

ONLINE SUPPLEMENTARY INFORMATION

***In vivo* modulation of locus coeruleus activity by light**

Fermin Balda Aizpurua¹, Roya Sharifpour⁴, Islay Campbell¹, Elise Beckers¹, Sara Lelot¹, Alexandre Berger¹, Nasrin Mortazavi¹, Ekaterina Koshmanova¹, Fabienne Collette¹, Laurent Lamalle¹, Heidi I.L. Jacobs^{2,3}, Mikhail Zubkov¹, Puneet Talwar^{1*}, Gilles Vandewalle^{1*#}

1. GIGA-Institute, CRC-Human Imaging, University of Liège, Liège, Belgium

2. Faculty of Health, Medicine and Life Sciences, School for Mental Health and Neuroscience, Alzheimer Centre Limburg, Maastricht University; Maastricht, The Netherlands

3. Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

4. Current address: Department of Translational Neuroimaging, Hôpital Erasme, Hôpital Universitaire de Bruxelles, Université libre de Bruxelles, Brussels, Belgium

Supplementary Table S1. Demographics of study sample.

	Total Sample
Number of Participants	35
Age	24.49 ± 3.32
Sex (M)	13
Mood (BDI-II)	6.93 ± 5.84
Anxiety (BAI)	5.37 ± 4.22
Sleep quality (PSQI)	4.17 ± 2.55
Seasonality (SPAQ)	1.1 ± 0.8
Chronotype (HO)	46.52 ± 8.93
Daytime sleepiness (ESS)	6.58 ± 3.07
Years of Education	14.7 ± 3.15
Sleep duration (night before fMRI protocol – sleep diary based)	7.51 ± 0.85
Number of participants per month (January to December)	j:1; f:2; m:2; a:5; m:4; j:3; j:1; a:3; s:5; o:4; n:4; d:1

Total number of participants included in the analyses. BDI-II, Beck's Depression Inventory; BAI, Beck Anxiety Inventory; PSQI, Pittsburgh Sleep Quality Index; SPAQ, Seasonal Pattern Assessment Questionnaire; HO, Horne and Östberg; ESS, Epworth Sleepiness Scale. Refer to the method for the references to the scales and questionnaires.

Supplementary Table S2. Light characteristics.

	Low BEL	Mid BEL	High BEL	Orange
Lux	47	116	240	7.5
Peak Spectral Irradiance (nm)	460	460	460	590
Melanopic EDI (lux; ipRGCs)	37	92	190	0.16
Rhodopic EDI (lux; Rods)	39	97	201	0.94
Cyanopic EDI (lux; S-cones)	32	79	163	0
Chloropic EDI (lux; M-cones)	44	110	227	5
Erythropic EDI (lux ; L-cones)	46	113	233	8
Irradiance ($\mu\text{W}/\text{cm}^2$)	15	36	75	1.4
Photon flux($1/\text{cm}^2/\text{s}$)	4.12E+13	1.02E+14	2.10E+14	4.24E+12
Log Photon Flux ($\log_{10} (1/\text{cm}^2/\text{s})$)	13.61	14.01	14.32	12.63
Narrowband peak	-	-	-	589
Narrowband FWHM	-	-	-	10

Detailed characteristics of the four conditions used in fMRI protocol. Blue enriched (BEL) (low, mid, and high) and monochromatic (589nm). ipRGCs: intrinsically photosensitive retinal ganglion cells. FWHM: full width at half maximum.

Supplementary Table 3. Effect of illuminance on performance to the emotional task and transient pupil dilation. F-statistics are reported for fixed effects with degrees of freedom (df) in parentheses, estimated using the Satterthwaite approximation. *P < .05, **P < .01, ***P < .001.

Emotional Task Performance with Light			
Effect	<i>F</i> (df)	<i>p</i>	η^2
Fixed Effects			
Light	2.41 (4,92)	0.054	0.095
Task	1.97 (1,19)	<0.001***	0.405
Age	0.53 (1,19)	0.176	0.094
Sex	1.37 (1,19)	0.475	0.027
BMI	0.11 (1,19)	0.256	0.067
Season	15.66 (1,23)	0.747	0.005
Light x Task	0.39 (4,92)	0.816	0.017
Emotional Transient Pupil Dialation with Light			
Effect	<i>F</i> (df)	<i>p</i>	η^2
Fixed Effects			
Light	9.2347 (4, 90.64)	0.003 ***	0.290
Age	0.1834 (1, 18.98)	0.673	0.010
Sex	3.0432 (1, 18.87)	0.097	0.139
BMI	0.1170 (1, 18.73)	0.736	0.006
Season	0.1460 (1, 18.73)	0.707	0.008
Task	1.5519 (1, 22.83)	0.225	0.064
Light x Task	0.2904 (4, 90.52)	0.884	0.013

Supplementary Table S4. Post hoc contrasts of the GLMM considering the activity of the LC under each illuminance (related to table 2 of the main text)

Condition	Comparison	<i>t</i>	<i>p</i> (<i>uncorrected</i>)
0 lux	Neut - Emo	0.125	0.9006
0.16 lux	Neut - Emo	0.206	0.8372
37 lux	Neut - Emo	-0.443	0.6583
92 lux	Neut - Emo	-1.342	0.1815
190 lux	Neut - Emo	-4.512	<0.001***
Neut	Darkness (0 lux) - Orange (0.16 lux)	-0.474	0.6357
Neut	Darkness (0 lux) - B1 (37 lux)	-0.22	0.8926
Neut	Darkness (0 lux) - B2 (92 lux)	-0.260	0.7953
Neut	Darkness (0 lux) - B3 (190 lux)	-1.548	0.1229
Neut	Orange (0.16 lux) - B1 (37 lux)	0.605	0.5457
Neut	Orange (0.16 lux) - B2 (92 lux)	0.219	0.8266
Neut	Orange (0.16 lux) - B3 (190 lux)	-1.046	0.2965
Neut	B1 (37 lux) - B2 (92 lux)	-0.393	0.6950
Neut	B1 (37 lux) - B3 (190 lux)	-1.670	0.0963
Neut	B2 (92 lux) - B3 (190 lux)	-1.288	0.1989
Emo	Darkness (0 lux) - Orange (0.16 lux)	-0.545	0.5468
Emo	Darkness (0 lux) - B1 (37 lux)	0.621	0.5350
Emo	Darkness (0 lux) - B2 (92 lux)	0.997	0.3200
Emo	Darkness (0 lux) - B3 (190 lux)	2.431	0.0158*
Emo	Orange (0.16 lux) - B1 (37 lux)	1.149	0.2516
Emo	Orange (0.16 lux) - B2 (92 lux)	1.516	0.1309
Emo	Orange (0.16 lux) - B3 (190 lux)	2.916	0.004**
Emo	B1 (37 lux) - B2 (92 lux)	0.372	0.7101
Emo	B1 (37 lux) - B3 (190 lux)	1.793	0.0742
Emo	B2 (92 lux) - B3 (190 lux)	1.424	0.1559

Supplementary Table S5. 4th Ventricle Main Stats. F-statistics are reported for fixed effects with degrees of freedom (df) in parentheses, estimated using the Satterthwaite approximation. *P < .05, **P < .01, ***P < .001.

Lightmod Model			
Emotional Task 4th Ventricle Analysis			
Effect	<i>F</i> (df)	<i>p</i>	η^2
Task	1.90 (1,34)	0.177	0.053
Age	1.83 (1,30)	0.186	0.058
Sex	0.34 (1,30)	0.564	0.011
BMI	0.07 (1,30)	0.793	0.002
Season	0.46 (1,30)	0.502	0.015

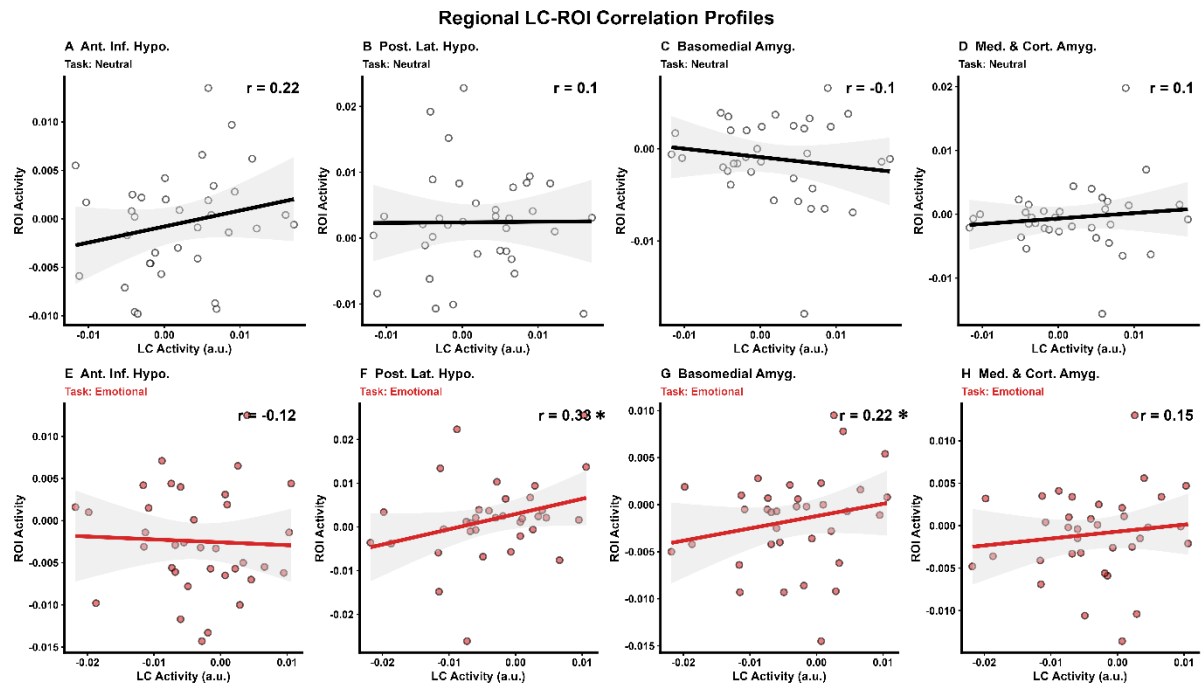
Supplementary Table 6. Association between performance to the task and pupil transient dilation to the emotional task and the activity of LC for each illuminance. F-statistics are reported for fixed effects with degrees of freedom (df) in parentheses, estimated using the Satterthwaite approximation. *P < .05, **P < .01, ***P < .001.

Effect	<i>F</i> (df)	<i>p</i>	η^2
Emotional Task Performance with LC			
Light	2.292 (4, 211.10)	.061	0.042
Age	1.982 (1, 18.99)	.175	0.095
Sex	0.518 (1, 19.01)	.480	0.027
BMI	1.373 (1, 18.98)	.256	0.067
Season	0.110 (1, 18.98)	.744	0.006
LC	0.044 (1, 217.63)	.834	0.000
Effect	<i>F</i> (df)	<i>p</i>	η^2
Emotional Transient Pupil Dialation with LC			
Light	10.219 (4, 206.42)	<.001***	0.165
Age	0.198 (1, 19.03)	.661	0.010
Sex	3.001 (1, 18.87)	.100	0.137
BMI	0.110 (1, 18.96)	.744	0.006
Season	0.150 (1, 18.79)	.703	0.008
LC	0.006 (1, 212.73)	.938	0.000

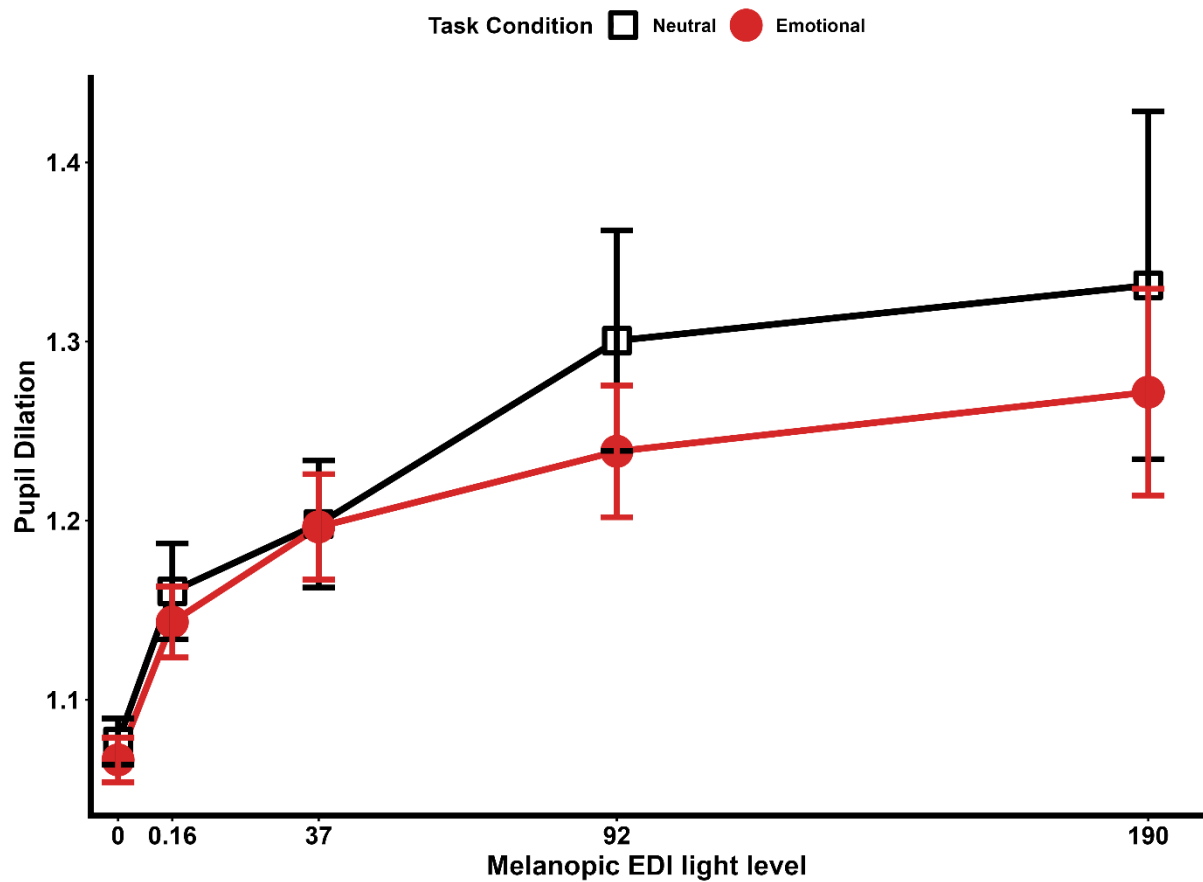
Supplementary Table 7. Association between LC activity and amygdala/hypothalamus subparts (related to Figure 3)

Task	Predictor Region	<i>t</i>	<i>p</i>	η^2
LC activity and Hypothalamus and Amygdala Relationships				
Neutral	Anterior Inferior Hypothalamus	1.756	0.09	0.154
	Posterior Lateral Hypothalamus	-0.388	0.701	0.069
	Basomedial Nuclei Amygdala	-0.749	0.460	0.082
	Medial and Cortical Nuclei Amygdala	0.807	0.426	0.085
Emotional	Anterior Inferior Hypothalamus	0.059	0.953	0.072
	Posterior Lateral Hypothalamus	2.181	0.037 *	0.203
	Basomedial Nuclei Amygdala	2.080	0.046 *	0.192
	Medial and Cortical Nuclei Amygdala	1.431	0.163	0.133

General Linear Models (GLM) examining subcortical predictors of Locus Coeruleus (LC) BOLD activity across Neutral and Emotional tasks. Relationships were examined using General Linear Models (GLM). To ensure the specificity of these associations, Age, Gender, BMI, and Season were included as covariates in all models to account for potential inter-individual variability and seasonal influences on LC and subcortical activity. Steiger's Z-tests revealed no significant differences in LC-association strength between subparts within tasks ($p > 0.19$). * $P < .05$, ** $P < .01$, *** $P < .001$.



Supplementary Figure 1. Regional LC-ROI Correlation Profiles. This eight-panel scatterplot matrix displays the relationship between Locus Coeruleus (LC) activity (a.u.; horizontal axes) and the activity of four specific brain Regions of Interest (ROIs; vertical axes): Anterior Inferior Hypothalamus (Ant. Inf. Hypo.), Posterior Lateral Hypothalamus (Post. Lat. Hypo.), Basomedial Amygdala (Basomedial Amyg.), and Medial & Cortical Amygdala (Med. & Cort. Amyg.). Panels **A–D** (top row, blue headers) illustrate correlations during the Neutral task condition, using black regression lines and open circles. Panels **E–H** (bottom row, red headers) show correlations during the Emotional task condition, utilizing red regression lines and red-filled circles. Spearman correlation coefficients (r) are displayed in the upper right of each panel; asterisks indicate significant correlations at $p < .05$. Bold, consistent lines signify mean trends, and the light grey shaded ribbons indicate the 95% confidence interval for each correlation. Data were processed to map discrete variables onto continuous plotting spaces and exclude outliers beyond strict axis limits for consistent visualization. (refer to Supplementary Table 7 for full statistics).



Supplementary Figure 2. Effect of Light Intensity and Task Condition on Pupil Dilation. This line graph illustrates the mean pupil dilation (ratio, a.u.) across five Melanopic EDI light levels (0, 0.16, 37, 92, and 190). Data are stratified by task condition: **Neutral** (black line, open squares) and **Emotional** (red line, filled circles). Error bars represent the standard error of the mean (SEM). The x-axis is plotted on a non-linear scale to reflect specific experimental light intensity intervals. Statistical analysis revealed a significant main effect of Light ($p = .003$), indicating a dose-dependent increase in pupil dilation as light intensity increases (refer to Supplementary Table 3 for full statistics).