Supplemental Data

Setting Clock Speed in Mammals: The CK1ε *tau* Mutation in Mice Accelerates Circadian Pacemakers by Selectively Destabilizing PERIOD Proteins

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Figure S1. Gene Targeting to Generate a Floxed *tau* Mutant Form of the *Ck1ε* Gene that Can Be Converted to a Knockout Allele by *Cre*-Mediated Recombination

(A) Targeting strategy. Schematic representation of the mouse WT *Ck1ε* gene locus (exons 2-7), targeting construct, homologously recombined allele, *tau* allele generated in ES cell clones by Cre-mediated deletion of the selection cassette (see methods); and the “knock-out” allele, generated in vivo by crossing the *tau* mutant line with a cre-deletor strain (see methods). Exons are depicted as filled blocks with numbers and adjoining lines represent introns. The location of the *tau* mutation is indicated (*). NeoTK - neomycin / thymidine kinase selection cassette. LoxP sites are shown as arrowheads and the external probe used for Southern blot is indicated as bar (probe).

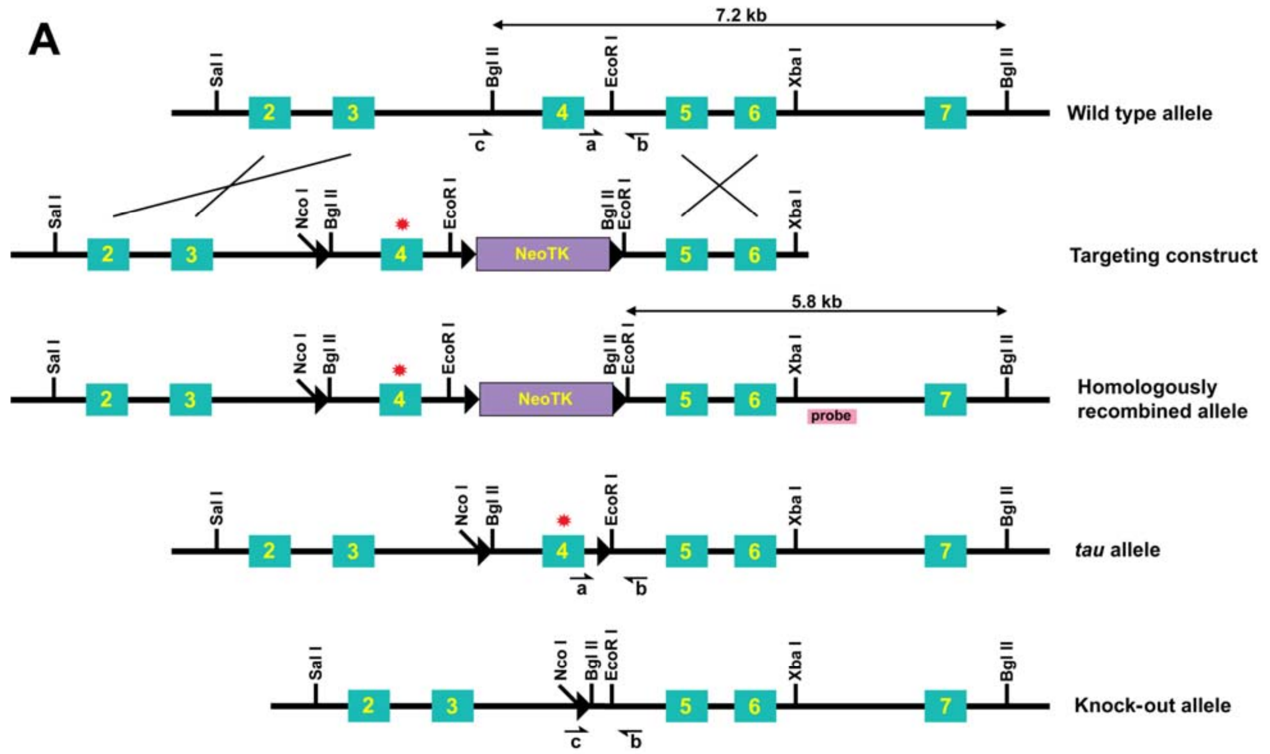
(B) Southern blot of Bgl II digested genomic DNA from G418 resistant ES cell clones hybridized with the ^{32}P-labelled external probe. Arrows indicate homologously recombined clones.

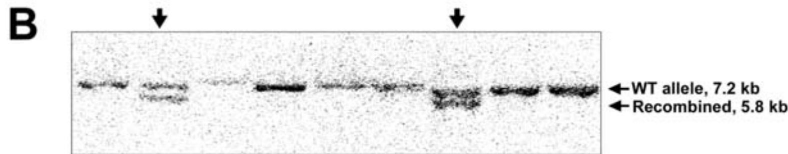
(C) Genotyping of mice carrying mutant forms of the *Ck1ε* gene. C1, Mice with WT (+) or *tau* (*tau*) alleles were genotyped using primers ‘a’ and ‘b’ (see Figure S1A above and methods). Genomic DNA amplified with the a/b primer combination yield ~600 bp fragment for the (+) allele and ~650 bp fragment for the (*tau*) allele. C2, The knockout (-) allele was identified by PCR using primers ‘b’ and ‘c’ (see Figure S1A). The (-) allele generates a DNA band of ~480 bp. This primer pair also generates a second band (~520 bp) from an entirely separate locus (chromosome 12 - determined by direct sequencing) which fortuitously acted as a positive control for the knock-out PCR.

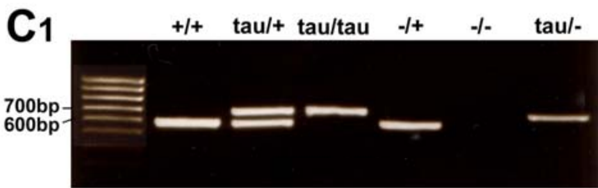
(D) *Tau* mutation determined by sequencing. Genomic DNA encoding exon 4 of *Ck1ε* gene was PCR amplified from WT (+/+) animals and mice homozygous for the *tau* mutation (*tau*/*tau*). Amplified products were sequenced and the relevant part of the chromatogram shows the C to T transition resulting in R178C substitution.

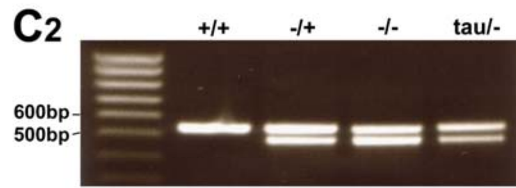
(E) RT-PCR demonstration of the absence of exon 4 in knockout mouse using brain cDNA as template. *Gapdh* gene was used as internal control.

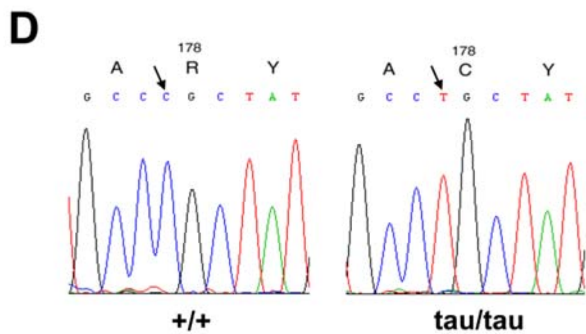
(F) Western blotting of CK1ε in whole brain extracts from +/+, *tau*/*tau* and -/- mice. α-Tubulin was used as loading control.

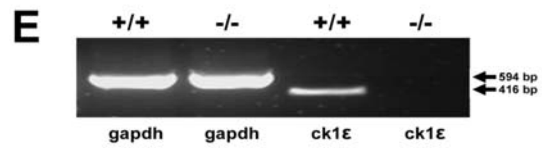

A
7.2 kb
Sal I
Bgl II
EcoR I
Xba I
Wild type allele
Targeting construct
Nco I
NeoTK
5.8 kb
Homologously recombined allele
probe
tau allele
Knock-out allele


B
WT allele, 7.2 kb
Recombined, 5.8 kb


C1
+/+
tau/+
tau/tau
-/+
-/-
tau/-
700bp
600bp


C2
+/+
-/+
-/-
tau/-
600bp
500bp


D
A R Y
178
A C Y
+/+
tau/tau


E
+/+
-/-
594 bp
416 bp
gapdh
ck1ε

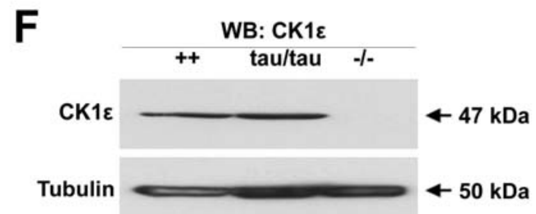

F
WB: CK1ε
++
tau/tau
-/-
CK1ε
47 kDa
Tubulin
50 kDa

Figure S2. Representative Actograms from Three *tau* Mutant Mice Exhibiting a Phase Advance to the Prevailing LD 12hrL:12hrD Cycle (A), Masking (B), or Absence of Entrainment (C)

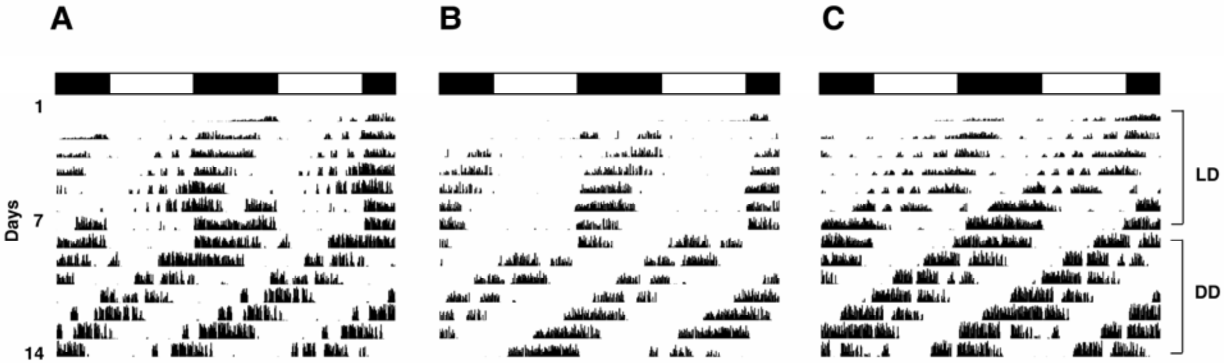
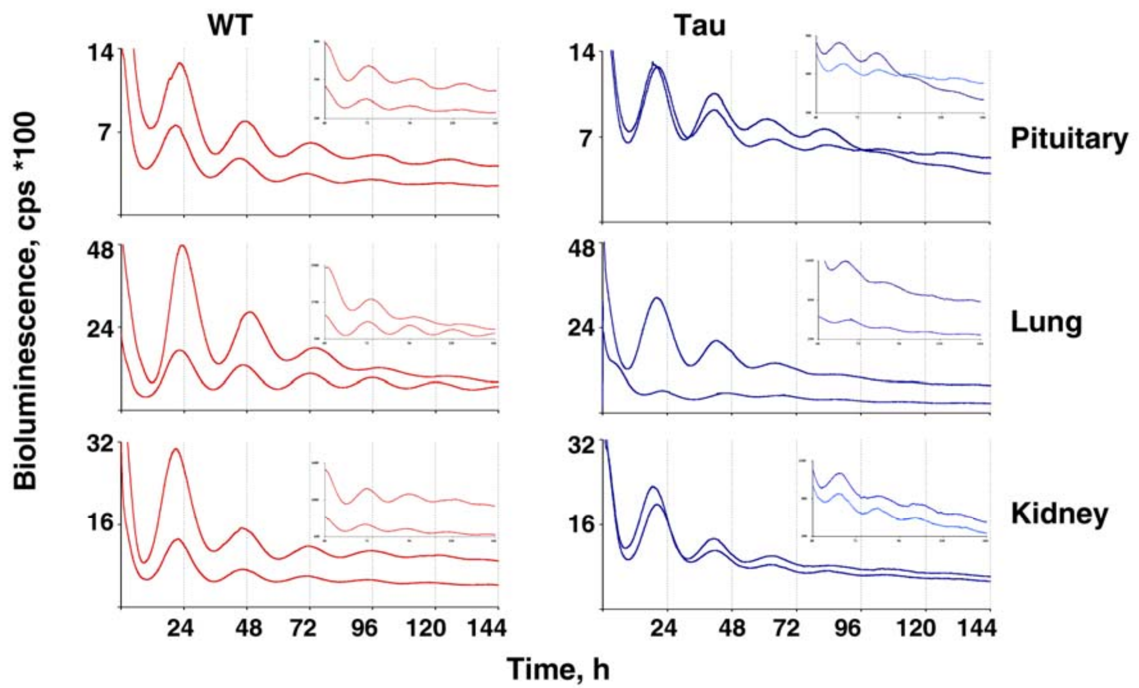


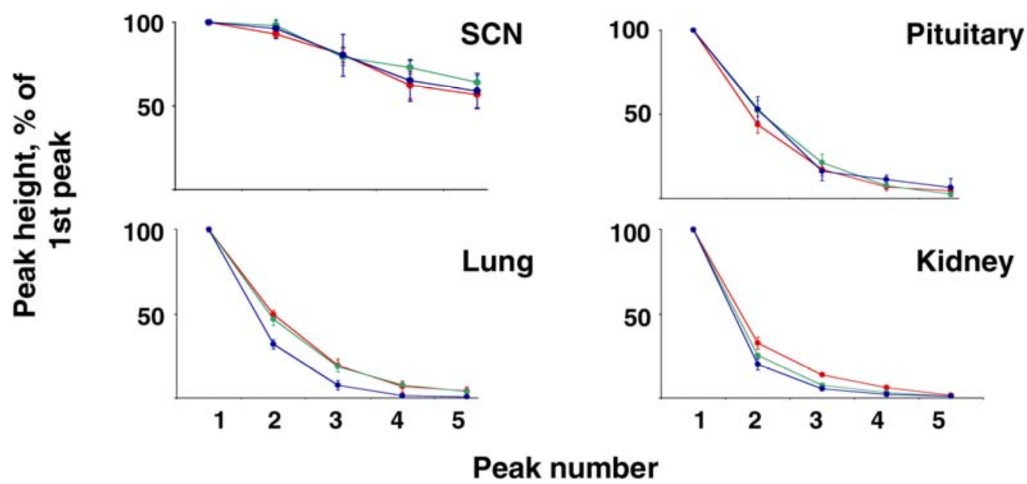
Figure S3. CK1ε^{tau} Mutation Shortens Period and Causes Damping of PER2::LUC Circadian Expression in Peripheral Tissues

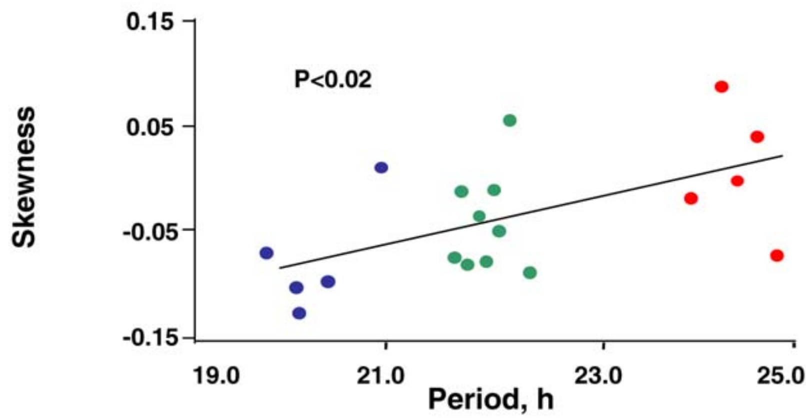
(A) Representative traces (2 per panel) of circadian bioluminescence from pituitary, lung and kidney explants of WT (left) and CK1ε$^{tau/tau}$ (Tau, right) mutant mice. Insets show data with expanded ordinate for corresponding time points to show continuing oscillation.

(B) Progressive damping of circadian PER2::LUC expression was more marked in pituitary, lung and liver than in SCN. Damping in the peripheral tissues was enhanced by the CK1ε^{tau} mutation (red= WT; green= heterozygote; blue= homozygote). Data are % normalized to the first peak of expression and plotted as group data (mean±SEM). All show a significant decline in peak amplitude with time, but only in lung and kidney is there a significant effect of genotype on damping (genotype and interaction effects, ANOVA: SCN: genotype ns; interaction ns. Lung: genotype $p<0.05$; interaction $p<0.01$. Kidney: genotype $p<0.01$; interaction $p<0.01$. Pituitary: genotype ns; repeated measure $p<0.001$; interaction ns).

(C) Correlation between circadian period and skewness of waveform from PER2::LUC bioluminescence recordings from SCN slices. CK1ε$^{tau/tau}$ slices (blue) with shorter periods also had greater negative (leftward) skew arising from more rapid decline in PER2::LUC signal than in heterozygous (green) or WT (red) slices.


A
WT
Tau
Bioluminescence, cps *100
14
7
48
24
32
16
Pituitary
Lung
Kidney
24
48
72
96
120
144
Time, h


B
Peak height, % of 1st peak
SCN
Pituitary
Lung
Kidney
100
50
1
2
3
4
5
Peak number


C
Skewness
P<0.02
0.15
0.05
-0.05
-0.15
19.0
21.0
23.0
25.0
Period, h