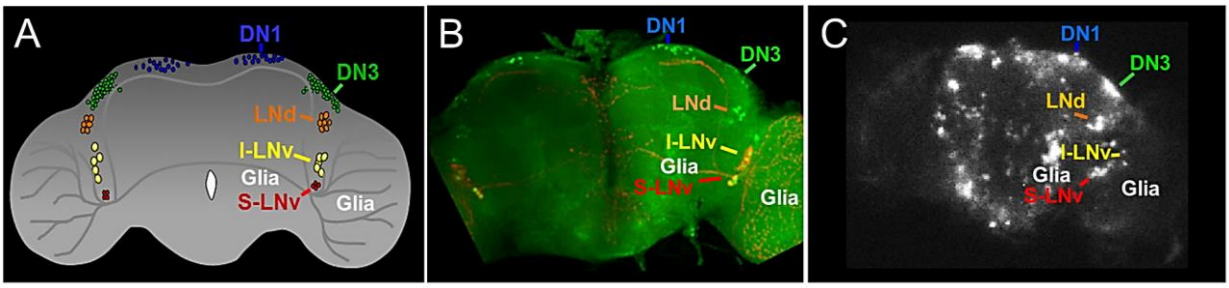
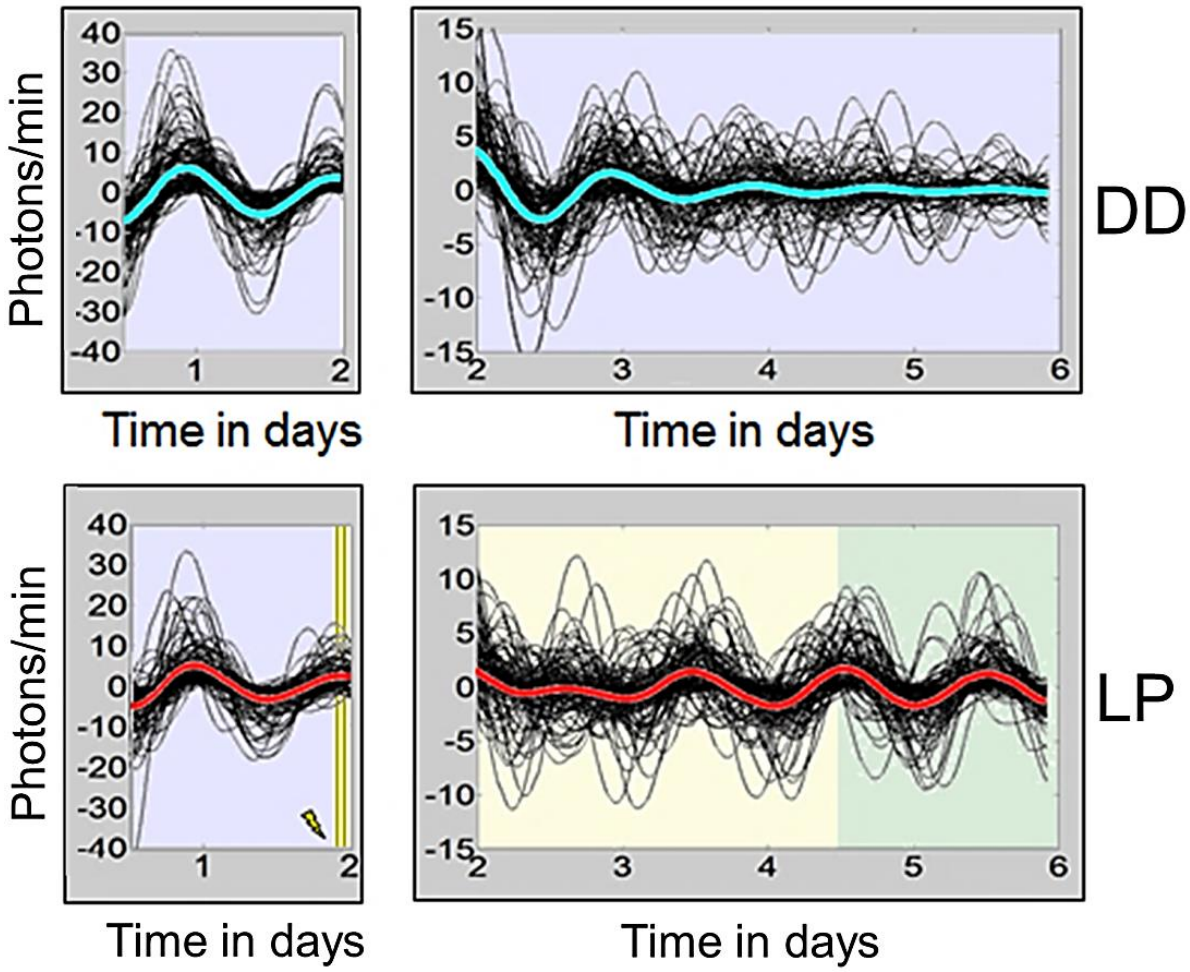
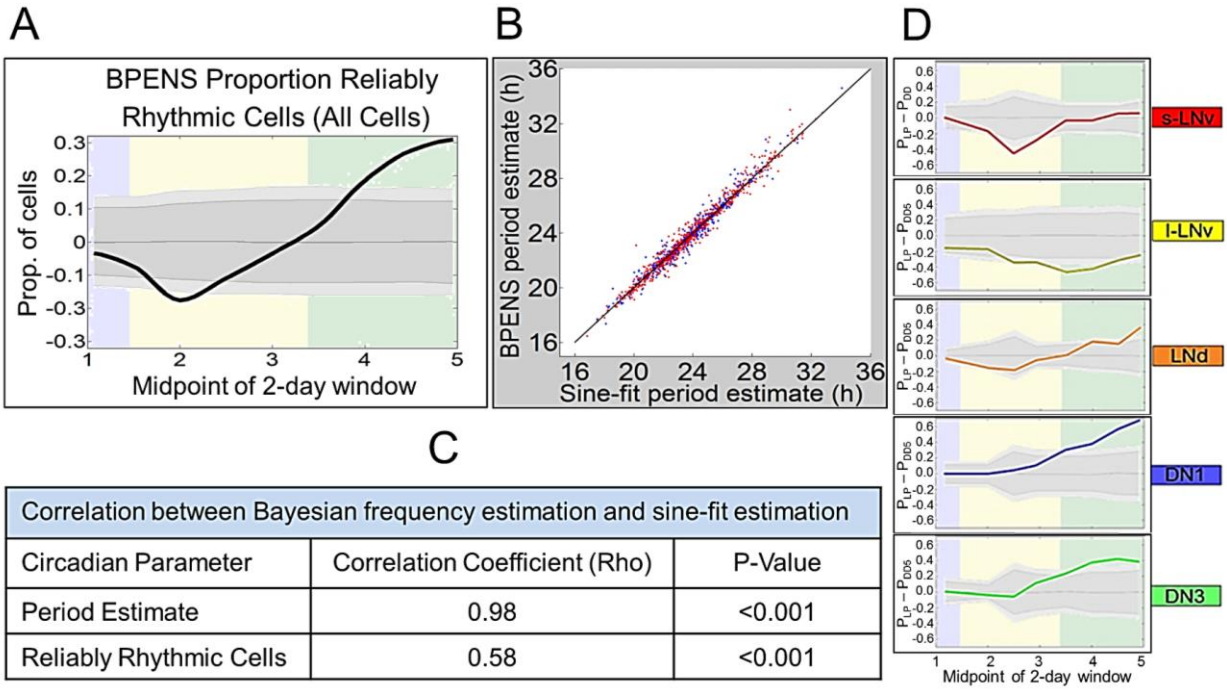


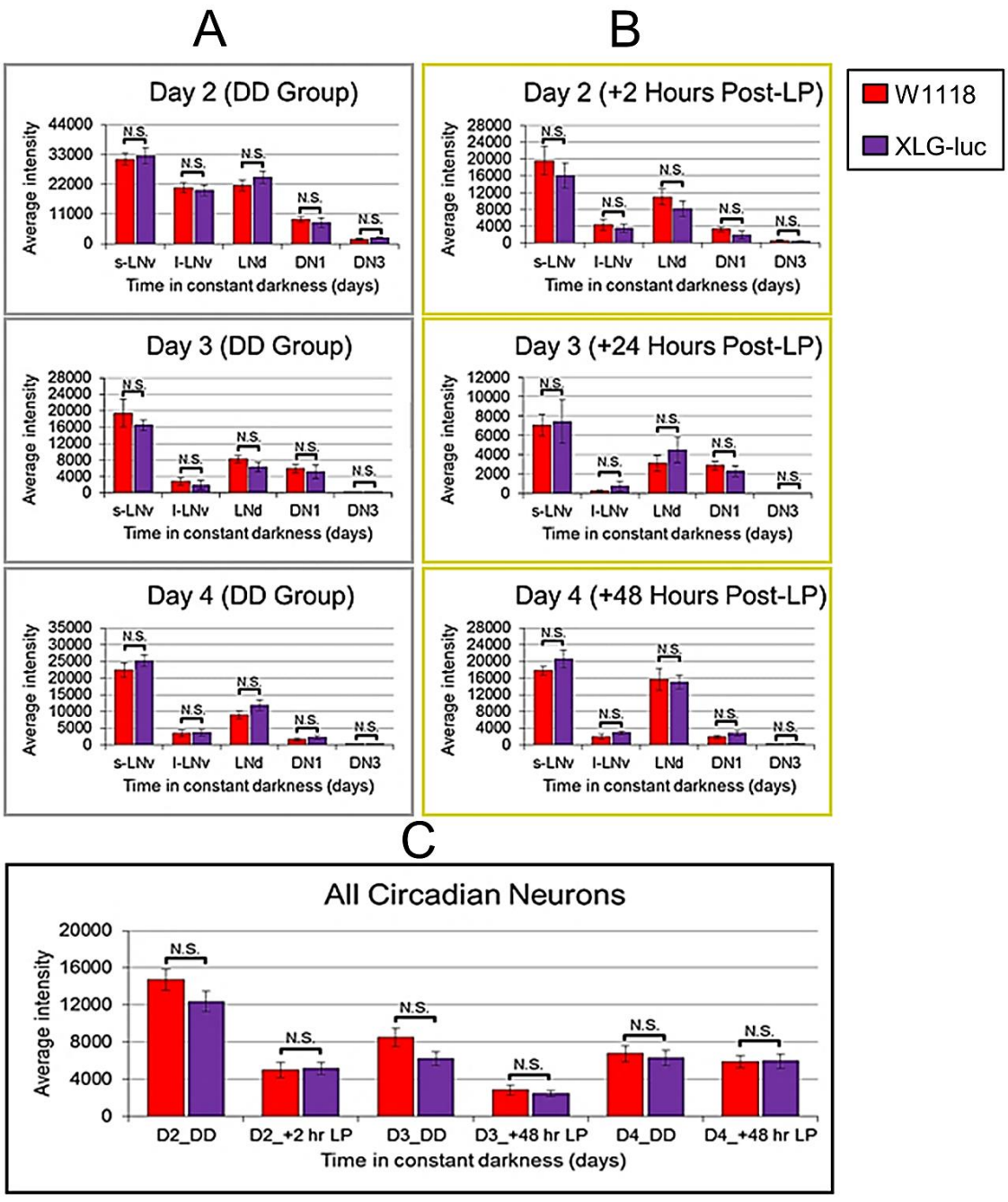


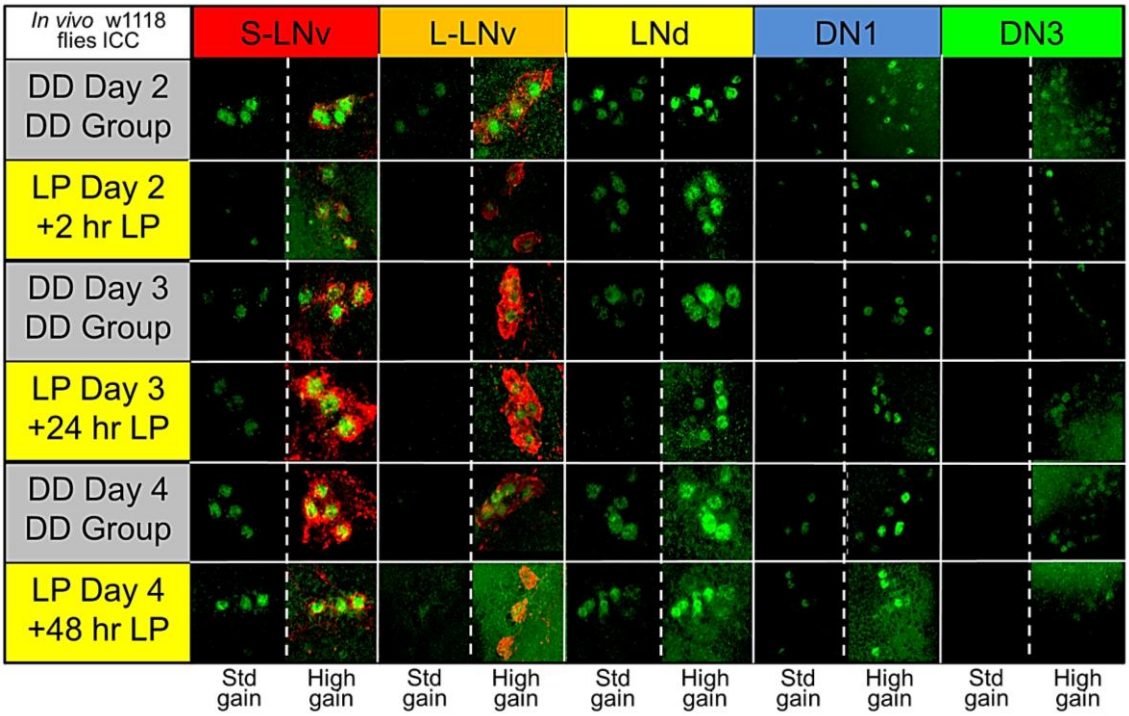
Constant Darkness	Post-light pulse Desynchrony	Post-light pulse Resynchrony

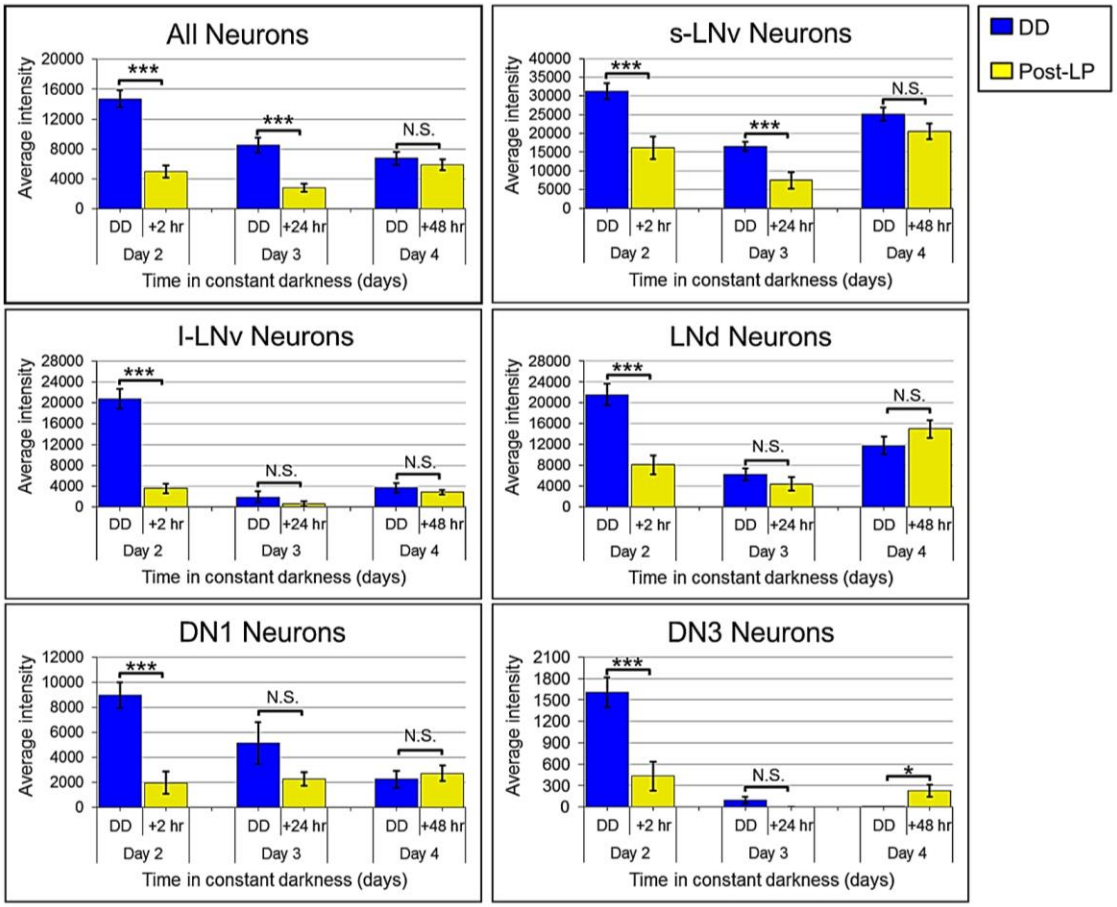


C

Correlation between Bayesian frequency estimation and sine-fit estimation		
Circadian Parameter	Correlation Coefficient (Rho)	P-Value
Period Estimate	0.98	<0.001
Reliably Rhythmic Cells	0.58	<0.001


A
B
W1118
XLG-luc
Day 2 (DD Group)
Day 2 (+2 Hours Post-LP)
Day 3 (DD Group)
Day 3 (+24 Hours Post-LP)
Day 4 (DD Group)
Day 4 (+48 Hours Post-LP)
Average intensity
N.S.
s-LNv
l-LNv
LNd
DN1
DN3
Time in constant darkness (days)
C
All Circadian Neurons
D2_DD
D2_+2 hr LP
D3_DD
D3_+48 hr LP
D4_DD
D4_+48 hr LP
Time in constant darkness (days)


In vivo w1118 flies ICC
S-LNv
L-LNv
LNd
DN1
DN3
DD Day 2 DD Group
LP Day 2 +2 hr LP
DD Day 3 DD Group
LP Day 3 +24 hr LP
DD Day 4 DD Group
LP Day 4 +48 hr LP
Std gain
High gain


All Neurons
s-LNv Neurons
l-LNv Neurons
LNd Neurons
DN1 Neurons
DN3 Neurons
DD
Post-LP
Average intensity
***
N.S.
*
DD
+2 hr
+24 hr
+48 hr
Day 2
Day 3
Day 4
Time in constant darkness (days)

Supplemental Figure Legends

Figure S1, Related to Figure 2. **Spatial mapping of cells expressing the *period* gene in the adult *Drosophila* brain**. S-LNv (red) and l-LNv (yellow) are the small and large ventral lateral neurons, respectively. LNd (orange) are the dorsal lateral neurons. DN1 (blue) and DN3 (green) are dorsal neurons subgroups 1 and 3, respectively. Clock glia cells (white text) are found throughout the brain circadian circuit. **A:** Schematic map depicting s-LNv, l-LNv, LNd, DN1, and DN3 subgroups. Neurites from the period-expressing cells are shown in gray. **B:** Confocal image of adult brain whole mount from male *XLG-Per-Luc* fly acutely fixed at ZT 23 and stained with anti-PER (green) and anti-PDF (red). Glia (white) are found throughout the brain but cannot be visualized in this image as they are dimmer and lie in different z-planes than the canonical neuronal subgroups. **C:** Bioluminescence image of single-cell *period* expression pattern in cultured adult whole-brain from male *XLG-Per-Luc* fly. The s-LNv, l-LNv, LNd, DN1, and DN3 neurons analyzed in further experiments for circadian time-course of PER expression are labeled with the same color scheme as above. DN2 neurons are not reliably detected in bioluminescence images of adult brains from *XLG-Per-Luc* flies, in agreement with [24, 40]. Glia (white) are found throughout the brain but cannot be seen in this image for the same reasons listed above.

Figure S2. Related to Figure 1. Oscillators exposed to a white light pulse exhibit qualitatively distinct patterns of loss, recovery then strengthening of synchrony over time relative to oscillators in DD. Averaged bioluminescence traces for DD cells (cyan; n = 122) and LP cells (red; n = 126) are superimposed on plots of detrended individual traces (black) of clock neurons seen in **Figure 1A**. Due to the transiently high amplitude bioluminescence at the start of recordings, the traces have been divided into two time frames (0.5-2 days and 2-6 days) with the vertical axis scaled separately for each time frame to allow for clearer visualization of changes in synchrony and amplitude over time in DD and in response to a light pulse. **Left**: It is qualitatively apparent that oscillators in the DD and LP groups show comparable synchrony and amplitude between 0.5-1.92 days (pre-LP). **Right**: Relative to the overall monotonic, gradual dampening observed by oscillators in DD **(top)** between 2-6 days, neurons exposed to a LP **(bottom)** generally exhibit qualitatively distinct patterns of synchrony tumbling followed by phase retuning (recovery/strengthening of synchrony along with phase shift).

Figure S3, Related to Figure 3. BPENS (Bayesian parameter estimation for noisy sinusoids) calculations confirm general trends in neuronal light response seen using sine-fit criterion. **A:** Bayesian frequency estimates of the proportion of reliably rhythmic cells for 'all neurons' (averaged from all neuronal subgroups) validates the general trend of transient reduction and delayed recovery in proportion of neurons following a light pulse observed by sine-fit estimates. Dark and light gray zones represent measures of uncertainty in the forms of 95% and 99% credible intervals, respectively. **B**: Scatter plot of sine-fit and Bayesian frequency estimates of period show strong positive linear correlation with particularly high clustering within the circadian range. Blue spots represent DD cells (688 contributions) while red spots represent LP cells (654 contributions) from all neuronal subgroups and 2-day windows of analyses. **C**: High correlation values between BPENS and sine-fit analyses of period and proportion of reliably rhythmic cells confirm that sine-fit analysis over 2-day windows, in combination with a discrete wavelet transform, is sufficiently consistent and reliable for our measures. **D:** Colored lines for each neuronal subgroup (using the same color scheme described in **Figure S1**) represent the difference in proportion of reliably rhythmic cells between corresponding neurons in LP and DD

conditions (PLP – PDD) over time. As observed by sine-fit estimates in Figure 3B, the ventral subgroups s-LNv and l-LNv exhibit significant reduction in this difference following a light pulse whereas the dorsal groups LNd, DN1 and DN3 exhibit a delayed increase following the LP. As DN3s lack CRY and share neurite connections with the s-LNvs, this provides evidence in support of previous studies [S1] that also support the idea that intercellular communication contributes to light-induced phase shifts Dark and light gray zones indicate 95% and 99% confidence intervals.

Figure S4, Related to Figure 6. Adult *w1118* and *XLG-Per-Luc* flies show no significant difference in PER staining intensities. Quantitative comparisons of PER staining average fluorescence using the software Volocity performed for **A:** neuronal subgroups maintained in DD (gray frames) and **B:** neuronal subgroups exposed to a 15 min 12.57 W/m2 light pulse at CT 22 of the second day in DD *in vivo* (yellow frames) indicated that there is no significant difference in intensities between corresponding neurons of *w1118* flies (violet) and *XLG-Per-Luc* flies (red) at matched time points and light regimes. **C:** Comparison of 'all circadian neurons' analyzed (i.e. cells from all neuronal subgroups averaged together) revealed no significant difference in overall staining intensity between *w1118* and *XLG-Per-Luc* flies at corresponding time points and conditions. Student's t-test was used to compare differences in average PER staining intensities between *w1118* and *XLG-Per-Luc* flies with $P>0.05$ considered not significant (N.S.). Laser intensity and other settings were always kept the same for comparison of PER staining intensities.

Figure S5, Related to Figure 5. *W1118* adult flies exposed to a light pulse exhibit the same trend as *XLG-Per-Luc flies* of transient loss and subsequent recovery/increase of PER staining intensity. Representative ICC images of neuronal subgroups in *W1118* flies show the same patterns of PER staining as *XLG-luc* ICC images shown in **Figure 5** for adult flies either maintained in DD or exposed to a 15 min 12.57 W/m2 (2,000 lux) light pulse at CT 22 of the second day in DD in vivo. See **Figure 5** for description and protocol.

Figure S6, Related to Figure 6. Quantification of changes in PER staining intensity for DD and LP cells qualitatively shown in Figure S5. Neuronal subgroups in adult *w1118* flies exhibit the same patterns of quantified changes in PER staining intensity as seen for *XLG-luc* flies in **Figure 6.** See **Figure 6** for description (same protocol and general results for both *w1118* and *XLG-luc* flies). **Movie S1, Related to Experimental Procedures. Raw time-lapse bioluminescence recordings of adult *XLG-Per-Luc Drosophila* whole-brain explants cultured for 6 days in darkness. A:** Whole brain culture maintained in constant darkness throughout recording. **B:** Whole brain culture exposed to a 15 minute 12.57 W/m2 light pulse at CT 22 of the second day in DD. A white flash at 1.92 days indicates the time at which the light pulse was administered *ex vivo*. The time stamp at the top indicates the number of days in DD that have elapsed (movies in **A** and **B** are time matched). The first 0.42 days are excluded due to saturating and highly variable bioluminescence when the explants are first cultured.

Supplemental Experimental Procedures

XLG-Per-Luc transgenic flies

Transgenic *XLG-Per-Luc* flies were generously provided by Dr. Ralf Stanewsky (University College London, UK). The *XLG-Per-Luc* flies express the XLG-*luc* construct which consists of most of the coding sequence of the *period* gene, excluding the last ten C-terminal amino acids, fused to the *luciferase* cDNA as previously described [24]. Expression of this fusion protein allows for measurement of PER activity as reported by LUC luminescence from cultured brains using a low light camera system (more below). A study using the BG-luc fusion protein, which shares the same 5'-upstream regulatory region of period as XLG-Per-Luc, showed that most of the bioluminescence signal in whole animal imaging is emitted from the abdomen and the eyes rather than the brain, and lacks single cell resolution [S2].

Culturing of adult Drosophila whole-brain explants

Fly brain explants were dissected and cultured using a modified version of a procedure previously described [9]. Prior to dissections, flies were entrained under standard 12 hr: 12 hr LD cycles at 25^{o}C and 50% humidity for at least 3 days. Under sterile conditions, Millicell-CM 0.4 µm culture plate inserts (Fisher) were placed in 35 mm Falcon culture dishes containing sterile PBS. The surfaces of culture plate inserts were coated with laminin and poly-D-lysine hydrobromide (BD Biosciences, NJ), each at 5 µg/cm2. Coated inserts were equilibrated for one hour at room temperature before aspirating excess coating, and then incubated at 37^{o}C overnight followed by extensive rinsing with sterile PBS the next day. Coated inserts were then stored at 4^{o}C for up to 3 weeks. Prior to dissection, adult male flies were anesthetized by placement on ice, and then washed in 70% ethanol for several seconds. Anesthetized flies were transferred to a sterile petri dish and dissected in ice-cold Schneider's Drosophila medium (Invitrogen, CA) containing 1% Antibiotic Antimycotic solution [10,000 U/ml penicillin, 10 mg/ml streptomycin, and 25 µg/ml amphotericin (Sigma, MO)]. Brains were carefully and cleanly dissected, removing cuticles and trachea but keeping optic lobes attached to the brain. The brains were then rinsed three times with a modified version of a previously described culture medium [9]: Schneider's *Drosophila* Medium containing 1% Antibiotic Antimycotic solution (see above), 10% fetal bovine serum, and 10 µg/ml insulin. For each experiment (3 experiments each for DD and LP condition), four brains were dissected and placed on a single insert, with ventral sides up and the brains in close proximity to allow for simultaneous imaging. Under sterile conditions, 1.2 ml of culture medium was filtered using Millex 33 mm sterile filter units into a fresh, sterile 35 mm petri dish. 1 mM luciferin (BioSynth) was filtered into the culture medium within 30 minutes of the start of recordings. A culture insert with 4 brains was transferred to this dish, which was then covered with a 40 mm circular coverslip (Thermo Scientific 40CIR1, Waltham, MA) and sealed with vacuum grease to prevent evaporation and contamination. Brain explants were maintained in the same culture medium throughout the recordings, as medium changes perturb PER activity (data not shown, see also [S3]).

Bioluminescence imaging

Bioluminescence imaging was performed using a modified version of a protocol previously described [S4]. The cultured brains were mounted on the stage of an inverted microscope (Olympus IX71, Tokyo, Japan) set on an antivibration table (TMC, Peabody, MA) in a dark

room isolated by black curtains with temperature maintained at 25^{o}C. The microscope focus was adjusted using bright-field illumination before turning off all lights and covering the brains with a small black Lucite box. Black plastic sheets (Thorlabs BK5, Newton, NJ) were then draped over the entire microscope to minimize stray light exposure. Cycling luminescence from the cultured whole brains was collected by an Olympus 4x XLFLUOR objective (NA 0.28) and transmitted directly to a cooled charge-coupled-device (CCD) camera (Series 800, Spectral Instruments) mounted on the bottom port of the microscope. Samples were obtained at 30 minute intervals with 29.5 minute exposure duration, no binning, and 50 KHz readout for ≥ 6 days of recording at single-cell resolution. Images were then transferred to a computer using SI Image SGL D software (Spectral Instruments) before using MetaMorph software (Molecular Devices, Sunnyvale, CA) and custom MATLAB scripts to measure bioluminescence with single cell resolution. In experiments analyzing light response, brains were exposed to a white light pulse at CT 22 of the second day in DD by turning on the microscope bright field light (Olympus, TH4-100) to an intensity of 12.57 W/m2 (2,000 lux) for 15 minutes. Only experiments with all four brains still healthy, contamination-free, intact, adherent to the substrate and exhibiting bioluminescence for ≥ 6 days were considered for analysis. The total number of cells analyzed in DD and LP conditions was 122 and 126 cells, respectively

Processing of Bioluminescence Images

Processing of bioluminescence images was performed as described previously [S4]. Briefly, cosmic ray artifacts were removed in MetaMorph by using a running minimum algorithm, i.e. a new image was constructed from each pair of sequential images using the minimum value of each pixel from the two images. A stack of images for each experiment was then generated and analyzed by measuring average luminescence intensity over time within regions of interest (ROIs) that were manually defined. ROIs were defined as borders of single neurons which could be clearly distinguished, isolated, anatomically identified, and exhibited bioluminescence cycling throughout the recordings. The positions of ROIs were easily tracked from frame to frame as needed to accommodate cell movement due to flattening of the brains over time with ROI sizes kept the same throughout the recording. ROIs that could not be easily distinguished and tracked were excluded from analysis. ROIs were categorized into canonical cell groups (colored-coded: red = s-LNv, yellow = l-LNv, orange = LNd, blue = DN1, green = DN3) based on classically recognized anatomical positions. Single neurons of neuronal subgroups actually became easier to identify over time as they spread out due to brain explants flattening. Raw luminescence data were then transferred to Microsoft Excel for additional processing. Single-cell luminescence was adjusted for background noise and converted to photons per minute. LumiCycle software (Dr. David Ferster, Actimetrics, IL) was used to generate clearly defined circadian peaks and troughs by subtracting linear trend (polynomial order = 0) and 13-point smoothing. For quantification of circadian parameters, smoothed data were analyzed with custom MATLAB scripts, with the first 10 hours of data excluded due to high intensities and variances in initial luminescence following dissection and addition of luciferin. Real-time bioluminescence data was analyzed for 6 day recordings.

Quantification of period activity dynamics

Custom MATLAB scripts (version 8.2) incorporating the Signal Processing and Statistics Toolboxes (MathWorks, MA) were used to analyze real-time bioluminescence data for quantification of circadian parameters including: order parameter, goodness-of-fit, period, and

amplitude. The scripts incorporated the WMTSA Wavelet Toolkit (C. Cornish, U. of Washington, WA) for application of a discrete wavelet transform to all time series. The wavelet transform served to remove high frequency noise and detrend the time series, providing clearer measures of circadian fluctuations and more accurate sine-fit estimates of rhythmic parameters [31]. The single-cell time series were also normalized with respect to variance for computation of the order parameter R, but were not altered for calculation of other circadian parameters. Boundary conditions for the wavelet filtering were carefully adjusted to minimize edge effects. Sliding 2-day windows were used to quantify properties of oscillations over time.

To quantify changes in synchrony over time, custom MATLAB scripts calculated an order parameter 'R,' defined as [40]

$$R = \frac{\langle X^2 \rangle - \langle X \rangle^2}{\frac{1}{N}\sum_{k=1}^{N}\left(\langle {X_k}^2 \rangle - \langle X_k \rangle^2\right)}$$

where angle brackets denote time average and $\frac{1}{N}\sum_{k=1}^{N} X_k$. If the phase, period and waveform of all N cells are in perfect synchrony then R=1, whereas uniform distribution would give R=0. Custom MATLAB scripts incorporating bootstrapping was used to compute significance in R estimates. In the bootstrapping procedure, we resampled with replacement from each data set, with the size of each bootstrap sample equal to the number of cells being sampled in each data set. This procedure was repeated 10,000 times to estimate 95% and 99% confidence intervals for the difference in R (RLP – RDD¬) between cells in constant darkness (DD) and cells exposed to a light pulse (LP), with the null hypothesis being no difference. For comparison of inter-subgroup kinetics after LP, changes in synchrony over time were considered significant if they were outside the bounds of the stricter 99% confidence interval. Graphs of DD and LP values of R plotted separately were also generated for qualitative comparisons.

Custom MATLAB scripts were used for discrete wavelet transform in combination with sine-fit-estimates of circadian parameters including: (1) the goodness-of-sine-fit, (2) the proportion of reliably rhythmic cells, (3) period and (4) amplitude. These parameters were measured with 2-day sliding windows to examine changes over time and in response to a light pulse. The goodness-of-fit was used as a measure of rhythmicity and was determined as the percentage of total variance accounted for by a fitted sine wave as described previously [S5]. The statistical significance of goodness-of-fit was determined by one-way ANOVA and Tukey post hoc test. Cells were considered to have reliably measurable circadian rhythms if their goodness-of-sine-fit measures were greater than a threshold value of 0.82. The criterion of 0.82 was determined by graphing all the time series against the best-fit sine curves and noting that sine-fits above 0.82 produced reliable estimates of period and amplitude. The 0.82 goodness-of-sine fit threshold was incorporated in custom MATLAB scripts to calculate changes in the proportion of reliably rhythmic cells ('P') over time and in response to the light pulse. The same 0.82 criterion was applied across all cell types and light conditions. Statistically significant changes in the proportion of reliably rhythmic cells over time were determined using bootstrapping (as described above). 95% and 99% confidence intervals were estimated by generating 10,000 bootstrap samples for each time series with the size of each bootstrap sample equivalent to the number of cells in each time series. For analysis of inter-subgroup kinetics after LP, changes in number of reliably rhythmic neurons over time were considered significant if they were outside

the bounds of the stricter 99% confidence interval. Custom MATLAB scripts incorporating direct sine-fitting were used to calculate periods and amplitudes for cells meeting the 0.82 criterion of reliably measurable rhythms [16]. Statistically significant changes in periods and amplitudes were determined by one-way ANOVA, Tukey post hoc test.

Animated Model Comparing Inter-Subgroup Kinetics of Synchrony Phase Retuning

A custom MATLAB script was used to generate an animation comparing inter-subgroup kinetics of changes in phase coherence after a light pulse. A color-coded heat map representing values of R for each neuronal subgroup was mapped onto a schematic model of an adult *Drosophila* brain to allow for visualization of spatiotemporal dynamics. The model was divided into two hemispheres to allow for comparisons of neuronal subgroups maintained in DD (left side) and neuronal subgroups exposed to a light pulse (right side). The background is color-coded to provide the same general frames of reference described above.

Validation of Sine-Fit Estimates

Custom MATLAB scripts for BPENS (Bayesian parameter estimation for noise sinusoid) calculations, employing a previously described procedure [16], were used to quantify confidence in our criterion for reliably rhythmic cells and our sine-fit estimates of periods based on measures of uncertainty in the form of 95% and 99% credible intervals (the Bayesian equivalent of confidence intervals). The direct sine-fit measure and the Bayesian frequency estimation method generate period estimates with correlation ρ=0.98 and $p<0.0001$ (688 contributions from the DD cells and 654 from the LP cells, combining across cell types and windows). The Bayesian frequency estimation of the proportion of cells with reliably measurable circadian rhythms and the goodness-of-sine-fit criterion share a correlation ρ=0.58 and $p<0.0001$ (combined again across cell types and windows). Custom MATLAB scripts also generated Bayesian plots for proportion of reliably rhythmic neurons for ‘all cells’ (from all neuronal subgroups) and individual neuronal subgroups (Figure S11). Qualitative comparison of these plots to sine-fit estimates of proportion of reliably rhythmic neurons confirms the same general trend of transient loss then delayed increase in proportion following a light pulse. It can be seen that the distinct patterns of light response for each subgroup are also seen by both Bayesian frequency and sine-fit estimates of the proportion of reliably rhythmic cells indicating that these trends are sufficiently reliably and consistent. Furthermore, tests were run with surrogate data from [16] using 2-day windows to check the accuracy of the wavelet-detrending + sine-fit method we are using. The resulting period estimates had mean absolute error 1.6% with a standard deviation of 1.2%. The results of this test, in combination with the correlation of sine-fit and BPENS measures, confirm that our sine-fit measures with discrete wavelet transform using 2-day sliding windows, though not as ideal as longer recordings would allow, is sufficiently reliably and consistent for our analysis.

Qualitative comparisons of inter-subgroup PER-luc kinetics and responses to light

Single-cell bioluminescence time series records were plotted to convey qualitatively distinct inter-subgroup kinetics and responses to light entrainment. Detrended records are shown for visualization of amplitude changes over time and to allow smooth continuity of the traces throughout the sliding 2-day windows used for quantitative analysis. Color-coded backgrounds were provided for general comparison of inter-subgroup kinetics. The backgrounds were color-

coded to indicate three general time frames of significant trends in light response as measured by phase coherence R values. The boundaries for these time frames were based on the time points at which the differences in R between corresponding DD and LP cells (RLP – RDD) crossed the 99% confidence interval. A blue-gray background denotes the time frame in which neurons have not been exposed to a light pulse, yellow denotes the time of significant desynchrony following light entrainment, and green denotes the time of significant phase retuning of cell synchrony. These general frames of reference were used to compare inter-subgroup kinetics for all circadian measures across all cell types. Plots of values of R for oscillators in DD versus LP were also plotted separated for qualitative comparison of trends in light response.

Immunocytochemistry

Immunostaining of whole brains from adult male flies was performed using a modified version of a previously described protocol [4]. Brains used in Figure 1 for spatial mapping of PER staining were fixed in 4% PFA at ZT 23 and 11. See description below for fixation times used for 'immunocytochemical analysis of *in vivo* light response by intact flies. Primary antibodies applied and incubated for ~48 hours at 4^{o}C included: anti-Per (rabbit, 1:10,000) and anti-Pdf (mouse, 1:10,000). Fluorescent secondary antibodies incubated with the brain explants overnight at 4^{o}C were: Alexa 488 (anti-rabbit, 1:3,000) and Alexa 594 (anti-mouse, 1:500). Brains were stored at 4^{o}C before obtaining images with a Zeiss LSM700 confocal microscope.

Immunocytochemical analysis of in vivo light response by intact flies

Adult male flies are entrained for ≥3 days under standard 12 hr/12 hr LD schedule before being placed in darkness for the rest of the experiment. Control flies (hereafter referred to as 'DD flies') were maintained in constant darkness (DD) for up to 4 days. PER staining for certain neuronal subgroups after four days became too dim to reliably measure. Adult *Drosophila* denoted as 'LP flies' were exposed to a 15 min 12.57 W/m2 (2,000 lux) white light pulse from a Nikon halogen lamp (Fostec 150W fiber optic light power supply) at CT 22 of the second day in DD. Whole brains were dissected in ≤5 minutes in chilled PBS under dim orange light in an otherwise completely dark room to prevent light-triggered degradation of the Period (PER) protein. Flies maintained in DD were fixed at CT 22 on DD day 2 and CT 0 on DD days 3 and 4. Flies exposed to the phase advance white light pulse were fixed 2, 24, and 48 hours after exposure to the light pulse at CT 2 for the first time point and CT 0 for the last two time points. Note that the CT was adjusted for LP flies such that CT 0 corresponds with the time at which the light pulse was administered. 3-4 fly whole brains were dissected, fixed and stained for PER and PDF for each group and time point using the protocol described above. Images of single neurons were obtained using a Zeiss LSM 700 confocal microscope with a 63x oil immersion objective (Plan-Apochromat 63x/1.4 NA). For quantitative comparison of PER staining fluorescence intensity, regions of interest (ROIs) for all DD and LP neuronal subgroups at every time point were imaged with the same standardized laser intensities and microscope settings ('std gain'; 488 nm laser intensity: 1.0%; gain: 740). For qualitative visualization of dimmer neuronal subgroups at later time points in DD, the same ROIs were subsequently imaged with higher gain settings ('high gain') optimized to allow the brightest and clearest visualization of individual neurons. Quantitative analysis of PER staining average fluorescence intensity was performed using the using the software Volocity (PerkinElmer). For dim images, ROIs were manually drawn using 'high gain' images with average fluorescence intensities obtained from corresponding 'standard gain' images. Statistical comparison of the average PER staining intensity of neuronal subgroups

in DD and LP conditions at each time point was performed using the Student's t-test (Sigmaplot version 11) with the null hypothesis that there is no significant difference in fluorescence intensity. The experiments were performed for both w1118 and XLG-Per-Luc flies using the same protocols and standardized intensity settings.

Supplemental References

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S4. Noguchi, T., Wang, L.L., and Welsh, D.K. (2013). Fibroblast PER2 circadian rhythmicity depends on cell density. Journal of Biological Rhythms 28, 183-192.

S5. Fowlkes DH, Karwoski CJ, & Proenza LM (1984) Endogenous circadian rhythm in electroretinogram of free-moving lizards. Investigative Ophthalmology & Visual Science 25(1):121-124.