subtracted from the artificial activity record generated in Step #7.
4. Noise was generated by creating a square wave with a random period between 0
5. and 1 minute. When the noise was in the OFF state the bin value = 0, and when
6. noise was in the ON state a random number ranging from 0 – 1 was selected.
7. The resultant random-period high-frequency time series values were then
8. subtracted from the artificial animal time series.
9. 9. Finally, to account for variability in activity level, we assumed the default time
10. series state to be high activity and multiplied each artificial activity record by a
11. decimal between 1.0 & .2 such that it somewhere between 100% to 20% of the
12. default level of activity. As this grouping of 10 artificial activity record is meant to
13. approximate group conditions that might lead to similar activity and temporal
14. structure we wanted groupings to have both similar activity levels while
15. maintaining some variance. To accomplish this for each set of 10 artificial activity
16. record, a random of number was chosen such that 50 % of the time they were
17. set to having high activity, a range of [1 - .6], and 50% of the time they were set
18. to having low activity, a range of [.6-.2]. For each individual artificial activity
19. record within the high and low grouping then a random number was chosen
20. within the corresponding range and the artificial activity record was multiplied by
21. it. Negative value bins resulting from noise subtraction were set to = 0.
22. 10. To evaluate face validity of artificial activity record, they were visually compared
23. to real locomotor activity time series data. See comparisons of artificial activity
24. record and actual activity data in Fig 2.

148

149

150

1. 1. Schwartz WJ, Zimmerman P. Circadian timekeeping in BALB/c and C57BL/6 inbred
2. mouse strains. J Neurosci. 1990;10(11):3685-94. PubMed PMID: 2230953; PubMed Central
3. PMCID: PMC6570095.

154