Supplementary material

Table S1. Summary of the experimental conditions, the measured factor, and the F-tests p-value assessing the alignment of experimental rhythmicity with the 24-hour cosinor model calculation.

Individual serial number	Experiment	Type of measurement	P-value	Color code
1’	24-hours – baseline- no stimulus	Normalized siphon opening	0.008	Blue-dashed
2’	24-hours – baseline- no stimulus	Normalized siphon opening	0.04	Green-dashed
3’	24-hours – baseline- no stimulus	Normalized siphon opening	-	Gray
5’	24-hours – baseline- no stimulus	Normalized siphon opening	0.51	Orange
6’	24-hours – baseline- no stimulus	Normalized siphon opening	0.0004	Black-dashed
8’	24-hours – baseline- no stimulus	Normalized siphon opening	$2*10^{-5}$	Cyan-dashed
9’	24-hours – baseline- no stimulus	Normalized siphon opening	$2.86*10^{-7}$	Red-dashed
4	24-hours stimulus every 2 hours	Normalized siphon opening	$1.9*10^{-5}$	Blue
5	24-hours stimulus every 2 hours	Normalized siphon opening	0.02	Black
6	24-hours stimulus every 2 hours	Normalized siphon opening	$4.76*10^{-5}$	Green
7	24-hours stimulus every 2 hours	Normalized siphon opening	0.7	Orange
8	24-hours stimulus every 2 hours	Normalized siphon opening	$1.68*10^{-5}$	Cyan
9	24-hours stimulus every 2 hours	Normalized siphon opening	-	-
10	24-hours stimulus every 2 hours	Normalized siphon opening	-	-
4	Stimulation every two hours	Recovery time	0.3	Blue
5	Stimulation every two hours	Recovery time	0.007	Black
6	Stimulation every two hours	Recovery time	$4*10^{-4}$	Green
7	Stimulation every two hours	Recovery time	0.64	Orange
8	Stimulation every two hours	Recovery time	0.9	Cyan

9	Stimulation every two hours	Recovery time	0.005	Purple
10	Stimulation every two hours	Recovery time	-	Pink
4	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	0.8	Blue
5	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	0.12	Black
6	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	0.22	Green
7	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	0.7	Orange
8	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	0.9	Cyan
9	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	-	-
10	Stimulation every two hours	Electrophysiology (activity in 200 seconds)	-	-

Table S2. Summary of the experimental conditions and the p-value for each spike type rhythmicity alignment with the 24-hour cosinor model calculation. Two values are presented when the experiment was run twice during separate 24-hour periods.

Individual serial number	Experiment	type 1	type 2	type 3	type 4	Collection date	Measurement starting date
1	Free running	0.09 ,0.19	0.09,0.16	0.08,0.18	0.006,$1.6*10^{-6}$	25.08.22	29.08.22
2	Free running	$6*10^{-7}$, 0.47	0.52, 046	0.009,0.88	0.01,0.82	25.08.22	01.09.22
3	Free running	0.04,0.65	0.56,0.03	0.01,0.008	0.66,0.85	25.08.22	08.09.22
4	24-hours	0.009	$9*10^{-5}$	0.0013	0.2	30.04.23	03.05.23
5	24-hours	0.61	0.06	0.27	0.17	30.04.23	07.05.23
6	24-hours	0.004	0.002	$1.4*10^{-5}$	0.0012	30.04.23	08.05.23
7	24-hours	0.002	0.003	0.0009	0.004	30.04.23	09.05.23
8	24-hours	0.21	0.04	0.04	0.1	30.04.23	10.05.23
9	24-hours	0.62	0.72	0.4	0.91	30.04.23	11.05.23
10	24-hours	0.004	0.02	0.5	0.008	30.04.23	14.05.23

Table S3. BLAST analysis of *Styela clava* candidate clock-gene homologs. Highly similar hits were retrieved with MegaBLAST, while more divergent hits were retrieved with Discontiguous MegaBLAST (BLASTn). Hits with E-value > 1×10^{-5} or query coverage < 20 % were excluded as below our biological significance thresholds.

Gene Name	Source	Level of BLAST	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Comments
Casein Kinase I epsilon	Ciona genome	More dissimilar sequences (discontiguous megablast)	737	737	27%	0	78.17%	2216	The genes with the highest similarity to the human sequqnce
E4BP4			49.1	49.1	8%	1.00E-08	71.28%	3616	
FWD1			870	870	55%	0	74.25%	2549	
HLF			58.1	58.1	9%	4.00E-11	66.49%	3170	
rev-erb			56.3	56.3	2%	2.00E-10	75.64%	2676	to LOC120325544
ROR-A			215	336	33%	1.00E-58	73.00%	3791	
RXR			372	372	50%	5.00E-106	68.33%	4312	
BMAL1	Human genome	N/A							
BMAL2									
CLOCK									
CRY1									
CRY2									
PER1									
PER2									
PER3									

Video S1. An example of stimulating *S. plicata* while recording behavior and electrophysiology (also be accessed via the following link: https://www.youtube.com/embed/IFIwt42Edg8?feature=oembed).

Video S2. Two-minute (downsampled) recording of siphon movement and tracking. The video was part of the basline behavior recordings, and includes no tactile stimulation in the experiment. The video includes a light transition, during which no behavioral response is observed (also be accessed via the following link: https://youtu.be/MCawKs2iVSs).

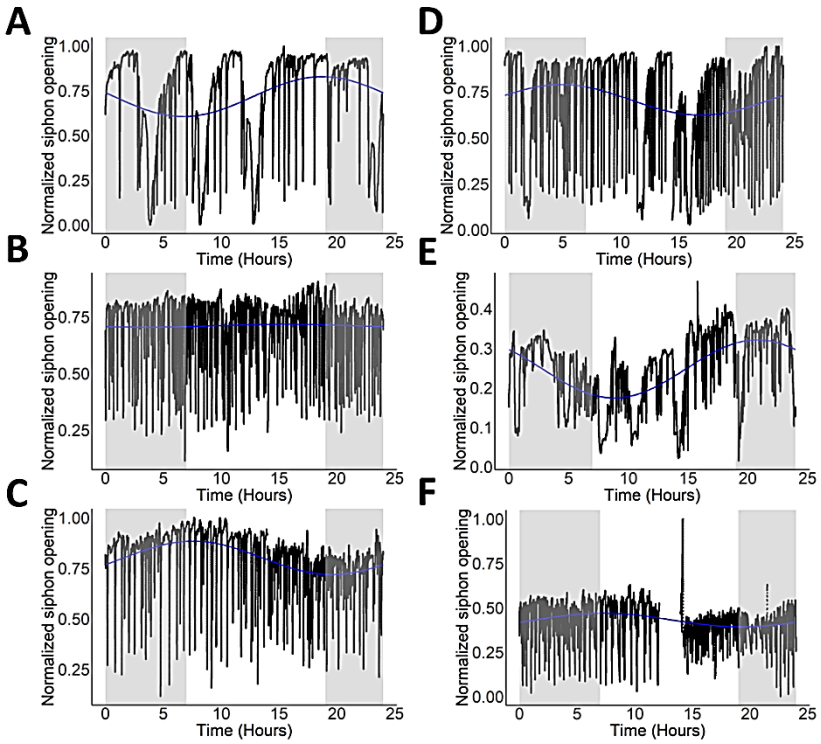


Figure S1. Cosinor fitting for behavioral analysis. Individuals normalized siphon opening of 1'-2',5'-9' are presented (black). Cosinor fitting is presented in blue. Gray shading indicates the night period

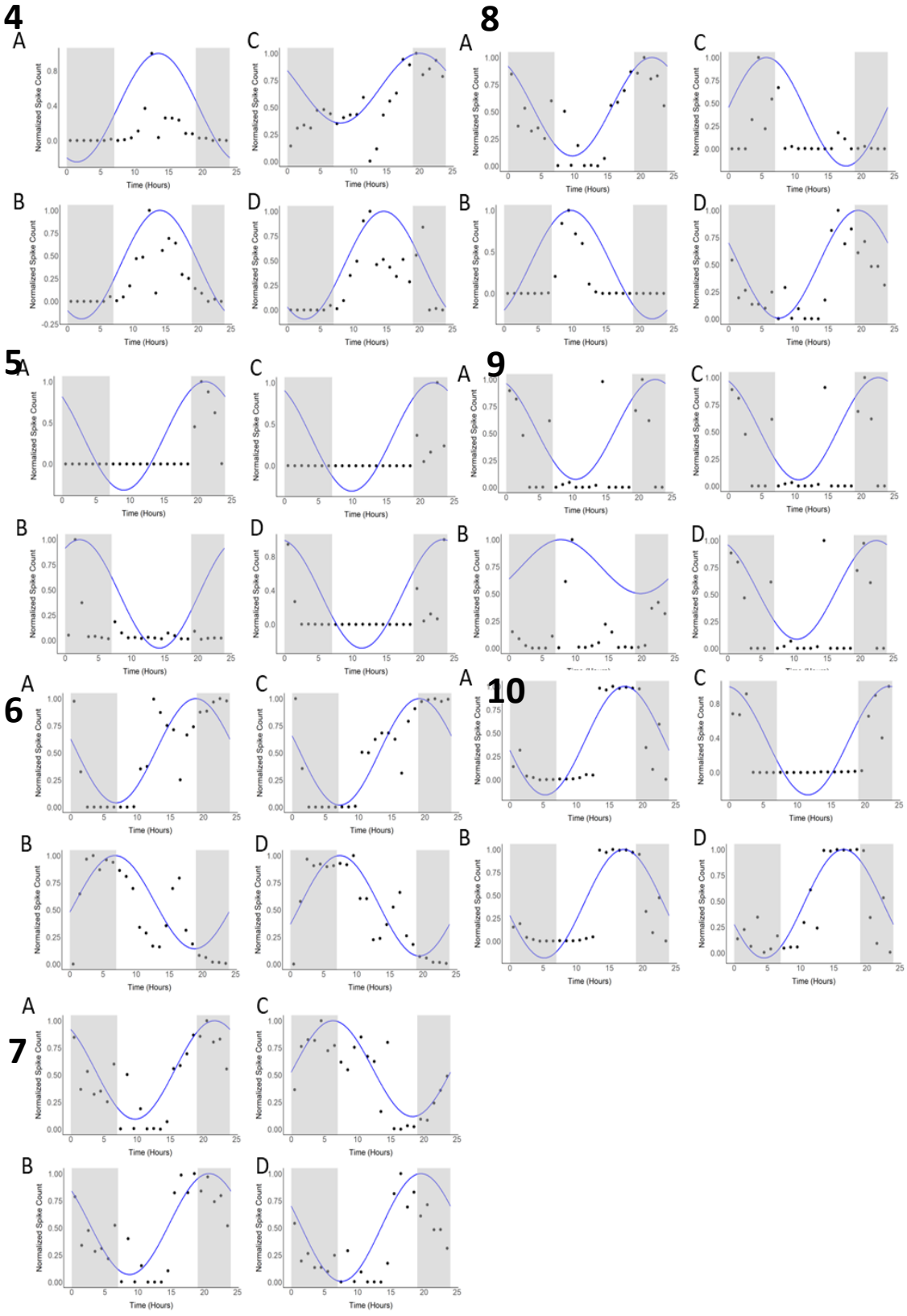


Figure S2. Cosinor fitting for spike analysis 24-hours. Indviduals 4-10 are presented with each point represents 1 hour bin and each panel A-D represents spike type 1-4 respectively. Gray shading indicates the night period

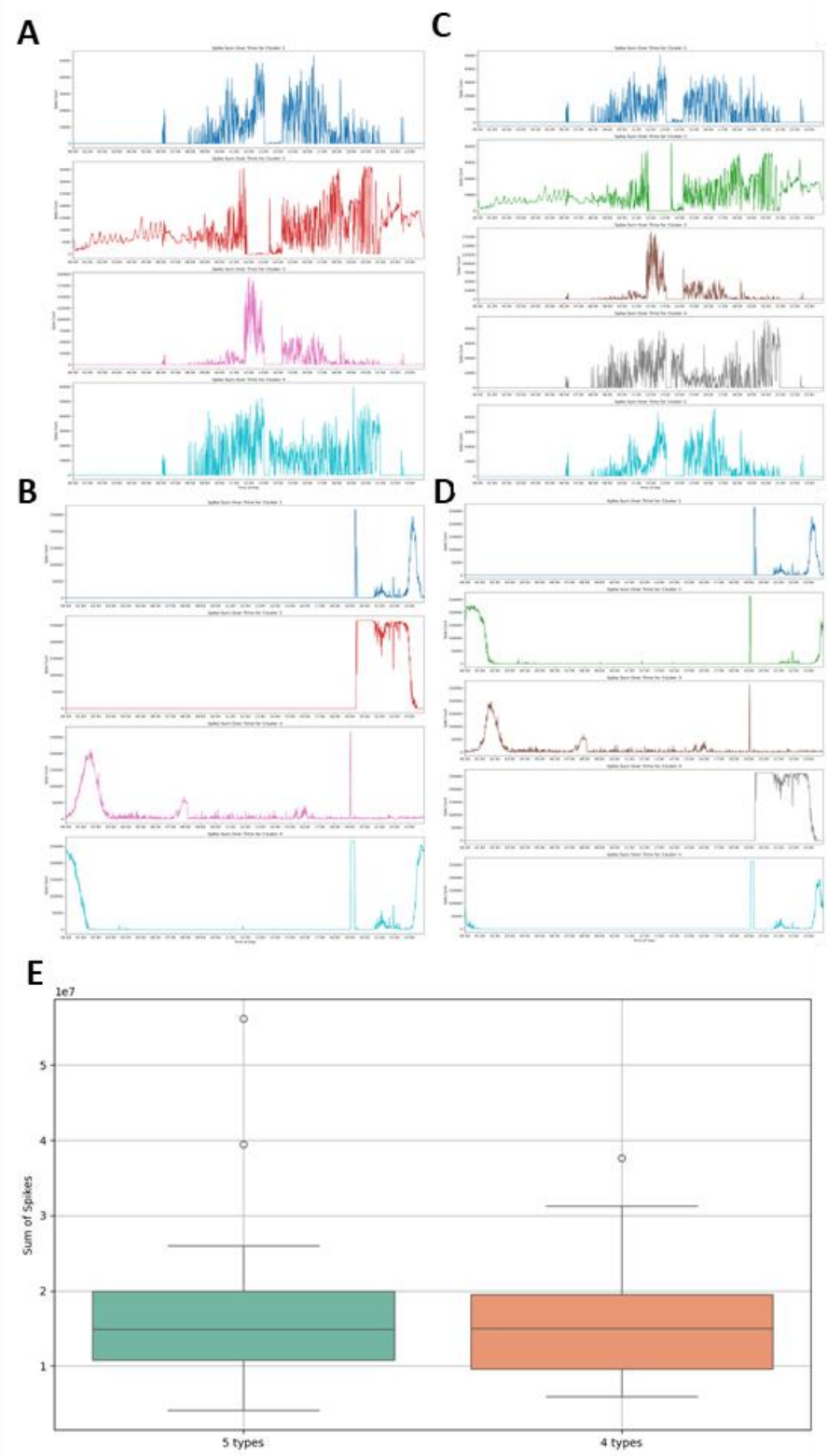


Figure S3. Comparison of raw spike counts using four- and five-type classifications. A&C): Spike count data from animal 4, displayed with four-type (A) and five-type (C) classifications. B&D) Spike count data from animal 5, divided into four types(B) and five-type (D) classifications. E) Total spike counts summed across all animals over a 24-hour period. While using a valley-seeking algorithm to decide how many spike types we have. This figure present visually that 5 types doesn't change the analysis significantly.

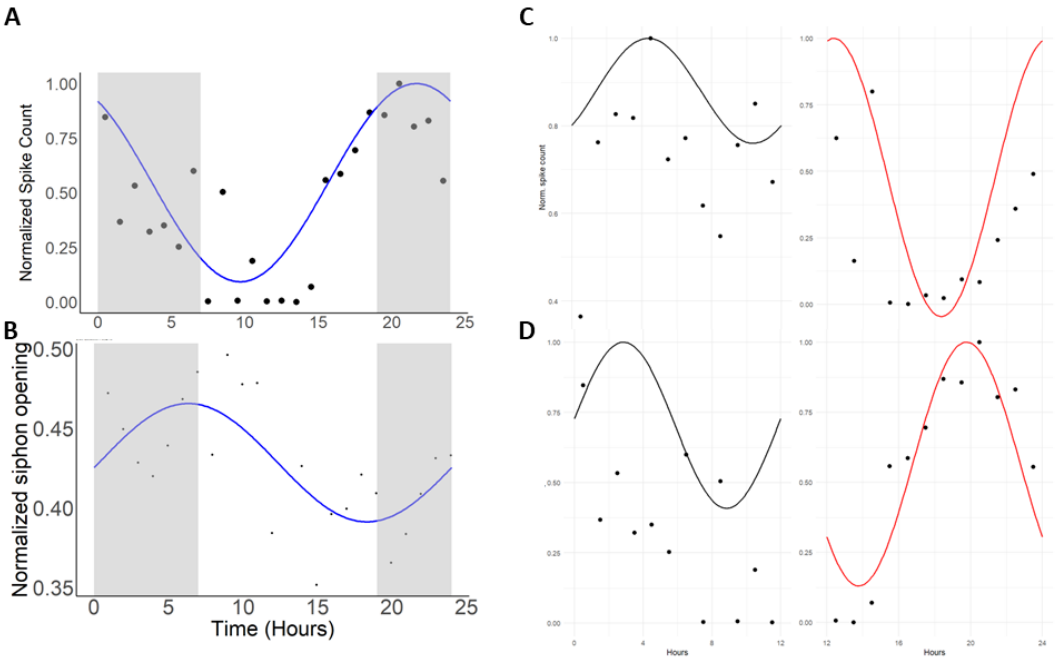


Figure S4. Comparison of 24-hour and 12-hour cycles in spike and behavioral data. A) Type 1 spike count from animal 4, analyzed over a 24-hour cycle. B) Behavioral data from animal 9', analyzed over a 24-hour cycle. C) Type 1 spike count from animal 4, analyzed over a 12-hour cycle (same data as in A). D) Behavioral data from animal 9', analyzed over a 12-hour cycle (same data as in B).

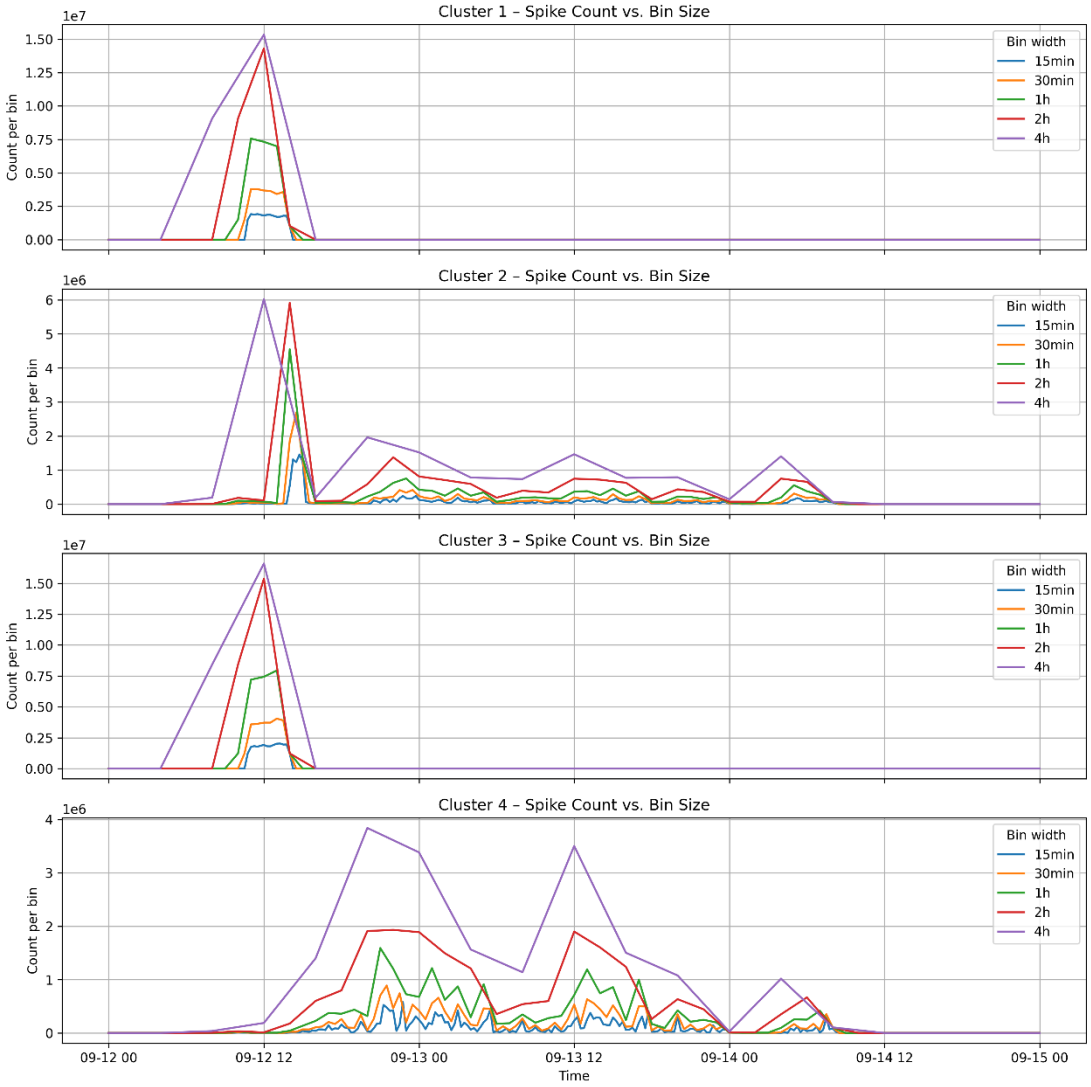
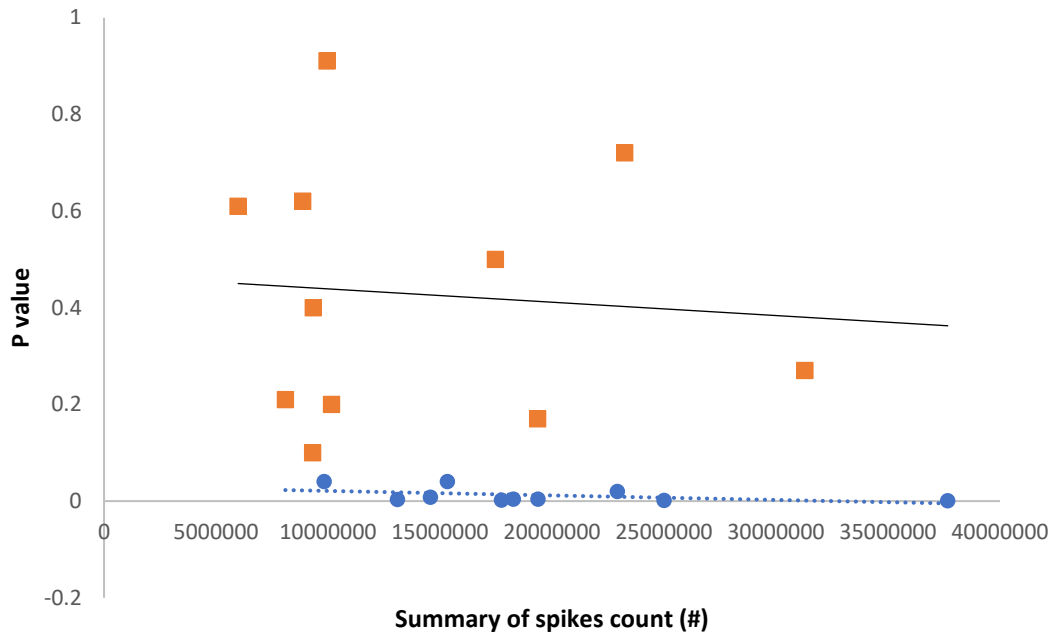


Figure S5. An example of bin-size sensitivity analysis across 48-hours recordings (experiment IV, animal 1) in the 4 spike types. We chose a 1-hour bin width for primary analyses, as it provided a balance between temporal resolution and noise reduction. Key trends were consistent across 1–2 hour bins, but smaller bins exhibited excessive variability and larger bins obscured event timing.



79
80 **Figure S6. Significance of cosinor analysis as a function of total spike count per**
81 **measurement.** In general, recordings with higher spike counts showed more
82 significant rhythmic trends. Orange indicates non-significant recordings ($p > 0.05$),
83 while blue indicates significant recordings ($p < 0.05$).
84