Natural selection against a circadian clock gene mutation in mice

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Circadian rhythms with an endogenous period close to or equal to the natural light–dark cycle are considered evolutionarily adaptive (“circadian resonance hypothesis”). Despite remarkable insight into the molecular mechanisms driving circadian cycles, this hypothesis has not been tested under natural conditions for any eukaryotic organism. We tested this hypothesis in mice bearing a short-period mutation in the enzyme casein kinase 1e (tau mutation), which accelerates free-running circadian cycles. We compared daily activity (feeding) rhythms, survivorship, and reproduction in six replicate populations in outdoor experimental enclosures, established with wild-type, heterozygous, and homozygous mice in a Mendelian ratio. In the release cohort, survival was reduced in the homozygote mutant mice, revealing strong selection against short-period genotypes. Over the course of 14 mo, the relative frequency of the tau allele dropped from initial parity to 20%. Adult survival and recruitment of juveniles into the population contributed approximately equally to the selection for wild-type alleles. The expression of activity during daytime varied throughout the experiment and was significantly increased by the tau mutation. The strong selection against the short-period tau allele observed here contrasts with earlier studies showing absence of selection against a Period 2 (Per2) mutation, which disrupts internal clock function, but does not change period length. These findings are consistent with, and predicted by the theory that resonance of circadian rhythms with frequencies that are not in close resonance with the 24 h cycle should be selected against in nature. The hypothesis that circadian clocks with abnormal periods out of resonance with the external cycle entail a real fitness cost has not been tested under natural conditions for any eukaryotic organism. The authors declare no conflict of interest.

Author contributions: K.S., M.W., and M.H. designed research; K.S., M.W., and M.H. performed research; A.S.I.L. contributed new reagents/analytic tools; K.S. and S.D. analyzed data; and K.S., S.D., A.S.I.L., and M.H. wrote the paper.

The authors declare no conflict of interest.

Significance

The circadian clock has evolved to anticipate daily events and is assumed to be important for Darwinian fitness. The endogenous period of the clock runs close to 24 h, permitting accurate entrainment to the natural light/dark cycle. Circadian clocks with abnormal periods are therefore predicted to have negative consequences for fitness. We compared the fitness of mice with deviant circadian periods in populations living in a seminatural environment. Mice with nearly 24 h “resonant” rhythms survived longer and reproduced more than mice with rhythms shortened by a mutation in the circadian Ck1e allele. Apart from the fundamental importance of such fitness effects in nature, this finding may have implications for humans subjected to circadian-rhythm deviations under abnormal work and lighting schedules.

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mice. In all trappings, a total of 853 new individuals were caught, genotyped, fitted with transponders, and released, so that analyses are based on a total of 1,088 mice.

**Table 1. Statistical evidence on tau genotype dependence of adult survival and juvenile recruitment in the combined six populations**

<table>
<thead>
<tr>
<th>Int. (d) and genotype</th>
<th>R (S)</th>
<th>S/R</th>
<th>χ² surv</th>
<th>P surv</th>
<th>fA*</th>
<th>Y</th>
<th>EY</th>
<th>χ² recr</th>
<th>P recr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int. 1 (157)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>52</td>
<td>23</td>
<td>0.44</td>
<td></td>
<td>74</td>
<td>41.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/+</td>
<td>135</td>
<td>61</td>
<td>0.45</td>
<td></td>
<td>64</td>
<td>73.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/tau</td>
<td>48</td>
<td>8</td>
<td>0.17</td>
<td></td>
<td>10</td>
<td>32.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>235</td>
<td>92</td>
<td>0.39</td>
<td>12.81</td>
<td>&lt;0.01</td>
<td>0.471</td>
<td>148</td>
<td>42.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Int. 2 (47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>97</td>
<td>76</td>
<td>0.78</td>
<td></td>
<td>102</td>
<td>84.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/+</td>
<td>125</td>
<td>82</td>
<td>0.66</td>
<td></td>
<td>70</td>
<td>82.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/tau</td>
<td>18</td>
<td>13</td>
<td>0.72</td>
<td></td>
<td>15</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>240</td>
<td>171</td>
<td>0.71</td>
<td>4.34</td>
<td>&gt;0.1</td>
<td>0.327</td>
<td>187</td>
<td>6.67</td>
<td>0.035</td>
</tr>
<tr>
<td>Int. 3 (114)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>178</td>
<td>43</td>
<td>0.24</td>
<td></td>
<td>232</td>
<td>229.2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>tau/+</td>
<td>152</td>
<td>26</td>
<td>0.17</td>
<td></td>
<td>178</td>
<td>177.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/tau</td>
<td>28</td>
<td>3</td>
<td>0.11</td>
<td></td>
<td>31</td>
<td>34.3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>All</td>
<td>358</td>
<td>72</td>
<td>0.20</td>
<td>4.21</td>
<td>&gt;0.1</td>
<td>0.279</td>
<td>441</td>
<td>0.36</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Int. 4 (106)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>+/+</td>
<td>275</td>
<td>16</td>
<td>0.058</td>
<td></td>
<td>530</td>
<td>453.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/+</td>
<td>204</td>
<td>9</td>
<td>0.044</td>
<td></td>
<td>272</td>
<td>325.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/tau</td>
<td>34</td>
<td>2</td>
<td>0.059</td>
<td></td>
<td>35</td>
<td>58.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>513</td>
<td>27</td>
<td>0.053</td>
<td>*</td>
<td>*</td>
<td>0.264</td>
<td>837</td>
<td>30.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ints. 1 3 (318)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>327</td>
<td>142</td>
<td>0.43</td>
<td></td>
<td>408</td>
<td>328.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau/+</td>
<td>412</td>
<td>169</td>
<td>0.41</td>
<td></td>
<td>312</td>
<td>353.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>tau/tau</td>
<td>94</td>
<td>24</td>
<td>0.26</td>
<td></td>
<td>56</td>
<td>94.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>833</td>
<td>335</td>
<td>0.40</td>
<td>9.94</td>
<td>&lt;0.01</td>
<td>776</td>
<td>40.16</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Data are listed in the table for the three genotypes over four intervals (1 4; Fig. 1) of the study. Headings from left to right are defined as follows: interval (d) and genotype, duration of interval (int.) between trapping in days and genotype; R, mice released with tags at the beginning of the interval (including those already tagged before); S, tagged mice caught alive at the end of the interval; S/R, fraction surviving the interval; χ² surv, survival; χ² recr, recruitment (recr) and P recr, test of the correspondence of the genotype in the prior (EY) and new (Y) cohort. In the bottom four rows, the data for intervals 1 3 are added and analyzed jointly. Note: In interval 4, the strongly reduced initial frequency of homozygote mice (6.6%) combined with the low winter population survival (5.3%) yielded an expected S for this genotype of only 2, too small to allow for χ² testing. For these reasons, interval 4 was not included in the overall joint analysis (for intervals 1 3) in the lower part of the table. Genotype dependence of recruitment can however be tested over all four intervals combined and yields χ² = 108.8 (P < 0.0001).
significantly affected by genotype (Table 1; \( P < 0.01 \)), with homozygous mutants having the lowest probability of survival. For the cohort initially released, for which the date of birth is known, the effect of genotype on survival had a clear effect on the mean life span: \(+/+\) mice, 160.5 d; \(tau/tau\) mice, 156.2 d; and \(tau/tau\) mice, 105.0 d. Homozygote mutant mice in this cohort exhibited a significantly increased mortality (Cox proportional hazard model; \( P < 0.005 \)). In all three genotypes, male mice lived significantly longer than female mice (\( P < 0.0001; \) Table 2); an interaction between genotype and sex was absent (\( P = 0.26 \)).

Genotypic variation in reproduction could not be assessed at the time of birth, but we estimated the variation in recruitment to the population from the genotypic distribution of newly appeared young mice that had not yet been fitted with tran sponders (Y) after each interval. The numbers of each genotype were compared with the number expected (\( E(Y) \)) on the basis of the Hardy–Weinberg equilibrium. This assessment of genotype effects on recruitment includes both differential reproduction and differential mortality in early life. Because both adult survival and recruitment were significantly affected by the \( tau \) mutant allele, we estimated their total effect on fitness i.e., the rate of gene propagation, along with the contributions of differential survival and differential recruitment. The effects on fitness were estimated by first computing the instantaneous rate loss in mutant allele frequency by differential survival (\( i_s, d^{-1} \)) and then by the combined effects of differential survival and recruitment together (\( i_s + i_r, d^{-1} \)). These two values turned out to be \(-0.0014 \) and \(-0.0028 \), respectively. Hence, effects on survival and recruitment contributed approximately equally to the rate of disappearance of the \( tau \) allele from the population.

### Table 2. Cox proportional hazard model on survival of the mice in the release cohort

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Hazard</th>
<th>Hazard ratio</th>
<th>SE</th>
<th>z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (( tau/tau ))</td>
<td>0.70</td>
<td>2.02</td>
<td>0.22</td>
<td>3.18</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Genotype (( tau/tau ))</td>
<td>0.56</td>
<td>1.87</td>
<td>0.15</td>
<td>4.18</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Enclosure was added as a random term; \( n = 211 \), model fit \( \chi^2 = 25.9 \) (df = 4), \( P < 0.0001 \). The hazard and hazard ratio values are relative to wild type and male mice; the positive values for homozgyous and male mice indicate a greater risk of death and relative death rate, respectively. n.s., not significant.

### Table 3. Estimation of the instantaneous rate of disappearance of the \( tau \) allele and the contribution of differential adult survival and juvenile recruitment

<table>
<thead>
<tr>
<th>Interval t</th>
<th>Days</th>
<th>R</th>
<th>S</th>
<th>Y</th>
<th>FR</th>
<th>FS</th>
<th>FY</th>
<th>( i_s )</th>
<th>( i_r )</th>
<th>( i_{prop} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>157</td>
<td>235</td>
<td>92</td>
<td>148</td>
<td>0.4915</td>
<td>0.4185</td>
<td>0.2838</td>
<td>0.0010</td>
<td>0.0035</td>
<td>0.0024</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>240</td>
<td>171</td>
<td>187</td>
<td>0.3354</td>
<td>0.3158</td>
<td>0.2674</td>
<td>0.0013</td>
<td>0.0048</td>
<td>0.0031</td>
</tr>
<tr>
<td>3</td>
<td>114</td>
<td>358</td>
<td>72</td>
<td>441</td>
<td>0.2905</td>
<td>0.2222</td>
<td>0.2721</td>
<td>0.0024</td>
<td>0.0006</td>
<td>0.0008</td>
</tr>
<tr>
<td>4</td>
<td>106</td>
<td>513</td>
<td>27</td>
<td>837</td>
<td>0.2651</td>
<td>0.2407</td>
<td>0.2043</td>
<td>0.0009</td>
<td>0.0025</td>
<td>0.0024</td>
</tr>
<tr>
<td>End</td>
<td>864</td>
<td></td>
<td></td>
<td></td>
<td>0.2054</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean: 0.0014 0.0028 0.0021

Headings from left to right are defined as follows: days, duration of interval between last days of each capture effort when all mice were released and the next capture effort; \( R \), total number of mice released (after last interval: killed); \( S \), number of individuals among \( R \) that were present alive at the end of the interval; \( Y \), number of (young) individuals present at the end that were not yet present at the beginning of the interval, i.e., born in the interval; \( FR \), relative frequency of the \( tau \) allele among \( R \); \( FS \), relative frequency of the \( tau \) allele among \( S \); \( FY \), relative frequency of the \( tau \) allele among \( Y \); \( i_s \), day \(^{-1} \), instantaneous rate of loss in relative \( tau \) allele frequency by differential survival, \( ln(\text{FS}/\text{FR})/d \); \( i_r \), day \(^{-1} \), instantaneous rate of loss in relative \( tau \) allele frequency by both differential survival and recruitment, \( ln(\text{FY}/\text{FR})/d \); \( i_{prop} \), day \(^{-1} \), instantaneous rate of overall loss in relative \( tau \) allele frequency observed in the population, \( ln(\text{RFy}/\text{RFx})/d \).

**Effect of the \( tau \) Allele on Feeding Activity Rhythms.** The tran sponder records permitted assessment of genotype effects on visits to the feeder and presumed feeding activity. Mice spent on average 37.4 min (interindividual SD 27.8 min) per day at a feeder. Feeding activity rhythms were variable, both within and between genotypes (Fig. 2 D F), but the mean activity patterns were comparable (composite actograms, Fig. 2 G I), and there was no significant effect of genotype on average total feeding activity per 24 h (ANOVA, \( F = 1.322, P = 0.25 \); Fig. 3 A). Under the strong natural LD cycle, periodicity in feeding activity was similar in all genotypes. In the Lomb Scargle power spectrum (12), averaged over all 14 d intervals within the lifespan of an individual, the period with the highest power was at or close to 24 h for virtually all mice, and the spectra of almost all mice had a second peak at a period <24 h (Fig. 4). We quantified each individual’s relative expression of feeding activity during daytime [Diurnality index \( D (13, 14); SI Appendix, SI Text \)]. \( D \) varied strongly over time (Fig. 2 F): In the summer of 2008, \( D \) reached levels above zero i.e., more activity during the day than at night. Throughout the study, homzygote \( tau \) mutants exhibited significantly more diurnal activity than wild types, with intermediate levels of \( D \) in the heterozygotes (\( \chi^2 = 94.7, df = 8, P < 0.001; \) Fig. 3 B). There was no significant interaction between sex and genotype (\( P = 0.92 \)).

**Discussion**

Our data show that the circadian \( tau \) mutant allele has a profound impact on both components of fitness, adult survival and juvenile recruitment, and affects entrainment of behavioral rhythms’ natural LD cycles. The strength of selection against the \( tau \) mutation is remarkable, and can be estimated by the overall instantaneous
rate of population decline in the relative frequency of the mutant allele, which is $i_{pop} = -0.0021 \text{ d}^{-1}$ (Table 3), or $\sim 19.6\%$ per 100 d. This value for strength of selection compares with competition.

Fig. 2. Effects of allele, which is rate of population decline in the relative frequency of the mutant cates darkness). under LD 12:12 h and constant darkness (DD; day 15 onward; gray shade indicates darkness from civil twilight (ct) at dusk. (J) Diurnality Index D over the entire course of the experiment.

Fig. 3. Variation in average daily feeding activity and average diurnality index between tau genotypes. Feeding activity, expressed in presence (in the number of 1 min bins per day per individual) at the feeders (no effect of genotype; ANOVA, $F = 1.322, P = 0.25$) (A) and the diurnality index D (B) estimates from a linear mixed effect model (LME) fitted with sex as fixed effect and date, individual, and enclosure as random effects (effect of genotype with both sexes combined, $\chi^2 = 94.7, df = 8, P < 0.0001$).

studies of circadian period mutations of Cyanobacteria (Synecho coccus) maintained in batch culture with wild type strains, which reported a net disappearance rate of 9.5% per generation (5). Collectively, these disappearance rates suggest that natural selection operates to eliminate genotypes expressing a circadian oscillation that is not in resonance with the period of the earth’s rotation. Our analyses suggested that both adult survival and juvenile recruitment contributed to selection for wild type alleles. To assess potential differences in recruitment among genotypes, we used the genotype distribution of known mice halfway through a trapping interval to predict the genotype distribution of offspring born in that interval and trapped at the end of it. Recruitment to the next generation encompasses both reproduction itself and juvenile survival until the next trapping effort. Recruitment could have been impaired in homozygous and heterozygous mutants through decreased mating success and lowered fecundity of parents, as well as increased intrauterine or juvenile mortality, both before and after weaning, none of which could be quantified in our field study. We nonetheless think it is useful to distinguish this episode of life history from adult mortality. The pathways through which the mutation affected adult survival likewise still have to be identified. In the initial release cohort the only cohort for which we could measure lifespan directly the $\tau$ mutation reduced lifespan of homozygous, but not of heterozygous, mice. We have no explanation as to why longevity in heterozygous mice was not intermediate; however, the reduced recruitment relative to the expected ($Y_{EX}$ in Table 1) shows intermediate values for heterozygote mice over intervals 1 3 (1.24, 0.88, and 0.59 for wild type, heterozygotes, and homozygotes, respectively).

The reduced lifespan in homozygous mice contrasts with laboratory studies of $\tau$ mutant hamsters kept in continuous darkness, and thus in the absence of an entraining LD cycle, in which the lifespan of male homozygote mutants was increased compared with wild types (15). In another study, heterozygote $\tau$ mutant hamsters (expressing a free running 22 h circadian period in absence of time cues) were kept on a 24 h LD cycle, to which these animals entrained with a phase advance to the LD cycle. The effects were also not observed after ablation of the central circadian pacemaker in the hypothalamus (suprachiasmatic nucleus) or in homozygous hamsters, which do not entrain to modulo 24, and free run across such lighting regimes. Thus, these physiological defects arose in $\tau$ hamsters as a consequence of abnormal entrainment and not as a result of a pleiotropic effect of the $\tau$ allele, an outcome also consistent with Pittendrigh’s circadian resonance hypothesis.

The circadian organization of behavior in the laboratory does not necessarily phenocopy behavior in the field (18). Our behavioral
to measure seasonal effects on reproductive success. Seasonal regu-
lation of reproduction may have been attenuated because we used
mice with a C57Bl6 background, which do not express melatonin, a
hormone known to be regulated by day length in wild derived mouse
strains and to have antagonodal effects (21).

The strong and persistent effect of the tau allele on fitness con-
trasts with data from a 2 \( \text{y} \) outdoor enclosure study of survivorship of mice bearing the circadian
Per2\( ^{Brdm1} \) mutant allele. Here, despite
laboratory studies showing an array of deleterious physiological
consequences for health in Per2\( ^{Brdm1} \) (22), there was no overall
effect on fitness in outdoor conditions. In contrast to the tau mu-
tation, the Per2\( ^{Brdm1} \) mutation does not strongly affect the circadian
period and had a negligible effect on activity patterns in outdoor
conditions (14). We cannot exclude the possibility that the effects
we report for the tau mutation may be due to an unknown non-
circadian pleiotropic effect, even though the earlier laboratory
studies on tau mutant hamsters have clearly revealed health con-
sequences as a result of the deleterious impact of inappropriate
entrainment to artificial lighting regimes (17).

In conclusion, our data, based on long term monitoring of rep-
licate seminatural populations of a vertebrate, show strong selection
against the tau mutation. The pathway of selection is not clear, but it
appears to involve both adult survival and juvenile recruitment. It is
consistent with the hypothesis proposing a positive role for circadian
resonance in fitness, although an unknown pleiotropic effect of the
mutation cannot be excluded.

Materials and Methods

Setup of Mouse Populations. Six replicate mouse (Mus musculus domestica,
C57Bl6 background) populations were established in enclosures at the Stony
Ford field station in Princeton (W074 40, N40 21). Each population was set up
with the release of \(~17~\) male and 22 female mice per enclosure. In each sex,
there was an initial approximate Mendelian ratio of 0.84:0.20:0.67 (females) and
0.72:0.20:0.74 (males) of wild type, heterozygous, and homozygous individuals,
respectively, with respect to the tau mutation generated in the laboratory of
A.S.I.L. (10). All mice were bred from heterozygote parents at the breeding
facility at the University of Groningen and raised in constant dim light. At \(~7~\) wk
of age, each mouse received a 90 mg transponder (Trovam ID100, Dorset ID) s.c.
The mice were released on November 2, 2007, at \( \sim \) 0.1 d of age, and lit-
termates were distributed over different enclosures. Each enclosure measured
180 m\(^2\) and was fenced by a 1.5 m high sheet metal wall with three electric
fences, keeping out terrestrial predators, without eliminating aerial pre-
dation. A small (180 \( \times \) 140 \( \times \) 70 cm) hay filled shed was present in each en-
closure. There were no trees, shrubs, or other vegetation. The experiment was
correlated with permission from the Princeton University Institutional Animal Care and Use Committee (Protocol 1626). All
procedures in the experiment were in accordance with national regulations
(New Jersey Fish & Wildlife Service).

Data Collection and Analysis. To obtain food, mice had to enter one of two
feeding stations, thereby passing through a decoder antenna coil (Ø 40 mm;
ANTC40, DorsetID). An individual was considered deceased if not recorded for a
week (SI Appendix, SI Text). Mice were fed breeder diet (Mouse Diet 9F; LabDiet)
to facilitate population growth, because a very low number of mice may cause
unwanted bias in allele frequency change (genetic drift); water was supplied ad
libitum. At three stages during the experiment, all mice were trapped at the
feeding stations, thereby passing through a decoder antenna coil (Ø 40 mm;
70 cm) hay filled shed was present in each en-
closure. Each enclosure measured
180 m\(^2\) and was fenced by a 1.5 m high sheet metal wall with three electric
fences, keeping out terrestrial predators, without eliminating aerial pre-
dation. A small (180 \( \times \) 140 \( \times \) 70 cm) hay filled shed was present in each en-
closure. There were no trees, shrubs, or other vegetation. The experiment was
correlated with permission from the Princeton University Institutional Animal Care and Use Committee (Protocol 1626). All
procedures in the experiment were in accordance with national regulations
(New Jersey Fish & Wildlife Service).

activity period, but also with a less prominent shorter period. These circa-
dian phenotypes may be attributed to the natural 24 h LD cycle
being strong enough to entrain mice of all genotypes, but it is
equally possible that natural illumination exerted a strong masking
effect on mouse activity. There was, however, a significant contri-
bution of circadian genotype to the extent of diurnality of feeding
activity, with homozygous mutant mice being the most and wild type
mice the least diurnal. We observed a clear increase in diurnality
index \( D \) during the summer months in all genotypes (Fig. 2 \( G \) I).
Increased \( D \) may reflect high flexibility in the feeding rhythm, which
may facilitate access to the feeders under strong competition. In the
release cohort, in which we know the age of all mice, the long lived
mice changed to more diurnal activity patterns at the end of their
lives. However, a causal relationship between diurnality and lon-
gevity is unlikely here: During the summer, when the long living
mice from the release cohort (the only ones still present from this
cohort) became more diurnal, the population density increased.
Diurnal behavior under high population densities is consistent with
earlier reports of mice maintained under field conditions (14).
Activity during the day may have led to a different predation risk
between genotypes. However, both nocturnal (great horned owls,
Bubo virginianus) and diurnal (red tailed hawks, Buteo jamaicensis)
predators were video recorded capturing mice during the experiment,
but the extent of the ensuing mortality by these predators could
not be quantified. Alternatively, entrainment of animals maintained
outdoors may have exacerbated the internal desynchronization be-
tween the light entrained neural pacemaker and that of other body
tissues (19, 20), which is known to occur in tau mutants. Although
breeding in mice may be modulated by photoperiod, we were unable

![Fig. 4. Distribution of the first and second period in feeding behavior. For each individual, these two periods were defined as the highest and second highest peak in the average 14 d Lomb Scargle (normalized) power spectrum. Each bar shows relative numbers of mice with their first (filled part of each bar) and the second (open part) most prominent rhythm at a specific period length (10 min resolution); \( n = 334/532 \) (first/second; \( \tau^{+/+} \)); \( n = 362/360 \) (tau\( ^{+} \)); \( n = 85/81 \) (tau\( ^{-} \)).](image-url)
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