Pharmacogenetics of Modafinil After Sleep Loss: Catechol-O-Methyltransferase Genotype Modulates Waking Functions But Not Recovery Sleep

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Sleep loss impairs waking functions and is homeostatically compensated in recovery sleep. The mechanisms underlying the consequences of prolonged wakefulness are unknown. The stimulant modafinil may promote primarily dopaminergic neurotransmission. Catechol-O-methyltransferase (COMT) catalyzes the breakdown of cerebral dopamine. A functional *Val158Met* polymorphism reduces COMT activity, and *Val/Val* homozygous individuals presumably have lower dopaminergic signaling in the prefrontal cortex than do *Met/Met* homozygotes. We quantified the contribution of this polymorphism to the effects of sleep deprivation and modafinil on subjective state, cognitive performance, and recovery sleep in healthy volunteers. Two-time 100 mg modafinil potently improved vigor and well-being, and maintained baseline performance with respect to executive functioning and vigilant attention throughout sleep deprivation in *Val/Val* genotype subjects but was hardly effective in subjects with the *Met/Met* genotype. Neither modafinil nor the *Val158Met* polymorphism affected distinct markers of sleep homeostasis in recovery sleep. In conclusion, dopaminergic mechanisms contribute to impaired waking functions after sleep loss.

Sleep and wakefulness form a daily continuum, yet the genetic and neurochemical mechanisms underlying sleep–wake regulation are largely unknown. Not only the clinically defined sleep disorders such as narcolepsy, obstructive sleep apnea, and behaviorally induced insufficient sleep syndrome ("sleep deprivation") but also shift work, jet lag, and voluntary sleep restriction are highly prevalent in the modern "24/7" society. In many people, non-restorative and insufficient sleep leads to excessive daytime sleepiness, which is strongly associated with the risk of accidents and human error.[1] The impact of insufficient sleep on health and well-being has developed into a major public health concern.[2] The availability of effective countermeasures to reduce sleepiness and the behavioral and cognitive consequences following sleep loss are of clinical and public importance. This fact is highlighted by the ubiquitous use of pharmacological agents to promote wakefulness and maintain alertness after insufficient sleep. Whereas caffeine is considered the most often consumed substance in the world, the stimulant modafinil, because of its apparently positive safety profile, is becoming increasingly popular.

Modafinil in daily doses of 100–400 mg is clinically used as the first-line treatment for pathological sleepiness in patients with narcolepsy.[3] Controlled studies also demonstrated its efficacy as an adjunct therapy for subjective sleepiness and fatigue in various diseases such as obstructive sleep apnea,[4] shift-work sleep disorder,[5] Parkinson's disease,[6] major depressive disorder,[7] and multiple sclerosis.[8] Although it is certain that the pharmacological profile of modafinil differs from those of amphetamine-like compounds, there is still controversy regarding its mode of action. The available evidence ranging from data from experiments in genetically modified mice to *in vitro* electrophysiology suggests that the drug stimulates wakefulness primarily by modifying dopaminergic and (nor)adrenergic neurotransmission.[9]

Consistent with this notion, clinical observations in narcolepsy indicate that the response to modafinil is modulated

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by a functional genetic variation in the important breakdown enzyme of cortical catecholamines, catechol-O-methyltransferase (COMT).[10] The human COMT gene is located on chromosome 22q11.2 and contains a functional single-nucleotide polymorphism (SNP) that alters the amino acid sequence of the membrane-bound COMT protein at codon 158 from valine (*Val*) to methionine (*Met*; SNP-ID: rs4680).[11] No equivalent COMT polymorphism was found in any other species examined to date, including non-human primates.[12] Europeans have nearly equal prevalence of *Val* (~48%) and *Met* (~52%) alleles, whereas the *Val* allele is much more common in populations from other parts of the world.[12] Individuals who are homozygous for the *Val* allele show more COMT protein in postmortem brain tissue than individuals with two *Met* alleles.[13] Moreover, *Val/Val* genotype subjects show three- to fourfold higher COMT activity and presumably lower dopaminergic signaling in the prefrontal cortex than do *Met/Met* genotype subjects.[13,14] There is strong evidence to suggest that the *Val158Met* polymorphism of COMT affects the prefrontal cortex and associated areas, modulating executive functioning, working memory, and measures of attention.[15]

The sleepiness in narcoleptic patients may be comparable to the sleepiness experienced by healthy subjects after 2 days without sleep.[16] Sleep loss not only increases sleepiness but also affects mood and impairs executive functions, working memory, and sustained attention.[17] Moreover, it is highly reliably compensated by enhanced non-rapid-eye-movement (non-REM) sleep intensity as measured by low-frequency (delta) electroencephalography (EEG) activity in recovery sleep.[18] A decrease in the activation and functioning of the prefrontal cortex may be critical for the waking consequences of sleep loss[19] (for recent review, see ref. 20). While the effects of modafinil on quantitative sleep EEG measures in humans are not known, the drug improves impaired alertness and performance on cognitive tasks after 36–85 h of prolonged waking.[9,21] Nevertheless, the subjective and objective efficacy are variable, and a return to pre-sleep-deprivation performance is not usually observed.[21,22]

We combined pharmacogenetic, pharmacokinetic, neuropsychologic, and polysomnographic methods to delineate a dopaminergic mechanism in human sleep–wake regulation. On the basis of the evidence described above, we hypothesized that sleep deprivation and modafinil affect the same neuronal processes and that the efficacy of the stimulant to reverse sleep loss–induced changes in subjective state, cognitive function, and sleep are modulated by the *Val158Met* polymorphism of COMT. Indeed, we found that, in *Val/Val* genotype subjects, modafinil potently improves vigor and subjective well-being and maintains stable performance with respect to executive function and vigilant attention throughout 40 continuous hours of wakefulness, whereas it is virtually ineffective in *Met/Met* genotype subjects. By contrast, modafinil does not affect well-established, wakefulness-induced sleep and sleep EEG changes in recovery sleep in subjects of either genotype. These findings show that mechanisms involving prefrontal cortex dopamine contribute to distinct aspects of impaired subjective state and cognitive performance after sleep deprivation but are not critically important for the homeostatic regulation of low-frequency delta activity in the non-REM sleep EEG.

RESULTS

Two groups of healthy men homozygous for the *Val/Val* ($n = 10$) and *Met/Met* ($n = 12$) genotypes of the functional *Val158Met* polymorphism of COMT were prospectively enrolled in this study (see **Supplementary Data** online). The groups were carefully matched for age, body mass index, habitual alcohol and caffeine consumption, anxiety (Trait Anxiety Inventory), subjective daytime sleepiness, and chronotype (**Table 1**; for nocturnal melatonin profile, see **Supplementary Figure S1** online). A screening night in the sleep laboratory demonstrated that all the participants were good sleepers with no sleep disturbances.

Modafinil concentration in saliva

Under constant supervision all volunteers completed two 40-h periods of continuous wakefulness in the sleep laboratory (**Figure 1**). After 11 and 23 h of wakefulness, they received 100 mg modafinil or placebo in randomized, double-blind, crossover fashion. Prior to the drug intake, modafinil was below the limit of detection in all subjects. Whereas there was no difference between subjects with the two genotypes with respect to the pharmacokinetics of the drug after the administration of the first capsule, the maximum concentration after the second capsule occurred earlier and was higher in *Val/Val* allele carriers than in the *Met/Met* group (**Figure 2**). Clearance from saliva 4 h after the second dose and the mean concentration of modafinil 1 h before initiation of recovery sleep (0.11 ± 0.01 μg/ml vs. 0.12 ± 0.01 μg/ml) were similar in the two groups.

Modafinil consistently improves subjective state after sleep deprivation in *Val/Val* genotype subjects only

Previous research has indicated that low-dose modafinil is not consistently associated with subjectively perceived elation or

Table 1 Demographics of study participants

	Val/Val	*Met/Met*	*P*
Caucasian/Asian ethnicity	8/2	12/0	
Age (years)	23.9 ± 0.8	23.0 ± 0.6	0.35
Body mass index (kg/m^2)	21.9 ± 0.6	22.3 ± 0.4	0.61
Alcohol consumption (drinks/week)	2.2 ± 0.6	3.0 ± 1.0	0.50
Caffeine consumption (mg/day)	137.0 ± 67.6	146.7 ± 36.0	0.90
Trait Anxiety Inventory[44]	38.6 ± 3.4	36.3 ± 2.2	0.57
Epworth Sleepiness Scale[45]	6.9 ± 0.7	7.2 ± 0.6	0.78
Chronotype			
MEQ	49.3 ± 3.0	47.5 ± 2.8	0.67
MCTQ	5.1 ± 0.4	4.9 ± 0.3	0.69

Values represent means ± SEM (*Val/Val* genotype, $n = 10$; *Met/Met* genotype, $n = 12$). *P* values: unpaired, two-tailed *t*-tests. Estimates of caffeine consumption were based on the following average caffeine content per serving: coffee, 100 mg; Ceylon or green tea, 30 mg; cola drink (2 dl), 40 mg; energy drink (2 dl), 80 mg; chocolate (100 g), 50 mg.

MEQ, Horne-Östberg Morningness-Eveningness Questionnaire;[46] MCTQ, Munich ChronoType Questionnaire.[47] The reported MCTQ values indicate the mid-sleep time on leisure days, including an estimated correction for the sleep debt accumulated during the work week.

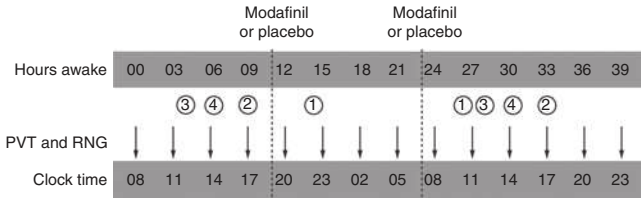

Figure 1 Schematic illustration of experimental protocol. Twenty-two healthy young men completed the experiment in two blocks separated by 1 week, consisting of adaptation and baseline nights, a prolonged period (40 h) of continuous wakefulness, and a recovery night. The sleep deprivation period is highlighted here. Time spent awake and clock time are rounded up to the nearest hour. After 11 and 23 h of wakefulness (vertical broken lines) the subjects received 100 mg modafinil or placebo according to a randomized, double-blind, crossover design. In the same block, subjects received either two capsules of modafinil or two of placebo. 1. Subjective stimulant effects scale—22:45/10:45 hours. 2. Profile of Mood States—16:45 hours. 3. Objective evaluation of subjective well-being (von Zerssen's Befindlichkeits-Skala)—10:45 hours. 4. Two-back task—14:45 hours. Arrows indicate test sessions comprising a psychomotor vigilance task (PVT) followed by a random-number-generation (RNG) task conducted at 3-h intervals beginning 30 min after awakening.

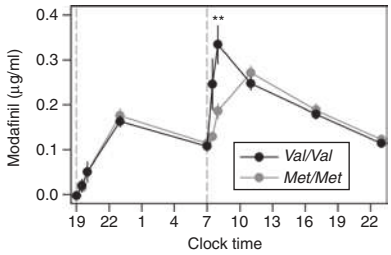

Figure 2 Time course of modafinil in saliva in catechol-O-methyltransferase *Val/Val* ($n = 10$, black circles) and *Met/Met* ($n = 12$, gray circles) allele carriers. The data represent mean values ± SEM. One data point is missing for the subjects with *Val/Val* genotype at 07:30 hours on day 2 of prolonged waking ($n = 9$). Broken vertical lines indicate 100 mg modafinil administration. The maximum concentration after administration of the second capsule occurred at an earlier time point and was higher in *Val/Val* than in *Met/Met* allele carriers ("time" × "genotype": $F_{9,104} = 6.74$, $P < 0.0001$). **$P < 0.01$ (unpaired, two-tailed *t*-test).

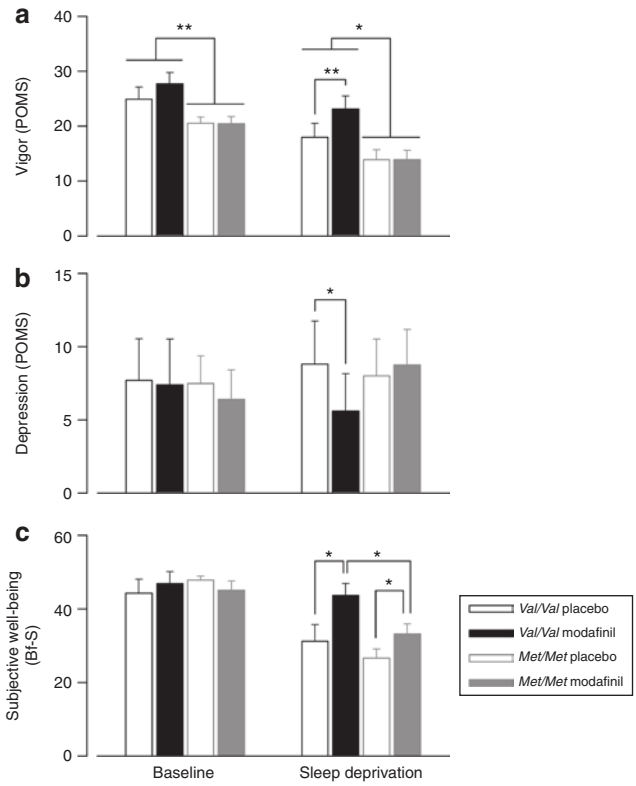

Figure 3 Modafinil consistently improves subjective state after sleep deprivation in *Val/Val* subjects only. The Profile of Mood States (POMS) was administered at 16:45 hours, and von Zerssen's "Befindlichkeits-Skala (Bf-S)" was administered at 10:45 hours on days 1 (baseline) and 2 (sleep deprivation). The data represent mean values + SEM in 10 men with the *Val/Val* genotype (open and filled black bars) and in 12 men with the *Met/Met* genotype (open and filled gray bars). (**a**) Modafinil attenuated the typical drop in vigor associated with prolonged wakefulness ("condition": $F_{2,27.8} = 23.08$, $P < 0.0001$). This effect was restricted to *Val/Val* subjects and was totally absent in *Met/Met* subjects ("genotype" × "condition": $F_{2,27.8} = 3.33$, $P = 0.05$). In both the rested (baseline) and sleep-deprived conditions, vigor was higher in *Val/Val* than in *Met/Met* subjects ("genotype": $F_{1,19.9} = 7.32$, $P < 0.02$). (**b**) Modafinil reduced depressive symptoms after sleep deprivation in *Val/Val* but not *Met/Met* subjects ("genotype" × "condition": $F_{2,32.8} = 4.11$, $P < 0.03$). (**c**) Modafinil improved subjective well-being after sleep deprivation in subjects of both genotypes, but the effect was stronger in *Val/Val* than in *Met/Met* subjects ("genotype" × "condition": $F_{2,39.6} = 3.38$, $P < 0.05$). The data in **c** are plotted on an inverse scale. **$P < 0.01$ (unpaired and paired, two-tailed *t*-tests); *$P < 0.05$ (unpaired and paired, two-tailed *t*-tests).

stimulation after sleep deprivation.[22] The subjective perception of the stimulant effects of modafinil was quantified 4 h after intake of each capsule, at the time of the expected maximum concentration of the drug in saliva.[9] The "stimulant effects score" was invariably higher after active treatment than after placebo in subjects with the *Val/Val* genotype (first capsule: 11.3 ± 2.2 vs. 5.7 ± 1.3, $P < 0.004$, paired *t*-test; second capsule: 15.2 ± 3.0 vs. 3.3 ± 1.2, $P < 0.002$). By contrast, there was no significant difference in scores after active treatment and after the placebo in *Met/Met* allele carriers (first capsule: 9.1 ± 2.4 vs. 4.7 ± 1.6, $P > 0.07$; second capsule: 7.8 ± 1.7 vs. 3.8 ± 1.6, $P > 0.2$). After sleep deprivation, the subjective effects of modafinil differed significantly between subjects with the two different genotypes ($P < 0.04$, unpaired *t*-test).

To examine whether the different subjective effects reflected the initially higher modafinil concentrations in *Val/Val* allele carriers, the Spearman rank-correlation coefficient between the mean concentration at 30 and 60 min after drug intake and the stimulant effect score after the second capsule were computed. No significant relationship emerged ($r = 0.1$, $P = 0.63$, $n = 22$). However, subjects with the *Val/Val* genotype tended to correctly assign verum and placebo capsules more often than subjects with the *Met/Met* genotype (90% vs. 66% correct, $P = 0.09$, Fisher's exact test).

Next, we analyzed the effects of sleep deprivation and modafinil on subjective symptoms of fatigue, anger, vigor, and depression, as quantified with a validated German translation of the Profile of Mood States.[23] These analyses revealed that sleep deprivation approximately doubled fatigue and slightly increased anger. Irrespective of genotype, modafinil was ineffective in mitigating these deteriorations in subjective state (data not shown). However, the drug attenuated the typical drop in

vigor associated with prolonged wakefulness. This effect was restricted to *Val/Val* genotype subjects and was completely lacking in those with the *Met/Met* genotype (**Figure 3a**). Moreover, in both baseline and sleep-deprived states, vigor was higher in *Val/Val* than *Met/Met* allele carriers. Neither sleep deprivation nor genotype affected symptoms of depression as measured using the Profile of Mood States. Nevertheless, depression after sleep deprivation was lower in the *Val/Val* genotype subjects after modafinil than after placebo (**Figure 3b**). The values did not differ in subjects with the *Met/Met* genotype.

To further investigate whether modafinil differently affects subjective well-being after sleep deprivation, the objective "Befindlichkeits-Skala" of von Zerssen was used.[24] Sleep deprivation reduced well-being, whereas modafinil improved it

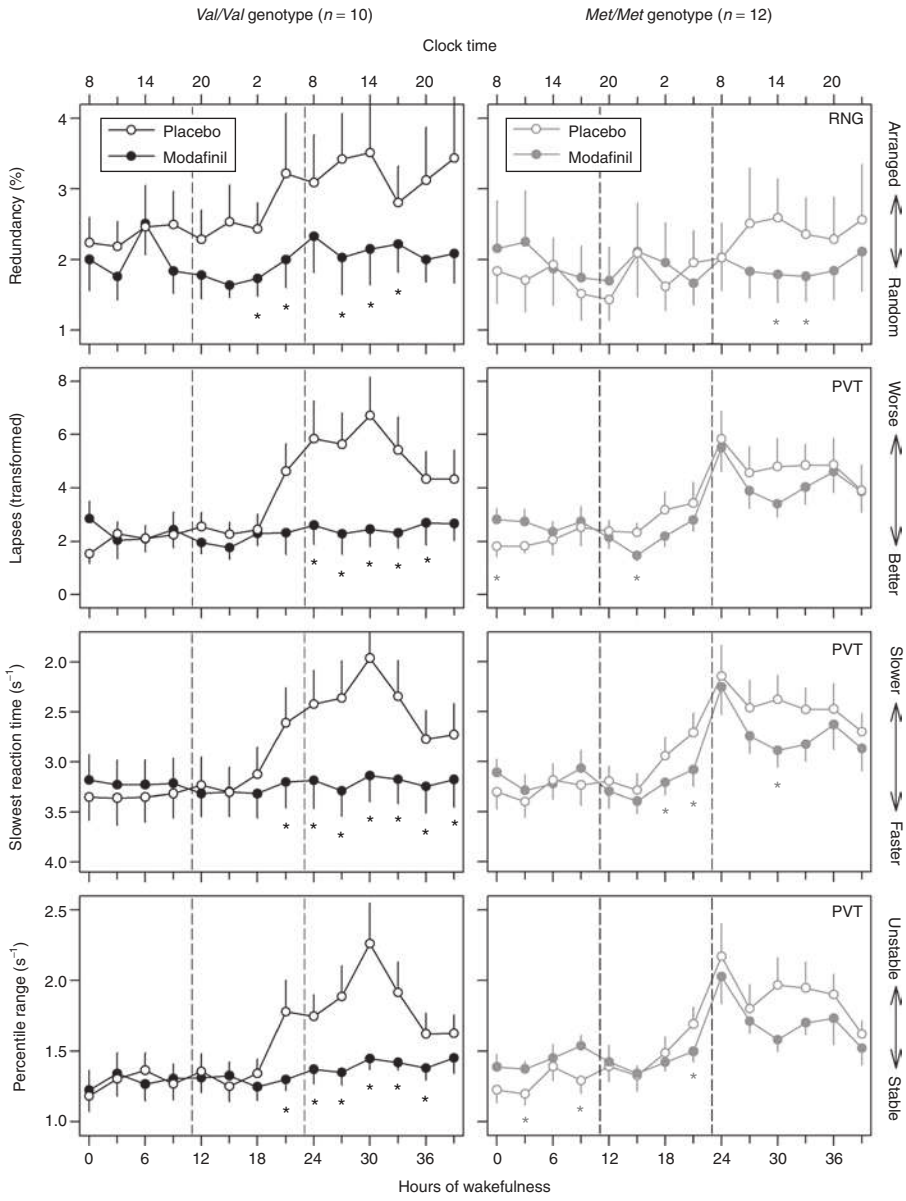

Figure 4 Modafinil maintains cognitive performance during sleep deprivation in *Val/Val* subjects only. Cognitive testing, including a 10-min random-number-generation (RNG) task preceded by a 10-min psychomotor vigilance task (PVT), was administered every 3 h across a 40 h waking period beginning 30 min after awakening. The tick marks on *x*-axes are rounded up to the nearest hour. The time course of RNG redundancy (zero-order response stereotypy), PVT lapses (reaction times (RTs) > 500 ms, transformed by $\sqrt{x} + \sqrt{1 + x}$), slowest 10th percentile of reaction times (expressed as speed, 1/RT), and the 90th–10th interpercentile range of speed are illustrated. All RTs < 100 ms ("errors of commission") were excluded from analyses. Broken vertical lines indicate 100 mg modafinil or placebo administration. Black symbols: *Val/Val* genotype (open circles: placebo condition; closed circles: modafinil condition). Gray symbols: *Met/Met* genotype (open circles: placebo condition; closed circles: modafinil condition). Top panels: modafinil reduced redundancy after sleep deprivation ("session": $F_{13,309} = 2.16$, $P < 0.02$; "treatment": $F_{1,96.8} = 16.93$, $P < 0.0001$), but the effect was higher in *Val/Val* than in *Met/Met* subjects ("genotype" × "treatment": $F_{1,96.8} = 9.94$, $P < 0.003$). Lower panels: during sleep deprivation modafinil maintained all measures of sustained vigilant attention (PVT lapses, slowest reaction times, interpercentile range) at baseline levels in *Val/Val* subjects, but was virtually ineffective in *Met/Met* subjects (lapses: "session" × "treatment" × "genotype": $F_{26,313} = 1.71$, $P < 0.02$; slowest reaction times: "session" × "treatment" × "genotype": $F_{26,321} = 2.07$, $P < 0.002$; interpercentile range: "session" × "treatment" × "genotype": $F_{26,317} = 1.69$, $P < 0.03$). Asterisks denote significant differences compared to the respective placebo values ($P \leq 0.05$, paired, two-tailed *t*-tests).

in subjects with either genotype. Interestingly, the presence of two *Val* alleles enhanced the drug's efficacy. Subjects with the *Val/Val* genotype had scores similar to those at baseline after drug intake following sleep deprivation, whereas, in subjects with the *Met/Met* genotype, modafinil produced significantly less improvement (**Figure 3c**).

Modafinil maintains cognitive performance during sleep deprivation in *Val/Val* genotype subjects only

Redundancy on a random-number-generation task provides a sensitive index of a subject's ability to update and monitor information.[25] This aspect of executive functioning is particularly responsive to the detrimental effects of prolonged wakefulness.[26] Compared to placebo, modafinil maintained low redundancy after sleep deprivation in subjects with either of the two genotypes (**Figure 4**, top panels). Nevertheless, the drug effect was more pronounced in *Val/Val* subjects than in *Met/Met* subjects.

Working memory is vulnerable to sleep loss.[17] To investigate whether modafinil affects working memory differently in *Val/Val* allele carriers than in *Met/Met* allele carriers after sleep deprivation, subjects performed a visual two-back task on days 1 and 2 of prolonged wakefulness. Irrespective of genotype, sleep deprivation reduced response speed and approximately doubled the percentage of incorrect responses (**Table 2**). Modafinil attenuated these impairments. After intake of the drug, the speed and accuracy of response in the sleep-deprived subjects no longer differed from baseline values in either genotype.

Performance on the psychomotor vigilance task (PVT) is a sensitive measure of sustained vigilant attention, which is normally impaired after prolonged wakefulness.[17] Across the first 16–20 h of wakefulness, *Val/Val* and *Met/Met* allele carriers maintained almost lapse-free, fast and stable PVT performance, under both placebo and modafinil conditions (**Figure 4**, lower panels). Afterward, in the placebo condition, the number of response lapses increased and reaction times became longer and more variable. Performance was worst when testing occurred in the morning of day 2 of prolonged wakefulness. Most intriguingly, low-dose modafinil fully eliminated the wakefulness-induced impairment of sustained vigilant attention in subjects with the *Val/Val* genotype ($P > 0.2$ for factor "session" for all PVT measures), whereas it was virtually ineffective in those with the *Met/Met* genotype ($P < 0.0001$). By contrast, the number of false responses in the absence of stimuli (sometimes referred to as "errors of commission") did not differ on the basis of either

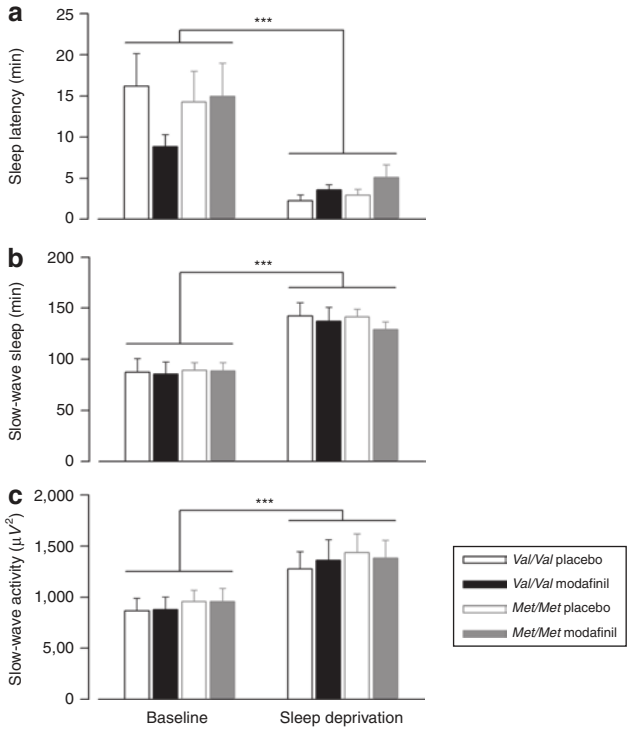

Figure 5 Irrespective of *Val158Met* genotype of catechol-O-methyltransferase and modafinil administration, prolonged wakefulness reduces sleep latency and increases slow-wave sleep and electroencephalography (EEG) low-frequency delta activity in subsequent non-rapid-eye-movement (non-REM) sleep. The data represent mean values + SEM in 10 men with the *Val/Val* genotype (open and filled black bars) and in 12 men with the *Met/Met* genotype (open and filled gray bars). (**a**) Sleep deprivation shortens the time between lights off and the first occurrence of stage 2 sleep. (**b**) Sleep deprivation prolongs the duration of slow-wave sleep (non-REM sleep stages 3 and 4). (**c**) Sleep deprivation increases all-night EEG spectral power within 0.75–2.0 Hz in non-REM sleep (stages 1–4). ***$P < 0.0001$ (factor "condition" of two-way analysis of variance with between-factor "genotype" (*Val/Val, Met/Met*) and within-factor "condition" (mean baseline, SD-placebo, SD-modafinil)).

Table 2 Modafinil attenuates sleep deprivation–induced impairment of accuracy and speed on a two-back task

	Placebo		Modafinil	
	Baseline	Sleep deprivation	Baseline	Sleep deprivation
Val/Val genotype				
Reaction time (ms)	690.6 ± 42.2	840.1 ± 65.3*	708.9 ± 44.6	772.3 ± 46.9
Incorrect (%)	6.3 ± 3.0	11.8 ± 4.0*	7.2 ± 2.8	8.5 ± 2.6
Met/Met genotype				
Reaction time (ms)	746.7 ± 45.1	808.5 ± 46.2	742.2 ± 50.1	747.9 ± 51.4
Incorrect (%)	4.3 ± 1.3	9.5 ± 2.9*	5.1 ± 2.0	7.5 ± 2.3

Values represent means ± SEM in 10 *Val/Val* subjects and 12 *Met/Met* subjects. The two-back task was performed at 6.75 (baseline) and 30.75 h (sleep deprivation) into prolonged waking. Accuracy: percentage of incorrect responses. Irrespective of genotype, sleep deprivation prolonged reaction time ("condition": $F_{2,25.4} = 4.7$, $P < 0.02$) and increased the number of incorrect responses ("condition": $F_{2,29.6} = 4.7$, $P < 0.02$). Modafinil attenuated the impairments induced by sleep deprivation.

*$P < 0.04$ (two-tailed, paired *t*-tests).

the treatments or the genotypes (data not shown). This observation suggests that lack of motivation was not a critical factor in the reduced efficacy of modafinil in subjects with the *Met/Met* genotype.

Modafinil does not affect established markers of sleep homeostasis in recovery sleep

The homeostatic facet of sleep regulation refers to the highly reliable finding in animals and humans that recovery sleep after prolonged waking occurs with reduced latency and is more intense than baseline sleep.[18] After sleep deprivation, in subjects with either genotype and irrespective of treatment, the reduction in the time taken for subjects to fall asleep and the lengthening of the duration of deep slow-wave sleep (non-REM sleep stages 3 and 4) were similar in relation to baseline values (**Figure 5a,b**).

Slow, rhythmic oscillations in EEG wave patterns during non-REM sleep are a well-established physiological marker of sleep need and sleep intensity. The prevalence and amplitude of oscillations in the 0.75–2.0 Hz range (low-frequency delta activity) were quantified with all-night spectral analysis in baseline as well as in recovery sleep. This frequency band most sensitively reflects the putative effects of genetic variation and caffeine on sleep homeostasis.[27–29] Sleep deprivation increased low-frequency delta activity to a similar extent of 47–50% in both *Val/Val* and *Met/Met* allele carriers (**Figure 5c**). Modafinil did not affect the magnitude of this increase in either genotype. These findings demonstrate that, in contrast to subjective state and distinct aspects of cognitive performance, recovery sleep was not significantly affected by modafinil or the *Val158Met* polymorphism of COMT after sleep deprivation.

DISCUSSION

In this study we showed that the functional *Val158Met* polymorphism of COMT strongly modulates the efficacy of low-dose modafinil with respect to subjective state and cognitive performance after sleep deprivation. The study provides the first example of a pharmacological intervention that sustains high and stable waking functions throughout 2 days and 1 night without sleep in a genetically distinct group of healthy individuals. Because both the COMT *Val158Met* polymorphism and modafinil changes dopaminergic signaling in the prefrontal cortex,[13,14,30,31] we conclude that altered dopaminergic neurotransmission contributes to impairment of well-being and cognitive performance after sleep loss. By contrast, neither COMT genotype nor modafinil were seen to affect well-established, sleep deprivation–induced changes in recovery sleep. These findings demonstrate that, in humans, the mechanisms that mediate the effects of sleep loss on waking neurobehavioral functions are different from those that mediate the effects of sleep loss on distinct characteristics of sleep physiology.

To follow the pharmacokinetics of modafinil in *Val/Val* and *Met/Met* allele carriers, we developed a novel liquid chromatography–mass spectrometry/mass spectrometry method for quantifying modafinil levels in saliva (see **Supplementary Data** online). Consistent with data from plasma, we found that in young men the drug is readily absorbed and peak concentration is reached at 2–4 h after oral administration.[3,9] There was a significant genotype-associated difference in modafinil levels 1 h after the second capsule intake. One cannot exclude the possibility that this pharmacokinetic difference contributes to some modafinil-induced variations in subjective state and performance after sleep deprivation. Nevertheless, modafinil is not known as a substrate of COMT, and the time course of drug concentration in the β-phase (final three measurements) indicates that modafinil metabolism and elimination are independent of COMT genotype. Esterase enzymes in the liver are primarily responsible for the hydrolytic deamination of the drug to modafinil acid, and cytochrome P450 (CYP) 3A4 converts this inactive metabolite to modafinil sulfone.[9] However, modafinil is a substrate of P-glycoprotein, which is encoded by the multidrug resistance gene *MDR1* and which inhibits and induces, respectively, the human CYP isoenzymes 2C19 and 3A4/5. The genes of these proteins are polymorphic and play important roles in mediating interindividual differences in absorption and metabolism of drugs. One cannot exclude the possibility that the distribution of alleles and genotypes in MDR1 and CYP isoenzymes differ between *Val/Val* and *Met/Met* allele carriers. The possibility that polymorphisms in these genes interact with the *Val158Met* polymorphism of COMT and modulate the pharmacodynamics and pharmacokinetics of modafinil is intriguing and warrants further investigation.

Low-dose modafinil does not reliably enhance cognitive task performance in non-sleep-deprived subjects.[32,33] Similarly, the drug's efficacy to reduce subjective and objective measures of sleepiness after sleep deprivation differs widely among individuals.[22] Our data provide the first demonstration that the *Val158Met* polymorphism of COMT contributes to these interindividual differences in healthy adults. Two-time 100 mg modafinil, the lowest recommended dose in narcolepsy, consistently improved subjective state and maintained executive functioning and vigilant attention throughout 40 h of continuous wakefulness in subjects with the *Val/Val* genotype but was virtually ineffective in those with the *Met/Met* genotype. Based on the hypothesis that this genetic variation affects synaptic dopamine levels in the prefrontal cortex,[13] our data suggest that prefrontal cortex dopamine is critically involved in subjective and objective impairment from sleep deprivation. Frontal brain structures and associated functions are especially vulnerable to the effects of sleep loss.[20] Medial frontal and prefrontal structures, together with the anterior cingulate cortex and the thalamus, are important for emotional processing, executive functions, and attentional control.[34] Modafinil increases blood flow in these cerebral regions in highly functional states[35] as well as in impaired cognitive states, such as after sleep loss.[36] The recruitment of cortical and subcortical activation by modafinil may primarily reflect dopaminergic effects,[30,31,37] although other mechanisms are also involved.[9] It had earlier been proposed that COMT genotype modulates the response of the prefrontal cortex to higher levels of dopamine according to an inverted U-shaped response curve.[38] Our observations are compatible with this hypothesis. They suggest that the drug efficiently mitigates impaired subjective state and cognitive performance after sleep loss in *Val/Val*

genotype subjects who, exhibit relatively deficient dopamine signaling. By contrast, the drug is barely effective in *Met/Met* genotype subjects who, have relatively higher dopaminergic tone at synapses where COMT activity is critical.

An important impact of the *Val158Met* polymorphism of COMT on daytime functioning was previously found in patients with narcolepsy. Specifically, a sex-related dimorphism and a strong effect of genotype on disease severity were reported.[39] Women narcoleptics with high COMT activity fall asleep twice as fast during the multiple sleep latency test (3 min) than those with low COMT activity (6 min). An opposite relationship, although less pronounced (5.6 min vs. 4.1 min), is observed in men.[39] An inspection of patient histories revealed that the response to treatment with modafinil to control excessive daytime sleepiness also differs between COMT genotypes. Patients (both female and male) with the *Val/Val* genotype need a higher daily dose (329.2 mg) than patients with the *Met/Met* genotype (241.0 mg).[10] A comparison of these data with our findings is difficult because different outcome variables and subject populations were studied. Nevertheless, COMT genotype may distinctly modulate the individual response to modafinil in narcolepsy patients undergoing long-term pharmacotherapy and in healthy men after acute drug administration. This notion is supported by functional brain imaging data showing that a 4-week intake of modafinil decreases cerebral blood flow in fronto-temporal cortices in narcoleptic patients,[40] whereas no such decrease is observed after single-dose administration in healthy volunteers.[35] The reasons for this discrepancy are unknown.

Previous reports postulate that the sleep rebound following modafinil-induced wakefulness is reduced or even absent compared to the sleep rebound after sleep deprivation.[41,42] Although there is some controversy regarding this finding,[43] it suggests that modafinil could compensate for changes in the brain that are the physiological consequences of prolonged wakefulness. These changes are reliably and predictably reflected in recovery sleep, in the form of shortened sleep latency, prolonged slow-wave sleep, and increased slow rhythmic oscillations in non-REM sleep EEG.[18] These can normally be reversed only by sleep. Modafinil had no effect on recovery sleep in either *Val/Val* subjects (who maintained baseline levels of executive functioning and sustained attention throughout 40 h without sleep) or *Met/Met* subjects (who showed wakefulness-induced impairment in waking functions). These observations challenge the hypothesis that modafinil inhibits the homeostatically regulated increase in deep non-REM sleep duration and intensity following prolonged wakefulness. In addition, the data suggest that the effects of sleep loss on daytime functioning and on the sleep EEG are separately regulated.

METHODS

Genotyping and subject recruitment. The study protocol and all experimental procedures were reviewed and approved by the local ethics committee for research on human subjects and conducted in accordance with the principles of the Declaration of Helsinki.

Blood samples for genotyping were obtained from 88 respondents to public advertisements seeking participants for this study. Genomic DNA was extracted from 3 ml fresh blood and used for the allelic identification of the *Val158Met* SNP (NCBI SNP-ID: rs4680) of the COMT gene. Twenty-two young men (age range: 20–29 years) were selected on the basis of their *Val158Met* genotype, and they were paid for participating in the study. Ten were homozygous *Val/Val* allele carriers, and 12 were homozygous *Met/Met* allele carriers. The two groups were carefully matched for age, body mass index, habitual alcohol and caffeine consumption, anxiety (Trait Anxiety Inventory[44]), subjective daytime sleepiness (Epworth Sleepiness Scale[45]), and chronotype (Horne-Östberg Morningness–Eveningness Questionnaire[46] and Munich ChronoType Questionnaire[47]). All screening and pre-experimental procedures, as well as the sleep and sleep deprivation protocol, were similar to or adapted from previous studies (see **Supplementary Data** online and refs. 26,48 for details).

Modafinil capsules and quantification of modafinil concentration in saliva. Two doses of 100 mg modafinil, in the form of capsules, were administered to all subjects after 11 and 23 h of prolonged wakefulness according to a randomized, double-blind, placebo-controlled, crossover design. The capsules were produced by homogenizing commercial Modasomil 100 tablets (distribution: Globopharm AG, Küsnacht, Switzerland) with mannitol (manufacturer: Siegfried Ltd., Zofingen, Switzerland). Placebo capsules of identical appearance contained only mannitol.

Saliva samples for modafinil quantification were collected in 15-ml centrifuge tubes (Techno Plastic Products AG, Trasadingen, Switzerland) immediately before administration of the first capsule and at defined time points during prolonged wakefulness. The samples were stored at −80 °C for later analyses with a novel liquid chromatography–mass spectrometry/mass spectrometry method developed in our institute (see **Supplementary Data** and **Supplementary Table S1** online).

Subjective state and cognitive performance. At 16:45 hours on days 1 and 2 of prolonged wakefulness, subjects completed a Profile of Mood States given in the form of a validated German translation[23] and at 10:45 hours, von Zerssen's Befindlichkeits-Skala for objective evaluation of subjective well-being was administered.[24] Four hours after ingesting the capsule, the participants filled in a 20-item questionnaire about the subjective stimulant effects of the drug. This scale was developed by our group for previous investigations on the effects of caffeine during sleep deprivation.[48] Possible answers to the questions relating to whether common effects of stimulants were present or not were "not at all" (scored as 0), "a little" (1), "quite a bit" (2), and "very much" (3) (total score: 0–60).

In each study block, the participants completed 14 sessions of cognitive testing at 3-h intervals during a prolonged 40-h waking period. Each session consisted of a random-number-generation task[26,49] preceded by a PVT[17] (for detailed information, see **Supplementary Data** online). On the eve of the adaptation night in each block, the subjects completed the tasks once to familiarize themselves with them.

A verbal two-back task to quantify working memory function was administered at 14:45 hours on days 1 and 2 of prolonged wakefulness. Performance on a task of an intermediate level of difficulty (two-back) was recently shown to be enhanced by 200 mg modafinil after overnight sleep deprivation.[36]

All-night polysomnography. Continuous all-night polysomnographic recordings were performed each night. The EEG (data of derivation C3A2 are reported here), electro-oculogram, mental electromyogram, and electrocardiogram were recorded using Rembrandt DataLab (version 8; Embla Systems, Broomfield, CO) and the polygraphic amplifier Artisan (Micromed, Mogliano Veneto, Italy). Analog signals were conditioned by a high-pass filter (EEG: −3 dB at 0.15 Hz; electromyogram: 10 Hz; electrocardiogram: 1 Hz) and an anti-aliasing low-pass filter (−3 dB at 67.2 Hz), digitized and transmitted via fiber optic cables to a personal computer. Data were sampled at a frequency of 256 Hz. The sleep stages were visually scored for 20-s epochs according to standard criteria,[50] using Rembrandt Analysis Manager (version 8; Embla Systems, Broomfield, CO). The EEG power spectra of consecutive 20-s

epochs (average of 5 4-s epochs, fast Fourier transform routine, Hanning window, frequency resolution 0.25 Hz) were calculated using MATLAB (The MathWorks, Natick, MA) and matched with the sleep scores. Twenty-second epochs with movement- and arousal-related artifacts were visually identified and excluded.

Data analyses and statistics. All statistical analyses were performed using SAS 8.02 software (SAS Institute, Cary, NC). The time courses of modafinil in saliva, subjective state, redundancy on a random-number-generation task, working memory, performance on PVT, sleep latency, and slow-wave sleep were analyzed. Data relating to changes in EEG low-frequency delta activity in non-REM sleep as a function of sleep deprivation and modafinil treatment in *Val/Val* and *Met/Met* allele carriers were also analyzed. Variables that were not normally distributed (absolute EEG power values, reaction times, and response lapses) were transformed so as to approximate a normal distribution. Two- and three-way, mixed-model analyses of variance were performed with the between-subject factor "genotype" (*Val/Val*, *Met/Met*) and the within-subject factors "condition" (mean baseline, SD-placebo, SD-modafinil), "treatment" (modafinil, placebo), "session" (14 assessments during prolonged waking), or "time" of saliva collection for modafinil determination (10 time points), as well as their interactions. Analyses involving the between-subject factor "order" (placebo-modafinil, modafinil-placebo) revealed no significant main effect or interaction. The significance level was set at $\alpha < 0.05$. Unless stated otherwise, only significant effects of factors and interactions are mentioned. Two-tailed, paired and unpaired *t*-tests to localize differences within and between groups were performed only if the respective main effects or interactions of the analysis of variance were significant.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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