Perception, particularly in the visual domain, is drastically influenced by rhythmic changes in ambient lighting conditions. Anticipation of daylight changes by the circadian system is critical for survival. However, the neural bases of time-of-day-dependent modulation in human perception are not yet understood. We used fMRI to study brain dynamics during resting-state and close-to-threshold visual perception repeatedly at six times of the day. Here we report that resting-state signal variance drops endogenously at times coinciding with dawn and dusk, notably in sensory cortices only. In parallel, perception-related signal variance in visual cortices decreases and correlates negatively with detection performance, identifying an anticipatory mechanism that compensates for the deteriorated visual signal quality at dawn and dusk. Generally, our findings imply that decreases in spontaneous neural activity improve close-to-threshold perception.
he day-night cycle leads to rhythmic changes in environmental conditions, particularly of ambient light. The circadian timing system anticipates such time of day (ToD)-dependent environmental rhythms and modulates physiology accordingly\(^1\). Animal models revealed ToD-dependent changes in genome readout, protein dynamics, and in electrophysiology that are regulated by the circadian system\(^3\) and homeostatic factors\(^4\). However, the neural bases of ToD-dependent changes in perception and cognition in humans are unclear\(^7\).

Perception can easily be studied in humans. An appropriate method to investigate the neural signatures of cognitive processes is the measurement of blood oxygen level dependent (BOLD) signal by functional magnetic resonance imaging (fMRI). fMRI during tasks can reveal brain activity associated with cognition, while fMRI during wakeful rest identifies more directly endogenous processes\(^5\). ToD modulates task-related BOLD-fMRI activity\(^6\) but more importantly also functional connectivity in resting-state networks\(^7\),\(^8\),\(^9\),\(^10\),\(^11\) suggesting that ToD modulates neural networks both during tasks but also independently of a cognitive task or sensory stimulation. Yet, the relationship between such network dynamics and ToD-dependent modulations of perception and behavior has not yet been shown. This lack of knowledge is surprising, because ToD dramatically influences the quality of the sensory input signals to the brain. Understanding the way ToD affects cortical dynamics may grant insights in how the brain deals with predictable challenges.

We investigated the ToD-dependency of BOLD signal variance – a quantification of the BOLD signal amplitude over time – as it provides important information about the relationship between brain activity and behavior\(^12\),\(^13\). BOLD signal variance was first analyzed during resting-state to detect endogenous ToD-dependent modulation of brain activity. In a second step, we investigated the consequences of such an endogenous modulation of cortical activity on visual perception. Our results reveal that BOLD signal variance drops endogenously at twilight compared to midday in sensory cortices while close-to-threshold visual perception improves in parallel. This identifies reductions in spontaneous sensory cortex activity as an anticipatory mechanism to compensate for the low sensory signal quality at twilight.

**Results**

**Time-of-day-effect in resting-state BOLD signal variance.** Fourteen healthy male participants were scanned using fMRI at six different ToD on two subsequent days (08:00, 11:00, 14:00, 17:00, 20:00, 23:00 h on each day). This was done to account for habituation effects to the experimental setting. Each scanning session included a resting-state measurement in constant dim light (< 0.1 lux). Voxel-wise BOLD variance was de-convolved using a published dataset in which the light conditions in the scanner were identical at each ToD. Although the 20:00 h scan was close to or matched sunset (start of civil evening twilight, range 16:24–21:39 h), this suggests a particular role of the visual compared to the other sensory cortices in ToD-dependent modulation of brain activity. Note that the light conditions in the scanner were identical at each ToD and participants adapted to the dim light before measurement.

**Potential masking factors.** A range of masking factors could have potentially influenced our observation. Yet, the main effect of ToD on BOLD SD in the visual cortex remained significant after accounting for potential confounders, including heart rate, breathing rate, body temperature, subjective sleepiness, and the amplitude and variance of head motion parameters, as well as the number of outliers in head motion parameters in a linear mixed model (main effect of ToD F\((5, 34.029) = 4.177, P = 0.005\), type III F-test, \(n = 14\)). This model also included chronotype, sleep debt, and scanning days to investigate effects of interindividual differences (see section Circadian and homeostatic factors) and habituation on resting-state BOLD SD. The repetitive measurements on two subsequent scanning days did not introduce habituation effects, because ToD remained significant when accounting for scanning days in the model and there was no significant interaction between ToD and scanning day (F\((5, 41.829) = 0.871, P = 0.509\), type III F-test, \(n = 14\)).

**Vigilance.** Because the main effect of ToD in BOLD SD remained significant when adjusting for subjective sleepiness, it is likely that the ToD-dependent modulation is not explained by vigilance states. We additionally investigated whether an objective marker of EEG-documented vigilance changes\(^14\),\(^16\),\(^17\) showed comparable ToD-effects. We tested the main effect of ToD in resting-state thalamo-cortical connectivity using correlation maps ofthalamic BOLD time series with all other brain voxels. This parameter was not affected by ToD (whole-brain repeated measures ANOVA, cluster size \(k = 3\), \(P > 0.999\), FWE cluster correction, \(n = 14\)), suggesting that both subjective and objective measures of vigilance states do not explain the diurnal modulation in resting-state BOLD SD in the sensory cortices. To ascertain that this negative finding did not result from insufficient statistical power, we performed a bootstrap analysis using a published dataset in which vigilance changes have been investigated\(^18\), testing whether a group size of 14, as used in the present study, is sufficient to detect vigilance changes. Indeed, vigilance-related changes in thalamo-cortical connectivity can reliably (1–8 = 0.8) be detected in sample sizes as the one used in this study.

**Circadian and homeostatic factors.** The lack of an association with vigilance state rather suggests an endogenous modulation of resting-state cortical activity by circadian and/or homeostatic factors. When we examined the region of the suprachiasmatic nucleus in the hypothalamus\(^20\), which is the central pacemaker of the circadian system in the brain, we did not observe a modulation of resting-state BOLD variance as a function of ToD (repeated measures ANOVA, all voxels F\((5, 65) < 4.25\) and \(P ≥ 0.09\), small volume corrected, \(n = 14\)). Still, it remains possible that the ToD effects in BOLD SD in sensory cortices are regulated by the circadian system, because the suprachiasmatic nucleus codes time at each ToD in the magnitude of firing rate rather than in a low frequency band around 0.01–0.1 Hz\(^16\),\(^17\), we replicated the BOLD SD results in the frequency domain by calculating the amplitude of low-frequency fluctuations (Supplementary Fig. 1, Supplementary Tables 1 and 2). BOLD SD decreases in sensory cortices at 08:00 h and 20:00 h temporally coincided with local times of civil twilight, as the 08:00 h scan was close to or matched sunrise (end of civil morning twilight, range = 05:16–08:24 h), while the 20:00 h scan was close to or matched sunset (start of civil evening twilight, range 16:24–21:39 h). This suggests that both subjective and objective measures of vigilance states do not explain the diurnal modulation in resting-state BOLD SD in the sensory cortices. To ascertain that this negative finding did not result from insufficient statistical power, we performed a bootstrap analysis using a published dataset in which vigilance changes have been investigated\(^18\), testing whether a group size of 14, as used in the present study, is sufficient to detect vigilance changes. Indeed, vigilance-related changes in thalamo-cortical connectivity can reliably (1–8 = 0.8) be detected in sample sizes as the one used in this study.
In firing rate variance \(^{21,22}\). Indeed, both, chronotype, a proxy for circadian phase, and sleep pressure, a homeostatic factor, explained part of the ToD modulation in BOLD variance (Supplementary Fig. 2). The linear mixed model on resting-state BOLD SD in the visual cortex showed a significant interaction between ToD and chronotype \((F(5, 34.603) = 2.738, P = 0.037, \text{type III } F\text{-test, } n = 14)\) and between ToD and sleep pressure \((F(5, 32.21) = 5.128, P = 0.001, \text{type III } F\text{-test, } n = 14)\). There was no significant interaction between ToD and sleep debt \((F(5, 38.554) = 1.922, P = 0.113, \text{type III } F\text{-test, } n = 14)\).

While our study was not originally designed to dissociate the specific contribution of circadian and homeostatic factors in the ToD-dependent modulation of sensory cortex activity, a sleep deprivation study in a larger sample documented interactions between circadian and homeostatic influences and BOLD activity during a visual psychomotor vigilance task\(^ {10}\). In this prior study, an interaction between homeostatic and circadian factors was particularly observed in posterior, mostly occipital cortices, while the circadian modulation seemed less important in the frontal cortices. This observation suggests that the circadian system modulates perceptual processes in the sensory cortices and could potentially be the primary mediator of the ToD effects observed here.

**Time-of-day-effects in close-to-threshold visual perception.** Because the ToD-dependent modulation of BOLD SD represents changes in the spontaneous neural activity during resting-state, independent of sensory stimulation and task demands, our results suggest a ToD-dependent endogenous regulation of cortical activity. To reveal the relationship between such neural ToD effects in sensory cortices and perception we examined ToD effects on close-to-threshold visual perception, because dawn and dusk affect the signal quality of primarily the visual input. During all measurements, participants performed also a close-to-threshold visual detection task while still being scanned in constant dim light. A repeated measures ANOVA showed a significant main effect of ToD on the number of omission errors \((F\)
(5, 40) = 2.67, \( P = 0.036 \); partial eta-squared = 0.250, \( n = 9 \); Fig. 1c). Participants performed best at 08:00 h (mean ± SD omission errors = 1.17 ± 1.17) and 20:00 h (mean ± SD omission errors = 0.56 ± 0.88) compared to midday (omission errors all \( \geq 2.00 \); Table 3). Reaction times were not significantly different throughout the day (\( F(5, 40) = 2.01, \ P = 0.097 \); partial eta-squared = 0.201, \( n = 9 \)), indicating that omission errors are a better measure for detection of brief stimuli than reaction times.

Good visual detection performance at 08:00 and 20:00 h (Fig. 1c) occurred in parallel to low resting-state BOLD SD (Fig. 1b), both coinciding with times of twilight (Pearson correlations accounting for the dependence among the repeated measures: \( r = 0.54, \ P < 0.001, \ n = 54 \)). We observed a positive, linear relationship between resting-state BOLD SD in the visual cortex and omission errors during visual detection at the same times of day (i.e., low resting-state BOLD SD was associated with good visual detection, Fig. 2a). This relationship between behavior during the visual detection task and prior task-independent resting-state activity points towards an anticipatory mechanism that facilitates visual perception. Yet, linking BOLD

| Table 1 Brain regions showing ToD-effects of resting-state BOLD variance |
|-----------------------------------------------|
| **Anatomical region** | **Cluster P-value (FWE)** | **Cluster size (in voxels)** | **Local maxima** | **MNI coordinates (x, y, z)** |
|-------------------------|--------------------------|------------------------------|------------------|-----------------------------|
| Cluster 1 Occipital cortex (visual cortex) | 0.001 | 9089 | L Cuneus | −8, −90, 20 |
| | | | L Cuneus | −8, −80, 22 |
| | | | R Lingual gyrus | −10, −70, −6 |
| | | | R Calcarine gyrus | −12, −68, 4 |
| | | | R Cuneus | 8, −88, 32 |
| | | | R Cuneus | 6, −84, 28 |
| | | | R Cuneus | 8, −86, 24 |
| | | | R Lingual gyrus | 20, −60, −4 |
| | | | L Cuneus | −4, −80, 30 |
| | | | L Lingual gyrus | −10, −74, −6 |
| | | | L Lingual gyrus | −16, −52, −4 |
| | | | L Superior occipital gyrus | −16, −82, 26 |
| | | | R Cuneus | 2, −86, 16 |
| | | | R Calcarine gyrus | 10, −78, 16 |
| | | | R Calcarine gyrus | −10, −66, 8 |
| | | | L Paracentral lobule | −4, −38, 68 |
| | | | R Paracentral lobule | 6, −26, 60 |
| | | | L Paracentral lobule | −4, −26, 80 |
| | | | R Paracentral lobule | 8, −44, 74 |
| | | | L Precuneus | −8, −48, 58 |
| | | | L Postcentral gyrus | −60, −8, 40 |
| | | | L Postcentral gyrus | −56, −8, 42 |
| | | | L Postcentral gyrus | −50, −10, 40 |
| | | | L Postcentral gyrus | −36, −32, 70 |
| | | | L Postcentral gyrus | −46, −12, 38 |
| | | | L Precentral gyrus | −26, −20, 76 |
| | | | L Precentral gyrus | −44, −22, 64 |
| | | | L Postcentral gyrus | −48, −20, 60 |
| | | | L Postcentral gyrus | −48, −18, 56 |
| | | | L Postcentral gyrus | −64, −16, 16 |
| | | | L Precentral gyrus | −46, 6, 26 |
| | | | L Precentral gyrus | −24, −24, 64 |
| | | | L Postcentral gyrus | −44, −12, 48 |
| | | | R Postcentral gyrus | 58, −8, 36 |
| | | | R Precentral gyrus | 44, −16, 66 |
| | | | R Precentral gyrus | 30, −24, 72 |
| | | | R Postcentral gyrus | 50, −28, 56 |
| | | | R Postcentral gyrus | 50, −22, 50 |
| | | | R Postcentral gyrus | 30, −34, 72 |
| | | | R Precentral gyrus | 26, −28, 64 |
| | | | R Precentral gyrus | 22, −28, 62 |
| | | | R Precentral gyrus | 34, −26, 60 |
| | | | R Postcentral gyrus | 40, −38, 62 |
| | | | R Precentral gyrus | 40, −18, 54 |
| | | | R Superior temporal gyrus | 64, −48, 20 |
| | | | R Superior temporal gyrus | 54, −40, 14 |
| | | | R middle temporal gyrus | 58, −50, 16 |
| | | | R middle temporal gyrus | 60, −40, 6 |
| | | | R Rolandic operculum | 44, −34, 22 |
| | | | R Superior temporal gyrus | 68, −34, 6 |
| | | | R Supramarginal gyrus | 46, −38, 24 |
| | | | L Superior temporal gyrus | −66, −42, 16 |

Denomination of local maxima was adopted from the SPM Anatomy toolbox. MNI-coordinates represent peak activations inside clusters.

Note that the left auditory cortex was slightly subthreshold.
Table 2 Repeated measures ANOVA post-hoc t-tests on resting-state BOLD SD

| Anatomical region         | Post-hoc t-test | t(13) | P-value (Bonf.) | Cohen’s d |
|---------------------------|-----------------|-------|-----------------|-----------|
| Cluster 1                 |                 |       |                 |           |
| Visual cortex             | 11:00-08:00 h   | 4.68  | 0.006           | 1.29      |
|                           | 14:00-08:00 h   | 4.05  | 0.021           | 1.15      |
|                           | 17:00-08:00 h   | 3.95  | 0.025           | 1.15      |
|                           | 11:00-20:00 h   | 4.84  | 0.005           | 1.59      |
|                           | 14:00-20:00 h   | 4.01  | 0.022           | 1.43      |
|                           | 17:00-20:00 h   | 5.04  | 0.003           | 1.41      |
| Cluster 2-4               |                 |       |                 |           |
| Somatosensory cortex      | 11:00-08:00 h   | 3.97  | 0.024           | 0.90      |
|                           | 14:00-08:00 h   | 3.75  | 0.037           | 0.87      |
|                           | 17:00-08:00 h   | 3.90  | 0.028           | 0.94      |
|                           | 11:00-20:00 h   | 3.68  | 0.042           | 1.21      |
|                           | 14:00-20:00 h   | 3.81  | 0.033           | 1.12      |
|                           | 17:00-20:00 h   | 4.21  | 0.015           | 1.17      |
| Cluster 5                 |                 |       |                 |           |
| Right auditory cortex     | 11:00-08:00 h   | 4.50  | 0.009           | 0.64      |
|                           | 14:00-08:00 h   | 5.03  | 0.003           | 0.69      |
|                           | 17:00-08:00 h²  | 3.06  | 0.138           | 0.56      |
|                           | 11:00-20:00 h   | 3.51  | 0.002           | 1.10      |
|                           | 14:00-20:00 h   | 4.39  | 0.011           | 1.01      |
|                           | 17:00-20:00 h   | 4.58  | 0.008           | 0.90      |

Paired samples t-tests for BOLD SD for each cluster (significance threshold at P < 0.05, Bonferroni corrected). The T-Test marked with “a” was subthreshold, but listed in the table for completeness. All other comparisons were P < 0.05.

Table 3 Repeated measures ANOVA post-hoc t-tests on omission errors

| Post-hoc t-test | Mean Δ in omission errors | t(8) | P-value | Cohen’s d |
|-----------------|---------------------------|------|---------|-----------|
| 11:00-08:00 h²  | 0.83                      | 2.00 | 0.081   | 0.52      |
| 14:00-08:00 h   | 1.44                      | 2.76 | 0.025   | 0.60      |
| 17:00-08:00 h²  | 0.83                      | 1.40 | 0.199   | 0.54      |
| 11:00-20:00 h   | 1.44                      | 2.69 | 0.027   | 0.98      |
| 14:00-20:00 h   | 2.06                      | 3.26 | 0.012   | 0.94      |
| 17:00-20:00 h   | 1.44                      | 2.83 | 0.022   | 0.91      |

Planned paired samples t-tests based on the results of the analysis of BOLD SD (significance threshold at P < 0.05). T-Tests marked with “a” were subthreshold, but listed in the table for completeness. All other comparisons were P < 0.05.

Variation reductions to performance requires correlating not only resting-state, but also task-related BOLD variance with visual detection performance.

**ToD-effects in BOLD signal variance during visual perception.** Therefore, we further analyzed fMRI data during the visual detection task. We first tested whether ToD modulates task-related BOLD SD in the visual cortex during visual detection in the same way as it modulates visual cortex BOLD SD during resting state using a t-test (cluster-extent based threshold: k = 112, P < 0.05, FWE corrected; cluster-defining primary threshold of t(40) > 3.31, P < 0.001, uncorrected; n = 9). Task-related BOLD SD in two clusters in the primary and secondary visual cortex was reduced at 8:00 and 20:00 h, as compared to midday measurements (Fig. 3a and b). Reductions ranged from 7.4 to 26.8% of BOLD SD values at 14:00 h. In comparison to resting state, BOLD SD during the visual detection task was overall decreased in both the primary and secondary visual cortex (Rest-task: t(8) = 3.82, P = 0.010, Cohen’s d = 0.99, mean difference = 1.26, n = 9) and the secondary visual cortex (Rest-task: t(8) = 4.75, P = 0.002, Cohen’s d = 1.30, mean difference = 1.63, n = 9); significance threshold was at P < 0.05, Bonferroni corrected. Task-related BOLD SD in both visual cortex clusters was also linearly and positively related to omission errors during visual detection at the same ToD (Pearson correlations accounting for the dependence among the repeated measures in primary and secondary visual cortex: r = 0.43, P = 0.003, n = 54 and secondary visual cortex: r = 0.42, P = 0.004, n = 54; Fig. 2b). The similar ToD-dependent effects during both, the visual detection task and rest, suggest that endogenous reductions in spontaneous cortical activity persist during active processing.

**Discussion**

Here we report reduced visual cortex BOLD signal variance during close-to-threshold perception at times of twilight that correlates with improved visual detection. Because activity is also reduced during prior resting-state and masking factors have been accounted for in our analyses, an endogenous source of this ToD-dependent modulation of cortical activity is likely.

Neuromodulatory systems such as the acetylcholine-basal forebrain system or the norepinephrine-locus coeruleus system are known to be under circadian influence and to modulate homeostatic processes. Both noradrenergic and cholinergic systems impact neural processing in sensory cortices by increasing the signal to noise ratio (SNR) and consequently facilitate processing of weak incoming signals. Neurmodulators could thus potentially mediate both, the SNR enhancements and ToD-dependency of endogenous resting-state and perception-related BOLD activity.

Although previous work has shown stimulus-induced decreases in BOLD variance when comparing task conditions to rest, our findings are novel in that they demonstrate BOLD variance reductions, occurring even in the absence of any external stimuli or task conditions. While endogenous fluctuations in neural activity in sensory cortices have been associated with subsequent visual and auditory perception, this study reveals a ToD-dependency of such relationship between endogenous BOLD variance and perception. A direct facilitatory role of BOLD variance reductions in visual perception is likely given the consistent association between visual perception and variance in visual cortex activity during both rest, and visual detection. We propose that this anticipatory reduction of ongoing neural activity in visual cortices at times of twilight represents a mechanism to increase the SNR for close-to-threshold visual perception.

In addition to ToD-dependent reductions of spontaneous neural activity in human visual cortices, we observed the same effect in somatosensory and auditory cortices. Such a parallel increase in SNR in these cortices may further enhance perception in general, since multisensory interactions improve the accuracy of sensory processing when vision alone provides insufficient sensory evidence. Indeed, an increased auditory stimulus discrimination performance in humans has been described to occur at early and late ToD (around 06:00 and 20:00 h).

From a dynamical systems perspective, BOLD variance can be interpreted as a “wandering” of the brain around attractor states. Within this framework, our results suggest that spontaneous exploration of internal brain states, involving sensory areas, is reduced at times of twilight, which in turn could facilitate detection of weak external stimuli. Because we did not investigate time points after 23:00 h, we cannot determine whether the observed BOLD variance reductions occur isolated at times of twilight or whether they also occur during later phases of the night. In addition, we did not measure light exposure prior to scanning and calorie intake, so that we could not examine the relationship between these factors and BOLD SD.

Humans, a day-active species, largely rely on the visual sense for spatial orientation. Yet, even in ancestral societies, human activity usually extended into morning and evening times of twilight, when natural illuminance is drastically reduced. An
anticipatory increase in the SNR in the visual system during these times, compensating for the environmental visual constraints, may thus have been crucial for survival until the introduction of electric light sources into social life. Beyond this vital importance, our findings may represent a new generic mechanism underlying improved perception of close-to-threshold sensory input.

Methods
Participants. Fourteen healthy male volunteers were included in this study (mean age = 23.8, range = 20–27). Four additional participants were recruited for this study, but had to be excluded from analysis because of non-completion of the entire study protocol or non-correctible imaging artifacts. To enable a quantification of potential habituation effects, participants came in on two days, and were assessed multiple times throughout the day. Because of this resource- and cost-intense study design, sample size was limited. No participant had a history of neuropsychiatric or vascular disease, took medication or psychoactive substances, had recent or actual sleep disturbances, worked shifts, or consumed caffeine excessively (>3 cups of coffee or other caffeinated beverages per day). We restricted this study to male participants because of reported gender variations in resting-state dynamics and possible interactions between the menstrual cycle and circadian rhythms. All participants gave their informed consent and received payment for their participation. This study has been approved by the ethics commission of the Medical Faculty of the Goethe University Frankfurt (GZ 244/09) in accordance with the Declaration of Helsinki.

We instructed all participants to follow regular bedtimes and wake-up-times (between 23:00–00:00 h and 7:00–8:00 h, respectively) for five days before scanning until completion of the experimental phase. Compliance was monitored with a diary of activities and by continuous wrist-actigraphy recordings. The actigraph (Actiwatch Mini; CamNtech Ltd., Papworth Everard, UK) was worn at all times on the non-dominant wrist except during scanning sessions. Individual differences in the phase of entrainment – i.e., the chronotype and increasing homeostatic sleep pressure during time spent awake, but also increasing sleep debt as a marker of sleep deprivation, are known to modulate the ToD-dependent profile of neurobehavorial variables. Therefore, the participants’ individual chronotype was assessed with the Munich Chronotype Questionnaire and characterized with a single value, the midpoint of sleep on free days, corrected for sleep debt accumulated during work days (MSFsc). We a priori excluded extreme chronotypes. The mean ± SD MSFsc of the participants was 4.87 ± 0.92 (range = 3.89–6.71). The MSFsc could not be assessed in two participants due to non-completion of the questionnaire.
Individual wake-up time on scan days was extracted from actigraphy and used as an indicator for sleep pressure in each participant at ToD\(25,26\). Since participants were always scanned at the same times, an earlier wake-up time indicates higher sleep pressure at each ToD compared to later wake-up times. Mean ± SD wake-up time of the participants was 06:10 ± 00:39 h (range = 05:00–07:00 h). Wake-up time could not be calculated in one participant. The cause for this could not be determined on the participants’ sleep debt, indexed by the difference between mean weekly sleep duration from the Munich Chronotype Questionnaire and sleep duration during scanning days extracted from actigraphy\(^{-25,26}\). Because of non-completion of the questionnaire and missing actigraphy data (see above), sleep debt could not be assessed in three participants. Mean ± SD sleep debt of the participants was 01:07 ± 03:09 h. Higher values indicate higher sleep debt. Because actigraphy-based sleep estimates often under-estimate the duration of sleep, as compared to self-reports\(^{45}\), this quantification possibly overestimates the true extent of sleep pressure in the population.

During fMRI scanning, body temperature was measured with an ear thermometer and the participants’ sleep propensity was assessed with a self-report questionnaire based on a modified version of the Epworth Sleepiness Scale (ESS) questionnaire\(^{44}\), in which participants were asked to rate their sleep propensity by projecting their actual vigilance state in the situations provided by the questionnaire to quantify diurnal sleepiness. Participants did not nap or consume stimulants during scanning days. Time in this paper is expressed in Central European Time (CET), the German local time. Times of twilight were obtained from the German Meteorological Office.

Resting-state fMRI. The participants underwent six fMRI scanning sessions at fixed times on two subsequent days (8:00, 11:00, 14:00, 17:00, 20:00, and 23:00 h on each day) to account for habituation effects to the experimental setting. To minimize interferences with the participants’ sleep, no measurements were scheduled between 24:00 and 07:00 h. Each fMRI session included a 7 min resting-state measurement in dim light. The participants were instructed to lie still and comfortably in a supine position, to keep their eyes open and fixate a white crosshair on a black background (< 1 lux, maximum illumination measured at corneal level using a WOLFCRAFT LX-1108 lux meter, Wolfcraft, Hirschau, Germany), not to fall asleep, and not to think of anything in particular and let their mind wander freely. A minimum of five minutes elapsed between switching off the main lights in the scanning room and the beginning of the functional measurement, allowing participants to adapt to the dim light conditions. Participants wore headphones and earplugs to protect against the scanner noise, and their heads were immobilized with foam cushions to minimize movement artifacts. If visual correction was necessary, participants were provided with appropriate MRI compatible corrective lenses. A coil-mounted mirror allowed viewing the projection screen and the white crosshair on a black background was displayed using Presentation software (Neurobehavioral Systems Inc., Berkely, CA, USA; http://www.neurobs.com). During fMRI scans, we monitored the participants visually and recorded their cardiorespiratory and movement parameters. Prior to the experimental phase, participants underwent an ‘acclimatization session’ in the scanner to acclimatize to the unfamiliar environment and experimental procedures.

Visual detection task fMRI. Nine of the original participants additionally performed a visual detection task in dim light while still being scanned for additional 4 min 52 s at each ToD. They were instructed to press a button with their right index finger as soon and as quickly as possible when they saw a low-contrast orange crosshair (< 1 lux) flashing shortly (500 ms) on the center of a black screen inside the scanner (background light in the dimly lit scanner room was <0.1 lux). Three one-minute task blocks with 11 crosshair presentations each (randomized interstimulus interval from 2–10 s) alternated with four 5 s fixation blocks (white crosshair, <1 lux, as during resting-state measurements).

Data acquisition and preprocessing of resting-state fMRI data. Resting-state data were collected using a 3 Tesla MRI-scanner (Siemens MAGNETOM Allegra, syno MR 2004A, Erlangen, Germany). A total of 12 resting-state sessions of a gradient-echo T2*–weighted transverse echo-planar imaging sequence (EPI) were acquired for each participant. A resting-state session comprised 210 EPI volumes (6 sessions on two subsequent days = 12 × 210 volumes for each participant). Each volume contained 30 axial slices acquired in a sequential manner, covering the whole brain (TR/TE/flip angle = 2000ms/30 ms/90°, FOV = 192 mm, matrix size (resolution) = 128 × 128 × 32, 3 mm slice distance). The first 4 volumes were discarded to avoid magnetic saturation effects. Spatial preprocessing was performed using standard algorithms implemented in SPM 12 (Statistical Parametrical Mapping 12, Version 6225; Wellcome Trust Center for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). Head-motion correction via intra-subject spatial Real Systems Analysis (least square approach and 6 parameter rigid body spatial transformation), spatial normalization to the standard EPI template of the Montreal Neurological Institute (MNI) and resampling to a voxel size of 2 × 2 × 2 mm\(^3\) (using a 12 parameter affine transformation), and spatial smoothing with an isotropic 8 mm full-width at half maximum (FWHM) Gaussian kernel\(^{16}\). Spatial smoothing improves the resolution of statistical T1 signal changes that was calculated after Dunlap et al.

Group analysis of resting-state fMRI data. Group statistical analyses were performed using SPM 12. First, for each participant, the BOLD SD, ALFF, and thalamic connectivity values acquired on the two consecutive days were averaged voxel-wise at each ToD separately, using the ImCalc tool in SPM, yielding BOLD SD, ALFF and thalamic connectivity whole brain maps for each of the six times of interest. Then we used whole-brain, random-effects, one-way repeated measures ANOVAs (ToD as independent variable with 6 levels, homoscedasticity and, midday measurements 11:00, 14:00, and 17:00 h). Again, clusters in right, left, and thalamus were investigated further, because we found no significant clusters in thalamic connectivity values for repeated measures ANOVA (ToD as independent variable with 6 levels). The high-resolution anatomical T1 sequence was used to exclude any structural anomalies and to map the functional results onto an averaged brain with higher anatomical resolution (see Supplementary Fig. 1).
sphere20 and served for a small volume correction in the BOLD SD voxel-wise Region of interest analysis in the suprachiasmatic region 8

NATURE COMMUNICATIONS |  (2018) 9:1274 | DOI: 10.1038/s41467-018-03660-8 | www.nature.com/naturecommunications

results for Supplementary Fig. 1 were rendered on the averaged T1-image using MRIcroGL (Version 1 Jan 2015; http://www.mccauslandcenter.sc.edu/mricrogl/).

Multi-variable adjustment of linear mixed models. Resting-state activity can be affected by a range of potential confounders. To test whether the observed ToD effects in BOLD SD were driven by such effects, we calculated a linear mixed model with resting-state BOLD SD in the visual cortex as dependent variable. This model included ToD and scanning day as repeated measures fixed effects (autoregressive covariance structure AR(1)), as well as heart rate, breathing rate, body temperature, subjective sleepiness, amplitude and variance of head motion parameters, and the number of outliers in head motion parameters (defined as values above the mean ± 2.5 standard deviations), chronotype, sleep pressure, and sleep debt as fixed effects. Subjects were included as a random effects variable. We used the restricted maximum likelihood estimation method and tested whether the main effect of ToD in BOLD SD in the visual cortex remained significant (P < 0.05), when including all these potential confounding factors. To study whether chronotype, sleep pressure, and sleep debt, as well as scanning day explained part of the ToD modulation in BOLD variance, we additionally included multiplicative interaction terms in the multi-variable adjusted mixed models. Interactions were considered significant at P < 0.05. A significant interaction between ToD and scanning day could potentially reveal habituation effects due to repetitive measurements on two subsequent scanning days. Yet, the interaction was not significant (F(5, 41.829) = 0.871, P = 0.509, type III F-test, n = 14).

Region of interest analysis in the suprachiasmatic nucleus. We additionally investigated ToD effects on resting-state BOLD SD in a region of interest in the suprachiasmatic nucleus, because this area controls the circadian pacemaker of the suprachiasmatic nucleus. The region of interest was defined using a published sphere 26 and served for a small volume correction in the BOLD SD voxel-wise group analysis. We tested for a main effect of ToD using an F-contrast with a statistical threshold of P < 0.05, small volume corrected.

Analysis of visual detection task fMRI data. The same data acquisition, preprocessing and single-subject statistical modeling parameters as for the resting-state BOLD SD analyses were applied to obtain voxel-wise BOLD SD for the whole brain, in each session (ToD) of the visual detection task blocks (see above). The first 5 volumes at the beginning of each block were removed to avoid carry-over effects from the previous block. Then, the three task blocks were concatenated and BOLD SD calculated separately for each ToD which resulted in a voxel-wise BOLD SD map for each ToD. Fixation blocks were not further analyzed, because the experimental conditions were similar to resting-state. Using SPM 12 for group statistical analyses, task-related BOLD SD in the visual cortex was probed for the ToD-dependent effects observed during resting-state. As with the resting-state data, task data on the two consecutive days were averaged voxel-wise for each ToD for each participant. We tested for BOLD SD reductions at 08:00 and 20:00 h compared to midday measurements (11:00, 14:00, and 17:00 h) using a one-sample t-test. The analysis was restricted to the visual cortex that showed a main effect of ToD during resting-state and results were thus corrected within this mask at P < 0.05, FWE cluster-corrected (cluster-forming threshold P < 0.001, uncorrected). Values from significant clusters were averaged within each cluster and extracted for each ToD and participant separately using MarsBaR and used for subsequent analyses (see below).

We ascertained the consistency of the effect by using the global conjunction hypothesis 25 of the contrasts (08:00 h separately against 11:00, 14:00, and 17:00 h) and (20:00 h separately against 11:00, 14:00, and 17:00 h).

Comparing resting-state and task-related BOLD SD. We tested for significant overall decreases in BOLD variance during visual detection compared to resting state in ToD-dependent, two-tailed, paired samples t-tests in SPSS (significance threshold at P < 0.05, Bonferroni corrected). The peak voxel BOLD SD values of significant clusters from the visual detection task were extracted for each ToD and participant using MarsBar, then averaged over all ToD and compared with the ToD-averaged resting-state BOLD SD values at the same coordinates.

Visual detection task performance. Omission errors (i.e., lapses) and reaction times for each participant and ToD were analyzed using SPSS Statistics. To test for ToD-dependent differences in visual detection mirroring the ToD-dependent changes of BOLD SD in sensory cortices, we performed a repeated measures ANOVA (ToD as independent variable with 6 levels, Mauchly’s test of sphericity was P = 0.05, unless otherwise stated) with subsequent planned pairwise comparisons using a Bonferroni adjusted, two-tailed t-test, testing the difference between 08:00 and 20:00 h and, midday measurements (11:00, 14:00, and 17:00 h), respectively (significance threshold set at P < 0.05).

Correlation analysis of omission errors and resting-state BOLD SD. A Pearson product-moment correlation analysis was performed to test for a linear relationship between BOLD SD in the visual cortex and omission errors in visual detection at the same times. Since an ordinary correlation is not appropriate for repeated measures 42, a Pearson correlation coefficient accounting for the dependence among the repeated measures was calculated, which removes the variance between subjects 43,44. Therefore, the resulting correlation coefficient represents the association between BOLD SD and omission errors over all ToD for all participants, corrected for interindividual differences. The significance threshold was set at P < 0.05.

Correlation analysis of omission errors and task-related BOLD SD. Using the same procedure as above, we also tested for a linear relationship between task-related BOLD SD in visual cortex and omission errors in visual detection at the same times. Since the analysis of task-related BOLD SD resulted in two significant clusters in the visual cortex, correlations were calculated for BOLD SD in the two clusters separately, significance threshold P < 0.05.

Power analysis forthalamic resting-state functional connectivity. To ascertain that the negative finding in ToD-dependent thalamicconnectivity as an objective marker of vigilance did not result from insufficient statistical power, we performed a power analysis using a bootstrapping method on a published dataset in which vigilance changes have been investigated 15,45. For this re-analysis we used exactly the same parameters that we applied in our study here and investigated whether vigilance changes (N1 sleep stage vs. wakefulness) could be detected in 14 subjects in the previous dataset. In detail, a total of 93 continuous (≈3.5 min) epochs of wakefulness and 54 of N1 sleep were selected from Tagliazucchi and Laufs 10. Two sets of 14 epochs were randomly selected (with replacement) from the wakefulness and N1 sleep sets and the voxel-wise thalamic functional connectivity (as determined by the AAL atlas 46) was computed. We tested for significant group differences using a mass-univariate two-sample and two-tailed Student’s t-test, thresholded at P < 0.001 cluster- and voxel-level. FWE corrected on the cluster level. For each iteration of the randomly selected 14 wakefulness/N1 sleep epochs, voxels were flagged as significant if their P-value fulfilled these criteria. This was repeated for 1000 iterations, and a voxel-wise map, showing the proportion of times a voxel was deemed significant, was obtained. Since significant clusters were observed, the group size of 14 participants as used in our study can be judged sufficiently large to be sensitive to detect vigilance changes.

Data availability. The imaging data that support the findings of this study are available at G-NODE with identifier https://doi.org/10.12751/g-node.16591b. The code to calculate voxel-wise variables of interest in fMRI data is available upon demand.

Received: 30 August 2017 Accepted: 1 March 2018
Published online: 10 April 2018

References
1. Ashcroft, J. (ed.) Handbook of behavioral neurobiology, Volume 4: biological rhythms. (Plenum Press, New York and London, 1981).
2. Mohawk, J. A., Green, C. B. & Takahashi, J. S. Central and peripheral circadian clocks in mammals. Annu. Rev. Neurosci. 35, 445–462 (2012).
3. Hastings, M. H., Reddy, A. B. & Maywood, E. S. A clockwork web: circadian timing in brain and periphery, in health and disease. Nat. Rev. Neurosci. 4, 649–661 (2003).
4. Brown, T. M. & Piggins, H. D. Electrophysiology of the suprachiasmatic circadian clock. Prog. Neurobiol. 82, 229–255 (2007).
5. Chang, D. & Reppert, S. The circadian clocks of mice and men. Neuron 29, 555–558 (2000).
6. Saper, C. B. & Fuller, P. M. Wake-sleep circuity: an overview. Curr. Opin. Neurobiol. 44, 186–192 (2017).
7. Schmidt, C., Collette, F., Cajoche, C. & Peigneur, P. A. time to think: circadian rhythms in human cognition. Cogn. Neurosci. 24, 755–789 (2007).
8. Silver, R. & Kriegsfeld, L. J. Circadian rhythms have broad implications for understanding brain and behavior. Eur. J. Neurosci. 39, 1866–1880 (2014).
9. Fox, M. D. & Raichle, M. E. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 8, 700–711 (2007).
10. Muto, V. et al. Local modulation of human brain responses by circadian rhythmicity and sleep debt. Science 353, 687–690 (2016).
11. Vandewalle, G. et al. Effects of light on cognitive brain responses depend on circadian phase and sleep homeostasis. J. Biol. Rhythms 26, 249–259 (2011).
12. Shannon, B. J. et al. Morning-evening variation in human brain metabolism and memory circuits. J. Neurophysiol. 109, 1444–1456 (2013).
13. Blautz, J., et al. Classifying fMRI-derived resting-state connectivity patterns according to their daily rhythmicity. *Neuroimage* 71, 298–306 (2013).

14. Garrett, D. B., et al. Moment-to-moment brain signal variability: a next frontier in human brain mapping? *Neurosci. Biobehav. Rev.* 37, 610–624 (2013).

15. Biancardi, M., et al. Modulation of spontaneous fMRI activity in human visual cortex by behavioral state. *Neuroimage* 45, 160–168 (2009).

16. Biswal, B. B., Van Keulen, J. & Hyde, J. S. Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps. *NMR Biomed.* 10, 165–170 (1997).

17. Cordes, D. et al. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. *Aurr. Am. J. Neuroradiol.* 22, 1326–1333 (2001).

18. Tagliazucchi, E. & Laufs, H. Decoding wakefulness levels from typical fMRI resting-state data reveals reliable drifts between wakefulness and sleep. *Neuron* 82, 695–708 (2014).

19. Picchioni, D., et al. Decreased connectivity between the thalamus and the neocortex during human nonrapid eye movement sleep. *Sleep* 37, 387–397 (2014).

20. Schmidt, C., et al. Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. *Science* 324, 516–519 (2009).

21. Shibata, S., Oommen, Y., Kita, H. & Hatton, K. Circadian rhythmic changes of neuronal activity in the suprachiasmatic nucleus of the rat hypothalamic slice. *Brain Res.* 247, 154–158 (1982).

22. Meijer, J. H., Schaap, J. & Albus, H. Multitunit activity recordings in the suprachiasmatic nucleus in vivo: versus in vitro models. *Brain Res.* 753, 322–327 (1997).

23. Aston-Jones, G., Chen, S., Zhu, Y., & Oshinsky, M. L. A neural circuit for control of behavior by time. *Science* 295, 1549–1552 (2002).

24. Kametani, H. & Kawamura, H. Circadian rhythm of cortical acetylcholine release as measured by in vivo microdialysis in freely moving rats. *Neurosci. Lett.* 132, 263–266 (1991).

25. Berrett, C. W. & Waterhouse, B. D. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Rev.* 42, 33–84 (2003).

26. Sato, H., Hata, Y., Masui, H. & Tsumoto, T. A functional role of cholinergic innervation to neurons in the cat visual cortex. *J. Neurophysiol.* 58, 765–780 (1987).

27. Ponce-Alvarez, A., He, B. J., Hagmann, P. & Deco, G. Task-driven activity reduces the cortical activity space of the brain: experiment and whole-brain modeling. *PLoS Comput. Biol.* 11, e1004445 (2015).

28. Sadaghiani, S., Hesselmann, G., Friston, K. J. & Kleinschmidt, A. The relation of ongoing brain activity, evoked neural responses, and cognition. *Front. Syst. Neurosci.* 4, 20 (2010).

29. Amedi, A., von Kriegstein, K., van Atteveldt, N. M., Beauchamp, M. S. & Naumer, M. J. Functional imaging of human crossmodal identification and object recognition. *Exp. Brain Res.* 166, 559–571 (2005).

30. Bullkin, D. A. & Grob, J. M. Seeing sounds: visual and auditory interactions in the brain. *Curr. Opin. Neurobiol.* 16, 415–419 (2006).

31. Lent, M., Wittmann, M., von Steinbach, N., Poppel, E. & Roenneberg, T. Daily rhythm of temporal resolution in the auditory system. *Cortex* 35, 89–100 (1999).

32. van Gelder, T. The dynamical hypothesis in cognitive science. *Behav. Brain Sci.* 21, 615–665 (1998).

33. Deco, G., Jirsa, V. K. & McIntosh, A. R. Resting brains never rest: computational insights into potential cognitive architectures. *Trends Neurosci.* 36, 268–274 (2013).

34. Yetish, G. et al. Natural sleep and its seasonal variations in three pre-industrial societies. *Curr. Biol.* 25, 2862–2868 (2015).

35. Packer, C., Swanson, A., Ilanda, D. & Kushnir, H. Fear of darkness, the full moon and the nocturnal ecology of african lions. *PLoS ONE* 6, e22285 (2011).

36. Filippi, M. et al. The organization of intrinsic brain activity differs between genders: a resting-state fMRI study in a large cohort of young healthy subjects. *Hum. Brain Mapp.* 34, 1330–1343 (2013).

37. Baker, F. C. & Driver, H. S. Circadian rhythms, sleep, and the menstrual cycle. *Sleep Med.* 8, 613–622 (2007).

38. Roenneberg, T. et al. Human activity and rest in sita. *Methods Enzymol.* 552, 257–283 (2015).

39. Roenneberg, T., Wirz-Justice, A. & Merrow, M. Life between clocks: daily patterns of human chronotypes. *J. Biol. Rhythms* 18, 80–90 (2003).

40. Roenneberg, T. et al. A marker for the end of adolescence. *Curr. Biol.* 14, R1038–R1039 (2004).

41. Roenneberg, T. et al. Epidemiology of the human circadian clock. *Sleep Med. Rev.* 11, 429–438 (2007).

42. Vetter, C., Juda, M. & Roenneberg, T. The influence of internal time, time awake, and sleep duration on cognitive performance in shiftworkers. *Chronobiol. Int.* 29, 1127–1138 (2012).

43. Crespedes, E. M. et al. Comparison of self-reported sleep duration with actigraphy: results from the Hispanic Community Health Study/Study of Latinos Sueno Ancillary Study. *Am. J. Epidemiol.* 183, 561–573 (2016).

44. Johns, M. W. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 14, 540–545 (1991).

45. Friston, K. J. et al. Spatial registration and normalization of images. *Hum. Brain Mapp.* 3, 165–189 (1995).

46. Weissenbacher, A. et al. Correlations and anticorrelations in resting-state functional connectivity MRI: a quantitative comparison of preprocessing strategies. *Neuroimage* 47, 1408–1416 (2009).

47. Tzourio-Mazoyer, N. et al. Automated anatomical labeling of activations in SPm using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289 (2002).

48. Zang, Y. F. et al. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev.* 29, 83–91 (2007).

49. Zuo, X. N. et al. The oscillating brain: complex and reliable. *Neuroimage* 49, 1432–1445 (2010).

50. Tagliazucchi, E., Carhart-Harris, R., Lecce, R., Nutt, D. & Chialvo, D. R. Enhanced repertoire of brain dynamical states during the psychedelic experience. *Hum. Brain Mapp.* 35, 5442–5456 (2014).

51. Eickhoff, S. B. et al. A new SPm toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335 (2005).

52. Dunlap, W. P., Cortina, J. M., Vaslow, J. B. & Burke, M. J. Meta-analysis of experiments with matched groups or repeated measures designs. *Psychol. Methods* 1, 170–177 (1996).

53. Friston, K. J., Penny, W. D. & Glaser, D. E. Conjunction revisited. *Neuroimage* 25, 661–667 (2005).

54. Bland, J. M. & Altman, D. G. Correlation, regression, and repeated data. *Br. Med. J.* 308, 896 (1994).

55. Bland, J. M. & Altman, D. G. Calculating correlation coefficients with repeated observations: Part 1-correlation within subjects. *Br. Med. J.* 310, 446 (1995).

56. Kriegeskorte, N., Simmons, W. K., Bellgowan, P. S. & Baker, C. I. Circular analysis in systems neuroscience: the dangers of double dipping. *Nat. Neurosci.* 12, 535–540 (2009).

Acknowledgements

This study was supported by the August Scheidel Foundation. C.A.K. is supported by an Emmy Noether grant of the German Research Foundation (KE 1514/2-1).

Author contributions

C.A.K. and J.H.S. conceptualized the study; L.C. and E.T. acquired the data; L.C., E.T., C.V., J.H.S. and C.A.K. wrote the manuscript. C.A.K. and J.H.S. conceptualized the study; L.C. and C.H. acquired the data; L.C., E.T., C.V., J.H.S. and C.A.K. wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-03660-8.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.