



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



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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 13 young and 12 older healthy participants during sleep deprivation. Although 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 neurobehavioral 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 behavior and address whether maintaining cortical excitability dynamics can counteract age-related cognitive decline.

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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 intracellular and extracellular milieu,

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.

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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 buildup of sleep needs 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 after 24 hours 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 buildup 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): although 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 arithmetic. 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—for example, 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. Because frontal brain regions are particularly prone to both aging (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, that is, 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 years \pm 3.8; 7 women) and 13 young (22.8 years \pm 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 2 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 \leq 14) (Beck et al., 1988); mood by the 21-item Beck Depression Inventory II (BDI-II \leq 14) (Steer et al., 1997); sleep quality by the Pittsburgh Sleep Quality Index Questionnaire (PSQI \leq 7) (Buysse et al., 1989); daytime sleepiness by the Epworth Sleepiness Scale (ESS \leq 11) (Johns, 1991); and 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.

2.2. Experimental protocol

At least a week before the experiment, participants completed a preparatory TMS-EEG session to determine optimal TMS parameters for artifact-free recordings. As in the studies by Huber et al. (2013) and Ly et al. (2016), the left or right superior frontal gyrus was set as the 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 (\pm 15 minutes; 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 \sim 19 °C, regular isocaloric liquid meals and water, semi-recumbent position, no time-of-day information, sound

Table 1
Sample characteristics (mean \pm SD)

Age group	Younger (18–30 y)	Older (50–70 y)	p Value
N	13	12	–
Women	5	6	0.96
Age (y)	22.8 \pm 2.9	62.3 \pm 3.7	–
Right handed	10	11	0.32
BMI (kg/m ²)	22.3 \pm 3	24.8 \pm 2.3	0.03
Anxiety level	2.6 \pm 3.9	3 \pm 3.8	0.8
Mood	2.8 \pm 2.7	3.2 \pm 2.8	0.77
Caffeine (cups/d)	1.1 \pm 1.9	2.2 \pm 1.3	0.12
Alcohol (doses/wk)	2.7 \pm 3.2	4.7 \pm 5	0.23
Subjective sleep quality	3.2 \pm 1	5.3 \pm 2.8	0.03
Subjective daytime sleepiness	3.6 \pm 2.8	4.8 \pm 4.3	0.41
Chronotype	56 \pm 6.1	59.6 \pm 7.2	0.58
Clock time of dim light melatonin onset (hh:min)	21:34 \pm 01:11	21:43 \pm 00:38	0.71
Clock time of dim light melatonin offset (hh:min)	08:21 \pm 01:01	07:55 \pm 01:05	0.31
In-lab baseline total time in bed (min, EEG)	509 \pm 19	502 \pm 18	0.21
In-lab baseline sleep duration (min, EEG)	456 \pm 45	405 \pm 67	0.01
In-lab baseline sleep efficiency (% EEG)	90 \pm 9	81 \pm 13	0.01
Baseline sleep time (hh:min)	23:20 \pm 00:48	23:21 \pm 00:30	
Baseline wake time (hh:min)	07:48 \pm 00:52	07:37 \pm 00:33	
Sleep duration for 7 preceding d (min, actigraphy)	511 \pm 30	490 \pm 32	0.18
Sleep time for 7 preceding d (hh:min, actigraphy)	23:28 \pm 00:43	23:35 \pm 00:28	
Wake time for 7 preceding d (hh:min, actigraphy)	08:04 \pm 00:53	07:48 \pm 00:44	
Intensity of TMS pulses (%)	54.2 \pm 4.5	55.2 \pm 5.2	0.66
Estimated electric field of TMS pulses (V/m) ^a	108.5 \pm 16	116.2 \pm 16.6	0.91
Distance from coil (scalp) and cortical hotspot (mm) ^a	17.9 \pm 2.2	17.5 \pm 2.2	0.87
Mean distance between individual hotspot location and group average hotspot location (MNI space, mm) ^b	7.3 \pm 4.3	10.94 \pm 6.93	0.18

N.B.: Sample of in-lab baseline sleep EEG: $N_{\text{young}} = 10$ (due to artifacted signal); $N_{\text{older}} = 12$.

Bolded values represent the significant p value ($p < 0.05$).

Key: TMS, transcranial magnetic stimulation; EEG, electroencephalogram; BMI, body mass index.

Anxiety was measured by the 21-item Beck Anxiety Inventory (BAI ≤ 14) (Beck et al., 1988); mood by the 21-item 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); chronotype by the Horne-Östberg Questionnaire (< 42 : evening types; 42 – 58 : intermediate types; > 58 : morning types) (Horne and Östberg, 1976).

^a As provided by the TMS-EEG system.

^b See section 2.3 for more details.

proofed rooms) sleep deprivation protocol, which has repeatedly been a successful means to assess in-lab interindividual differences in sleep homeostatic and circadian interplay (Duffy and Dijk, 2002). Participants were maintained in dim light for 5.5 hours (< 5 lux), during which they were trained to the cognitive test batteries, before sleeping at their habitual bedtime, for their habitual duration (in complete darkness) (Fig. 1A). The TMS-compatible electrode cap was placed on awaking before sustained wakefulness period under 34 hours of constant routine conditions. TMS-evoked EEG potentials were recorded 9 times (1000, 1600, 2000, 2200, 0100, 0500, 0700, 1000, and 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 1500 protocol hours with multiple measures including 225 TMS-EEG sessions derived from 13 young and 12 older participants.

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 2 additional bipolar electrodes. Participants wore the EEG cap during the entire constant routine protocol, and electrodes impedance was set below 5 k Ω before 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 (AEPs) evoked by TMS and bone conduction were minimized by diffusing a continuous loud white masking noise through ear-plugs 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 and 500 ms after 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 artifact, rereferencing 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), and robust averaging. Cortical excitability was inferred from the slope of the first EEG component (0 – 35 ms) of the TMS-evoked potential (~ 250 trials per session), measured at the artifact-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

during the training before baseline sleep, 3 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 analysis, 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

This task probes vigilant attention (Basner and Dinges, 2011) and requires participants to press a computer space bar as soon as a chronometer pseudorandomly starts on the screen (random interval of 2–10 seconds; 48 trials per task; 5 minutes). Performance was inferred from the mean reaction time after removal of anticipation (<100 ms) and lapses (>500 ms) [and error (>3000 ms)].

2.4.4. Visuomotor vigilance compensatory 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 psychomotor vigilance task (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, after 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 second from a lapse period were discarded from analyses.

2.5. Salivary melatonin and cortisol samples

Salivary melatonin and cortisol samples were first placed at 4 °C, before centrifugation and congelation at –20 °C within 12 hours. 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 μ L volumes, whereas it was 0.37 ± 0.05 nmol/L using 500 μ L 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 hours” to “wake-up time +10 hours.” DLMO was computed as the time at which melatonin level reached 20% of the baseline to maximum level (linear interpolation).

2.6. Sleep EEG

Sleep EEG data were recorded using M7000 amplifiers (EMBLA, NATUS, Planegg, Germany) according to the 10/20 system. The habituation night montage consisted of a full polysomnography with 5 EEG channels (Fz, Cz, Pz, Oz, C3) referenced to left and right mastoids (A1, A2), 2 bipolar EOG, 2 bipolar electrocardiogram channels, 2 bipolar electrodes placed on the chin (electromyogram), 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 electromyogram channels. EEG data were digitized at a sampling rate of 200 Hz. Sleep EEG recordings were automatically scored using a validated algorithm (ASEEGA, PHYSIP, Paris, France), including artifact rejection (Berthomier et al., 2007). Three recordings of young participants were rejected because of artifacted signal. Total time spent in bed (TIB), total sleep time, sleep efficiency (the ratio between total sleep time 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 hour). All data points were resampled after 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 hour), such that resampling at 300° could not be carried out for most participants and was advanced at 270° instead. For analyses only including cognitive test batteries, data were resampled every 30°, after linear interpolation, from –135° to 255°. Data points situated 3 interquartile ranges above or below the 25th and 75th percentile were defined as extreme outliers and removed (up to 2 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 (χ^2 test 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 (GLMMs) (PROC GLIMMIX) were applied to compute all statistics after determination of the dependent variable distribution (using Allfitdist MATLAB function). Subject (intercept) effect was included as a 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 assessed 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 applying 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 included circadian phase, age group, and their interaction. When seeking for associations between cortical excitability (slope of the first TMS evoked EEG response) and behavior, GLMM included cortical excitability, the 4 circadian periods of the protocol (first early waking day, evening, end of the biological night, second early waking day after sleep loss), age group and all double/triple interactions. Each circadian period gathered 2 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 and 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 group 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 2-tailed *t*-test on beta coefficients; this finding was then used as prior for subsequent tests of beta group difference (one-tailed *t*-test).

Semipartial R^2 (R_{sp}^2) was reported for each significant effect of interest as described in the study by Jaeger et al., 2017. Generalization of the R^2 statistic to GLMMs remains an unresolved problem, with several methods proposed (Jaeger et al., 2017; Nakagawa and Schielzeth, 2013). We opted for the approach proposed and validated in the study by Jaeger et al., 2017 because it allows for a simple computation of semipartial 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 [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 GLMMs.

Before the wakefulness extension, participants slept in the laboratory under polysomnography (Fig. 1A). TIB did not differ between age groups (Wilcoxon rank-sum test: $Z = 0.79$, $p = 0.21$; Table 1) but, as expected (Klerman and Dijk, 2008), sleep efficiency

was significantly lower in older compared to young participants (Wilcoxon rank-sum test: $Z = 2.47$, $p = 0.01$; Table 1). Also as expected (Sagaspe et al., 2007), during the following 34 hours of prolonged wakefulness, older participants did not feel sleepier than younger participants [main effect of age group, $F(1, 21.51) = 0.46$, $p = 0.5$; main effect of circadian phase, $F(30, 583.5) = 11.72$, $p < 0.0001$; age group \times circadian phase interaction, $F(30, 583.5) = 1.10$, $p = 0.33$; 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 versus younger group (area under the curve, Wilcoxon rank-sum test: $Z = -1.55$, $p = 0.06$). 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: $Z = 3.4$, $p < 0.0007$), 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 aging.

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 \times age group interaction, $F(8,128.1) = 2.09$, $p = 0.04$; Fig. 3]. A significant simple effect of circadian phase was also detected [$F(8,128.1) = 2.37$, $p = 0.02$]. 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°, $p < 0.015$), whereas in older, cortical excitability was void of any robust changes over the protocol ($p > 0.05$ for all comparisons). Furthermore, cortical excitability was higher in younger versus older individuals at the end of the biological night (young > older: 135°, $p = 0.02$; 165°, $p = 0.06$), when the circadian signal does not counter high sleep pressure, suggesting that high sleep homeostatic 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, $F(1,24) = 1.56$, $p = 0.22$). Analyses of the amplitude of the earliest EEG response evoked by the TMS

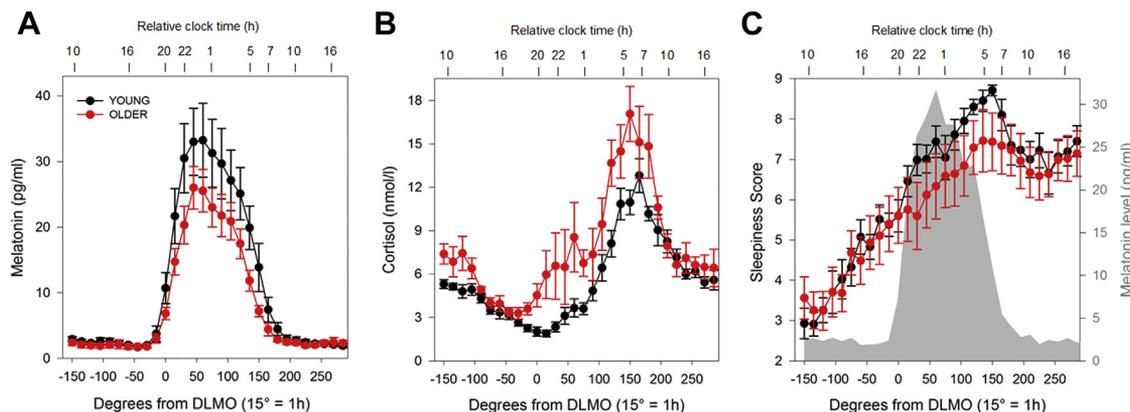


Fig. 2. Endocrine and sleepiness time course during 34 hours of prolonged wakefulness in young and older adults (mean \pm SE). (A–C) Time course of melatonin, cortisol, and subjective sleepiness (mean \pm SE; $N_{\text{young}} = 13$; $N_{\text{older}} = 12$) relative to individual melatonin onset (phase 0°; 15° = 1h). Average melatonin profile is displayed in gray on panel C. Refer to main text for differences between circadian phases. Abbreviation: DLMO, dim light melatonin onset.

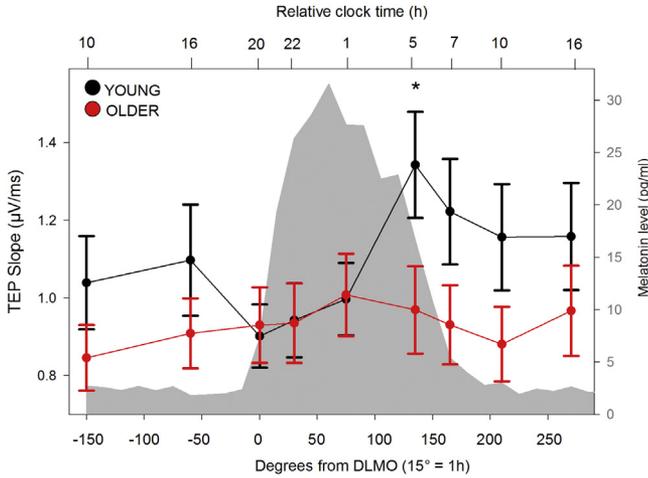


Fig. 3. Cortical excitability dynamics during 34 hours of prolonged wakefulness in young and older adults (mean ± SE). Time course of cortical excitability (slope of the first TMS-evoked EEG response; $N_{\text{young}} = 13$; $N_{\text{older}} = 12$): a circadian profile is visible in young, whereas 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 gray. * significant group differences ($p = 0.04$) at circadian phase 135°, that is, around the end of the biological night. Abbreviations: TMS, transcranial magnetic stimulation; DLMO, dim light melatonin onset; EEG, electroencephalogram; TEP, TMS-evoked potential.

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).

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 into 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, whereas the visuomotor compensatory 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, $F(13,240.7) = 6.97$, $p < 0.0001$; 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 ($75^\circ\text{--}210^\circ > -135^\circ\text{--}0^\circ, 270^\circ$, $p < 0.05$). 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 was detected [as in (Buyse et al., 2005), but see (Sagaspe et al., 2012)]. Compensatory tracking task performance yielded a circadian phase × age-group interaction ($F(8,131.9) = 1.99$, $p = 0.05$; Fig. 4B). Group differences were detected at all circadian phases except the last 3 assessments (young < older; $-150^\circ\text{--}135^\circ$, $p < 0.05$; $165^\circ\text{--}270^\circ$, $p > 0.05$), indicating a differential response to sleep loss, leading to less pronounced differences in performance between age groups toward the end of the protocol. An overall simple effect of circadian phase was also found [$F(8, 131.9) = 9.64$, $p < 0.0001$], with worse performance at the end of the biological night as compared to the first and second circadian day ($-150^\circ\text{--}0^\circ, 210^\circ, 270^\circ < 135^\circ, 165^\circ$, $p <$

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

GLMM predictor factors	PVT performance (mean reaction times ^a)	CTT performance (distance from target)	2-Back performance (D-prime)	3-Back performance (D-prime)	GO/NO-GO performance (commission error rate)
<i>Cortical excitability</i>	$F(1,146.1) = 0.28$ $p = 0.59$	$F(1,122.6) = 0.17$ $p = 0.68$	$F(1,92.63) = 1.32$ $p = 0.25$	$F(1,101.7) = 0.10$ $p = 0.75$	$F(1,138.3) = 3.90$
Circadian period	$F(3,82.82) = 5.32$ $p = 0.002$ $R_{sp}^2 = 0.16$	$F(3,78.78) = 2.06$ $p = 0.11$	$F(3,55.4) = 1.16$ $p = 0.33$	$F(3,62.69) = 0.39$ $p = 0.76$	$p = 0.051$ $R_{sp}^2 = 0.03$ $F(3,72.72) = 1.00$ $p = 0.40$
Age group	$F(1,66.67) = 0.82$ $p = 0.37$	$F(1,82.93) = 1.07$ $p = 0.30$	$F(1,73.2) = 11.67$ $p = 0.001$ $R_{sp}^2 = 0.14$	$F(1,75.06) = 2.65$ $p = 0.11$	$F(1,79.54) = 1.44$ $p = 0.23$
<i>Cortical excitability × age group</i>	$F(1,146.1) = 1.06$ $p = 0.30$	$F(1,122.6) = 0.01$ $p = 0.93$	$F(1,92.63) = 5.67$ $p = 0.02$ $R_{sp}^2 = 0.06$	$F(1,101.7) = 0.03$ $p = 0.86$	$F(1,138.3) = 0.02$ $p = 0.89$
<i>Cortical excitability × circadian period</i>	$F(3,79.35) = 0.43$ $p = 0.73$	$F(3,74.66) = 0.50$ $p = 0.68$	$F(3,52.74) = 0.26$ $p = 0.85$	$F(3,59.99) = 0.68$ $p = 0.57$	$F(3,75.37) = 0.40$ $p = 0.75$
<i>Age group × circadian period</i>	$F(3,82.82) = 2.66$ $p = 0.05$ $R_{sp}^2 = 0.09$	$F(3,78.78) = 2.66$ $p = 0.05$ $R_{sp}^2 = 0.09$	$F(3,55.4) = 0.07$ $p = 0.98$	$F(3,62.96) = 0.72$ $p = 0.54$	$F(3,72.72) = 3.25$ $p = 0.03$ $R_{sp}^2 = 0.12$
<i>Cortical excitability × age group × circadian period</i>	$F(3,79.35) = 0.89$ $p = 0.45$	$F(3,74.66) = 0.91$ $p = 0.44$	$F(3,52.74) = 0.47$ $p = 0.70$	$F(3,59.99) = 2.87$ $p = 0.04$ $R_{sp}^2 = 0.13$	$F(3,75.35) = 3.89$ $p = 0.01$ $R_{sp}^2 = 0.13$

Factors including cortical excitability are in italic. Statistically significant results are in bold.

GLMMs including first row variable as dependent variables and left column variable as predictors. Degrees of freedom are indicated between brackets and were estimated using Kenward-Roger's correction. Dependent variable sample: PVT, CTT, GO/NO-GO tasks; $N_{\text{young}} = 13$; $N_{\text{older}} = 12$. 2-back, 3-back tasks; $N_{\text{young}} = 13$; $N_{\text{older}} = 9$ (refer to Methods for details).

Key: TMS, transcranial magnetic stimulation; PVT, psychomotor vigilance task; CTT, compensatory tracking task; GLMM, Generalized linear mixed model.

^a 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) (not shown).

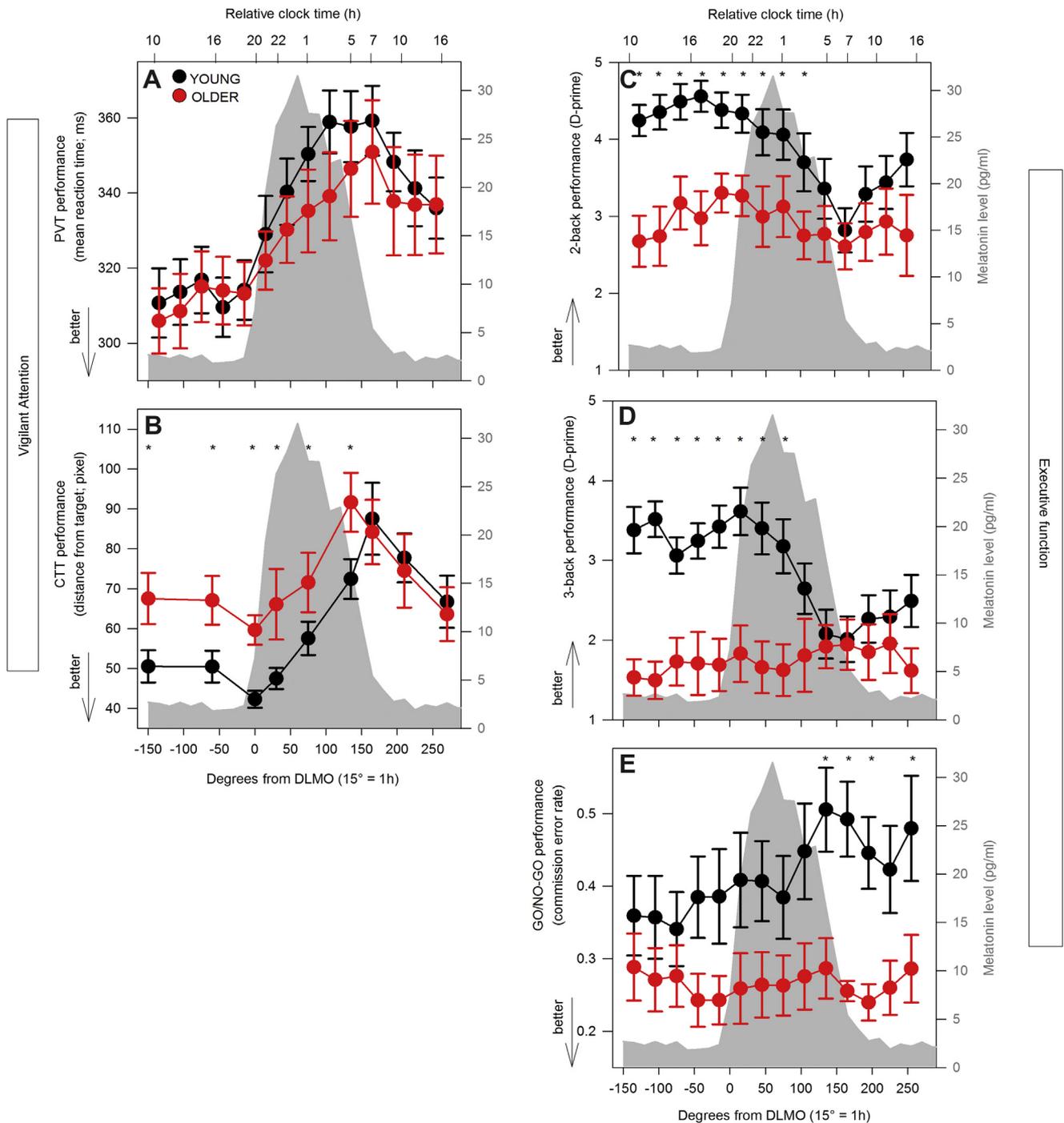


Fig. 4. Cognitive performance dynamics during 34 hours of prolonged wakefulness in young and older adults (mean \pm SE). (A, B) Time course of vigilant attention performance [psychomotor Vigilance Task (PVT), mean reaction times; visuomotor compensatory tracking task (CTT), distance from target; $N_{\text{young}} = 13$; $N_{\text{older}} = 12$]. (C–E) Time course of executive performance (2-back and 3-back task, D-prime (Ingleby, 1967); $N_{\text{young}} = 13$; $N_{\text{older}} = 9$; GO/NO-GO task, commission error rate; $N_{\text{young}} = 13$; $N_{\text{older}} = 12$). Time course of all measures is expressed relative to individual melatonin onset (DLMO = phase 0°; 15° = 1h). Average melatonin profile is displayed in gray. Vertical black arrows indicate the direction of performance improvement. * significant group differences ($p < 0.05$). Refer to main text for differences between circadian phases. Abbreviation: DLMO, dim light melatonin onset.

0.05). A trend for an age group difference was found [young < older, $F(1, 23.92) = 3.74$, $p = 0.07$].

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), that is, 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). GLMM

statistical outcomes are reported in Table 2. These analyses 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 3 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 [$F(13,191.7) = 2.30, p = 0.007$], and according to the age group [young > older, $F(1,20.27) = 8.01, p = 0.01$], but without a circadian phase \times age group interaction [$F(13,191.7) = 1, p = 0.45$]. Performance to the 3-back task showed a significant circadian phase \times age group interaction [$F(13,221.1) = 3.29, p = 0.0001$], a simple effect of age [$F(1,19.96) = 11.96, p = 0.03$], but no simple effect of circadian phase [$F(13,221.1) = 1.43, p = 0.15$]. 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° – $105^\circ, p \leq 0.05$; 3-back: young > older, -135° – $75^\circ, p \leq 0.05$). In addition, in young individuals, performance was significantly worse during the end of the biological night and early morning after sleep loss compared with all prior measurements (2-back: young, -135° – $75^\circ > 165^\circ, -75^\circ$ to $-15^\circ > 195^\circ, -75^\circ$ to $-45^\circ > 135^\circ, p < 0.05$; 3-back: young, -135° – $75^\circ > 105^\circ$ – $225^\circ, p < 0.05$), whereas no differences between circadian phases were detected in older individuals ($p > 0.05$ for all comparisons). GO/NO-GO performance (Fig. 4E) yielded a significant main effect of circadian phase [$F(13,234.8) = 1.84, p = 0.04$], a trend for a main effect of age group [$F(1,23.21) = 3.99, p = 0.057$], with higher commission error rate in younger individuals but no circadian phase \times age group interaction [$F(13,234.8) = 0.79, p = 0.67$]. 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° – $195^\circ, 255^\circ, p < 0.05$).

These results show that overall performance to an n-back task is lower in older individuals, whereas it is higher for the GO/NO-GO, as in the study by 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

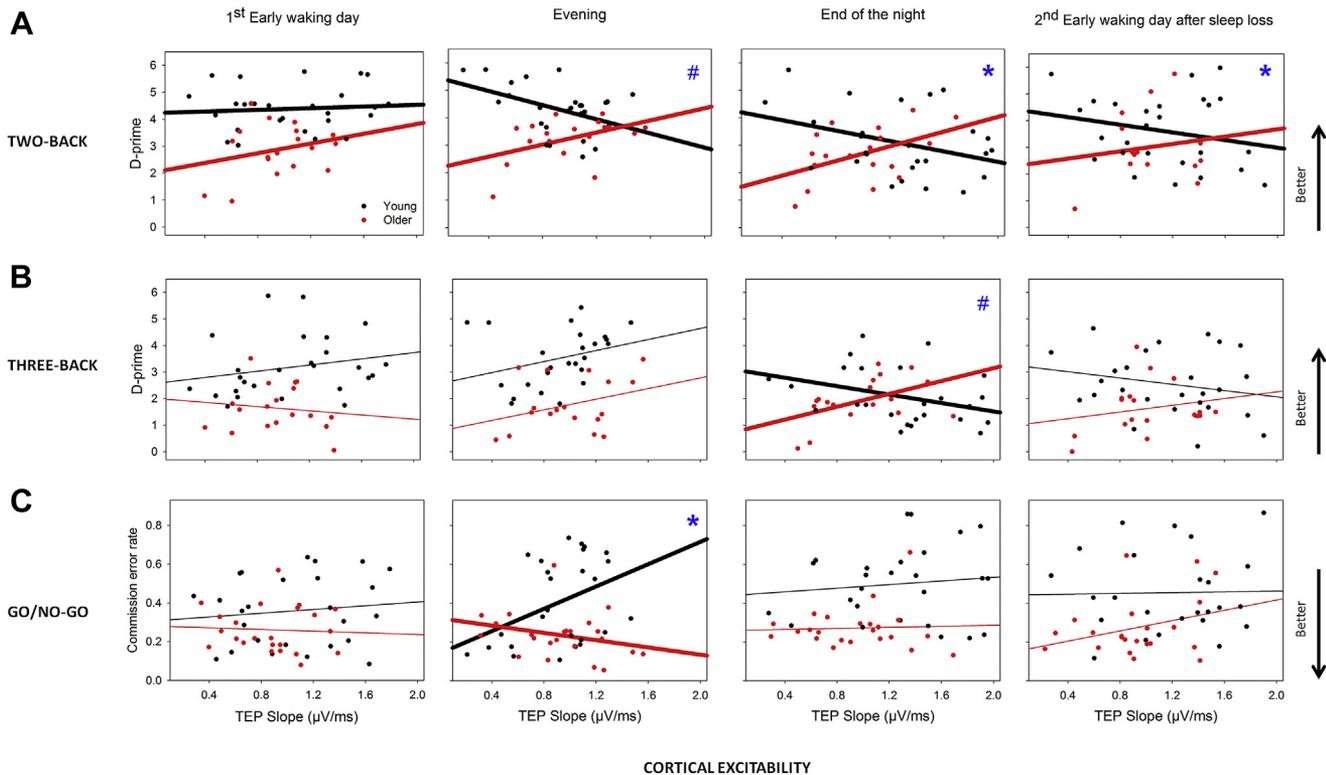


Fig. 5. Associations between executive performance and cortical excitability in young and older individuals during prolonged wakefulness. Regression display between executive performance measures to the 2-back ($N_{\text{young}} = 13; N_{\text{older}} = 9$) (A), 3-back ($N_{\text{young}} = 13; N_{\text{older}} = 9$) (B) and GO/NO-GO ($N_{\text{young}} = 13; N_{\text{older}} = 12$) (C) tasks and cortical excitability (measured as the slope of the first TMS-evoked response), across the 4 circadian periods of the protocol (i.e., first early waking day, evening, end of the biological night and second 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 group difference of beta, $p \leq 0.05$; # trend for age group difference of beta, $p \leq 0.07$. 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 across all circadian periods; refer to Supplementary Fig. S3 for associations between executive performance and cortical excitability irrespective of circadian period. Abbreviations: GLMM, Generalized linear mixed model; TEP, TMS-evoked potential.

similarity, we computed GLMMs to address whether executive task performance was associated with cortical excitability over the 4 circadian periods of the protocol (first early waking day, evening, end of the biological night, second early waking day after sleep loss). Statistical outcomes are reported in [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 \times 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 = -0.41 ; beta older = 1.17 ; young vs. older, $p = 0.02$; [Fig. 5A](#) and [Supplementary Fig. S3A](#)). Analyses yielded similar results when considering the 3-back and GO/NO-GO tasks but at specific circadian periods (significant cortical excitability \times age group \times 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 groups 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 = -0.36 ; beta older = 0.6 ; young vs. older, $p = 0.07$; [Fig. 5B](#)). Considering the GO/NO-GO task, higher cortical excitability was associated with poorer and better performance, respectively, in the young and older groups during the evening, when the circadian alerting signal maximally counteracts the need for sleep ([Dijk and Czeisler, 1994](#)) (beta young = 0.73 ; beta older = -0.19 ; young vs. older, $p = 0.02$; [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, $F(1,138.3) = 3.90$, $p = 0.05$; [Table 2](#)].

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 aging, with only minor variations during the protocol. The age-related decrease in the buildup 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 neuro-rehabilitation, 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 aging, 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, has, 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 gliotransmission regulation ([Meyer et al., 2007](#)) were detected. Interestingly, neuronal desynchrony in the aged suprachiasmatic nucleus, that is, the circadian

master clock in mammals, was found in an animal model, resulting in an overall dampening of suprachiasmatic nucleus 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, that is, 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 buildup ([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 aging ([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). Although we do not demonstrate that physiological aging 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 nonlinear 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 behavior 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 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 buildup of the need for sleep, are reduced or compromised in older individuals: the optimal level beyond which the association becomes negative is not reached. Because the association between cortical excitability and executive performance was positive in older adults, it may imply that cognition could be improved in aging 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 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 reside 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 among those most vulnerable to the aging process. Our cortical measure may have caught these subtle age-related differences in measures of executive performance, especially when considering early stages of cognitive decline (our age sample was ~60 years old).

5. Conclusions

Herein, we tested whether sleep-wake regulation of basic cortical function changed across young adults (<30 years) to late middle-aged individuals (50–70 years). We demonstrate that the dynamics of cortical excitability during prolonged wakefulness dampens in older individuals, presumable because of the age-related changes in the interplay between circadian rhythmicity and sleep homeostasis (Schmidt et al., 2012). We further provide preliminary evidence that the lessened clockwork of the circadian and sleep homeostasis processes in aging may act on cognition through a reduction of cortical excitability during extended wakefulness. It is likely that this process does not suddenly change at the age range of 60 years but gradually abate from the middle year of life (Carrier et al., 2001). The current results provide a framework

for future studies that should address whether preserved cortical excitability dynamics during sustained wakefulness may not only counteract cognitive decline into advanced age but also protect against neurodegenerative diseases, such as Alzheimer's disease.

Disclosure

The authors declare no competing financial interests.

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Authors' contribution: GG and GV designed the experiment, acquired and analyzed the data, and wrote the article. JQML and SLC designed the experiment and acquired the data. VM, MJ, and CM acquired the data. TD, RD, and AV acquired and analyzed data. JN analyzed data. MV, AL, ES, and FC provided expertise for statistical analysis and cognitive tests. CB computed automatic sleep scoring. DC and CP provided expertise for EEG analyses. CS designed the experiment and acquired and analyzed the data. All authors edited the article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.neurobiolaging.2019.02.004>.

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