Increased Metabotropic Glutamate Receptor Subtype 5 Availability in Human Brain After One Night Without Sleep

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Background: Sleep deprivation (wake therapy) provides rapid clinical relief in many patients with major depressive disorder (MDD). Changes in glutamatergic neurotransmission may contribute to the antidepressant response, yet the exact underlying mechanisms are unknown. Metabotropic glutamate receptors of subtype 5 (mGluR5) are importantly involved in modulating glutamatergic neurotransmission and neuronal plasticity. The density of these receptors is reduced in the brain of patients with MDD, particularly in brain structures involved in regulating wakefulness and sleep. We hypothesized that prolonged wakefulness would increase mGluR5 availability in human brain.

Methods: Metabotropic glutamate receptor subtype 5 binding was quantified with positron emission tomography in 22 young healthy men who completed two experimental blocks separated by 1 week. Two positron emission tomography examinations were conducted in randomized, crossover fashion with the highly selective radioligand, ^{11}C-ABP688, once after 9 hours (sleep control) and once after 33 hours (sleep deprivation) of controlled wakefulness. ^{11}C-ABP688 uptake was quantified in 13 volumes of interest with high mGluR5 expression and presumed involvement in sleep-wake regulation.

Results: Sleep deprivation induced a global increase in mGluR5 binding when compared with sleep control ($p < .006$). In anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala, this increase correlated significantly with the sleep deprivation-induced increase in subjective sleepiness.

Conclusions: This molecular imaging study demonstrates that cerebral functional mGluR5 availability is increased after a single night without sleep. Given that mGluR5 density is reduced in MDD, further research is warranted to examine whether this mechanism is involved in the potent antidepressant effect of wake therapy.

Key Words: Antidepressant, ^{11}C-ABP688, major depression, molecular brain imaging, sleepiness, wake therapy

Sleep and mood regulation are tightly associated. Disrupted sleep is an important diagnostic criterion and risk factor for major depressive disorder (MDD) (1). Insomnia symptoms often precede the onset of depression by several months, are resistant to treatment, and increase the risk of relapse in remitted patients. Intriguingly, whereas sleep-wake disturbances are highly prevalent in MDD, sleep deprivation (wake therapy) provides rapid clinical relief in many patients (2). Changes in glutamatergic neurotransmission have been proposed to contribute to the antidepressant response (2), but the exact underlying mechanisms are unknown.

Metabotropic glutamate receptors, including metabotropic glutamate receptors of subtype 5 (mGluR5), play an important role in regulating glutamatergic neurotransmission (3). The density of mGluR5 is reduced in various cortical and subcortical brain regions in patients with MDD when compared with healthy control subjects (4). These receptors are present on postsynaptic neurons and glia cells and contribute to long-term depression (LTD), as well as long-term potentiation (5,6). Moreover, they are involved in sleep-wake related postsynaptic plasticity in rats (7) and interact directly or indirectly with different molecular markers of sleep-wake regulation in animals and humans. These markers include Homer 1a (8,9), fragile X mental retardation protein (FMRP) (10–12), brain-derived neurotrophic factor (BDNF) (13,14), adenosine deaminase (15,16), and the adenosine A_{2A} receptor (17,18).

Prolonged wakefulness not only affects mood and other daytime functions, including sleepiness, but also elevates sleep need in a recovery night when compared with a baseline night. The most reliable markers of sleep need are the amount of slow wave sleep and electroencephalographic (EEG) slow-wave activity (SWA) in non-rapid-eye-movement (NREM) sleep (19). Functional brain imaging studies consistently suggest that ventromedial prefrontal cortex, basal forebrain, insula, anterior cingulate cortex, striatum, parahippocampal gyrus, precuneus, and other regions are importantly involved in the regulation of slow waves and NREM sleep (20–22). Interestingly, mGluR5 are preferentially expressed in most of these brain regions (23). In addition, they are important for shaping the EEG slow oscillation in NREM sleep (24) and synchronized theta mode network activity in wakefulness (25). Based on this convergent evidence for a possible involvement of mGluR5 in

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sleep-wake regulation, we aimed to quantify mGluR5 availability in rested and sleep-deprived state in humans.

We used the recently developed, selective mGluR5 radioligand, ^{11}C-ABP688, to directly visualize mGluR5 availability in the living human brain (26,27). We quantified in healthy adults ^{11}C-ABP688 uptake in sleep control and sleep deprivation conditions in 13 brain regions with high mGluR5 expression and presumed involvement in sleep-wake regulation. Given the recent observation that mGluR5 density is reduced in depressed patients (4) and the suggested role of mGluR5 in sleep-wake related postsynaptic plasticity (7), we predicted that prolonged wakefulness may increase mGluR5 availability in the brain.

Methods and Materials

Study Participants

The study protocol and all experimental procedures were approved by the cantonal and federal authorities for research on human subjects and carried out in accordance with the Declaration of Helsinki (1964).

Twenty-two healthy young men completed the 2-week study after giving written informed consent and being extensively screened for medical history and psychological state. Individuals with a significant medical history (e.g., loss of consciousness) or past psychiatric illness were excluded. A prestudy screening night was performed in the sleep laboratory to exclude subjects with unknown sleep disturbances and low sleep efficiency (< 85%). All participants were nonsmokers, right-handed, and abstinent from any medication; denied previous and current illicit drug use; and had not crossed time zones or done shift work during the last 3 months before the experiment. Validated questionnaires to assess lifestyle and demographic characteristics demonstrated the presence of a healthy study sample (Table 1).

Table 1. Demographic Characteristics of Study Participants

	Mean ± SD
Age (Years)	23.4 ± 2.1
Body Mass Index (kg/m^2)	22.1 ± 1.9
Alcohol Consumption (Drinks/Week)	3.5 ± 3.3
Caffeine Consumption (mg/day)	153.5 ± 160.9
Diurnal Preference	49.7 ± 8.4
Daytime Sleepiness	7.1 ± 3.3
Trait Anxiety	34.5 ± 7.0
Depression Score	3.2 ± 3.8
Eysenck Personality Traits	
Extraversion	8.6 ± 2.8
Neuroticism	3.7 ± 2.8
Lie scale	2.5 ± 1.9
Psychoticism	3.1 ± 1.4
Cloninger Personality Traits	
Novelty seeking	14.7 ± 5.6
Harm avoidance	11.8 ± 4.7
Reward dependence	16.0 ± 5.2

Twenty-two male participants completed the study; one participant had to be excluded from positron emission tomography analyses because of strong head movement. German versions and validated German translations of questionnaires were used to assess lifestyle and personality traits. Caffeine consumption was estimated based on the following average caffeine contents per serving: coffee: 100 mg; ceylon or green tea: 30 mg; cola drink: 40 mg (2 dL); energy drink: 80 mg (2 dL); chocolate: 50 mg (100 g). Diurnal preference: Horne-Östberg Morningness-Eveningness Questionnaire (61); daytime sleepiness: Epworth Sleepiness Scale (62); trait anxiety: State-Trait Anxiety Inventory (33); depression score: Beck Depression Inventory (63); personality traits: Eysenck Personality Questionnaire (64) and Cloninger's Tridimensional Personality Questionnaire (65).

Prestudy Procedures and Experimental Protocol

Three days before each study block, participants abstained from caffeine and alcohol and adhered to a 16-hour wake/8-hour sleep schedule. Caffeine, alcohol, and sleep logs; breath-alcohol measurements upon arrival in the laboratory; and wrist-actigraphy were used to verify adherence to these instructions.

All subjects completed two experimental blocks, referred to as sleep control and sleep deprivation conditions. The two conditions occurred in randomized, cross-over fashion, typically 1 week apart, and included polysomnographically recorded adaptation, baseline, and experimental nights (time in bed: 11:30 PM–07:30 AM). In the sleep deprivation condition, the baseline night was followed by 40 hours continuous wakefulness, during which the participants were under constant supervision of the research team. All subjects underwent two positron emission tomography (PET) examinations with ^{11}C-ABP688 to quantify mGluR5 availability in the brain (Division of Nuclear Medicine, University Hospital Zürich). These assessments occurred in random order, either ~9 or ~33 hours after awakening from the baseline night. The time of day of the PET examinations in sleep control (4:36 PM ± 9 minutes) and sleep deprivation (4:34 PM ± 8 minutes) conditions did not differ ($p > .4$).

Magnetic Resonance and PET Image Acquisition

A T1-weighted, whole-brain, three-dimensional magnetic resonance (MR) image (resolution: 1 × 1 × 1 mm) was obtained for each subject (Philips Achieva 3T whole-body MR unit equipped with transmit/receive head coil; Philips Healthcare, Best, The Netherlands), to exclude morphological abnormalities and as anatomical standard for the quantification of the PET images.

Tracer synthesis and PET brain imaging with ^{11}C-ABP688 using a bolus/infusion protocol were performed as previously described (4,26–28). Catheters were placed into the antecubital veins of each participant's arms, one for tracer injection and one for blood sampling. Venous blood was collected at 42 and 58 minutes after the start of the bolus infusion.

All PET examinations were performed in three-dimensional mode on a DVCT PET/computed tomography scanner (GE Medical Systems, Glattbrugg, Switzerland) or a DSTx PET/computed tomography scanner (B. Braun Medical, Sempach, Switzerland). A low-dose computed tomography scan was performed before the PET examination for photon attenuation correction. The emission scans were initiated simultaneously with the start of the injection of radioligand using an infusion pump (Perfusor FM, Braun Medical). Injected activity (sleep control: 582.9 ± 23.7 MBq; sleep deprivation: 543.5 ± 19.5 MBq) and mass of cold compound (sleep control: 6.2 ± .7 nmol; sleep deprivation: 6.9 ± .7 nmol) did not differ between the conditions ($p_{all} > .39$; two-tailed, paired t tests). Of the total activity, 47.6% (sleep control: 294.8 ± 9.9 MBq; sleep deprivation: 288.9 ± 9.8 MBq) was injected as a bolus over 2 minutes ($K_{bol} = 53$ min) and the rest was continuously infused over 58 minutes (sleep control: 283.7 ± 9.6 MBq; sleep deprivation: 278.0 ± 9.5 MBq). Specific activity of ^{11}C-ABP688 at the end of syntheses was also very similar in sleep control (132.6 ± 15.4 GBq/μmol; range: 71–289 GBq/μmol) and sleep deprivation conditions (109.0 ± 13.1 GBq/μmol; range: 61–243 GBq/μmol). Twenty frames were collected during the 60-minute protocol (10 × 60 sec and 10 × 300 sec). The images were reconstructed using filtered back

projection and displayed over 47 transaxial slices. Using a 128 × 128 matrix, the resulting voxel size was 2.3 × 2.3 × 3.2 mm.

Subjects were instructed to not fall asleep during image acquisition and the EEG was simultaneously recorded, according to established procedures (16,29), to verify wakefulness during the PET examinations. As soon as sleep-like EEG activity was noted, subjects were alerted via an intercom. Direct contact with them was avoided, to minimize movement artifacts.

Image Processing and Quantification

Image processing consisted of within-subject motion correction by realigning the average of frames 17 to 19 to the average of frames 2 to 10 (rigid matching) and spatial normalization of averaged frames 17 to 19 to the Montreal Neurological Institute template brain for definition of volumes of interest (VOI). The averaged frames 17 to 19 of the PET images were then co-registered to the corresponding MR image to delineate the cerebellum (28). These steps were performed with the PMOD software package, version 3.1 (PMOD Technologies, Zürich, Switzerland). Standard VOIs for the Montreal Neurological Institute template brain available in PMOD were used to measure radioactivity concentration in the normalized PET images (30). Because PMOD provides no single VOI for cerebellum, this region was defined manually in each subject on the MR image and subsequently transferred to the corresponding PET images to measure radioactivity concentration in this region. Tissue time activity curves (TAC) were generated for cerebellum, anterior cingulate cortex, superior frontal cortex, putamen, and thalamus in both hemispheres to confirm that steady state of receptor binding was reached in frames 17 to 19 (45–55 min) of image acquisition. The TAC for cerebellum and anterior cingulate cortex in two representative individuals in sleep control and sleep deprivation conditions are illustrated in Supplement 1.

The average radioactivity concentration between 45 and 55 minutes was calculated in each VOI ($C_{t[VOI]}$). Because some venous blood metabolite analyses were unreliable due to technical difficulties, regional V_t values could not be obtained in all subjects. Instead, quantification of the PET images was done by dividing the regional radioactivity concentration values with the corresponding value in the cerebellum ($C_{t[Cb]}$) to obtain V_{norm} ($V_{norm} = C_{t[VOI]}/C_{t[Cb]}$). This method was successfully used in previous studies to quantify mGluR5 availability in the brain (4,28). Furthermore, a preclinical study showed that ^{11}C-ABP688 binding in the cerebellum is negligible and that this region can thus be used as a reference region (31). To address the question whether sleep deprivation changed ^{11}C-ABP688 binding in the cerebellum, we calculated the nondisplaceable volume of distribution (V_{ND}) in sleep control and sleep deprivation conditions in those nine study participants in whom reliable plasma radioactivity concentration and metabolite concentrations were available. These analyses confirmed that ^{11}C-ABP688 binding in the cerebellum was not changed after sleep deprivation when compared with the control condition (control: $V_{ND} = 1.98 \pm .14$; sleep deprivation: $V_{ND} = 2.18 \pm .16$; $p = .19$, two-tailed paired t test).

Behavioral and Cognitive Effects of Sleep Deprivation

To document that sleep deprivation was successful, validated questionnaires were employed before each PET scan to assess subjective sleepiness (Karolinska Sleepiness Scale) (32), state anxiety (State Trait Anxiety Inventory) (33), and affective state (Profile of Mood States) (34). Approximately 3 hours before each scan, the subjects also completed a cognitive test session consisting of the psychomotor vigilance task (PVT) (35) and the Deese-Roediger-McDermott false memory paradigm (36,37). To approach a normal distribution, mean reaction time (RT) on the PVT was expressed as speed (1/RT) and the number of lapses (RT $>$ 500 msec) was transformed by ($\sqrt{x}+\sqrt{[x+1]}$).

Cortisol Concentration in Saliva

Immediately before each PET examination, all study participants provided a saliva sample to determine the cortisol concentration as a measure of acute stress. Unstimulated saliva was collected by placing a salivette (Sarstedt, Sevelen, Switzerland) under the tongue and keeping the head slightly inclined for 1 to 2 minutes. The samples were stored at −20 °C until the biochemical analysis took place. The saliva was centrifuged at 3000 rpm for 5 minutes before free cortisol was analyzed using an immunoassay with time-resolved fluorescence detection (38).

Statistical Analyses

All statistical analyses were performed with SAS 9.1 software (SAS Institute, Cary, North Carolina). Mixed-effect analysis of variance models included the factors condition (sleep control, sleep deprivation), region (13 VOIs), and hemisphere (left, right), as well as their interactions. Two-tailed, paired t tests were conducted to localize significant differences. To limit the number of comparisons and to control for type I errors, analyses of PET data were restricted to the predefined VOIs and the significance level was set at $\alpha < .0038$ (Bonferroni correction: $\alpha = .05/13$). To estimate the possible associations between the effects of sleep deprivation on mGluR5 availability and changes in behavioral and cognitive variables, regression analyses were performed and

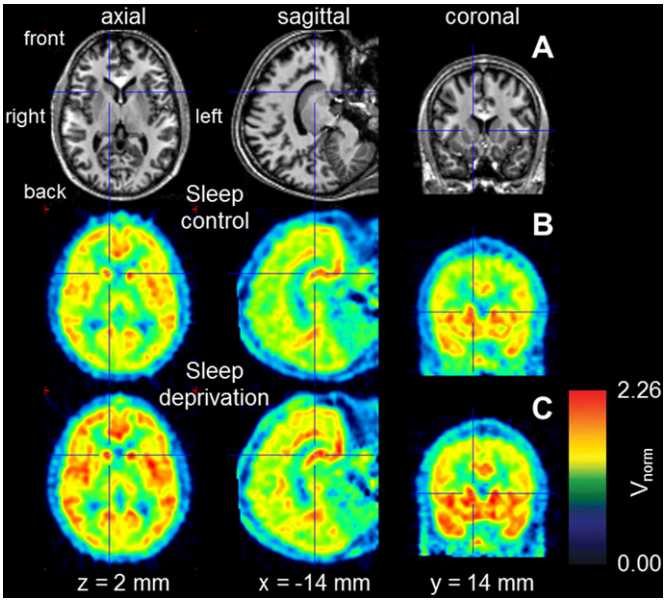

Figure 1. Axial, sagittal, and coronal views of ^{11}C-ABP688 binding in a representative individual. **(A)** Magnetic resonance image template for anatomical reference. **(B)** Color-coded normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 after ~9 hours of wakefulness (sleep control condition). **(C)** Color-coded V_{norm} of ^{11}C-ABP688 after ~33 hours of wakefulness (sleep deprivation condition). The crosshair was placed in the right caudate nucleus (coordinates according to the Montreal Neurological Institute brain atlas: −14, 14, 2).

Spearman rank correlation coefficients were calculated. If not stated otherwise, only significant results are reported.

Results

Regional Uptake of ^{11}C-ABP688 in Human Brain

Axial, sagittal, and coronal views of ^{11}C-ABP688 binding in the brain of one single individual in sleep control and sleep deprivation conditions are presented in Figure 1. Consistent with previous studies (27,39), regional uptake of ^{11}C-ABP688 reflected the known distribution of mGluR5 with most pronounced binding in anterior cingulate, insula, medial temporal lobe, medial prefrontal cortex, striatum, and amygdala. Lower radioligand binding was present in thalamus and substantially lower radioligand binding was present in cerebellum, which was used to calculate the normalized volumes of distribution (V_{norm}). Visual inspection of TAC in distinct brain regions confirmed that a steady state of ^{11}C-ABP688 uptake was reached 45 minutes after tracer injection in all participants and scans.

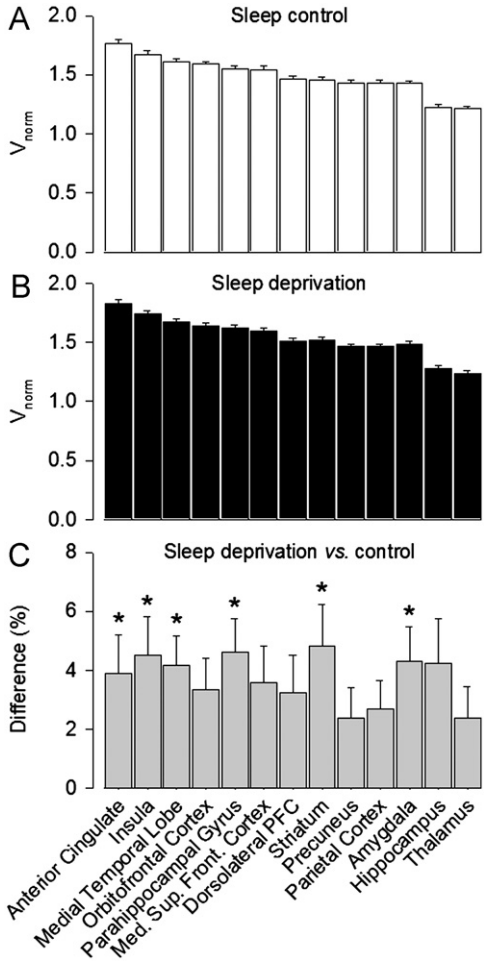

Figure 2. Regional differences in metabotropic glutamate receptor subtype 5 density and effect of sleep deprivation. **(A)** Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake in 13 regions of interest after ~9 hours of wakefulness (sleep control). **(B)** V_{norm} after ~33 hours of wakefulness (sleep deprivation). **(C)** Percent difference in V_{norm} between sleep deprivation and sleep control conditions. The two measurements occurred in random order and were separated by 1 week. Data represent means+1 SEM ($n = 21$). *Significant differences between sleep deprivation and sleep control conditions (condition: $p < .0038$). Med. Sup. Front. Cortex, medial superior frontal cortex; PFC, prefrontal cortex.

Sleep Deprivation Increases Functional mGluR5 Availability in Human Brain

Regional V_{norm} values of ^{11}C-ABP688 in sleep control and sleep deprivation conditions are shown in Figure 2. Prolonged wakefulness increased global V_{norm} by 3.5 ± 1.1% when compared with control (1.50 ± .02 vs. 1.55 ± .02, $p < .006$). Mixed-model analysis of variance with the factors condition, region (VOIs), and hemisphere revealed highly significant main effects of condition [$F(1,20) = 184.5$, $p < .0001$] and region [$F(25,500) = 257.6$, $p < .0001$], yet no significant effect of hemisphere [$F(1,20) = .1$, $p > .7$]. Thus, the data of left and right hemispheres were averaged. The increase in mGluR5 binding was significant in anterior cingulate cortex (3.9 ± 1.3%, $p < .0006$), insula (4.5 ± 1.3%, $p < .0001$), medial temporal lobe (4.2 ± 1.0%, $p < .0005$), parahippocampal gyrus (4.6 ± 1.1%, $p < .0002$), striatum (4.8 ± 1.4%, $p < .0003$), and amygdala (4.3 ± 1.2%, $p < .002$). No VOI showed a reduction in ^{11}C-ABP688 binding after sleep deprivation when compared with control.

Increase in ^{11}C-ABP688 Binding Correlates with Increase in Subjective Sleepiness

Sleep deprivation impaired subjective state and cognitive performance. Sleepiness, state anxiety, fatigue, lapses on the PVT, and false alarms on the Deese-Roediger-McDermott false memory paradigm were increased after prolonged wakefulness when compared with control (Table 2). By contrast, vigor was reduced and PVT reaction times were prolonged.

Correlation analyses between the sleep deprivation-induced change in subjective sleepiness and the percent change in mGluR5 availability revealed consistent associations in all brain regions showing a significant increase in ^{11}C-ABP688 binding. In other words, those subjects who were most affected by sleep deprivation exhibited the largest increase in mGluR5 binding in anterior cingulate cortex, insula, medial temporal lobe, parahippocampal gyrus, striatum, and amygdala (Figure 3). A similar association was also found for fatigue on the Profile of Mood States questionnaire

Table 2. Behavioral and Cognitive Effects of Sleep Deprivation

	Sleep Control	Sleep Deprivation	$p <$
Karolinska Sleepiness Scale	3.13 ± .28	5.04 ± .43	.001[a]
State Anxiety Inventory	36.45 ± 2.07	40.73 ± 2.47	.001[a]
Profile of Mood States			
Fatigue	5.77 ± 1.49	13.86 ± 2.22	.001[a]
Vigor	18.54 ± 1.35	10.54 ± 1.38	.001[a]
Depression/anxiety	3.32 ± 1.42	4.14 ± 2.08	.506
Irritability	.91 ± .53	1.68 ± .72	.284
Psychomotor Vigilance Task			
Mean reaction speed (s^{-1})	3.79 ± .07	3.50 ± .07	.001[a]
Lapses (transformed)	2.29 ± .25	4.71 ± .62	.001[a]
Deese-Roediger-McDermott Paradigm			
False alarms	5.25 ± .99	8.40 ± .86	.001[a]
False memory	12.25 ± .55	13.80 ± .68	.067
Hit rate	40.35 ± 1.51	41.15 ± 1.80	.638

Values represent means ± SEM ($n = 22$). p values refer to two-tailed, paired t tests.

[a]Significant differences between sleep control and sleep deprivation conditions.

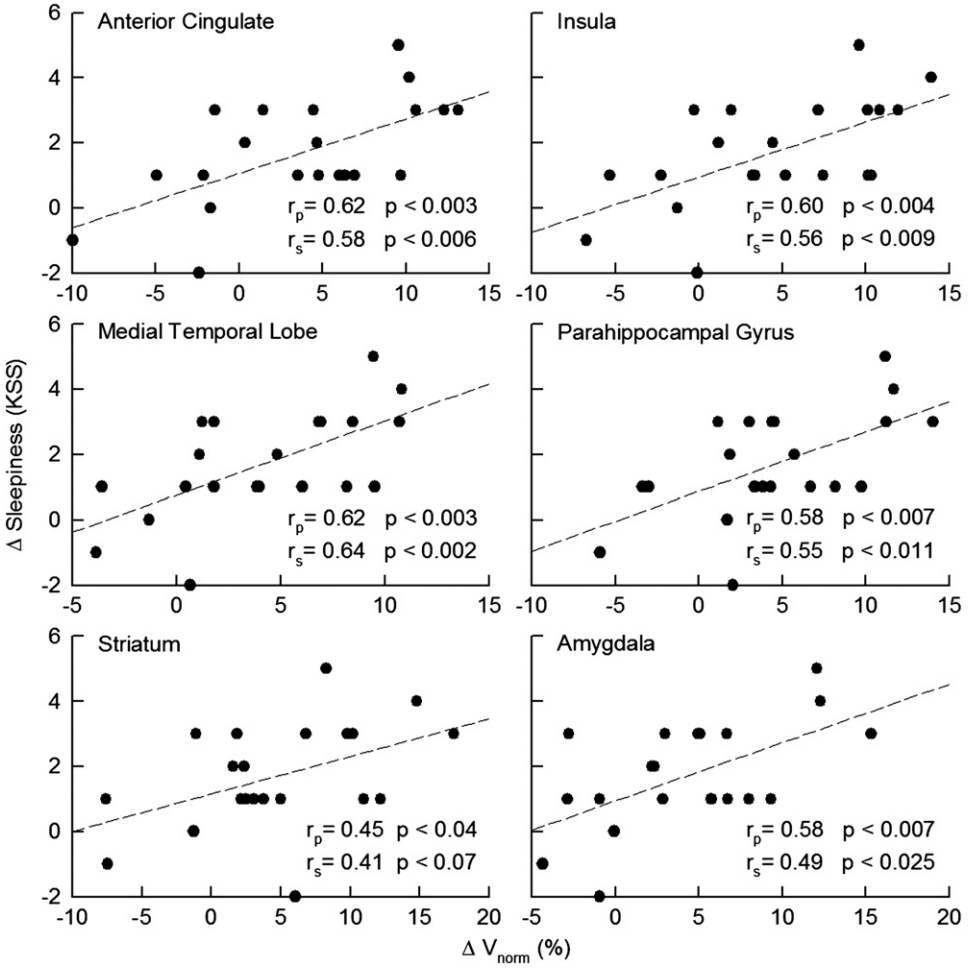

Figure 3. Relationship between the individual subjects' difference in subjective sleepiness and the difference in metabotropic glutamate receptor subtype 5 availability between sleep deprivation and sleep control conditions. Individual percent changes in normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 in those brain regions that showed a significant increase after sleep deprivation are illustrated. A linear regression line was fitted through the 21 individual data points. KSS, Karolinska Sleepiness Scale; r_p, Pearson's product-moment correlation coefficient; r_s, Spearman rank correlation coefficient.

and mGluR5 availability in medial temporal lobe ($r_p = .43$, $p = .053$; $r_s = .45$, $p < .04$; $n = 21$). The other behavioral and cognitive effects of sleep deprivation revealed no consistent correlation with the changes in mGluR5 binding in the significant VOIs.

Cortisol Concentration in Saliva

Prolonged wakefulness tended to slightly increase salivary cortisol concentration when compared with the sleep control condition (9.4 ± .6 vs. 7.0 ± .6 mmol/L, $p < .08$). The individual changes in salivary cortisol were not associated with individual changes in mGluR5 binding.

Discussion

This in vivo molecular imaging study in humans reveals the first evidence for increased functional cerebral mGluR5 availability after prolonged wakefulness. After a single night without sleep, binding of ^{11}C-ABP688 was significantly increased on a global level and in distinct brain regions that were previously shown to reflect physiological changes after sleep deprivation (for recent review, see [40]). These effects of short-term sleep loss are intriguing. The expression of G-protein coupled receptors on the cell surface is tightly regulated and provides a powerful mechanism of signal amplification (41). A recent study demonstrated that regional ^{11}C-ABP688 binding may reflect mGluR5 protein expression (4). Because mGluR5 density is reduced in major depression (4), our findings suggest that changes in functional mGluR5 availability may be involved in the rapid antidepressant effect of sleep deprivation in MDD patients.

The mGluR5 are mostly expressed on postsynaptic membranes of neurons and astrocytes in corticolimbic areas of the brain, including medial-prefrontal and orbitofrontal cortex, cingulate, striatum, amygdala, and hippocampus (23). They may be very well positioned to integrate and modulate the expression of established molecular markers of wakefulness and sleep. Indeed, mGluR5 interact either directly or indirectly with Homer 1a, FMRP, BDNF, adenosine deaminase, and A_{2A} receptors. Convergent evidence obtained from studies in invertebrates, rodent models, and humans strongly indicate that all these molecules play a causal role in sleep-wake regulation (9,11–14,16,18,42).

It was previously suggested that sleep is crucial for protecting and recovering the brain from increased intracellular calcium concentrations imposed by prolonged wakefulness (9).

The immediate early gene Homer 1a is a proposed core molecular marker of sleep homeostasis, i.e., the sleep-wake dependent facet of sleep regulation (9). Homer 1a selectively uncouples mGluR5 from effector targets in the membrane of the postsynaptic density and attenuates the mGluR5-mediated rise in intracellular calcium levels (43). The interaction between Homer 1a and mGluR5 is necessary for mGluR5-dependent synaptic LTD (44) and may promote synaptic changes during sleep (9). Interestingly, Homer 1a was shown to alter mGluR5 signaling without inducing large-scale changes in mGluR5 distribution (43).

Not only Homer 1a, but also FMRP, interacts with mGluR5. A genetic defect in the X chromosome-linked human *FMR1* (fragile X mental retardation 1) gene encoding FMRP gives rise to increased mGluR5 signaling and Fragile X syndrome (FXS), the most common form of inherited mental retardation and a leading cause of autism. Brain slices of *FMR1* knockout mice, an established model of FXS, show enhanced mGluR5-mediated synaptic LTD (45). In vitro as well as in vivo, these mice show prolonged spontaneous UP-states, which predominantly occur in slow wave sleep. This altered neocortical rhythmic activity is due to enhanced mGluR5 signaling (10). Sleep-wake regulation was studied in Drosophila mutants carrying dFmr1 loss-of-function (amorphs) and gain-of-function (hypermorphs) mutations (11). The dFmr1 amorphs were long sleepers, whereas dFmr1 hypermorphs were short sleepers. A recent study further demonstrated that dFmr1 is important for sleep-dependent synaptic normalization (12).

Also, interactions of mGluR5 with BDNF and adenosinergic neurotransmission may be important for sleep-wake regulation. Expression of BDNF in cerebral cortex is high during wakefulness, low during sleep, and increased after sleep deprivation (46). Cortical injection of BDNF to awake animals promotes synaptic strength and enhances SWA in subsequent NREM sleep. This effect is reversible and opposite to the changes in local SWA after pharmacologic inhibition of BDNF-tyrosine-kinase-β-receptor stimulation (13). Pharmacologic activation of mGluR5 induces BDNF expression in rat cortical neurons and glia cells (47,48). It may, thus, be speculated that enhanced mGluR5-induced BDNF secretion after prolonged wakefulness contributes to the antidepressant response to sleep deprivation. Indeed, patients suffering from MDD typically exhibit reduced serum BDNF levels, which may normalize after antidepressant therapies (49).

The facilitatory action of BDNF on hippocampal long-term potentiation requires adenosine A_{2A} receptor activation by endogenous adenosine (50). A primary role for adenosine and adenosine receptors in sleep regulation is well established in animals and humans (51,52). In an approach similar to the present study, it was recently shown that sleep deprivation increases A_1 receptor binding in human brain (53). Nevertheless, accumulating evidence indicates that also A_{2A} receptors contribute to sleep-wake regulation (17,18). These receptors are colocalized with mGluR5, dopamine D_2, and *N*-methyl-D-aspartate receptors and functionally interact in vitro and in vivo (54–57). Importantly, a recent PET study showed that one night without sleep reduced dopamine $D_{2/3}$ receptor availability in the ventral striatum by roughly 5% (nondisplaceable binding potential of ^{11}C-raclopride: 2.80 ± .37 vs. 2.95 ± .37) (58). After the same experimental procedure, we found an increase in mGluR5 availability in the striatum by 4.8 ± 1.4% ($p < 0.0003$). Given these results, it is intriguing to note that mGluR5 and D_2 receptor signaling in the striatum functionally counteract each other by means of intramembrane interactions (59). The almost identical magnitude of sleep-deprivation induced changes in mGluR5 and $D_{2/3}$ receptor availability and the significant correlation in both studies with increased sleepiness after the night without sleep strongly support the notion that the reported changes are real and have more than mere statistical significance.

Because plasma samples were not available for calculating regional V_t in all subjects, the cerebellum was used to calculate V_{norm}. The density of mGluR5 in this region is unlikely to produce a specific binding signal with PET. Preclinical and clinical studies demonstrated that the cerebellum is suitable to quantify ^{11}C-ABP688 binding in the brain (4,28,31). Another possible limitation of our study may be due to the fact that two different PET scanners were employed for examining the subjects. Because each volunteer was examined on the same scanner in sleep control and sleep deprivation conditions and a within-subject design was used, it is improbable that differences between PET scanners have biased the findings.

In conclusion, the current observations indicate that mGluR5 may be involved in the effects of sleep deprivation. To corroborate this notion and to address the question whether changes in glutamate concentration could underlie the current observations, multimodal imaging studies combining PET and magnetic resonance spectroscopy are warranted to simultaneously measure functional mGluR5 availability and glutamine/glutamate levels in the brain. The mGluR5 has been implicated in various central nervous system pathologies, and pharmacologic agents targeting this receptor currently provide promising future treatments for psychiatric and neurological disorders, including schizophrenia, anxiety, FXS, substance abuse, and drug withdrawal (23,60). Studies also suggest that mGluR5 agonists have antidepressant-like properties (3). Blockade of glutamatergic neurotransmission with the *N*-methyl-D-aspartate receptor antagonist ketamine and sleep deprivation therapy both rapidly reverse depression in a large subgroup of patients (2). Given the reduced mGluR5 density in depressed patients (4) and the enhanced functional mGluR5 availability after prolonged wakefulness, it is tempting to hypothesize that mGluR5 are involved in the rapid mood-enhancing effects of sleep deprivation. Because the neurobiology of depressed patients is likely to differ from healthy volunteers, future research is needed to confirm this possible mechanism in patients with depression. Such an approach may lead to novel treatments of depression and other mental disorders, which, according to the World Health Organization, will become the biggest health burden on society within the next 20 years.

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Increased Metabotropic Glutamate Receptor Subtype 5 Availability in Human Brain After One Night Without Sleep

Supplemental Information

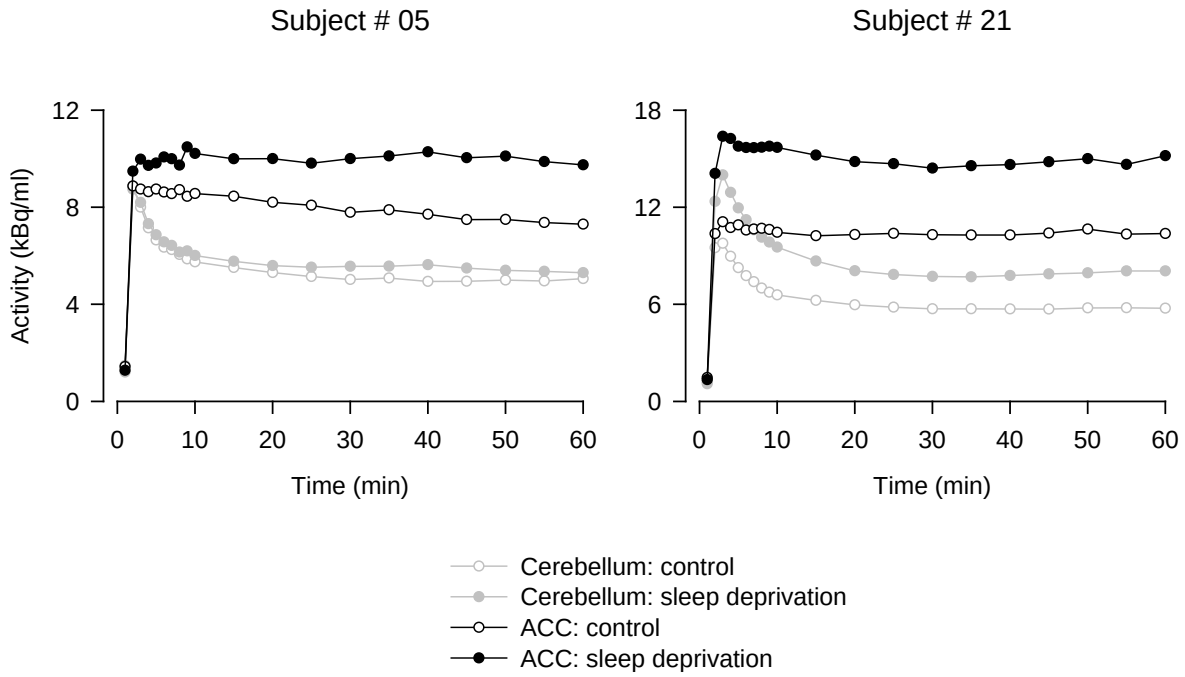

Figure S1. Tissue time-activity-curves (TAC) of cerebellum (gray symbols) and anterior cingulate cortex (ACC; black symbols) of representative subjects # 05 and 21 show that in sleep control (open symbols) and sleep deprivation (filled symbols) conditions a steady state of receptor binding was reached after 45-55 min of image acquisition. The ACC was chosen because this region showed the highest ^{11}C-ABP688 binding in both conditions.

Sleep Deprivation Induced No Change in Cerebellar V_{ND}

To address the question whether sleep deprivation changed ^{11}C-ABP688 binding in the cerebellum, which could have driven the results in the other regions, we calculated the non-displaceable volume of distribution (V_{ND}) in sleep control and sleep deprivation conditions in those 9 study participants in whom reliable blood metabolite concentrations were available. These analyses confirmed that ^{11}C-ABP688 binding in the cerebellum was not changed after sleep deprivation when compared to the control condition.

$V_{ND} = C_{Cb}/C_{plasma}$, where C_{plasma} is the ^{11}C-ABP688 concentration (kBq/mL) in plasma:

Sleep control: $V_{ND} = 1.98 \pm 0.14$

Sleep deprivation: $V_{ND} = 2.18 \pm 0.16$ $p = 0.19$ (2-tailed, paired *t*-test, $n = 9$)

Table S1. Individual normalized volumes of distribution (V_{norm}) in sleep control and sleep deprivation conditions.

#	Ant. cingulate cortex		Insula		Medial temporal lobe		Orbitofrontal cortex		Parahippo-campal gyrus		Med. sup. frontal gyrus		Dorsolat. pre-frontal cortex		Striatum		Precuneus		Parietal cortex		Amygdala		Hippocampus		Thalamus	
	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD	Ctrl.	SD
2	2.044	1.995	1.882	1.880	1.769	1.781	1.728	1.754	1.777	1.814	1.759	1.793	1.598	1.643	1.561	1.655	1.556	1.542	1.537	1.524	1.656	1.640	1.319	1.369	1.248	1.305
3	1.778	1.861	1.710	1.785	1.620	1.698	1.605	1.655	1.550	1.639	1.605	1.639	1.503	1.557	1.460	1.495	1.477	1.506	1.447	1.519	1.420	1.454	1.218	1.243	1.186	1.177
4	1.692	1.854	1.593	1.747	1.581	1.731	1.531	1.651	1.488	1.654	1.427	1.546	1.374	1.500	1.404	1.520	1.329	1.429	1.319	1.446	1.425	1.597	1.228	1.347	1.201	1.280
5	1.648	1.726	1.526	1.640	1.523	1.667	1.528	1.617	1.482	1.604	1.455	1.516	1.365	1.427	1.314	1.380	1.338	1.417	1.342	1.433	1.398	1.478	1.203	1.253	1.173	1.224
6	1.790	1.816	1.760	1.794	1.627	1.657	1.622	1.650	1.672	1.692	1.574	1.585	1.485	1.510	1.514	1.542	1.484	1.467	1.448	1.436	1.530	1.487	1.368	1.331	1.255	1.281
7	1.542	1.731	1.460	1.608	1.405	1.502	1.382	1.529	1.460	1.525	1.370	1.503	1.274	1.381	1.291	1.418	1.268	1.327	1.248	1.327	1.276	1.340	1.129	1.141	1.175	1.218
8	1.618	1.831	1.491	1.652	1.475	1.633	1.447	1.581	1.378	1.571	1.453	1.582	1.372	1.488	1.314	1.447	1.308	1.383	1.306	1.412	1.276	1.362	1.072	1.161	1.170	1.217
10	1.813	1.787	1.750	1.745	1.615	1.635	1.624	1.617	1.530	1.576	1.579	1.589	1.494	1.487	1.404	1.389	1.451	1.438	1.479	1.456	1.398	1.440	1.144	1.138	1.127	1.092
11	1.786	1.698	1.635	1.598	1.651	1.591	1.628	1.546	1.555	1.508	1.525	1.508	1.420	1.400	1.312	1.340	1.477	1.440	1.515	1.487	1.428	1.415	1.212	1.217	1.187	1.169
12	1.799	1.914	1.798	1.892	1.686	1.753	1.649	1.701	1.606	1.676	1.602	1.679	1.554	1.631	1.624	1.685	1.532	1.573	1.494	1.489	1.385	1.496	1.324	1.362	1.270	1.292
13	1.647	1.705	1.582	1.635	1.517	1.545	1.485	1.530	1.376	1.429	1.432	1.481	1.395	1.432	1.362	1.404	1.350	1.378	1.390	1.425	1.287	1.362	1.081	1.119	1.009	1.049
14	1.666	1.500	1.559	1.455	1.470	1.413	1.462	1.357	1.490	1.402	1.419	1.320	1.358	1.246	1.372	1.270	1.286	1.237	1.285	1.249	1.321	1.263	1.164	1.104	1.134	1.053
15	1.915	2.118	1.731	1.939	1.678	1.820	1.657	1.773	1.622	1.805	1.603	1.821	1.531	1.701	1.579	1.855	1.440	1.576	1.453	1.520	1.475	1.701	1.270	1.574	1.319	1.444
16	1.586	1.739	1.486	1.636	1.494	1.617	1.453	1.561	1.387	1.523	1.340	1.436	1.316	1.403	1.264	1.403	1.324	1.417	1.354	1.441	1.343	1.434	1.110	1.205	1.095	1.201
17	1.926	1.893	1.872	1.849	1.762	1.739	1.769	1.741	1.636	1.664	1.701	1.602	1.681	1.532	1.637	1.617	1.568	1.437	1.489	1.413	1.491	1.490	1.354	1.325	1.336	1.282
18	1.969	1.976	1.946	1.969	1.838	1.858	1.780	1.798	1.687	1.719	1.757	1.776	1.659	1.675	1.660	1.686	1.642	1.673	1.577	1.579	1.559	1.593	1.339	1.348	1.289	1.285
19	2.014	1.972	1.850	1.751	1.786	1.794	1.754	1.697	1.760	1.700	1.772	1.653	1.660	1.558	1.737	1.605	1.608	1.568	1.605	1.568	1.605	1.559	1.386	1.294	1.370	1.305
20	1.730	1.906	1.568	1.786	1.540	1.707	1.542	1.687	1.491	1.665	1.546	1.695	1.461	1.603	1.438	1.650	1.465	1.596	1.526	1.637	1.422	1.596	1.211	1.363	1.265	1.400
21	1.771	1.894	1.604	1.770	1.594	1.690	1.592	1.686	1.617	1.726	1.484	1.645	1.411	1.535	1.398	1.569	1.316	1.404	1.358	1.451	1.399	1.530	1.215	1.369	1.217	1.287
22	1.728	1.832	1.668	1.722	1.617	1.680	1.612	1.669	1.517	1.567	1.614	1.670	1.516	1.576	1.443	1.479	1.517	1.532	1.467	1.535	1.460	1.502	1.220	1.266	1.224	1.217
23	1.593	1.664	1.634	1.751	1.573	1.680	1.514	1.626	1.523	1.593	1.408	1.484	1.370	1.455	1.376	1.470	1.339	1.404	1.374	1.432	1.374	1.444	1.191	1.284	1.181	1.249
Mean	1.765	1.829	1.672	1.743	1.611	1.676	1.589	1.639	1.553	1.621	1.544	1.596	1.467	1.511	1.451	1.518	1.432	1.464	1.429	1.466	1.425	1.485	1.227	1.277	1.211	1.239
Std Dev	0.144	0.137	0.142	0.125	0.114	0.106	0.112	0.101	0.114	0.109	0.130	0.124	0.117	0.110	0.135	0.142	0.114	0.102	0.100	0.085	0.102	0.107	0.094	0.112	0.084	0.098
		*		*		*				*						*						*				

Values represent cerebellum-normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake in 13 volumes-of-interest (VOI) after 9 (control, Ctrl.) and 32 hours (sleep deprivation, SD) of wakefulness. Mean values + SEM are also presented in Fig. 2. Asterisks indicate those six VOI that showed a significant increase in V_{norm} after sleep deprivation (Bonferroni-corrected *p*-value of factor 'condition': $p < 0.0038$). Ant. cingulate cortex, anterior cingulate cortex; Med. sup. frontal cortex, medial superior frontal cortex; Dorsolat. prefrontal cortex, dorsolateral prefrontal cortex; Std Dev, standard deviation.