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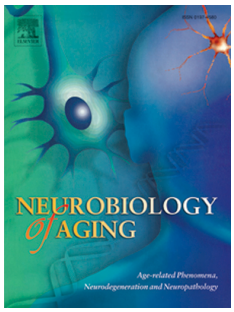

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PII: S0197-4580(19)30044-2

DOI: https://doi.org/10.1016/j.neurobiolaging.2019.02.004

Reference: NBA 10502

To appear in: *Neurobiology of Aging*

Received Date: 5 October 2018

Revised Date: 24 January 2019

Accepted Date: 2 February 2019

Please cite this article as: Gaggioni, G., Ly, J.Q.M., Muto, V., Chellappa, S.L., Jaspar, M., Meyer, C., Delfosse, T., Vanvinckenroye, A., Dumont, R., Coppieters ‘t Wallant, D., Berthomier, C., Narbutas, J., Van Egroo, M., Luxen, A., Salmon, E., Collette, F., Phillips, C., Schmidt, C., Vandewalle, G., Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition, *Neurobiology of Aging* (2019), doi: https://doi.org/10.1016/j.neurobiolaging.2019.02.004.

Age-related decrease in cortical excitability circadian variations during sleep loss and its links with cognition

Giulia Gaggioni[a,b], Julien Q.M. Ly[a,b,c], Vincenzo Muto[a,b], Sarah L. Chellappa[a,b,#], Mathieu Jaspar[a,b,d], Christelle Meyer[a,b], Tillo Delfosse[a], Amaury Vanvinckenroye[a], Romain Dumont[a], Dorothée Coppieters 't Wallant[a], Christian Berthomier[e], Justinas Narbutas[a], Maxime Van Egroo[a], Andé Luxen[a], Eric Salmon[a,c,d], Fabienne Collette[a,d], Christophe Phillips[a,f], Christina Schmidt[a,d], Gilles Vandewalle[a,b,*]

[a] GIGA-Institute, Cyclotron Research Center/In Vivo Imaging, University of Liège, 4000 Liège, Belgium

[b] Walloon Excellence in Life sciences and Biotechnology (WELBIO), Belgium

[c] Department of Neurology, CHU Liège, 4000 Liège, Belgium

[d] Psychology and Neuroscience of Cognition Research Unit, Faculty of Psychology and Educational Sciences, University of Liège, 4000 Liège, Belgium

[e] Physip, 75011 Paris, France

[f] GIGA- Institute, In silico Medicine, University of Liège, 4000 Liège, Belgium

[#] Current address: Medical Chronobiology Program, Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, MA 02115, USA

[*] Correspondence to: Gilles Vandewalle, GIGA-Institute, Cyclotron Research Center/In Vivo Imaging, Bâtiment B30, Allée du VI Août, 8, 4000 Liege, Belgium, +3243662316, gilles.vandewalle@uliege.be

ABSTRACT

Cortical excitability depends on sleep-wake regulation, is central to cognition and has been implicated in age-related cognitive decline. The dynamics of cortical excitability during prolonged wakefulness in aging are unknown, however. Here, we repeatedly probed cortical excitability of the frontal cortex using transcranial magnetic stimulation and electroencephalography in thirteen young and twelve older healthy participants during sleep deprivation. While overall cortical excitability did not differ between age groups, the magnitude of cortical excitability variations during prolonged wakefulness was dampened in older individuals. This age-related dampening was associated with mitigated neurobehavioural consequences of sleep loss on executive functions. Furthermore, higher cortical excitability was potentially associated with better and lower executive performance, respectively in older and younger adults. The dampening of cortical excitability dynamics found in older participants likely arises from a reduced impact of sleep homeostasis and circadian processes. It may reflect reduced brain adaptability underlying reduced cognitive flexibility in aging. Future research should confirm preliminary associations between cortical excitability and behaviour, and address whether maintaining cortical excitability dynamics can counteract age-related cognitive decline.

Keywords: ageing, circadian, cognition, cortical excitability, sleep

1. Introduction

The intrinsic excitability, or reactivity, of cortical neuronal cells is a basic, yet essential, feature of brain function (Rizzo et al., 2015). Cortical excitability reflects inherent cellular properties of neurons that arise from the combined impacts of multiple parameters [e.g. ion concentration in the intra- and extracellular milieus, neuromodulator actions, membrane potential, action potential threshold] (Bushey et al., 2015; Frank and Cantera, 2014; Meisel et al., 2015; Rizzo et al., 2015; Tononi and Cirelli, 2014). Cortical excitability is grounded in the responsiveness and response selectivity of cortical neurons which determines, at least in part, how an input is processed by the brain and is therefore central to cognition. In fact, a decrease in neuron excitability has been implicated in the cognitive decline found in normal and pathological aging (Chang et al., 2005; Rizzo et al., 2015). Critically, cortical excitability was recently demonstrated to vary substantially during wakefulness and following sleep (Huber et al., 2013; Ly et al., 2016). Yet, the regulation of sleep and wakefulness profoundly change in aging (Schmidt et al., 2012). Whether these age-related changes affect cortical excitability is unknown.

Two fundamental mechanisms regulate sleep and wakefulness and their associated cognitive functions: sleep homeostasis and the circadian system (Dijk and Czeisler, 1995; Schmidt et al., 2012). During the day, the circadian signal opposes the homeostatic build-up of sleep need to maintain wakefulness and cognition, up to the evening, shortly before habitual sleep onset (Dijk and Czeisler, 1995). At night, the circadian system promotes sleep to favor sleep continuity, up to the end of the biological night, shortly before habitual wake up time (Dijk and Czeisler, 1995). Any disturbance in this fine-tuned interplay is detrimental for cognition (Lo et al., 2012; Schmidt et al., 2012). An extreme disruption consists in prolonging wakefulness overnight: cognition is greatly compromised because the circadian system promotes sleep at a time of high sleep need (Lo et al., 2012; Schmidt et al., 2012). If wakefulness is further prolonged the next day, the wake-promoting signal of the circadian system rescues in part cognition (Lo et al., 2012). Thus, because of the interplay between the homeostatic and circadian processes, all periods of prolonged wakefulness are not equivalent or linearly related to one another. Likewise all aspects of cognition are also not equally affected by sleep loss: the magnitude of the detrimental impact of insufficient sleep and prolonged wakefulness during the biological night has been most repeatedly observed and showed strongest effect sizes for monotonous tasks with high attentional demands, at least in young adults (Lo et al., 2012). At the level of cortical excitability, the interplay between sleep homeostasis and the circadian system is reflected in

young individuals in an overall increase in excitability following 24 h of continuous wakefulness – attributed to the build-up of sleep need (Huber et al., 2013; Ly et al., 2016) – and in more local variations around the evening and early morning – attributed to the influence of the circadian system (Ly et al., 2016).

Even in the absence of clinically significant sleep disorders, aging is characterized by deterioration in sleep-wake regulation. In healthy older individuals, sleep intensity, duration and continuity decrease (Dijk et al., 1999; Klerman and Dijk, 2008; Schmidt et al., 2012; Van Cauter, 2000), but these changes are not systematically accompanied by increased daytime sleepiness (Klerman and Dijk, 2008). In fact, sleep need and its build-up during wakefulness decrease as one gets older (Landolt et al., 2012; Schmidt et al., 2012). Concomitantly, the timing of the circadian system is advanced and the strength of the circadian signal has been suggested to decrease (Dijk et al., 1999; Kondratova and Kondratov, 2012; Münch et al., 2005). Overall, these combined changes lead to changes in cognition. The acute detrimental cognitive effect of sleep loss is reduced in aging (Landolt et al., 2012; Sagaspe et al., 2012; Schmidt et al., 2012): even though they may achieve overall lower performance than young adults, older individuals suffer relatively less during a night without sleep, at least over several cognitive domains, including vigilant attention, executive function (inhibitory motor control) and mental arithmetics. Whether these changes in cognition regulation during wakefulness may arise from alterations in the impact of sleep homeostasis and of the circadian system on cortical excitability is unknown, however. This question is important because long-term age-related sleep-wake changes lead to a fragmentation of the normal waking-rest cycle - e.g. more wakefulness during night-time sleep – that is associated with an overall decline of cognitive abilities in older individuals (Lim et al., 2013; Oosterman et al., 2009).

Here, we repeatedly probed cortical excitability in healthy older and younger individuals during prolonged wakefulness. We used Transcranial Magnetic Stimulation (TMS) coupled to Electroencephalogram (EEG) to record direct perturbations of cortical neuron activity - bypassing sensory systems - using identical stimulations delivered over the exact same brain location. Since frontal brain regions are particularly prone to both ageing (Reuter-Lorenz and Park, 2014) and the interplay between circadian and homeostatic processes (Landolt et al., 2012; Schmidt et al., 2012), cortical excitability was assessed over the frontal cortex. We hypothesized that fluctuations in cortical excitability during prolonged wakefulness would be reduced in older participants, particularly at critical

time-points for the interplay between the circadian alerting signal and the homeostatic sleep pressure, i.e. in the evening and the end of the biological night – when the circadian signal maximally/minimally opposes high sleep pressure, respectively. Our protocol also included repeated cognitive test batteries, spanning executive and attentional domains. We therefore explored whether a lower but stable cortical excitability profile in older individuals during wake extension would be associated with reduced performance impairment during sleep loss.

2. Material and Methods

2.1 Participants. The study was approved by the Ethics Committee of the Medicine Faculty of the University of Liège. Participants gave their written informed consent and received a financial compensation. Twenty-six healthy participants were enrolled, 13 older adults (62.6 y ± 3.8; 7 women) and 13 young (22.8 y ± 2.9; 5 women). Exclusion criteria included: 1) Body Mass Index (BMI) < 18 and > 28; 2) recent psychiatric history, severe trauma, sleep disorders; 3) addiction, chronic medication; 4) smokers, excessive alcohol (> 14 doses/week) or caffeine (> 3 cups/day) consumption; 5) night shift workers during the last year; 6) transmeridian travel during the last two months; 7) anxiety or depression; 8) poor sleep quality; 9) excessive self-reported daytime sleepiness; 10) early signs of dementia (in older participants). Anxiety was measured by the 21 item Beck Anxiety Inventory (BAI ≤ 14) (Beck et al., 1988); mood by the 21 items Beck Depression Inventory II (BDI-II ≤ 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index Questionnaire (PSQI ≤ 7) (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (ESS ≤ 11) (Johns, 1991); early signs of dementia using Mattis scale (Mattis, 1988). Chronotype was also assessed using the Horne-Östberg Questionnaire (Horne and Östberg, 1976). One older participant was removed because his performance was 3 interquartile ranges above or below the 25th and 75th percentile of the older participant sample across all cognitive tasks. **Table 1** summarizes the demographic characteristics of the final study sample.

Insert Table 1

2.2 Experimental protocol. At least a week before the experiment, participants completed a preparatory TMS-EEG session to determine optimal TMS parameters for artefact-free recodings. As in

(Huber et al., 2013; Ly et al., 2016), the left or right superior frontal gyrus was set as stimulation target for right or left-handed, respectively. Participants also completed a screening night of sleep to exclude major sleep disorders (periodic leg movement; apnea-hypopnea index > 15/h). During the 7 days preceding the study, they kept a regular sleep-wake schedule (± 15 min; verified using wrist actigraphy - actiwatch, Cambridge Neurotechnology, UK - and sleep diaries). Schedule and duration were based on at least 10 days of unconstrained actimetry recordings and/or self-reported sleep times and duration. Participants were requested to abstain from all caffeine and alcohol-containing beverages for 3 days preceding the study.

The experiment consisted in a constant routine (i.e. light < 5 lux, temperature ~19°C, regular isocaloric liquid meals and water, semi-recumbent position, no time-of-day information, sound proofed rooms) sleep deprivation protocol, which has repeatedly been a successful mean to assess in-lab inter-individual differences in sleep homeostatic and circadian interplay (Duffy and Dijk, 2002). Participants were maintained in dim light for 5.5 h (< 5 lux), during which they were trained to the cognitive test batteries, prior to sleeping at their habitual bedtime, for their habitual duration (in complete darkness) (**Fig. 1a**). The TMS-compatible electrode cap was placed upon awaking prior to sustained wakefulness period under 34 h of constant routine conditions. TMS-evoked EEG potentials were recorded 9 times (1000, 1600, 2000, 2200, 0100, 0500, 0700, 1000, 1600 for a subject sleeping from 2300 to 0700). Cognitive test batteries were carried out 13 times during the protocol in between TMS-EEG sessions (1100, 1500, 1700, 1900, 2100, 2300, 0200, 0400, 0600, 0800, 1100, 1300, 1500). Overall, the study included 1,500 protocol hours with multiple measures including 225 TMS-EEG sessions derived from 13 young and 12 older participants.

Insert Fig.1

2.3 TMS-evoked EEG response acquisitions and processing. Stimulation target was located in the superior frontal cortex on individual structural MRI by means of a neuronavigation system (Navigated Brain Stimulation; Nexstim) (**Fig. 1b**). This device allows for reproducible evoked EEG responses and precise target location (FDA approval for presurgery). TMS pulses were generated by a Focal Bipulse 8-coil (Nexstim, Helsinki, Finland). Each TMS-EEG session included 250-300 trials. Interstimulus intervals were randomly jittered between 1900 and 2200 ms. TMS responses were recorded with a 60-channel TMS-compatible EEG amplifier (Eximia; Nexstim), equipped with a proprietary sample-and-

hold circuit that provides TMS artifact free data from ~5 ms post-TMS (Virtanen et al., 1999). Electrooculogram (EOG) was recorded with two additional bipolar electrodes. Participants wore the EEG cap during the entire constant routine protocol, and electrodes impedance was set below 5 kΩ prior to each recording session. Signal was band-pass-filtered between 0.1 and 500 Hz and sampled at 1450 Hz. Each TMS-EEG session ended with a neuronavigated digitization of the location of each electrode. Auditory EEG potentials (AEP) evoked by TMS and bone conductance were minimized by diffusing a continuous loud white masking noise through earplugs, and applying a thin foam layer between the EEG cap and the TMS coil. Each session was followed by a sham session consisting in 30-40 TMS pulses delivered parallel to the scalp while white noise was diffused at the same level. Absence of AEP was checked online on Cz between 0-500 ms post-TMS (all sessions were AEP-free). Data of sham sessions were not considered any further.

EEG data were processed using SPM12 (Statistical Parametric Mapping 12, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 2015 (The Mathworks Inc, Natick, MA). Processing included the following: visual rejection of artefact, re-referencing to average of good channels, low-pass filtering at 80 Hz, resampling from 1450 to 1000 Hz, high-pass filtering at 1 Hz, epoching between –100 and 300 ms around TMS pulses, baseline correcting (-100 to -1 ms pre-TMS), robust averaging. Cortical excitability was inferred from the slope of the first EEG component (0-35 ms) of the TMS evoked potential (TEP; ~ 250 trials per session), measured at the artefact free electrode closest from the frontal hotspot (i.e. the brain location with highest TMS-induced electrical field estimated by the neuronavigation system) (**Fig. 1b**). This electrode was always located in the stimulated brain hemisphere. It could vary across participants but remained constant at the individual level.

The neuronavigation system ensured that hotspot location remained constant across sessions within an individual (± 2 mm). Across individuals, hotspot location varied. The mean coordinates (x, y, z ± SD; MNI space) of the hotspot across all subjects was [-6.6 ± 3.2, 10.1 ± 9.8, 71 ± 4.3], while across young or older individuals only, it was [-6.1 ± 3.6, 11.8 ± 7.5, 70 ± 2.8] and [-7.1 ± 2.9, 8.3 ± 11.9, 72.1 ± 5.5], respectively [nb: coordinates of the right hemisphere (case of 3 volunteers) were transpose to the homologue location in the left hemisphere, for average location computation]. Averages in each group are therefore < 1.8mm in either direction from the overall average, indicating that the area of the superior frontal cortex stimulated was similar in each group. To further assess

whether hotspot location could contribute to potential group differences, we computed the distance between individual hotspot (median location across all TMS sessions) and average location within each group. Statistical analyses (Wilcoxon rank-sum test) revealed no significant difference between both groups (**Table 1**).

2.4 Cognitive test batteries, placed in between TME-EEG recordings, were administered in the same following order to all participants:

2.4.1) GO/NO-GO task. This tasks probes motor inhibition (Sagaspe et al., 2012) and requires to press a keypress as quickly as possible for the frequent letter “M”, and to refrain from responding for the target “W” (320 trials; 20% of NO-GO targets; ~ 8.5 min). Letters were displayed for 200 ms and stimulus onset asynchrony randomly varied between 1500 and 1900 ms. Our main performance measure consisted in the number of false alarm (i.e. commission error rate of NO-GO trials, keyboard response).

2.4.2) N-back tasks. These tasks require continuous updating of presented information (Lo et al., 2012). Participants were instructed to state whether or not the current letter was identical to the consonant presented 2 and 3 stimuli earlier, respectively for the 2-back and 3-back tasks, by pressing one of two possible keys of the keyboard (75 trials per task; 30% of targets; 2.5 min). Stimulus onset asynchrony was 2 s and letter was displayed for the entire 2 s. D-prime - a response discriminability index (i.e. a measure of sensitivity, following the signal detection theory (Ingleby, 1967)) - was computed for both versions of the task. The n-back task is sensitive to ageing (De Beni and Palladino, 2004) and is a difficult task for older individuals, particularly the 3-back version. Although comprehension of the instructions and accuracy was verified during the training prior to baseline sleep, three older subjects did not apply the instructions correctly (e.g. they only responded every 2 or 3 items or less), or did not do the task at all, as indicated by a D-prime value close to zero. These subjects were removed from the analyses leaving, for this analyze, 13 young individuals and 9 older individuals. Thus, associations between cortical excitability and behavior are to be considered as preliminary results.

2.4.3) Psychomotor Vigilance Task (PVT). This task probes vigilant attention (Basner and Dinges, 2011) and requires participants to press a computer space bar as soon as a chronometer pseudo-randomly starts on the screen (random interval of 2-10 s; 48 trials per task; 5 min). Performance was

inferred from the mean reaction time following removal of anticipation (< 100 ms), and lapses (> 500 ms) [and error (> 3000 ms)].

2.4.4) Visuomotor vigilance continuous tracking task (CTT). This task also probes vigilant attention and was performed during the TMS-EEG recordings (as in (Huber et al., 2013; Ly et al., 2016)). It consists of keeping a constantly randomly moving cursor on a target located in the center of a computer screen, using a trackball device. The task was preferred to PVT during TMS-EEG recordings because it only requires continuous smooth and limited movement of a single finger and allows for continuous vigilance monitoring. Performance was computed as the average distance (in pixels) between the cursor and the target during TMS-EEG recordings, following removal of lapses. If signs of drowsiness were detected while performing the task during TMS-EEG sessions, the experimenter briefly touched the participant. Transitory lapses of vigilance resulted in temporary increases of the target-cursor distance, and could be automatically detected offline. A lapse was identified when the cursor was located outside a central 200 by 200 pixel box surrounding the target for > 500 ms from the last trackball movement. The lapse period ranged from the last trackball movement until the lapse detection. TMS evoked responses occurring during and < 1 s from a lapse period were discarded from analyses.

2.5 Salivary melatonin and cortisol samples were first placed at 4°C, prior centrifugation and congelation at -20°C within 12 h. Salivary melatonin and cortisol were measured by radioimmunoassay (Stockgrand Ltd, Guildford, UK), as previously described (English et al., 1993). Most samples were analyzed in duplicate. The limit of detection of the assay for melatonin was 0.8 ± 0.2 pg/ml using 500 µL volumes, while it was 0.37 ± 0.05 nmol/L using 500 µL volumes (Read et al., 1977). Estimation of individual's dim light melatonin onset (DLMO = phase 0°) was determined based on raw values. The 4 first samples were disregarded and maximum secretion level was set as the median of the 3 highest concentrations. Baseline level was set to be the median of the values collected from "wake-up time + 5 h" to "wake-up time + 10 h". DLMO was computed as the time at which melatonin level reached 20% of the baseline to maximum level (linear interpolation).

2.6 Sleep EEG data were recorded using a M7000 amplifiers (EMBLA, NATUS, Planegg, Germany) according to the 10/20 system. The habituation night montage consisted of a full polysomnograpy with

5 EEG channels (Fz, Cz, Pz, Oz, C3) referenced to left and right mastoids (A1, A2), 2 bipolar EOG, 2 bipolar electrocardiogram (ECG) channels, 2 bipolar electrodes place on the chin (electromyogram – EMG), 2 bipolar electrodes placed on a leg to check for periodic movements, thoracic and stomach respiratory belts, nasal cannula and an oximeter for sleep related breathing disorder detection. Baseline night montage consisted of 11 EEG channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, O2) referenced to left and right mastoids (A1, A2), 2 bipolar EOG and 2 bipolar EMG channels. EEG data were digitized at a sampling rate of 200 Hz. Sleep EEG recordings were automatically scored using a validated algorhythm (ASEEGA, PHYSIP, Paris, France), including artefact rejection (Berthomier et al., 2007). Three recordings of young participants were rejected because of artefacted signal. Total time spent in bed (TIB), total sleep time (TST), sleep efficiency (SE; the ratio between TST and TIB in %) are reported in **Table 1**. The other aspects related to sleep will be reported elsewhere.

2.7 Statistics. The circadian phase of all data points was estimated relative to individual DLMO (i.e. phase 0°, 15° = 1 h). All data points were resampled following linear interpolation at the theoretical phases of the TMS-EEG sessions in the protocol (**Fig. 1a**): -150°, -60°, 0°, 30°, 75°, 135°, 165°, 210° and 270°. Data were not extrapolated beyond 15° (i.e. 1 h), such that resampling at 300° could not be carried out for the majority of the participants and was advanced at 270° instead. For analyses only including cognitive test batteries, data were resampled every 30°, following linear interpolation, from -135° to 255°. Data points situated 3 interquartile ranges above or below the 25^{th} and 75^{th} percentile were defined as extreme outliers and removed (up to two data points were removed per analyses, i.e. 1-2% data points per analyze).

Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA). *T*-test on independent samples compared group characteristics (Chi squared for proportion comparisons; **Table 1**). Wilcoxon rank-sum test compared sleep, melatonin, cortisol and relative distance mean values by group (non-normal distribution). Generalized linear mixed models (PROC GLIMMIX) were applied to compute all statistics following determination of the dependent variable distribution (using Allfitdist Matlab function). Subject (intercept) effect was included as random factor. Circadian phase was included as the repeated measure together with an autoregressive estimation of autocorrelation of order 1 [AR(1)], and the covariance structure specified both the subject and group effect. In all GLMMs, degrees of freedom were estimated using Kenward-Roger's correction (they are

reported between brackets for each test). If an interaction term was significant, simple effects were assed using post-hoc contrasts (difference of least square means) adjusted for multiple testing with Tukey's procedure. Betas (i.e. regression coefficient) were derived by applied the ESTIMATE statement. Differences of beta between age groups were not corrected for multiple comparisons. Regressions were used for visual display only, and not as a substitute of the full GLMM statistics.

When analyzing the time course of a given variable (i.e. cortical, behavioral and endocrine measures), GLMM model included circadian phase, age group and their interaction. When seeking for associations between cortical excitability (slope of the first TMS evoked EEG response) and behaviour, GLMM model included cortical excitability, the four circadian periods of the protocol (1st early waking day, evening, end of the biological night, 2nd early waking day after sleep loss), age group and all double/triple interactions. Each circadian period gathered two circadian phases (phase 75° was excluded to provide a clear distinction as in (Shekleton et al., 2013)) to identify over what part of the circadian cycle associations were detected - rather than specific phase – and to increase statistical power. Circadian phase was included as the repeated measure (i.e. the smallest experimental unit) and an interaction between subject x circadian period was included in the covariance structure to specify that measures from the same subject should be correlated within the same circadian period. Betas in each group are only reported for completeness as the age groups difference in beta was considered for statistics. *T*-tests on beta coefficients were performed when seeking for group differences in the link between cortical excitability and performance. The association between cortical excitability and 2-back performance significantly diverged across age groups, irrespective of circadian period, in a two-tailed *t*-test on beta coefficients; this finding was then used as prior for subsequent tests of beta group difference (one-tailed *t* test).

Semi-partial R^2 ($\mathbf{R_{sp}^2}$) was reported for each significant effect of interest as described in (Jaeger et al., 2017). Generalization of the R^2 statistic to GLMMs remains an unresolved problem, with several method proposed (Jaeger et al., 2017; Nakagawa and Schielzeth, 2013). We opted for the approach proposed and validated in (Jaeger et al., 2017), because it allows for a simple computation of semi-partial R^2 as [Sum of Squares/(1+Sum of Square)], with [Sum of Squares = NumDF * FValue / DenDF] (NumDF: numerator degrees of freedom (DF); DenDF: denominator DF), provided that DF are estimated using Kenward-Roger's methods.

3. Results

3.1 Endocrine and sleepiness measures in older and young participants

The sleep deprivation protocol was performed under strictly controlled constant environmental conditions to detect both the influence of sleep homeostasis and of the circadian system on our measures of interest (Duffy and Dijk, 2002). Melatonin levels were assayed in hourly saliva samples, and all data were subsequently realigned relative to the onset of melatonin secretion [dim-light melatonin onset (DLMO) = circadian phase 0°], a gold standard marker of endogenous circadian phase (Pevet and Challet, 2011). Thus, all data are reported with respect to individual's internal circadian clock (and expressed in degrees; 15° = 1h), instead of the external clock time. Statistical analyses sought for effects of circadian phase, age group, and their interaction on the measures of interest through general linear mixed models (GLMMs).

Prior to the wakefulness extension, participants slept in the laboratory under polysomnography (**Fig. 1a**). Time in bed did not differ between age groups (Wilcoxon rank-sum test: $Z = 0.79$, $P = .21$; **Table 1**) but, as expected (Klerman and Dijk, 2008), sleep efficiency was significantly lower in older compared to young participants (Wilcoxon rank-sum test: $Z = 2.47$, $P = .01$; **Table 1**). Also as expected (Sagaspe et al., 2007), during the following 34 h of prolonged wakefulness, older participants did not feel sleepier that younger participants (main effect of age group, $F(1, 21.51) = .46$, $P = .5$; main effect of circadian phase, $F(30, 583.5) = 11.72$, $P < .0001$; age group x circadian phase interaction, $F(30, 583.5) = 1.10$, $P = .33$; **Fig. 2c**). In addition, melatonin showed its typical night time secretion profile in both age groups (**Fig. 2a**), but levels tended to be lower in the older vs. younger group (area under the curve, Wilcoxon rank-sum test: $Z = -1.55$, $P = .06$). This may reflect the previously reported reduction in the strength of the circadian signal (Münch et al., 2005). Hourly saliva samples were also assayed for cortisol, which is under strong circadian control as well (**Fig. 2b**). Cortisol level was significantly higher in older compared with younger individuals (area under the curve, Wilcoxon rank-sum test: $Z = 3.4$, $P < .0007$), in line with previous findings (Van Cauter, 2000). Our sample of younger and older healthy individuals appears therefore in line with previous studies on the impact of prolonged wakefulness in ageing.

Insert Fig. 2

3.2 Age-related dampening of the dynamics in cortical excitability during prolonged wakefulness

When focusing on cortical excitability measures (i.e. the slope of the earliest EEG response evoked by the TMS pulses), GLMM analyses revealed that its modulation across circadian phases differed between older and young participants (circadian phase x age group interaction, $F(8,128.1) = 2.09$, $P = .04$; **Fig. 3**). A significant simple effect of circadian phase was also detected ($F(8,128.1) = 2.37$, $P = .02$). Subsequent post-hoc comparisons indicated that cortical excitability was lower in the evening and first part of the biological night when compared to the end of the biological night in young individuals (0°, 30°, 75° < 135°, $P < .015$), while in older, cortical excitability was void of any robust changes over the protocol ($P > .05$ for all comparisons). Furthermore, cortical excitability was higher in younger vs. older individuals at the end of the biological night (young > older: 135°, $P = .02$; 165°, $P = .06$), when the circadian signal does not counter high sleep pressure, suggesting that high sleep homeostat and circadian misalignment do not impact equally cortical excitability of older and young participants. No significant simple effect of age group was found (i.e. irrespective of circadian phase, $F(1,24) = 1.56$, $P = .22$). Analyses of the amplitude of the earliest EEG response evoked by the TMS pulses, as an alternative measure of cortical excitability (Ly et al., 2016), led to similar statistical outcomes (**Fig. S1**). Importantly, these differences were detected while intensity of TMS pulses, estimated electric field generated by TMS, and the distance between the TMS coil and cortical hotspot did not differ between age groups (**Table 1**).

Insert Fig.3

3.3 No significant association between cortical excitability and performance to vigilant attention tasks

We then switched to exploratory analyses including measures of cognitive performance to gain insight in the potential impact of cortical excitability dynamics on the outputs of brain function. We first considered the 'simpler' tasks of the protocol, which probed vigilant attention. The PVT (Basner and Dinges, 2011) was administered 13 times during the protocol *in between* TMS-EEG recordings, while the visuomotor constant tracking task [CTT; (Ly et al., 2016)] was administered 9 times *during* TMS-EEG recordings (Fig. 1a). PVT performance significantly changed across circadian phases (main

effect of circadian phase, $F(13,240.7) = 6.97$, $P < .0001$; **Fig. 4a**): it remained stable during a normal waking day and then sharply deteriorated (i.e. reaction time increased) during the biological night and early morning hours (75° to 210° > -135° to 0°, 270°, $P < .05$). Although qualitative inspection of data may suggest that older individuals suffered less from night time prolonged wakefulness, no significant age group difference nor any circadian phase by group interaction were detected [as in (Buysse et al., 2005), but see (Sagaspe et al., 2012)]. CTT performance yielded a circadian phase x age-group interaction ($F(8,131.9) = 1.99$, $P = .05$; **Fig. 4b**). Group differences were detected at all circadian phases except the last three assessments (young < older; -150° to 135°, $P < .05$; 165° to 270°, $P > .05$), indicating a differential response to sleep loss, leading to less pronounced differences in performance between age groups towards the end of the protocol. An overall simple effect of circadian phase was also found ($F(8, 131.9) = 9.64$, $P < .0001$), with worse performance at the end of the biological night as compared to the first and second circadian day (-150° to 0°, 210°, 270° < 135°, 165°, $P < .05$). A trend for an age group difference was found (young < older, $F(1, 23.92) = 3.74$, $P = .07$).

We asked whether variations in performance to each vigilant attention task were significantly associated with cortical excitability changes during the protocol. Associations between cortical excitability and vigilant attention measures were investigated over 4 broad circadian periods of the protocol (instead of single circadian phase), known to be critical for the interplay between the sleep homeostasis and the circadian timing system (Dijk and Czeisler, 1995), i.e. the first early waking day, the evening period, the end of the biological night, and the second early waking day after sleep loss (Fig. 1a; see **2.7 Statistics**). GLMMs statistical outcomes are reported in **Table 2**. These analyzes did not reveal any significant association (**Supplementary Fig. S2**). In our sample, cortical excitability is therefore not significantly associated with performance to tasks relying primarily on vigilant attention.

3.4 Significant association between the dynamics of cortical excitability and executive performance during prolonged wakefulness

Our focus then switched to the cognitive tasks with a higher executive load: the 2-back and 3-back versions of the n-back task and the GO/NO-GO task, which were administered during the cognitive test batteries (**Fig. 1a**; right before the PVT). The 2- and 3-back tasks are more resource-demanding than the GO/NO-GO, such that three older individuals were removed from the n-back

analyses because task instructions were not applied correctly (De Beni and Palladino, 2004) (see *2.4.2) N-back tasks*). The 2- and 3-back tasks showed overall similar performance profiles (Fig. **4c-d**). Performance to the 2-back task changed across circadian phases ($F(13,191.7) = 2.30$, $P = .007$), and according to the age group (young > older, $F(1,20.27) = 8.01$, $P = .01$), but without a circadian phase x age group interaction ($F(13,191.7) = 1$, $P = .45$). Performance to the 3-back task showed a significant circadian phase x age group interaction ($F(13,221.1) = 3.29$, $P = .0001$), a simple effect of age ($F(1,19.96) = 11.96$, $P = .03$), but no simple effect of circadian phase ($F(13,221.1) = 1.43$, $P = .15$). For both tasks, post-hoc comparisons revealed that young individuals performed significantly better than older adults from the beginning of the protocol to the middle of the night (2-back: young > older, -135° to 105°, $P \leq .05$; 3-back: young > older, -135° to 75°, $P \leq .05$). In addition, in young individuals, performance was significantly worse during the end of the biological night and early morning following sleep loss compared to all prior measurements (2-back: young, -135° to 75° > 165°, -75° to -15° > 195°, -75° to -45° > 135°, $P < .05$; 3-back: young, -135° to 75° > 105° to 225°, $P < .05$), while no differences between circadian phases were detected in older individuals ($P > .05$ for all comparisons). GO/NO-GO performance (**Fig. 4e**) yielded a significant main effect of circadian phase ($F(13,234.8) = 1.84$, $P = .04$), a trend for a main effect of age group ($F(1,23.21) = 3.99$, $P = .057$), with higher commission error rate in younger individuals, but no circadian phase x age group interaction ($F(13,234.8) = .79$, $P = .67$). Post-hoc contrasts yielded significant differences between age groups, with better performance in the older group from the end of the biological night until the end of the protocol (older < younger: 135° to 195°, 255°, $P < .05$).

Insert Fig. 4

These results show that overall performance to an n-back task is lower in older individuals, while it is higher for the GO/NO-GO, as in (Sagaspe et al., 2012). Better age-related performance to the GO/NO-GO may arise from a speed-accuracy trade-off (Staub et al., 2015) (**Supplementary Fig. S3d**). The results further confirm that, for both types of executive tasks, older individuals suffer relatively less from sleep loss as compared with the younger group (Sagaspe et al., 2012), a pattern that is reminiscent of the dynamics in the underlying cortical excitability. To formally test this similarity, we computed GLMMs to address whether executive task performance was associated with cortical

excitability over the four circadian periods of the protocol (1st early waking day, evening, end of the biological night, 2nd early waking day after sleep loss). Statistical outcomes are reported in **Table 2**.

Insert Table 2

We found that the direction of the association between executive performance and cortical excitability differed between age groups. For the 2-back, this association was irrespective of the circadian period (significant cortical activity x age group interaction; **Table 2**). Higher cortical excitability was associated with better performance in the older group, whereas the inverse was true for young adults (beta young = -.41; beta older = 1.17; young vs. older, *P* = .02; **Fig. 5a** and **Supplementary Fig. S3a**). Analyses yielded similar results when considering the 3-back and GO/NO-GO tasks, but at specific critical circadian periods (significant cortical excitability x age group x circadian period interaction; **Table 2**). For the 3-back, higher cortical excitability was associated with poorer and better performance, respectively, in the young and older group at the end of the biological night, when the circadian signal maximally promotes sleep at a time of very high sleep need (Dijk and Czeisler, 1995) (beta young = -.36; beta older = .6; young vs. older, *P* = .07; **Fig. 5b**). Considering the GO/NO-GO task, higher cortical excitability was associated with poorer and better performance, respectively, in the young and older group during the evening, when the circadian alerting signal maximally counteracts the need for sleep (Dijk and Czeisler, 1994) (beta young = .73; beta older = -.19; young vs. older, *P* = .02; **Fig. 5c**). GO/NO-GO performance was also positively related to cortical excitability, irrespective of age group and circadian period (main effect of cortical excitability, $F(1,138.3) = 3.90$, $P = .05$; **Table 2**).

Insert Fig. 5

4. Discussion

Elucidating the bases of age-related changes in brain function is a crucial scientific challenge. Here we focused on cortical excitability, an essential aspect of basic brain function previously implicated in age-related cognitive decline (Rizzo et al., 2015). The data reveal that cortical excitability dynamics during prolonged wakefulness dampens in ageing, with only minor variations during the protocol. The age-related decrease in the build-up of sleep pressure and in the amplitude of the circadian signal,

previously detected in EEG synchrony, behavior and endocrine measures (Dijk et al., 1999; Landolt et al., 2012; Münch et al., 2005; Schmidt et al., 2012), are therefore also reflected in the dynamics of a basic aspect of brain function, making cortical excitability of older adults less susceptible to sleep loss and circadian misalignment. This finding alone may have implications for neurostimulation and neurorehabilitation, which are therapies commonly provided for age-related neurological disorder (Di Pino et al., 2014).

There are several potential mechanisms underlying the progressive change in cortical excitability dynamics in ageing, and we are not in a position to isolate them. Recent mouse data indicate that the repertoire of single neuron activity during wakefulness and sleep in the motor cortex is stable in aging, suggesting that single neuron functional characteristics change very little over the lifespan (McKillop et al., 2018). Change in threshold and amplitude of action potentials, as well as in their frequency have, however, been reported in aging (Rizzo et al., 2015). Similarly, ion channel function and neuromodulator concentrations are progressively altered over the lifespan (Mather and Harley, 2016; Raz and Rodrigue, 2006; Rizzo et al., 2015). In addition, age-related reduction in clock gene expression (Chen et al., 2016; Kondratov et al., 2006) or alterations in homeostatic sleep-dependent gliotansmission regulation (Meyer et al., 2007) were detected. Interestingly, neuronal desynchrony in the aged suprachiasmatic nucleus (SCN), i.e. the circadian master clock in mammals, was found in an animal model, resulting in an overall dampening of SCN activity fluctuation over the circadian cycle (Farajnia et al., 2012). Our findings suggest that reduced circadian variation in neuronal function also takes place within the frontal cortex, i.e. outside the master circadian clock.

Cortical excitability may ultimately be related to synaptic strength (Rossini and Rossi, 2007). If true, we could infer that, in young individuals, extended wakefulness during the biological night prevent sleep-dependent synaptic downscaling (Tononi and Cirelli, 2006) and increases overall synaptic strength (de Vivo et al., 2017), concomitantly to a strong circadian modulation. In older individuals, we barely detected any changes in cortical excitability when wakefulness was prolonged from one day to the next day (cf. Fig.3, -150° vs. 210° or -60° and 270°). This could be due to age-related synaptic changes (Morrison and Baxter, 2012), which would lead to overall reduced experience-dependent synaptic modification so that sleep would be less required for maintaining synaptic function in aging. This is in line with the age-related reduction in sleep need build-up (Klerman and Dijk, 2008; Shiromani et al., 2000). In vitro research suggests that TMS triggers

responses mainly arising from neuron somas (Pashut et al., 2014), such that age-related changes in cortical excitability may also be driven, at least in part, by neuron cell-body.

Importantly, we do not find significant difference between age groups irrespective of circadian phase. This is in line with another study (Casarotto et al., 2011), but is contradicting other previous indications of a reduced cortical and neuronal excitability in ageing (Ferreri et al., 2017). Discrepancies between studies may in fact reside, at least in part, in the differential impact of sleep need and circadian phase on cortical excitability as one gets older (if prior sleep-wake history or time-of-day were not properly controlled for). While we do not demonstrate that physiological ageing has no impact on overall cortical excitability, our results strongly suggest that, in comparison, the age-related changes in the dynamics of cortical excitability during prolonged wakefulness are more important.

Change in cortical excitability represents part of one's capacity to adapt to daily challenges. We confirm that, in young individuals, this adaptation takes the form of a non-linear circadian modulation of cortical excitability (i.e. significant difference between the evening vs. early morning) likely reflecting combined circadian and sleep homeostasis influences (Huber et al., 2013; Ly et al., 2016). The dampening of cortical excitability dynamics during prolonged wakefulness in older participants might therefore reflect less adaptable brain underlying reduced cognitive flexibility in aging. In other words, the flexibility in cortical excitability and behaviour seen in young during prolonged wakefulness might be a positive allostatic response to acute disruption of the sleep-wake cycle, and ultimately an indicator of cognitive fitness.

Exploratory analyses show that cortical excitability may be differentially related to different aspects of cognition as in our data set it was significantly related to performance to executive tasks, but not to vigilant attention tasks. Using a larger sample of younger individuals, we did find, however, an association between cortical excitability dynamics during sleep loss and vigilant attention (Ly et al., 2016). Our data further suggest that the direction of the association between cortical excitability and executive performance may change across the age groups: in our data set, older individuals' increased cortical excitability is associated with better performance, whereas in young adults it is associated with worse performance. This may again be related to specific and relatively subtle synaptic alterations which are associated with impairments in cognitive function, rather than to a merely loss of neurons in the neocortex (Morrison and Baxter, 2012). This preliminary finding may also indicate that older participants displaying a margin ability in increasing cortical excitability (i.e. cortical

resilience) perform better in task requiring a high degree of cognitive flexibility, such as executive function (Gajewski and Falkenstein, 2018). It is important to stress, however, that no causal link can be drawn for the present study. Our findings may point toward a role for the dynamics of cortical excitability during prolonged wakefulness in driving age-related variations in cognitive performance, at least for executive processes. We surmise that this link would follow two different trajectories depending on age: an inverted U-shape for the young, with an optimal level of cortical excitability beyond which performance would be negatively related to higher cortical excitability. In young individuals, cortical excitability would be close to this optimal level during the circadian day while well rested, as indicated by mostly high and stable performance, but the significant rise in cortical excitability found during the biological night would be detrimental for cognition. In contrast, in older individuals, the link between cortical excitability and performance would be linear. Modifications of cortical excitability, through changes in the circadian system and in the build-up of the need for sleep, are reduced or compromised in older individuals: the optimal level beyond which the association becomes negative is not reached. Since the association between cortical excitability and executive performance was positive in older adults, it may imply that cognition could be improved in ageing by acting on neuron excitability, but this remains to be formally tested with a large sample size. Herein, we observed an association between cortical excitability and executive performance at specific circadian periods for two out of the three executive tasks. Future investigations, in larger sample size, are required to confirm these preliminary findings and address notably whether the association between cortical excitability and executive performance is specific to certain circadian periods or is present at all circadian phases with variable strength.

The reason for the unequal association between cortical excitability and different cognitive domains may resides in part on the distinct brain regions sustaining them: executive function rely heavily (but not exclusively) on the frontal cortex, the region probed with TMS in the present study, while the cortical substrates of attentional processes are more posterior and depend more substantially on the parietal cortex and on subcortical areas (Fan et al., 2005; Schmidt et al., 2009). Furthermore, evidence suggest that early age-specific and subtle neural changes are nested primarily in the frontal cortex areas (Daigneault et al., 1992; Masliah et al., 1993) sustaining high order abilities (Wang et al., 2011), so that executive functions are amongst those most vulnerable to the ageing process. Our cortical measure may have caught these subtle age-related differences in measures of

553 executive performance, especially when considering early stages of cognitive decline (our age sample
554 was ~ 60 y old).
555
556 **5. Conclusions**
557 Herein, we tested whether sleep-wake regulation of basic cortical function changed across
558 young adults (< 30 y) to late middle-aged individuals (50-70 y). We demonstrate that the dynamics of
559 cortical excitability during prolonged wakefulness dampens in older individuals, presumable because
560 of the age-related changes in the interplay between circadian rhythmicity and sleep homeostasis
561 (Schmidt et al., 2012). We further provide preliminary evidence that the lessened clockwork of the
562 circadian and sleep homeostasis processes in ageing may act upon cognition through a reduction of
563 cortical excitability during extended wakefulness. It is likely that this process does not suddenly
564 change at the age range of 60 years, but gradually abate from the middle year of life (Carrier et al.,
565 2001). The current results provide a framework for future studies that should address whether
566 preserved cortical excitability dynamics during sustained wakefulness may counteract cognitive
567 decline into advanced age, but also protect against neurodegenerative diseases, such as Alzheimer's
568 disease.
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Acknowledgements

We thank P. Maquet for enriching discussions, and E. Balteau, A. Claes, C. Degueldre, J. Devillers, B. Herbillon, P. Hanwotte, E. Lambot, B. Lauricella for their help in different steps of the study. The study was funded by Wallonia Brussels International (WBI), Fonds Léon Fredericq (FLF), Fonds National de la Recherche Scientifique (FRS-FNRS, FRSM 3.4516.11, Belgium), Actions de Recherche Concertées (ARC 09/14-03) of the Fédération Wallonie-Bruxelles, University of Liège (ULiège), Fondation Simone et Pierre Clerdent, AXA Foundation, Fondation Médicale Reine Elisabeth (FMRE, Belgium), European Regional Development Fund (ERDF; Radiomed project), and Walloon excellence in life sciences and biotechnology (WELBIO-CR-2010-06E, Belgium). M.V., F.C., C.P., C.S., G.V. are supported by the FNRS-Belgium.

Authors' contribution

G.G., G.V. designed the experiment, acquired and analyzed the data and wrote the paper. J.Q.M.L., S.L.C. designed the experiment and acquired the data. V.M., M.J., C.M. acquired the data. T.D., R.D., A.V. acquired and analyzed data. J.N. analyzed data. M.V., A.L., E.S., F.C. provided expertise for statistical analysis and cognitive tests. C.B. computed automatic sleep scoring. D.C., C.P. provided expertise for EEG analyses. C.S. designed the experiment, acquired and analyzed the data. All authors edited the manuscript.

Disclosure statement

The authors declare no competing financial interests.

References

Basner, M., Dinges, D.F., 2011. Maximizing sensitivity of the psychomotor vigilance test (PVT) to sleep loss. Sleep 34, 581–91. https://doi.org/10.1111/jsr.12322

Beck, A.T., Brown, G., Epstein, N., Steer, R.A., 1988. An Inventory for Measuring Clinical Anxiety: Psychometric Properties. J. Consult. Clin. Psychol. 56, 893–897. https://doi.org/10.1037/0022-006X.56.6.893

Berthomier, C., Drouot, X., Herman-Stoïca, M., Berthomier, P., Prado, J., Bokar-Thire, D., Benoit, O., Mattout, J., D'Ortho, M.-P., 2007. Automatic analysis of single-channel sleep EEG: validation in healthy individuals. Sleep 30, 1587–1595.

Bushey, D., Tononi, G., Cirelli, C., 2015. Sleep- and wake-dependent changes in neuronal activity and reactivity demonstrated in fly neurons using in vivo calcium imaging. Proc. Natl. Acad. Sci. U. S. A. 112, 4785–90. https://doi.org/10.1073/pnas.1419603112

Buysse, D.J., Monk, T.H., Carrier, J., Begley, A., 2005. Circadian patterns of sleep, sleepiness, and performance in older and younger adults. Sleep 28, 1365–1376.

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213. https://doi.org/10.1016/0165-1781(89)90047-4

Carrier, J., Land, S., Buysse, D.J., Kupfer, D.J., Monk, T.H., 2001. The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). Psychophysiology 38, 232–42.

Casarotto, S., Mä Ä Ttä, S., Herukka, S.-K., Pigorini, A., Napolitani, M., Gosseries, O., Niskanen, E., Kö Nö Nen, M., Mervaala, E., Rosanova, M., Soininen, H., Massimini, M., 2011. Transcranial magnetic stimulation-evoked EEG/cortical potentials in physiological and pathological aging. Wolters Kluwer Heal. NeuroReport 22. https://doi.org/10.1097/WNR.0b013e328349433a

Chang, Y.-M., Rosene, D.L., Killiany, R.J., Mangiamele, L.A., Luebke, J.I., 2005. Increased Action Potential Firing Rates of Layer 2/3 Pyramidal Cells in the Prefrontal Cortex are Significantly Related to Cognitive Performance in Aged Monkeys. Cereb. Cortex 15, 409–418. https://doi.org/10.1093/cercor/bhh144

622 Chen, C.-Y., Logan, R.W., Ma, T., Lewis, D.A., Tseng, G.C., Sibille, E., McClung, C.A., 2016. Effects
623 of aging on circadian patterns of gene expression in the human prefrontal cortex. Proc. Natl.
624 Acad. Sci. U. S. A. 113, 206–11. https://doi.org/10.1073/pnas.1508249112

625 Daigneault, S., Braun, C.M.J., Whitaker, H.A., 1992. Early effects of normal aging on perseverative
626 and non□perseverative prefrontal measures. Dev. Neuropsychol. 8, 99–114.
627 https://doi.org/10.1080/87565649209540518

628 De Beni, R., Palladino, P., 2004. Decline in working memory updating through ageing: Intrusion error
629 analyses. Memory 12, 75–89. https://doi.org/10.1080/09658210244000568

630 de Vivo, L., Bellesi, M., Marshall, W., Bushong, E.A., Ellisman, M.H., Tononi, G., Cirelli, C., 2017.
631 Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. Science (80-.). 355,
632 507–510. https://doi.org/10.1126/science.aah5982

633 Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., Ranieri, F., Tombini, M.,
634 Ziemann, U., Rothwell, J.C., Di Lazzaro, V., 2014. Modulation of brain plasticity in stroke: a novel
635 model for neurorehabilitation. Nat. Rev. Neurol. 10, 597–608.
636 https://doi.org/10.1038/nrneurol.2014.162

637 Dijk, D., Duffy, J.F., Riel, E., Shanahan, T.L., Czeisler, C.A., 1999. Ageing and the circadian and
638 homeostatic regulation of human sleep during forced desynchrony of rest , melatonin and
639 temperature rhythms.

640 Dijk, D.J., Czeisler, C.A., 1995. Contribution of the circadian pacemaker and the sleep homeostat to
641 sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity
642 in humans. J. Neurosci. 15, 3526–3538.

643 Dijk, D.J., Czeisler, C.A., 1994. Paradoxical timing of the circadian rhythm of sleep propensity serves
644 to consolidate sleep and wakefulness in humans. Neurosci. Lett. 166, 63–68.
645 https://doi.org/10.1016/0304-3940(94)90841-9

646 Duffy, J.F., Dijk, D.J., 2002. Getting through to circadian oscillators: why use constant routines? J Biol
647 Rhythm. 17, 4–13.

648 English, J., Middleton, B.A., Arendt, J., Wirz-Justice, A., 1993. Rapid direct measurement of melatonin

649 in saliva using an iodinated tracer and solid phase second antibody. Ann Clin Biochem 30 (Pt 4),
650 415–416.

651 Fan, J., McCandliss, B.D., Fossella, J., Flombaum, J.I., Posner, M.I., 2005. The activation of
652 attentional networks. Neuroimage 26, 471–479.
653 https://doi.org/10.1016/j.neuroimage.2005.02.004

654 Farajnia, S., Michel, S., Deboer, T., vanderLeest, H.T., Houben, T., Rohling, J.H.T., Ramkisoensing,
655 A., Yasenkov, R., Meijer, J.H., 2012. Evidence for neuronal desynchrony in the aged
656 suprachiasmatic nucleus clock. J. Neurosci. 32, 5891–9.
657 https://doi.org/10.1523/JNEUROSCI.0469-12.2012

658 Ferreri, F., Vecchio, F., Guerra, A., Miraglia, F., Ponzo, D., Vollero, L., Iannello, G., Maatta, S.,
659 Mervaala, E., Rossini, P.M., Di Lazzaro, V., 2017. Age related differences in functional
660 synchronization of EEG activity as evaluated by means of TMS-EEG coregistrations. Neurosci.
661 Lett. 647, 141–146. https://doi.org/10.1016/j.neulet.2017.03.021

662 Frank, M.G., Cantera, R., 2014. Sleep, clocks, and synaptic plasticity. Trends Neurosci. 37, 491–501.
663 https://doi.org/10.1016/j.tins.2014.06.005

664 Gajewski, P.D., Falkenstein, M., 2018. ERP and Behavioral Effects of Physical and Cognitive Training
665 on Working Memory in Aging: A Randomized Controlled Study. Neural Plast. 2018, 3454835.
666 https://doi.org/10.1155/2018/3454835

667 Horne, J.A., Östberg, O., 1976. A self-assessment questionnaire to determine morningness-
668 eveningness in human circadian rhythms. Int. J. Chronobiol. 4, 97–110.

669 Huber, R., Mäki, H., Rosanova, M., Casarotto, S., Canali, P., Casali, A.G., Tononi, G., Massimini, M.,
670 2013. Human cortical excitability increases with time awake. Cereb. Cortex 23, 332–338.
671 https://doi.org/10.1093/cercor/bhs014

672 Ingleby, J.D., 1967. Signal detection theory and psychophysics, Journal of Sound and Vibration. Wiley,
673 New-York. https://doi.org/10.1016/0022-460X(67)90197-6

674 Jaeger, B.C., Edwards, L.J., Das, K., Sen, P.K., 2017. An R 2 statistic for fixed effects in the
675 generalized linear mixed model. J. Appl. Stat. 44, 1086–1105.

https://doi.org/10.1080/02664763.2016.1193725

Johns, M.W., 1991. A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale. Sleep. https://doi.org/10.1093/sleep/14.6.540

Klerman, E.B., Dijk, D.J., 2008. Age-Related Reduction in the Maximal Capacity for Sleep-Implications for Insomnia. Curr. Biol. 18, 1118–1123. https://doi.org/10.1016/j.cub.2008.06.047

Kondratov, R. V, Kondratova, A.A., Gorbacheva, V.Y., Vykhovanets, O. V, Antoch, M.P., 2006. Early aging and age-related pathologies in mice deficient in BMAL1, the core componentof the circadian clock. Genes Dev. 20, 1868–73. https://doi.org/10.1101/gad.1432206

Kondratova, A. a, Kondratov, R. V, 2012. The circadian clock and pathology of the ageing brain. Nat. Rev. Neurosci. 13, 325–35. https://doi.org/10.1038/nrn3208

Landolt, H.P., Rétey, J. V, Adam, M., 2012. Reduced neurobehavioral impairment from sleep deprivation in older adults: Contribution of adenosinergic mechanisms. Front. Neurol. APR, 1–11. https://doi.org/10.3389/fneur.2012.00062

Lim, A.S.P., Kowgier, M., Yu, L., Buchman, A.S., Bennett, D.A., 2013. Sleep Fragmentation and the Risk of Incident Alzheimer's Disease and Cognitive Decline in Older Persons. Sleep 36, 1027–1032. https://doi.org/10.5665/sleep.2802

Lo, J.C., Groeger, J.A., Santhi, N., Arbon, E.L., Lazar, A.S., Hasan, S., von Schantz, M., Archer, S.N., Dijk, D.-J., 2012. Effects of Partial and Acute Total Sleep Deprivation on Performance across Cognitive Domains, Individuals and Circadian Phase. PLoS One 7, e45987. https://doi.org/10.1371/journal.pone.0045987

Ly, J.Q.M., Gaggioni, G., Chellappa, S.L., Papachilleos, S., Brzozowski, A., Borsu, C., Rosanova, M., Sarasso, S., Middleton, B., Luxen, A., Archer, S.N., Phillips, C., Dijk, D.-J., Maquet, P., Massimini, M., Vandewalle, G., 2016. Circadian regulation of human cortical excitability. Nat. Commun. 7, 11828. https://doi.org/10.1038/ncomms11828

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Terry, R.D., 1993. Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43, 192–7.

Mather, M., Harley, C.W., 2016. The Locus Coeruleus: Essential for Maintaining Cognitive Function

703 and the Aging Brain. Trends Cogn. Sci. https://doi.org/10.1016/j.tics.2016.01.001

704 Mattis, S., 1988. Dementia Rating Scale: Professional Manual.

705 McKillop, L.E., Fisher, S.P., Cui, N., Peirson, S.N., Foster, R.G., Wafford, K.A., Vyazovskiy, V. V,
706 2018. Effects of ageing on cortical neural dynamics and local sleep homeostasis in mice. J.
707 Neurosci. 38, 2513–17. https://doi.org/10.1523/JNEUROSCI.2513-17.2018

708 Meisel, C., Schulze-Bonhage, A., Freestone, D., Cook, M.J., Achermann, P., Plenz, D., 2015. Intrinsic
709 excitability measures track antiepileptic drug action and uncover increasing/decreasing
710 excitability over the wake/sleep cycle. Proc. Natl. Acad. Sci. 112, 14694–14699.
711 https://doi.org/10.1073/pnas.1513716112

712 Meyer, P.T., Elmenhorst, D., Boy, C., Winz, O., Matusch, A., Zilles, K., Bauer, A., 2007. Effect of aging
713 on cerebral A1 adenosine receptors: A [18F]CPFPX PET study in humans. Neurobiol. Aging 28,
714 1914–1924. https://doi.org/10.1016/j.neurobiolaging.2006.08.005

715 Morrison, J.H., Baxter, M.G., 2012. The ageing cortical synapse: hallmarks and implications for
716 cognitive decline. Nat. Rev. Neurosci. 13, 240–250. https://doi.org/10.1038/nrn3200

717 Münch, M., Knoblauch, V., Blatter, K., Schröder, C., Schnitzler, C., Kräuchi, K., Wirz-Justice, A.,
718 Cajochen, C., 2005. Age-related attenuation of the evening circadian arousal signal in humans.
719 Neurobiol. Aging 26, 1307–1319. https://doi.org/10.1016/j.neurobiolaging.2005.03.004

720 Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized
721 linear mixed-effects models. Methods Ecol. Evol. 4, 133–142. https://doi.org/10.1111/j.2041-
722 210x.2012.00261.x

723 Oosterman, J.M., Van Someren, E.J.W., Vogels, R.L.C., Van Harten, B., Scherder, E.J.A., 2009.
724 Fragmentation of the rest-activity rhythm correlates with age-related cognitive deficits. J. Sleep
725 Res. 18, 129–135. https://doi.org/10.1111/j.1365-2869.2008.00704.x

726 Pashut, T., Magidov, D., Ben-Porat, H., Wolfus, S., Friedman, A., Perel, E., Lavidor, M., Bar-Gad, I.,
727 Yeshurun, Y., Korngreen, A., 2014. Patch-clamp recordings of rat neurons from acute brain
728 slices of the somatosensory cortex during magnetic stimulation. Front. Cell. Neurosci. 8, 145.
729 https://doi.org/10.3389/fncel.2014.00145

730 Pevet, P., Challet, E., 2011. Melatonin: Both master clock output and internal time-giver in the
731 circadian clocks network. J. Physiol. 105, 170–182.
732 https://doi.org/10.1016/j.jphysparis.2011.07.001

733 Raz, N., Rodrigue, K.M., 2006. Differential aging of the brain: Patterns, cognitive correlates and
734 modifiers. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2006.07.001

735 Read, G.F., Fahmy, D.R., Walker, R.F., 1977. Determination of cortisol in human plasma by
736 radioimmunoassay. Use of the 125I-labelled radioligand. Ann. Clin. Biochem. 14, 343–9.

737 Reuter-Lorenz, P.A., Park, D.C., 2014. How Does it STAC Up? Revisiting the Scaffolding Theory of
738 Aging and Cognition. Neuropsychol. Rev. 24, 355–370. https://doi.org/10.1007/s11065-014-
739 9270-9

740 Rizzo, V., Richman, J., Puthanveettil, S. V., 2015. Dissecting mechanisms of brain aging by studying
741 the intrinsic excitability of neurons. Front. Aging Neurosci.
742 https://doi.org/10.3389/fnagi.2014.00337

743 Rossini, P.M., Rossi, S., 2007. Transcranial magnetic stimulation: Diagnostic, therapeutic, and
744 research potential. Neurology 68, 484–488. https://doi.org/10.1212/01.wnl.0000250268.13789.b2

745 Sagaspe, P., Taillard, J., Amiéva, H., Beck, A., Rascol, O., Dartigues, J.F., Capelli, A., Philip, P., 2012.
746 Influence of age, circadian and homeostatic processes on inhibitory motor control: A Go/Nogo
747 task study. PLoS One 7. https://doi.org/10.1371/journal.pone.0039410

748 Sagaspe, P., Taillard, J., Chaumet, G., Moore, N., Bioulac, B., Philip, P., 2007. Aging and nocturnal
749 driving: better with coffee or a nap? A randomized study. Sleep 30, 1808–13.

750 Schmidt, C., Collette, F., Leclercq, Y., Sterpenich, V., Vandewalle, G., Berthomier, P., Berthomier, C.,
751 Phillips, C., Tinguely, G., Darsaud, A., Gais, S., Schabus, M., Desseilles, M., Dang-Vu, T.T.,
752 Salmon, E., Balteau, E., Degueldre, C., Luxen, A., Maquet, P., Cajochen, C., Peigneux, P., 2009.
753 Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area.
754 Science 324, 516–9. https://doi.org/10.1126/science.1167337

755 Schmidt, C., Peigneux, P., Cajochen, C., 2012. Age-related changes in sleep and circadian rhythms:
756 Impact on cognitive performance and underlying neuroanatomical networks. Front. Neurol. JUL,

1–11. https://doi.org/10.3389/fneur.2012.00118

Shekleton, J.A., Rajaratnam, S.M.W., Gooley, J.J., Van Reen, E., Czeisler, C.A., Lockley, S.W., 2013. Improved neurobehavioral performance during the wake maintenance zone. J. Clin. Sleep Med. 9, 353–362. https://doi.org/10.5664/jcsm.2588

Shiromani, P.J., Lu, J., Wagner, D., Thakkar, J., Greco, M.A., Basheer, R., Thakkar, M., 2000. Compensatory sleep response to 12 h wakefulness in young and old rats. Am. J. Physiol. Integr. Comp. Physiol. 278, R125–R133. https://doi.org/10.1152/ajpregu.2000.278.1.R125

Staub, B., Doignon-Camus, N., Marques-Carneiro, J.E., Bacon, É., Bonnefond, A., 2015. Age-related differences in the use of automatic and controlled processes in a situation of sustained attention. Neuropsychologia 75, 607–616. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2015.07.021

Steer, R.A., Ball, R., Raneeri, W.F., Beck, A.T., 1997. FURTHER EVIDENCE FOR THE CONSTRUCT VALIDITY OF THE BECK DEPRESSION INVENTORY-II WITH PSYCHIATRIC OUTPATIENTS. Psychol. Rep. 80, 443–446. https://doi.org/10.2466/pr0.1997.80.2.443

Tononi, G., Cirelli, C., 2014. Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron. https://doi.org/10.1016/j.neuron.2013.12.025

Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med. Rev. 10, 49–62. https://doi.org/10.1016/j.smrv.2005.05.002

Van Cauter, E., 2000. Age-Related Changes in Slow Wave Sleep and REM Sleep and Relationship With Growth Hormone and Cortisol Levels in Healthy Men. JAMA 284, 861. https://doi.org/10.1001/jama.284.7.861

Virtanen, J., Ruohonen, J., Naatanen, R., Ilmoniemi, R.J., 1999. Instrumentation for the measurement of electric brain responses to transcranial magnetic stimulation. Med Biol Eng Comput 37, 322–326.

Wang, M., Gamo, N.J., Yang, Y., Jin, L.E., Wang, X.-J., Laubach, M., Mazer, J.A., Lee, D., Arnsten, A.F.T., 2011. Neuronal basis of age-related working memory decline. Nature 476, 210–3. https://doi.org/10.1038/nature10243

784 **Table 1.** Sample characteristics (mean ± SD).

785

Age group	Younger (18-30 y)	Older (50-70 y)	*P* value
N	13	12	-
Women	5	6	.96
Age (yr.)	22.8 ± 2.9	62.3 ± 3.7	-
Right handed	10	11	.32
BMI (kg/m^2)	22.3 ± 3	24.8 ± 2.3	**.03**
Anxiety level	2.6 ± 3.9	3 ± 3.8	.8
Mood	2.8 ± 2.7	3.2 ± 2.8	.77
Caffeine (cups/day)	1.1 ± 1.9	2.2 ± 1.3	.12
Alcohol (doses/week)	2.7 ± 3.2	4.7 ± 5	.23
Subjective sleep quality	3.2 ± 1	5.3 ± 2.8	**.03**
Subjective daytime sleepiness	3.6 ± 2.8	4.8 ± 4.3	.41
Chronotype	56 ± 6.1	59.6 ± 7.2	.58
Clock time of dim light melatonin onset (hh:min)	21:34 ± 01:11	21:43 ± 00:38	.71
Clock time of dim light melatonin offset (hh:min)	08:21 ± 01:01	07:55 ± 01:05	.31
In-lab baseline total time in bed (min, EEG)	509 ± 19	502 ± 18	.21
In-lab baseline sleep duration (min, EEG)	456 ± 45	405 ± 67	**.01**
In-lab baseline sleep efficiency (%, EEG)	90 ± 9	81 ± 13	**.01**
Baseline sleep time (hh:min)	23:20 ± 00:48	23:21± 00:30	
Baseline Wake time (hh:min)	07:48 ± 00:52	07:37 ± 00:33	
Sleep duration for 7 preceding days (min, actigraphy)	511 ± 30	490 ± 32	.18
Sleep time for 7 preceding days (hh:min, actigraphy)	23:28 ± 00:43	23:35 ± 00:28	
Wake time for 7 preceding days (hh:min, actigraphy)	08:04 ± 00:53	07:48 ± 00:44	
Intensity of TMS pulses (%)	54.2 ± 4.5	55.2 ± 5.2	.66
Estimated electric field of TMS pulses (V/m) *	108.5 ± 16	116.2 ± 16.6	.91
Distance from coil (scalp) and cortical hotspot (mm) *	17.9 ± 2.2	17.5 ± 2.2	.87
Mean distance between individual hotspot location and group average hotspot location (MNI space, mm) **	7.3 ± 4.3	10.94 ± 6.93	.18

786 *As provided by the TMS-EEG system.

787 **See section 2.3 for more details.

788 *N.B.*: Sample of in-lab baseline sleep EEG: N_{young} = 10 (due to artefacted signal); N_{older} = 12.

789 Anxiety was measured by the 21 item Beck Anxiety Inventory (BAI ≤ 14) (Beck et al., 1988); mood by the 21 items

790 Beck Depression Inventory II (BDI-II ≤ 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index

791 Questionnaire (PSQI ≤ 7) (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (ESS ≤ 11)

792 (Johns, 1991); chronotype by the Horne-Östberg Questionnaire (< 42: evening types; 42-58: intermediate types; >

793 58: morning types) (Horne and Östberg, 1976).

794 **Table 2.** Association between cortical excitability (measured as the slope of the first TMS-evoked potentials) and cognitive performance. Factors including cortical

795 excitability are in italic. Statistically significant results are in bold.

	PVT performance (mean reaction times*)	**CTT performance** (distance from target)	**2-back performance** (D-prime)	**3-back performance** (D-prime)	**GO/NO-GO performance** (commission error rate)
Cortical excitability	*F*(1,146.1) = .28 *P* = .59	*F*(1,122.6) = .17 *P* = .68	*F*(1,92.63) = 1.32 *P* =.25	*F*(1,101.7) = .10 *P* = .75	***F(1,138.3) = 3.90*** ***P = .051*** ***R_{sp}^2 = .03***
Circadian period	***F*(3,82.82) = 5.32** ***P* =.002** **R_{sp}^2 = .16**	*F*(3,78.78) = 2.06 *P* = .11	*F*(3,55.4) = 1.16 *P* = .33	*F*(3,62.69) = .39 *P* = .76	*F*(3,72.72) = 1.00 *P* = .40
Age group	*F*(1,66.67) = .82 *P* = .37	*F*(1,82.93) = 1.07 *P* = .30	***F*(1,73.2) = 11.67** ***P* = .001** **R_{sp}^2 = .14**	*F*(1,75.06) = 2.65 *P* = .11	*F*(1,79.54) = 1.44 *P* = .23
Cortical excitability x age group	*F*(1,146.1) = 1.06 *P* =.30	*F*(1,122.6) = .01 *P* = .93	***F(1,92.63) = 5.67*** ***P = .02*** ***R_{sp}^2 = .06***	*F*(1,101.7) = .03 *P* = .86	*F*(1,138.3) = .02 *P* = .89
Cortical excitability x circadian period	*F*(3,79.35) = .43 *P* = .73	*F*(3,74.66) = .50 *P* = .68	*F*(3,52.74) = .26 *P* = .85	*F*(3,59.99) = .68 *P* = .57	*F*(3,75.37) = .40 *P* = .75
Age group x circadian period	*F*(3,82.82) = .78 *P* = .51	***F*(3, 78.78) = 2.66** ***P* = .05** **R_{sp}^2 = .09**	*F*(3,55.4) = .07 *P* = .98	*F*(3,62.96) = .72 *P* = .54	***F*(3,72.72) = 3.25** ***P* = .03** **R_{sp}^2 = .12**
Cortical excitability x age group x circadian period	*F*(3,79.35) = .89 *P* = .45	*F*(3,74.66) = .91 *P* = .44	*F*(3,52.74) = .47 *P* = .70	***F(3,59.99) = 2.87*** ***P = .04*** ***R_{sp}^2 = .13***	***F(3,75.35) = 3.89*** ***P = .01*** ***R_{sp}^2 = .13***

796 GLMMs including first row variable as dependent variables and left column variable as predictors. Degrees of freedom are indicated between brackets and were estimated

797 using Kenward-Roger's correction.

798 Dependent variable sample: PVT, CTT, GO/NO-GO tasks: N_{young} = 13; N_{older} = 12. 2-back, 3-back tasks: N_{young} = 13; N_{older} = 9 (refer to Methods for details).

799 * All statistical outcomes are identical when considering other metrics of the PVT such as 10% slowest/fastest/median reaction times or lapses (Basner and Dinges, 2011)

800 (not shown).

Figure 1. Experimental protocol and TMS-evoked potentials.

a. After a baseline night of sleep, 12 older and 13 young healthy participants underwent 34 h of sustained wakefulness under constant routine conditions. Cortical excitability was assessed 9 times using TMS-EEG (▲), over the 1st early waking day, evening, biological night, and 2nd early waking day after sleep loss. During TMS-EEG sessions, a visuomotor constant tracking task (CTT) was administered. In-between, 13 behavioural test batteries were administered (○) - including the psychomotor vigilance task (PVT), and executive tasks (2-back, 3-back, GO/NO-GO). Saliva samples were collected hourly for melatonin and cortisol assays, allowing a posteriori data realignment and interpolation based on individual endogenous circadian timing (inferred based on dim light melatonin onset – DLMO). Time is expressed in circadian phase (degrees - °; 15° = 1h), and equivalent elapsed time awake (h). Representative clock time is for a participant with a 2300–0700 sleep-wake schedule.

* Data were not extrapolated > 15° from the last recording: resampling at 300° could not be carried out in most participants, and was done at 270° instead.

b. *Left panel:* MRI based head reconstruction together with the neuronavigated position of the electrodes. Representative location of a TMS hotspot over the superior frontal gyrus as provided by the neuronavigation system. The arrows represent the direction of the generated electric field.

Middle panel: A butterfly plot of all electrodes of a representative TMS-evoked potential.

Right panel: Representative average TMS-evoked potentials measured at the electrode closest to the hotspot (-2 - 32 ms post-TMS) in each of the nine sessions of the protocol.

Figure 2. Endocrine and sleepiness time course during 34 h of prolonged wakefulness in young and older adults (mean ± SE).

a-c. Time course of melatonin, cortisol and subjective sleepiness (mean ± SE; N_{young} = 13; N_{older} = 12) relative to individual melatonin onset (phase 0°; 15° = 1h). Average melatonin profile is displayed in grey on panel c. Refer to main text for differences between circadian phases.

Figure 3. Cortical excitability dynamics during 34 h of prolonged wakefulness in young and older adults (mean ± SE).

Time course of cortical excitability (slope of the first TMS-evoked EEG response; N_{young} = 13; N_{older} = 12): a circadian profile is visible in young, whereas is dampened in older participants. Time course is expressed relative to individual melatonin onset (DLMO = phase 0°; 15° = 1h). Average melatonin profile is displayed in grey. * significant group differences (*P* = .04) at circadian phase 135°, i.e. around the end of the biological night.

Figure 4. Cognitive performance dynamics during 34 h of prolonged wakefulness in young and older adults (mean ± SE).

a-b. Time course of vigilant attention performance [Psychomotor Vigilance Task (PVT), mean reaction times; visuomotor constant tracking task (CTT), distance from target; N_{young} = 13; N_{older} = 12].

c-e. Time course of executive performance (2-back and 3-back task, D-prime (Ingleby, 1967): N_{young} = 13; N_{older} = 9; GO/NO-GO task, commission error rate: N_{young} = 13; N_{older} = 12).

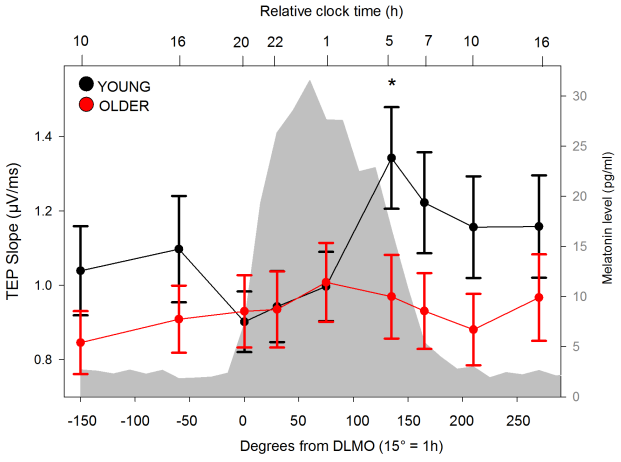
Time course of all measures is expressed relative to individual melatonin onset (DLMO = phase 0°; 15° = 1h). Average melatonin profile is displayed in grey. Vertical black arrows indicate the direction of performance improvement. * significant group differences (*P* < .05). Refer to main text for differences between circadian phases.

Figure 5. Associations between executive performance and cortical excitability in young and older individuals during prolonged wakefulness.

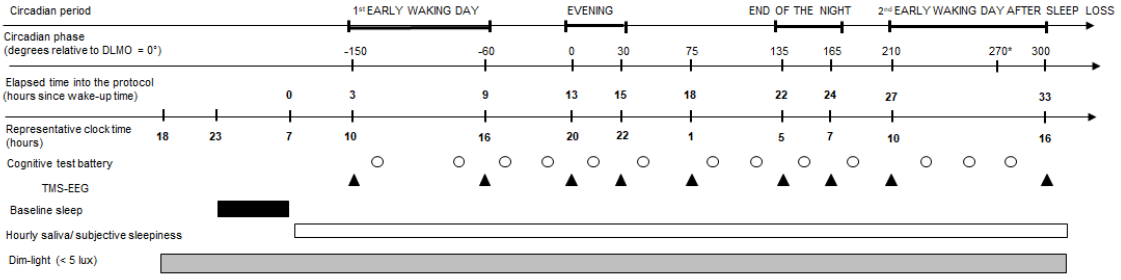
Regression display between executive performance measures to the 2-back (N_{young} = 13; N_{older} = 9) (**a**), 3-back (N_{young} = 13; N_{older} = 9) (**b**) and GO/NO-GO (N_{young} = 13; N_{older} = 12) (**c**) tasks and cortical excitability (measured as the slope of the first TMS-evoked response), across the four circadian periods of the protocol (i.e. 1st early waking day, evening, end of the biological night and 2nd early waking day after sleep loss). Vertical black arrows indicate the direction of performance improvement. Thicker regression lines highlight the significant associations found in the GLMM analyses; ***** age groups difference of beta, *P* ≤ .05; $^{\#}$ trend for age groups difference of beta, *P* ≤ .07. Regressions were used for visual display only, and not as a substitute of the full GLMM statistics presented in Table 2. For consistency, cortical excitability and 2-back association was also displayed

859 across all circadian periods; refer to Supplementary Fig. S3 for associations between executive

860 performance and cortical excitability irrespective of circadian period.



a



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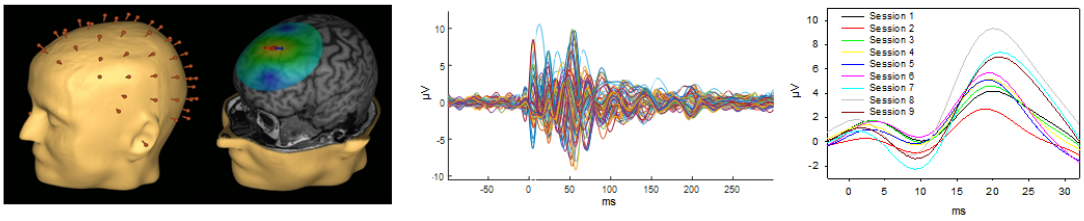

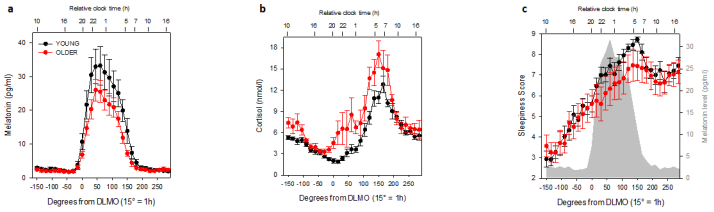

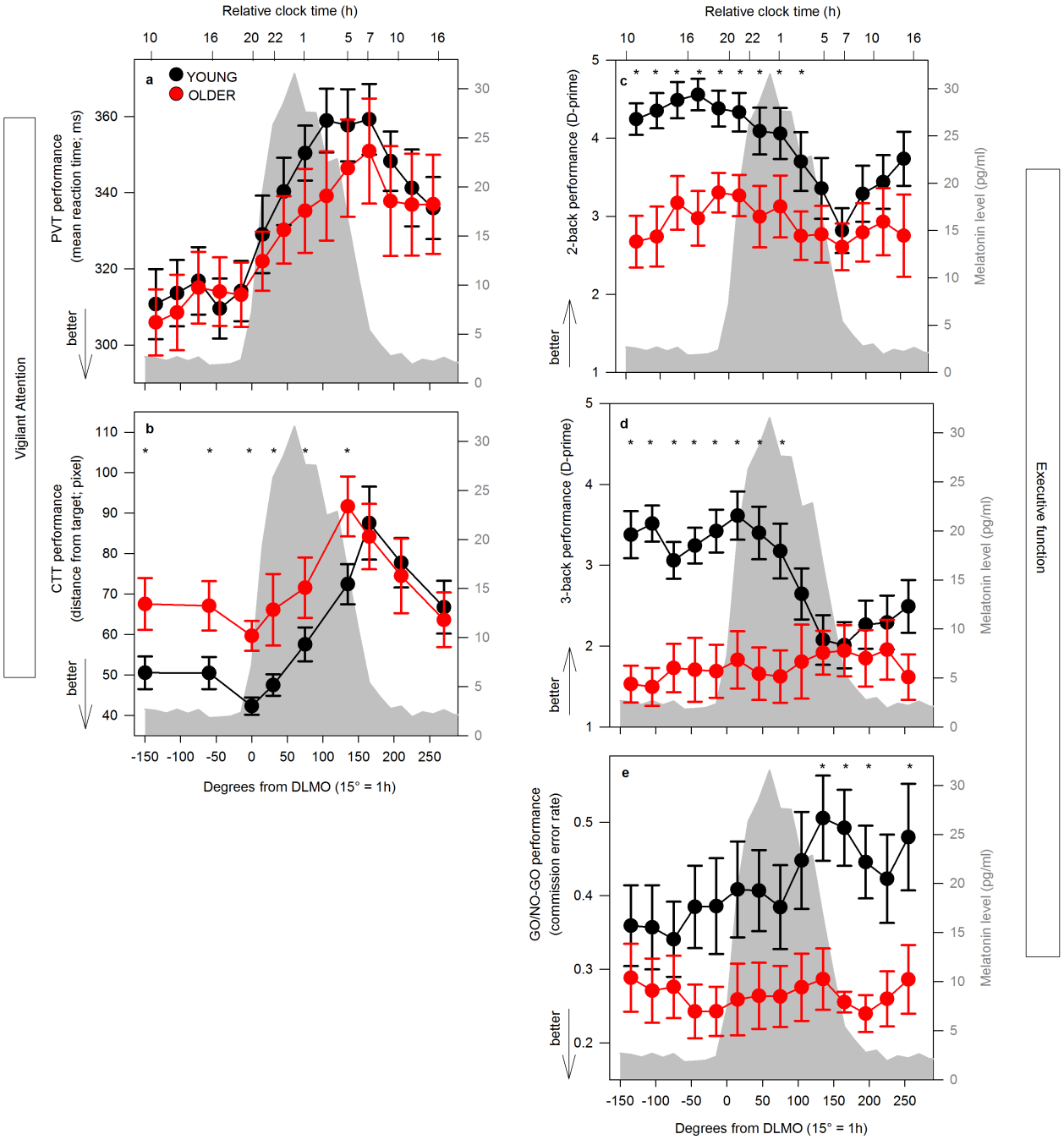

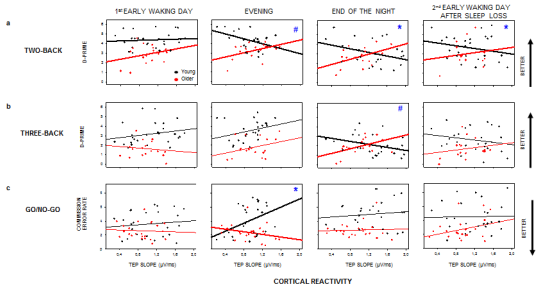

Highlights

- Overall cortical excitability levels are similar in younger and older individuals
- Circadian dynamics in cortical excitability is dampened in older vs. young adults
- Cortical excitability dynamics is associated with variation in executive performance
- Higher cortical excitability is associated with better performance in older adults
- In contrast, high cortical excitability correlates with low performance in the young

Liège, 04 October 2018

Dr. Peter R. Rapp
Editor-in-Chief
Neurobiology of Aging

Dear Dr. Rapp and Editorial board,

1) All the authors declare no actual or potential conflict of interests.

2) The study was funded by Wallonia Brussels International (WBI), Fonds Léon Fredericq (FLF), Fonds National de la Recherche Scientifique (FRS-FNRS, FRSM 3.4516.11, Belgium), Actions de Recherche Concertées (ARC 09/14-03) of the Fédération Wallonie-Bruxelles, University of Liège (ULiège), Fondation Simone et Pierre Clerdent, AXA Foundation, Fondation Médicale Reine Elisabeth (FMRE, Belgium), European Regional Development Fund (ERDF; Radiomed project), and Walloon excellence in life sciences and biotechnology (WELBIO-CR-2010-06E, Belgium). M.V., F.C., C.P., C.S., G.V. are supported by the FNRS-Belgium.

3) This manuscript is not under consideration elsewhere. The data contained in the manuscript have not been previously published and have not been and will not be submitted elsewhere while under consideration at Neurobiology of Aging.

4) All experimental procedures were conducted in agreement with our local Ethic Committee, and all participants provided written informed consent.

5) All co-authors had an active part in the study, approved the content of the manuscript, and validated the accuracy of the data.

Yours sincerely,

Giulia Gaggioni and Gilles Vandewalle

giulia.gaggioni@doct.uliege.be
gilles.vandewalle@uliege.be

GIGA-R – Cyclotron Research Centre/In Vivo Imaging Unit
B30, 8 Allée du VI Août, Quartier Agora, B-4000 Liège, Belgium
Tel +32 4 366 23 16 Fax +32 4 366 29 46
www.giga.ulg.ac.be/sleep