Section: Behavioral/Systems/Cognitive Neuroscience

Functional MRI assessed brain responses during an executive task depend on interaction of sleep homeostasis, circadian phase, and PER3 genotype

Gilles Vandewalle[1], Simon N. Archer[2], Catherine Wuillaume[1], Evelyne Balteau[1], Christian Degueldre[1], André Luxen[1], Pierre Maquet[1], Derk-Jan Dijk[2]

***1**. Cyclotron Research Centre, University of Liège, Belgium;*

***2**. Surrey Sleep Research Centre, University of Surrey, Guildford, UK.*

Online Supplemental Material

Number of pages: 16

Number of supplemental figures: 3

Number of supplemental tables: 5

Supplemental Methods

Genotyping

Genotyping was carried out as described previously (Viola et al., 2007), with some modifications. Briefly, buccal swab (Sarstedt, Nümbrecht) samples were collected from 254 volunteers and genomic DNA was extracted using the QuickExtract system (Epicentre Biotechnologies, Madison, Wisconsin). The *PER3* 4/5 variable number tandem-repeat polymorphism was genotyped by PCR DNA amplification followed by fragment length analysis with agarose gel electrophoresis. PCR was carried out using GoTaq Green Master Mix (Promega, Madison, Wisconsin) with the following cycling conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min. 254 DNA samples were successfully genotyped. Of these, 105 were homozygous for the 4-repeat allele (41.4%), 29 were homozygous for the 5-repeat allele (11.4%), and 120 were heterozygous (47.2%).

Sleep-wake cycles

Volunteers were requested to refrain from all caffeine and alcohol-containing beverages and intense physical activity for 3 days before participating in the study.

After both segments of the experiment, actigraphy data were visually inspected to determine sleep onset and wake times, sleep midpoint and sleep durations of all nights during the 7 days of scheduled sleep preceding each experimental segment (Table S2). Repeated measure ANOVAs with genotype ($PER3^{4/4}$ and $PER3^{5/5}$) as between-subject factor, and segment (Sleep and Sleep Deprivation - SD) as within-subject factor, revealed no main effect of segment ($p > 0.1$), or genotype ($p > 0.1$), or genotype-by-segment interaction ($p > 0.1$) for any of the parameters, demonstrating, in accordance with our previous study (Viola et al 2007), no significant differences in sleep-wake habits between the genotypes in the selected sample.

Timing of fMRI sessions

To verify that the timing of fMRI sessions relative to the actigraphically assessed sleep-wake parameters did not differ between segments and genotypes, we computed repeated

measure ANOVAs with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and segment (Sleep and SD) as within-subject factor. This analysis revealed no main effect of segment ($p > 0.1$), and of genotype ($p > 0.1$), and no genotype-by-segment interaction ($p > 0.1$) for any of the parameters (clock times of fMRI sessions, time awake before each fMRI session, time of fMRI sessions relative to actigraphy-assessed sleep midpoint).

fMRI sessions

Each fMRI session consisted of two consecutive fMRI runs. The first run (~10 minutes) was acquired in complete darkness and is reported in the present manuscript. The second run (~14 minutes) investigated the effects of light exposure. These data will be reported in another article. In both runs, subjects performed an auditory 3-back task (Cohen et al., 1997). Three drops of tropicamidum 0.5% (Tropicol®) were administered in the eyes 20 min before entering the scanner to inhibit pupillary constriction.

During the data acquisition period, all subjects interacted with the same investigator, who used a standardized set of sentences before and between each run. This protocol was implemented in order to minimize variation in motivational state due to social interactions [*e.g.* encouragement by an investigator which may modify brain responses (Grandjean et al., 2005)]. No feedback was given on performance. Volunteers were trained on a shortened version of the protocol and habituated to the experimental conditions during a training session taking place at least a week before the first experimental segment.

3-back task

The stimuli consisted of nine French monosyllabic consonants that are phonologically different, for easier identification. Stimuli were 500ms long and stimulus onset asynchrony was 2500ms. Seven blocks consisting of series of 26 to 30 consonants were presented in each session for a total of 65 to 75s. Series of stimuli were pseudo-randomly constructed with ~30% positive answers. Blocks were separated by a rest period which lasted 10 to 26s (complete runs lasted 9.5 to 10 minutes). Stimuli were produced using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) implemented in MATLAB 6.1 (Mathworks Inc., MA),

and transmitted to the subjects using MR CONTROL amplifier and headphones (MR Confon, Germany). The first session was preceded by a short session, during which participants adjusted the volume level to ensure optimal auditory perception during scanning.

A priori location of interest for fMRI multiple comparison correction over small spherical volume

Frontal cortex: **inferior frontal gyrus/sulcus** (Thomas et al., 2000)**; middle frontal gyrus/sulcus** (Drummond et al., 2001; Koechlin et al., 2003; Drummond et al., 2005a; Collette et al., 2007)**; superior frontal gyrus/sulcus** (Drummond et al., 2000)

Occipital cortex: **middle occipital cortex** (Chee and Chuah, 2007)**; fusiform gyrus** (Majerus et al., 2006; Chee and Chuah, 2007)

Parietal cortex: **superior parietal cortex** (Drummond et al., 2005a; Tomasi et al., 2008)**; intraparietal sulcus** (Drummond et al., 2005a)

Temporal cortex: **inferior temporal gyrus/sulcus; middle temporal gyrus/sulcus; superior temporal gyrus/sulcus** (Chee and Chuah, 2007) (Drummond et al., 2001)

Subcortical areas: **globus pallidus** (Drummond et al., 2005b); **putamen** (Thomas et al., 2000; Drummond et al., 2004); **superior colliculus** (Morris et al., 1999); **thalamus** (Portas et al., 1998; Coull, 2004; Habeck et al., 2004; Kastner et al., 2004; Choo et al., 2005);

Cingulate cortex (Thomas et al., 2000; Drummond et al., 2001)

Cerebellum (Habeck et al., 2004)

Others: **anterior insula** (Cohen et al., 1997; Thomas et al., 2000); **parahippocampus** (Habeck et al., 2004)

Melatonin assay

Salivary melatonin was measured by radioimmunoassay (Stockgrand Ltd, University of Surrey, Guildford, United Kingdom), as previously described (English et al., 1993). Saliva samples were collected before the evening fMRI session and then at ten hourly intervals afterwards until the morning fMRI session. Thus, 11 samples in total were collected from the 27 subjects, except for three individuals in whom the first, second and last samples,

respectively, were missing. Of the total 293 samples collected, 143 samples were analyzed in duplicate for melatonin concentration. For each sample, 500 µL volumes were analyzed for melatonin concentration, except for 17 samples where between 200 – 400 µL were used due to sample volume limitations. Final melatonin concentrations for these samples were adjusted accordingly. The limit of detection of the assay was 0.7 pg/ml. The intra-assay coefficients of variation (CV) were: 3.2 ± 7.9%, 5.0 ± 5.0%, 25.2 ± 6.2%, and 41.4 ± 5.3%, and the inter-assay CVs were: 3.2 ± 11.4%, 5.1 ± 13.1%, 22.8 ± 12.5%, and 40.5 ± 9.4%.

Other statistical analyses

All other statistical analyses were computed with STATISTICA 6.1 (StatSoft France, France). Repeated measure ANOVAs with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and session (MS, ES, ESD and MSD) as within-subject factor, were computed on accuracy and reaction times for the 3-back tests, and the KSS scores recorded around the fMRI sessions. Repeated measure ANOVAs with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and segment (Sleep and SD) as within-subject factor, were computed on actigraphy-assessed sleep parameters (Table S2), time awake before each fMRI session, clock times of fMRI, and time relative to actigraphy-assessed sleep midpoint (Table S3). Repeated measure ANOVAs with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and repetition as within-subject factor, were computed on melatonin concentrations and KSS scores obtained during the sleep deprivation. Two-sample t-tests on independent samples (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) were computed on subjects' characteristics as assessed during the screening (Table S1).

Supplemental Results

Demographics

The *PER3*$^{4/4}$ and *PER3*$^{5/5}$ populations were not significantly different for any of the measured characteristics, except for the ESS scores (Table S1). The ESS requires subjects to self-assess the probability of falling asleep in various non-stimulating situations (Johns, 1991). On this scale, *PER3*$^{5/5}$ individuals rated themselves more likely to fall asleep than *PER3*$^{4/4}$ did. It is

worth emphasizing that these scores were obtained at least one week before the experiment, i.e. before being assigned to a regular habitual sleep schedule for 7 days.

Melatonin

Repeated measure ANOVAs with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor and melatonin sample concentration (11 samples) as within-subject factor revealed a main effect of sample ($F = 7.9$; $df = 10, 250$; $p < 10^{-6}$), but no main effect of genotype ($F = 0.01$; $df = 1, 25$; $p = 0.92$) and no genotype-by-sample interaction ($F = 0.29$; $df = 10, 250$; $p > 0.98$) (Figure 2).

Subjective sleepiness

Subjective sleepiness scores, as assessed by the KSS scale, were analyzed in 2 steps. First, we considered the 5 KSS scores collected during the 60 minutes of strictly controlled activity that preceded each fMRI session (3 KSS scores) and while subjects were in the scanner, i.e. the scores collected after the first and second runs (the second run is not included in this paper) (Figure S1a, 4 left panels). Repeated measure ANOVA on these KSS scores with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and repetition of KSS score (5) and session (MS, ES, ESD, MSD) as within-subject factors revealed a main effect of session ($F = 77.57$; $df = 3, 75$; $p < 10^{-6}$), and a significant session-by-repetition interaction ($F = 1.83$; $df = 4, 100$; $p < 10^{-4}$). However, no main effect of repetition ($F = 1.83$; $df = 4, 88$; $p = 0.13$) and of genotype ($F = 0.003$; $df = 1, 25$; $p = 0.96$), and no repetition-by-genotype interaction ($F = 1.15$; $df = 4, 100$; $p = 0.94$), as well as no repetition-by-session-by-genotype interaction ($F = 0.57$; $df = 12, 300$; $p = 0.86$) were detected. Planned comparison showed that KSS scores of MS vs. ES, and ES vs. ESD sessions were not significantly different ($F < 2.8$, $df = 1, 25$; $p > 0.1$) but that those of ESD vs. MSD, and MS vs. MSD sessions were significantly different ($F > 186.28$, $df = 1, 25$; $p < 10^{-6}$), demonstrating that subjects were equally sleepy in the morning and in the evening, unless they were sleep deprived, in which case sleepiness in the morning was increased.

We then considered the KSS scores collected between the ESD and MSD sessions of the SD segment (Figure S1a, right panel), i.e. the 18 scores collected between the end of ESD

session and the beginning of the 60 minutes of strictly controlled activity preceding MSD session (values already analyzed above were not included). Repeated measure ANOVA on these 18 KSS scores with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor, and repetition of KSS score (18) as within-subject factor revealed a main effect of repetition ($F = 22.93$; $df = 17, 408$; $p < 10^{-6}$), but no main effect of genotype ($F = 0.013$; $df = 1, 24$; $p = 0.97$), and no repetition-by-genotype interaction ($F = 1.01$; $df = 17, 408$; $p = 0.55$).

To summarize, subjective sleepiness scores increased during the course of the sleep deprivation night but were not significantly different between genotypes.

Behavior

Accuracy

Performance accuracy was computed for each of the 7 blocks of 3-back (i.e. series of consonant) performed in each session (Figure S1b). Repeated measures ANOVA on accuracy with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$) as between-subject factor and repetition of blocks (7) and session (MS, ES, ESD, MSD) as within-subject factors revealed main effects of block ($F = 2.39$; $df = 6, 150$; $p = 0.03$) and of session ($F = 6.41$; $df = 3, 75$; $p = 0.0006$), and a significant block-by-session interaction ($F = 1.9$; $df = 18, 450$; $p = 0.01$), but no main effect of genotype ($F = 0.004$; $df = 1, 25$; $p = 0.95$), no block-by-genotype interaction ($F = 1.88$; $df = 6, 150$; $p = 0.09$), no session by genotype interaction ($F = 1.03$; $df = 3, 75$; $p = 0.38$), as well as no repetition-by-session-by-genotype interaction ($F = 0.89$; $df = 18, 450$; $p = 0.6$).

Planned comparison showed that accuracy during ES vs. ESD, and ESD vs. MSD sessions were not significantly different ($F < 1.98$, $df = 1, 25$; $p > 0.17$), but that accuracy during ES vs. MS, and MS vs. MSD sessions were significantly different ($F > 6.21$, $df = 1, 25$; $p < 0.02$). Planned comparisons also revealed that that accuracy significantly decreased over time only in the MSD session, i.e. after sleep deprivation ($F = 3.39$; $df = 6, 150$; $p = 0.004$), but not in the other sessions ($F < 1.61$; $df = 6, 150$; $p > 0.15$).

To summarize, performance was significantly better after sleep than in the other sessions, and deteriorated significantly with time only after SD, but was not significantly different between *PER3*$^{4/4}$ and *PER3*$^{5/5}$.

Reaction times

Subjects were instructed to respond as fast as possible but to prefer accuracy over speed. Mean reaction times in each session were nevertheless analyzed for completeness. Repeated measures ANOVA on mean reaction times with genotype (*PER3*$^{4/4}$ and *PER3*$^{5/5}$*)* as between-subject factor and session (MS, ES, ESD, MSD) as within-subject factor revealed a main effect of session ($F = 4.09$; $df = 3, 75$; $p = 0.009$) but not of genotype ($F = 0.87$; $df = 1, 25$; $p = 0.36$), and no session-by-genotype interaction ($F = 2.1$; $df = 3, 75$; $p = 0.11$). Planned comparison showed that reaction times of ES vs. ESD, and of ESD vs. MSD sessions were not significantly different ($F < 2.9$, $df = 1, 25$; $p > 0.1$), but that reaction times of ES vs. MS, and MS vs. MSD sessions were significantly different (ES [1105ms ± 201; mean ± SD] vs. MS [1050ms ± 209]: $F = 12.69$, $df = 1, 25$; $p < 0.001$; MS [1050ms ± 209] vs. MSD [1092ms ± 215]: $F = 3.55$, $df = 1, 25$; $p = 0.07$).

To summarize, reaction times were significantly shorter after sleep than in the other sessions, but were not significantly different between *PER3*$^{4/4}$ and *PER3*$^{5/5}$.

Table S1: Subjects characteristics [mean +/- SD]

PER3 genotype	*PER3* $^{4/4}$	*PER3* $^{5/5}$	P_{value}
N	15	12*	
AGE	24.13 ± 0.95	24.17 ± 1.17	0.98
BODY MASS INDEX	21.9 ± 0.59	21.9 ± 0.59	0.49
SEX (M/F)	8 / 7	7 / 5	0.55
SLEEP DISTURBANCE	3.33 ± 0.51	4.17 ± 0.53	0.24
DAYTIME PROPENSITY TO FALL ASLEEP	3.70 ± 0.64	6.96 ± 1.07	0.008
CHRONOTYPE	49.80 ± 3.80	52.33 ± 2.90	0.59
ANXIETY LEVEL	5.13 ± 1.05	5.33 ± 1.16	0.89
MOOD	3.6 ± 0.83	5.42 ± 1.33	0.22
YEARS OF EDUCATION	17.1 ± 0.68	16.5 ± 0.6	0.52
IQ	125.5 ± 2.85	125.9 ± 3.19	0.72
ORDER: Sleep segment before Sleep Deprivation segment	6/15	6/12	0.45
Women using oral contraceptive	6/7	5/5	0.58
Women in luteal phase	0/7	0/5	
Right handed participants	15/15	12/12	
Ethnicity	Caucasian (all)	Caucasian (all)	

SLEEP DISTURBANCE was determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse et al., 1989); *DAYTIME PROPENSITY TO FALL ASLEEP* during daytime non-stimulating situations was assessed by the Epworth Sleepiness Scale (ESS) at least one week prior to the first segment of the protocol (i.e. before subject followed a regular sleep schedule for 7 days prior to each segment) (Johns, 1991); *CHRONOTYPE* was assessed by the Horne-Östberg Questionnaire (Horne and Ostberg, 1976); *ANXIETY LEVEL* was measured on the 21 item Beck Anxiety Inventory (BAI) (Beck et al., 1988); *MOOD* was assessed using the 21 item Beck Depression Inventory II (BDI-II) (Steer et al., 1997); *IQ* (intellectual quotient) was assessed by the Advanced Progressive Matrices (Raven et al., 1998). The Edinburgh Inventory (Oldfield, 1971) was administered to verify that the participants were right-handed.

* 13 *PER3*$^{5/5}$ completed the protocol but one fell asleep during the MSD session and was removed from all analyses. Note that levels of statistical significance in this table were not affected if the subject was included.

Table S2: Actigraphy-assessed sleep parameters during the 7 day of scheduled sleep prior to each experiment segment [Mean ± SD].

PER3 genotype	*PER3* $^{4/4}$	*PER3* $^{5/5}$
Sleep duration (min)		
Sleep segment	470.2 ± 17.1	456.4 ± 23
SD segment	459.9 ± 30.1	450.6 ± 20.8
Clock time of sleep onset (h:min)		
Sleep segment	23:45 ± 49min	00:01 ± 49min
SD segment	23:50 ± 52min	00:00 ± 54min
Clock time of wake (h:min)		
Sleep segment	07:35 ± 56min	07:38 ± 56min
SD segment	07:30 ± 54min	07:32 ± 51min
Clock time of sleep midpoint (h:min)		
Sleep segment	03:55 ± 8min	03:49 ± 52min
SD segment	03:40 ± 52min	03:46 ± 10min
Sleep duration between ES and MS session in the sleep segment (min)	449.4 ± 5.4min	451.2 ± 3min

Table S3: Timing of fMRI sessions relative to actigraphy-assessed sleep parameters during the 7 days preceding each segment [Mean ± SD].

PER3 genotype	*PER3* $^{4/4}$	*PER3* $^{5/5}$
Time awake before each fMRI session (h:min)		
MS	01:30 ± 1min	01:32 ± 8min
ES	13:50 ± 35min	13:47 ± 32min
ESD	14:01 ± 41min	14:02 ± 21min
MSD	24:53 ± 29min	25:01 ± 21min
Clock time of fMRI sessions (hrs)		
MS	08:20 ± 56min	08:31 ± 46min
ES	21:25 ± 58min	21:31 ± 58min
ESD	21:30 ± 71min	21:30 ± 48min
MSD	08:22 ± 65min	08:30 ± 49min
Time of fMRI session relative to actigraphy-assessed sleep midpoint		
MS	04:25 ± 54min	04:41 ± 21min
ES	-06:30 ± 57min	-06:18 ± 21min
ESD	-06:10 ± 33min	-06:16 ± 50min
MSD	04:29 ± 23min	04:44 ± 50min

Table S4: Significant brain activity related to the 3-back task after 1.5h (MS) and 14h (ES) of wakefulness, common to both genotypes.

Brain areas	*Side*	*X Y Z*	*Z score*	*P value*
Anterior cingulate cortex / Superior frontal gyrus (a)	R	2 16 52	5.69	0.001
Middle frontal gyrus (b – d)	L	-48 6 34	5.49	0.001
	R	32 2 60	5.24	0.005
	L	-28 4 58	5.16	0.008
	L	-48 0 56	5.14	0.009
	R	48 32 24	4.97	0.018
Inferior frontal gyrus (e – f)	L	-54 16 34	5.59	0.001
	R	58 18 22	4.69	0.003
Superior temporal sulcus / middle temporal gyrus (g)	R	62 -18 -6	6.12	<0.001
Superior temporal gyrus (h – i)	L	-52 -24 0	5.23	0.006
	L	-64 -32 2	5.01	0.015
Intraparietal sulcus / anterior IPS (j – k)	R	42 -48 52	5.86	<0.001
	L	-34 -48 46	5.84	<0.001
	L	-44 -40 48	5.34	0.004
	R	38 -60 54	4.89	0.025
Anterior insula (l – m)	L	-28 22 -6	5.00	0.016
	R	28 24 -8	4.82	0.034
Putamen (n – o)	L	-20 6 4	3.93	0.005
	R	20 10 6	3.87	0.005
Cerebellum (p – r)	R	30 -66 -32	6.13	<0.001
	R	32 -56 -34	5.90	<0.001
	L	-30 -68 -32	5.73	0.001
	L	-6 -80 -32	5.06	0.012
	R	6 -56 -20	5.06	0.012
	R	6 -68 -10	5.09	0.011

Results of the (ES+MS) * $PER3^{5/5}$ contrast inclusively masked by the (ES+MS) * $PER3^{4/4}$ (p = 0.001 inclusive mask).

Table S5: Significant brain activity related to the 3-back task after 25.5h (MSD) of wakefulness, common to both genotypes.

Brain areas	***Side***	***X Y Z***	***Z score***	$P_{corrected}$ ***value***
Anterior cingulate cortex (a)	R	6 22 40	5.52	0.001
Superior frontal gyrus (b)	R	0 12 54	6.03	<0.001
Middle frontal gyrus (c – e)	R	50 34 32	6.07	<0.001
	L	-22 0 50	5.19	0.006
	R	30 14 66	4.84	0.027
	R	32 14 54	4.81	0.030
Inferior frontal gyrus (f)	L	-50 30 32	5.06	0.011
	L	-48 16 28	4.76	0.037
Intraparietal sulcus / anterior IPS (g – h)	R	42 -50 50	5.29	0.004
	L	-36 -46 46	5.14	0.008
	L	-44 -46 54	4.72	0.044
Inferior parietal lobule	R	52 -36 46	5.57	0.001
	R	48 -42 52	5.41	0.002
Anterior insula (i – j)	L	-30 22 0	5.58	0.001
	R	32 24 -2	4.59	<0.001
Thalamus (k)	L	-18 -6 8	6.06	<0.001
Putamen (l)	R	20 6 8	4.59	<0.001
Cerebellum (m – n)	R	30 -60 -38	5.88	<0.001
	L	-30 -70 -30	5.46	0.002
	L	-32 -62 -38	5.22	0.004

Results of the (MSD) * $PER3^{5/5}$ contrast inclusively masked by the (MSD) * $PER3^{4/4}$ (p = 0.001 inclusive mask).

References

Beck AT, Epstein N, Brown G, Steer RA (1988) An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol 56:893-897.

Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ (1989) The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 28:193-213.

Chee MW, Chuah YM (2007) Functional neuroimaging and behavioral correlates of capacity decline in visual short-term memory after sleep deprivation. Proc Natl Acad Sci U S A 104:9487-9492.

Choo WC, Lee WW, Venkatraman V, Sheu FS, Chee MW (2005) Dissociation of cortical regions modulated by both working memory load and sleep deprivation and by sleep deprivation alone. Neuroimage 25:579-587.

Cohen JD, Perlstein WM, Braver TS, Nystrom LE, Noll DC, Jonides J, Smith EE (1997) Temporal dynamics of brain activation during a working memory task. Nature 386:604-608.

Collette F, Van der Linden M, Laureys S, Arigoni F, Delfiore G, Degueldre C, Luxen A, Salmon E (2007) Mapping the updating process: common and specific brain activations across different versions of the running span task. Cortex 43:146-158.

Coull JT (2004) fMRI studies of temporal attention: allocating attention within, or towards, time. Brain Res Cogn Brain Res 21:216-226.

Drummond SP, Gillin JC, Brown GG (2001) Increased cerebral response during a divided attention task following sleep deprivation. J Sleep Res 10:85-92.

Drummond SP, Brown GG, Salamat JS, Gillin JC (2004) Increasing task difficulty facilitates the cerebral compensatory response to total sleep deprivation. Sleep 27:445-451.

Drummond SP, Meloy MJ, Yanagi MA, Orff HJ, Brown GG (2005a) Compensatory recruitment after sleep deprivation and the relationship with performance. Psychiatry Res 140:211-223.

Drummond SP, Brown GG, Gillin JC, Stricker JL, Wong EC, Buxton RB (2000) Altered brain response to verbal learning following sleep deprivation. Nature 403:655-657.

Drummond SP, Bischoff-Grethe A, Dinges DF, Ayalon L, Mednick SC, Meloy MJ (2005b) The neural basis of the psychomotor vigilance task. Sleep 28:1059-1068.

English J, Middleton BA, Arendt J, Wirz-Justice A (1993) Rapid direct measurement of melatonin in saliva using an iodinated tracer and solid phase second antibody. Ann Clin Biochem 30 (Pt 4):415-416.

Grandjean D, Sander D, Pourtois G, Schwartz S, Seghier ML, Scherer KR, Vuilleumier P (2005) The voices of wrath: brain responses to angry prosody in meaningless speech. Nat Neurosci 8:145-146.

Habeck C, Rakitin BC, Moeller J, Scarmeas N, Zarahn E, Brown T, Stern Y (2004) An event-related fMRI study of the neurobehavioral impact of sleep deprivation on performance of a delayed-match-to-sample task. Brain Res Cogn Brain Res 18:306-321.

Horne JA, Ostberg O (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol 4:97-110.

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545.

Kastner S, O'Connor DH, Fukui MM, Fehd HM, Herwig U, Pinsk MA (2004) Functional imaging of the human lateral geniculate nucleus and pulvinar. J Neurophysiol 91:438-448.

Koechlin E, Ody C, Kouneiher F (2003) The architecture of cognitive control in the human prefrontal cortex. Science 302:1181-1185.

Majerus S, Poncelet M, Van der Linden M, Albouy G, Salmon E, Sterpenich V, Vandewalle G, Collette F, Maquet P (2006) The left intraparietal sulcus and verbal short-term memory: Focus of attention or serial order? Neuroimage 32:880-891.

Morris JS, Ohman A, Dolan RJ (1999) A subcortical pathway to the right amygdala mediating "unseen" fear. Proc Natl Acad Sci U S A 96:1680-1685.

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97-113.

Portas CM, Rees G, Howseman AM, Josephs O, Turner R, Frith CD (1998) A specific role for the thalamus in mediating the interaction of attention and arousal in humans. J Neurosci 18:8979-8989.

Raven J, Raven J, Court J (1998) Raven Manual: Sec. 4. Advanced Progressive Matrices. Oxford: Oxford Psychologists Press.

Steer RA, Ball R, Ranieri WF, Beck AT (1997) Further evidence for the construct validity of the Beck depression Inventory-II with psychiatric outpatients. Psychol Rep 80:443-446.

Thomas M, Sing H, Belenky G, Holcomb H, Mayberg H, Dannals R, Wagner H, Thorne D, Popp K, Rowland L, Welsh A, Balwinski S, Redmond D (2000) Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. J Sleep Res 9:335-352.

Tomasi D, Wang RL, Telang F, Boronikolas V, Jayne MC, Wang GJ, Fowler JS, Volkow ND (2008) Impairment of Attentional Networks after 1 Night of Sleep Deprivation. Cereb Cortex.

Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ (2007) PER3 polymorphism predicts sleep structure and waking performance. Curr Biol 17:613-618.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Behavior

a. **Subjective sleepiness (Karolinska Sleepiness Scale).** *Left panels:* mean (± SD) subjective sleepiness reported by the participants of both genotypes prior to and during each fMRI session, i.e. the 3 scores collected during the 60 minutes of strictly controlled activity that preceded each fMRI session and the 2 scores while subjects were in the scanner. *Right panel:* mean (± SD) subjective sleepiness as reported by the participants during the total sleep deprivation segment (TSD).
b. **Mean (± SD) accuracy to the 3-back task in the different sessions in both genotypes.**

Time (hrs) relative to sleep midpoint of scheduled sleep prior to each segment

ES: evening session, sleep segment; ESD: evening session sleep deprivation segment; MS: morning session, sleep segment; MSD: morning session, sleep deprivation segment.

Figure S2: Significant common brain activity related to the 3-back during a normal sleep wake cycle irrespective of the genotype.

Results of the (ES+MS) * *PER3*$^{5/5}$ contrast inclusively masked by the (ES+MS) * *PER3*$^{4/4}$ (p = 0.001 inclusive mask). Statistical results are overlaid to the population mean structural image ($p_{uncorrected}$ <0.001). See Table S4 for the names of the brain areas corresponding to the letters.

Figure S3: Significant common brain activity related to the 3-back task after 25.5h of wakefulness irrespective of the genotype.

Results of the MSD * *PER3*$^{5/5}$ contrast inclusively masked by the MSD * *PER3*$^{4/4}$ (p = 0.001 inclusive mask). Statistical results are overlaid to the population mean structural image ($p_{uncorrected}$ <0.001). See Table S5 for the names of the brain areas corresponding to the letters.