Supplementary Online Material

Lab Mice in the Field: Unorthodox Daily Activity and Effects of a Dysfunctional Circadian Clock Allele

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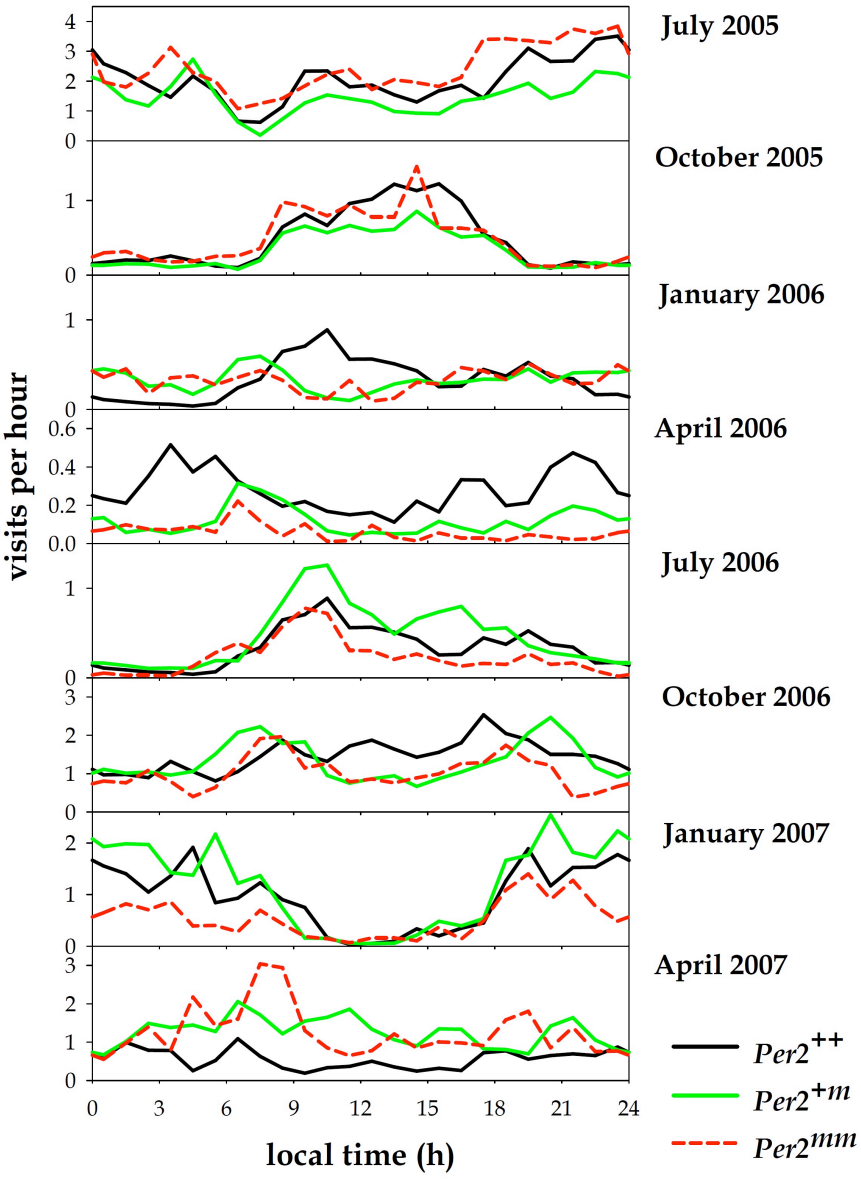
1. Genotyping:

Mouse tail tips (about 3 mm) were placed into a 1.5 ml Eppendorf tube containing 500 µl of Lysis buffer (20mM Tris/HCl, pH 8, 5mM EDTA, pH8, 400 mM NaCl, 1%SDS). 10 µl of proteinase K (20 mg/ml) were added and the samples were stored at room temperature for transport. Upon arrival in the lab 10 µl of proteinase K (20 mg/ml) were added to each tube and digestion continued overnight at 60°C. After 2 min. of centrifugation in an Eppendorf centrifuge at 13'000 rpm the genomic DNA in the supernatant was precipitated with ethanol and washed once in 70% ethanol. The genomic DNA was resuspended in TE buffer (10mM Tris/HCl, 1mM EDTA, pH 8) to a concentration of 0.3 mg/ml. 0.3 µg genomic DNA was added to a final volume of 50 µl containing Qiagen PCR buffer (Cat. No 201207) with 6nm $(NH_4)_2SO_4$, 0.2 mM dNTP (Roche), 2.5 units of Taq polymerase (Qiagen) and primers 0.7 µM each (*Per2* forward: 5'-GCT GGT CCA GCT TCA TCA ACC-3', *Per2* reverse: 5'-GAA CAC ATC CTC ATT CAA AGG-3' and PKG hprt reverse: 5'-CGC ATG CTC CAG ACT GCC TTG-3'). PCR cycles were as follows: 2 min. at 94°C initial denaturation, then 30 s at 94°C, 30 s at 56°C and 1 min. at 72°C for 32 cycles. The PCR products were analysed on a 1.5 % agarose gel detecting a 381 nt long product for wild type and a 120 nt long product for mutant *Per2*.

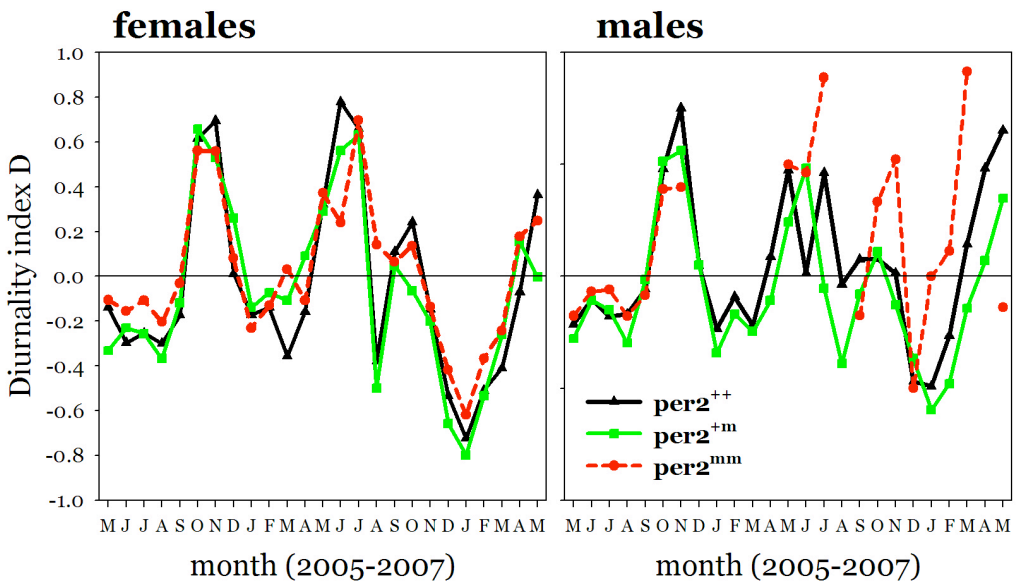
2. Seasonal change in activity profiles of three mouse genotypes

We quantified the activity for all mice in the months of January, April, July and October in both years of the study. Lines connect average counts per hour per individual mouse present for the three genotypes separately. Note the difference in scaling of the figures per month.

There is little systematic difference between the genotypes, but pronounced variation between the seasons. The records for October 2005 and July 2006 show that mice of all three genotypes under these seminatural conditions can spontaneously be nearly exclusively diurnal instead of nocturnal.



3. Changes in monthly diurnality index (D) for the three genotypes in females and males separately.



Note that no values are given for male homozygote mutants December 2005- April 2006 when they were not present in the population, and again in April 2007, when no activity was recorded for homozygous mutant males.

Overall average monthly values for D were:

F Per2^{++}:	- 0.04 (sd 0.41)	M Per2^{++}:	+0.05 (sd 0.33)
F Per2^{+m}:	- 0.06 (sd 0.39)	M Per2^{+m}:	- 0.06 (sd 0.31)
F Per2^{mm}:	+0.02 (sd 0.32)	M Per2^{mm}:	+0.17 (sd 0.39)

As covariates two meteorological variables recorded in Velikie Luki (56°21'N; 30°43'E; weather station 264770 ULOL) were derived from www.TuTiempo.net. Variables used were snowcover (the number of snow cover days/total days per month), and mean daily temperature per month.

Generalized linear models (SPSS) were used to simultaneously test effects of sex, genotype (presence or absence of at least one Per2^{+} allele), snowcover, temperature. Wald χ^2 values were 8.530 (snowcover p=0.003), 2.444 (sex; p=0.118), 0.793 (temperature; p=0.373), 0.106 (genotype; p=0.745). Interactions did not contribute significantly to the explained variance. Thus snowcover is a strong predictor of reduced diurnality.

To further test for differences between sexes and genotypes, we omitted the weather data and used month (n=25) as a random factor in analysis of variance: Month F_{24} = 8.76 (p=0.007); sex F_1 = 4.31 (p=0.048); genotype F_2 = 6.83 (p=0.015). Thus while season had an overriding influence, males were slightly more diurnal than females and homozygous per2 mutants slightly more diurnal than wildtype and heterozygotes.

4. Effect of genotype and yearclass on median life span.

To test whether differences in genotypic effects on survival rates between the two years of the study are systematic, we assessed the median life span after release per sex for each genotype. The table presents median lifespans for all mice that were recorded at least 10 days following release for both sexes in two year classes (2005: cohorts 1,2; 2006: cohorts 3,4). Standard errors of the medians are provided in parentheses. P-values are given for the effect of genotype (number of $Per2^{Brdm}$ alleles as ordinal variable) according to the Kaplan-Meijer (log rank Mantel-Cox) procedure in SPSS.

year-class	**genotype**	**females**	**n**	**males**	**n**	**Both sexes**	**n**
2005	$Per2^{++}$	150 (20.6)	35	63 (34.5)	23	127 (37.8)	58
	$Per2^{+m}$	132 (22.4)	77	56 (20.3)	48	94 (13.6)	125
	$Per2^{mm}$	63 (12.0)	64	50 (8.7)	27	59 (3.3)	91
	p	0.007		0.025		0.004	
2006	$Per2^{++}$	64 (15.4)	28	42 (9.8)	21	58 (14.2)	49
	$Per2^{+m}$	137 (10.1)	57	48 (8.6)	32	110 (28.9)	89
	$Per2^{mm}$	> 241 (-)	18	45 (6.9)	8	127 (14.2)	26
	p	0.018		0.648		0.018	

The table shows that the median life span strongly depended on genotype. In the year class of 2005 both females and males had a significantly longer life expectancy after release in wildtype than in homozygous $Per2^{mm}$ mutants, with the heterozygotes in between (SPSS Kaplan-Meijer/log rank Mantel Cox life table analysis). This effect was reversed for the sexes combined in the year class of 2006. Here the effect was solely due to the females.