

Chapter 6

Disturbed intracortical excitability is a stable trait of chronic insomnia

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ABSTRACT

Chronic insomnia is a poorly understood disorder. Risk factors for developing chronic insomnia are largely unknown, yet disturbances in arousal seem to accompany the disorder. We here investigate whether insomnia patients and control participants differ with respect to one operationalization of arousal, i.e. cortical excitability. A method to directly investigate the excitability level of the human cerebral cortex is offered by transcranial magnetic stimulation (TMS). We used single- and double-pulse TMS to investigate cortical excitability in 16 insomnia patients and 14 carefully matched control participants. Non-pharmacological insomnia patients showed, first, an exaggerated *absolute* response to both suprathreshold single- and double-pulse stimulation compared to control participants; secondly, a reduced *relative* response to double-pulse stimulation at long inter-pulse intervals. The abnormal excitability persisted despite sleep therapy that effectively improved sleep quality as well as behavioral and neuroimaging indices of brain function. The results suggest that a subtly disturbed intracortical excitability characterizes patients with chronic insomnia: a relatively reduced intracortical facilitation in the context of a globally increased absolute excitability. The findings do not resemble TMS-findings after sleep deprivation or in sleep apnea and thus seem specific to insomnia. They may offer diagnostic value and implications for assessment of risk to develop this common and disabling disorder.

INTRODUCTION

Chronic insomnia is a disorder with profound impact on well-being, social and cognitive functioning¹. Despite its profundity, differences between insomnia patients and normal sleepers have been notoriously hard to establish, e.g. at the level of their scalp EEG^{2,3} and in their cognitive skills⁴. More recently, however, we and others have shown differences between the brains of insomnia patients and controls without sleep complaints both at the structural and functional level⁵⁻⁹. Such investigations suggest a dysregulation of arousal systems in the brain of patients with chronic insomnia⁸. Yet, performance differences on cognitive tasks are subtle and sometimes revealed only when modulating task demands, such as an accelerated response to unambiguous, simple, stimuli but a slowed response to more complex stimuli¹⁰. Our findings therefore indicate that the disturbance of arousal in the brain of insomnia patients may result in different effects, dependent on the nature of the task and the measure obtained.

Transcranial magnetic stimulation (TMS) offers the possibility of assessing degrees of arousal in the human brain, in particular the excitability of the cerebral cortex. The technique consists of delivering short-lived pulses of a strong magnetic field over the scalp of a subject, inducing local electrical currents in the brain through electromagnetic induction¹¹. The response to the induced brain activation depends on the region targeted; typically, the magnetic coil is held over the location of the primary motor cortex and pulses of sufficient intensity lead to a muscle response that can be quantified using electromyographic recording. The size of the evoked muscle response (MEP) to single-pulse stimulation is then taken as an index of excitability. Double-pulse TMS allows a further refinement of excitability measurement by modulating the MEP size by means of preceding the TMS pulse with a sub-threshold pulse. The timing of the pre-pulse relative to the test-pulse determines the degree of excitability (showing either inhibition or facilitation of the MEP), presumably through activation of interneuron populations that control the activation of pyramidal output neurons¹². The double-pulse technique is a test of intracortical control over excitability, and has revealed state- and trait-specific abnormalities and responsiveness to pharmacological intervention¹¹. To date, no studies of cortical excitability have been performed in chronic insomnia. We here report a study of 16 non-pharmacological patients meeting established criteria for chronic insomnia and 14 age-matched controls without sleep complaints, using double-pulse TMS¹². The patients were randomly assigned to a group receiving sleep therapy and a waitlist control group, after which we performed repeated testing.

METHODS

All procedures complied with the declaration of Helsinki and approval was obtained from the medical ethical committee of the VU University medical centre. Informed consent was obtained from all participants.

PARTICIPANTS

We recruited insomnia patients through the sleep disorders outpatient clinic at the VU University medical center in Amsterdam, The Netherlands. In addition, we recruited both patients and control participants through door-to-door leaflets and advertisements in local and national newspapers, magazines for the elderly and the internet site of the Dutch Society for Sleep-Wake Research (NSWO). All patients matched the DSM-IV criteria for primary insomnia¹³: 1) a complaint of difficulty falling asleep or maintaining sleep or of non-restorative sleep; 2) duration of this complaint of more than 1 month; 3) the sleep disturbance causes significant distress or impairment; 4) insomnia does not occur exclusively during the course of a mental disorder; 5) insomnia is not due to another medical or sleep disorder or effects of medications/drug abuse. We carefully selected the patients to exclude other neurological, psychiatric or metabolic disease that could account for their sleep complaints. Patients underwent polysomnography, a physical exam and an interview by a clinical neurophysiologist specialized in sleep disorders (RS). Patients with sleep apnea, restless legs, periodic limb movement during sleep, or sleep state misperception were excluded. Control participants were similarly screened for sleep problems and additional health criteria. Of note, in order to attain good matching, we carefully selected patients and controls on the absence of even slightly increased ratings on symptoms of cognitive decline. Thus, all participants scored within normal limits on tests of cognition and general intellectual functioning, i.e. the Dutch version of the Adult Reading Test (DART)^{14,15}, the shortened version of the Groninger Intelligentie Test (GIT)¹⁶ for general intellectual functioning, the Mini Mental State Examination (MMSE)¹⁷ for possible indications of dementia, and the Boston Naming Task (BNT)¹⁸ testing the subject's object naming and visual abilities. Scores of all participants were within normal limits on the Geriatric Depression Scale (GDS)¹⁹, on sleep-related subscales of the Symptoms Check List (SCL-90)²⁰, and on quality of life as judged by the Short Form-36 health survey (SF-36)²¹. Participation required abstinence from hypnotic medication for at least two months.

Upon the several stages of screening, 373 participants (patients and controls) were excluded; 16 participants diagnosed with primary insomnia without comorbidity and normal ratings on the mentioned symptom scales entered the

study (4 men, 12 women; 60.6 ± 7.0 years of age (mean \pm sd)). Patients suffered from insomnia for at least 2.5 years (range 2.5-50 years, average 17.4 ± 15.6 years (mean \pm sd)). A total of 14 controls was included (4 men, 10 women; mean age 60.4 ± 8.3). We randomly assigned patients to a sleep therapy group or a waitlist control group (see below) and re-tested the patients after, on average, six weeks of treatment (range 5-7 weeks). We were able to obtain data from 6 patients post-therapy and 7 patients post waitlist; due to technical difficulties, data of the second TMS session could not be retrieved in three patients.

SLEEP THERAPY

Therapy comprised a combination of interventions that improved sleep quality and are known to be effective for insomnia treatment²². As an extended form of cognitive behavioral sleep therapy²³⁻²⁷, it consisted of sleep restriction, cognitive restructuring and sleep hygiene^{28,29}, bright light exposure³⁰⁻³², body temperature manipulations^{33,34} and structured physical activity²³, as previously described in more detail^{6,10}. All patients received the complete set of interventions. After explaining the interventions and their purpose to the patient, the 'therapy contract', a list of action points was established. The therapists kept once weekly scheduled telephone contact with the patients, to answer questions, reinforce interventions and adjust sleep restriction schedules. Therapy lasted for a minimum of 6 weeks in all patients and improved sleep quality as well as behavioral and neuroimaging (fMRI) indices of brain function, as described before^{6,10}.

TMS

Double-pulse stimulation was performed between 4 and 6 pm while participants were semi-reclined on a hospital bed with their head leaning backwards on a pillow and their arms supported and extended comfortably alongside their legs with palms facing up, to ensure complete muscle relaxation. The target for stimulation was then localised, by moving the coil over the left hemisphere and delivering single pulses, under constant online electromyography (EMG) verification. The optimal location showing the largest motor evoked potentials (MEPs) and corresponding to the hand area of the left primary motor cortex was then marked on the skin to allow visual control over the coil position throughout the recording. The experimenter stood behind the participants and held the stimulating coil (figure-of-eight coil) with its center over this location. The TMS coil was hand-held by a trained TMS experimenter (YvdW) at an angle of approximately 45° with the midline, perpendicular to the assumed orientation of the central sulcus, with its center over the left primary motor cortex. MEPs were measured from the first dorsal interosseus muscle as described below. Magnetic pulses were

delivered using a MagPro X100 stimulator (Medtronic, Minneapolis, MN, USA). We first determined the resting motor threshold (rMT) of the subject as the lowest intensity of stimulation that resulted in at least 5 MEPs of 50 microvolts or larger in a series of 10 consecutive single-pulse stimulations. An experiment consisted of applying 15 trials each of double-pulse stimulations with inter-pulse-intervals (IPI) of 1, 3, 5, 7, 9, 11, 13, 15 milliseconds; this range spans the most inhibitory (i.e. 1 and 3 milliseconds) to the most excitatory inter-pulse intervals (13 and 15 milliseconds). The pre-pulse was always 80% of rMT, the test-pulse 120%. In addition, we administered 30 single pulses of TMS at 120% of rMT as a reference. The double and single-pulse trials were administered in pre-randomized order with pre-randomized intervals of 4, 5, 6, or 7 seconds.

MEASUREMENTS

Motor evoked potentials (MEPs) were recorded from the first dorsal interosseus muscle using a belly-tendon montage referenced to the joint of the index finger using a Keypoint EMG recorder (Danica, Leusden, The Netherlands) sampling at 5000 Hz. Continuous recordings were stored and exported for subsequent offline analysis of the area under the curve of the MEPs as a measure of MEP size, using software developed in-house. We excluded the first ten trials from subsequent analyses to account for any habituation or novelty responses of the participants to the stimulations; this effectively removed initial muscle contractions and tension in the participants at onset of testing. Any subsequent trials with baseline muscle contraction or large artefacts were excluded through visual inspection by a trained rater, blind to the diagnosis or patient group (KvD). For the analysis of relative MEP sizes, we divided double-pulse MEP sizes by the subject-specific average of the single-pulse supra-threshold MEP sizes.

STATISTICAL ANALYSIS

We analysed the relative MEP sizes using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). We analyzed the data in three ways.

First, we investigated group differences in **absolute** values of the MEPs to single- and double-pulse stimulations. We performed an analysis of variance across all inter-pulse intervals (8 levels) to investigate group differences in absolute MEP size (Huynh-Feldt corrected where appropriate). We used unpaired t-tests to directly compare the MEPs to double-pulse stimulations and to single-pulse suprathreshold stimulations (unequal variances assumed where appropriate).

Second, we analyzed group differences in **relative** MEP responses to double-pulse stimulation, i.e. divided by the reference MEP response to single-pulse stimulation at 120% resting motor threshold. We similarly conducted an analysis of

variance across all inter-pulse intervals to investigate group differences in relative MEP size and performed directed t-tests for every inter-pulse interval separately.

Third, for investigation of the effects of therapy vs. waitlist on the MEP sizes of patients upon retesting we used a three-way repeated-measures analysis of variance with both session (two levels: first and second session) and IPI (eight levels) as within-subject factors and group as between-subject factor (two levels: therapy vs. waitlist).

RESULTS

RESTING MOTOR THRESHOLDS AND RESPONSES TO SINGLE-PULSE STIMULATION

Insomnia patients and control participants did not differ in their resting motor threshold (51.8 ± 1.2 and 54.1 ± 2.3 of % stimulator output, mean \pm sem, respectively; $p=0.35$). They did, however, differ in their response to single-pulse suprathreshold stimuli, i.e. 120% of resting motor threshold: the area under the curve (AUC) for insomnia patients was 1511.4 ± 208.6 vs. 790.0 ± 128.0 microvolt² for the controls (unpaired t-test: $p=0.008$). This indicates that the patients show a greater 'recruitment' in terms of their MEP size as compared to control participants, when going from 100% to 120% of resting motor threshold, while the actual stimulator output is the same between the two groups. No changes were observed in the MEP sizes for insomnia patients post-therapy (1660.4 ± 114.1) or post-waitlist (1687.6 ± 149.3).

DOUBLE-PULSE TMS: ABSOLUTE MEP SIZES

Analysis of variance showed a strongly significant main effect of IPI on absolute MEP size ($F=32.412$ $df=3.227$, $p<0.001$) across patients and controls, reflecting an increase in MEP with longer IPIs. There was a main effect of group bordering significance ($F=4.073$, $df=1$, $p=0.05$), indicative of a global excitability difference and a significant effect of interaction between IPI and group ($F=2.961$, $df=3.227$, $p=0.033$), indicating a stronger effect with longer IPIs. Directed unpaired t-tests showed that the MEP sizes in response to double-pulse stimulation were larger for the insomnia patients as compared with control participants for the long IPIs (IPI=7-11 millisecon.: $p<0.05$), less robustly so for IPI=5 ($p=0.095$) and IPI=13 millisecon. ($p=0.087$) and not significant for the longest IPI, i.e. 15 milliseconds ($p=0.230$). No changes were found after the intervention, in either the therapy or waitlist control groups (See figure 1).

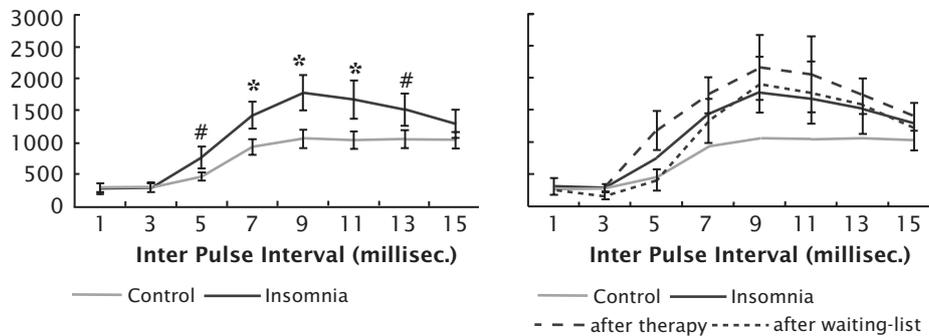
MicroVolt*millisecond

Figure 1. Left: absolute MEP sizes in response to double-pulse TMS for insomnia patients vs. well sleeping control participants. There is a main effect of group and an interaction between group and inter-pulse interval (IPI), such that no difference exists for the short IPIs, a difference emerges for the successively longer IPIs, with a drop-off towards the longest IPIs. Overall, the insomnia patients show an increased absolute excitability ('hyperarousal') relative to control participants, but this increase is not consistent across the IPIs studied. * denotes a significant difference at $p < 0.05$, # denotes a trend at $p < 0.10$, error bars denote standard errors of the mean.

Right: sleep therapy, nor the placebo control, i.e. waitlist procedure, results in a change in the increased absolute excitability. For comparison, the curves of the figure on the left are shown in light grey, for controls (lower curve) and insomnia patients (upper curve); error bars omitted for clarity. Error bars denote standard errors of the mean.

DOUBLE-PULSE TMS: RELATIVE MEP SIZES

After normalizing all MEP sizes by dividing all values with the subject-specific single-pulse suprathreshold MEP size, all participants showed the characteristic profile of relative inhibition at short IPIs (significant at 1, 3 and 5 millisecond IPIs across insomnia patients and controls, all $p < 0.001$). Intracortical facilitation on the other hand was seen only in the group of control participants: an interaction between group and IPI occurred ($p = 0.05$), with reduced MEP sizes in the insomnia group relative to controls reaching significance for the 11 ($p = 0.04$) and 15 millisecond inter-pulse-intervals (IPIs) ($p = 0.031$). There was no significant main effect of group, supporting a selectively altered intracortical facilitation only in insomnia. No effects of treatment, session or interactions between IPI*treatment, IPI*session or IPI*session*treatment were observed (all $p > 0.50$; figure 2).

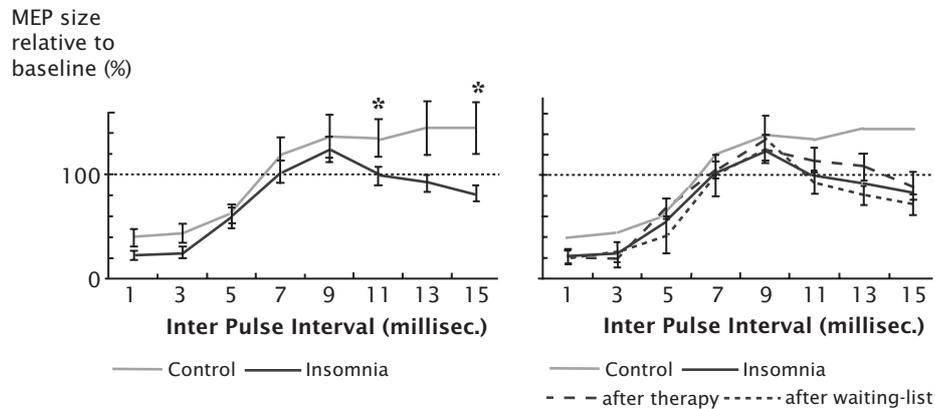


Figure 2. Left: MEP sizes after double-pulse TMS, relative to single-pulse suprathreshold pulses (120% of resting motor threshold) show a selective reduction of intracortical excitability with increasing inter-pulse intervals (IPI) for insomnia patients as compared with controls. There is a significant interaction between group and IPI, such that progressively longer IPIs show an increasingly smaller response in insomnia patients, indicative of a relatively reduced intracortical facilitation. * denotes a significant difference at $p < 0.05$, error bars denote standard errors of the mean.

Right: The pattern of a relatively decreased excitability at higher IPIs does not change after intervention, i.e. either sleep therapy or waitlist placebo condition. For comparison, the curves of the figure on the left are shown in light grey, for controls (upper curve) and insomnia patients (lower curve); error bars omitted for clarity. Error bars denote standard errors of the mean.

DISCUSSION

Our study demonstrates that patients with chronic insomnia show an increase in excitability as evidenced by larger MEP sizes to both single-pulse and double-pulse TMS, as compared to control participants without sleep complaints. This indicates a stronger 'recruitment' of MEP size. The global differences in MEP size cannot be accounted for simply by an increased muscle tone in the insomnia patients, as borne out by the equivalent MEP sizes at lower inter-pulse intervals and the comparable resting motor thresholds. In spite of this general absolute hyperexcitability, the increased responses are not maintained for the longest inter-pulse intervals. Relatively spoken, therefore, insomnia patients show a reduced intracortical facilitation. The control participants without sleep complaints, on the other hand, show the characteristic intracortical inhibition and facilitation processes previously described for paired-pulse TMS^{11,12}, i.e. conditioning pulses

preceding the test-pulse with a short IPI (<7 milliseconds) induce inhibition of the MEP in response to the test-pulse, whereas long IPIs (>7 milliseconds) result in a facilitation of MEP size. Both the absolute increase in excitability and the relative reduction of intracortical facilitation in insomniacs proved to be a robust finding and to persist upon re-testing; the effects did not respond to successful non-pharmacological sleep therapy, that improved not only sleep quality but also normalized brain activation and task performance, as demonstrated previously^{6,10}. In normal healthy subjects, the intracortical facilitation appears maximal with an inter-pulse interval up to approximately 15 milliseconds, before disappearing at longer inter-pulse intervals^{35,36}. The shape of the intracortical inhibition-facilitation curve in our insomnia patients therefore appears as if it is shifted to the left, relative to that seen in control subjects; insomnia patients, due to abnormal processes governing intracortical excitability, would show maximal facilitation at inter-pulse intervals shorter than healthy control participants and the drop-off with longer inter-pulse intervals would resemble that seen in controls at even longer intervals.

The intracortical inhibition and facilitation phenomena appear to result from different mechanisms and are differentially sensitive to modulation of central nervous system activity³⁷. Although specific pharmacological modulations of these phenomena have most elaborately been described for intracortical inhibition, it has been shown that intracortical facilitation can be attenuated with glutamatergic antagonists, which may act directly on the pyramidal neurons or through mediation of GABAergic inhibitory interneurons³⁸. Similarly, noradrenergic and dopaminergic stimulants tend to enhance, while antagonists reduce intracortical facilitation, although findings remain somewhat equivocal³⁷⁻⁴². Interestingly, dopaminergic and GABAergic drugs are powerful modulators of sleep and wakefulness. Dopamine agonists enhance wakefulness whereas antagonists induce drowsiness; GABA agonists enhance sleep⁴³.

Possibly, insomnia patients have a reduction in glutamatergic neurotransmission or a dysbalance in the multisynaptic interaction of glutamatergic and GABAergic neurotransmission; this would be consistent with a recent finding of a reduced GABA concentration in the brains of insomnia patients⁴⁴. Alternatively, the modulating influence of the monoamines NA and DA on intracortical glutamatergic/GABAergic mechanisms might be affected.

Modulation of facilitation has also been reported in relation to sleep/wake states: Salih and colleagues reported absent intracortical facilitation during REM sleep⁴⁵, but upon awakening from REM sleep, enhanced facilitation was found⁴⁶. TMS-studies after acute experimental sleep disruption, however, show equivocal results, and in none of them a specific intracortical facilitation (ICF)-attenuation was reported⁴⁷⁻⁴⁹. Several studies investigated excitability in patients with

chronically disturbed sleep due to obstructive sleep apnea syndrome and reported an increased duration of the cortical silent period reflecting increased intracortical inhibition following single pulses of TMS^{50,51}. It is unclear whether these changes reflect disturbed sleep, the effects of repeated hypoxic episodes on the brain, common physiological factors underlying the nocturnal pharyngeal muscle relaxation or a combination of these factors. In patients with sleep-associated restless legs syndrome a reduced inhibition and increased facilitation has been described; again, effects due to sleep disturbances are hard to disentangle from physiological or neural changes that might underlie the abnormality rather than reflect the accompanying sleep disturbance⁵².

In conclusion, our finding of both an increased absolute excitability, yet an attenuated intracortical facilitation appears highly specific to chronic insomnia. Since it could, moreover, robustly be replicated in both waitlist placebo control and treated patients, we regard it unlikely to have resulted from acute effects of disrupted sleep. Our findings suggest that the altered excitability reflects disturbed intracortical interactions between the principal and interneuron populations that resist effective treatment. The mechanism of such a possibly phenotypical trait in insomnia remains to be determined. Because family-studies and twin-sib studies indicate heritability of problematic sleep complaints^{53,54}, follow-up twin-sib studies are warranted to evaluate whether a disturbed pattern of excitability could be an endophenotype that paves the way for genetic studies to elucidate insomnia.

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