Neuroscience

Regional response to light illuminance across the human hypothalamus

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Abstract

Light exerts multiple non-image-forming biological effects on physiology including the stimulation of alertness and cognition. However, the subcortical circuitry underlying the stimulating impact of light is not established in humans. We used 7 Tesla functional magnetic resonance imaging to assess the impact of variations in light illuminance on the regional activity of the hypothalamus while healthy young adults (N=26; 16 women; 24.3 ± 2.9y) were completing two auditory cognitive tasks. We find that, during both the executive and emotional tasks, higher illuminance triggered an activity increase over the posterior part of the hypothalamus, which includes part of the tuberomamillary nucleus and the posterior part of the lateral hypothalamus. In contrast, increasing illuminance evoked a decrease in activity over the anterior and ventral parts of the hypothalamus, encompassing notably the suprachiasmatic nucleus and another part of the tuberomammillary nucleus. Critically, performance of the executive task was improved under higher illuminance and was negatively correlated with the activity of the posterior hypothalamus area. These findings reveal the distinct local dynamics of different hypothalamus regions that underlie the impact of light on cognition. They may suggest that light acts on the orexin and histamine system to affect the quality of wakefulness.

eLife assessment

This **fundamental** work describes the complex interplay between light exposure, hypothalamic activity, and cognitive function. The evidence supporting the conclusion is **compelling** with potential therapeutic applications of light modulation. The work will be of broad interest to basic and clinical neuroscientists.

Introduction

Light exerts multiple non-image-forming (NIF) biological effects that influence the quality of sleep and wakefulness, and higher illuminance is known to stimulate alertness and cognition.[1] The biological effects of light primarily rely on a subclass of retinal ganglion cells that are intrinsically photosensitive (ipRGCs) because they express the photopigment melanopsin, which is maximally sensitive to photons with wavelength ~480nm. IpRGCs combine the light signalling of rods and cones to their intrinsic photosensitivity and, collectively, the biological effects of light present a maximal sensitivity to the shorter blue wavelength of visible light.[2] IpRGCs project to multiple subcortical brain areas and their denser projections are found within the hypothalamus, particularly in nuclei involved in sleep and wakefulness regulation.[2,3] The suprachiasmatic nucleus (SCN), which is the site of the principal circadian clock, receives the strongest inputs from ipRGC inputs, over the anterior part of the hypothalamus.[4] Other nuclei also receive ipRGC projections: the subparaventricular zone, one of the main output routes of the SCN, the ventrolateral preoptic nucleus (VLPO) and the preoptic nucleus (PON), involved in sleep initiation and also found in the anterior part of the hypothalamus; the lateral hypothalamus (LH), site of the orexinergic wake-promoting neurons and melanin-concentrating hormone sleep-promoting neurons, found in contrast over the lateral and posterior parts of the hypothalamus.[2,3]

The brain circuitry underlying the biological effects of light has mostly been uncovered in nocturnal rodent models.[1,2] Translation to diurnal human beings, where the later maturation of the cortex allows for complex cognitive processing,[5] remains scarce. In particular, whether hypothalamus nuclei contribute to the stimulating impact of light on cognition in humans is not established.

We addressed this question using ultra-high-field (UHF) 7 Tesla (7T) functional magnetic resonance imaging (fMRI) in healthy young adults exposed to light of various illuminance while engaged in two different auditory cognitive tasks. We find that higher illuminance increased the activity of the posterior part of the hypothalamus encompassing the mamillary bodies (MB) and parts of the LH and tuberomammillary nucleus (TMN). In contrast, higher illuminance decreased the activity over the anterior and ventral parts of the hypothalamus encapsulating notably the SCN and another part of the TMN. Critically, the pattern of modulation was consistent across the two cognitive tasks. Importantly, performance of the complex cognitive task was improved under higher illuminance while the activity of the posterior part of the hypothalamus was correlated to task performance. The findings reveal the distinct local dynamics of different hypothalamus areas in response to changing illuminance that may contribute to light's impact on cognition.

Results

Twenty-six healthy young adults (16 women; 24.3 ± 2.9 y; **Suppl. Table S1**) completed two auditory cognitive tasks encompassing, respectively, the executive[6] and emotional[7] domains, while alternatively maintained in darkness or exposed to short periods (< 1 min) of light of four different illuminances (0.16, 37, 92, 190 melanopic equivalent daylight illuminance - mel EDI-lux; **Suppl. Table S2**) [**Fig. 1**]. The hypothalamus of each participant was segmented into 5 subparts – inferior-anterior, superior-anterior, inferior-tubular, superior-tubular, and posterior [**Fig. 2A**] – so we could consistently extract the regional effect of illuminance change on fMRI blood-oxygen-level-dependent (BOLD) signal over most of the hypothalamus volume.

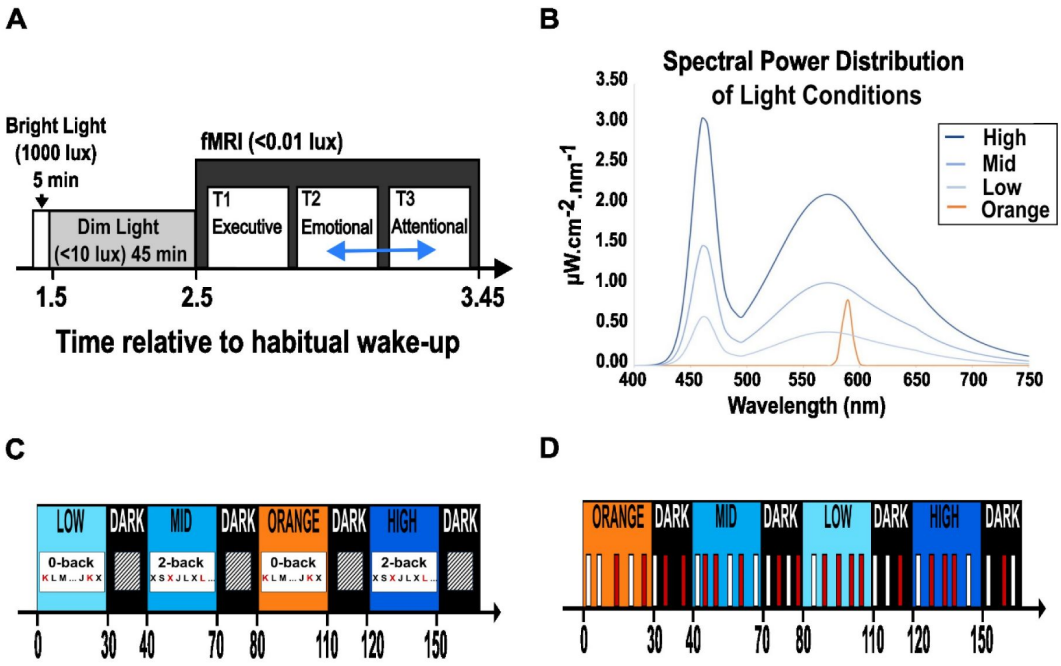
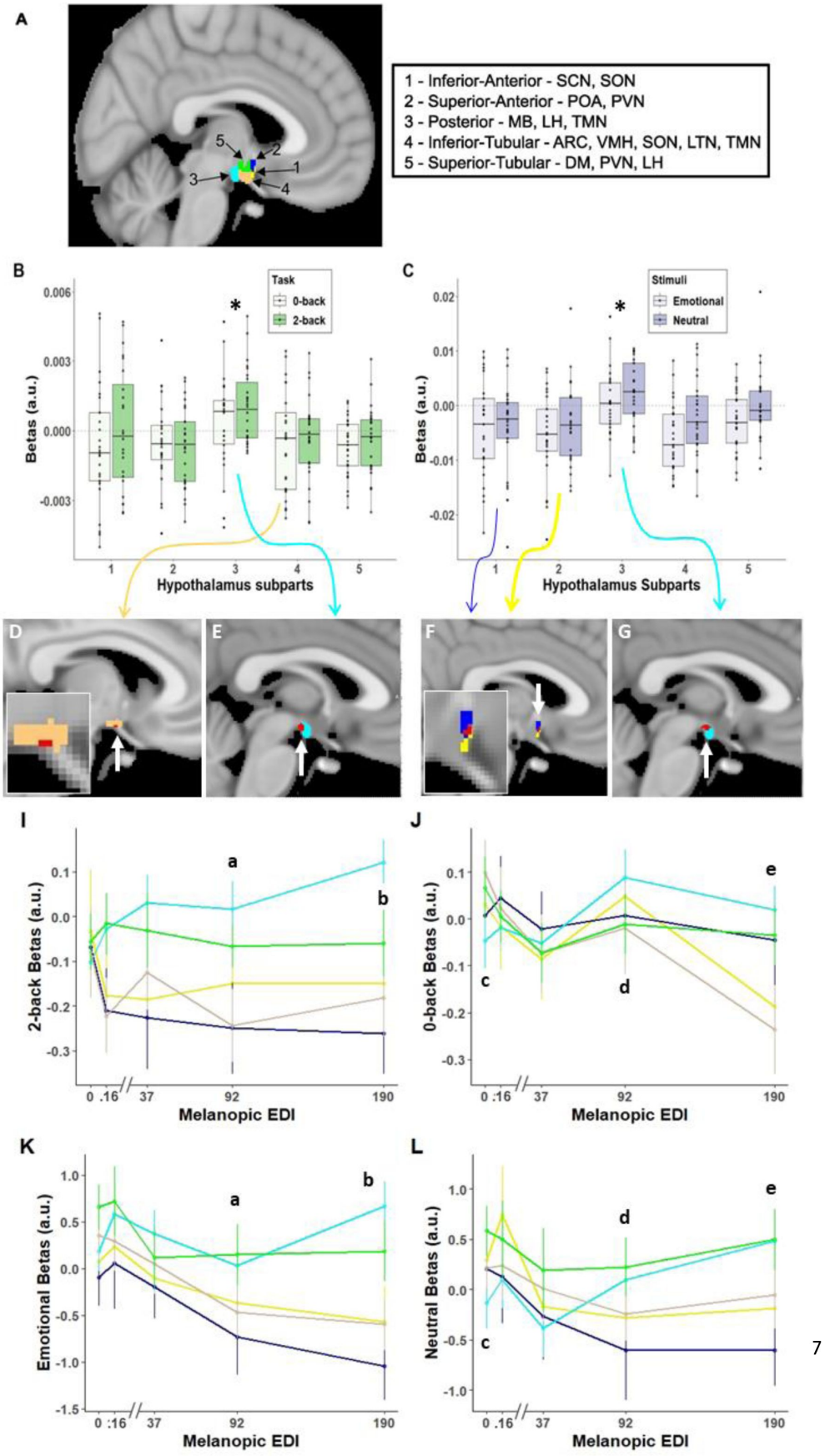


Figure 1.

Experimental protocol.

(A) Overall timeline. After prior light history standardisation, participants performed executive (always first), emotional and attentional tasks (pseudo-randomly 2nd or 3rd, blue arrow). As the attentional task included fewer light conditions, it is not considered in the present manuscript (see methods for more details)
(B) Spectral power distribution of light exposures. Monochromatic orange: 0.16 mel EDI lux; Polychromatic, blue-enriched light (6500K); LOW, MID, HIGH: 37, 92, 190 mel EDI lux. For the present analyses, we discarded colour differences between the light conditions and only considered illuminance as indexed by mel EDI lux, constituting a limitation of our study. See Suppl. Table S2 for full details
(C-D) Tasks procedures. Time is reported in seconds relative to session onset; participants were pseudo-randomly exposed to the 4 light conditions. **(C)** Executive task: alternation of letter detection blocks (0-back) and working memory blocks (2-back). **(D)** Emotional task: lure gender discrimination of vocalizations (50% angry (red), 50% neutral (white).


A
1 - Inferior-Anterior - SCN, SON
2 - Superior-Anterior - POA, PVN
3 - Posterior - MB, LH, TMN
4 - Inferior-Tubular - ARC, VMH, SON, LTN, TMN
5 - Superior-Tubular - DM, PVN, LH
B
Task
0-back
2-back
Betas (a.u.)
Hypothalamus subparts
C
Stimuli
Emotional
Neutral
Betas (a.u.)
Hypothalamus Subparts
D
E
F
G
I
2-back Betas (a.u.)
Melanopic EDI
J
0-back Betas (a.u.)
Melanopic EDI
K
Emotional Betas (a.u.)
Melanopic EDI
L
Neutral Betas (a.u.)
Melanopic EDI

7

Figure 2.

Illuminance impact on the hypothalamus subparts.

(A) Segmentation of the hypothalamus in five subparts in a representative participant. The nuclei encompassed by the different subparts are indicated in the right inset – according to [8]. ARC: arcuate nucleus; DMH; dorsomedial nucleus; LH lateral hypothalamus; LTN: lateral tubular nucleus; MB: mamillary body; POA: preoptic area; PVN: paraventricular nucleus; PNH: posterior nucleus of the hypothalamus; SCN: suprachiasmatic nucleus; SON: supraoptic nucleus; TMN: tuberomammillary nucleus; VMN: ventromedial nucleus

(B-C) Estimates (beta; arbitrary unit – a.u.) of the collective impact of illuminance variation on the activity of each hypothalamus subpart. (Refer to **Table 1** full statistics)

(B) Executive task: significant main effect of hypothalamus subparts (p=0.002), no significant main effect of task type (p=0.4) or subpart-by-task-type interaction (p=0.61).

(C) Emotional task: significant main effect of hypothalamus subparts (p<.0001), no significant main effect of stimulus type (p=0.053) or subpart-by-stimulus-type interaction (p=0.7).

(D-G) Whole brain analyses of the collective impact of the variations in illuminance over the hypothalamus area - for illustration.

A local positive peak (red; $p_{uncorrected}$<0.001) was detected over the posterior hypothalamus subpart (light blue) in executive **(E)** and emotional **(G)**.

A local negative peak (red; $p_{uncorrected}$<0.001) was detected over the inferior-tubular hypothalamus subparts (light orange) during the executive task **(D)**, while local negative peak (red; $p_{uncorrected}$<0.001) was detected over the inferior-anterior (yellow) and superior-anterior §blue) hypothalamus subparts during the emotional task **(F)** – insets correspond to enlargements over the hypothalamus area.

Arrows from panels B and C arise from and are colour coded according to the hypothalamus subpart that is displayed in panels D to G.

These results indicate that our finding does not arise from a nearby “leaking” activation/deactivation.

(I-L) Estimates of the impact of each illuminance on the activity of the hypothalamus subparts. (Refer for **Table 2** and **Suppl. Tables S3-S7** for full statistics)

Activity dynamics across illuminance for each subpart (colour code as in A). Results are displayed per task or stimulus type although no interactions with task or stimulus type were detected. Significant illuminance-by-hypothalamus-subpart interactions were detected for **(I-J)** the executive task (p=0.041) and **(K-L)** the emotional task (p=0.041).

Small letter indicate significant difference ($p < 0.05$) between the following subparts at illuminance: **a.** 92 mel EDI lux: posterior vs. superior-anterior; posterior vs. inferior-tubular; **b.** 190 mel EDI lux: posterior vs. superior-anterior; posterior vs. inferior-tubular; **c.** 0 mel EDI lux: posterior vs. superior-tubular; **d.** 92 mel EDI lux: posterior vs. superior-anterior; superior-anterior vs. inferior-tubular; **e.** 190 mel EDI lux: posterior vs. inferior-anterior; posterior vs. superior-anterior; posterior vs. inferior-tubular; superior-anterior vs. superior-tubular; inferior-tubular vs. superior-tubular.

Executive task							
Main GLMM				Pairwise comparisons			
Effect	F value	P value*	Partial R^2	Contrast#	t-value	$P_{uncorrected}$	$P_{corrected}$
Hypothalamus subparts	4.36	**0.002**	**0.08**	1 vs. 2	-0.30	0.76	0.99
				1 vs. 3	-3.48	**0.0006**	**0.0056**
				1 vs. 4	< 0.01	0.99	1
Task	0.74	0.4		1 vs. 5	-0.57	0.57	0.98
Hypothalamus subparts x task type	0.68	0.61		2 vs. 3	-3.17	**0.0017**	**0.015**
				2 vs. 4	0.31	0.76	0.99
				2 vs. 5	-0.27	0.79	0.99
Age	0.33	0.57		3 vs. 4	3.48	**0.0006**	**0.0055**
BMI	0.59	0.45		3 vs. 5	2.54	**0.0041**	**0.033**
Sex	0.01	0.91		4 vs. 5	-0.5	0.57	0.98
Emotional task							
Main GLMM				Pairwise comparisons			
Effect	F Value	P value*	Partial R^2	Contrast#	t-value	$P_{uncorrected}$	$P_{corrected}$
Hypothalamus subparts	9.38	**<.0001**	**0.22**	1 vs. 2	0.32	0.75	0.99
				1 vs. 3	-4.76	**< 0.0001**	**< 0.0001**
				1 vs. 4	-0.05	0.96	1
Task	4.13	0.053		1 vs. 5	-2.24	**0.025**	0.17
Hypothalamus subparts x stimulus type	0.55	0.7		2 vs. 3	-5.09	**< 0.0001**	**0.0001**
				2 vs. 4	-0.37	0.71	0.99
				2 vs. 5	-2.57	**0.011**	0.081
Age	0.18	0.67		3 vs. 4	4.71	**< 0.0001**	**< 0.0001**
BMI	0.05	0.82		3 vs. 5	2.52	**0.013**	0.091
Sex	1.54	0.23		4 vs. 5	-2.19	**0.03**	0.19

Outputs of generalized linear mixed model (GLMM) with subject as random factor (intercept and slope), and task and subpart as repeated measures (ar(1) autocorrelation).

* The corrected p-value for multiple comparisons over 2 tests is $p < 0.025$.

refer to Figure 2A for correspondence of subpart numbers

Table 1.

Differences between hypothalamus subparts in the collective impact of the variation in illuminance on their activity.

Executive task							
Main GLMM				Comparisons between subparts per illuminance #			
Effect	F-value	P value	Partial R^2	Illuminance*	contrast	t-value	p-value
Subpart	1.4	0.23		92	2 vs. 3	-2.25	**0.025**
Illuminance	2.15	0.073		92	3 vs. 4	2.58	**0.01**
				190	1 vs. 3	-2.80	**0.0053**
Task	3.24	0.073		190	2 vs. 3	-2.24	**0.025**
Subpart x Illuminance	1.7	**0.041**	**0.09**	190	3 vs. 4	3.15	**0.0017**
Age	1.19	0.29					
BMI	0.01	0.9					
Sex	0.38	0.54					
Emotional task							
Main GLMM				Comparisons between subparts per illuminance #			
Effect	F-value	P value	Partial R^2	Illuminance*	contrast	t-value	p-value
Subpart	4.29	**0.0023**	**0.07**	0	3 vs. 5	-2.05	**0.04**
				92	2 vs. 3	-2.53	**0.012**
Illuminance	9.41	**< 0.0001**	**0.035**	92	2 vs. 5	-2.96	**0.0032**
				190	1 vs. 3	-3.31	**0.001**
				190	2 vs. 3	-4.75	**< 0.0001**
Task	0.13	0.72		190	1 vs. 5	-2.5	**0.013**
				190	2 vs. 5	-4.04	**<0.0001**
				190	3 vs. 4	3.13	**0.0018**
Subpart x Illuminance	1.7	**0.041**	**0.026**	190	4 vs. 5	-2.32	**0.021**
Age	0.59	0.45					
BMI	1.54	0.23					
Sex	0.05	0.83					

* illuminance in mel EDI lux

only significant comparisons are reported in the main text. For the full table, including post hocs comparing light levels within a subpart, refer to Suppl. Table S3 to S6

Table 2.

Statistical outputs of GLMM testing for differences between the activity of each subpart of the hypothalamus under each illuminance

The impact of illuminance variations on the activity of the hypothalamus is not uniform

The main analyses aimed at isolating differences in the overall impact of illuminance changes among the 5 hypothalamus subparts. For each subpart, we extracted an index of the illuminance impact as their average regression coefficients between their responses to the tasks and the illuminance levels. These analyses showed significant differences between the hypothalamus subparts for the executive (generalized linear mixed models (GLMM); main effect of the subparts; $p = 0.002$) and emotional (GLMM; main effect of the subparts; $p < 0.0001$) tasks, revealing that, during both tasks, the variations in illuminance affected the activity of the 5 hypothalamus subparts differently [**Fig.2 B-C**, **Table 1**]. For both tasks, there was no significant main effect for any of the covariates and post hoc analyses showed that the index of the illuminance impact was consistently different in the posterior hypothalamus subpart compared to the other subparts [$p_{corrected} \leq 0.05$, except for the difference with superior tubular hypothalamus subpart during the emotional task: $p_{corrected} = 0.09$; **Table 1**]. Importantly, whole-brain analyses confirmed that increasing illuminance resulted in a local increase and decrease of activity that could be detected, respectively, over the posterior and inferior subparts of the hypothalamus [**Fig. 2 D-G**]. This shows that our results do not come from a relatively unspecific and widespread increase in BOLD signal surrounding the hypothalamus subparts and that the effect of light was most prominent over the posterior and inferior-anterior subparts.

Opposite dynamics between the posterior and inferior/anterior hypothalamus at higher illuminance

This prompted us to assess the activity of the hypothalamus subparts under each illuminance to detail the different regional activity dynamics across the hypothalamus. The statistical analyses confirmed that the activity dynamics across illuminance levels differed between the 5 subparts during the executive and the emotional tasks (GLMM; subparts-by-illuminance interaction; $p = 0.041$) tasks [**Fig. 2 I-L**; **Table 2**]. Post hoc contrasts first considered the impact of the changes in illuminance within each subpart (**Suppl. Tables S3-4**). The activity of the posterior hypothalamus subpart significantly ($p < 0.05$) increased under the highest illuminance (190 mel EDI) compared with darkness for both tasks and with the lower illuminances (37 and 92 mel EDI lux) for the emotional task. In contrast, for both tasks, the activity in the inferior-anterior and inferior-tubular hypothalamus subparts significantly ($p < 0.05$) decreased under the highest illuminance compared with darkness, and with lower illuminances for the emotional task. Finally, the activity of the superior anterior hypothalamus subpart decreased under higher illuminance during the emotional but not the executive task, while the activity of the fifth hypothalamus subpart, the superior tubular subpart, was not significantly affected by illuminance changes in either task.

Post hoc analyses also yielded several significant differences between hypothalamus subparts ($p < 0.05$) (**Table 2; Suppl. Tables S5-6**). For both tasks, the activity of the posterior hypothalamus subpart was consistently significantly higher than the activity inferior-tubular subpart under the highest illuminances (92 and 190 mel EDI lux). For the executive task, the activity of the posterior hypothalamus subpart was also significantly higher than the superior-anterior subpart under the highest illuminances (92 and 190 mel EDI lux). For the emotional task, the activity of the posterior hypothalamus subpart was also significantly higher than the superior-anterior subpart under the highest illuminances (190 mel EDI lux), while the activity superior-tubular hypothalamus subpart was significantly higher than the activity of the inferior-tubular, inferior-anterior and superior-anterior hypothalamus subparts (92 and/or190 mel EDI lux).

The overall picture arising from these comparisons is that higher illuminance increased the activity of the posterior and superior hypothalamus subparts while it decreased the activity of the inferior and anterior hypothalamus subparts.

Performance to the executive task is improved by light and related to the activity of the posterior hypothalamus

Following these analyses, we explored whether the changes in activity across illuminances were related to cognitive performance. We first considered the more difficult (2-back) subtask of the executive task as it requires higher cognitive functions (see method for a full rationale).[6] The analysis revealed that accuracy to the executive task was high in all participants, but accuracy to the more difficult subtask (2-back) improved with increasing illuminance (GLMM; main effect of illuminance; F = 2.72; **p = 0.034**; Partial R^2 = 0.1; **Fig. 3A**), controlling for age, sex and BMI. Critically, the analysis also showed that performance under each illuminance was significantly related to the activity of the posterior hypothalamus subpart (GLMM; main effect of posterior subpart activity; F = 9.43; **p = 0.0027**; Partial R^2 = 0.09). Surprisingly, the association was negative (**Fig. 3B**), suggesting that the part of variance explained by the hypothalamus subpart is distinct from the impact of light on performance. In contrast, no significant association was found when considering the activity of the other four subparts (GLMM; main effect of posterior subpart activity; F < 0.62; p > 0.4; **Fig. 3 C-D**, **Suppl. Table S7**). We went on and found that the accuracy to the simpler control subtask of the executive tasks (0-back, see method) was not associated with the activity of the posterior hypothalamus subpart (GLMM controlling for age, sex and BMI; main effect of subpart activity; F = 0.57; p = 0.45; **Fig. 3E**), suggesting that the association with performance is specific to the 2-back subtask.

In the last step, we explored the reaction times during the emotional task (accuracy to the lure task is not meaningful). We found that reaction times to the emotional stimuli were not significantly affected by illuminance (GLMM controlling for age, sex and BMI; main effect of illuminance; F = 1.01; p = 0.41; **Fig. 3F**) and yet, they were significantly associated with the activity of the posterior hypothalamus subpart across each illuminance (GLMM; main effect of subpart activity; F = 4.34; **p = 0.04**; **Fig. 3G**). The association was positive meaning that reaction times were longer if activity estimates were higher, which could indicate a reinforcement of the emotional response characterized by longer reactions.[7] No such significant association was detected when considering reaction times to the neutral items of the task (GLMM controlling for age, sex and BMI; main effect of illuminance; F = 1.5; p = 0.21; main effect of subpart activity; F = 0.28; p = 0.6; **Fig. 3H**).

Discussion

Animal research has established that the biological impact of light illuminance impinges on many subcortical structures, many of which regulate sleep and wakefulness.[1–4] How these findings translate to human beings is not established. Here, we took advantage of the relatively high resolution and signal-to-noise ratio of UHF 7T fMRI to determine how illuminance affects the activity of the hypothalamus as, based on animal research, it receives the densest projections from ipRGCs.[2,4] We find that the activity of the posterior part of the hypothalamus increases with increasing illuminance while, in contrast, the inferior and anterior hypothalamus show a seemingly opposite pattern and see their activity decrease under higher illuminance. Importantly, performance to the complex cognitive task was improved under higher illuminance and was correlated to the activity of the posterior part of the hypothalamus under the different illuminance, though negatively. The results demonstrate that the human hypothalamus does not respond uniformly to variations in illuminance while engaged in a cognitive challenge, and suggest that the posterior part of the hypothalamus may be key in mediating the stimulating impact of light on cognition.

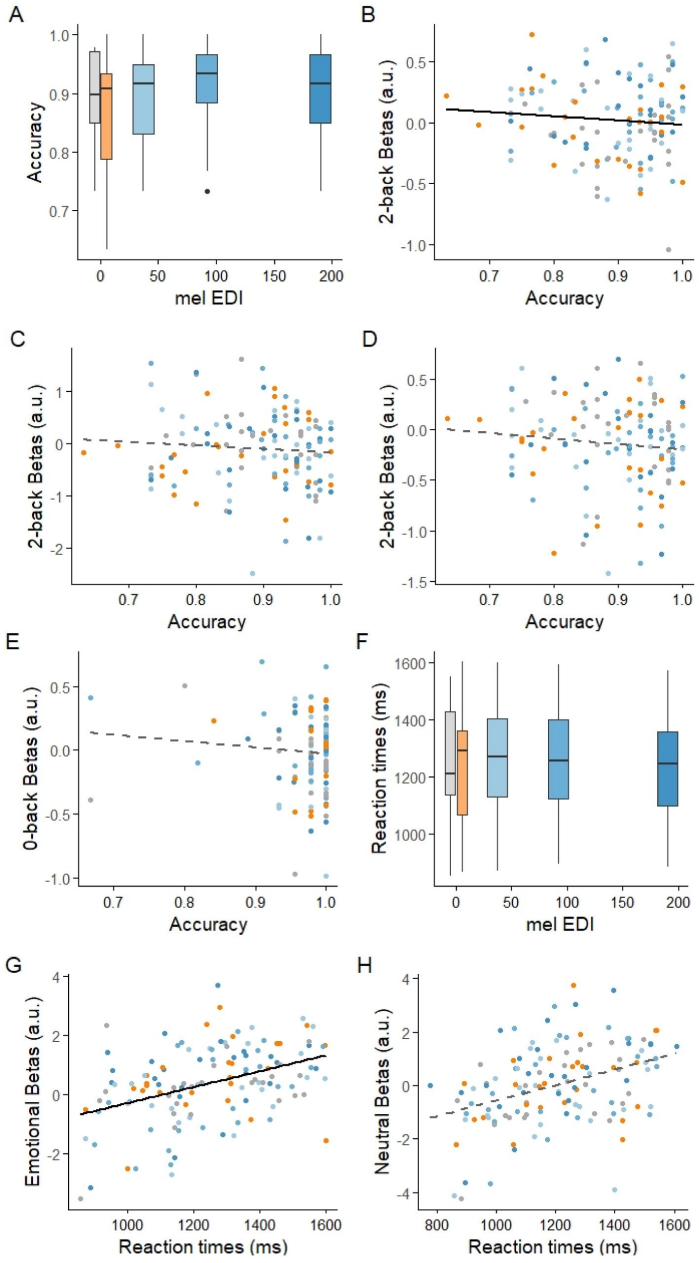


Figure 3.

Impact of illuminance on performance and relationships with the activity of the posterior hypothalamus subpart.

(A) Accuracy (percentage of correct responses) to the 2-back increased with increasing illuminance ($p = 0.034$).
(B) Accuracy to the 2-back task is negatively correlated to the activity of the posterior hypothalamus subpart ($p = 0.0027$).
(C-D) Accuracy to the 2-back task is not correlated to the activity of the inferior-anterior
(C) and inferior-tubular **(D)** hypothalamus subparts ($p > 0.4$). Association between superior-anterior and superior-tubular subparts are not displayed but were not significant ($p > 0.6$). See Suppl. Table S7 for full details.
(E) – Accuracy to the 0-back task is not correlated to the activity of the posterior hypothalamus subpart ($p = 0.45$).
(F) Reaction times to the emotional stimuli did not significantly change with increasing illuminance ($p = 0.41$).
(G) Reaction times to the emotional stimuli are correlated to the activity of the posterior hypothalamus subpart ($p = 0.04$) with higher activity associated to slower reaction times.
(H) Reaction times to the neutral stimuli are not correlated to the activity of the posterior hypothalamus subpart ($p = 0.6$). Solid and dashed lines correspond to the significant and not significant linear regression lines, respectively.

The different nuclei of the hypothalamus do not have clear contrast boundaries based on MRI signals.[8] As a result, achieving nucleus resolution over the human hypothalamus even using UFH MRI remains out of reach.[9] Therefore, we cannot assign the effects we report to a specific nucleus. We can only speculate and present a selection of plausible scenarios that would need to be tested. The posterior part of the hypothalamus - delineated in each participant based on an automatic reproducible procedure[8] - encompasses the MB as well as parts of the LH and the TMN. All these nuclei could participate to the increased BOLD signal we detect under higher illuminance. The LH and TMN, respectively, produce orexin and histamine, which are both known to promote wakefulness, while animal histology reports direct projection of the ipRGCs to the LH.[2–4] Orexin is a good candidate to constitute the circadian signal that promotes wakefulness and to counter the progressive increase in sleep needs with prolonged wakefulness.[10] Our data may therefore be compatible with an increase in orexin release by the LH with increasing illuminance. This would stimulate cognition and maintain or increase alertness[1] and may also be part of the mechanisms through which daytime light increases the amplitude in circadian variations of several physiological features.[11,12] It could then also be part of the mechanisms through which evening light may disturb subsequent sleep[13] when illuminance is higher than the recommended maximum of 10 mel EDI lux for evening light.[14] If the TMN was the hypothalamus nucleus underlying the regional increase in the BOLD signal we report, it could confer a role to histamine in mediating the stimulating impact of light. Of interest, the TMN receives orexin signal from the LH.[15] Alternatively, our findings may suggest a role for the MBs in mediating the impact of light on ongoing cognition, potentially influenced through its innervation by the TMN.[16]

Previous research indicated that increasing illuminance reduced the activity of the anterior part of the hypothalamus encompassing the SCN, either following the exposure to light[17] or during the exposure.[18] We extend this finding by showing that the significant decrease in activity extends beyond the inferior anterior hypothalamus and therefore much beyond the SCN. The inferior-tubular and inferior-anterior subparts of the hypothalamus – also isolated based on an automatic reproducible procedure[8] - encompass several nuclei such as notably the SCN, SON, ventromedial nucleus of the hypothalamus, arcuate nucleus and part of the TMN. Again, all these nuclei may be involved in the reduction in BOLD signal we observe at higher illuminance. In terms of chemical communication, these changes in activity could correspond to a modification of the γ-aminobutyric acid (GABA) internal or external signalling of the SCN which has been implicated in its response to light.[19] GABA produced by the SCN has also been reported to have excitatory properties such that the BOLD signal changes we report may correspond to a reduction in excitation.[19] Likewise, the SCN is also producing other neuropeptides that could affect its downstream targets. As the inferior-tubular subpart of the hypothalamus also includes part of the TMN it may be the TMN and its GABA production that is decreased by higher illuminance.[15] The decrease in BOLD signal with increasing illuminance we report could therefore arguably reflect a decreased inhibitory signal arising from the anterior and inferior nuclei of the hypothalamus.

Importantly, none of the scenarios we elaborated on are mutually exclusive and we stress overlooked the potential implication of several nuclei as well as the cellular diversity of the nuclei of the hypothalamus.[3,20] We further note that the anterior-superior hypothalamus subpart of the hypothalamus encompassing the VLPO and PON sees its activity decreasing under higher illuminance during the emotional task, similarly to the anterior-inferior and inferior-tubular areas. Likewise, similar to the posterior subpart, the activity of the superior-tubular hypothalamus subpart may be increased under higher illuminance during the emotional task. Whether this represents a task-specific effect arising, for instance, from differences in the salience of the auditory stimulus, remains to be determined.

A critical aspect of our results is that the performance to the 2-back (executive) subtask was significantly increased when exposed to higher illuminance light. The extent of this increase was limited, likely because performance was overall high at all illuminances, but was not detected for

the simpler detection letter subtask (0-back). The result contrasts with many previous 3T MRI investigations on the biological effects of light on human brain function which did not report behavioural changes induced by repeated short exposures to light (e.g. [21–23] but see [24,25]). Our 7T MRI study, which includes a sample size larger than many of these previous studies, supports that BOLD fMRI is sensitive in detecting subtle impacts of light on the brain and that these detected changes can arguably contribute to the behavioural changes others reported using longer light exposure and other approaches (e.g. [26,27] but see [28]).

Importantly, we find that activity of the posterior hypothalamus subpart is negatively related to the performance to the executive task, making it unlikely that it mediates directly the positive impact of light on performance. The activity of the posterior hypothalamus was, however, associated with an increased behavioural response to the emotional stimuli. The association between behaviour and the posterior hypothalamus is therefore likely to be complex and may depend on the context, with for instance different nuclei or neuronal populations contributing in some instances but not in others.[15,20] It is likely also to work jointly with the decreased activity of the anterior/inferior hypothalamus we detected as well as with other non-hypothalamus subcortical structures regulating wakefulness to influence behaviour, which intrinsically primarily depends on cortical activity. Future research should assess the impact of light on other subcortical structures and on entire subcortical network to determine how illuminance modifies their crosstalk as well as their interaction with the cortex, to eventually lead to behavioural impacts. These analyses could for instance address whether the regional changes in activity of the hypothalamus we find are upstream of the repeatedly reported impact of light illuminance on the activity of the pulvinar in the thalamus.[1,24] Although it does not receive direct dense input from ipRGCs, it is likely to indirectly mediate the biological impact of light on ongoing cognitive activity.[29]

These knowledge gaps are important to address because acting on light stands as a promising means to reduce high sleepiness and improve cognitive deficits during wakefulness as well as to facilitate sleep in the few hours preceding bedtime.[14,30–35] Light therapy is also a validated means to improve mood and treat mood disorders.[36,37] Light administration can also be considered a simple means to disturb the brain circuitry regulating sleep and wakefulness such that it can provide insights about novel means to improve their quality. For instance, if orexin and histamine were part of the mechanism through which natural light affects brain functions, their administration may be the most ecological and/or natural means to affect alertness and cognition. Likewise, as both orexin and histamine are targets for the treatment of brain disorders, our findings could suggest that light may constitute a non-pharmacological complementary intervention to compounds that are being developed to treat arousal, sleep, or cognitive dysfunction in brain disorders.[38] It remains, however, premature in our view to base recommendations on the therapeutic use of light based on the MRI findings gathered to date. Targeted lighting for interventions or for precise interference of subcortical circuits will require a full understanding of how light affects the brain, particularly at the subcortical level. Our findings represent an important step towards this goal, at the level of the hypothalamus.

Methods

The data used in this paper arise from a large study that is leading to several publications and part of the methods have been published previously.[29,39,40] The protocol was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège. Participants gave their written informed consent to take part in the study and received monetary compensation for their participation.

Participants

Thirty healthy young adults (19 women; 24.3 ± 2.9 y; **Suppl. Table S1**) were included in the analyses. Exclusion criteria were assessed through questionnaires and a semi-structured interview: history of psychiatric and neurological disorders, sleep disorders, use of psychoactive drugs or addiction; history of ophthalmic disorders or auditory impairments; colour blindness; night shift work during the last year or recent trans-meridian travel during the last 2 months; excessive caffeine (>4 caffeine units/day) or alcohol consumption (>14 alcohol units/week); medication affecting the central nervous system; smoking; pregnancy or breast-feeding (women); counter indication for MRI-scanning. All participants had to score < 18 on the 21-item Beck Anxiety Inventory (up to mild anxiety),[41] and on the Beck Depression Inventory-II (up to mild depression),[42] < 12 on the Epworth Sleepiness Scale,[43] and < 8 on the Pittsburgh Sleep Quality Index.[44] Questionnaires further assessed chronotype with the Horne-Östberg questionnaire[45] and seasonality with the Seasonal Pattern Assessment Questionnaire,[46] but the latter two questionnaires were not used for the inclusion of the participants.

For each task, 4 datasets were missing or corrupted data such that 26 participants were included in the analyses of each task (23 participants had valid datasets for both tasks). For the emotional task, two participants' data failed the MRI quality control (QC) check, and the other two participants were excluded as they did not complete the entire task. For the executive task, four of the participants' data failed the MRI QC check. **Supplementary Table S1** summarises participants' characteristics respective to each task. **Overall Protocol.** Participants completed an MRI session at least one week before the experiment during which structural images of the brain were acquired and which served as habituation to the experimental conditions. Participants then maintained a loose sleep-wake schedule (± 1h from the habitual sleep/wake-up time) during the 7 days preceding the fMRI experiment to warrant similar circadian entrainment across participants and avoid excessive sleep loss while maintaining realistic real-life conditions (verified using sleep diaries and wrist actigraphy - AX3 accelerometer, Axivity, United Kingdom). Volunteers were requested to refrain from all caffeine and alcohol-containing beverages, and extreme physical activity for 3 days before participating in the fMRI acquisitions. Data acquisitions took place in Liège, Belgium, between December 2020 and May 2023.

Participants arrived at the laboratory 1.5 to 2h after habitual wake time for the fMRI scan. They were first exposed for 5 min to a bright polychromatic white light (1000 lux) and then maintained in dim light (< 10 lux) for 45 min to standardise the participant's recent light history. During this period participants were given instructions about the fMRI cognitive tasks and completed practice tasks on a luminance-controlled laptop (< 10 lux). The fMRI session consisted of participants completing three auditory cognitive tasks while alternatively maintained in darkness or exposed to light: an executive task (25 min), an emotional task (20 min) and an attentional task (15 min) [**Fig. 1**]. The executive task was always completed first, as it was the most demanding task. The order of the following two tasks was counterbalanced. Because it included only 3 light conditions (see below) instead of 5 for the other two tasks, the attentional task was not included in the present analyses. An eye-tracking system (EyeLink 1000Plus, SR Research, Ottawa, Canada) was monitored for proper eye opening during all data acquisitions.

Light exposure

An 8-m long MRI-compatible optic fibre (1-inch diameter, Setra Systems, MA, USA) transmitted light from a light box (SugarCUBE, Ushio America, CA, USA) to the dual end of the fibre which was attached to a stand fitted at the back of the MRI coil that allowed reproducible fixation and orientation of the optic fibre ends. The dual branches illuminated the inner walls of the head coil to ensure relatively uniform and indirect illumination of participants' eyes. A filter wheel (Spectral Products, AB300, NM, USA) and optical fibre filters (monochromatic narrowband orange filter -

589mn; full width at half maximum: 10 nm - or a UV highpass filter - 433–1650nm) were used to create the light conditions needed for the experiment (see **Fig. 1B** and **Suppl. Table S2** for in-detail light characteristics).

For the executive and emotional task, the light conditions consisted of three different illuminance of a white, blue-enriched polychromatic LED light (37, 92, 190 mel EDI lux; 6500K) and one illuminance level of monochromatic orange light (.16 mel EDI lux; 590nm full width at half maximum - FWHM: 10 nm). For the present analyses, we discarded colour differences between the light conditions and only considered illuminance as indexed by mel EDI lux, constituting a limitation of our study. In the executive task, participants were exposed to 30s to 70s (median 30s) of light blocks separated by 10s of darkness (< 0.1 lux) and the light blocks were repeated 11 times for each light condition. For the emotional task, participants were exposed to 30 to 40s (median 35s) light blocks separated by 20s of darkness (< 0.1 lux) and the light blocks were repeated five times for each light condition.

The attentional task only included a single illuminance level of the blue-enriched polychromatic LED light (92 mel EDI lux) and one illuminance level of the monochromatic orange light (.16 mel EDI lux), otherwise the task would have been too long (> 30 min). Participants were exposed to 30s of light blocks separated by 10s of darkness (<0.1 lux). The light blocks were repeated 7 times for each light condition. As mentioned above it is not considered for the present analyses.

Auditory cognitive tasks

The tasks were programmed with Opensesame (3.2.8).[47] Participants heard the auditory stimuli through MR-compatible earbuds (Sensimetrics, Malden, MA). Before starting the tasks, to ensure optimal auditory perception of task stimuli, participants set the volume through a volume check procedure. Participants used an MRI-compatible keypad to respond to task items (Current Designs, Philadelphia, PA), which was placed in the participant's dominant hand. The tasks were separated by about 5 minutes in near darkness, to recalibrate the eye tracking system and to clarify instructions about the next task to the participant.

Executive Task

The task consisted of an auditory variant of the n-back task[6] with a working memory 2-back task and a control letter detection 0-back task. Participants were either asked to detect whether the current item was identical to the letter presented 2 items earlier (2-back) or whether the current item consisted of the letter "K" (0-back) or using the keypad (one button for "yes", one button for "no"). A block design was used for this task in which each block included 15 items and lasted 30s. Task blocks were separated by 10-20s rest periods and were preceded by an auditory instruction (500 ms) indicating the type of task to be completed. Task levels were pseudo-randomised across the 4 light conditions with 3 blocks of 0-back and 4 blocks of 2-back per light condition. ***Emotional Task.*** The task consisted of gender discrimination of auditory vocalizations that were either pronounced with emotional or neutral prosody.[7] Participants were asked to use the keypad to indicate what they believed the gender of the person pronouncing each token was. The gender classification was a lure task ensuring participants paid attention to the auditory stimulation. The purpose of the task was to trigger an emotional response as participants were not told that part of the stimuli was pronounced with angry prosodies. The 240 auditory stimuli were pronounced by professional actors (50% women) and consisted of three meaningless words ("*goster*", "*niuvenci*", "*figotleich*"). The stimuli were expressed in either an angry or neutral prosody, which has been validated by behavioural assessments[48]Click or tap here to enter text. and in previous experiments.[7,49,50] The stimuli were also matched for the duration (750 ms) and mean acoustic energy to avoid loudness effects. During each 30 to 40-s light block, four angry prosody stimuli and four neutral prosody stimuli were presented in a pseudorandom order and delivered every 3 to 5 seconds. A total of 160 distinct voice stimuli (50% angry; 50% neutral) were distributed

across the four light conditions. The darkness period separating each light block contained two angry and two neutral stimuli. A total of 80 distinct voice stimuli (50% angry; 50% neutral) were distributed across the darkness periods.

Data acquisition

The MRI data were acquired in a 7T MAGNETOM Terra MR scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receive and 1-channel transmit head coil (Nova Medical, Wilmington, MA, USA). Dielectric pads (Multiwave Imaging, Marseille, France) were placed between the subject's head and receiver coil to homogenize the magnetic field of Radio Frequency (RF) pulses.

Multislice T2*-weighted fMRI images were obtained with a multi-band Gradient-Recalled Echo - Echo-Planar Imaging (GRE-EPI) sequence using axial slice orientation (TR = 2340 ms, TE = 24 ms, FA = 90°, no interslice gap, in-plane FoV = 224 mm × 224 mm, matrix size = 160 × 160 × 86, voxel size = 1.4 × 1.4 × 1.4 mm^3). To avoid saturation effects, the first three scans were discarded. To correct for physiological noise in the fMRI data the participants' pulse and respiration movements were recorded using a pulse oximeter and a breathing belt (Siemens Healthineers, Erlangen, Germany). Following the fMRI acquisition a 2D GRE field mapping sequence to assess B0 magnetic field inhomogeneities with the following parameters: TR = 5.2 ms, TEs = 2.26 ms and 3.28 ms, FA = 15°, bandwidth = 737 Hz/pixel, matrix size = 96 × 128, 96 axial slices, voxel size = (2x2x2) mm^3, acquisition time = 1:38 min, was applied.

For the anatomical image, a high-resolution T1-weighted image was acquired using a Magnetization-Prepared with 2 RApid Gradient Echoes (MP2RAGE) sequence: TR = 4300 ms, TE = 1.98 ms, FA = 5°/6°, TI = 940ms/2830 ms, bandwidth = 240 Hz, matrix size = 256x256, 224 axial slices, acceleration factor = 3, voxel size = (0.75x0.75x0.75) mm^3.

Data processing

For the MP2RAGE images, the background noise was removed using an extension (extension: *https://github.com/benoitberanger/mp2rage*) of Statistical Parametric Mapping 12 (SPM12; *https://www.fil.ion.ucl.ac.uk/spm/software/spm12/*) under Matlab R2019 (MathWorks, Natick, Massachusetts).[51] Then the images were reoriented using the 'spm_auto_reorient' function (*https://github.com/CyclotronResearchCentre/spm_auto_reorient*) and corrected for intensity non-uniformity using the bias correction method implemented in the SPM12 "unified segmentation" tool.[52] To ensure optimal co-registration, brain extraction was done using SynthStrip[53] in Freesurfer (*http://surfer.nmr.mgh.harvard.edu/*). The brain-extracted T1-images were used to create a T1-weighted group template using Advanced Normalization Tools (ANTs, *http://stnava.github.io/ANTs/*) prior to normalization to the Montreal Neurological Institute (MNI) space using ANTs (1mm^3 voxel; MNI 152 template). The hypothalamus of each participant was segmented within 1mm^3 MNI 152 template into 5 subparts - inferior anterior, superior anterior, inferior tubular, superior tubular, posterior [cf. **Fig. 2a**] using an automatic computational approach.[8]

For the EPI images, auto reorientation was applied on the images first. Then, voxel-displacement maps were computed from the phase and magnitude images associated with B0 map acquisition (taken right after the task), using the SPM fieldmap toolbox. To correct for head motion and static and dynamic susceptibility-induced variance, the "Realign & Unwarp" of SPM12 was then applied to the EPI images. The realigned and distortion-corrected EPI images then underwent brain extraction using the SynthStrip and then the final images were smoothed with a Gaussian kernel characterized by a FWHM = 3mm. The first level analyses were performed in the native space to prevent any possible error that may be caused by co-registration.

Statistical analyses

The whole-brain univariate analyses consisted of a general linear model (GLM) computed with SPM12. For the executive task, task blocks and light blocks were modelled as block functions. For the emotional task, the auditory stimuli were modelled as stick functions. For both tasks, a high-pass filter with a 256 s cut-off was applied to remove low-frequency drifts. For both tasks, stick or block functions were convolved with the canonical hemodynamic response function. Movement and physiological parameters (cardiac, and respiration), which were computed with the PhysIO Toolbox (Translational Neuromodeling Unit, ETH Zurich, Switzerland), were included as covariates of no interest.[54]

Two separate analyses were completed. In the main analyses, we sought to test whether brain responses during the tasks were modulated by overall changes in illuminance level. The regressors of task blocks or events were accompanied by a single parametric modulation regressor corresponding to the light melanopic illuminance level (.16, 37, 92, 190 mel EDI). The contrasts of interest consisted of the main effects of the parametric modulation. In the subsequent post hoc analysis, we estimated the responses to the stimuli under each light condition. Separate regressors modelled each task's block or event type under each light condition (0, 0.16, 37, 92, 190 mel EDI). The contrasts of interest consisted of the main effects of each regressor.

The output masks of the segmentation procedure we used to extract regression betas associated with each of the hypothalamus subpart using the REX Toolbox (*https://web.mit.edu/swg/software.htm*),[55] Betas were averaged (mean) within each subpart and then across the homologous subparts of each hemisphere. In the main analyses this yielded 1 activity estimate per stimulus type and per hypothalamus subpart (i.e. 10 per individual), while in the subsequence analyses, we obtained 5 activity estimates per stimulus type and per subpart (50 per individual).

For visualization of whole-brain results over the entire sample, all statistical maps obtained from the first level analysis were first transferred to the group template space and then the MNI space ($1x1x1mm^3$ image resolution). All the registration steps were performed with ANTs. The visualisation was focussed on the hypothalamus regions to assess whether increasing illuminance resulted in local increase and decrease of beta estimates within the hypothalamus or whether beta estimates were mainly influenced by a relatively unspecific and widespread increase in BOLD signal surrounding the hypothalamus.

Statistical analyses of the activity of the hypothalamus subparts were performed in SAS 9.4 (SAS Institute, NC, USA). Analyses consisted of Generalised Linear Mixed Models (GLMM) with the subject as a random factor (intercept and slope) and were adjusted for the dependent variable distribution. As the main statistical analysis was completed for each task, the significance threshold was corrected for multiple comparisons and was set at $p < 0.025$. Direct post hoc of the main analyses were corrected for multiple comparisons using a Tukey adjustment. The subsequent more detailed analyses were considered as post hoc that were not corrected for multiple comparisons ($p < 0.05$). To detect outlier values within the data sets, Cook's distance > 1 was used for exclusion. No outliers were detected for activity estimates of both tasks, while four outlier values were removed from the analyses of the 2-back and 0-back performance.

The main analyses included the activity estimates modulated by light illuminance as a dependent variable and the hypothalamus subpart and stimulus type (2-back/0-back – neutral/emotional) as repeated measures (autoregressive (1) correlation), together with age, sex and BMI as covariates. The second set of post hoc GLMM analyses included the activity estimates of the hypothalamus subparts as the dependent variable and hypothalamus subpart, stimulus type and illuminance (0, 0.16, 37, 92, 190 mel EDI lux) as the repeated measures (autoregressive (1) correlation), together with age, sex, and BMI as covariates and interaction term between illuminance and hypothalamus subpart. The final set of analyses included performance metrics as dependent variables (accuracy

to the 2-back or 0-back task - as percentage of correct responses; reaction time – ms - to emotional or neutral stimuli during the emotional task) and included the same repeated measures and covariates ads in the preceding set as well as activity of the relevant hypothalamus subpart.

Optimal sensitivity and power analyses in GLMMs remain under investigation (e.g. [56]). We nevertheless computed a prior sensitivity analysis to get an indication of the minimum detectable effect size in our main analyses given our sample size. According to G*Power 3 (version 3.1.9.4),[57] taking into account a power of 0.8, an error rate α of 0.025 (correcting for 2 tasks), and a sample of 26 allowed us to detect large effect sizes $r > 0.54$ (two-sided; absolute values; CI: 0.19–0.77; $R^2 > 0.29$, R^2 CI: 0.04–0.59) within a multiple linear regression framework including one tested predictor (illuminance effect) and three covariates (age, sex and BMI).

Data and code availability statement

The processed data and analysis scripts supporting the results included in this manuscript are publicly available via the following open repository: *https://gitlab.uliege.be/CyclotronResearchCentre/Public/xxxx* (the repository will be created following acceptance / prior to publication of the paper).

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Author Contributions

I.C., R. S. and G.V. designed the research. I.C., R.S., J.F.B.A., E.B. and I.P. acquired the data. A.B., E.K., N.M., J.R., M.Z., P.T., F.C., S.S., C.P., and L.L. provided valuable insights while acquiring, interpreting, and discussing the data. I.C. and R.S. analysed the data supervised by G.V. I.C. and G.V. wrote the paper. All authors edited and approved the final version of the manuscript.

Competing Interest Statement

The authors declare no conflict of interest.

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Reviewer #1 (Public Review):

Summary:

Campbell et al investigated the effects of light on the human brain, in particular the subcortical part of the hypothalamus during auditory cognitive tasks. The mechanisms and neuronal circuits underlying light effects in non-image forming responses are so far mostly studied in rodents but are not easily translated in humans. Therefore, this is a fundamental study aiming to establish the impact light illuminance has on the subcortical structures using the high-resolution 7T fMRI. The authors found that parts of the hypothalamus are differently responding to illuminance. In particular, they found that the activity of the posterior hypothalamus increases while the activity of the anterior and ventral parts of the hypothalamus decreases under high illuminance. The authors also report that the performance of the 2-back executive task was significantly better in higher illuminance conditions. However, it seems that the activity of the posterior hypothalamus subpart is negatively related to the performance of the executive task, implying that it is unlikely that this part of the hypothalamus is directly involved in the positive impact of light on performance observed. Interestingly, the activity of the posterior hypothalamus was, however, associated with an increased behavioural response to emotional stimuli. This suggests that the role of this posterior part of the hypothalamus is not as simple regarding light effects on cognitive and emotional responses. This study is a fundamental step towards our better understanding of the mechanisms underlying light effects on cognition and consequently optimising lighting standards.

Strengths:

While it is still impossible to distinguish individual hypothalamic nuclei, even with the high-resolution fMRI, the authors split the hypothalamus into five areas encompassing five groups of hypothalamic nuclei. This allowed them to reveal that different parts of the hypothalamus respond differently to an increase in illuminance. They found that higher illuminance increased the activity of the posterior part of the hypothalamus encompassing the MB and parts of the LH and TMN, while decreasing the activity of the anterior parts encompassing the SCN and another part of TMN. These findings are somewhat in line with studies in animals. It was shown that parts of the hypothalamus such as SCN, LH, and PVN receive direct retinal input in particular from ipRGCs. Also, acute chemogenetic activation of ipRGCs was shown to induce activation of LH and also increased arousal in mice.

Weaknesses:

While the light characteristics are well documented and EDI calculated for all of the photoreceptors, it is not very clear why these irradiances and spectra were chosen. It would be helpful if the authors explained the logic behind the four chosen light conditions tested. Also, the lights chosen have cone-opic EDI values in a high correlation with the melanopic EDI, therefore we can't distinguish if the effects seen here are driven by melanopsin and/or other photoreceptors. In order to provide a more mechanistic insight into the light-driven

effects on cognition ideally one would use a silent substitution approach to distinguish between different photoreceptors. This may be something to consider when designing the follow-up studies.

https://doi.org/10.7554/eLife.96576.1.sa2

Reviewer #2 (Public Review):

Summary:

The interplay between environmental factors and cognitive performance has been a focal point of neuroscientific research, with illuminance emerging as a significant variable of interest. The hypothalamus, a brain region integral to regulating circadian rhythms, sleep, and alertness, has been posited to mediate the effects of light exposure on cognitive functions. Previous studies have illuminated the role of the hypothalamus in orchestrating bodily responses to light, implicating specific neural pathways such as the orexin and histamine systems, which are crucial for maintaining wakefulness and processing environmental cues. Despite advancements in our understanding, the specific mechanisms through which varying levels of light exposure influence hypothalamic activity and, in turn, cognitive performance, remain inadequately explored. This gap in knowledge underscores the need for high-resolution investigations that can dissect the nuanced impacts of illuminance on different hypothalamic regions. Utilizing state-of-the-art 7 Tesla functional magnetic resonance imaging (fMRI), the present study aims to elucidate the differential effects of light on the hypothalamic dynamics and establish a link between regional hypothalamic activity and cognitive outcomes in healthy young adults. By shedding light on these complex interactions, this research endeavors to contribute to the foundational knowledge necessary for developing innovative therapeutic strategies aimed at enhancing cognitive function through environmental modulation.

Strengths:

(1) Considerable Sample Size and Detailed Analysis:
The study leverages a robust sample size and conducts a thorough analysis of hypothalamic dynamics, which enhances the reliability and depth of the findings.

(2) Use of High-Resolution Imaging:
Utilizing 7 Tesla fMRI to analyze brain activity during cognitive tasks offers high-resolution insights into the differential effects of illuminance on hypothalamic activity, showcasing the methodological rigor of the study.

(3) Novel Insights into Illuminance Effects:
The manuscript reveals new understandings of how different regions of the hypothalamus respond to varying illuminance levels, contributing valuable knowledge to the field.

(4) Exploration of Potential Therapeutic Applications:
Discussing the potential therapeutic applications of light modulation based on the findings suggests practical implications and future research directions.

Weaknesses:

(1) Foundation for Claims about Orexin and Histamine Systems:
The manuscript needs to provide a clearer theoretical or empirical foundation for claims regarding the impact of light on the orexin and histamine systems in the abstract.

(2) Inclusion of Cortical Correlates:
While focused on the hypothalamus, the manuscript may benefit from discussing the role of

cortical activation in cognitive performance, suggesting an opportunity to expand the scope of the manuscript.

(3) Details of Light Exposure Control:
More detailed information about how light exposure was controlled and standardized is needed to ensure the replicability and validity of the experimental conditions.

(4) Rationale Behind Different Exposure Protocols:
To clarify methodological choices, the manuscript should include more in-depth reasoning behind using different protocols of light exposure for executive and emotional tasks.

https://doi.org/10.7554/eLife.96576.1.sa1

Reviewer #3 (Public Review):

Summary:

Campbell and colleagues use a combination of high-resolution fMRI, cognitive tasks, and different intensities of light illumination to test the hypothesis that the intensity of illumination differentially impacts hypothalamic substructures that, in turn, promote alterations in arousal that affect cognitive and affective performance. The authors find evidence in support of a posterior-to-anterior gradient of increased blood flow in the hypothalamus during task performance that they later relate to performance on two different tasks. The results provide an enticing link between light levels, hypothalamic activity, and cognitive/affective function, however, clarification of some methodological choices will help to improve confidence in the findings.

Strengths:

* The authors' focus on the hypothalamus and its relationship to light intensity is an important and understudied question in neuroscience.

Weaknesses:

* I found it challenging to relate the authors' hypotheses, which I found to be quite compelling, to the apparatus used to test the hypotheses - namely, the use of orange light vs. different light intensities; and the specific choice of the executive and emotional tasks, which differed in key features (e.g., block-related vs. event-related designs) that were orthogonal to the psychological constructs being challenged in each task.

* Given the small size of the hypothalamus and the irregular size of the hypothalamic parcels, I wondered whether a more data-driven examination of the hypothalamic time series would have provided a more parsimonious test of their hypothesis.

https://doi.org/10.7554/eLife.96576.1.sa0

Author response:

> ***Reviewer #1 (Public Review):***
>
> *Summary:*
>
> *[...] This study is a fundamental step towards our better understanding of the mechanisms underlying light effects on cognition and consequently optimising lighting standards.*

> *Strengths:*
>
> *While it is still impossible to distinguish individual hypothalamic nuclei, even with the high-resolution fMRI, the authors split the hypothalamus into five areas encompassing five groups of hypothalamic nuclei. This allowed them to reveal that different parts of the hypothalamus respond differently to an increase in illuminance. They found that higher illuminance increased the activity of the posterior part of the hypothalamus encompassing the MB and parts of the LH and TMN, while decreasing the activity of the anterior parts encompassing the SCN and another part of TMN. These findings are somewhat in line with studies in animals. It was shown that parts of the hypothalamus such as SCN, LH, and PVN receive direct retinal input in particular from ipRGCs. Also, acute chemogenetic activation of ipRGCs was shown to induce activation of LH and also increased arousal in mice.*
>
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>
> *While the light characteristics are well documented and EDI calculated for all of the photoreceptors, it is not very clear why these irradiances and spectra were chosen. It would be helpful if the authors explained the logic behind the four chosen light conditions tested. Also, the lights chosen have cone-opic EDI values in a high correlation with the melanopic EDI, therefore we can't distinguish if the effects seen here are driven by melanopsin and/or other photoreceptors. In order to provide a more mechanistic insight into the light-driven effects on cognition ideally one would use a silent substitution approach to distinguish between different photoreceptors. This may be something to consider when designing the follow-up studies.*

We thank the reviewer for acknowledging the quality and interest of our work and agree with the weaknesses they pointed out.

Blue-enriched light illuminances were set according to the technical characteristics of the light source and to keep the overall photon flux similar to prior 3T MRI studies of our team (between ~1012 and 1014 ph/cm²/s) (Vandewalle et al. 2010 PNAS, Vandewalle et al. 2011 Biol. Psy.). The orange light was introduced as a control visual stimulation for potential secondary whole-brain analyses. It's photopic illuminance should ideally have been set similar to the low illuminance blue-enriched light condition, but it was not the case. For the present region of interest analyses, we discarded colour differences between the light conditions and only considered illuminance as indexed by mel EDI lux. This constitutes indeed a limitation of our study as it does not allow attributing the findings to a particular photoreceptor class.

The revised version of the manuscript will include a better explanation as to the choice of illuminances and spectra. The discussion will make clear that these choices limit the interpretation about the photoreceptors involved. The discussion will also point out that silent substitution could be used in the future to resolve such question.

> ***Reviewer #2 (Public Review):***
>
> *[...] By shedding light on these complex interactions, this research endeavors to contribute to the foundational knowledge necessary for developing innovative therapeutic strategies aimed at enhancing cognitive function through environmental modulation.*
>
> *Strengths:*
>
> *(1) Considerable Sample Size and Detailed Analysis: The study leverages a robust sample size and conducts a thorough analysis of hypothalamic dynamics, which enhances the reliability and depth of the findings.*

> *(2) Use of High-Resolution Imaging: Utilizing 7 Tesla fMRI to analyze brain activity during cognitive tasks offers high-resolution insights into the differential effects of illuminance on hypothalamic activity, showcasing the methodological rigor of the study.*
>
> *(3) Novel Insights into Illuminance Effects: The manuscript reveals new understandings of how different regions of the hypothalamus respond to varying illuminance levels, contributing valuable knowledge to the field.*
>
> *(4) Exploration of Potential Therapeutic Applications: Discussing the potential therapeutic applications of light modulation based on the findings suggests practical implications and future research directions.*
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> *(1) Foundation for Claims about Orexin and Histamine Systems: The manuscript needs to provide a clearer theoretical or empirical foundation for claims regarding the impact of light on the orexin and histamine systems in the abstract.*
>
> *(2) Inclusion of Cortical Correlates: While focused on the hypothalamus, the manuscript may benefit from discussing the role of cortical activation in cognitive performance, suggesting an opportunity to expand the scope of the manuscript.*
>
> *(3) Details of Light Exposure Control: More detailed information about how light exposure was controlled and standardized is needed to ensure the replicability and validity of the experimental conditions.*
>
> *(4) Rationale Behind Different Exposure Protocols: To clarify methodological choices, the manuscript should include more in-depth reasoning behind using different protocols of light exposure for executive and emotional tasks.*

We thank the reviewer for recognising the interest and strength of our study. We agree that corrections and clarifications to the text were needed. We will address the weaknesses they pointed out as follows:

(1) As detailed in the discussion, we do believe orexin and histamine are excellent candidates for mediating the results we report. As also pointing out, however, we are in no position to know which neurons, nuclei, neurotransmitter and neuromodulator underlie the results. We will therefore remove the last sentence of the abstract as we agree our final statement in the abstract was too strong. We will carefully reconsider the discussion to avoid such overstatements.

(2) We are unsure at this stage how to address the comment of the reviewer without considerably lengthening the manuscript with statements which can only be putative. Hypothalamus nuclei are connected to multiple cortical (and subcortical) structures. The relevance of these projections will vary with the cognitive task considered. In addition, we have not yet considered the cortex in our analyses such that truly integrating cortical structures appears premature. We will nevertheless refer to the general statement that subcortical structures (and particularly those receiving direct retinal projections) are likely to receive light illuminance signal first before passing on the light modulation to the cortical regions involved in the ongoing cognitive process.

(3) Illuminance and spectra could not be directly measured within the MRI scanner due to the ferromagnetic nature of measurement systems. The MR coil and the associated optic fibre stand, together with the entire lighting system were therefore placed outside of the MR room to reproduce the experimental conditions of the in a completely dark room. A sensor was placed 2 cm away from the mirror of the coil (mounted at eye level), i.e. where the eye of the

first author of the paper would be positioned, to measure illuminance and spectra. The procedure was repeated 4 times for illuminance and twice for spectra and measurements were averaged. This procedure does not take into account inter-individual variation in head size and orbit shape such that the reported illuminance levels may have varied slightly across subjects. The relative differences between illuminance are very unlikely to vary substantially across participants such that statistics consisting of tests for the impact of relative differences in illuminance were not affected. We will report these methodological details in the supplementary material file associated to the paper.

(4) The comment is similar to the issue raised by reviewer 1 (and reviewer 3) so we refer to the response provided to reviewer 1 to address the final comment of reviewer 2.

> ***Reviewer #3 (Public Review):***
>
> *[...] The authors find evidence in support of a posterior-to-anterior gradient of increased blood flow in the hypothalamus during task performance that they later relate to performance on two different tasks. The results provide an enticing link between light levels, hypothalamic activity, and cognitive/affective function, however, clarification of some methodological choices will help to improve confidence in the findings.*
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> *Strengths:*
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> *The authors' focus on the hypothalamus and its relationship to light intensity is an important and understudied question in neuroscience.*

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> *I found it challenging to relate the authors' hypotheses, which I found to be quite compelling, to the apparatus used to test the hypotheses - namely, the use of orange light vs. different light intensities; and the specific choice of the executive and emotional tasks, which differed in key features (e.g., block-related vs. event-related designs) that were orthogonal to the psychological constructs being challenged in each task.*
>
> *Given the small size of the hypothalamus and the irregular size of the hypothalamic parcels, I wondered whether a more data-driven examination of the hypothalamic time series would have provided a more parsimonious test of their hypothesis.*

We thank the reviewer for acknowledging the originality and interest of our study. We agree that some methodological choices needed more explanations. We will address the weaknesses they pointed out as follows:

The first comment questions the choices of the light conditions and of the tasks. Regarding light conditions, since reviewer 1 (and reviewer 2) raised a similar issue, we refer to the response provided to reviewer 1. We agree that many different tasks could have been used to test our hypotheses. Prior work of our team showed that the n-back task and emotional task we used were successful probes to demonstrate that light illuminance modulates cognitive activity, including within subcortical structures (though resolution did not allow precise isolation of nuclei or subparts). When taking the step of ultra-high field imaging we therefore opted for these tasks as our goal was to show that illuminance affects subcortical brain activity across cognitive domains in general and we were not interested in tasks that would test specific aspects of these domains. The fact that one task is event-related while the other consists of a block design adds, in our view, to the robustness of our finding that a similar anterior-posterior gradient of activity modulation by illuminance is present in hypothalamus. We will update the discussion to highlight this aspect.

As mentioned in the text, the protocol also included an auditory attentional task that could have further broadened the potential generalisability of our findings, but it was not part of the analyses as it could only include 2 illuminance levels due to time constrains.

We agree that a data driven approach could have constituted an alternative means to tests our hypothesis. We opted for an approach that we mastered best while still allowing to conclusively test for regional differences in activity across the hypothalamus. Examination of time series of the very same data we used will mainly confirm the results of our analyses – an anterior-posterior gradient in the impact of illuminance - and may yield slight differences in the limits of the subparts of the hypothalamus undergoing decreased or increased activity with increasing illuminance. While the suggested approach may have been envisaged if we had been facing negative results (i.e. no differences between subparts, potentially because subparts would not correspond functional differences in response to illuminance change), it would now constitute a circular confirmation of our main findings (i.e. using the same data). While we truly appreciate the suggestion, we do not consider that it would constitute a more parsimonious test of our hypothesis now that we successfully applied GLM/parcellation and GLMM approaches.