SUPPLEMENTAL INFORMATION

Optimization of Circadian Responses with Shorter and Shorter Millisecond Flashes

Sevag Kaladchibachi, David C. Negelspach, Jamie M. Zeitzer, and Fabian Fernandez

Supplementary Table 1. Light Exposure Conditions

Protocol ID		Luminance in cd/m^2	Pulse Duration (ms)	Pulse Frequency (Hz)	Total No. Pulses Delivered	Total Exposure Time (s)	Darkness between Pulses (s)
Blue	1	200	120	1.00	900	108	0.88
Blue	2	160	120	1.00	900	108	0.88
Blue	3	160	120	0.25	225	27	3.88
Blue	4	160	120	0.13	117	14	7.58
Blue	5	90	120	1.00	900	108	0.88
Blue	6	90	120	0.25	225	27	3.88
Blue	7	90	120	0.13	117	14	7.58
Blue	8	27	120	1.00	900	108	0.88
Blue	9	27	120	0.25	225	27	3.88
Blue	10	27	120	0.13	117	14	7.58
Blue	11	27	120	0.06	54	6.5	16.6
Blue	12	12	120	1.00	900	108	0.88
Blue	13	12	120	0.25	225	27	3.88
Blue	14	12	120	0.13	117	14	7.58
Blue	15	12	120	0.06	54	6.5	16.6
Blue	16	6	120	1.00	900	108	0.88
Blue	17	6	120	0.25	225	27	3.88
Blue	18	6	120	0.13	117	14	7.58
Blue	19	6	120	0.06	54	6.5	16.6
Blue	20	2	120	1.00	900	108	0.88
Blue	21	2	120	0.25	225	27	3.88
Blue	22	2	120	0.13	117	14	7.58
Blue	23	950	16	1.00	900	14.4	0.984

Protocol ID		Luminance in cd/m^2	Pulse Duration (ms)	Pulse Frequency (Hz)	Total No. Pulses Delivered	Total Exposure Time (s)	Darkness between Pulses (s)
Blue	24	950	16	0.25	225	3.6	3.984
Blue	25	950	16	0.13	117	1.87	7.68
Blue	26	200	16	1.00	900	14.4	0.984
Blue	27	200	16	0.25	225	3.6	3.984
Blue	28	200	16	0.13	117	1.87	7.68
Blue	29	200	16	0.06	54	0.864	16.6
Blue	30	90	16	1.00	900	14.4	0.984
Blue	31	90	16	0.25	225	3.6	3.984
Blue	32	90	16	0.13	117	1.87	7.68
Blue	33	90	16	0.06	54	0.864	16.6
Blue	34	45	16	1.00	900	14.4	0.984
Blue	35	45	16	0.25	225	3.6	3.984
Blue	36	45	16	0.13	117	1.87	7.68
Blue	37	45	16	0.06	54	0.864	16.6
Blue	38	2	16	1.00	900	14.4	0.984
Blue	39	2	16	0.25	225	3.6	3.984
Blue	40	2	16	0.13	117	1.87	7.68
Blue	41	950	8	1.00	900	7.2	0.992
Blue	42	950	8	0.25	225	1.8	3.992
Blue	43	950	8	0.13	117	0.936	7.68
Blue	44	950	8	0.06	54	0.432	16.7
Blue	45	950	8	0.04	36	0.288	24.992
Blue	46	950	8	0.03	27	0.216	32.25
Blue	47	950	8	0.02	15	0.120	58.82
Blue	48	400	8	1.00	900	7.2	0.992
Blue	49	400	8	0.25	225	1.8	3.992

Protocol ID	Luminance in cd/m^2	Pulse Duration (ms)	Pulse Frequency (Hz)	Total No. Pulses Delivered	Total Exposure Time (s)	Darkness between Pulses (s)
Blue 50	400	8	0.13	117	0.936	7.68
Blue 51	400	8	0.06	54	0.432	16.7
Blue 52	400	8	0.04	36	0.288	24.992
Blue 53	400	8	0.03	27	0.216	32.25
Blue 54	180	8	1.00	900	7.2	0.992
Blue 55	180	8	0.25	225	1.8	3.992
Blue 56	180	8	0.13	117	0.936	7.68
Blue 57	180	8	0.06	54	0.432	16.7
Blue 58	90	8	1.00	900	7.2	0.992
Blue 59	90	8	0.25	225	1.8	3.992
Blue 60	90	8	0.13	117	0.936	7.68
Blue 61	90	8	0.06	54	0.432	16.7
Blue 62	2	8	1.00	900	7.2	0.992
Blue 63	2	8	0.25	225	1.8	3.992
Blue 64	2	8	0.13	117	0.936	7.68

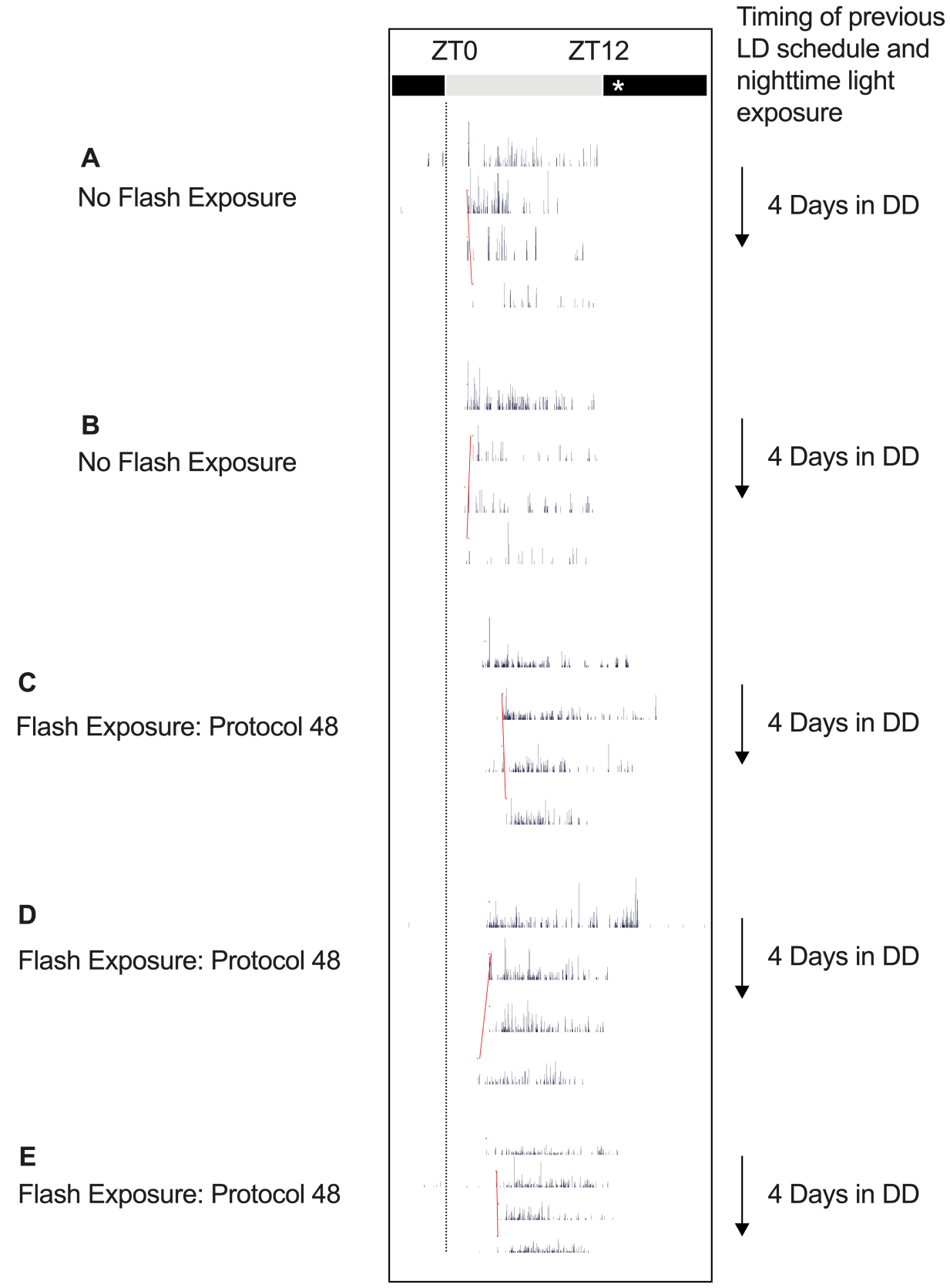
Supplementary Table 2. Energy-Response Values

Wavelength Emission		**Rate**	**Total Light Energy**			**Behavior**
Protocol ID	Irradiance in $\mu W/cm^2$	Photon flux in photons/cm^2/s	Quanta density in photons/cm^2	Energy density in $\mu J/cm^2$	Absolute energy in μJ	Magnitude of Delay in hours, SEM (*n*)
Blue 1	34.1	7.75×10^{13}	8.36×10^{15}	3683	18046	1.25 ± 0.17 (28)
Blue 2	27.22	6.18×10^{13}	6.68×10^{15}	2940	14405	1.50 ± 0.14 (32)
Blue 3	27.22	6.18×10^{13}	1.67×10^{15}	735	3601	1.30 ± 0.12 (32)
Blue 4	27.22	6.18×10^{13}	8.38×10^{14}	369	1809	1.27 ± 0.13 (26)
Blue 5	14.5	3.29×10^{13}	3.56×10^{15}	1566	7673	1.56 ± 0.18 (27)
Blue 6	14.5	3.29×10^{13}	8.89×10^{14}	392	1918	1.34 ± 0.10 (26)
Blue 7	14.5	3.29×10^{13}	4.62×10^{14}	204	998	0.99 ± 0.11 (32)
Blue 8	4.01	9.11×10^{12}	9.84×10^{14}	433	2122	1.82 ± 0.10 (32)
Blue 9	4.01	9.11×10^{12}	2.46×10^{14}	108	531	1.72 ± 0.11 (32)
Blue 10	4.01	9.11×10^{12}	1.28×10^{14}	56	276	1.41 ± 0.14 (32)
Blue 11	4.01	9.11×10^{12}	5.90×10^{13}	26	127	1.67 ± 0.11 (32)
Blue 12	2.3	5.22×10^{12}	5.64×10^{14}	248	1217	1.58 ± 0.14 (23)
Blue 13	2.3	5.22×10^{12}	1.41×10^{14}	62	304	1.43 ± 0.17 (24)
Blue 14	2.3	5.22×10^{12}	7.33×10^{13}	32	158	1.13 ± 0.16 (32)
Blue 15	2.3	5.22×10^{12}	3.39×10^{13}	15	73	1.05 ± 0.13 (32)
Blue 16	1.56	3.54×10^{12}	3.83×10^{14}	168	826	1.50 ± 0.13 (31)
Blue 17	1.56	3.54×10^{12}	9.57×10^{13}	42	206	1.36 ± 0.10 (32)
Blue 18	1.56	3.54×10^{12}	4.97×10^{13}	22	107	1.03 ± 0.11 (32)
Blue 19	1.56	3.54×10^{12}	2.30×10^{13}	10	50	0.84 ± 0.15 (32)
Blue 20	1.44	3.27×10^{12}	3.53×10^{14}	156	762	0.81 ± 0.15 (38)
Blue 21	1.44	3.27×10^{12}	8.83×10^{13}	39	191	0.87 ± 0.12 (30)
Blue 22	1.44	3.27×10^{12}	4.59×10^{13}	20	99	0.29 ± 0.13 (32)
Blue 23	16.4	3.73×10^{13}	5.36×10^{14}	236	1157	1.81 ± 0.09 (96)
Blue 24	16.4	3.73×10^{13}	1.34×10^{14}	59	289	1.65 ± 0.11 (62)

Wavelength Emission			**Rate**	**Total Light Energy**			**Behavior**
Protocol ID		Irradiance in $\mu W/cm^2$	Photon flux in photons/cm^2/s	Quanta density in photons/cm^2	Energy density in $\mu J/cm^2$	Absolute energy in μJ	Magnitude of Delay in hours, SEM (*n*)
Blue	25	16.4	3.73×10^{13}	6.97×10^{13}	31	150	1.40 ± 0.08 (63)
Blue	26	4.82	1.09×10^{13}	1.58×10^{14}	69	340	1.52 ± 0.10 (63)
Blue	27	4.82	1.09×10^{13}	3.94×10^{13}	17	85	1.48 ± 0.10 (53)
Blue	28	4.82	1.09×10^{13}	2.05×10^{13}	9.0	44	1.44 ± 0.12 (51)
Blue	29	4.82	1.09×10^{13}	9.46×10^{12}	4.2	20	1.33 ± 0.11 (32)
Blue	30	2.81	6.38×10^{12}	9.19×10^{13}	40	198	1.84 ± 0.15 (30)
Blue	31	2.81	6.38×10^{12}	2.30×10^{13}	10	50	1.36 ± 0.13 (31)
Blue	32	2.81	6.38×10^{12}	1.19×10^{13}	5.3	26	0.98 ± 0.12 (32)
Blue	33	2.81	6.38×10^{12}	5.51×10^{12}	2.4	12	0.97 ± 0.14 (32)
Blue	34	1.62	3.68×10^{12}	5.30×10^{13}	23	114	1.58 ± 0.12 (31)
Blue	35	1.62	3.68×10^{12}	1.32×10^{13}	5.8	29	1.35 ± 0.10 (32)
Blue	36	1.62	3.68×10^{12}	6.89×10^{12}	3.0	15	1.57 ± 0.13 (32)
Blue	37	1.62	3.68×10^{12}	3.18×10^{12}	1.4	6.9	0.88 ± 0.10 (32)
Blue	38	0.2	4.54×10^{11}	6.54×10^{12}	2.9	14	0.89 ± 0.17 (22)
Blue	39	0.2	4.54×10^{11}	1.64×10^{12}	0.7	3.5	0.62 ± 0.11 (32)
Blue	40	0.2	4.54×10^{11}	8.50×10^{11}	0.4	1.8	0.23 ± 0.12 (32)
Blue	41	8.64	1.96×10^{13}	1.41×10^{14}	62	305	1.46 ± 0.18 (26)
Blue	42	8.64	1.96×10^{13}	3.53×10^{13}	16	76	1.62 ± 0.17 (32)
Blue	43	8.64	1.96×10^{13}	1.84×10^{13}	8.1	40	1.57 ± 0.11 (27)
Blue	44	8.64	1.96×10^{13}	8.48×10^{12}	3.7	18	1.68 ± 0.13 (32)
Blue	45	8.64	1.96×10^{13}	5.65×10^{12}	2.5	12	1.94 ± 0.12 (32)
Blue	46	8.64	1.96×10^{13}	4.24×10^{12}	1.9	9.1	1.62 ± 0.10 (32)
Blue	47	8.64	1.96×10^{13}	2.35×10^{12}	1.0	5.1	1.17 ± 0.21 (17)
Blue	48	3.33	7.56×10^{12}	5.45×10^{13}	24	117	1.92 ± 0.13 (32)
Blue	49	3.33	7.56×10^{12}	1.36×10^{13}	6.0	29	2.01 ± 0.11 (32)

Wavelength Emission			**Rate**	**Total Light Energy**			**Behavior**
Protocol ID		Irradiance in $\mu W/cm^2$	Photon flux in photons/cm^2/s	Quanta density in photons/cm^2	Energy density in $\mu J/cm^2$	Absolute energy in μJ	Magnitude of Delay in hours, SEM (*n*)
Blue	50	3.33	7.56×10^{12}	7.08×10^{12}	3.1	15	1.61 ± 0.10 (32)
Blue	51	3.33	7.56×10^{12}	3.27×10^{12}	1.4	7.0	1.37 ± 0.08 (32)
Blue	52	3.33	7.56×10^{12}	2.18×10^{12}	1.0	4.7	1.08 ± 0.12 (32)
Blue	53	3.33	7.56×10^{12}	1.63×10^{12}	0.7	3.5	1.03 ± 0.12 (32)
Blue	54	2.96	6.72×10^{12}	4.84×10^{13}	21	104	1.79 ± 0.11 (32)
Blue	55	2.96	6.72×10^{12}	1.21×10^{13}	5.3	26	1.28 ± 0.12 (32)
Blue	56	2.96	6.72×10^{12}	6.29×10^{12}	2.8	14	1.74 ± 0.11 (32)
Blue	57	2.96	6.72×10^{12}	2.90×10^{12}	1.3	6.3	0.92 ± 0.12 (32)
Blue	58	1.33	3.02×10^{12}	2.18×10^{13}	9.6	47	1.41 ± 0.12 (31)
Blue	59	1.33	3.02×10^{12}	5.44×10^{12}	2.4	12	1.05 ± 0.11 (32)
Blue	60	1.33	3.02×10^{12}	2.83×10^{12}	1.2	6.1	0.95 ± 0.10 (32)
Blue	61	1.33	3.02×10^{12}	1.31×10^{12}	0.6	2.8	0.87 ± 0.10 (32)
Blue	62	0.1	2.27×10^{11}	1.64×10^{12}	0.7	3.5	0.02 ± 0.09 (64)
Blue	63	0.1	2.27×10^{11}	4.09×10^{11}	0.2	0.9	0.37 ± 0.14 (32)
Blue	64	0.1	2.27×10^{11}	2.13×10^{11}	0.1	0.5	0.08 ± 0.11 (31)

Supplementary Figure 1.



Legend: Calculating phase-shifts to blue LED flashes. Representative actograms taken from flies receiving no light (**A, B**) or a flash treatment employing 8-ms, 3.33 μW/cm^2 pulses delivered once per second for 15 min (**C-E**; protocol #48 in Supplementary Tables 1 and 2). Post treatment, from the last night of the light-dark (LD) cycle, animals were left to free-run in constant darkness (DD) for 4 days. Grey and black bars seated at the top of the figure show the timing of the previous LD schedule (ZT0, lights-on at 01.00; ZT12, lights-off at 13.00), while an asterisk marks the timing of light exposure (beginning at ZT13, 14.00). The 4 days proceeding from the last night of the LD cycle are depicted in each line of the example actograms. Within each actogram, dark blue bars indicate raw 30-s epochs where the animals registered breaks in the activity-tracking beams, smoothed over each minute of recording. Data are vertically aligned, such that one 24-h day of movement is shown per line, with successive days appearing one below the other. Red lines locate a regression fit for the daily activity onsets estimated by ClockLab (Actimetrics, Wilmette, IL). In keeping with previous literature, the size of each phase shift was determined by calculating the delay in activity onset that occurred on the second day in DD after light treatment relative to the timing of lights-on in the previous LD schedule (the activity onset of *Drosophila ananassae* residing in a fixed LD cycle is invariably entrained to the time of lights-on). To help visualize shifts in locomotor rhythms, example actograms are organized in a temporally aligned column, with a dotted line marking the timing of ZT0 in the previous LD schedule. Animals transitioning from LD to DD but receiving no light treatment exhibit phase movements (delays) that average 1.53 h (SEM, 0.13 h, n = 28). Those submitted to flash protocol #48 exhibit 1.92 h delays (SEM, 0.13 h, n = 32) after discounting/subtracting-out the average phase movements accompanying the LD schedule transition. In all cases, individual flies demonstrate a stable periodicity of the activity rhythm from days 2-4 in DD.