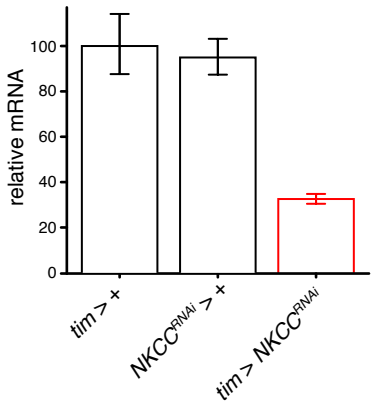

Figure S1. *Related to Figures 1-5*.

RT-qPCR verification of *NKCC^{RNAi}* kock-down.

qPCR analysis of relative mRNA levels in whole heads showing the efficiency of RNAi-mediated knock-down of *NKCC* (*tim* > *NKCC^{RNAi}*) compared to controls (either *gal4* or *uas* alone). Bars are means, whiskers min-max.

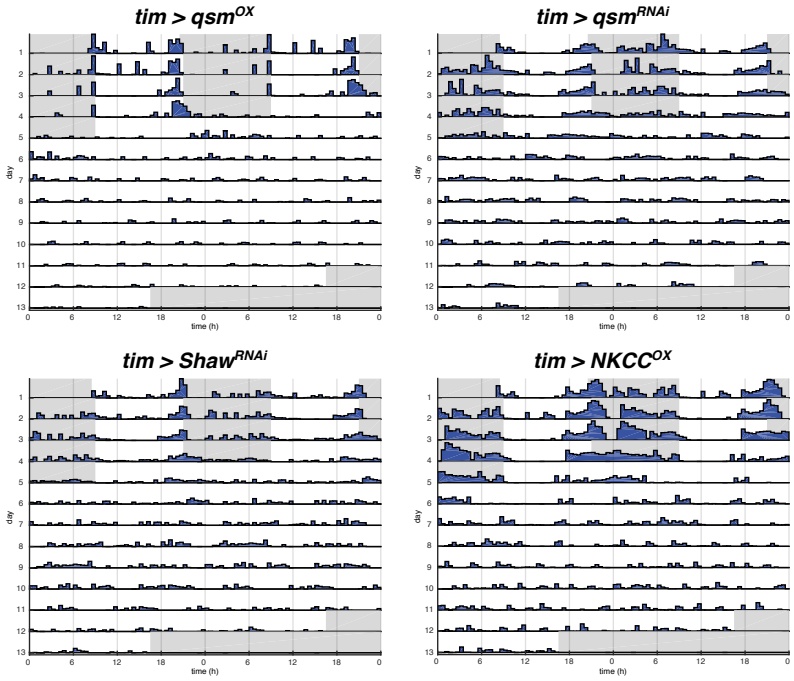

Figure S2. *Related to Figure 1 and Table S1*.

LL behavior of flies carrying the *s-tim* allele.

Double-plotted actograms of exemplary individual flies of the indicated genotypes recorded for the first 4 days in LD (grey — lights off; white — lights on) followed by 9 days of constant dim light.

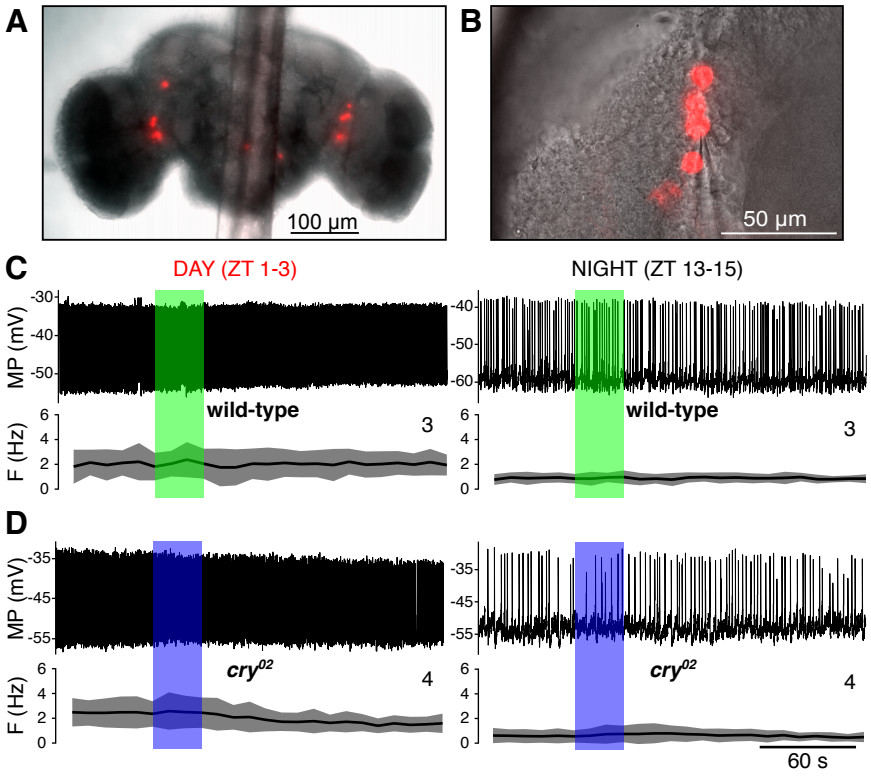

Figure S3. *Related to Figures 2, 4 and 5 and Table S2.*
Whole-cell recording preparation and response of l-LNvs to acute light.
(**A**) Whole brain preparation showing the Pdf-RFP labeled l-LNvs for recording (the band in the middle is a nylon thread holding the brain down in the recording chamber) and (**B**) detail of the l-LNvs with the recording electrode (from below). (**C**) In wild-type control flies green light (555 nm) had no effect on wild-type l-LNvs neither in the day nor at night time. (**D**) *cry*^{02} l-LNvs did not respond to blue light (470 nm) with a change of firing both in the day and at night. In each panel the top traces show an example of a whole cell current clamp recording of a l-LNv for 1 min before, 30 s during and the 2.5 min after exposure to light (indicated by green or blue bar). The bottom graphs show a quantification of the light response from multiple recordings. Mean, solid line; SD, pale background; MP, membrane potential; F, firing frequency; n, indicated.

Table S1. *Related to Figures 1 and S2.*
Circadian behavior of flies in constant light (LL).

genotype	*tim* allele	% rhythmic	period (h)	R.S.	n
***tim* > + (wild-type)**	*ls*	4.5	- - -	- - -	44
***tim* > *qsm*^{OX}**	*ls*	75.0	13.4 (1.8)	2.2 (0.7)	32
***tim* > *qsm*^{RNAi}**	*ls*	89.7	26.6 (1.6)	2.4 (0.6)	29
***tim* > *qsm*$^{RNAi(2)}$**	*ls*	98.1	27.6 (2.0)	3.3 (1.0)	104
***tim* > *Shaw*^{OX}**	*ls*	72.7	4.2 (0.6)	***	22
***tim* > *Shaw*^{RNAi}**	*ls*	91.3	12.5 (0.8)	2.0 (0.7)	23
***tim* > *NKCC*^{OX}**	*ls*	83.0	28.9 (0.8)	2.4 (1.0)	53
***tim* > *NKCC*^{RNAi}**	*ls*	73.9	13.3 (1.1)	2.3 (0.8)	23
***tim* > *qsm*^{OX}**	*s*	34.0	30.3 (5.6)	2.1 (0.4)	50
***tim* > *qsm*^{RNAi}**	*s*	31.4	30.2 (5.4)	2.5 (1.2)	35
***tim* > *qsm*$^{RNAi(2)}$**	*s*	40.0	24.6 (2.5)	2.3 (0.6)	20
***tim* > *Shaw*^{RNAi}**	*s*	58.3	29.8 (5.4)	2.5 (1.2)	24
***tim* > *NKCC*^{OX}**	*s*	26.6	34.1 (3.9)	2.4 (1.2)	30
***cry*^{02}**	*s*	96.6	24.3 (1.3)	3.1 (1.0)	29
***gl*^{60J}**	*ls*	0	- - -	- - -	22
***norpA*^{P41}**	*s*	0	- - -	- - -	32
***Hdc*^{JK910}**	*s/ls*	16.6	25.6 (4.3)	2.3 (0.8)	36
***qsm*^{105} > +**	*ls*	36.8	27.4 (1.9)	2.4 (0.8)	19
***qsm*^{105} > *Shaw*^{OX}**	*ls*	2.2	- - -	- - -	45
***qsm*^{105} > *Shaw*^{RNAi}**	*ls*	54.2	26.6 (2.9)	2.3 (0.7)	24
***qsm*^{105} > *NKCC*^{OX}**	*ls*	81.3	29.1 (1.2)	3.3 (0.8)	16
***qsm*^{105} > *NKCC*^{RNAi}**	*ls*	2.2	- - -	- - -	45

For each genotype, the percentage of the overall rhythmic flies, the mean (SD) of the prevalent rhythmic category, the rhythmic statistics (R.S.) and the number of flies (n) are given.
*** R.S. values could not be calculated for periods of this length using the signal-processing tool-box in MatLab. Rather, rhythmicity for each fly was deemed significant based on periodogram analysis using Actogram J with a p-level set to 0.001. Each period was subsequently verified by manual inspection of individual actograms.

Table S2. *Related to Figure 1.*
Circadian behavior of flies in constant darkness (DD).

genotype		*tim* allele	% rhythmic	period (h)	R.S.	n
***tim* > + (wild-type)**		*ls*	93.8	23.6 (0.4)	2.8 (0.8)	16
***tim* > *qsm*^{OX}**		*ls*	100	24.1 (0.2)	3.9 (0.4)	8
***tim* > *qsm*^{RNAi}**		*ls*	100	24.1 (0.2)	4.0 (0.4)	8
***tim* > *Shaw*^{OX}**	*	*ls*	13	24.4 (0.4)	2.5 (0.2)	39
***tim* > *Shaw*^{RNAi}**	*	*ls*	97	24.3 (0.1)	4.8 (0.3)	32
***tim* > *NKCC*^{OX}**		*ls*	85.7	24.2 (0.3)	2.9 (0.6)	14
***tim* > *NKCC*^{RNAi}**		*ls*	81.3	24.6 (0.5)	2.7 (0.7)	16

For each genotype, the percentage of the overall rhythmic flies, the mean (SD) of the prevalent rhythmic category, the rhythmic statistics (R.S.) and the number of flies (n) are given.
* *Shaw* data taken from (22)

Table S3. *Related to Figures 2, 3 and 5.*
Physiological parameters (RMP, SFR) and acute light response (F_{on}-F_{off}) at day and night.

genotype		RMP (mV)	SFR (Hz)	F_{on}-F_{off} (Hz)	n
***Pdf > +* (wild-type)**	day	-51.2 (4.6)	2.27 (0.90)	-0.23 (0.84)	15
	night	-57.7 (2.7)	0.60 (0.41)	1.61 (1.17)	11
Pdf > qsm^{OX}	day	-57.6 (5.3)	0.54 (0.46)	0.17 (0.22)	10
	night	-56.0 (2.6)	0.74 (0.73)	0.06 (0.52)	9
Pdf > qsm^{RNAi}	day	-51.6 (4.5)	1.56 (0.67)	1.50 (1.31)	11
	night	-52.9 (2.5)	2.15 (0.56)	1.41 (0.98)	10
Pdf > Shaw^{OX}	day	-61.1 (6.3)	0.34 (0.47)	-0.02 (0.17)	9
	night	-58.3 (4.3)	0.35 (0.39)	-0.06 (0.14)	6
Pdf > Shaw^{RNAi}	day	-51.0 (3.4)	1.93 (0.82)	1.56 (1.61)	10
	night	-51.7 (3.3)	1.76 (0.68)	1.21 (0.69)	6
Pdf > NKCC^{OX}	day	-51.7 (4.1)	2.94 (1.66)	1.52 (0.99)	7
	night	-50.7 (2.7)	5.30 (1.74)	1.20 (1.14)	6
Pdf > NKCC^{RNAi}	day	-61.7 (7.1)	0.56 (0.87)	0.11 (0.15)	10
	night	-61.3 (6.4)	0.51 (0.61)	-0.02 (0.21)	7
cry^{02}	day	-53.3 (5.1)	2.47 (1.11)	-0.06 (0.38)	4
	night	-56.5 (2.4)	0.63 (0.53)	0.11 (0.19)	4
tim > qsm^{RNAi}/GFP	day	-54.9 (2.7)	5.78 (0.84)	- - -	9
	night	-55.8 (2.1)	5.70 (1.26)	- - -	8
tim > qsm^{RNAi}/Shaw^{OX}	day	-61.2 (3.1)	0.47 (0.57)	- - -	9
	night	-59.0 (2.5)	0.90 (0.84)	- - -	7
tim > qsm^{RNAi}/NKCC^{RNAi}	day	-57.6 (1.8)	1.90 (0.72)	- - -	7
	night	-55.7 (3.5)	2.56 (0.56)	- - -	7

RMP, resting membrane potential; SFR, spontaneous firing rate; F_{on}-F_{off}, firing frequency prior to lights on subtracted from firing rate at lights on. For each parameter, the mean (SD) and number of measured neurons (n) are given.

Table S4. *Related to Figure 2.*
Sustained current density at +100 mV.

genotype	current (pA/pF)	n
***Pdf* > + (wild-type) day**	37.1 (12.0)	6
***Pdf* > + (wild-type) night**	69.9 (21.6)	8
***Pdf* > *Shaw*^{OX}**	81.9 (11.7)	6
***Pdf* > *Shaw*^{RNAi}**	46.1 (16.7)	6
***Pdf* > *qsm*^{OX}**	82.5 (19.2)	5
***Pdf* > *qsm*^{RNAi}**	45.8 (12.4)	5

The mean (SD) and number of measured neurons (n) are given.

Table S5. *Related to Figure 2.*
GABA reversal potential.

genotype	E_{GABA} (mV)	n
***Pdf* > + (wild-type) day**	-63.8 (4.3)	7
***Pdf* > + (wild-type) night**	-74.6 (4.2)	8
***Pdf* > *NKCC*^{OX}**	-59.0 (5.0)	5
***Pdf* > *NKCC*^{RNAi}**	-74.8 (5.3)	5
***Pdf* > *qsm*^{OX}**	-72.5 (7.2)	5
***Pdf* > *qsm*^{RNAi}**	-55.6 (4.9)	5

E_{GABA}, GABA reversal potential; the mean (SD) and number of measured neurons (n) are given.