

# High intention to fall asleep causes sleep fragmentation

ELENA RASSKAZOVA<sup>1</sup>, IRINA ZAVALKO<sup>2</sup>, ALEXANDER TKHOSTOV<sup>1</sup> and VLADIMIR DOROHOV<sup>3</sup>

<sup>1</sup>M.V. Lomonosov Moscow State University, Moscow, Russia, <sup>2</sup>SSC RF Institute for Bio-Medical Problems, Russian Academy of Science, Moscow, Russia and <sup>3</sup>Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Science, Moscow, Russia

## Keywords

good sleeper, intention to sleep, intention-related arousal, nap, polysomnography, psychological regulation of sleep

## Correspondence

Elena Rasskazova, PhD, Department of Psychology, Lomonosov Moscow State University, Mohovaya str., 11, str. 9, Moscow, Russia.

Tel.: +7-9263830945;

fax: +7-4959390126;

e-mail: e.i.rasskazova@gmail.com

Accepted in revised form 25 October 2013;  
received 23 May 2013

DOI: 10.1111/jsr.12120

## SUMMARY

The aim of this study was to investigate the effects of intention to fall asleep on sleep quality in good sleepers using polysomnographic and subjective nap parameters. We hypothesized that high intention to sleep would lead to arousal, worsening sleep quality. A counterbalanced 2 × 2 experimental design with one intra-individual (neutral versus motivating instruction) and one inter-individual (instruction sequence) variable was used. Thirty-three good sleepers (22 females; mean age: 24.1 ± 8.4 years) each attended two 1-h daytime polysomnographic recording sessions in the laboratory. When providing motivating instruction, the experimenter insisted on the importance of falling asleep as quickly as possible and promised a financial reward. Compared with neutral instruction, motivating instruction was associated with increased waking after sleep onset, number of awakenings and arousal index during napping. No relationship between instruction and subjective nap appraisal was found. The effect of high intention on sleep fragmentation remained significant after controlling for habitual napping, depression, anxiety and sleepiness. Thus, our findings suggest that high intention to fall asleep worsened sleep quality, especially in terms of sleep fragmentation, in good sleepers.

## INTRODUCTION

Empirical research based on physiological and cognitive models has demonstrated that high levels of neurocognitive arousal can lead to sleep disturbance (Harvey and Espie, 2004; Perlis *et al.*, 2011), and are more typical in patients with insomnia than in good sleepers (Kronholm *et al.*, 2007). Several psychological and behavioural factors (e.g. stress, dysfunctional beliefs, self-restrictive behaviour, clock monitoring) have been considered as candidates triggering such arousal (Harvey and Espie, 2004; Jansson-Fröjmark and Linton, 2007, 2008; Kovrov and Vein, 2005; Levin *et al.*, 1984; Mayers *et al.*, 2009; Morin, 1993; Perlis *et al.*, 2011; Tang *et al.*, 2007).

Despite the promising results of previous research, little is known about mechanisms that create arousal and 'translate' in some people to sleep disturbance. Most studies have focused on insomnia, but common psychological mechanisms of sleep regulation (e.g. dysfunctional beliefs about sleep) should produce sleep disturbance in good and poor sleepers. In this context, models and studies recreating

connections among explanatory variables, arousal and sleep in normative and clinical samples would be very useful.

A psychological model of body function regulation (Nikolaeva and Arina, 2009; Tkhostov, 2002) can be applied to sleep regulation to explain the mechanisms of insomnia perpetuation. According to this model, social demand regulates the development of partially voluntary control over physiological functions. Social and cultural beliefs about the need for and value of such control have led to the subjective perception that most functions are totally manageable. As a result, any problem (e.g. poor sleep) perceived as a loss of control provokes anxiety and rumination, as well as hypertrophied activity to restore voluntary control (including attempts to fall asleep, daytime napping and self-restrictive behaviour). However, an individual has only partial control over sleep and this activity paradoxically perpetuates the functional disturbance (Tkhostov and Rasskazova, 2011). The model was used to explain perpetuation of the wide range of illnesses, including functional somatic illnesses (functional impotence, gastralgia; Nikolaeva and Arina, 2009; Tkhostov, 2002), psychosomatic illnesses in children and adolescents

(Nikolaeva and Arina, 2009), and psychophysiological insomnia (Tkhostov and Rasskazova, 2011).

The application of this model to sleep provides a framework that corresponds strongly with Espie *et al.*'s (2006) and Morin's (1993) models of insomnia, but additionally explains the sources of attention activation and dysfunctional beliefs from social and cultural perspectives, and the ways in which conscious intention to fall asleep directly and indirectly (through behaviour) disturbs sleep structure.

Paradoxical intention (based on the instruction 'try to remain awake as long as possible') suggested by V. Frankl (Frankl, 1965) is one of the recommended treatments for chronic insomnia according to American Academy of Sleep Medicine practice parameters (Morgenthaler *et al.*, 2006). Few randomized controlled trials have demonstrated that this technique reduces subjective sleep latency and number of awakenings (Ascher and Turner, 1979; Broomfield and Espie, 2003; Espie *et al.*, 1989; Ladouceur and Gros-Louis, 1986; Turner and Ascher, 1979). Only one of these trials investigated objective measurements, but it failed to demonstrate any effect of paradoxical intention on actigraphic sleep latency and sleep efficiency (Broomfield and Espie, 2003). However, all these studies focused on patients with insomnia.

Only one study to date (Ansfield *et al.*, 1996) has focused on the role of intention to sleep in good sleepers, demonstrating longer subjective sleep latency in subjects who were instructed to fall asleep as quickly as possible while listening to 'sleep-inhibiting music'. To our knowledge, no study has examined the effect of intention to fall asleep on sleep function in good sleepers using polysomnography.

The aim of this study was to assess the role of intention to sleep in the objective and subjective quality of naps in good sleepers. We hypothesized that the excessive intention to fall asleep would disturb sleep structure in these good sleepers, as it does in poor sleepers, and that the effect of the intention to fall asleep would remain significant after adjusting for anxiety, depression and sleepiness levels.

## MATERIALS AND METHODS

### Design and participants

This study had a 2 × 2 experimental design with one intra-individual (neutral versus motivating instruction) and one inter-individual (instruction sequence) variable. We recruited participants by advertisement on the campuses of two universities in Moscow. The announced requisition was the absence of 'sleep disturbance', described as typically requiring <30 min to fall asleep and the absence or rarity (0–3) of nocturnal awakening. Each participant was paid 1000 rubles (approximately 23 euro). Thirty-five good sleepers took part in the study, which comprised two 1-h sessions.

Upon arrival at the laboratory, participants were screened for sleep disorder symptoms (sleep apnoea, narcolepsy and restless legs syndrome) by a neurologist experienced in Sleep Medicine (IZ), and were interviewed about their

habitual sleep patterns, behaviours on the previous day and night, previous night's sleep, and that morning's physical activity. To avoid social desirability biases, the experimenter explained that the participants' responses were needed as control data and would not impact their participation or monetary reward. Exclusion criteria were: symptoms of any sleep disorder; ≥2-h difference in sleep patterns (onset, duration and/or time of awakening) on the nights before experimental sessions; different or unusual patterns of physical activity on the mornings before experimental sessions (physical activity before one visit but not the other or sports activity that was unusual for a participant); consumption of alcohol, coffee or an excessive amount of food on the evening, night or morning before an experimental session; and <15 min rapid eye movement (REM) latency (an analogue of the sleep-onset REM period of the Multiple Sleep Latency Test), used as an additional sign of possible sleep disturbance (e.g. narcolepsy, obstructive sleep apnoea; Arand *et al.*, 2005).

We excluded one participant with 2 h difference in the sleep duration on the nights previous to the experiments, and more sports activity during the second visit in comparison with the first one. Another participant's data were excluded due to short REM latency. Thus, the final sample consisted of 33 good sleepers (22 females; mean age: 24.1 ± 8.4 years).

### Procedure

Each participant visited the laboratory twice at a 1-week interval at a randomly assigned afternoon time (12:30 or 14:30 hours) on a weekend day.<sup>1</sup> These times were chosen using data about daytime sleepiness fluctuation to coincide with daytime peaks of sleepiness (Lavie and Scherson, 1981). Participants were instructed to sleep for the usual period (but <9 h) on the nights before their visits, and to wake up at or before 09:00 hours, to allow a substantial time period between awakening and the experiment. Upon arrival to the laboratory all participants provided written informed consent.

The experimental procedure had two components. The first component, completed during the first laboratory visit, comprised a complete description of the study, an interview about habitual sleep patterns and daytime sleep, and completion of the Hospital Anxiety and Depression (Zigmond and Snaith, 1983) and Epworth Sleepiness (Johns, 1991) scales (Table 1). Given the lack of published normative data on these scales for Russian versions, we could not use them as inclusion/exclusion criteria, but we controlled for their effects in statistical analyses. We expected that typical students would not get the required amount of sleep, and thus would report high levels of sleepiness. To control for this possibility, we asked 'How much do you need to sleep to feel fresh and vital?' during the interview.

<sup>1</sup>Two times were used for technical convenience. This parameter had no main effect or interaction with instruction effects on subjective and objective nap variables.

**Table 1** Participants' characteristics

Parameter	n or Mean $\pm$ SD
Gender (male/female)	11/22
Age (years)	24.1 $\pm$ 8.4
Subjective habitual sleep duration (h)	7.5 $\pm$ 1.2
Subjective sleep duration necessary to feel vital (h)	8.2 $\pm$ 1.0
Subjective sleep latency (min)	11.2 $\pm$ 10.7
Subjective typical number of nocturnal awakenings (0/1/2)	18/13/2
Habitual napping (yes/no)	14/19
Sleep duration on the night before neutral instruction (h)	7.7 $\pm$ 1.4
Sleep duration on the night before motivating instruction (h)	7.9 $\pm$ 1.1
HADS anxiety subscale score	6.6 $\pm$ 3.2
HADS depression subscale score	4.0 $\pm$ 3.0
Epworth Sleepiness Scale score	8.1 $\pm$ 3.7

HADS, Hospital Anxiety and Depression Scale.

The second component consisted of sleep recording and participants' subjective appraisal. The sleep recording procedure was the same for the first and second laboratory visits. Each participant was randomly assigned to one of two sequences of experimental conditions: 17 participants received neutral instruction during the first visit and motivating instruction during the second visit (neutral–motivating sequence); and 16 participants received a motivating–neutral instruction sequence. The neutral instruction was: 'You should just lie down as if you decided to have some rest during the day. Whether or not you fall asleep doesn't matter. Just relax'. The motivating instruction was: 'It's very important for you to make yourself fall asleep as quickly as possible. Use all the methods you know. If you can fall asleep really quickly, you will get an additional monetary reward. For 5 min, you'll get 300 rubles (approximately 7 euro); for 10 min, 200 rubles; for 15 min, 100 rubles'. We confirmed that these reward amounts would motivate typical Russian students in a discussion held with another group of students before the experiment.

After electrodes were fixed to the participant and the instruction was provided, he or she lay for 1 h in an acoustically insulated room. Polysomnography was performed using a 54-channel somnological polygraph (Sagura Medizintechnik GmbH, Muehlheim am Main, Germany) and included six electroencephalogram channels (F3/A2, F4/A1, C3/A2, C4/A1, O1/A2, O2/A1), a submental electromyogram and an electrooculogram. The recordings were scored manually following the standard criteria of the American Association of Sleep Medicine (Iber *et al.*, 2007). We calculated all standard sleep parameters (Iber *et al.*, 2007) and additionally computed the following values. The number of macro-awakenings was calculated as amount of awakenings longer than 3 min. The longest period of continuous

awakening after sleep onset, and the longest period of continuous (uninterrupted by awakening) sleep were also calculated.

After 1 h, participants were awakened and interviewed about nap characteristics and the subjective intention to sleep. Participants subjectively appraised time in bed, sleep time, sleep latency, and the number and duration of awakenings. We also assessed subjective sleep quality and post-nap vitality using a scale ranging from 1 (very poor) to 5 (excellent). The subjective intention to sleep was measured by a single question ('How strong was your intention to fall asleep?') using a Likert scale ranging from 0 (no intention) to 10 (high intention) to control for the effectiveness of instruction manipulation. To prevent intention appraisal from influencing naps, we asked participants to appraise this parameter after polysomnographic recording had been completed.

### Ethical approval

The study was approved by the Ethical Committee of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences.

### Statistical analysis

Data were analysed using SPSS software (ver. 17.0; SPSS, Chicago, IL, USA). Student's *t*-test was used to determine whether experimental manipulation increased subjects' intention to fall asleep as quickly as possible, and to investigate the effects of instruction on subjective and objective nap parameters. Due to the well-known positive skewing of the distribution of sleep latency, log-10 transformation (with the addition of a small constant) was performed to achieve approximate normality for this parameter. Then, we examined whether control variables (habitual napping, duration of sleep on the night before the experiment, anxiety, depression and sleepiness score) moderated the effects of instruction on nap structure and its subjective appraisal. Correlation analysis or Student's *t*-test was performed for each control variable and nap parameter separately for each instruction, and differences between instruction conditions were also examined. Control variables demonstrating significant relationships with nap parameters were included as covariates in a repeated-measures analysis of variance (ANOVA).

## RESULTS

After the exclusion of one participant with a 2-h difference in sleep duration on the nights before experimental sessions who had engaged in more sports activity before the second visit than before the first session, and one participant with short REM latency, the final sample consisted of 33 good sleepers (22 females; mean age: 24.1  $\pm$  8.4 years). Participants' characteristics are shown in Table 1.

### Effectiveness of experimental manipulation

The aim of instruction manipulation was to increase participants' intention to sleep by provoking them to try to force themselves to fall asleep. After neutral instruction, self-reported scores for the intention to sleep ranged from 0 to 9 (median, 4; mean  $\pm$  SD,  $4.1 \pm 3.0$ ). After motivating instruction, these scores increased significantly (median, 8; range, 5–10; mean  $\pm$  SD,  $7.7 \pm 1.7$ ;  $t = -6.49$ ;  $df = 32$ ;  $P < 0.01$ ). These results confirmed the effectiveness of manipulation.

### Effects of instruction type on subjective sleep appraisal

Participants' subjective appraisals of nap parameters were unrelated to instruction type (motivating versus neutral; Table 2).

### Effects of instruction type on objective sleep parameters

Unexpectedly, instruction type did not affect sleep latency (Table 2). However, motivating instruction led to more

fragmented sleep: it significantly increased awakening after sleep onset, the number of macro-awakenings and the arousal index. It also tended to increase the total number and maximum duration of awakenings, although these effects were not significant. The total sleep time and duration of longest continuous sleep episode tended to be shorter under motivating instruction.

### Effects of control variables

Under neutral instruction, participants who napped habitually ( $n = 14$ ) reported longer durations of awakening than did those who did not nap habitually ( $t = -2.28$ ,  $df = 31$ ,  $P < 0.05$ ). Total sleep time on the night before nap recording was not correlated with any relevant objective or subjective nap parameter. Depression scale scores were related to the number of macro-awakenings (neutral instruction only:  $r = 0.44$ ,  $df = 31$ ,  $P < 0.05$ ), but anxiety and sleepiness scores were not. The depression scale score was negatively correlated with the difference in the number of macro-awakenings between instruction conditions ( $r = -0.38$ ,  $df = 31$ ,  $P < 0.05$ ), and related to the subjectively appraised number of awakenings after neutral instruction ( $r = 0.47$ ,  $df = 31$ ,  $P < 0.05$ ). Sleepiness level was correlated negatively with post-nap vitality (neutral instruction:  $r = -0.48$ ,  $df = 31$ ,  $P < 0.01$ ; motivating instruction:  $r = -0.30$ ,  $df = 31$ ,  $P < 0.1$ ).

Thus, some relationships were observed between nap parameters and depression scores, as well as subjective appraisals, habitual napping and sleepiness. However, habitual napping and sleepiness were related to subjective parameters that were not affected by instruction. Therefore, they were not included in further analysis. To control for the possible influence of the depression score on sleep fragmentation after motivating instruction, we performed repeated-measures ANOVA of the number of macro-awakenings with the depression score serving as a covariate. In repeated analysis, the number of macro-awakenings remained affected by instruction type ( $F = 10.34$ ,  $df = 1$ ,  $P < 0.01$ ). No independent effect of the depression score on macro-awakenings was observed, but depression score  $\times$  instruction type showed a significant interaction effect ( $F = 5.13$ ,  $df = 1$ ,  $P < 0.05$ ). Thus, depression scores did not explain sleep fragmentation after motivating instruction.

### DISCUSSION

This study demonstrated the deteriorating role of high intention to fall asleep in napping in good sleepers. Participants' intention to fall asleep increased after motivating instruction. In support of our hypothesis, high intention worsened sleep and particularly led to sleep fragmentation, as demonstrated by increased awakening after sleep onset, arousal index, and numbers of awakenings and macro-awakenings. High intention also resulted in marginally significant reductions in total sleep time and the duration of

**Table 2** Objective and subjective sleep parameters under neutral and motivating instructions

Parameter	Neutral instruction ( $n = 33$ )	Motivating instruction ( $n = 33$ )	Student's $t$ ( $df = 32$ )
Subjective time in bed (min)	46.9 $\pm$ 21.8	50.3 $\pm$ 17.3	-1.06
Subjective sleep duration (min)	22.4 $\pm$ 17.7	24.5 $\pm$ 16.2	-0.57
Subjective sleep latency (min)	22.2 $\pm$ 16.3	22.8 $\pm$ 19.2	-0.15
Subjective number of awakenings	1.4 $\pm$ 1.8	1.1 $\pm$ 1.1	0.61
Subjective duration of awakenings	1.1 $\pm$ 2.3	2.4 $\pm$ 3.8	-1.27
Subjective sleep quality	3.7 $\pm$ 1.0	3.6 $\pm$ 1.0	0.27
Subjective post-nap vitality	3.4 $\pm$ 1.1	3.7 $\pm$ 1.2	-1.09
Sleep latency (min)	13.7 $\pm$ 10.9	14.1 $\pm$ 13.1	-0.27
Total sleep time (min)	38.5 $\pm$ 15.8	33.6 $\pm$ 16.9	1.92*
Stage 1 sleep (min)	11.3 $\pm$ 9.4	10.7 $\pm$ 7.1	0.39
Stage 2 sleep (min)	19.7 $\pm$ 10.6	17.8 $\pm$ 12.9	1.10
Delta sleep (min)	7.5 $\pm$ 10.1	5.2 $\pm$ 8.3	1.55
Wakening after sleep onset (min)	6.5 $\pm$ 9.0	13.1 $\pm$ 14.2	-2.66**
Number of awakenings	2.7 $\pm$ 2.0	3.6 $\pm$ 2.5	-1.92*
Number of macro-awakenings	0.6 $\pm$ 0.9	1.0 $\pm$ 1.0	-2.20*
Longest awakening (min)	4.6 $\pm$ 8.2	7.86 $\pm$ 8.3	-2.07*
Longest continuous sleep period (min)	28.4 $\pm$ 15.6	23.5 $\pm$ 14.7	2.02*
Arousal index (/h)	29.9 $\pm$ 24.1	45.8 $\pm$ 46.8	-2.86***

Values are presented as means  $\pm$  SD.

\* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$ .



the longest continuous period of sleep, as well as prolonging the longest period of awakening.

Within the context of the psychological model of body function regulation (Nikolaeva and Arina, 2009; Tkhostov, 2002) and the attention-intention-effort model (Espie *et al.*, 2006), two mechanisms may underlie this effect. First, high intention increases attention bias to sleep-related stimuli (Jansson-Fröjmark *et al.*, 2013; Spiegelhalder *et al.*, 2008, 2010) and arousal, disturbing sleep directly. Arousal is a common factor in sleep disturbances (Harvey and Espie, 2004; Perlis *et al.*, 2011), but it remains unclear whether different arousal types (cognitive, emotional, physiological) reflect the same underlying process or have different impacts on sleep. Further research is needed to clarify the nature of intention-related arousal in good and poor sleepers. Individual differences in the 'translation' of intention into arousal may also increase some people's vulnerability to insomnia (e.g. in patients with insomnia the same levels of intention to fall asleep lead to higher arousal than in good sleepers). The investigation of factors potentially moderating this relationship (e.g. sleep-related beliefs, anxiety, depression) would further elucidate the psychological mechanisms of insomnia. In a wider perspective, these data are in line with the studies (Nikolaeva and Arina, 2009; Tkhostov, 2002) suggesting that high intention to control physiological functions could trigger and/or perpetuate symptoms in functional diseases (e.g. functional impotence and gastralgia).

Second, the impact of intention may be mediated by inappropriate behaviour aimed at controlling sleep (e.g. trying to fall asleep as quickly as possible). Empirical studies have shown that patients with insomnia typically use behavioural strategies that may perpetuate sleep problems in attempts to control sleep (e.g. self-restrictive, rather than active, behaviours; MacDonald *et al.*, 2008; Robertson *et al.*, 2007). Although our sample was too small to compare the effectiveness of behavioural strategies, in the interview participants reported using various strategies, including relaxation, visualization, attempts to stop thinking or to make oneself fall asleep, and prayer. Further experimental research is needed to clarify whether these behaviours have different impacts on sleep and to identify factors influencing behavioural choices under high-intention conditions. According to the body function regulation model, behavioural strategy choices are determined by social and cultural beliefs about sleep regulation. In line with this suggestion, the role of dysfunctional beliefs about sleep in insomnia is well established (Jansson-Fröjmark and Linton, 2008; Morin, 1993; Perlis *et al.*, 2011). A cross-sectional study (Tkhostov and Rasskazova, 2011) identified several dysfunctional beliefs about the causes of insomnia and effective coping strategies (e.g. that sleep problems could be resolved only by voluntary control or medication, that they are not related to daily behaviour/events), which were common among good sleepers in Russia and even more prominent in patients with insomnia. Such beliefs may determine the choice of a dysfunctional behaviour when the intention to fall asleep is high.

Contrary to our expectations, there was no effect of instruction type on sleep latency. A possible explanation might be that sleep latency was highly influenced by other factors (individual differences, instruction sequence, using nap instead of nocturnal sleep, etc.). Further research could control some of these factors (see limitations of the study below).

Surprisingly, we found that instruction type had no effect on participants' subjective appraisals of nap characteristics. This result may be interpreted with respect to three factors. First, from a social perspective, the subjective definition of a 'good nap' seems to be more ambiguous than that of a 'good night's sleep'. Subjective criteria depend on whether the person is a habitual napper and on his/her point of reference (e.g. vitality after a nap versus after a night of sleep). Individual differences in these criteria may have influenced appraisals of nap quality. Second, the five-point scale used to characterize sleep quality may have been insufficiently sensitive. Third, given the nature of subjective appraisal, participants who believed that the intention to fall asleep quickly would lead to shorter sleep latency may have underestimated latency periods. Additionally, the promise of a monetary reward under the motivating condition may have led participants to (un)consciously improve their appraisals of sleep after motivating instruction. Thus, individual differences in the discrepancy between objective sleep characteristics and subjective appraisals (Bianchi *et al.*, 2012) may have been prominent in our sample. Nevertheless, the observed absence of any effect of instruction on subjective appraisals of sleep quality should be explored further in future research.

Possible alternative explanations of our results are related to the control variables (habitual napping; sleep duration on the night before a laboratory visit; anxiety, depression and excessive sleepiness scale scores). Consistent with previous data showing a negative correlation between depression and sleep (Jansson-Fröjmark *et al.*, 2008), higher depression (but not anxiety or excessive sleepiness) scores were slightly related to poorer nap quality. In our study no control variable moderated the effect of motivating instruction on sleep fragmentation. However, this study was conducted with a normative sample of good sleepers, and results may differ in clinical samples (patients with insomnia or high anxiety/depression levels). Although sleepiness had no impact on the relationship between instruction and sleep fragmentation, further studies and normative data for the Russian version of the Epworth Sleepiness scale would aid our understanding of whether the high mean sleepiness scale score in our sample was typical for students, or whether it reflects item translation and scale validation problems. As in a previous study (Tkhostov and Rasskazova, 2011), our participants reported that they typically slept less than they needed to feel vital, but not because of sleep disturbance. These data support the hypothesis that mild sleepiness could be explained by the typical sleep-related behaviours of Russian students.

The use of polysomnographic recordings to verify the effect of manipulation in this study constitutes an important

advantage over many studies of the psychological regulation of sleep. However, our experiment had several limitations that should be taken into account in the interpretation of the results. First, as in most laboratory studies, our sample was small, increasing the likelihood of interfering effects of individual differences. Although we controlled for some variables (habitual napping, depression, anxiety, sleepiness, sleep time before the experiment), further research is needed to test alternative explanations of the observed effects. Second, we examined the manipulation of daytime naps instead of nocturnal sleep. Although naps are frequently used as sleep models, differences between naps and nocturnal sleep may restrict the applicability of our results. Third, for technical reasons we performed no pre-experiment recording that could eliminate the impact of adaptation to the laboratory. To control for the effect of instruction sequence, each participant was randomly assigned to one of two sequences. Further research could control for the effect of the first visit to the laboratory and investigate this interaction in greater depth. Fourth, multiple comparisons could lead to statistical mistakes that were not controlled for in this study and should be addressed in the future.

Despite these limitations, our results indicate the important role of the intention to fall asleep in sleep quality in good sleepers. They provide indirect support for the existence of common psychological mechanisms of sleep regulation among people with insomnia and the non-clinical population. Given this common basis, criteria for vulnerability to sleep problems in the normative population should be proposed, studied and used for insomnia risk prevention in sleep medicine. For instance, prevention efforts could address arousal (and related coping strategies), the processes by which intention leads to higher arousal, and/or behavioural choices that perpetuate sleep problems.

## ACKNOWLEDGEMENTS

The authors thankfully acknowledge Prof. Vladimir Kovalzon for providing wise advice about study design and organization. This research was supported by a grant from the RFH project (no. 11-36-00242a).

## AUTHORS' CONTRIBUTIONS

ER created the study design, data collection, interpretation of the results, manuscript writing, data analysis. IZ created the study design, data collection, interpretation of the results, manuscript writing. AT created the study design, interpretation of the results, manuscript review. VD created the study design, interpretation of the results, manuscript review.

## CONFLICT OF INTEREST

No conflicts of interest declared.

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