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Supplemental Information

White and Amber Light at Night

Disrupt Sleep Physiology in Birds

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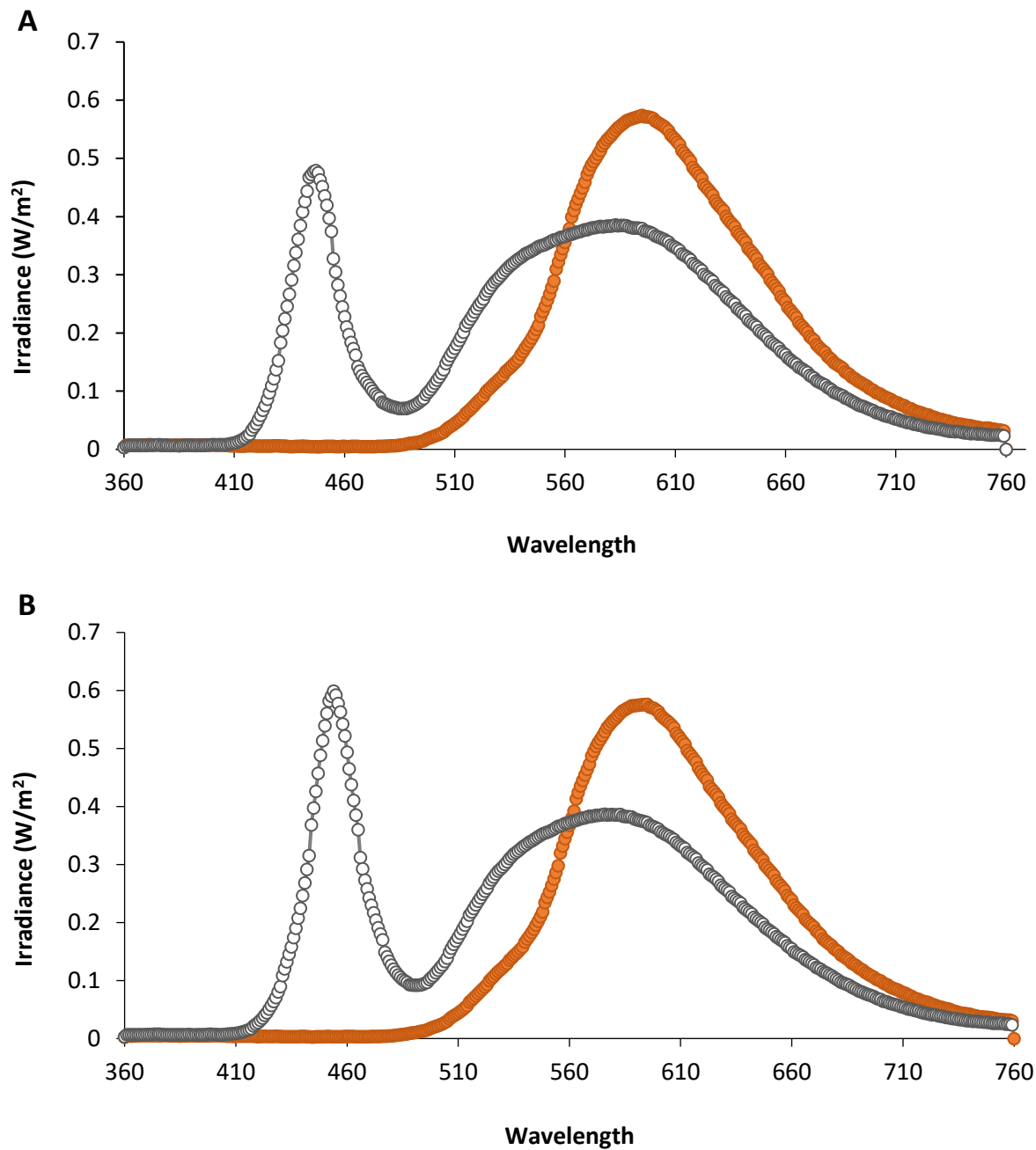


Figure S1: Spectral Output of White Lighting (White) and Amber Lighting (Orange) for (A) *Experiment 2* (pigeons) and (B) *Experiment 3* (Australian magpies), related to STAR Methods. Light spectra were recorded using a MK350 LED Meter (UPRtek Zhunan, Taiwan) 2 m from the light and are therefore relative.

Table S1: Effects of White Light at Night on Night-Time and Daytime Sleep in *Experiment 1*, and White and Amber Light at Night on Night-Time Sleep in *Experiment 2* in Pigeons, Related to Figures 1-4.

	dfn ^a	NREM sleep			REM sleep			NREM SWA			% REM sleep			NREM bout duration			REM bout duration		
		dfd ^b	F	p	dfd	F	p	dfd	F	p	dfd	F	p	dfd	F	p	dfd	F	p
<i>Night-time sleep (Experiment 1)</i>																			
Night ^c	2	64	204.02	<0.001	64	117.47	<0.001	64	95.77	<0.001	72	66.39	<0.001	64	72.54	<0.001	64	119.28	<0.001
Third of night ^d	2	64	7.16	0.002	64	21.34	<0.001	64	42.26	<0.001	72	23.11	<0.001	64	10.05	<0.001	64	35.14	<0.001
Night x third	4	64	3.31	0.016	64	1.88	0.124	64	18.86	<0.001	72	2.97	0.025	64	5.74	0.001	64	6.97	<0.001
<i>Daytime sleep (Experiment 1)</i>																			
Day ^c	2	64	204.02	<0.001	64	117.47	<0.001	64	95.77	<0.001									
Third of day ^d	2	64	7.16	0.002	64	21.34	<0.001	64	42.26	<0.001									
Day x third	4	64	3.16	0.020	64	2.20	0.079	64	1.60	0.185									
<i>Night-time sleep (Experiment 2)</i>																			
Night ^c	1	77	287.58	<0.001	77	114.36	<0.001	77	80.11	<0.001	77	19.14	<0.001	77	62.54	<0.001	77	54.25	<0.001
Third of night ^d	2	77	8.42	<0.001	77	23.41	<0.001	77	42.26	<0.001	77	23.11	<0.001	77	10.05	<0.001	77	35.14	<0.001
Color of light ^e	1	77	3.86	0.053	77	2.80	0.098	77	0.10	5.09	77	0.027	1.35	77	0.25	2.88	77	1.36	0.247
Night x third	2	77	4.98	0.009	77	1.24	0.296	77	11.28	<0.001	77	1.72	0.185	77	6.05	0.004	77	2.10	0.129
Night x color	1	77	2.41	0.125	77	3.80	0.055	77	5.31	0.024	77	2.41	0.124	77	0.82	0.368	77	0.76	0.387
Third x color	2	77	0.34	0.716	77	0.12	0.886	77	0.12	0.889	77	0.24	0.790	77	0.18	0.835	77	1.40	0.252
Night x third x color	2	77	0.24	0.787	77	0.80	0.455	77	0.08	0.923	77	0.36	0.701	77	0.62	0.539	77	0.27	0.766

Fitted models are linear mixed effects models with bird identity as a random intercept. Night-time (12 h) and daytime (12 h) sleep were modelled separately. For *Experiment 1*, night/day (baseline, treatment/post-treatment, or recovery), third of the night/day, and the interaction between night/day and third were included as categorical fixed effects. For *Experiment 2*, night (baseline or light at night), third of the day/night, light color, and the interactions between these factors were included as categorical fixed effects. Results presented are omnibus tests performed using a type three analysis of variance. Statistically significant results are highlighted in bold.

^a dfn is degrees of freedom numerator

^b dfd is degrees of freedom denominator. Degrees of freedom were calculated using the Satterthwaite method and can vary depending on the response variable.

^c Night is baseline (no artificial light at night), treatment (white light throughout the night) and recovery (night after treatment night; no artificial light at night). Day is the 12 h day after each of these nights (baseline, post-treatment, recovery).

^d Third of night/day represents a 4 h period of the night/day

Table S2: Effect of White Artificial Light at Night on Night-time and Daytime Sleep in Pigeons (Post-Hoc Comparisons), Related to Figures 1 and 2.

	Third	df	Treatment Night – Baseline Night			Recovery Night – Baseline Night		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
Night-time sleep								
NREM sleep	T1	64	-0.97 ± 0.07	-13.52	<0.001	-0.20 ± 0.07	-2.75	0.012
	T2	64	-0.75 ± 0.07	-10.54	<0.001	-0.20 ± 0.07	-2.79	0.011
	T3	64	-0.63 ± 0.07	-8.82	<0.001	-0.04 ± 0.07	-0.53	0.631
REM sleep	T1	64	0.83 ± 0.12	-4.90	<0.001	-0.04 ± 0.12	4.80	<0.001
	T2	64	1.00 ± 0.12	-6.40	<0.001	0.10 ± 0.12	2.85	0.01
	T3	64	0.59 ± 0.12	-5.94	<0.001	0.10 ± 0.12	1.22	0.286
NREM SWA	T1	64	-0.08 ± 0.02	-3.54	0.001	-0.16 ± 0.02	-6.46	<0.001
	T2	64	-0.20 ± 0.02	-8.48	<0.001	-0.14 ± 0.02	-5.91	<0.001
	T3	64	-0.28 ± 0.02	-11.67	<0.001	-0.06 ± 0.02	-2.67	0.014
NREM Bout Length	T1	64	-0.61 ± 0.07	-9.20	<0.001	-0.49 ± 0.07	-7.36	<0.001
	T2	64	-0.31 ± 0.07	-4.66	<0.001	-0.31 ± 0.07	-4.65	<0.001
	T3	64	-0.43 ± 0.07	-6.52	<0.001	-0.14 ± 0.07	-2.06	0.062
REM Bout Length	T1	64	-0.05 ± 0.03	-1.78	0.108	0.15 ± 0.03	5.76	<0.001
	T2	64	-0.19 ± 0.03	-7.46	<0.001	0.11 ± 0.03	4.33	<0.001
	T3	64	-0.16 ± 0.03	-6.19	<0.001	0.03 ± 0.03	1.12	0.319
Percent Sleep REM	T1	72	-0.16 ± 0.11	-1.54	0.169	0.61 ± 0.11	5.69	<0.001
	T2	72	-0.47 ± 0.11	-4.40	<0.001	0.36 ± 0.11	3.32	0.002
	T3	72	-0.39 ± 0.11	-3.63	0.001	0.15 ± 0.11	1.38	0.22
	Third	df	Post-treatment Day – Baseline Day			Recovery Day – Baseline Day		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
Daytime sleep								
NREM	T1	64	0.38 ± 0.10	3.99	<0.001	-0.19 ± 0.10	-1.95	0.076
	T2	64	0.36 ± 0.10	3.77	0.001	-0.10 ± 0.10	-1.07	0.337
	T3	64	0.11 ± 0.10	1.17	0.303	0.00 ± 0.10	-0.05	0.959
REM	T1	64	0.83 ± 0.12	6.83	<0.001	-0.04 ± 0.12	-0.29	0.787
	T2	64	1.00 ± 0.12	8.20	<0.001	0.10 ± 0.12	0.83	0.472
	T3	64	0.59 ± 0.12	4.88	<0.001	0.10 ± 0.12	0.81	0.472
NREM SWA	T1	64	-0.18 ± 0.03	-5.65	<0.001	0.01 ± 0.03	0.32	0.779
	T2	64	-0.13 ± 0.03	-3.85	0.001	0.02 ± 0.03	0.62	0.591
	T3	64	-0.08 ± 0.03	-2.44	0.025	0.02 ± 0.03	0.57	0.616

After determining that there were significant effects of light at night on pigeon sleep, as well as time of day/night (*Experiment 1*; Table S1), we conducted post-hoc comparisons between each third (4 h) of each night/day and the equivalent baseline period. Df is degrees of freedom; estimate is unstandardized regression co-efficient (± standard error). To control for the false discovery rate, we adjusted p-values across each experiment using a Benjamini-Hochberg correction [S2, S3]. Significant differences are shown in bold font.

Table S3: Effects of White and Amber Artificial Light at Night on Night-time Sleep in Pigeons (Post-Hoc Comparisons), Related to Figures 3 and 4.

	Third	df	White: Treatment – Baseline			Amber: Treatment – Baseline		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
NREM	T1	77	-1.00 ± 0.10	-9.61	<0.001	-0.82 ± 0.10	-7.88	<0.001
	T2	77	-0.71 ± 0.10	-6.85	<0.001	-0.55 ± 0.10	-5.25	<0.001
	T3	77	-0.65 ± 0.10	-6.21	<0.001	-0.60 ± 0.10	-5.74	<0.001
REM	T1	77	-0.65 ± 0.13	-4.81	<0.001	-0.37 ± 0.13	-2.79	0.013
	T2	77	-0.72 ± 0.13	-5.32	<0.001	-0.37 ± 0.13	-2.75	0.014
	T3	77	-0.72 ± 0.13	-5.35	<0.001	-0.70 ± 0.13	-5.18	<0.001
NREM SWA	T1	77	-0.11 ± 0.04	-2.64	0.016	-0.02 ± 0.04	-0.39	0.769
	T2	77	-0.17 ± 0.04	-4.21	<0.001	-0.09 ± 0.04	-2.27	0.039
	T3	77	-0.29 ± 0.04	-6.94	<0.001	-0.23 ± 0.04	-5.48	<0.001
NREM Bout Length	T1	77	-0.62 ± 0.10	-6.14	<0.001	-0.42 ± 0.10	-4.13	<0.001
	T2	77	-0.17 ± 0.10	-1.69	0.124	-0.18 ± 0.10	-1.78	0.104
	T3	77	-0.30 ± 0.10	-2.97	0.008	-0.27 ± 0.10	-2.66	0.016
REM Bout Length	T1	77	-0.08 ± 0.04	-2.17	0.049	-0.06 ± 0.04	-1.58	0.143
	T2	77	-0.17 ± 0.04	-4.62	<0.001	-0.11 ± 0.04	-3.13	0.005
	T3	77	-0.12 ± 0.04	-3.29	0.004	-0.12 ± 0.04	-3.24	0.004
Percent Sleep REM	T1	77	-0.19 ± 0.12	-1.57	0.144	0.00 ± 0.12	0.04	0.973
	T2	77	-0.36 ± 0.12	-2.96	0.008	-0.13 ± 0.12	-1.07	0.333
	T3	77	-0.34 ± 0.12	-2.73	0.014	-0.30 ± 0.12	-2.42	0.028

After determining that there were significant effects of light at night on pigeon sleep, as well as time of night (*Experiment 2*; Table S1), we conducted post-hoc comparisons between each third (4 h) of each night for each light type and the equivalent baseline period. Df is degrees of freedom; estimate is unstandardized regression co-efficient (± standard error). To control for the false discovery rate, we adjusted p-values across each experiment using a Benjamini-Hochberg correction [S1, S2]. Significant differences are shown in bold font.

Table S4: Effects of White and Amber Artificial Light on Night-time and Daytime Sleep in Magpies (Experiment 3), Related to Figures 3 and 4.

	dfn ^a	NREM sleep			REM sleep			NREM SWA			% REM sleep			NREM bout duration			REM bout duration		
		dfd ^b	F	p	dfd	F	p	dfd	F	p	dfd	F	p	dfd	F	p	dfd	F	p
Night-time sleep																			
Night ^c	2	100	2.36	0.100	99	49.74	<0.001	101	12.05	<0.001	98	38.96	<0.001	101	5.92	0.004	97	11.33	<0.001
Third of night ^d	2	100	2.36	0.100	99	49.74	<0.001	101	12.05	<0.001	98	38.96	<0.001	101	5.92	0.004	97	11.33	<0.001
Color of light ^e	1	107	6.10	0.015	106	3.11	0.081	106	6.49	0.012	106	3.35	0.070	107	6.14	0.015	99	12.02	0.001
Night x third	4	100	79.41	<0.001	99	15.00	<0.001	101	7.86	<0.001	98	7.38	<0.001	101	38.71	<0.001	97	2.10	0.087
Night x color	2	100	0.56	0.572	99	4.08	0.020	101	2.81	0.065	98	2.68	0.074	101	0.91	0.407	97	1.91	0.154
Third x color	2	100	1.57	0.213	99	1.65	0.198	101	2.88	0.061	98	1.38	0.257	101	7.88	0.001	97	1.36	0.261
Night x third x color	4	100	0.91	0.461	99	0.24	0.914	101	2.28	0.066	98	0.19	0.941	101	5.15	0.001	97	0.01	>0.999
Daytime sleep																			
Day ^c	1	65	4.33	0.041	64	3.21	0.078	62	3.85	0.054									
Third of day ^d	2	65	33.50	<0.001	64	0.67	0.517	62	0.30	0.739									
Color of light	1	70	2.60	0.111	69	1.43	0.236	68	5.84	0.018									
Day x third	2	65	1.61	0.209	64	0.44	0.644	62	0.07	0.929									
Day x color	1	65	1.98	0.164	64	0.14	0.714	62	2.00	0.162									
Third x color	2	65	0.25	0.776	64	0.85	0.433	62	0.13	0.875									
Day x third x color	2	65	0.72	0.492	64	2.12	0.128	62	1.71	0.188									

Fitted models are Linear mixed effects models with bird identity as a random intercept. Night-time (12 h) and daytime (12 h) sleep were modelled separately, with night (baseline, treatment, recovery), third of the day/night, light color, and the interactions between these factors as categorical fixed effects. Results presented are omnibus tests performed using a type three analysis of variance. Statistically significant results are highlighted in bold.

^a dfn is degrees of freedom numerator

^b dfd is degrees of freedom denominator. Degrees of freedom were calculated using the Satterthwaite method and can vary depending on the response variable.

^c Night is baseline (no artificial light at night), treatment (light during the first 4 h of the night), and recovery (night after treatment night; no artificial light at night). Day is the 12 h day after each of these nights (baseline, post-treatment, recovery).

^d Third of night/day represents a 4 h period of the night/day

^e Color of light was white (blue-rich) or amber (blue-reduced)

Table S5: Effects of White Artificial Light in the Early Night on Sleep in Night-time and Daytime sleep in Magpies (Post-Hoc Comparisons), Related to Figures 3 and 4.

	Third	df	Treatment night – Baseline night			Recovery night – Baseline night		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
Night-time sleep								
NREM	T1	100	-1.69 ± 0.16	-10.56	<0.001	-0.39 ± 0.16	-2.44	0.071
	T2	100	0.90 ± 0.16	5.63	<0.001	-0.17 ± 0.16	-1.09	0.576
	T3	100	0.55 ± 0.16	3.43	0.006	-0.06 ± 0.16	-0.35	0.880
REM	T1	99	-1.16 ± 0.19	-6.02	<0.001	0.26 ± 0.19	1.33	0.434
	T2	99	-1.18 ± 0.19	-6.11	<0.001	0.12 ± 0.19	0.60	0.828
	T3	99	-0.13 ± 0.19	-0.68	0.812	-0.12 ± 0.19	-0.61	0.828
NREM SWA	T1	102/101*	-0.29 ± 0.08	-3.68	0.003	-0.13 ± 0.08	-1.67	0.275
	T2	101	0.27 ± 0.08	3.52	0.005	-0.13 ± 0.08	-1.74	0.264
	T3	101	0.05 ± 0.08	0.66	0.812	-0.03 ± 0.08	-0.34	0.880
NREM Bout Length	T1	101	-1.69 ± 0.20	-8.28	<0.001	-0.41 ± 0.20	-2.10	0.143
	T2	101	1.32 ± 0.20	6.76	<0.001	-0.11 ± 0.20	-0.56	0.838
	T3	101	0.33 ± 0.20	1.67	0.275	-0.09 ± 0.20	-0.45	0.843
REM Bout Length	T1	97	-0.21 ± 0.07	-2.52	0.061	0.04 ± 0.07	0.53	0.838
	T2	97	-0.20 ± 0.07	-2.76	0.038	0.04 ± 0.07	0.54	0.838
	T3	97	-0.11 ± 0.07	-1.49	0.346	-0.04 ± 0.07	-0.63	0.825
Percent Sleep REM	T1	98	-0.61 ± 0.21	-2.92	0.026	0.08 ± 0.21	0.36	0.880
	T2	98	-0.90 ± 0.21	-4.29	<0.001	-0.11 ± 0.21	-0.53	0.838
	T3	98	-0.04 ± 0.21	-0.21	0.923	-0.07 ± 0.21	-0.32	0.890
Post-treatment day – Baseline day								
	Third	df	Estimate ± SE	t-ratio	p-value			
Daytime sleep								
NREM	T1	65	-0.08 ± 0.37	-0.21	0.923			
	T2	65	1.00 ± 0.37	2.71	0.042			
	T3	65	0.65 ± 0.37	1.78	0.257			

After determining that there were significant effects of light at night on magpie sleep, as well as time of night/day (*Experiment 2*; Table S4), we conducted post-hoc comparisons between each third (4 h) of each night/day and the equivalent baseline period. To control for the false discovery rate, we adjusted p-values across each experiment using a Benjamini-Hochberg correction [S1, S2]. Df is degrees of freedom; estimate is unstandardized regression co-efficient (± standard error). Significant differences are shown in bold font. *Degrees of freedom differ because one magpie did not sleep during the first third of the treatment night (therefore we had no SWA data for this bird).

Table S6: Effects of Amber Artificial Light in the Early Night on Night-time and Daytime Sleep in Magpies (Post-Hoc Comparisons), Related to Figures 3 and 4.

	Third	df	Treatment – Baseline			Recovery – Baseline		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
Night-time sleep								
NREM	T1	100	-1.27 ± 0.16	-7.94	<0.001	-0.03 ± 0.16	-0.16	0.947
	T2	100	0.84 ± 0.16	5.23	<0.001	-1.27 ± 0.16	-0.57	0.838
	T3	100	0.43 ± 0.16	2.69	0.042	-0.09 ± 0.16	-0.56	0.838
REM	T1	99	-0.81 ± 0.19	-4.21	<0.001	0.09 ± 0.19	0.45	0.843
	T2	99	-0.88 ± 0.19	-4.54	<0.001	-0.14 ± 0.19	-0.74	0.790
	T3	99	0.13 ± 0.19	0.67	0.812	-0.09 ± 0.19	-0.49	0.841
NREM SWA	T1	101	0.08 ± 0.08	1.03	0.621	-0.05 ± 0.08	-0.67	0.812
	T2	101	0.26 ± 0.08	3.40	0.006	-0.05 ± 0.08	-0.61	0.828
	T3	101	0.12 ± 0.08	1.64	0.281	-0.05 ± 0.08	-0.66	0.812
NREM Bout Length	T1	101	-0.55 ± 0.20	-2.82	0.034	-0.23 ± 0.20	-1.19	0.508
	T2	101	0.88 ± 0.20	4.49	<0.001	-0.10 ± 0.20	-0.51	0.841
	T3	101	0.25 ± 0.20	1.26	0.455	-0.15 ± 0.20	-0.78	0.786
REM Bout Length	T1	97	-0.13 ± 0.07	-1.69	0.274	0.01 ± 0.07	0.17	0.943
	T2	97	-0.12 ± 0.07	-1.69	0.274	0.00 ± 0.07	-0.01	0.994
	T3	97	0.00 ± 0.07	-0.04	0.983	-0.05 ± 0.07	-0.77	0.786
Percent Sleep REM	T1	98	-0.61 ± 0.21	-2.92	0.026	0.08 ± 0.21	0.36	0.880
	T2	98	-0.90 ± 0.21	-4.29	<0.001	-0.11 ± 0.21	-0.53	0.838
	T3	98	-0.04 ± 0.21	-0.21	0.923	-0.07 ± 0.21	-0.32	0.890
Post-treatment day – Baseline day								
	Third	df	Estimate ± SE	t-ratio	p-value			
Daytime sleep								
NREM	T1	65	-0.03 ± 0.37	-0.07	0.983			
	T2	65	0.18 ± 0.37	0.49	0.841			
	T3	65	0.15 ± 0.37	0.41	0.862			

After determining that there were significant effects of light at night on magpie sleep, as well as time of night/day (*Experiment 2*; Table S4), we conducted post-hoc comparisons between each third (4 h) of each night/day and the equivalent baseline period. Df is degrees of freedom; estimate is unstandardized regression co-efficient (± standard error). To control for the false discovery rate, we adjusted p-values across each experiment using a Benjamini-Hochberg correction [S1, S2]. Significant differences are shown in bold font.

Table S7: Comparing Amber and White Artificial Light Treatments on Night-time Sleep in Magpies (Post-Hoc Comparisons), Related to Figures 3 and 4.

	Third	df	Amber – White: Baseline Night			Amber – White: Treatment Night			Amber – White: Recovery Night		
			Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value	Estimate ± SE	t-ratio	p-value
NREM	T1	102	0.01 ± 0.16	0.05	0.983	0.43 ± 0.16	2.65	0.044	0.37 ± 0.16	2.31	0.089
	T2	102	0.04 ± 0.16	0.25	0.918	-0.02 ± 0.16	-0.14	0.952	0.12 ± 0.16	0.76	0.786
	T3	102	0.15 ± 0.16	0.94	0.674	0.03 ± 0.16	0.21	0.923	0.12 ± 0.16	0.74	0.790
REM	T1	100	-0.19 ± 0.19	-0.96	0.670	0.16 ± 0.19	0.84	0.748	-0.36 ± 0.19	-1.84	0.232
	T2	100	0.01 ± 0.19	0.06	0.983	0.31 ± 0.19	1.61	0.292	-0.25 ± 0.19	-1.27	0.455
	T3	100	-0.36 ± 0.19	-1.82	0.233	-0.09 ± 0.19	-0.48	0.841	-0.33 ± 0.19	-1.70	0.274
NREM SWA	T1	103	0.00 ± 0.08	0.02	0.988	0.37 ± 0.08	4.69	<0.001	0.08 ± 0.08	1.02	0.621
	T2	103	-0.02 ± 0.08	-0.24	0.920	-0.03 ± 0.08	-0.37	0.880	0.07 ± 0.08	0.90	0.699
	T3	103	0.02 ± 0.08	0.28	0.897	0.10 ± 0.08	1.27	0.455	0.00 ± 0.08	-0.04	0.983
NREM Bout Length	T1	102	0.09 ± 0.20	0.48	0.841	1.23 ± 0.20	6.01	<0.001	0.27 ± 0.20	1.39	0.402
	T2	102	0.08 ± 0.20	0.40	0.862	-0.36 ± 0.20	-1.85	0.232	0.09 ± 0.20	0.45	0.843
	T3	102	0.09 ± 0.20	0.44	0.843	0.01 ± 0.20	0.04	0.983	0.02 ± 0.20	0.12	0.967
REM Bout Length	T1	97	-0.11 ± 0.07	-1.55	0.317	-0.02 ± 0.07	-0.28	0.897	-0.14 ± 0.07	-1.91	0.212
	T2	97	-0.06 ± 0.07	-0.77	0.786	0.02 ± 0.07	0.29	0.897	-0.09 ± 0.07	-1.32	0.438
	T3	97	-0.17 ± 0.07	-2.37	0.080	-0.07 ± 0.07	-0.93	0.674	-0.18 ± 0.07	-2.51	0.061

After determining that there were significant effects of light at night on magpie sleep, as well as time of night/day (*Experiment 2*; Table S4), we conducted post-hoc comparisons between each third (4 h) of each night/day and the equivalent baseline period. Df is degrees of freedom; estimate is unstandardized regression co-efficient (± standard error). To control for the false discovery rate, we adjusted p-values across each experiment using a Benjamini-Hochberg correction [S1, S2]. Significant differences are shown in bold font.

SUPPLEMENTAL REFERENCES

- S1. Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. Ser. B. (Stat. Method.)* 57, 289-300.
- S2. Yekutieli, D., and Benjamini, Y. (1999). Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. *J. Stat. Plan. Infer.* 82, 171–196.