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Mood, season and light within the amygdala

Gilles Vandewalle

gilles.vandewalle@uliege.be

University of Liège https://orcid.org/0000-0003-2483-2752

Islay Campbell

Oxford

Jose Balda

University of Liège

Roya Sharifpour

Université Libre de Bruxelles

Ilenia Paparella

University of Liège

Elise Beckers

University of Liège https://orcid.org/0000-0002-5523-1741

Nasrin Mortazavi

University of Liège

John Read

University of Liège https://orcid.org/0009-0009-3269-4362

Christophe Phillips

University of Liège https://orcid.org/0000-0002-4990-425X

Fabienne Collette

University of Liège https://orcid.org/0000-0001-9288-9756

Puneet Talwar

University of Liège

Laurent Lamalle

University of Liège https://orcid.org/0000-0002-8930-7337

Mikhail Zubkov

University of Liège

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Mood, season and light within the amygdala

Islay Campbell[1*#], Fermin Balda Aizpurua[1*], Roya Sharifpour[1&], Ilenia Paparella[1], Elise Beckers[1], Nasrin Mortazavi[1], John Read[1], Christophe Phillips[1], Fabienne Collette[1], Puneet Talwar[1], Laurent Lamalle[1], Mikhail Zubkov[1], Gilles Vandewalle[1**]

Short Title: Mood, season, light and the amygdala

[1]GIGA-CRC-Human Imaging, University of Liège, Liège, 4000 Belgium

[#]Current address: Sir Jules Thorn Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK

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

* shared first authorship

****Corresponding author**: Gilles Vandewalle, GIGA-Cyclotron Research Centre-In Vivo Imaging, Bâtiment B30, 8 Allée du Six Août, University of Liège-Sart Tilman, 4000 Liège, Belgium.
Email: gilles.vandewalle@uliege.be

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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 of the paper / upon request of the editor).

Abstract

The brain mechanisms through which changes in seasonality and light exposure modulate affective state may involve different nuclei of the amygdala. We aimed to test this hypothesis using 7 Tesla functional magnetic resonance imaging in 29 healthy young adults. We first considered the seasonal changes in activity, that are related to the slow change in photoperiod. We find that the activity of medial and superior nuclei of the amygdala is related to season and affective state. We further assessed how alternating short exposures to light of different illuminance acutely affected the regional activity of the amygdala. We show that the same medial and superior nuclei showed a linear reduction of activity when exposed to increasing light illuminance, specifically when processing emotional stimulations. Importantly also, the impact of light on part of these nuclei varied with season and affective state. These findings provide new evidence that humans show seasonality and that for affective states it involved parts of the amygdala. The results bring novel insights into the mechanisms that underlie the long-term and acute impact of light on the affective system and that may contribute to the benefits of light therapy in the treatment of mood disorders.

Introduction

Affective state and the symptoms of many psychiatric disorders show seasonal variations.[1] These variations are driven in part by the reduced shorter photoperiod taking place during the fall and winter seasons. Light therapy is established as a non-pharmacological treatment for seasonal affective disorder (SAD), including its subclinical forms and the relatively common winter blues.[2,3] In addition, it is also considered an efficient adjunct therapy for other non-seasonal psychiatric disorders.[2–4] Beyond light's long-term seasonal effects, light can also trigger acute (or immediate) responses that can modulate emotional processing.[5] The daily repetition of these acute responses is plausibly contributing to the long-term impact of light on affective states.

The mechanism of light's influence on affective state and emotional processing is most likely mediated through the light-sensitive pathway of the retina that detects environmental irradiance to regulate multiple biological functions not directly related to image formation,[6–9] often referred to as the non-image forming (NIF) pathway.[10] Intrinsically photosensitive retinal ganglion cells (ipRGCs) are the main photoreceptors of the NIF system and express the photopigment melanopsin, which is maximally sensitive to blue-wavelength light (~480nm).[11] IpRGCs combine their intrinsic photosensitivity with inputs from rods and cones[12] to influence multiple brain targets with a maximal efficiency shifted towards blue-wavelength light.[13]

The amygdala coordinates emotional signals.[11,14] It is a complex structure that is divided into several nuclei – likely 13 nuclei in humans, though their exact classification is still debated.[15] Rodent data has shown that the amygdala receives direct inputs from ipRGCs and may play a key role in mediating light exposure behaviour.[11] In rodents, ipRGCs project to the central nucleus of the amygdala and this pathway was found to mediate anxiety-related behaviour after acute light exposure.[16,17] Lesions of the medial amygdala, to which ipRCGs

also project, induced altered light-enhanced startle and open-field behaviour, suggesting that the effects of light on anxiety may also be mediated by the ipRGCs innervation of the medial amygdala.[18] In addition, increased blue wavelength light at night was further reported to increase the activity of the basolateral amygdala in rodents.[19] Short photoperiod in rodent models was further reported to modify fear conditioning behaviour while increasing the dendritic spine density of the neurons of the basolateral amygdala.[20] The translation of these acute effects of light in rodents to humans is not fully established.

In vivo, functional Magnetic Resonance Imaging (fMRI) in humans suggested that blue-wavelength light enhanced the responses to emotional stimuli in the amygdala as well as its crosstalk with the hypothalamus.[5] In contrast, another resting-state fMRI study found that exposure to a warm polychromatic white light suppressed amygdala activity and connectivity between the amygdala and the ventromedial prefrontal cortex during light exposure.[21] The discrepancies may arise from differences in the light sources or from being engaged in a cognitive task vs. at rest. They may also be due to the context-dependent response of the different nuclei of the amygdala that could not be addressed given the data resolution. Furthermore, prolonged light therapy in humans increased amygdala connectivity to the prefrontal cortex.[22] The amygdala volume was further related to depressed mood and season changes across all the subparts.[23]

Overall animal models and human research suggest the amygdala is an excellent candidate to mediate part of the impact of light exposure, photoperiod and seasonality have on the affective state. Given the known involvement of the amygdala nuclei in psychiatric disorders,[24–26] a precise understanding of the mechanisms at play, may have implications beyond the healthy individuals and could help to improve the management of seasonal

symptoms in psychiatric disorders, the use of light therapy and/or extend it to more brain disorders.

Here, we aimed to determine whether the regional activity of the amygdala varied with seasons and whether light exposure affected the activity of its different nuclei. We took advantage of the higher resolution of ultra-high-field (UHF) 7 Tesla (7T) fMRI to record the activity of the amygdala in healthy young adults exposed to the light of various illuminances while engaged in an auditory emotional task. We find that the regional activity of the same four subparts of the amygdala shows an association with season or affective state and with the acute impact of illuminance specifically when processing emotional stimulations. In addition, the impact of light on part of these nuclei varied with season and affective state. These findings provide new insights into the mechanisms that may underlie the impact of photoperiod and light exposure on affective state and psychiatric symptoms.

Results

The brain activity of 29 healthy young participants (24y ±3.1; 18 women) was recorded during an fMRI protocol conducted in Liège (Belgium) in the morning, and including three different cognitive tasks, one of which is an emotional auditory task and the sole focus of this analysis (**Fig. 1, Suppl. Table S1**). During the task, participants were alternatively maintained in darkness or exposed to different light consisting of a blue-enriched polychromatic white light of three different illuminance levels (37, 92, 190 melanopic Equivalent Daylight Illuminance – mel EDI - lux) and a low illuminance monochromatic orange light (0.16 mel EDI lux).

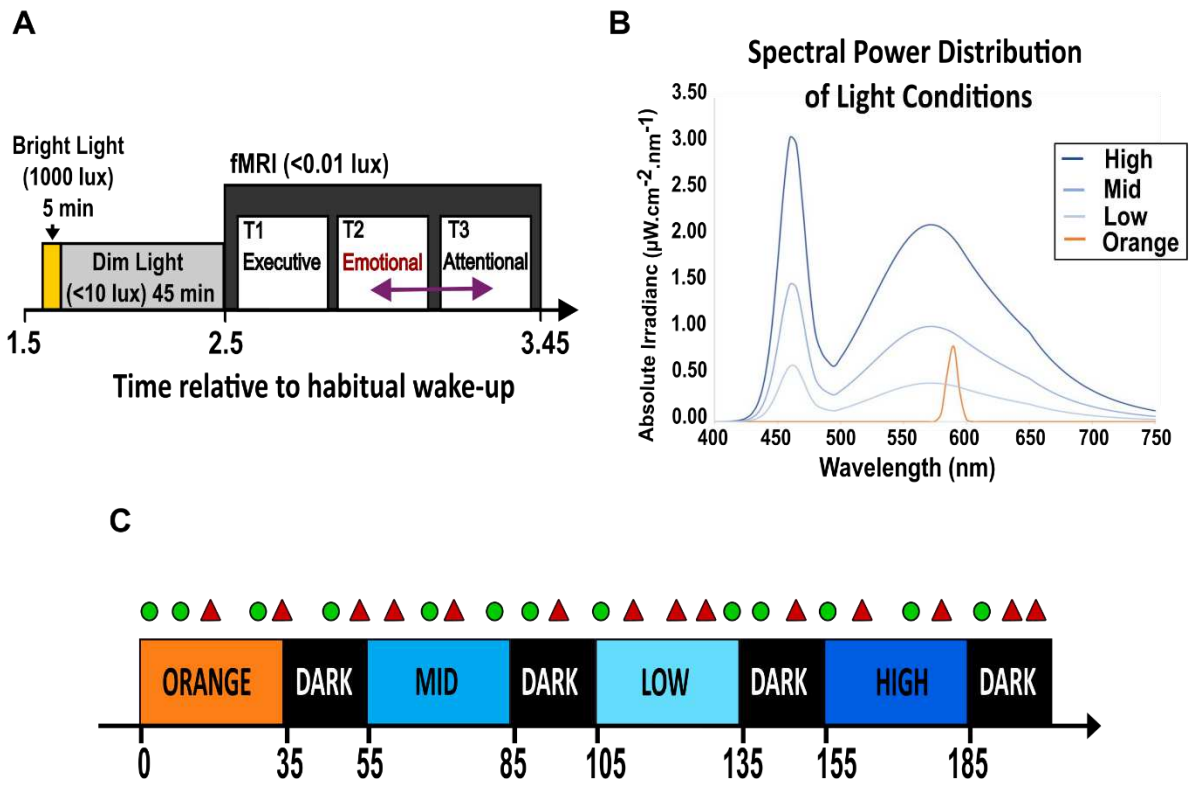


Figure 1. Experimental protocol

(A) Overall timeline. Following 7 days of regular lose sleep-wake schedule (+- 1h, verified using actigraphy) and after prior light history standardisation, 29 participants (24y ±3.1; 18 women) performed an executive (always first), an emotional and an attentional task (pseudo-randomly 2nd or 3rd, purple arrow). Only the emotional task is considered in the present manuscript. The protocol was administered at different times of the year and completed once by each participant (Winter: 4; Spring: 10; Summer: 6; Fall: 9; **Supplementary Table S1**)

(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 (**Suppl. Table S2** for full details). Blue-enriched 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.[5,27] The orange light was introduced as a control visual stimulation for potential secondary whole-brain analyses. 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, constituting a limitation of our study.

(C) Emotional task and light blocks. The task consisted of a lure gender classification of meaningless vocalisation.[28] Untold to the participants, vocalizations were pronounced with neutral (50%, green circle) or angry/emotional (50%, red triangle) prosody by professional actors (50% women). Participants were pseudo-randomly exposed to the four light conditions. Time is reported in seconds relative to session onset.

Participants achieved 92 ±7.3% (mean ±SD) button response, while the accuracy to the lure gender classification task[29] was 79.7% ±9.9%, which is slightly lower than previous studies

using the same task.[28] Critically, the reaction times were faster for the neutral (1203 ±183ms) as compared with the emotional stimuli (1249 ±184ms) (main effect, stimulus type; $F_{1,28}$=14.6, **p=.0007**) (**Fig. 2C**). This is in line with previous literature[5,28] and confirms that the emotional content of the stimuli was successful in triggering a behavioural response. Reaction times were not significantly affected by illuminance (main effect, illuminance; $F_{4,221}$= .92, p= .45) or by season (main effect, season; $F_{1,25}$= .12, p= .73) and there was no interaction between illuminance and stimulus type ($F_{4,221}$= .35, p= .85). This does not, in principle, preclude illuminance or season from affecting the underlying brain activity and ensures that neuroimaging findings related to light exposure are not merely the results of a change in reaction times. We further assessed variation in the affective state in our sample – based on a questionnaire,[30] and found no association with season, age, sex and BMI ($F_{1,20}$ <.65, p >.4). The questionnaire-based seasonality score [31] was low in all participants and was not used in the analyses (**Suppl. Table S1**).

<u>The activity of the medial-superior amygdala is related to season and affective state</u>

We used a template that consistently divided the amygdala into 10 subparts[32] (**Fig. 2A**) to extract the regional activity within the amygdala. The statistical analysis, which controlled for age, sex and BMI, first confirmed that the task was successful in triggering emotional responses in the amygdala with higher response to emotional than to neutral stimuli (GLMM; main effect, stimulus type, $F_{1,\ 27.74}$= 9.93; **p= .0039**, R^{2*}= .26; **Fig. 2C**) (**Suppl. Table S3**). This is supported by the visualization of the whole brain group analysis ($p_{uncorrected}$ <.001) which yielded local positive peaks bilaterally in the amygdala (mostly over the medial and superior subparts – ~subparts 1, 3, 5 and 10) indicating that our finding does not arise from a nearby "leaking" activation (**Fig. 2B**). As suggested by this visualization, responses significantly

differed across amygdala subparts (GLMM; main effect, subpart, $F_{9, 499.9}$= 2.13; **p= .0256,** R^{2*}= .04), though not in interaction with stimulus type (GLMM; interaction, stimulus type by subpart: $F_{9, 499.9}$= .56, p= .85) (**Suppl. Table S3**). Post hoc tests indicated that three subparts did not respond significantly to the auditory stimulations (p >.05, **Suppl. Table S3**; Intermediate and dorsal basolateral nucleus - subpart 2, blue on Fig. 2A, Central nucleus - subpart 4, purple on Fig. 2A, and Ventral basolateral nucleus and Paralaminar nucleus - subpart 6, red on Fig. 2A). The remaining of the analyses only considered the 7 other subparts.

Although low affective state was among our exclusion criteria, it varied across our participants which were recorded at all times of the year (Winter: 4; Spring: 10; Summer: 6; Fall: 9; **Suppl. Table S1**). We therefore then asked whether the activity of the amygdala subparts was associated with the season/time-of-the-year and affective state. The statistical analysis provided a positive answer to the question (GLMM; main effect, time-of-year: $F_{1, 17.85}$= 12.6, **p = .0023**, R^{2*}= .41; main effect, affective state: $F_{1, 17.8}$= 17.5, **p= .0006**, R^{2*}= .495) and further revealed that the association with season/time-of-the-year and affective state value differed across amygdala subparts (GLMM; three-way interaction, subpart by time-of-year by affective state: $F_{6,272.4}$= 4.03, **p= .0007**, R^{2*}= .08; interaction, subpart by time-of-year: $F_{6, 272.6}$= 4.66, **p= .0002**, R^{2*}= .09; interaction, subpart by affective state: $F_{6, 272.4}$= 2.99, **p=.0076**, R^{2*}= 0.062) (**Suppl. Table S4**).

Considering first season/time-of-the-year, post hoc tests showed that the activity of the basomedial nucleus (BMN; t= 3.54, $\mathbf{p_{corrected}}$**= .004**, subpart 3, dark blue, **Fig. 2A**) and of the anterior amygdaloid area (AAA; t= 4.96, $\mathbf{p_{corrected}}$ **<.0001**, subpart 9, light green on **Fig. 2A**) significantly varied with time-of-year (both subparts were also different from part of the other subparts in their association with time-of-year) (**Fig. 2F-G, Suppl. Table S4**). For both areas, responses were stronger during winter (**Fig.2H-I**). Post hoc tests then revealed worse affective

state was significantly associated with a higher activity of medial and cortical nuclei (t= 4.28, $\mathbf{p_{corrected}= .0002}$, subpart 5, violet, Fig. 2A) and amygdala transition areas (ATA, t= 4.64, $\mathbf{p_{corrected}}$ **<.0001**, subpart 7, orange, Fig. 2A) (both subparts were also different from part of the other subparts in their association with time-of-year) (**Fig. 2J-K, Suppl. Table S4**). A statistical trend for an association with affective state was further detected for the AAA (t= 2.6, $p_{uncorrected}$= .011, $p_{corrected}$= .063, subpart 9, light green on Fig. 2A) and the intercalated nuclei (t=2.46, $p_{uncorrected}$=.016, $p_{corrected}$=.094, subpart 10, green on Fig. 2A) (**Fig. 2L-M, Suppl. Table S4**). Overall, the results suggest that the activity of the nuclei composing the medial and superior region of the amygdala (encompassing 4 to 5 subparts of our template) is related to season and/or affective state.

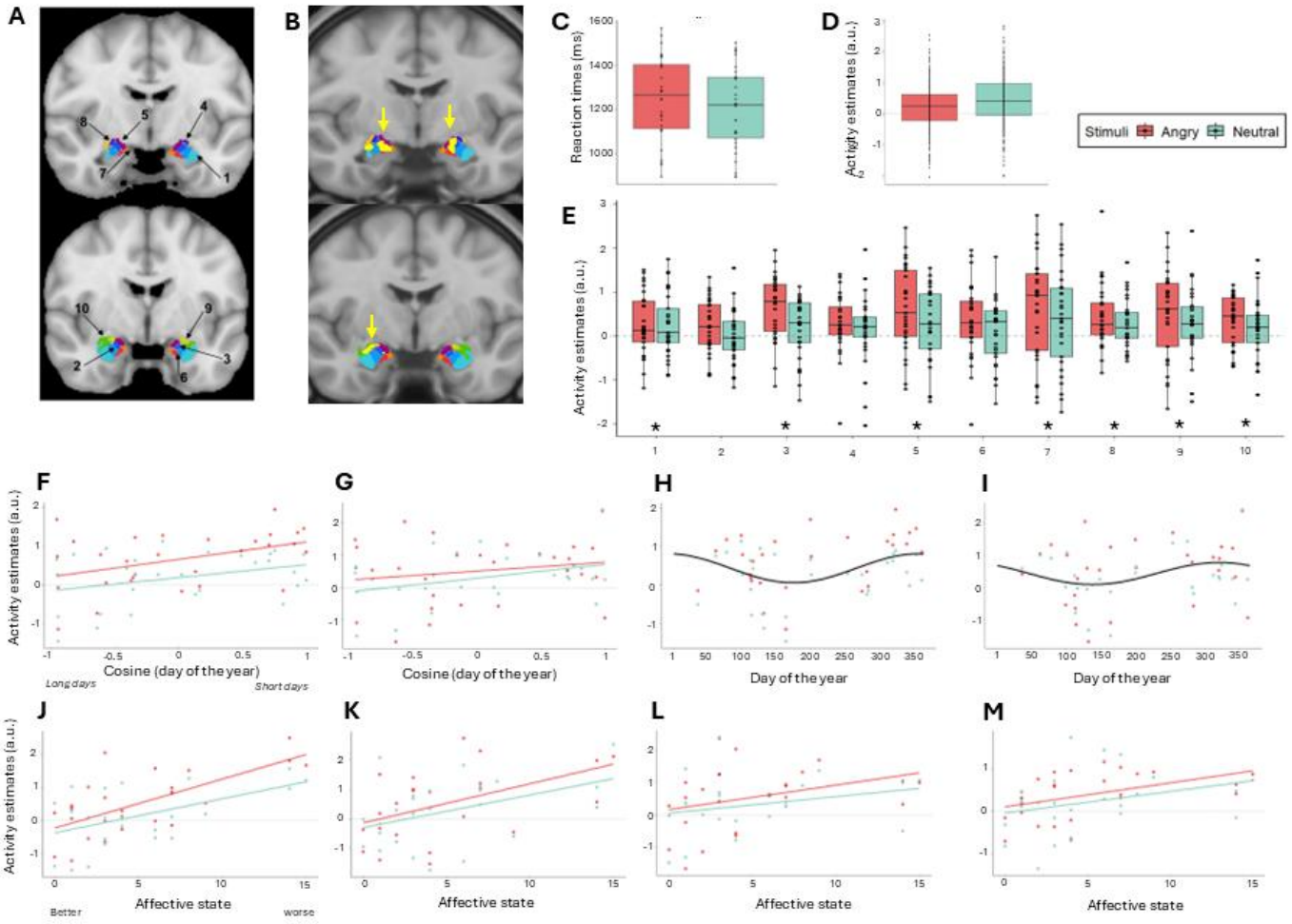


Figure 2. Regional difference in the response of amygdala subparts to emotional and neutral auditory stimulations and relationships with season and affective state.
(A) Segmentation of the amygdala into ten subparts. The amygdala template isolate nuclei and nucleus groups as follows: (1) Lateral nucleus, (2) Intermediate and dorsal basolateral nucleus, (3) Basomedial nuclei, (4) Central nucleus, (5) Medial and Cortical nuclei, (6) Ventral basolateral nucleus and Paralaminar nucleus, (7) amygdala transition area composed of Amygdalocortical area, Amygdalohippocampal area, Periamygdaloid cortex, (8) Amygdalostriatal, (9) Anterior amygdaloid area, (10) Intercalated nuclei[32].
(B) Visualization of the whole brain analyses of the difference between emotional and neutral auditory stimuli over the amygdala area. A local positive peak (yellow arrow and yellow cluster; $p_{uncorrected}$<.001) was detected bilaterally in the amygdala mostly over the subparts 1, 3, 5 and 10. These results indicate that our finding does not arise from a nearby "leaking" activation.
(C) Significant difference in reaction times to neutral and angry stimuli.
(D-E) Impact of stimuli type on the activity of the amygdala subparts. Activity estimates (arbitrary unit – a.u.) averaged over the entire amygdala (D) and for each subpart (E). Refer to panel A for names corresponding to subpart numbers and refer to Suppl. Tables S3 for full statistics.
(F-I) Seasonal variation in the activity of the amygdala subparts. Activity estimates in the basomedial nucleus (F; subpart 3) and anterior amygdaloid area (G; subpart 9) vs. cosine of degree transformation of day of year (0° = January 1st; 1 day = 0.98°) (i.e. the value used in the GLMMs) and vs. day of the year for visualization - basomedial nucleus (H; subpart 3) and anterior amygdaloid area (I; subpart 9). averaged over the entire amygdala (D) and for each subpart €. Refer to panel A for name of subpart numbers. Refer to Suppl. Tables S4 for full statistics.
(J-M) Association between affective state and the activity of the amygdala subparts. Activity estimates in the medial and cortical nuclei (J; subpart 5), and in the amygdala transition areas (K, subpart 7) was higher when affective state was worse according a questionnaire score. A similar statistical trends was detected in the anterior amygdaloid area (L; subpart 9) and the intercalated nuclei (M; subpart 10). Refer to Suppl. Tables S4 for full statistics.

The next statistical analysis included the impact of illuminance variation on the activity of the amygdala subparts, still controlling for age, sex and BMI. It revealed that the illuminance affected differently the activity of the different subparts (GLMM; main effect, subpart: $F_{6, 331.4}$= 7.05, **p <.0001**, R^{2*}= .11) (**Fig. 3B, Suppl. Table S5**). The impact of light further depended on the stimulus types (GLMM; main effect, stimulus type: $F_{1, 27.2}$= 6.32, **p= .0181**, R^{2*}= .19), while there was no interaction between stimulus type and subparts (GLMM; interaction, subpart by stimulus type: $F_{6, 331.4}$=1.42, p=.21) (**Suppl. Table S5**).

Post hoc analyses found that the BMN (**$p_{corrected}$= .0001**, subpart 3, dark blue, Fig. 2A), medial and cortical nuclei (**$p_{corrected}$ <.0001**, subpart 5, violet, Fig. 2A), ATA (**$p_{corrected}$= .0005**, subpart 7, orange, Fig. 2A) and the AAA (**$p_{corrected}$= .0002**, subpart 9, light green on Fig. 2A) were significantly affected by illuminance changes, and differently so compared to many

other amygdala subparts (**Suppl. Table S5**). Visualization of the whole brain group analysis ($p_{uncorrected}$<.001) yielded local negative peaks bilaterally in the amygdala when comparing emotional and neutral items, mostly over the left subparts 3, 5 and 7 and the right subparts 3, 5 and 9, again supporting the finding and indicating that it does not arise from a nearby "leaking" activation (**Fig. 3A**). When investigating in more detail the regional impact of light in the 4 subparts, results are compatible with a linear decrease in activity with increasing illuminance in the 4 subparts (**Fig. 3C-D**; **Suppl. Table S6**). The regions that were related to season and/or mood independently of illuminance, are therefore also linearly affected by illuminance variations when processing emotional stimulations.

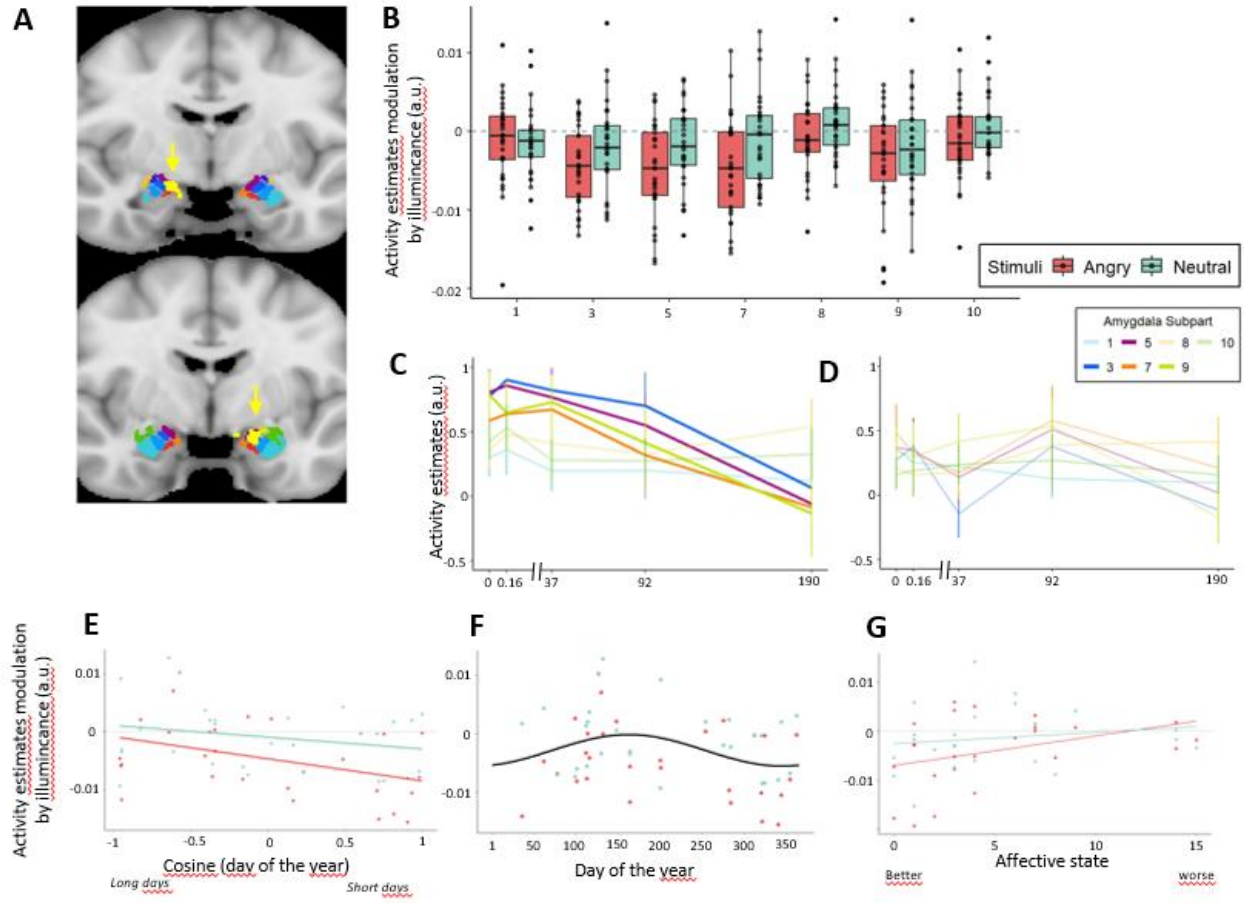


Figure 3 Regional difference in the impact of light illuminance on the responses of amygdala subparts and relationships with season and affective state.
(A) Visualization of the impact of illuminance change over the whole brain of the entire sample. Visualization of the whole brain analyses of the collective impact of the emotional variations in

illuminance over the amygdala area. A local negative peak (yellow arrow and yellow cluster; $p_{uncorrected}$<.001) was detected bilaterally in the amygdala mostly over the subparts 3, 5 and 7 in the left hemisphere and over subparts 3, 5 and 9 in the right hemisphere. These results indicate that our finding does not arise from a nearby "leaking“ deactivation.
(B) Collective impact of illuminance variation on the activity of the amygdala subpart. Parametric modulation of the activity estimates (arbitrary unit - a.u.) by the variations in illuminance. Refer to panel Fig. 2A for names corresponding to subpart numbers and refer to Suppl. Tables S5 for full statistics.
(C-D) Impact of each illuminance on the activity of the amygdala subparts. Results are displayed for emotional (C) and neutral (D) stimulus for all 7 subparts that responded to the task (cf. Fig 2E). The small inset provides the number corresponding to the colour code. The four subparts showing significant impact of the collective change in illuminance are highlighted on panel C (only those 4 regions were used for statistics, the others three are shown for comparisons). Refer to panel Fig. 2A for names corresponding to subpart numbers and refer to Suppl. Tables S6 for full statistics.
(E-F) Seasonal variation in the impact of light on the activity of the amygdala transition areas (subpart 7). Activity estimates vs. cosine of degree transformation of day of year (0° = January 1st; 1 day = 0.98°) (i.e. the value used in the GLMMs) (E) and vs. day of the year for visualization (F). Refer to Suppl. Tables S7 for full statistics.
(G) Association between affective state and the impact of light on the anterior amygdaloid area (subpart 9). Activity estimates vs. questionnaire affective state score. Refer to Suppl. Tables S7 for full statistics.

The regional impact of light on the amygdala depends on season and affective state

In the final steps, we asked whether the impact of illuminance on the different amygdala subparts varied with time-of-year and affective state. The statistical analysis (controlling for age, sex and BMI), provided again a positive answer such that the association with time of the year and affective state value differed across amygdala subparts (GLMM; interaction, subpart by time-of-year: $F_{6, 272}$= 2.94, **p= .0085**, R^{2*}= .06; interaction, subpart by affective state: $F_{6, 272}$= 2.24, **p= .04**, R^{2*}= 0.05; three-way interaction, subpart by time-of-year by affective state: $F_{6,272}$= .87, p= .52) (**Suppl. Table S7**). Post hoc tests did not point to a subpart significantly driving the associations but rather yielded statistical trends suggesting that the impact of illuminance varied with time-of-year within the ATA (t= -1.73, $p_{uncorrected}$= .096, $p_{corrected}$= .32, the subpart differed significantly from several other subparts) while it depended on affective state in the AAA (t= 2.21, $p_{uncorrected}$= .033, $p_{corrected}$= .12, the subpart differed significantly from several other subparts) (**Suppl. Table S7**). Visualisation of the data indicates that light impact

on the ATA is maximum around summer (**Fig.3 E-F**), while the worse affective state is associated with a larger impact of illuminance on the AAA (**Fig.3 G**).

Discussion

Affective state varies with seasons in a large portion of the healthy population, while psychiatric symptoms in patients also show seasonal variations.[1] Light therapy is further established as an effective and established treatment used in treating several mood disorders.[2,3] The brain mechanisms underlying seasonality and light impacts have not been fully resolved, though they are arguably involving nuclei of the amygdala.[33] Considering the connection between the neural pathways involved in emotional and mood regulation,[34] we aimed to determine which subparts of the amygdala were affected by light during an emotional task using fMRI and an automatic parcellation of the amygdala in 10 subparts (corresponding to a unique nucleus or several nuclei).

Our analysis was twofold: we first considered the seasonal changes in activity, that are related to the slow change in photoperiod, before assessing how alternating short exposures to light of different illuminance acutely affected the regional activity of the amygdala. We found that the activity of medial and superior nuclei of the amygdala was related to either season or affective state. We further found that the same medial and superior nuclei showed a linear reduction of activity with increasing illuminance, specifically when processing emotional stimulations. Importantly also, the impact of light on part of these nuclei varied with time-of-year or with affective state. These findings provide new evidence of seasonality in humans.[35,36] They further bring new insight into the mechanisms that underlie the long-term and acute biological impact of light on the brain and that may contribute to the benefits of light therapy in the treatment of mood disorders.

315 The task we administered was successful in triggering a differential response between
316 neutral and emotional stimuli in most of the subparts of the amygdala isolated by our
317 segmentation procedure. Among these, the response of the BMN and of the AAA is higher
318 around winter solstice as compared with summer solstice, at least in the morning in the
319 context of our experiment. It is therefore tempting to speculate that their time-of-year
320 variation underlies seasonal variations in affective state and potentially psychiatric symptoms.
321 In addition, although affective state did not show significant seasonal variation in our sample,
322 responses were higher when affective state was worse within a subpart of our template
323 encompassing the medial and cortical nuclei (as their boundaries are not well-defined enough
324 on MRI images to separate them)[32] and the ATA, which includes several small nuclei.
325 The BMN and medial nuclei send reciprocal projections to each other and both project
326 to the cortical nucleus.[37] According to a simplified overview of sensory information flow
327 through the amygdala, information enters through the BMN and then progresses through to
328 the cortical and medial nuclei which act as an output station for downstream targets.[14]
329 Activation of the medial nucleus is associated with stress and leads to the secretion of
330 adrenocorticotropic hormone and the activation of the hypothalamic-pituitary axis (a
331 neuroendocrine system that maintains physiological homeostasis).[14] The medial nuclei mainly
332 contain GABAergic neurons[38] suggesting that it fulfils its functions mainly through an
333 inhibitory influence on downstream areas, within and outside the amygdala.
334 The AAA, which is less developed in primates, could be among the downstream targets
335 of the medial nucleus, although it rather receives projections from the lateral and central
336 nuclei.[14,37] Likewise, the amygdalohippocampal area and the periamygdaloid cortex, which
337 compose part of the ATA (together with the Amygdalocortical area), both receive projections
338 from the BMN and medial nuclei and projects back to the medial nuclei,[37] such that the ATA,

339 which has received little scientific focus, could be among the downstream targets of the BMN.
340 We speculate therefore that the BMN is in an ideal position to affect multiple amygdala-
341 dependent processes, including the seasonal regulation of affective state, through the medial
342 and cortical nuclei and ATA. This speculation is only partially supported by our data as activity
343 of the AAA, but not of the BMN, was weakly associated with affective state in our data
344 (association was significant prior to correction for multiple comparisons). This somewhat
345 partially supported scenario may be due to the healthy nature of our sample of relatively
346 limited size, warranting further investigation in larger and more diverse samples.

347 Interestingly, the same four nuclei/subparts that are related to season or affected
348 state were also acutely affected by light illuminance. Research in rodents reported that the
349 central and medial amygdala receives direct functional inputs from ipRGCs, and both
350 projections were implicated in the impact of light on anxiety-related behaviours.[16–18,39,40]
351 Neuroimaging research reported that light influences the activity of the amygdala including
352 during emotional processing, but did not isolate the nuclei involved.[5,21] Our results support
353 that in humans it is the medial nuclei (together with the BMN maybe) that mediates the
354 impact of light on emotional state. This provides support to the existence of a functional
355 projection from ipRGCs to the medial parts of the amygdala in humans, similar to rodents.[11]
356 This would mean that alike mice, light can influence emotional status independent of the
357 suprachiasmatic nucleus (SCN), which receives the strongest inputs from ipRGCs based on
358 studies in animals and is the site of the principal circadian clock.[11,41] Our data are, however,
359 also compatible with the effect of light on the BMN and medial nucleus activity through the
360 SCN or other nuclei. For instance, according to data collected in rodents, several nuclei of the
361 amygdala, including the 4 nuclei/subparts we isolated, receive inputs from the orexinergic
362 neurons of the lateral hypothalamus.[42] The latter nucleus is known to promote wakefulness

as part of the ascending activating system[43] and also receives direct projection from ipRGCs [39], while it is likely affected by light illuminance in humans.[44] In fact, one could postulate that the impact of light following both direct and indirect routes depends on the timescale. The acute impact could be mediated by a direct projection to the medial amygdala while the longer-term impact of photoperiod would indirectly reach the BMN through the SCN and/or the lateral hypothalamus. The fact that the winter symptomatic period of patients suffering from seasonal affective disorders was found to be associated with a misalignment of the circadian clock that can be corrected by light therapy[1,45] may bring support to the latter assumption. Future connectivity analyses would however help verify these hypotheses.

Importantly, the impact of light on the amygdala varied with seasons over some of its nuclei/subparts, at least when light is delivered in the morning. This seasonal variation may be driven by the ATA which showed a lower impact of light around the winter solstice. In addition, the impact of light was stronger over some parts of the amygdala when affective state was worse, particularly over the AAA. These variations related to season/time-of-year and to affective state may contribute to the effectiveness of light therapy or the need for it and/or to seasonal variations in affective state. This will, however, require further investigations. Apart from this question, our results indicate that the short procedure to standardise recent light history included in our protocol did not eliminate all seasonal changes, implying that a "memory" for longer light history or a true endogenous seasonal oscillator may underlie our findings.[46]

Given the decreased activity and GABAergic activity of the medial nucleus mentioned above,[38] one could postulate that the decrease in activity induced by light over the medial and superior nuclei of the amygdala triggers a reduction or inhibition of this inhibitory influence. The hypothesis that ipRGCs have the potential to be inhibitory is not without evidence, as a

rodent study found that a subset of ipRGCs release the inhibitory neurotransmitter GABA at brain targets (e.g. SCN), leading to reduced sensitivity of the pupil to light and circadian photoentrainment.[47] Overall, although we do not have empirical data to support this hypothesis, we tentatively speculate that given the high interconnectivity of the amygdala nuclei, the deactivation in the ATA and AAA in response to illuminance change reflects the downstream propagation of signals from the output of the BMN and medial/cortical nuclei.

Ultimately, although we favour an impact of light on the amygdala through the direct projection of the retina to the medial nuclei, all four subparts could equally contribute to the decrease BOLD signal we detect under higher illuminance.[37] Again, complex connectivity analyses will be required to test these hypotheses and to assess how our findings fit within a larger network of small brain regions including the perihabenular zone, ventral lateral geniculate nucleus and intergeniculate leaflet, all receiving ipRGCs innervation and implicated in pathways mediating light's influence on depressive-like behaviours in animal models.[48,49] The cross-talk with the prefrontal cortex is also of interest given the changes in functional connectivity previously reported following light therapy when considering the amygdala as whole.[22] Future research will also determine how our findings parallel the production of serotonin and dopamine known to be induced by light and to show seasonal variation.[1,50,51]

The processing of negative stimuli has been associated with increased activity in the amygdala whereas positive stimuli have been associated with decreased activity in the amygdala.[52] A functional neuroimaging study that focused on bright light therapy in healthy young participants found that after three weeks of light therapy, there was a dose-dependent decrease in the response to emotional stimuli in the amygdala and the medial prefrontal cortex.[22] Our findings support that the reduced responsiveness of (part of) the amygdala is already present acutely, during the exposure. The sizes of the effects we detected are small

such that light exposure may be beneficial to the emotional state through a repeated reduction of the response of the BMN and medial parts of the amygdala. It is rather striking that successful repeated electroconvulsive therapy in major depression can restore normal volume of the BMN in major depression[53]. Based on our findings, one could speculate that light therapy could contribute to the improvement of symptoms in major depression [4] through repeated action on the BMN.

The decrease of activity we find in several subparts of the amygdala contrasts with previous research done using 3T fMRI in healthy participants that found that exposure to light increased the response of the amygdala to emotional stimuli using the same emotional task.[5] The conflicting findings may be due to the differences in light sources used as it was a monochromatic blue light (corresponding to an illuminance of ~22 and 93 mel EDI lux). In contrast, another 3T resting state MRI, which did not involve a cognitive task, found that the activity of the amygdala was decreased under polychromatic white light (~35 mel EDI lux; 2800k) in comparison to darkness.[21] In the present study, we used a polychromatic white, blue-enriched light source of three different intensities (37, 92, 190 mel EDI; 6500k), i.e. the lowest light level we used has a similar mel EDI lux to the resting state study. It may be that the amygdala sees its activity increase or decrease in response to light depending on illuminance and spectrum, depending potentially on the type of retinal photoreceptor recruited.

The BMN, medial nuclei, AAA and the periamygdaloid cortex project downstream to the frontal cortex,[37] which governs cognition. In addition, one of the main targets of the medial nucleus is the hypothalamus, specifically over the anterior paraventricular nucleus. The cortical nuclei also project to the brainstem locus coeruleus, producing norepinephrine and the dopaminergic ventral tegmental area.[37,42] Light could affect behaviour both acutely

and in the long run through some or all of these projections. We stress, however, that although the task we used[5,28,29] was successful in triggering acute behavioural responses to the emotional content vocalizations when considering reaction times, these were not significantly different across the light exposures of different illuminance. How our fMRI findings explain how longer/repeated light exposures may acutely affect behaviour is not straightforward and we suspect that longer light exposures and/or other cognitive tasks would be required to trigger clear acute behavioural responses.[10]

In terms of photoreceptors, we cannot conclusively say that the ipRGCs are underlying the decrease in activity in the subparts we see at higher light levels. Given the projections of ipRGCs to the medial amygdala and to the hypothalamus reported in rodents [39] and the light level we used, their implication is very likely. Rods and cones may however also be contributing – e.g. as a normalisation of their function was suggested following light therapy,[45] and there may be differential recruitment of photoreceptors at different illuminance levels.[54] Future studies in humans could determine the photoreceptor underlying the impact of light on emotion processing and affective state by using, for instance, silent substitutions and metameric light exposures to selectively affect one photoreceptor type.[55] Our protocol bears other limitations including the inherent non-ecological experiment condition of an MR apparatus, the lack of investigation at other times-of-day and other light spectra when it is established that time-of-day and light content in short wavelength photons affect the acute and longer-term biological NIF responses to light.[10] Our protocol also only included negative (and neutral) valence vocalization which fits well with the important involvement of the amygdala in negative affect.[56,57] Positive stimulation should be included in the future to make investigating the relationship with the positive effect of light therapy on

affective state more straightforward. While none of these limitations invalidate our observations, they restrain their generalisability.

Emotional regulation is vital and evolutionary critical. The amygdala fulfils part of this regulation to allow for adaptation to the changing environment. We find, in a healthy sample devoid of comorbidities related to a specific brain disorder, that several subregions of the amygdala, including the medial nucleus that receives direct projection from ipRGCs, showed seasonal variation in activity as well as reduction of activity with increasing illuminance that depends on time-of-year/season when processing emotionally charged stimuli. We speculate that it is through the BMN and medial that light affects the emotional state in healthy individuals as well as in psychiatric patients in which different amygdala nuclei may contribute to disorders.[24,58–63]

Methods and Materials

This paper arises from a larger study and part of the methods have been described previously.[64–67] The protocol was approved by the Ethics Committee of the Faculty of Medicine at the University of Liège. Participants gave their written informed consent and received monetary compensation. Data acquisitions took place in Liège, Belgium, between December 2020 and August 2023. Additional methodological details are provided as supplementary methods.

Participants

Thirty-six healthy participants (23.9y ±2.8; 23 women) took part in the study. The exclusion criteria were assessed through questionnaires and a semi-structured interview and were as follows: 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). Their scores on the 21-item Beck Anxiety Inventory [68] and the Beck Depression Inventory-II [30] were minimal or mild (< 18) and minimal (< 14), respectively. Questionnaires further assessed chronotype with the Horne-Östberg questionnaire [69] and seasonality with the Seasonal Pattern Assessment Questionnaire [31], but the latter two questionnaires were not used for the inclusion of the participants. Participants refrained from caffeinated and alcohol-containing beverages and excessive exercise for at least 3 days before the experiment. The scores to the Beck Depression Inventory-II [30] were used to assess the influence of affective state on the regional activity of the amygdala.

Seven datasets were missing or corrupted such that 29 participants (24y±3.1; 18 women; **Supplementary Table 1**) were included in the present analyses (three participants did not complete the entire task because of scan technical issue interrupting acquisition or subject willing to exit the scanner before the end of the session, while quality check revealing an important ghosting artefact for 2 datasets and important mismatch in the coregistration of subject space to the MNI space for 2 datasets which could not be resolved following multiple attempts at the time of submitting the manuscript). At least one valid data set was acquired each month of the year and participants are relatively well spread across the 4 seasons (Winter: 4; Spring: 10; Summer: 6; Fall: 9)

Protocol

Structural brain images were acquired 1 to 2 weeks before the experiment, during a visit which served as habituation to the experimental conditions. Participants then followed a loose sleep-wake schedule (±1h from habitual sleep/wake-up time) for 7 days, to maintain realistic entrained life conditions and avoid excessive sleep restriction (verified using wrist actigraphy -AX3, Axivity, UK- and sleep diaries). Participants arrived at the laboratory 1.5 to 2h after their habitual wake time. To standardise participants' recent light history, they were exposed to 5min of bright white light (1000 lux) and were then maintained in dim light (<10 lux) for 45min. During the dim light exposure, participants were given instructions about the fMRI cognitive tasks and completed practice tasks on a luminance-controlled laptop (<10 lux). The fMRI sessions consisted of an executive task (25min), an attentional task (15min), and an emotional task (20min), which is the only task included in the present paper [**Fig.1A**]. Participants always completed the executive task first as it was the most demanding task. The order of the following two tasks was counterbalanced across participants. While in the MR-scanner, participants were asked to keep their eyes open and try not to blink too much during

517 the cognitive tasks. An eye-tracking system (EyeLink 1000Plus, SR Research, Ottawa, Canada)
518 was monitored for proper eye-opening during all data acquisitions.

519 **Light Exposure**

520 An MRI-compatible light system designed-in-lab was developed to ensure relatively uniform
521 and indirect illumination of participants' eyes whilst in the MRI scanner. An 8-m long MRI-
522 compatible dual-branched optic fibre (1-inch diameter, Setra Systems, MA, USA) transmitted
523 light from a light box (SugarCUBE, Ushio America, CA, USA), that was stored in the MRI control
524 room. The dual end of the optic fibre was attached to a light stand fitted at the back of the
525 MRI coil, allowing for reproducible fixation and orientation of the optic fibre ends. The dual
526 branches illuminated the inner walls of the head coil to illuminate indirectly the participants'
527 eyes. A filter wheel (Spectral Products, AB300, NM, USA) and optical fibre filters to switch
528 between a narrowband 589nm filter (full width at half maximum: 10 nm) and a UV long-
529 bypass filter (433 – 1650nm) filter) and alternate between a monochromatic orange light and
530 the full output of the polychromatic blue-enriched LEDs (6500K) (**Fig. 1B** and **Supplementary**
531 **Table S2** for in-detail light characteristics). The spectra of the lights were assessed at the level
532 of the end of the optic fibre (AvaSpec-2048, Avantes, The Netherlands). Illuminance could not
533 be measured directly in the magnet, but the light source was calibrated prior to the
534 experiment (840-C power meter, Newport, Irvine, CA).

535 Blue-enriched light illuminances were set according to the technical characteristics of
536 the light source and to keep the overall photon flux similar to prior 3T MRI studies of our team
537 using the same emotional task (between ~10^{12} and 10^{14} ph/cm²/s).[70] The orange light was
538 introduced as a control visual stimulation for potential secondary whole-brain analyses. For
539 the present region of interest 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.

Emotional Task

The task consisted of gender discrimination of auditory vocalizations [**Fig. 2C**].[71] Participants were asked to use a keypad to indicate what they believed the gender of the person pronouncing each token was. The gender classification was a lure task: its purpose was to trigger an emotional response as participants were not told that 50% of the stimuli were 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 (validated by behavioural assessments[71] and in previous experiments[28,29]). 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 periods separating light blocks contained two angry and two neutral stimuli (80 stimuli in total). The emotional task (together with light exposure changes) was programmed with Opensesame (3.2.8).[72] Participants heard the auditory stimuli through MR-compatible headphones (Sensimetrics, Malden, MA) and the volume was set by the participant before starting the tasks to ensure a good auditory perception. Participants used an MRI-compatible keypad to respond to task items (Current Designs, Philadelphia, PA). The instruction was to privilege accuracy over rapidity when responding. The auditory stimuli used in the task were matched for the duration (750 ms) and mean acoustic energy to avoid loudness effects.

Data acquisition

MRI data were acquired in a 7T MAGNETOM Terra MR (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. The multi-band Gradient-Recalled Echo - Echo-Planar Imaging (GRE-EPI) sequence with axial slice orientation was set as follows: TR=2340ms, TE=24ms, FA=90°, no interslice gap, in-plane FoV =224mm×224mm, 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). Following the fMRI acquisition a 2D GRE field mapping sequence to assess B0 magnetic field inhomogeneities with the following parameters: TR=5.2ms, TEs =2.26ms and 3.28ms, FA=15°, bandwidth = 737Hz/pixel, matrix size = 96×128, 96 axial slices, voxel size = (2x2x2)mm^3, acquisition time=1:38min. The Magnetization-Prepared with 2 RApid Gradient Echoes (MP2RAGE) sequence was set as follows: 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 pre-processing

For the MP2RAGE images, the background noise was removed using an 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) [73]. 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

587 "unified segmentation" tool [74]. To ensure optimal co-registration, brain extraction was done
588 using SynthStrip [75] in Freesurfer (http://surfer.nmr.mgh.harvard.edu/). The brain-extracted
589 T1-images were used to create a T1-weighted group template using Advanced Normalization
590 Tools (ANTs, http://stnava.github.io/ANTs/) before normalization to the Montreal
591 Neurological Institute (MNI) space using ANTs (1mm³ voxel; MNI 152 template).

592 For the EPI images, auto reorientation was applied on the images first. Then, voxel-
593 displacement maps were computed from the phase and magnitude images associated with
594 B0 map acquisition, using the SPM fieldmap toolbox. To correct for head motion and static
595 and dynamic susceptibility-induced variance, "Realign & Unwarp" of SPM12 was then applied
596 to the EPI images. The realigned and distortion-corrected EPI images then underwent brain
597 extraction using the SynthStrip and then the final images were smoothed with a Gaussian
598 kernel characterized by a full width at half maximum of 3mm.

599 **Data analysis**

600 The brain-extracted anatomical T1-images were used to create a T1-weighted group template
601 using Advanced Normalization Tools (ANTs, http://stnava.github.io/ANTs/) before
602 normalization to the Montreal Neurological Institute (MNI) space using ANTs (1mm³ voxel;
603 MNI 152 template). For each subject, the first level analysis of fMRI data was performed in
604 the native space. Before the group analysis, statistical maps were first transferred to the
605 group template space and then the MNI space (1x1x1mm^3 resolution; MNI 152 template)
606 using ANTs. The subject level GLM included a high-pass filter with a 256s cut-off to remove
607 low-frequency drifts as well as movement and physiological parameters (cardiac, and
608 respiration), which were computed with the PhysIO Toolbox (Translational Neuromodeling
609 Unit, ETH Zurich, Switzerland) [76]. All these additional regressors were included as covariates
610 of no interest.

Our statistical analyses consisted of an a priori region of interest focused on the activity of the amygdala which was estimated as part of a whole brain general linear mixed model computed with SPM12. The auditory stimuli were modelled as stick functions convolved with a canonical hemodynamic response function. There were two parts to the whole-brain analysis. In the main analysis, we assessed brain responses during the task and how they were modulated by overall changes in illuminance level. The two regressors of task events (neutral, angry) were each accompanied by a parametric modulation corresponding to the light melanopic illuminance level (0, .16, 37, 92, 190 mel EDI). The contrasts of interest consisted of the main effect of the task (emotional vs. neutral stimuli) and their parametric modulations (emotional vs. neutral stimuli x illuminance). In the next whole-brain analysis, we assessed the responses to the stimuli under each light condition. Separate regressors modelled each task's event type under each light condition. The contrasts of interest consisted of the main effects of each regressor. Whole-brain group results over the entire sample were used for visualization purposes only.

We used an amygdala atlas to segment the region into 10 subparts (bihemispheric) in the MNI subject space[32], corresponding to nuclei or nucleus groups (**Fig 2.A**). The REX Toolbox (https://web.mit.edu/swg/software.htm) was used to extract the activity estimates (betas) from contrast of interest in each amygdala subpart.[77] In the first analysis, this yielded 1 activity estimate per contrast, per stimulus type and per amygdala subpart and in the second analysis, we obtained 5 activity estimates per stimulus and subpart.

Statistics

Statistical analyses of the activity of each subpart were computed in SAS 9.4 (SAS Institute, NC, USA) and 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 main the statistical analysis included all subparts, light conditions and stimulus types in a single model, the significance threshold was not corrected for multiple comparisons and was set at $p < 0.05$. Direct post hoc of the main analyses were corrected for multiple comparisons using a simulated adjustment. Activity estimates were considered outliers if > ±3 SDs across emotional stimuli and light level and were removed.

The two main analyses included the average activity estimates to emotional and neutral stimuli i) independent of light and ii) modulated by illuminance as a dependent variable and the amygdala subparts and stimulus type (neutral/angry; as repeated measure with compound symmetry correlation), together with their interaction and age, sex and BMI as covariates. The first main analysis indicated that 3 areas did not respond to the task, these were therefore discarded from all further analyses related to season, affective state and light acute impact.

To test for variations related to season/time-of-year and affective state, both models were recomputed respectively with a season covariate consisting of the cosine value of the day of the year expressed in degrees of the 360-day year (365 day = 360°; 1 day = .986°; December 21st = 0°) and the score of Beck Depression Inventory-II [30]. Three-way interaction terms were included in the model [subpart x season x affective state] as well as the three simple interactions composing the three-way interaction.

The main analysis focusing on light exposure impact used a linear parametric modulation of the changes in activity with increasing illuminance to grasp the overall impact of illuminance change. Since the later may have missed non-linear changes in activity with changes in illuminance, a second GLMM included the four amygdala subparts that were showing an impact of overall illuminance change. The GLMM included the activity estimates of the four subparts as the dependent variable and amygdala subpart, stimulus type

(neutral/angry; as repeated measure with compound symmetry correlation) and illuminance (0, 0.16, 37, 92, 190 mel EDI lux; as the repeated measures nested within stimulus type - compound symmetry correlation), together with age, sex and BMI as covariates. A three-way interaction term was included in the model [subpart x illuminance x stimulus type] as well as the three simple interactions composing the three-way interaction.

A final set of exploratory GLMMs reported in the discussion section included performance metrics as dependent variables (reaction time – ms) and included the activity of each of the four amygdala subparts that were showing an impact of overall illuminance change in separated model together with stimulus type and illuminance (repeated measures nested within stimulus type - compound symmetry correlation), together with age, sex and BMI as covariates.

Optimal sensitivity and power analyses in GLMMs remain under investigation (e.g. [78]). 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), [79] taking into account a power of 0.8, an error rate α of 0.05, and a sample of 29 allowed us to detect medium effect sizes $r > 0.47$ (two-sided; absolute values; CI: 0.12–0.71; $R^2 > 0.22$, R^2 CI: 0.02–0.5) within a linear multiple regression framework including two tested predictor (illuminance effect, amygdala subpart) and four covariates (stimulus type, 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). The raw data could be identified and linked to a single subject and represent a large amount of data. Researchers willing to access to the raw data should send a request to the corresponding author (GV). Data sharing will require evaluation of the request by the local Research Ethics Board and the signature of a data transfer agreement (DTA).

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Author contributions

Study concept and design by IC and GV. Data acquisition and analysis by IC, FB, RS, NM, PT, EB, & IP. Methodological support and/or support in the interpreting the data by CP, FC, MZ, LL. Funding was mostly obtained by FC, CP, and GV. IC and GV drafted the first version of the manuscript. All authors revised the manuscript and had final responsibility for the decision to submit for publication.

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