Effects of COMT genotype and tolcapone on lapses of sustained attention after sleep deprivation in healthy young men

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EFFECTS OF *COMT* GENOTYPE AND TOLCAPONE ON LAPSES OF SUSTAINED ATTENTION AFTER SLEEP DEPRIVATION IN HEALTHY YOUNG MEN

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Abstract

Tolcapone, a brain penetrant selective inhibitor of catechol-O-methyltransferase (COMT) devoid of psychostimulant properties, improves cognition and cortical information processing in rested volunteers, depending on the genotype of the functional Val158Met polymorphism of *COMT*. The impact of this common genetic variant on behavioral and neurophysiological markers of increased sleep need after sleep loss is controversial. Here we investigated the potential usefulness of tolcapone to mitigate consequences of sleep deprivation on lapses of sustained attention, and tested the hypothesis that dopamine signaling in the prefrontal cortex (PFC) causally contributes to neurobehavioral and neurophysiological markers of sleep homeostasis in humans. We first quantified in 73 young male volunteers the impact of *COMT* genotype on the evolution of attentional lapses during 40 hours of extended wakefulness. Subsequently, we tested in an independent group of 30 young men whether selective inhibition of COMT activity with tolcapone counteracts attentional and neurophysiological markers of elevated sleep need in a genotype-dependent manner. Neither *COMT* genotype nor tolcapone affected brain electrical activity in wakefulness and sleep. By contrast, *COMT* genotype and tolcapone modulated the sleep-loss induced impairment of vigilant attention. More specifically, Val/Met heterozygotes produced twice as many lapses after a night without sleep than Met/Met homozygotes. Unexpectedly, tolcapone further deteriorated the sleep loss-induced performance deficits when compared to placebo, particularly in Val/Met and Met/Met genotypes. The findings suggest that PFC dopaminergic tone regulates sustained attention after sleep loss according to an inverse U-shape relationship, independently of neurophysiological markers of elevated sleep need.

Introduction

Elevated sleepiness and impaired vigilance due to medical disorders, shift work or jetlag are highly prevalent in society. Despite the risk of adverse effects, abuse and dependence, modafinil and psychostimulant medications are frequently used to treat pathological sleepiness in patients suffering from narcolepsy, attention-deficit-hyperactivity disorder, circadian sleep-wake rhythm disorders, or behaviorally-induced insufficient sleep (Farah, 2015). Furthermore, pronounced inter-individual variation exists in the response to psychostimulants in sleep-disordered or sleep-deprived individuals. It was previously proposed that functional variation in the catechol-O-methyltransferase (*COMT*) gene may contribute to these differences (Bodenmann *et al*, 2009; Holst *et al*, 2016), yet evidence for a causal genotype-phenotype association is currently lacking.

Prefrontal cortex (PFC) dopaminergic neurotransmission contributes to a wide range of cognitive and neuropsychiatric phenotypes (Goldman-Rakic *et al*, 2000; Tunbridge *et al*, 2006), and the *COMT* gene encodes the main enzyme degrading catecholamines in the PFC (Matsumoto *et al*, 2003). A common valine-to-methionine substitution at codon 158 of COMT protein (Val158Met single nucleotide polymorphism [SNP]; SNP-Id: rs4680) drastically reduces enzymatic activity (Chen *et al*, 2004), leading to elevated PFC dopaminergic tone in Met/Met homozygotes when compared to Val/Val homozygotes (Tunbridge *et al*, 2006). While *COMT* genotype modulates PFC functioning (Egan *et al*, 2001; Tunbridge *et al*, 2006), some effects of this polymorphism may only become prominent when the dopamine system is challenged, such as with behavioral or pharmacological interventions including sleep deprivation (Bodenmann *et al*, 2009; Satterfield *et al*, 2017) and administration of the brain penetrant COMT inhibitor tolcapone (Barkus *et al*, 2016; Farrell *et al*, 2012; Giakoumaki *et al*, 2008). Tolcapone is a non-stimulant drug thought to lead to relatively specific increases in PFC dopamine (Apud *et al*, 2007; Farrell *et al*, 2012; Giakoumaki *et al*, 2008).

Because it hardly affects striatal dopaminergic transmission, tolcapone may mitigate subjective and behavioral consequences of sleep deprivation without unwanted psychostimulant

adverse effects. In addition, it offers the unique opportunity to dissect distinct roles of dopaminergic circuities in sleep-wake regulation. The impact of mesocortical dopaminergic neurotransmission for sleep-wake phenotypes in humans is controversial (Dauvilliers *et al*, 2015). More specifically, by studying dopaminergic mechanisms of sustained attention after sleep deprivation, the wake promoting agent modafinil was virtually ineffective in Met/Met homozygotes of *COMT* (Bodenmann *et al*, 2009). By contrast, in homozygous Val/Val allele carriers, modafinil maintained optimal performance on the psychomotor vigilance test (PVT) throughout 40 hours of prolonged wakefulness (Bodenmann *et al*, 2009). These results were compatible with a hypothesized inverse U-shape relationship in the response of the PFC to elevated dopaminergic neurotransmission (Mattay *et al*, 2003). Intriguingly, neither *COMT* genotype nor modafinil altered the waking-induced rebound in electroencephalographic (EEG) slow-wave activity (SWA, spectral power within the 0.75-4.5 Hz range) in non-rapid-eye-movement (NREM) sleep (Bodenmann *et al*, 2009; Bodenmann and Landolt, 2010). In contrast to this observation, another study suggested that the Val158Met polymorphism of *COMT* could serve as a genetic biomarker for predicting individual differences in sleep physiology (Goel *et al*, 2011). Reconciling this discrepancy is important because EEG SWA is the best established neurophysiologic biomarker of the homeostatic facet of sleep-wake regulation (Borbély, 1982).

Here we aimed at clarifying the role of COMT in sleep-wake regulation and at testing the causality of the suggested genotype-phenotype associations. We first investigated the impact of *COMT* genotype on sustained attention after one night without sleep in 73 male volunteers. Subsequently, we examined the interaction of *COMT* genotype, tolcapone and sleep deprivation on PVT performance and neurophysiological markers of sleep-wake regulation in 30 young men. Given their baseline differences in COMT enzymatic activity, we hypothesized that sleep loss would differently impair sustained attention in Val/Val, Val/Met and Met/Met allele carriers, and that Val/Val homozygotes would benefit from tolcapone because it counteracts the overactive COMT and associated deficit in PFC dopaminergic tone. Our results show that tolcapone had no beneficial

effect in any genotype, but further deteriorated attentional lapses after sleep deprivation in Val/Met and Met/Met allele carriers without affecting EEG markers of sleep homeostasis. The data are consistent with an inverted U-shape relationship between mesocortical dopaminergic signaling and sustained attention after sleep loss.

Materials and Methods

Sleep deprivation (SD) study

Participants and genotyping

The analysis of sustained vigilant attention during extended wakefulness relied on the data of 52 male participants of three previously reported sleep deprivation studies (Hefti *et al*, 2013; Holst *et al*, 2014), as well as 21 additional male volunteers completing an analogous protocol. Female subjects were excluded because *COMT* gene expression is modulated by estrogen-regulating sequences (Xie *et al*, 1999) and interactions between sex and genotype were previously reported in humans and mice (Dauvilliers *et al*, 2002; Gogos *et al*, 1998). No study participant worked shifts or at night, travelled more than two time zones in the last three months, nor suffered from any known disorder of the nervous system or an acute medical condition. Participants were free of medication and recreational drug use and consumption of excessive amounts of alcohol (< five “drinks” per week) or caffeine (< three cups of coffee or equivalent per day). They were enrolled into the studies when a pre-study screening night in the laboratory confirmed the absence of any sleep disorder, and sleep efficiency defined as the percentage of polysomnographically recorded total sleep time per time in bed (i.e., elapsed time between ‘lights-off’ and ‘lights-on’) in the screening night equaled > 80 %. According to the principles in the Declaration of Helsinki, all study protocols were approved by the ethics committee of the Canton of Zürich for human research. Written informed consent was obtained from all volunteers, who received a financial compensation for their participation.

In the 52 participants of the previous studies, genomic DNA was extracted from 3 ml fresh EDTA-blood (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI). The Val158Met polymorphism of *COMT* was determined using Taqman® SNP Genotyping Assays (Life Technologies Europe B.V.). Allelic discrimination analysis was performed with SDS v2.2.2 software (Applied Biosystems, Foster City, CA, USA). In 30 participants, DNA was isolated from 200 µl blood using NucleoSpin® Blood (Macherey Nagel GmbH Co. KG). In the 21 additional participants, genomic DNA was extracted from 2 ml saliva samples using Oragene® DNA kit (DNA Genotek Inc., Ottawa, Canada). The Val158Met genotypes of *COMT* were determined by PCR followed by sequencing. Primers used for amplification on MJ Research PTC-225 thermal cyclers (MJ Research/Bio-Rad, Reno, USA) were 5'-GAT CCA AGT TCC CCT CTC TC-3' and 5'-GGT GGT CCA CAA GTG TGG T-3'. The amplified DNA sequences were then sequenced using the Sanger chain-termination method. A Sephadex G-50 purification step was applied according to the manufacturer's instructions. The capillary gel electrophoresis and laser detection of the purified DNA products (351 bp) was performed with an ABI PRISM 3100 or ABI PRISM 3730 Genetic Analyser (Life Technologies, Zug, Switzerland). The DNA reads from the sequencer were analyzed using the Sequencing Analysis 5.2 and SeqScape version 2.5 (both Life Technologies, Zug CH) softwares. All genotypes were replicated at least once for independent confirmation.

The demographic characteristics of the participants in the 'SD study' are summarized in Table 1. The Val/Val, Val/Met and Met/Met genotype groups did not differ with respect to age, BMI, habitual alcohol and caffeine consumption, chronotype, subjective daytime sleepiness and anxiety.

Study protocol

After 8-hour adaptation and baseline nights (00:00-8:00), subjects were kept awake for 40 hours, and given a 10-hour sleep opportunity for recovery. During prolonged wakefulness, subjects were constantly supervised and completed questionnaires, cognitive and neurobehavioral tasks, and

waking EEG recordings at regular time intervals. All participants abstained from caffeine during two weeks prior to the start of the experiments, and from alcohol and daytime naps during at least three days before the studies. They were required to spend 8 hours in bed (00:00-8:00) on the three nights before each study block. Compliance with these instructions was verified by actigraphy and sleep-wake diaries, as well as determination of saliva caffeine and breath alcohol levels upon arrival in the laboratory for sleep recordings. All studies were completed in the sleep lab of the Institute of Pharmacology and Toxicology at the University of Zürich.

Psychomotor vigilance test (PVT)

A computerized version of the Psychomotor Vigilance Test (PVT) (e-Prime software, Psychology Software Tools Inc., Pittsburgh, PA, USA) was used to assess sustained vigilant attention (Basner and Dinges, 2011). Each test consisted of 100 trials (lasting approximately 10 min), in which a numeric count-up timer appeared at random intervals on the computer screen. Participants had to press the "space" key as soon as possible upon detection of the stimulus. They completed a training session before the first adaptation night. Lapses of attention (reaction times [RT] > 500 ms), 'errors of commission' (RT < 100 ms) and 'correct' responses (100 ms < RT < 500 ms) were considered. Speed, defined as the reciprocal values of RT, was calculated for correct responses only. Outliers were defined as values below and above 2 standard deviations of the mean, and incomplete sessions (< 100 trials) were excluded from analysis. To assess 'time on task' (TOT) dependent behavior, the task was subdivided into 5 equal quintiles, each containing 20 trials.

Sleep deprivation and tolcapone (SD&T) study

Participants and genotyping

To test causality in the observed *COMT* genotype-phenotype association, 30 participants were enrolled for a separate study ('SD&T study'; see supplementary Fig. S1). The study protocol was approved by the ethics committee of the Canton of Zürich for human research, as well as the Swiss Agency for Therapeutic Products. Written informed consent was obtained from all participants who received financial compensation for their participation. The Phase I Clinical Trial was registered on www.clinicaltrials.gov (identifier: NCT02080715). Data collection was completed between August 2013 and April 2014.

The DNA for genotyping was isolated from 200 µl blood using NucleoSpin® Blood (Macherey Nagel GmbH Co. KG). The Val158Met polymorphism of *COMT* was determined as described above.

The Val/Val, Val/Met and Met/Met genotype groups did not differ with respect to age, BMI, habitual alcohol and caffeine consumption, chronotype, subjective daytime sleepiness and sleep quality, as well as neuropsychiatric personality traits (Table 2).

Study protocol

Study protocol and testing of performance on the PVT during prolonged waking were the same as described above. In addition, mood, tiredness and sleepiness were quantified at 3-hour intervals with the following validated questionnaires: Profile of Mood States (POMS; fatigue, vigor, irritability, depression) (McNair *et al*, 1992); Visual Analogue Scales (VAS; mood, energy, motivation and excitation); Tiredness Symptom Scale (TSS) (Schulz *et al*, 1991) and Karolinska Sleepiness Scale (KSS) (Åkerstedt and Gillberg, 1990). A morning questionnaire was administered shortly after each night to rate subjective sleep quality, including number of awakenings, total duration of time spent awake, subjective sleep latency, sleep intensity and mood upon awakening.

Waking EEG recordings

In each study block, participants also completed at 3-hour intervals 14 wake EEG recordings, the first initiated 15 min after 'lights on' after the baseline nights. Recordings consisted of a 3-min period with eyes closed and a 5-min period with eyes open, while sitting immobile and fixating a dot on the wall. Subjects were alerted through the intercom whenever signs of drowsiness were detected (e.g., reduced EEG alpha activity or rolling eyes). Continuous EEG, bipolar electrooculogram (EOG), electromyogram (EMG), and electrocardiogram (ECG) were recorded with Rembrandt® Datalab (Version 8; Embla Systems, Broomfield, CO, USA) 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; EMG: 10 Hz; ECG: 1 Hz) and an antialiasing low-pass filter (-3 dB at 67.2 Hz), digitized and stored with a resolution of 256 Hz (sampling frequency of 256 Hz). Ten bipolar EEG derivations along the right and left anteroposterior axes were recorded. The data of the C3A2 derivation are reported here; artifacts were visually identified. Power spectra (Fast Fourier Transform, Hanning window) of artifact-free, 50%-overlapping 2-s epochs were computed with MATLAB® (The MathWorks Inc., Natick, MA, USA), resulting in a 0.5 Hz frequency resolution. Frequency bins between 1 and 20 Hz were analyzed. To compare the waking EEG between baseline and after sleep deprivation, mean values recorded at 8:00, 11:00, 14:00 and 17:00 on the second day of prolonged wakefulness were expressed as a percentage of the respective mean values of the baseline day.

Polysomnographic recordings

Continuous recording of EEG, EOG, EMG, and ECG was performed during all nights. Standard sleep stages (Rechtschaffen and Kales, 1968) were visually scored in 20-s epochs (C3A2 derivation) with Rembrandt® Analysis Manager (Version 8; Embla Systems, Broomfield, CO, USA). Movement- and arousal-related artifacts were visually identified and eliminated. EEG spectra (FFT routine, Hanning window, 50% overlapping 4-s epochs, 0.25 Hz resolution) were calculated with MATLAB®, averaged over 5 consecutive 4-s epochs, and matched with the sleep scores. To compute all-night

power spectra in NREM sleep (stages 2, 3 and 4) and REM sleep, all artifact-free 20-s values were averaged. NREM/REM sleep cycles were defined according to Feinberg and Floyd (1979). Data analyses were restricted to the first 8 hours of the recovery nights. Relative EEG power values in the recovery nights were normalized to the average all-night EEG power of the two baseline nights.

Tolcapone

Tolcapone is an orally active, reversible, competitive inhibitor of COMT. Following oral administration, the compound crosses the blood brain barrier and has a specific effect on PFC dopamine neurotransmission (Napolitano *et al*, 1995; Rojo *et al*, 2001). Two doses of 100 mg tolcapone, in the form of capsules, were administered to all subjects after 11 (at 7:00 pm) and 23 hours (at 7:00 am) of prolonged wakefulness according to a randomized, double-blind, placebo-controlled, crossover design. *In vitro* work suggests that at a dose of 100 mg, tolcapone induces maximum inhibition (72 %) of COMT enzymatic activity after approximately 1-1.5 hours, while activity returns to baseline levels after roughly 13 hours (Dingemanse *et al*, 1995). These pharmaco-dynamic properties of tolcapone permitted us to address the question whether COMT inhibition during prolonged wakefulness affects the sleep EEG independently of an acute pharmacological effect. Capsules and random allocation sequence were produced by the 'Kantonsapotheke Zürich' by homogenizing commercial Tasmar® Tolcaponum 100 mg tablets (distribution: MEDA Pharma GmbH, Wangen-Brüttisellen, Switzerland) with mannitol (Siegfried Ltd., Zofingen, Switzerland); placebo capsules of identical appearance contained only mannitol. Verum and placebo were administered one week apart to two randomization groups, containing 5 individuals of each *COMT* genotypes and differing by the sequence of drug administration.

Due to the potential hepatotoxicity of tolcapone, liver functions were assessed before and after the experiment. They were required to lay within normal range for study participation (alanine transaminase: < 82 U/l; aspartate transaminase: < 76 U/l).

Statistical analyses

Performance on the PVT was assessed with SAS 9.2 software (SAS Institute, Cary, North Carolina) similarly to previous studies (Holst *et al*, 2017). Linear mixed models for repeated measures with the random effect *'subject'*, the between-subjects factor '*genotype*' (Val/Val, Val/Met, Met/Met), and the within-subjects factors '*day*' (baseline, sleep deprivation), '*time'* (14 sessions), *'time on task'* (quintiles 1-5), *'treatment'* (placebo, tolcapone), *'condition'* (baseline night, placebo recovery night, tolcapone recovery night), and *'NREM sleep episode'* (1-4) were conducted as specified in text and Figure legends. If not stated otherwise, estimated means and standard errors of corresponding mixed models are illustrated. Numbers of lapses were defined as percentage within each quintile ('lapse frequency') and absolute EEG power densities were log-transformed before statistical analyses, to approximate a normal distribution. Effect sizes (partial eta squared: η_P^2) were calculated from corresponding ANOVA F-values and degrees of freedom. Effect sizes of 0.01, 0.059 and 0.138 are considered small, moderate and large (Cohen, 2013; Richardson, 2011). Significance level was set at $\alpha < 0.05$. Bonferroni-corrected post-hoc tests to correct for multiple comparisons are reported where appropriate.

Results

COMT genotype contributes to individual differences in attentional lapses after sleep deprivation

Prolonged wakefulness and time on task increased the number of lapses on the PVT, reflecting well-established homeostatic and circadian influences (*'time'*: $F_{5,3720} = 12.00$, $p < 0.0001$, $\eta_P^2 = 0.016$; *'day'*: $F_{1,3720} = 500.9$ $p < 0.0001$, $\eta_P^2 = 0.119$; *'time on task'*: $F_{4,3720} = 22.0$, $p < 0.0001$, $\eta_P^2 = 0.023$) (Fig. 1A). Moreover, *COMT* genotype modulated the impairment in PVT performance over time (*'genotype'* x *'time'* interaction: $F_{10,3720} = 2.47$, $p < 0.006$, $\eta_P^2 = 0.007$). To better illustrate this main finding of the 'SD study', Fig. 1B depicts mean lapse frequency on the days before and after

sleep deprivation. While all genotypes performed equally well without virtually any lapses in baseline, they clearly differed on the second day (*'genotype'* x *'day'* interaction: $F_{2,3720} = 19.16$, $p < 0.0001$, $\eta_P^2 = 0.010$). Specifically, Val/Met heterozygotes produced almost the double number of lapses (11.8 ± 1.0, n = 35) on the day after sleep loss when compared to Met/Met homozygotes (6.2 ± 1.3, n = 21) (Fig. 1B). A similar yet less striking modulation by *COMT* genotype was observed in the placebo condition in the 30 independent participants of the 'SD&T study' (*'genotype'* x *'day'* interaction: $F_{2,1524} = 6.0$, $p < 0.003$, $\eta_P^2 = 0.008$) (see Fig. 3B).

COMT genotype and COMT inhibition have no impact on neurophysiological and behavioral markers of elevated sleep pressure

To tackle the question whether the build-up of homeostatically regulated sleep pressure is modulated by *COMT* genotype and selective pharmacological inhibition of COMT enzymatic activity, detailed quantitative analyses of the EEG in wakefulness and sleep were performed. On the baseline day, absolute spectral power between 1 and 20 Hz averaged over all waking EEG recordings from 8:00 to 17:00 was similar in all genotypes *('genotype'*: $F_{2,25} < 3.8$, $p_{all} > 0.06$). Sleep deprivation increased EEG activity in all bins below 9 Hz and above 11.5 Hz ('*condition*': $F_{1,72} > 3.89$, $p_{all} < 0.05$, $\eta_P^2 = 0.051$). Fig. 2a illustrates the evolution of delta-theta activity (1-9 Hz band) across prolonged wakefulness in Val/Val, Val/Met and Met/Met genotypes of *COMT*. In all genotypes, the evolution of EEG activity reflected similar homeostatic and circadian influences (*'time'*: $F_{13,261} = 20.3$, $p < 0.0001$, $\eta_P^2 = 0.502$*; 'genotype'*: $F_{2,26} = 2.49$, $p > 0.1$), which was indistinguishable between tolcapone and placebo (*treatment'*: $F_{1,71} = 0.18$, $p > 0.6$; *'genotype'* x *'treatment'* interaction: $F_{2,71} = 0.69$, $p > 0.5$).

Compared to the baseline night, sleep deprivation increased EEG SWA, slow wave sleep, total sleep time and sleep efficiency in the recovery night irrespectively of *COMT* genotype and tolcapone treatment (Fig. 2B and supplementary Table S1). Absolute SWA values, the dissipation of SWA across consecutive NREM sleep episodes in baseline and recovery nights, as well as the relative

increase in SWA after sleep deprivation were similar among genotypes and treatment conditions. More specifically, 3-way mixed-model ANOVA with the factors *'genotype*', '*condition*' and *'NREM sleep episode'* yielded significant main effects and interaction of *'condition*' ($F_{2,295}$ = 84.3, p < 0.0001, η_P^2 = 0.363) and *'episode'* ($F_{3,293}$ = 488.7, p < 0.0001, η_P^2 = 0.833; '*condition'* x *'episode*' interaction: $F_{6,293}$ = 11.8, p < 0.0001, η_P^2 = 0.195), yet no effects involving '*genotype*'. Furthermore, no significant main effects of *'genotype'* or *'treatment'*, or interactions with the factor *'condition'* were observed in visually scored sleep variables and subjective ratings of sleep quality (supplementary Table S1).

Sleep deprivation also increased sleepiness, fatigue, tiredness and irritability, and reduced response speed on the PVT, vigor, energy, motivation, and mood. These typical effects of prolonged wakefulness were not modulated by *COMT* genotype nor tolcapone treatment (supplementary Fig. S2).

Tolcapone enhances attentional lapses after sleep deprivation depending on COMT genotype

Although they were devoid of effects on neurophysiological and state markers of elevated sleepiness after sleep loss, both *COMT* genotype and tolcapone affected attentional lapses after sleep deprivation. Prolonged wakefulness and time-on-task enhanced lapsing also in the 'SD&T study' (*'time'*: $F_{5,2937}$ = 12.7, p < 0.0001, η_P^2 = 0.021; *'day'*: $F_{1,2937}$ = 768.0, p < 0.0001, η_P^2 = 0.207; *'time on task'*: $F_{4,2937}$ = 11.1, p < 0.0001, η_P^2 = 0.015). Unexpectedly, however, rather than improving performance, tolcapone further increased lapse frequency on the day following sleep loss when compared to placebo (20.3 ± 1.4 *vs.* 15.8 ± 1.4; *'treatment'*: $F_{1,2937}$ = 27.8, p < 0.0001, η_P^2 = 0.009; '*treatment' x 'day'* interaction: $F_{1,2937}$ = 7.1, p = 0.009, η_P^2 = 0.002; Fig. 3A). When analyzing the data on a finer time scale, a significant *'genotype' x 'treatment' x 'time'* interaction was observed ($F_{10,2937}$ = 3.2, p < 0.0005, η_P^2 = 0.011). More specifically, intake of tolcapone at 7 am after the night without sleep significantly deteriorated performance in Val/Met and Met/Met genotypes when compared to placebo (p < 0.05; Fig. 3B). The difference between the treatment conditions was insignificant in the

Val/Val genotype. By contrast, in Val/Met heterozygotes, performance between tolcapone and placebo still differed at 5 pm, 10 hours after the second tolcapone ingestion.

Discussion

Because tolcapone appears to be free of major psychostimulant properties and abuse potential (Apud *et al*, 2007), the pharmacodynamic profile of this COMT inhibitor may be beneficial to improve vigilance in sleepy patients and healthy individuals undergoing sleep deprivation. Indeed, previous research consistently concluded that this compound may improve distinct aspects of cognitive functions in Parkinson's patients (Gasparini *et al*, 1997), healthy volunteers (Apud *et al*, 2007; Farrell *et al*, 2012; Giakoumaki *et al*, 2008), as well as rats and mice (Barkus *et al*, 2016; Risbrough *et al*, 2014; Tomlinson *et al*, 2015). While we expected that especially individuals with high COMT activity (Val/Val genotype) may benefit from pharmacological COMT inhibition, we found that tolcapone does not improve sustained attention after prolonged wakefulness in any *COMT* genotype. On the contrary, it increased attentional lapses in Val/Met and Met/Met allele carriers.

Sustaining focused attention while performing a 10-min PVT is a challenging task, especially when sleep deprived. Increased lapsing compared to the rested state has been assumed to reflect transient cognitive disruptions referred to as "wake-state instability" (Lim and Dinges, 2008). The precise neural bases of how acute total sleep loss impairs sustained attention are currently unknown (Ma *et al*, 2015). Nevertheless, a core brain network comprising dorsomedial, mid- and ventrolateral prefrontal cortex, anterior insula, parietal areas, as well as subcortical structures including cerebellar vermis, thalamus, basal ganglia, and midbrain subserves vigilant attention (Langner and Eickhoff, 2013). The midbrain is the origin of three major dopaminergic pathways (Haber, 2014). The *nigrostriatal pathway* projects from the substantia nigra to the striatum (caudate/putamen) and plays an important role in motor control. The *mesolimbic pathway* is associated with the reward circuit. This pathway originates in the ventral tegmental area (VTA), connects to several structures of

the limbic system, and is important for memory, reward and motivation behaviors. Similarly, the *mesocortical pathway* also originates in VTA, but projects to the frontal cortex and surrounding structures. Given that dopaminergic neurotransmission in the mesocortical pathway is primarily controlled by COMT (Käenmäki *et al*, 2010; Sesack, 2014), the present genetic and pharmacogenetic data suggest that the mesocortical dopaminergic system importantly contributes to the regulation of lapses in sustained attention after sleep deprivation. Nevertheless, it needs to be kept in mind that vigilant attention during sleep deprivation is regulated by several neurotransmitters, and yet unknown factors and their interactions could influence the current findings. For example, previous reports have shown that benefits of the cholinesterase inhibitor, donepezil, were most pronounced in study participants whose vigilance clearly declined in the placebo condition when sleep deprived, whereas those participants who maintained vigilance when receiving placebo worsened under donezepil (Chuah and Chee, 2008; Chuah *et al*, 2009). Apart from increasing cholinergic neurotransmission, donepezil secondarily also influences dopaminergic and noradrenergic signaling. In addition, the interpretation of the results should consider that the COMT enzyme, which is present in the brain and the periphery, not only metabolizes dopamine but also various other compounds including the dopamine derivatives norepinephrine and epinephrine.

Sleep deprivation and tolcapone modulate attentional lapses according to an inverse U-shaped relationship

It is well established that genetic variation of *COMT* modulates body and brain functions according to U-shape relationships (e.g., Mattay *et al*, 2003; Apud *et al*, 2007; Giakoumaki *et al*, 2008; Sesack, 2014). Corroborating the view that dopaminergic agonists can correct behavioral consequences of suboptimal PFC dopaminergic signaling, metamphetamine and tolcapone reliably improved different aspects of memory, executive functioning, and risk taking in non-sleep-deprived

Val/Val allele carriers, but were ineffective or even impaired performance in Met/Met individuals (Apud *et al*, 2007; Farrell *et al*, 2012; Giakoumaki *et al*, 2008; Mattay *et al*, 2003). The present study demonstrates that beyond and above *COMT* genotpye, a pre-existing sleep debt strongly impacts on the effects of a pharmacological increase of dopaminergic neurotransmission on brain functions. This conclusion is consistent with a previous study, showing that a low dose of modafinil maintained optimal sustained attention, executive functioning and subjective well-being for as long as two days and one night of prolonged wakefulness in Val/Val homozygotes, yet was ineffective in matched Met/Met allele carriers (Bodenmann *et al*, 2009). The current work extends these findings and suggests that similar to other PFC dependent processes such as working memory and risky decision making (Farrell *et al*, 2012; Goldman-Rakic *et al*, 2000; Tunbridge *et al*, 2006), there is an inverted U-shape relationship between dopamine activity in PFC and attentional lapses, and this relationship is modulated by sleep loss.

As schematically illustrated in Fig. 4, the data suggest that lapse frequency on a 10-min PVT is optimal in well-rested study participants and indistinguishable among Val/Val, Val/Met and Met/Met genotypes of *COMT* (also see Bodenmann *et al*, 2009; Goel *et al*, 2011). Wakefulness increases dopamine concentrations in periphery and central nervous system, including nucleus accumbens and medial PFC, in rats (Andersen *et al*, 2005; Léna *et al*, 2005), as well as human plasma (McMorris *et al*, 2006). Thus, sleep deprivation can be assumed to increase PFC dopamine to super-optimal levels. Due to the genotype-dependent differences in COMT enzymatic activity and associated differences in baseline dopaminergic neurotransmission, the relative increase in dopaminergic tone after prolonged waking may be most pronounced in Val/Val, intermediate in Val/Met, and weakest in Met/Met genotype, leading to the observed differences in PVT lapses after sleep loss. Instead of mitigating the consequences of sleep loss, tolcapone treatment induced even more pronounced impairment. Future studies are warranted to probe the dose-effect relationships of the interaction between total and partial sleep deprivation and tolcapone, to test whether there is an additive interaction of sleep loss and inhibition of COMT. This work will have considerable

translational relevance because COMT inhibitors are used as adjunctive treatment in Parkinson's disease and have been considered for patients and people with impaired vigilance due to insufficient sleep.

Tolcapone and sleep-wake regulation

While it is well established that serotonin, histamine and norepinephrine importantly contribute to sleep-wake regulation (for recent review, see Holst *et al*, 2016), the exact roles of dopamine in regulating sleep remain incompletely understood. Recent studies in flies, mice and humans accumulated convergent evidence that mesolimbic dopaminergic pathways importantly contribute to the maintenance of wakefulness, the homeostatic regulation of sleep, arousal, as well as sleep-wake dependent behaviors (Andretic *et al*, 2008; Eban-Rothschild *et al*, 2016; Holst *et al*, 2014, 2017; Oishi *et al*, 2017; Ueno *et al*, 2012; Valomon *et al*, 2014; Volkow *et al*, 2012; Wisor *et al*, 2001). By contrast, the available studies on the impact of the mesocortical pathway and COMT activity on sleep-wake regulation are inconclusive. For example, research in large samples of individuals studied under field conditions with questionnaires, actigraphy and waking EEG revealed no consistent influence on daytime sleepiness and physiological markers of circadian and homeostatic sleep-wake regulation (Dauvilliers *et al*, 2001; Jawinski *et al*, 2016; Valomon *et al*, 2014). Nevertheless, a laboratory study employing a chronic partial sleep restriction protocol suggested that Met/Met homozygotes of the Val158Met polymorphism of *COMT* exhibit a steeper decline in EEG slow-wave energy during sleep than Val/Met and Val/Val allele carriers (Goel *et al*, 2011). This observation was interpreted to reflect a differential homeostatic response to sleep loss among different *COMT* genotypes. However, another well-controlled study found no differences between Val/Val and Met/Met homozygotes in established sleep and EEG markers of sleep homeostasis in baseline and after acute total sleep deprivation (Bodenmann *et al*, 2009; Bodenmann and Landolt, 2010). The current investigation confirmed that functional genetic variation and

selective pharmacological inhibition of COMT enzymatic activity do not affect established waking and sleep EEG markers of sleep homeostasis when challenged by acute total sleep deprivation. The data, thus, indicate that the mesocortical dopaminergic system has no major role in regulating sleep homeostasis. Supporting this conclusion, the monoaminergic wake-promoting agent, modafinil, and the irreversible monoamine oxidase inhibitor, phenelzine, do not alter the dynamics of EEG SWA in NREM sleep (Bodenmann and Landolt, 2010; Holst *et al*, 2014; Landolt *et al*, 2001). The dissociation between sleep-wake dependent changes in neurobehavioral performance, subjective sleepiness and neurophysiologic markers of sleep pressure has been highlighted in various previous publications (e.g., Leproult *et al*, 2003; Galliaud *et al*, 2008; Lim and Dinges, 2008; Holst *et al*, 2017), suggesting that these markers of elevated sleep drive are regulated by different underlying mechanisms.

Main conclusions

In summary, the current results demonstrate that PFC dopaminergic neurotransmission contributes to the regulation of attentional lapses during sleep deprivation without changing neurophysiological markers of sleep homeostasis. Intriguingly, selective inhibition of COMT can even reverse the expected beneficial effects of pharmacologically enhanced dopaminergic tone after sleep deprivation. This finding has important implications, given the increased use of stimulants in healthy people aiming at improved vigilance and cognitive functions (Farah, 2015; Smith and Farah, 2011). Nevertheless, the data presented here were obtained in healthy young men and the results of this study may not be generalized to women and older individuals. Future pharmacogenetics studies are warranted to disentangle the complex roles of the dopaminergic system in regulating human sleep-wake behavior and physiology.

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Table 1. Demographic characteristics of participants in 'Sleep Deprivation' (SD) study.

	Val/Val	Val/Met	Met/Met	$F_{2,70}$	p
Sample size	17	35	21		
Age (years)	24.6 ± 4.0	23.8 ± 3.1	23.5 ± 2.3	0.68	0.58
Body mass index (kg/m^2)	22.5 ± 2.2	22.3 ± 1.6	22.6 ± 2.2	0.18	0.83
Alcohol consumption (drinks/week)	2.4 ± 2.6	3.6 ± 2.8	3.3 ± 3.5	0.87	0.42
Caffeine consumption (mg/day)	147 ± 186	121 ± 119	148 ± 109	0.35	0.7
Epworth Sleepiness Scale	7.9 ± 2.6	6.5 ± 2.5	6.3 ± 2.5	1.7	0.19
Munich ChronoType Questionnaire	4.3 ± 1.0	4.3 ± 0.9	4.2 ± 0.9	0.18	0.83
Trait Anxiety	36.1 ± 9.6	33.9 ± 7.8	34.3 ± 6.8	0.46	0.63

F- and p-values from ANOVA with factor *'genotype'*. Estimates of caffeine consumption were based on the following average caffeine content per serving: coffee, 100 mg; energy drink (2 dl), 80 mg; cola drink (2 dl), 40 mg; black or green tea, 30 mg; chocolate (100 g), 50 mg [same as: (Valomon *et al*, 2014)]. Higher Epworth Sleepiness Scale scores (Johns, 1991) indicate higher daytime sleepiness. Munich ChronoType Questionnaire score (Wirz-Justice *et al*, 2003) indicates the mid-sleep time on leisure days, including an estimated correction for the sleep debt accumulated during the work week, age and sex. Trait Anxiety Inventory (Spielberger *et al*, 1970). Means ± SD.

Table 2. Demographic characteristics of participants in 'Sleep Deprivation and Tolcapone' (SD&T) study.

	Val/Val	Val/Met	Met/Met	$F_{2,27}$	p
Sample size	10	10	10		
Age (years)	23.7 ± 2.4	23.3 ± 1.6	23.3 ± 2.0	0.11	0.89
Body mass index (kg/m^2)	21.9 ± 1.4	22.4 ± 1.4	22.9 ± 1.4	1.16	0.33
Alcohol consumption (drinks/week)	1.8 ± 1.6	2.9 ± 2.8	2.7 ± 2.0	0.71	0.50
Caffeine consumption (mg/day)	110 ± 112	66 ± 47	134 ± 106	1.38	0.23
Epwort Sleepiness Scale	6.1 ± 3.5	5.8 ± 3.2	7.1 ± 3.3	0.42	0.66
Pittsburgh Sleep Quality Index	3.6 ± 2.0[1]	3.4 ± 1.7	4.7 ± 1.6	1.57	0.23
Munich ChronoType Questionnaire	4.0 ± 0.9	4.2 ± 1.2	4.4 ± 1.2	0.38	0.69
Trait Anxiety	32.9 ± 9.2	32.8 ± 7.6	33.1 ± 8.6	0	1
Beck Depression Inventory (BDI-II)	5.7 ± 4.7	4.1 ± 3.7	3.9 ± 4.7	0.5	0.61
Extraversion (EPQ)	6.5 ± 3.6	8.6 ± 2.7	7.8 ± 3.4	1.07	0.36
Neuroticism (EPQ)	2.8 ± 2.6	1.9 ± 1.9	3.2 ± 2.4	0.82	0.45
Psychoticism (EPQ)	3.5 ± 2.3	4.3 ± 2.6	3.0 ± 2.3	0.76	0.48

F- and p-values from ANOVA with factor *'genotype'*. Estimates of caffeine consumption were based on the following average caffeine content per serving: coffee, 100 mg; energy drink (2 dl), 80 mg; cola drink (2 dl), 40 mg; black or green tea, 30 mg; chocolate (100 g), 50 mg [same as: (Valomon *et al*, 2014)]. Higher Pittsburgh Sleep Quality Index scores (Buysse *et al*, 1989) indicate lower sleep quality. Higher Epworth Sleepiness Scale scores (Johns, 1991) indicate higher daytime sleepiness. Munich ChronoType Questionnaire score (Wirz-Justice *et al*, 2003) indicates the mid-sleep time on leisure days, including an estimated correction for the sleep debt accumulated during the work week, age and sex. Trait Anxiety Inventory (Spielberger *et al*, 1970). EPQ, Eysenck Personality Questionnaire, revised short German version (Ruch, 1999). Beck Depression Inventory (BDI-II). Means ± SD.

[1] For the PSQI, one study participant missing.

Figure Legends

Figure 1. Lapses of attention (i.e., trials on a 10-min PVT with reaction times > 500 ms, expressed as a percentage of all trials in a quintile) across 40 hours of sustained wakefulness, split by *COMT* genotypes. (**A**) PVT lapses (y-axis) as a function of time on task (z-axis) and time awake (x-axis). The warmer the colors, the higher the lapse frequency. The 3D plots are split by *COMT* genotypes (left panel: Val/Val; middle panel: Val/Met; right panel: Met/Met). (**B**) Comparison of lapse frequencies in baseline (day 1) and after sleep deprivation (day 2), split by *COMT* genotypes (Val/Val: red line; Val/Met: black line; Met/Met: blue line). Values represent estimated means ± standard error; p-value ($p < 0.0006$) withstands Bonferroni correction ($p < 0.016$).

Figure 2. The homeostatic build-up in sleep pressure is indistinguishable among *COMT* genotypes and not affected by tolcapone. (**A**) Evolution of 1-9 Hz activity in the waking EEG across 40 hours prolonged wakefulness. EEG activity in placebo (open circles) and tolcapone (filled circles) conditions was expressed as a percentage of the mean values at 08:00, 11:00, 14:00, and 17:00 on the baseline day (dashed horizontal line), split by *COMT* genotypes (Val/Val: red line; Val/Met: black line; Met/Met: blue line). Administration of tolcapone at 7:00 pm and 7:00 am is indicated with capsule and vertical dashed lines. Three-way mixed-model ANOVA with the factors *'genotype', 'treatment*' and '*time*' yielded significant effects of '*time*', yet no significant effects and interactions with '*genotype*' or '*treatment*' (see main text). (**B**) Dynamics of mean SWA across NREM sleep episodes 1-4 in baseline (mean of two baseline nights) and recovery nights. EEG activity (means ± SEM) in placebo (open circles) and tolcapone conditions (filled circles) was expressed as a percentage of the mean all-night values in baseline (dashed horizontal line), split by *COMT* genotypes (Val/Val: red line; Val/Met: black line; Met/Met: blue line). Three-way mixed-model ANOVA with the factors '*genotype*', '*condition*' and '*NREM sleep episodes'* yielded significant effects of *'episode'*, *'condition*' and '*cycle' x 'condition*', yet no significant interactions involving '*genotype*' (see main text). Values represent estimated means ± standard error.

* $p < 0.05$ (tolcapone recovery *vs.* mean baseline; paired, 2-tailed *t*-tests)

$^{+}$ $p < 0.05$ (placebo recovery *vs.* mean baseline; paired, 2-tailed *t*-tests).

Figure 3. The increase in attentional lapses on a 10-min PVT after sleep deprivation is exacerbated by the COMT inhibitor tolcapone and modulated by *COMT* genotype. (**A**) Average lapse frequencies after sleep deprivation (day 2) in placebo (white) and tolcapone (black) conditions. Bars represent means ± SEM (n = 30). (**B**) Evolution of lapse frequency on the PVT, split by treatment (placebo: open circles; tolcapone: filled circles) and genotype (Val/Val: red; Val/Met: black; Met/Met: blue). Estimated means ± standard error (n = 10 per group). Tolcapone administration is indicated with vertical lines.

* $p < 0.0004$ (tolcapone vs. placebo; withstanding Bonferroni correction)

Figure 4. Schematic illustration of the proposed relationships between PFC dopamine transmission and attentional lapses in baseline (green shading), after sleep deprivation (orange shading), and after sleep deprivation and tolcapone administration (yellow shading), split by *COMT* genotypes (Val/Val: red; Val/Met: black; Met/Met: blue). The data suggest an inverse U-shape relationship between PFC dopamine transmission and lapse frequency. While the increase in PFC dopamine transmission with sleep deprivation may be relatively more pronounced in Val/Val than in Val/Met and Met/Met allele carriers, tolcapone reduces COMT activity to roughly the same level in all three genotypes (Apud *et al*, 2007). Asterisks indicate impacts of genotype and tolcapone treatment after sleep deprivation. SD = 'sleep deprivation study'; SD&T = 'sleep deprivation and tolcapone study'. See Discussion for details.

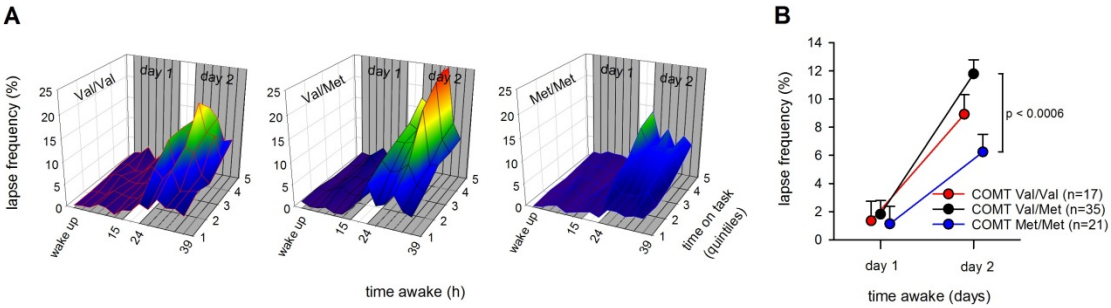
A
lapse frequency (%)
Val/Val
Val/Met
Met/Met
day 1
day 2
wake up
15
24
39
time on task (quintiles)
time awake (h)
B
lapse frequency (%)
p < 0.0006
COMT Val/Val (n=17)
COMT Val/Met (n=35)
COMT Met/Met (n=21)
day 1
day 2
time awake (days)

Figure 1

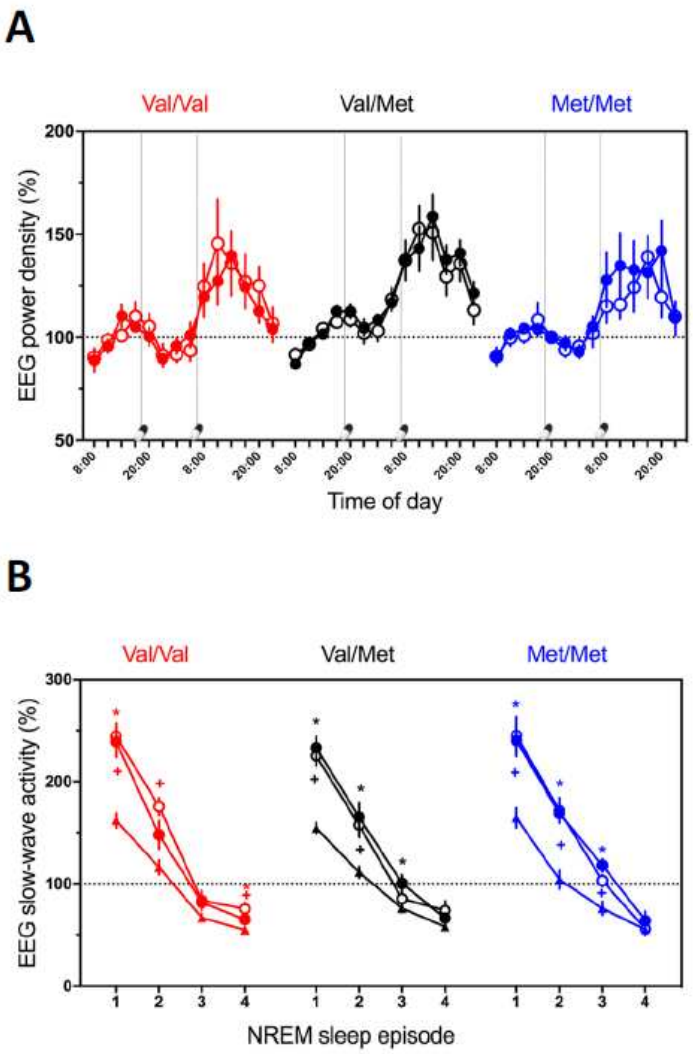
A
Val/Val
Val/Met
Met/Met
EEG power density (%)
200
150
100
50
8:00
20:00
Time of day
B
Val/Val
Val/Met
Met/Met
EEG slow-wave activity (%)
300
200
100
0
1
2
3
4
NREM sleep episode

Figure 2

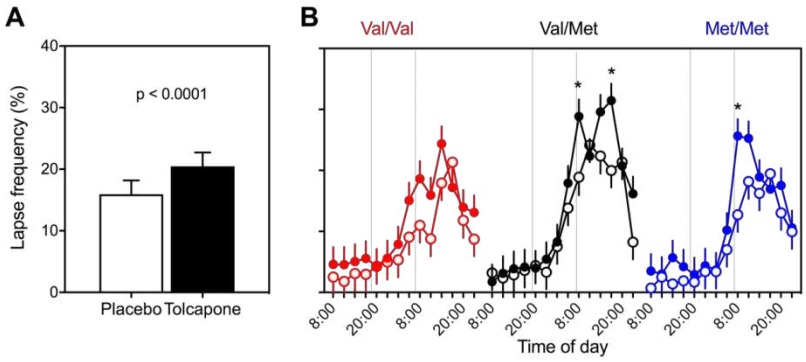
A
B
Lapse frequency (%)
40
30
20
10
0
p < 0.0001
Placebo
Tolcapone
Val/Val
Val/Met
Met/Met
*
*
*
8:00
20:00
Time of day

Figure 3

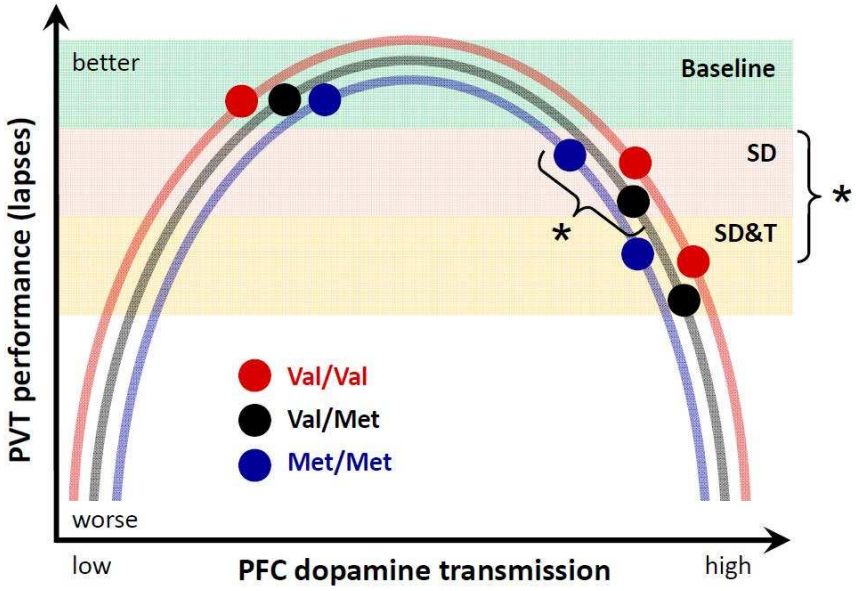
better
Baseline
SD
SD&T
*
*
PVT performance (lapses)
Val/Val
Val/Met
Met/Met
worse
low
PFC dopamine transmission
high

Figure 4

EFFECTS OF *COMT* GENOTYPE AND TOLCAPONE ON LAPSES OF SUSTAINED ATTENTION AFTER SLEEP DEPRIVATION IN HEALTHY YOUNG MEN

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Supplementary Information

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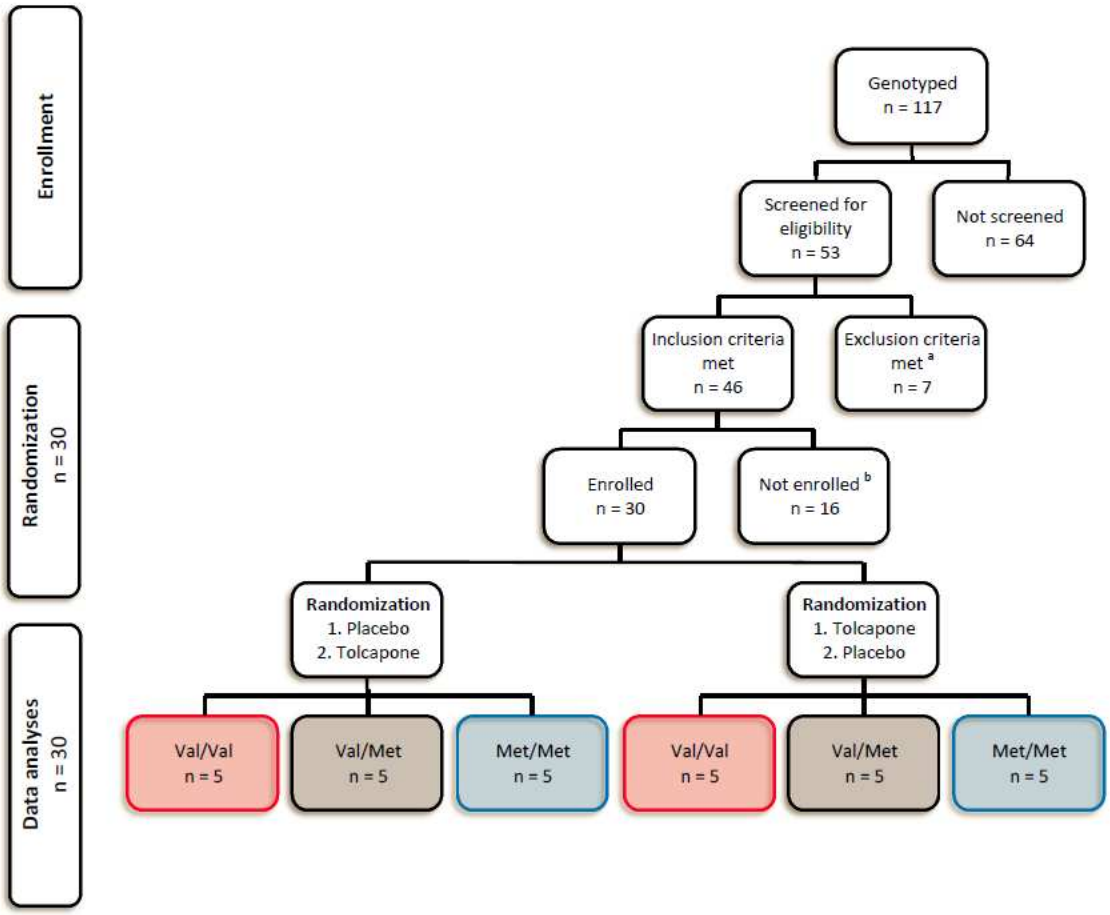
Supplementary Table S1: Tolcapone has no effect on polysomnographic and subjective markers of sleep quality in baseline and recovery nights.

	Placebo condition		**Tolcapone condition**		**Factor *'condition'***	
	Baseline	Recovery	Baseline	Recovery	$F_{1,79}$	p
Stage 1 (min)	30.1 ± 10.9	14.6 ± 8.0	30.3 ± 16.2	15.4 ± 8.4	131.2	< 0.0001
Stage 2 (min)	219.7 ± 29.2	207.7 ± 31.6	221.5 ± 29.2	202.6 ± 32.3	17.0	< 0.0001
Slow wave sleep (min)	89.8 ± 23.0	141.5 ± 35.9	89.4 ± 25.1	138.6 ± 23.6	367.5	< 0.0001
NREM sleep (min)	309.5 ± 23.7	349.2 ± 28.1	310.9 ± 24.4	341.1 ± 26.2	122.1	< 0.0001
REM sleep (min)	110.8 ± 22.9	104.7 ± 24.7	110.3 ± 21.2	112.5 ± 23.9	1.5	0.22
WASO (min)	7.4 ± 10.1	1.6 ± 2.3	8.3 ± 13.1	1.3 ± 2.2	27.2	< 0.0001
Total sleep time (min)	450.4 ± 16.5	468.6 ± 6.2	451.5 ± 20.2	469.0 ± 5.4	41.4	< 0.0001
Sleep episode (min)	462.7 ± 14.9	475.7 ± 5.4	463.7 ± 15.9	475.7 ± 3.6	20.6	< 0.0001
Sleep efficiency (%)	93.9 ± 3.4	97.6 ± 1.3	94.1 ± 4.2	97.7 ± 1.1	61.2	< 0.0001
Sleep latency (min)	15.3 ± 10.4	4.3 ± 5.4	16.2 ± 16.0	4.3 ± 3.6	42.4	< 0.0001
REM sleep latency (min)	75.7 ± 24.7	72.1 ± 29.2	68.4 ± 18.7	71.2 ± 25.7	0.0	0.85
EEG SWA (μV^2)	131.1 ± 8.1	164.9 ± 10.9	128.0 ± 8.2	168.7 ± 11.1	189.4	< 0.0001
Subjective sleep quality						
Sleep latency (min)	16.1 ± 9.2	6.4 ± 4.7	17.0 ± 9.3	8.5 ± 9.0	43.5	< 0.0001
Number of awakenings	2.2 ± 2.2	0.8 ± 0.7	2.1 ± 1.4	1.2 ± 1.0	22.3	< 0.0001
Time spent awake (min)	19.9 ± 26.1	7.1 ± 11.8	16.1 ± 25.4	4.8 ± 6.2	14.8	0.0002
Sleep intensity (VAS)	50.0 ± 16.3	73.5 ± 12.9	47.8 ± 17.8	66.0 ± 17.8	67.9	< 0.0001
Mood state (VAS)	53.1 ± 14.6	55.1 ± 13.1	50.3 ± 11.4	51.7 ± 14.8	1.2	0.27

Values represent means ± SD. Visually scored sleep variables ('polysomnography') are derived from the first 480 minutes from lights-off, in 10 individuals of each genotype groups (except for n = 9 in Val/Val genotype in recovery nights due to artefacts in recordings). Stages 1 and s2 = NREM sleep stages 1 and 2; Slow wave sleep = combined NREM sleep stages 3 and 4; WASO = wakefulness after sleep onset; TST = total sleep time; Sleep episode = time after sleep onset until final awakening; Sleep efficiency = percentage of TST per 480 min; Sleep latency = time from lights-off to first occurrence of stage 2; REM sleep latency = time from sleep onset to first occurrence of REM sleep; EEG SWA = slow-wave activity, spectral power in 0.5-4.5 Hz range. Subjective sleep quality was assessed with 100-mm visual analogue scales (VAS) administered upon awakening. Subjectively perceived sleep intensity was estimated from two questions about how calm and deep sleep was, and subjective mood state represents an average score of five ratings about feeling recovered, happy, energetic, calm, and focused.

F- and p-values: mixed-model ANOVA with the factors *'condition'*, *'COMT genotype'*, and *'treatment'*. No significant main effects and interactions with factors *'COMT genotype'* and *'treatment'* were found.

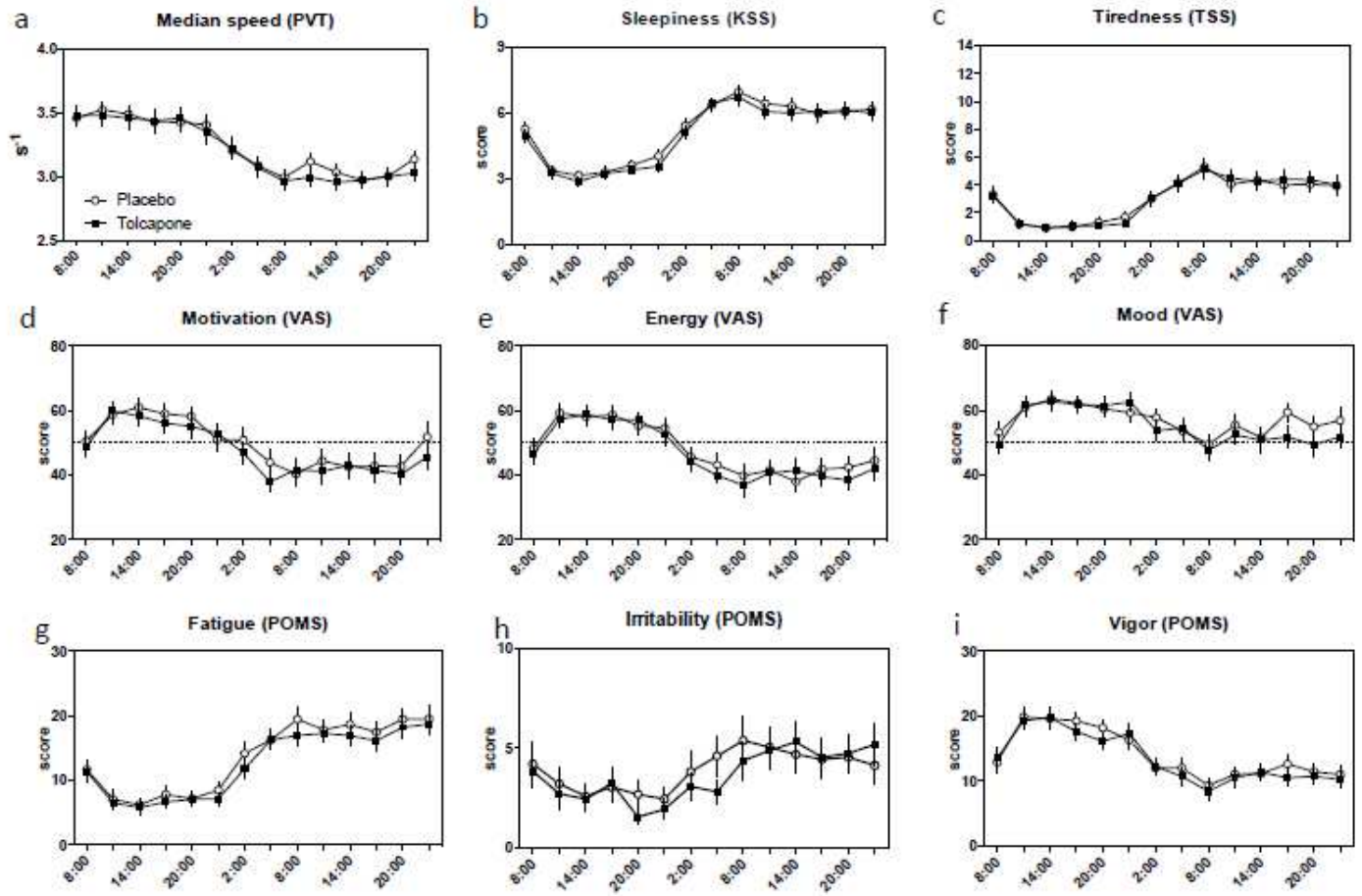
Supplementary Figure S1. Flow chart for enrollment of study participants and randomization into drug order groups.



[a] Reasons for exclusion after screening: < 80 % sleep efficiency in screening night, n = 6; positive drug screen, n = 1.

[b] Reasons for no enrollment despite successful screening: scheduled study dates were not possible due to work obligations or illness, loss of contact with potential study participants, objective of 10 participants per genotype was reached.

Supplementary Figure S2. Evolution of median PVT response speed and subjective state during prolonged waking.



Data represent means ± SEM in 30 study participants, split by treatment (placebo: white symbols; tolcapone: black symbols). Mixed-model ANOVA indicated highly significant effects of *'time'* ($p_{all} < 0.0001$), yet no significant main effects or interactions of the factors *'COMT genotype'* and *'treatment'*.

The following task and questionnaires were employed: (a) median speed on the psychomotor vigilance task (PVT); (b) (a) Karolinska Sleepiness Scale (KSS; range 0-9); (c) Tiredness Symptom Scale (TSS; range 0-14); (d-f) Visual Analogue Scales (VAS; ranges 0-100); (g-i) Profile of Mood States (POMS; ranges 0-42).