Title: REM sleep quality is associated with balanced tonic activity of the locus coeruleus during wakefulness

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Abstract

Background

Animal studies established that the locus coeruleus (LC) plays important roles in sleep and wakefulness regulation. Whether it contributes to sleep variability in humans is not yet established. Here, we investigated if the *in vivo* activity of the LC is related to the variability in the quality of Rapid Eye Movement (REM) sleep.

Methods

We assessed the LC activity of 34 healthy younger (~22y) and 18 older (~61y) individuals engaged in bottom-up and top-down cognitive tasks using 7-Tesla functional Magnetic Resonance Imaging (fMRI). We further recorded their sleep electroencephalogram (EEG) to evaluate associations between LC fMRI measures and REM sleep EEG metrics.

Results

Theta oscillation energy during REM sleep was positively associated with LC response in the top-down task. In contrast, REM sleep theta energy was negatively associated with LC activity in older individuals during the bottom-up task. Importantly, sigma oscillations power immediately preceding a REM sleep episode was positively associated with LC activity in the top-down task.

Conclusions

LC activity during wakefulness was related to REM sleep intensity and to a transient EEG change preceding REM sleep, a feature causally related to LC activity in animal studies. The associations depend on the cognitive task, suggesting that a balanced level of LC tonic activity during wakefulness is required for optimal expression of REM sleep. The findings may have implications for the high prevalence of sleep complaints reported in aging and for disorders such as insomnia, Alzheimer's, and Parkinson's disease, for which the LC may play pivotal roles through sleep.

Keywords: Locus coeruleus, sleep, 7 Tesla functional magnetic resonance imaging, aging

Background

Up to 35% of the general population report unsatisfying sleep[1] and one out of two adults aged over 50y complains about sleep or daytime sleepiness.[2] Insomnia is the obvious extreme form of these complaints and constitutes the second most prevalent psychiatric disorder.[3] Beyond the behaviors that do not favor appropriate sleep, some people are more vulnerable to poor sleep or more at risk of developing insomnia.[4,5] The biological origin of sleep variability is however not fully established.[6]

The locus coeruleus (LC) is a small nucleus of the dorsal pons – cylinder-like shape of approximately 15 mm with ~2.5 mm diameter.[7] It is responsible for producing norepinephrine (NE) as a part of the ascending arousal system and projects to the neocortex, hippocampus, amygdala, thalamus, and cerebellum.[8] It plays a crucial role in the control of behavioral states, including alertness and attention,[9] as well as in the regulation of sleep.[6] LC-NE tone must decrease to allow sleep onset, while LC activity dynamically changes during sleep to shape the alternation between Rapid Eye Movement (REM) and non-REM sleep and some of the microstructure elements of sleep.[10,11] A lot of our understanding of the role of the LC in sleep regulation arises from experiments conducted in rodents. Therefore translation to diurnal human beings, with a more developed cortex, is not straightforward[6]. In addition, imaging the LC *in vivo* remains difficult because of its deep location and small size, as well as because of the difficulty to image human sleeping brains. Recent advances in neuroimaging techniques have lifted part of these limitations.[12] For instance, degeneration of the LC was recently suggested to contribute to alteration in rest-activity patterns.[12] We further reported that a higher response of the LC to a salience detection task during wakefulness, which presumably reflects in

part LC activity during sleep, was associated with lower intensity of REM sleep in healthy older individuals.[13] REM sleep intensity does not however solely depend on LC activity, and no association with sleep features shown to be under the direct control of the LC in animal were reported in the study.

LC neurons follow phasic and tonic activity patterns, which lead to distinct release of NE.[14] Phasic activity typically occurs in response to transient salient stimulation, while the tonic mode is mostly required for sustained processes. The dynamics of the tonic mode is considered to follow an inverted U-shape where low LC tonic discharge is associated with low arousal and poor performance, moderate LC tonic activity allowing pronounced phasic activity leads to optimal arousal and performance, and high tonic activity hinders phasic activity and leads to anxiety-like behavior and poorer performance .[15] Likewise, during sleep the LC undergoes periods of higher or lower tonic activity.[10,11]

How the balance between phasic and tonic modes of activity of the LC during wakefulness may be related to REM sleep is not known. In the present study, we used Ultra-High-Field (UHF) 7-Tesla (7T) Magnetic Resonance Imaging (MRI) to extract *in vivo* the LC activity in healthy younger and older adults during two cognitive tasks that rely on different tonic tones of the LC. We related the LC activity during both tasks to electrophysiology metrics of REM sleep. We hypothesized that the LC activity in both tasks would be mainly associated with REM sleep intensity. Moreover, we expected that the associations would differ between two tasks, and would be more pronounced in older individuals. To test for associations with sleep features under direct control of the LC, we further tested the association between LC activity and the

power of the sigma band of the EEG that preceded REM sleep episodes, because it is causally related to NE tone in rodents[16].

Methods

This study was approved by the faculty-hospital ethics committee of ULiège. All participants provided written informed consent and received financial compensation. The study is part of a larger project that has led to previous publications.[13,17] Part of the results dealing with one cognitive task were reported in one of these publications[13] and are included for comparisons purposes and to conduct additional analyses. Most of the methods were described in details in[13]. More details are also available in the **Supplementary Methods**.

Participants

Fifty-two healthy participants including 34 healthy young (22.2±3.1y, 28 women) and 18 late middle-aged (60.9±5.4y, 14 women) individuals, completed the study **(Table 1)**. The exclusion criteria were as follows: history of major neurologic/psychiatric diseases or stroke; recent history of depression/anxiety; sleep disorders; medication affecting the central nervous system; smoking, excessive alcohol (>14 units/week) or caffeine (>5 cups/day) consumption; night shift work in the past 6 months; BMI ≤18 and ≥29 (for older individuals) and ≥25 (for younger individuals). All older participants had to show normal performance on the Mattis Dementia Rating Scale (score > 130/144).[18]

112 **Table 1. Characteristics of the study sample**

	Young individuals (n = 34)				Late middle-aged individuals (n = 18)				P-value
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	
Age (years)	22.20	3.17	18.00	29.00	60.88	5.41	53.00	70.00	**<.0001**
BMI (kg/m2)	21.90	2.74	17.20	28.40	24.75	3.40	19.40	30.90	**0.0047**
Education (years)	14.46	2.31	12.00	20.00	14.61	2.70	9.00	19.00	0.85
Depression level (BDI)	6.64	4.17	0	20.00	5.33	4.01	0	14.00	0.27
Anxiety level (BAI)	4.00	2.92	0	11.00	3.05	3.18	0	9.00	0.30
TIV (cm^3)	1402.1	136.6	1203.3	1946.7	1420.5	125.5	1167.6	1600.1	0.627
Daytime sleepiness (ESS)	6.93	3.90	0	14.00	5.77	3.76	0	13.00	0.30
Sex (F–M)	28 F – 6 M				12 F – 6 M				0.20
Habitual Subjective sleep quality (PSQI)	4.79	2.18	1.00	10.00	3.83	2.22	0	8.00	0.14
TST (min)	454.80	30.97	379.50	499.50	394.63	48.16	282.50	495.50	**<.0001**
Theta power in REMS (μV^2)	7281.5	5061.7	1162.4	22346.4	4789.5	3508.2	765.3	13051.8	**0.0449**
REMS percentage (%)	26.63	4.41	19.93	36.45	21.52	5.50	13.11	35.39	**0.002**
REMS latency (min)	103.0	47.834	0	213.5	81.944	56.806	38.000	295.5	0.190
Sigma power prior to REMS (μV^2)	15.7615	8.8957	5.0656	44.356	16.0544	6.795	3.654	27.856	0.896
SWE (μV^2)	175531	171295	14259.2	555453	126388	109290	23293.3	470116	0.2146

113

114 The p-values shown in the table correspond to two-sample t-tests except for sex that were compared
115 using a Chi-square test. BMI: Body Mass Index; BDI: Beck Depression Inventory; BAI: Beck Anxiety
116 Inventory; TIV: Total Intracranial Volume; PSQI: Pittsburgh Sleep Quality Index; ESS: Epworth Sleepiness
117 Scale; TST: Total Sleep Time; SWE: slow wave energy; REMS: rapid eye movement sleep. See
118 supplementary method for references of the questionnaires.

119

120 *Protocol*

121 Participants' sleep was recorded in the lab twice. First, participants completed a night of sleep
122 under polysomnography (called habituation night) to screen and exclude for sleep abnormalities
123 (apnea hourly index and periodic leg movement >15; no parasomnia or REM behavioral
124 disorder). All participants further completed a whole-brain structural MRI (sMRI) acquisition and
125 a specific acquisition centered on the LC. Participants were then requested to sleep regularly for
126 7 days before the baseline night (±30min from their sleep schedule) based on their preferred
127 schedule (compliance was verified using sleep diaries and wrist actigraphy - Actiwatch and AX3,
128 AXIVITY LTD, Newcastle, UK). For the baseline night, participants first remained awake for 3h
129 under dim light (<10 lux) then their habitual sleep was recorded in darkness (N7000 amplifier,
130 EMBLA, Natus, Middleton, WI). Approximately 3h after wake-up time under dim light (<10 lux),
131 participants completed a functional MRI (fMRI) session that included 3 tasks (**Figure 1A**). This
132 paper is centered on the analyses of the first two tasks, the perceptual rivalry and auditory
133 salience detection tasks.

134 Younger participants completed the fMRI session immediately following the baseline night but
135 older participants were initially part of a different study[19,20] and completed the MRI
136 procedures in addition to their initial engagement, which included the baseline night recording.
137 Consequently, the baseline nights of sleep and MRI sessions were completed about 1.25y apart
138 (mean±SD: 15.5±5.3 months). Prior to the fMRI session, older participants slept regularly for 1
139 week and were maintained in dim light (<10 lux) for 45 minutes.

140 *Sleep EEG metrics*

Sleep was staged in 30s-epochs using an automatic algorithm (ASEEGA, PHYSIP, Paris)[21] to provide REM sleep percentage and total sleep time (TST). Averaged power was computed per 30-min bin, adjusting for the proportion of rejected data and it was subsequently aggregated in a sum for REM[22] to provide REM theta energy (overnight cumulated 4.25-8Hz power). Sigma power (12.25–16 Hz) prior to REMS was calculated as the mean sigma power before REMS episodes, obtained by dividing the total sigma power in the 1-minute period preceding each REMS episode by the number of REMS episodes. Sigma power was computed as the weighted sum of 4s artifact-free windows (2s overlap per 30s epoch).

Cognitive tasks

Visual perceptual rivalry task – high-tonic LC. The task consisted of watching a 3D Necker cube, which can be perceived in two different orientations (**Figure 1B**), for 10 blocks of 1min separated by 10s of screen-center cross fixation (~12min). Participants were instructed to report switches between the two percepts through a button press. Given the top-down nature of the task, we considered that if the task recruited the LC, it would trigger a relatively lower phasic response over a relatively larger tonic tone (high-tonic LC).

Auditory salience detection task – low-tonic LC. The task consisted of an oddball paradigm requiring reports on the perception of rare deviant target tones (1,000Hz, 100ms, 20% of tones) that were pseudo-randomly interleaved within a stream of standard stimuli (500Hz, 100ms) through a button press (**Figure 1C**). The task included 270 stimuli (54 targets; ~10min). Given the bottom-up nature of the task, we considered that if the task recruited the LC, it would trigger a relatively larger phasic response over a relatively lower tonic tone (low-tonic LC).

Pupil size was recorded (Eyelink-1000, SR Research, Osgoode, ON, Canada; sampling rate: 1000Hz) during both cognitive tasks only in a subset of participants due to technical difficulty in maintaining stable signal (in part because of the small window between receive and transmit parts of the MR head coils through which the eye is within camera view) leading to missing/corrupted data (> 25% of missing/corrupted). Analyses of pupil data of the perceptual rivalry and salience detection tasks respectively included 32 (23 women; 23 young individuals) and 27 (20 women; 19 young individuals) individuals with 22 participants common to both tasks (15 women; 15 young individuals).

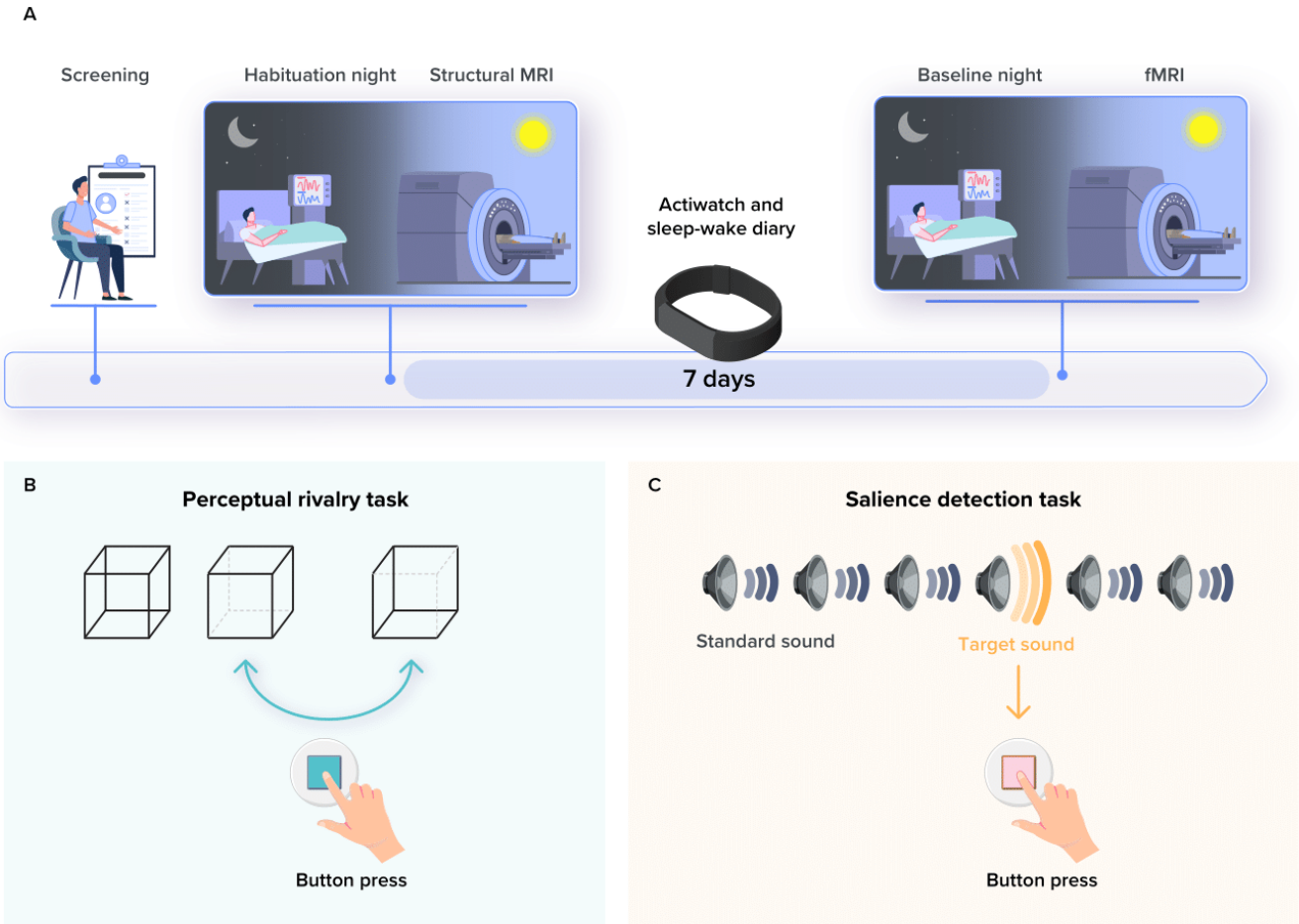

Figure 1. Overview of the study protocol.

(A) After screening, participants completed an in-lab screening and habituation night under polysomnography to minimize the effect of the novel environment for the subsequent baseline night and to exclude volunteers with sleep disorders. They further completed a structural 7T MRI session including a whole-brain structural MRI and a LC-specific sequence. The latter was used to create individual LC masks in each participant's brain space. After 7 nights of regular sleep-wake time at home, which was confirmed by actigraphy data and/or sleep-wake diary, participants came to the lab three hours before their sleep time and were maintained in dim light (<10 lux) until sleep time. Participants' habitual baseline sleep were recorded overnight in-lab under EEG to extract our main sleep features of interest. All participants underwent an fMRI session approximately 3h after wake-up time (following ≥45min in dim light - < 10lux), during which they completed the visual perceptual rivalry task and the auditory salience detection task. **(B)** The Visual perceptual rivalry task consisted of watching a 3D Necker cube, which can be perceived in two different orientations (blue arrow), for 10 blocks of 1min separated by 10s of screen-center cross fixation (total duration ~12min). Participants reported switches in perception through a button press. We considered that task would involve a relatively lower phasic response over a relatively larger tonic tone (high-tonic LC). **(C)** The auditory salience detection task consisted of an oddball paradigm requiring button-press reports on the perception of rare deviant target tones (20% occurrence) within a stream of frequent tones (total duration ~10min). We considered that task would involve a relatively larger phasic response over a lower relatively tonic tone (low-tonic LC).

MRI data acquisition and *preprocessing*

MRI data were acquired using a MAGNETOM Terra 7T MRI system (Siemens Healthineers, Erlangen, Germany). FMRI and sMRI data were preprocessed using SPM12, ANTs and SynthStrip brain extraction tool[23], as fully described previously[13]. The preprocessed data were resampled to a 1mm^3 resolution. Individual statistical analyses included one regressor of interest consisting of a switch in perception (perceptual rivalry) or the occurrence of a target deviant tone (salience detection) modeled as an event (convolved with the canonical hemodynamic response function - HRF). Participant movement, respiration, and heart rate were used as covariates of no interest (physiological data of 4 volunteers were not available).

Individual LC masks were manually delineated by 2 experts based on LC-specific images (as in[13]) to extract LC activity estimate or LC responses to the task (i.e., in arbitrary units – a.u. – as the mean value of the of the multiple regression fits over the LC mask) during both tasks and to compute a LC probabilistic map in the group space for visualization. The T1 structural whole-

brain image was used to extract individual total intracranial volume (TIV) using CAT12 toolbox.[24]

Statistics

Statistical analyses were performed in SAS 9.4 (SAS Institute, NC, USA) and consisted of Generalized Linear Mixed Models (GLMM) with 4 main sleep features of interest (REM sleep latency, REM sleep percentage, REM Theta energy, sigma power prior to each REM sleep episode) as the dependent variables (with adjusted distribution), LC activity as an independent variable, as well as age group (younger and older individuals), sex, TST and TIV. Outliers among all variables lying beyond four times the standard deviation were removed from the analysis (maximum 2 data points were removed). The final number of individuals included in each analysis is reported in **table 2** and **table 3**. Partial R^2 (R^{2*}) values were computed as previously described.[13] We used Pearson correlations to assess the association between transient pupil dilation and estimates of LC activity and for visualization of the GLMM outputs. The analyses included 4 main dependent variables of interest: Benjamini and Hochberg False Discovery Rate (FDR) correction considering 4 independent tests was used to test for significant associations [$p<.0125$ (for rank 1/4); $p<.025$ (for rank 2/4); $p<.0375$ (for rank 3/4); $p<.05$ (for rank 4/4)]. All models included an interaction term between LC activity and age group. If the Bayesian Index Criterion (BIC) of the model without the interaction was better (i.e. lower) and the interaction term was not significant it was removed from the model.

Importantly, statistical analyses are slightly modified compared to[13] with the inclusion of TIV as a covariate (rather than BMI) to take into account non-specific variation in EEG power

computations. Statistical outcomes related to the auditory salience detection task may therefore slightly vary compared with our previous publication.[13]

We 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),[25] taking into account a power of .8, an error rate α=.05, and a sample size of 52, we could detect medium effect sizes r>.39 (2-sided; CI:.13–.6; R^2>.15, CI:.02–.36) within a linear multiple-regression framework including 2 tested predictor (LC activity, age group) and 2/3 covariates (sex, TST, TIV where relevant).

Results

Fifty-two healthy individuals aged 18 to 31y (N=34; 28 women) and 50 to 70y (N=18; 14 women) completed an fMRI protocol consisting of a top-down visual perceptual rivalry task[26], which we considered could trigger a lower phasic response of the LC over a relatively larger tonic tone (high-tonic LC), and a bottom-up auditory salience detection task[27], which we considered could trigger a larger phasic response of the LC over a relatively lower tonic tone (low-tonic LC task). Both tasks successfully triggered a phasic response of the left LC when inspecting the whole-brain statistical analyses over the entire sample (**Figure 2A and B**). The recruitment of the LC was further supported by the fact that, in a subset of participants (see methods), we successfully detected significant transient pupil dilation around the event of interest (**Figure 2C, D, E and G**). The LC is indeed considered to drive part of the transient pupil dilation associated with the detection of salient stimuli or with changes in perception in a perceptual rivalry context.[27,28]

We therefore extracted the activity of the left LC in all individuals. We first found that transient pupil dilation during the salience detection task was significantly correlated to the activity estimate of the left LC (Pearson's r=.4, **p=.03**; **Figure 2H**), while it was not for the perceptual rivalry task (**Figure 2F**, Pearson's r=-.20, p=.29). Since pupil responses are associated with phasic responses of LC,[29] the absence of significant correlation in the perceptual rivalry task is in line with our hypothesis that this task involved more tonic LC activity. We further found that LC responses during both tasks were not correlated (Figure 3A) (Pearson's r= .08, p=.54), indicating that the activity of LC in one task did not linearly predict its activity in the other task and supporting that both tasks probe different aspects of LC activity. We did find a significant positive Pearson correlation (whole sample: r=0.357; p=0.010; in the young only: r=0.415; p=0.018; in the older only: r=-0.457, p=0.056) between LC activity estimate during the salience detection task and squared LC activity estimate in the perceptual rivalry task. This suggests a U-shape relationship between LC activity estimate in two tasks and may support the idea that tonic and phasic activities interact in a nonlinear manner. This would deserve future investigations.

We then targeted our main goal: testing relationships between LC activity and canonical characteristics of REM sleep estimated based on a night of sleep in the lab under EEG. Considering first the perceptual rivalry task, we found a significant positive association between REM theta energy and high-tonic LC activity (t=3.38; **p= .001**; **Figure 3B**) on top of the main effect of TST, while the other covariates were not significant (**Table 2)**. As previously reported,[13] we then found a significant association between REM theta energy and the interaction between low-tonic LC activity during the salience detection task and age group (t=2.68; **p=.01; Table 3**) that came on top of a main effect of LC activity (t=-2.78; **p=.008**) (**Figure**

265 **3C**) while the other covariates were not significant (**Table 3**). Post hoc contrasts revealed that
266 higher REM theta energy was associated with a lower response of the LC in older (t= -2.78;
267 **p=.008**) but not in younger (t=.33; p=.74) individuals, indicating that the main effect of LCactivity
268 was mainly driven by the older participants.

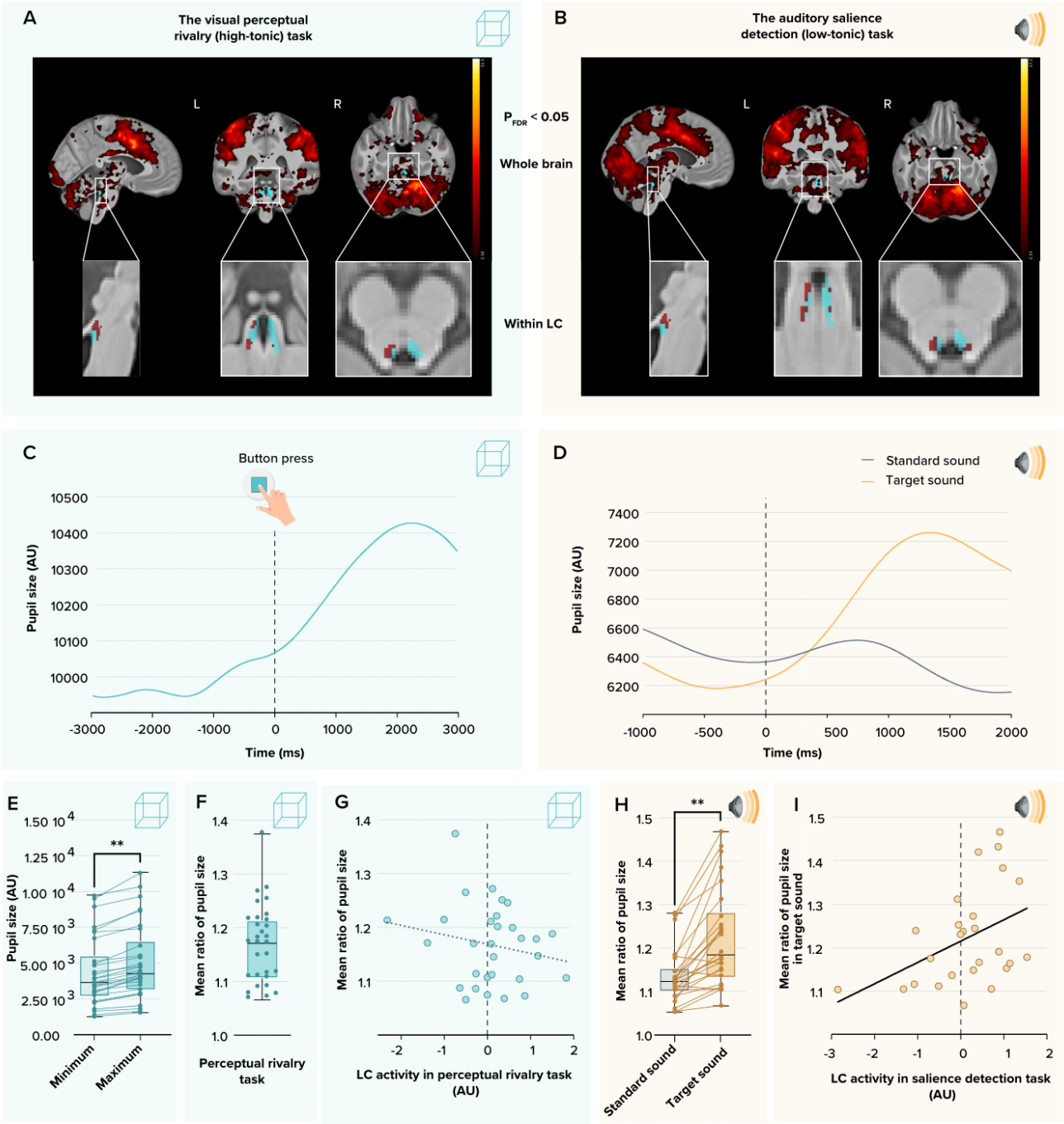

Figure 2. (A) Whole-brain and LC responses to the perceptual switches during the visual perceptual rivalry task. Sagittal, coronal, and axial views [MNI coordinates: (−4 −37 -21 mm)]. The images at the top show the whole-brain results using significance for a threshold of p< 0.05 FDR-corrected (t > 2.16) over the group average brain structural image coregistered to the MNI space. Insets at the bottom show the LC probabilistic template (blue) created based on individual LC masks and the significant activation detected within this mask (dark grey). **(B)** Whole-brain and LC responses to the target sound during the auditory salience detection task. Sagittal, coronal, and axial views [MNI coordinates: (−4 −34 -21 mm)]. The images at the top show the whole-brain results using significance for a

threshold of $p < 0.05$ FDR-corrected ($t > 2.33$) over the group average brain structural image coregistered to the MNI space. Insets at the bottom show the LC probabilistic template (blue) and the significant activation detected within this mask (dark grey). The legend shows the t-values associated with color maps. **(C)** A representative example of the pupil dilation in response to the perceptual switches during the visual perceptual rivalry task in one participant. **(D)** A representative example of the pupil dilation in response to the standard and target sounds during the auditory salience detection task in the same subject as in panel C. **(E)** Minimum and maximum pupil size before and after the perceptual switches during the visual perceptual rivalry task. Maximum pupil size was significantly higher than the minimum ($N=32$, $p < 0.0001$). Arbitrary units (AU) of the pupil size in the figures C, D and E are based on pixel number following proprietary algorithms application yet do not correspond exactly to pixel number. **(F)** Mean ratio of the pupil size in the perceptual rivalry task. The mean ratio of pupil size was computed as the change in the pupil diameter from before to after the perceptual switches. **(G)** Association between LC activity during visual perceptual rivalry task and mean ratio of pupil size in response to the perceptual switches. The association was not significant (Pearson's correlation $r=-0.201$, $P=0.29$). **(H)** Mean ratio of pupil size in response to standard sound and target sound during the auditory salience detection task. The mean ratio of pupil size was computed as the change in the pupil diameter from before to after the auditory stimulus presentation. The change in pupil size was significantly higher for target versus standard sound ($N=27$, $p < 0.0001$). (**I**) Association between LC activity during auditory salience detection task and mean ratio of pupil size in response to the target sound. We found a significant positive Pearson's correlation ($r=0.4$, $P=0.03$).

For both low-tonic and high-tonic LC activity the inclusion of REM sleep duration and/or REM sleep percentage instead of or on top of TST did not modify the statistical outputs of the models (data not shown). Furthermore, REM theta energy associations with the activity of the LC during both tasks remain similar when including them simultaneously in the same GLMM, i.e. a significant main effect of high-tonic LC activity ($F_{(1,41)}=9.52$, **p=.0036**) and a weak trend for interaction between low-tonic LC activity and age: $F_{(1,41)}=2.72$, $p=.1$), supporting that they explain different parts of the variance in REM theta energy (using squared values of LC activity estimates of each task separately or together estimates did not yield significant outputs – not shown).

Table 2. Associations between REM sleep metrics and LC activity estimated via the visual perceptual rivalry (high-tonic) task.

Sleep metric (dependent variable)	LC activity	Age group	Sex	TIV	Total sleep time
REM Theta energy (N=51)	F(1,45)=11.39 **P=0.001*** **R^{2*}=0.201**	F(1,45)=0.14 P=0.709	F(1,45)=0.22 P=0.645	F(1,45)=0.08 P=0.781	F(1,45)=5.15 **P=0.028** **R^{2*}=0.102**
Sigma power prior to REM (N=52)	F(1,46)=5.55 **P=0.023*** **R^{2*}=0.107**	F(1,46)=0.60 P=0.442	F(1,46)=3.64 P=0.062	F(1,46)=2.18 P=0.147	
REMS percentage (N=52)	F(1,47)=2.05 P=0.159	F(1,47)=13.27 **P=0.0007** **R^{2*}=0.220**	F(1,47)=1.66 P=0.204	F(1,47)=3.15 P=0.082	
REMS latency (N=52)	F(1,46)= 0.10 P= 0.750	F(1,46)= 2.01 P= 0.163	F(1,46)= 1.27 P= 0.265	F(1,46)= 0.27 P= 0.602	F(1,46)= 0.74 P= 0.392

Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).

* Significant following Hochberg False Discovery Rate (FDR) correction for 4 independent tests (see methods)

In all models the interaction between LC activity and age group was not significant. Goodness of fit metric (BIC) indicated that the interaction term should be removed. LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep.

No association was found when considering the other macroscopic REM sleep metrics (REM sleep onset latency, REM sleep percentage) (**Suppl. Figure S1, Table 2 and 3**). Further analyses indicated that the associations with REM theta energy were specific to this particular band as no significant association was uncovered between the energy of the other spectral bands of the EEG during REM sleep (except for a significant main effect of low tonic LC activity [t= -2.33; p=.025) and of an age-group-by-low tonic LC activity interaction (t= 2.14; p=.037) when using REM alpha energy as the dependent variable - **Suppl. Figure S2, Suppl. Table S1 and S2**]. The significant associations we detected were also specific to REM sleep as NREM slow wave energy

(SWE), i.e. the cumulated overnight power over the delta band (.5-4Hz) typical of NREM sleep, was not significantly associated with either low-tonic and high-tonic LC activity (**Suppl. Figure S2, Suppl. Table S1 and S2**).

Table 3. Associations between REM sleep metrics and LC activity estimated via <u>the auditory salience detection (low-tonic) task</u>.

Sleep metric (dependent variable)	LC activity	Age group	LC activity*age group	Sex	TIV	Total sleep time
REM Theta energy (N=51)	F(1,44)=6.74 **P=0.012*** **R^{2*}=0.132**	F(1,44)=0.54 P=0.468	F(1,44)=7.19 **P=0.010*** **R^{2*}=0.140**	F(1,44)=0.14 P=0.707	F(1,44)=0.10 P=0.750	F(1,44)=3.48 P=0.068
Sigma power prior to REM (N=50)	F(1,44)=1.90 P=0.175	F(1,44)=0.26 P=0.611	F(1,44)=2.35 P=0.132	F(1,44)=8.01 **P=0.007** **R^{2*}=0.154**	F(1,44)=3.11 P=0.084	
REMS percentage (N=52)	F(1,46)=1.38 P=0.245	F(1,46)=15.60 **P=0.0003** **R^{2*}=0.253**	F(1,46)=2.07 P=0.157	F(1,46)=0.23 P=0.635	F(1,46)=5.56 **P=0.022** **R^{2*}=0.107**	
REMS latency (N=52)	F(1,45)= 2.88 P= 0.096	F(1,45)= 0.75 P= 0.389	F(1,45)=1.40 P=0.242	F(1,45)= 2.07 P= 0.157	F(1,45)= 0.88 P= 0.351	F(1,45)= 0.96 P= 0.331

Prior to the analysis, we removed the outliers among all variables by excluding the samples lying beyond four times the standard deviation (the final number of individuals included in each analysis is reported below each dependent variable).

* Significant following Hochberg False Discovery Rate (FDR) correction for 4 independent tests (see methods)

In all models, the results without the interaction between LC activity and age group were not significant. Goodness of fit metric (BIC) indicated that the interaction term provides a better fitness.

LC: locus coeruleus; TIV: total intracranial volume; REM: rapid eye movement; REMS: rapid eye movement sleep.

345 In the next steps, we turned towards sigma power prior to REM sleep as it has been causally
346 related to LC activity during sleep in animal models.[16] Statistical analyses yielded a significant
347 positive main effect of high-tonic LC activity during the perceptual rivalry task (t=2.36; **p=0.022**;
348 **Table 2; Figure 3D**) and no significant interaction between LC activity and age group. In contrast,
349 no significant association with low-tonic LC activity during the salience detection task (p=0.17)
350 and no significant interaction between LC activity and age group were detected (**Table 3; Figure**
351 **3E**). In both analyses, we did not detect significant effects of the other covariates except for
352 main effect of sex: a statistical trend in the perceptual rivalry task (t=-1.91; p= 0.062; Table 2)
353 and a significant main effect in the salience detection task (t=-2.83; p= 0.007; Table 3), resulting
354 from men presented less sigma power compared to women (Suppl. Figure S4). As it is not the
355 main focus of our study, sex difference will not be discussed in detail. We note that the sex
356 difference in sigma power prior to REM may be related to the reduced percentage of REM sleep
357 sometimes found in other studies [30] (though REM sleep percentage did not differ between
358 sexes in our sample: t = -0.63; p = 0.52).

359 Finally, when including the activity of both tasks in the same GLMM with sigma power prior to
360 REM as the dependent variable, the analysis yielded a significant main effect of high-tonic LC
361 activity ($F_{(1,42)}$=7.62,**p=.0085**) as well as a trending interaction between low-tonic LC activity and
362 age ($F_{(1,42)}$=3.77, p=.058). As for REM theta energy, this result suggests that both types of LC
363 activity estimates explain different parts of the variance in sigma power prior to REM.

364 For completeness, the final steps of our analyses consisted of exploratory analyses for
365 associations between the activity of LC estimated during both tasks and other REM sleep

metrics, i.e. REM sleep duration, REM bouts duration, and number of arousals during REM sleep. These led to no significant association (**Suppl. Figure S3, Suppl. Table S1 and S2**).

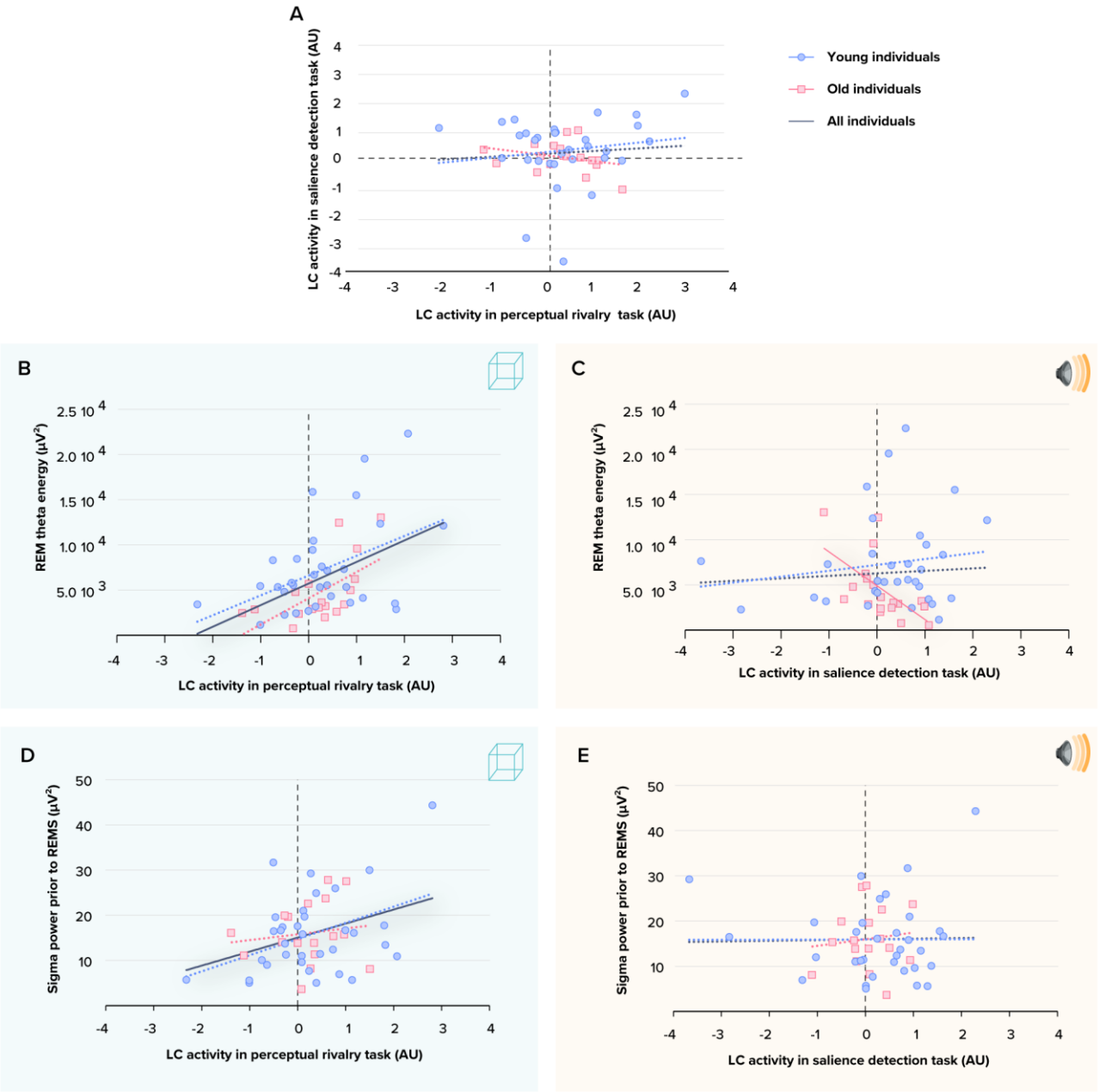

Figure 3. (A) LC activity estimates during the visual perceptual rivalry task and auditory salience detection task were not significantly correlated (p=0.54). **(B)** Association between REM theta energy and the LC activity estimates during the visual perceptual rivalry task. The GLMM yielded a significant main effect of LC activity (p=0.0015). **(C)**

Association between REM theta energy and the LC activity estimates during the auditory salience detection task. The GLMM yielded a significant age group by LC activity interaction (p=0.0103), and post hoc analyses led to a significant association for the older (p=0.008) but not the young group (p=0.75). **(D)** Association between sigma power prior to REM sleep and the LC activity estimates during the visual perceptual rivalry task. The GLMM yielded a significant main effect of LC activity (p=0.026). **(E)** Association between sigma power prior to REM sleep and the LC activity estimates during the auditory salience detection task. The GLMM showed neither a significant main effect of LC activity (p=0.859) nor a significant age group by LC activity interaction (p=0.132).

Simple regression lines are used for a visual display and do not substitute the GLMM outputs. The black line represents the regression irrespective of age groups (young + old, n = 52). Solid and dashed regression lines represent significant and non-significant outputs of the GLMM, respectively.

Discussion

Whether the variability in the functioning of human LC may contribute to the interindividual variability in sleep is not established. In the present cross-sectional study, we show that the activity of the LC, assessed during wakefulness through 2 distinct cognitive tasks, is related to the variability of REM sleep intensity, as reflected by the energy of the theta band of the EEG. We find that the associations depend on the task considered - presumably because they do not rely on the same balance between phasic and tonic LC activity, and on age - when considering LC activity during the low-tonic task. In addition, we find that the amount of sigma band oscillations immediately preceding REM sleep is associated with the activity of the LC assessed during the high-tonic task. These findings add to the existing view that LC function is essential for sleep regulation that was mainly brought by animal experiments. We show that the *in vivo* variability in the activity of the human LC may govern part of the variability in sleep quality assessed using electrophysiology. The findings may have implications for the neuropathology of several brain disorders such as insomnia, Alzheimer's disease (AD), and Parkinson's disease (PD) for which the LC may play a pivotal role through sleep.

398 LC cells discharge action potentials in both tonic and phasic modes during wakefulness. The
399 tonic mode consists of irregular but constant baseline activity (1-6 spikes/s), while phasic activity
400 is characterized by short (<300ms) bursts of high-frequency activity followed by a long period of
401 sustained inhibition of spontaneous activity.[31] A balanced tonic activity is required for an
402 optimal arousal level allowing the expression of phasic activity in response to salient stimuli or
403 behavioral changes.[15] While the LC was considered for long as a quiet region during sleep,
404 recent animal studies revealed that the activity of the LC varies importantly during NREM sleep
405 and may even transiently reach activity levels similar to wakefulness.[10] NE level, driven by LC
406 neuron activity, fluctuates during NREM sleep between periods of higher and lower NE tone
407 following an infraslow rhythm of ~50s.[10] This periodic decrease in NE tone must reach levels
408 that are low enough to open the gate for REM sleep, which is considered to require sustained
409 NE-free periods to allow, for instance, synaptic pruning.[4] The periodic decrease of the LC
410 activity is reflected in periodic increases in the expression of sleep spindles and in sigma power
411 such that each REM episode is preceded by a pronounced transient increase in sigma power[32]
412 that is well known to somnologists. The overall picture emerging from this recent literature is
413 that LC activity shapes the macro- and microstructure of sleep and that inappropriate
414 fluctuations in LC tonic activity can disturb sleep and in particular REM sleep.[4,10]

415 We find that a higher phasic response of the LC during the top-down perceptual rivalry task,
416 arguably characterized by a relatively high tonic activity of the LC is associated with a larger
417 expression of theta oscillations during REM sleep and of sigma power prior to REM sleep
418 episodes. We interpret this finding as the reflection of a more appropriate lower tonic activity of
419 the LC which allows a better expression of its phasic response during the task. In contrast, if

420 tonic activity during the task is too high, it partly masks the phasic response of the LC. We detect
421 this during wakefulness but surmise that it constitutes a trait such that the more appropriate
422 tonic activity of the LC present during wakefulness would also be present during sleep. This
423 would mean that, in the case of a low tonic LC activity allowing for a large dynamic range of
424 phasic LC oscillation, NE tone reaches a lower level prior to a REM sleep episode, allowing a
425 large expression of sigma oscillation and the initiation of a REM sleep episode and complete
426 silencing during REM. This would in turn enhance the expression (intensity) of REM theta
427 energy, the most typical oscillatory mode of REM sleep. Theta activity during REM sleep is
428 considered to reflect hippocampus activity and plays key roles in memory consolidation during
429 REM sleep.[33] The putative more appropriate tonic activity of the LC we report could therefore
430 reflect a better expression of REM sleep functions.

431 In contrast, we find that a larger expression of the phasic response of the LC during the bottom-
432 up salience detection task, which is arguably underlined by a lower tonic activity of the LC, is
433 associated with lower expression of theta oscillations during REM sleep, particularly in older
434 individuals. We link our results to the recent report in rodents that the so-called startle effect,
435 which is triggered by abrupt sensory changes, is attenuated under higher vs. lower arousal levels
436 as well as when LC activity is induced prior to the salient stimulation.[34] This was in line with
437 the established observation that elevated tonic firing of the LC-NE neurons makes the LC
438 sensory-evoked response less pronounced.[31] This would mean that in individuals with lower
439 tonic activity of the LC during wakefulness, the dynamic range of the phasic response of the LC is
440 potentially large. This trait-like phasic LC activity could become maladaptive and during sleep,
441 perturb the activity of limbic areas, thereby resulting in a reduction in REM theta oscillations.

This process becomes particularly manifest in aging which is characterized by increased sleep fragility and degradation of sleep quality.[35]

Overall, we interpret our findings as a reflection of the requirement for a balanced tonic LC activity for optimal REM sleep. This notion is supported, in our view, by the fact that our statistical outputs remain similar (either significant or as weak trends) when including concomitantly the activity of the LC as assessed in a low-tonic and high-tonic context. Our findings may further be related to the diversity in neuron types within the LC that is emerging in the literature, for instance, if both tasks were recruiting different proportions of LC neurons co-expressing or not dopamine or projecting to different brain territories[6] and were therefore characterizing the activity of these different neurons during sleep.

We stress that, although plausible, our interpretations remain putative and that measures of LC activity during sleep are required to confirm our hypotheses. Future studies could assess the connectivity of the LC with other brain structures in humans, as sleep and wakefulness regulation relies on a network of subcortical nuclei rather than on the LC alone.[36] Furthermore, while we interpret our finding in “LC centric” manner, i.e. the activity of the LC during wakefulness reflects its activity during sleep, it may also be that the activity of the LC during wakefulness shapes the activity of other brain areas during sleep. Sleep oscillations depend indeed in prior wakefulness in a regional use-dependent manner[37] such that the impact of NE tone and LC activity on other regions, and particularly over cortical regions, may be reflected in a larger or lower expression of theta oscillation during REM.

462 Beyond the healthy population, our findings are in line with the view that inappropriate LC
463 activity during both wakefulness and sleep can contribute to insomnia disorder.[4] Insomnia is
464 characterized by a hyperarousal state that is presumably due in part to inappropriately high
465 tonic activity of the LC during wakefulness, contributing to delayed sleep onset, and during
466 sleep, contributing to early and/or multiple awakenings. This would also lead to disturbed REM
467 sleep and unresolved emotional distress.[4] Our participants being healthy and devoid of sleep
468 disorders, our finding could reflect the continuum between normal sleep and insomnia. They
469 could also be indicative of the neuropathological mechanisms eventually leading to insomnia,
470 i.e. a prolonged inappropriate LC tonic activity and REM sleep disturbance are consequences or
471 contribute to the progressive changes leading to insomnia or maintaining the disorder. LC is also
472 central to several neurodegenerative diseases characterized by preclinical alteration in sleep.
473 The LC is indeed among the first sites showing abnormal tau and alpha-synuclein aggregates,
474 hallmarks of AD and PD, respectively.[38,39] Abnormal tau inclusions can even be detected post-
475 mortem as early as during the second decade of life[38] and were suggested to be associated
476 with larger cortical excitability in late middle-aged healthy individuals.[40] In addition, *post-*
477 *mortem* degeneration of the human LC was recently associated with alteration in the rest-
478 activity cycle over the ~7y preceding death, particularly in those with higher cortical AD
479 pathology.[41] Future research could try to link our findings to assessments of tau and alpha-
480 synuclein burden within the LC, though the level of protein aggregate would go undetected *in*
481 *vivo* in young individuals, and would remain challenging to assess over such a deep and small
482 nuclei in older individuals.

483 As previously mentioned,[13] although our study provides new insights into the associations
484 between LC activity and sleep variability in humans, it bears limitations. The baseline night of
485 sleep was followed by the fMRI acquisition the next day in young individuals, while in the older
486 group, there was a ~1y gap between the baseline night and the fMRI session. Although sleep
487 changes over the lifespan,[42] it is stable over a short life period (e.g. a few years).[43] We
488 therefore consider that this important limitation is unlikely to fully explain our results,
489 particularly given the fact that no age-related differences were found for the high-tonic
490 perceptual rivalry task. Moreover, our sample was primarily composed of women. Although it
491 was considered in our statistical analyses, this limits the generalizability of our results. In
492 addition, although it represents a considerable data collection effort, the size of our sample
493 remains modest, particularly for the older subsample. Replication in a larger sample is therefore
494 warranted. Future studies could also apply individually tailored HRF to assess LC response. We
495 used the canonical HRF to model activity over the entire brain to model average LC response,
496 while individual LC responses can differ across individuals.[44] Likewise, we used multiple linear
497 regression approaches for the statistical, mostly precluding isolation of non-linear relationships.
498 Finally, the LC activity was measured using task-evoked fMRI BOLD signals, which reflect changes
499 in neural activity relative to a baseline condition. Although, on average responses were positive
500 (cf. Figure 2 A-B), the negative values in LC activity detected in some participants (cf. Figure 2 G
501 & I) represent instances where the BOLD signal in the LC during a task condition is lower than
502 the baseline signal. The meaning of negative BOLD value is unclear. They may reflect differences
503 in individual task engagement, arousal levels, or baseline LC activity. Further investigation is

needed to better understand the underlying physiological or methodological factors contributing to this phenomenon.

Conclusions

In summary, we provide original evidence that, aside from its integrity, the functioning of the LC shapes part of the quality of sleep. Our data suggest that an optimal level of LC tonic activity is required for an optimal expression of REM sleep and its functions. This reinforces the view that the LC-NE is promising for interventions aiming at improving sleep quality, including preventing and/or delaying brain disorders. Our findings suggest that these interventions could aim at restoring optimal LC functioning during sleep as well as during wakefulness.

515 **List of abbreviations**

516 **AD** - Alzheimer's disease

517 **BAI** - Beck Anxiety Inventory

518 **BDI**- Beck Depression Inventory

519 **BMI** - Body Mass Index

520 **CI** - confidence intervals

521 **EEG** - electroencephalogram

522 **ESS**- Epworth Sleepiness Scale

523 **FDR** - False Discovery Rate

524 **fMRI** - functional Magnetic Resonance Imaging

525 **GLMM** - Generalized Linear Mixed Models

526 **LC** - locus coeruleus

527 **NE** - norepinephrine

528 **PD** - Parkinson's disease

529 **PSQI**- Pittsburgh Sleep Quality Index

530 **REM** - Rapid Eye Movement

531 **REMS**- rapid eye movement sleep.

532 **SD** – standard deviation

sMRI - structural MRI

SWE- slow wave energy

TIV - Total Intracranial Volume

TST - Total Sleep Time

Declarations

Ethics approval and consent to participate

This study was approved by the faculty-hospital ethics committee of ULiège. All participants provided written informed consent and received financial compensation.

Consent for publication

Not applicable.

Availability of data and materials

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/fasst/Balanced_LC_activity_and_REM_sleep. 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 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).

552 **Competing interests**

553 All authors declare no conflict of interest.

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569 **Authors' contributions**

570 Study concept and design by NM, GV and PM. Data acquisition and analysis by NM, PT, EK, RS,
571 EB, AB, IC, IP, FB, ID & SS. Methodological support and/or support in the interpreting the data by
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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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