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Original Article

Selective slow-wave sleep suppression affects glucose tolerance and melatonin secretion. The role of sleep architecture☆

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ABSTRACT

Objectives: Our study aimed to assess the impact of one night of slow-wave sleep (SWS) suppression on glucose tolerance, and explore whether melatonin plays a role in glucose tolerance impairment after SWS suppression.
Methods: In sum, 20 volunteers participated in two experimental sessions: a session with SWS suppression during one night's sleep and a session with a regular night's sleep (control). Each session included collecting seven salivary samples. The following morning, an oral glucose tolerance test (OGTT) was performed.
Results: SWS suppression effects depended on the individual blood glucose response to the OGTT. During the control session, 'responders' (N = 11), already presented with low glucose tolerance, which further declined after SWS suppression. 'Non-responders' (N = 9) experienced high glucose tolerance in both conditions. Among the responders, SWS suppression led to an increase in melatonin at the moment of awakening, while in non-responders melatonin increased during the first half of the night. In both conditions, responders were characterized by a shorter total sleep time (TST) and less rapid eye movement (REM) sleep. During SWS suppression, they had more non-rapid eye movement (NREM) stage 1 and longer nocturnal wakefulness. Responders and non-responders showed a comparable amount of SWS.
Conclusions: This study highlights three key findings: first, SWS suppression leads to an increase in salivary melatonin; second, melatonin's effect on glucose tolerance depends on its secretion timing; and third, durations of REM sleep and nocturnal awakenings, appear to play an important role in melatonin secretion and glucose tolerance, indicating the potential clinical relevance of these findings for type 2 diabetes risk assessment.

1. Introduction

It has been demonstrated repeatedly that insufficient or fragmented sleep adversely affects glucose tolerance [1,2], thus increasing the risk of type 2 diabetes [3–5]. There is evidence that the deepest stage of non-rapid eye movement (NREM) sleep, also known as slow-wave sleep (SWS), plays a crucial role in glucose regulation, and that SWS suppression has a negative impact on glucose homeostasis [6,7]. The exact neurophysiological and hormonal underpinnings of these effects, however, remain unclear. Among the most evident candidates are changes in corticotropic activity and sympathovagal balance.

Indeed, it has been shown that sleep disturbance, possibly acting on the body as a stressor, can increase cortisol secretion [2,8]. A major function of cortisol is mobilizing energy resources in the presence of increased energy needs [9]. Cortisol promotes gluconeogenesis and suppresses glucose uptake in skeletal muscle and adipocytes [10–12], thus contributing to insulin resistance. However, data on the relationship between cortisol secretion and time spent in SWS, are inconsistent [6,13–15].

Another possible effect of SWS suppression is a shift in sympathovagal balance. SWS is characterized by the prevalence of parasympathetic activity of the autonomic nervous system (ANS)

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List of abbreviations

ANS	autonomic nervous system
AUC	area under the curve
BMI	body mass index
ECG	electrocardiogram
EEG	electroencephalography
ESS	Epworth Sleepiness Scale
HF	high frequency of heart rate variability
HRV	heart rate variability
LF	low frequency of heart rate variability
MCTQ	Munich ChronoType Questionnaire
NREM	non-rapid eye movement sleep
OGTT	oral glucose tolerance test
PSG	polysomnography
PSQI	Pittsburgh Sleep Quality Index
REM	rapid eye movement sleep
SPT	sleep period time
SSS	Stanford Sleepiness Scale
SWS	slow wave sleep
TST	total sleep time
VASS	Visual Analog Sleepiness Scale
WASO	wake after sleep onset

[16,17]. Therefore, its suppression could prevent a shift in the sympathovagal balance toward parasympathetic predominance and lead to a persistent increase in sympathetic activity. Since excessive sympathoadrenal activation can cause metabolic dysregulation, namely hyperglycemia and insulin resistance [18,19], we assume that it can contribute to reduced glucose tolerance after SWS suppression.

A less evident, but from our point of view a very likely link between sleep disturbance and impaired glucose tolerance, is a change in melatonin secretion. Melatonin influences the synthesis, secretion, and action of insulin [20]. Restricted sleep has been shown to significantly phase-delay the melatonin rhythm in humans, independent of bedtime [21,22]. Zeitzer et al., reported elevated levels of melatonin during extended wakefulness (constant routine conditions) in young subjects [23]. Similarly, in a study by Ackermann et al., one night of total sleep deprivation increased the amplitude of the melatonin rhythm [24]. However, although melatonin is widely used in sleep-related studies, data regarding the acute effects of SWS suppression on its amplitude and timing, remain scarce. We found only one study on the effect of SWS deprivation on melatonin secretion, conducted by Rao and colleagues [25]. Their study showed that nocturnal serum melatonin was significantly lower during the night with selective SWS disturbance than during the control night. The present study aimed to explore whether melatonin plays a role in glucose tolerance impairment after SWS suppression. To more clearly identify the impact of melatonin per se, we also measured changes in sympathovagal balance and cortisol secretion.

2. Methods

2.1. Participants

Healthy males (N = 23) participated in a balanced crossover study. All participants were undergraduate volunteers from Lomonosov Moscow State University. Study eligibility was determined via written questionnaires about sleep quality, habitual sleep time, daytime sleepiness, physical and mental health, medication use, and health behaviors (eg, smoking, alcohol consumption, and work and study schedules). Exclusion criteria included a history of head injury, chronic or acute illness, current medication of any kind, alcohol or drug abuse, smoking, shift work, excessive daytime somnolence (ie, an Epworth Sleepiness Scale [ESS] score >11) [26], sleep complaints (ie, a Pittsburgh Sleep Quality Index [PSQI] score $\geq$ 5) [27], and the presence of any oral inflammatory processes with or without evident bleeding.

One to two weeks before the first session, the volunteers visited the laboratory to familiarize themselves with the experimental conditions and equipment, as well as undergo daytime nap polysomnography (PSG) for 1.5 h to adapt to the PSG recording procedure. None of the participants had difficulty falling asleep or with sleep maintenance. Participants were required to maintain a strict regular sleep-wake schedule for seven days prior to entering the laboratory, with bedtimes between 23:00 and 24:00 h, and wake-up times between 07:00 and 08:00 h, as well as refrain from taking naps during the day. Compliance with these instructions was confirmed by daily sleep diaries and accelerometric recordings (Xiaomi Mi band 2). Caffeine and alcohol consumption was prohibited during the final three days of this baseline period.

The study was performed according to the Declaration of Helsinki on research involving human participants. The ethics committee of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, approved the study protocol. Written informed consent was obtained before the study procedures began, and participants were allowed to withdraw at any time. Each participant received 2000 RUB (32 USD) in financial compensation.

One participant failed to keep the prescribed sleep-wake cycle before the experiment; therefore, he was excluded from the study. Saliva samples of one volunteer showed signs of coloration potentially due to contamination with blood, and he was also excluded. One participant was not included in the analysis due to symptoms of sleep disorder identified during the first experimental session. Thus, only 20 subjects were included in the current study analysis.

According to the sleep diaries and accelerometric recordings, all the participants had a regular sleep-wake cycle during the week prior to taking part in the experimental sessions, with a habitual sleep duration of 7.82 ± 0.15 min (mean ± SEM; range = 7–9 h), bedtimes of 23:18 ± 0:11 (mean ± SEM; range = 22:00–24:00 h), and wake times of 06:51 ± 0:12 (mean ± SEM; range = 06:00–08:00 h). Their average age was 22.50 ± 0.40 years (mean ± SEM), and their average body mass index (BMI) was 22.80 ± 0.73 kg/m2 (mean ± SEM).

2.2. Procedure

Due to the study's randomized, balanced crossover design, each volunteer participated in two experimental sessions: a session with selective SWS suppression during one night's sleep, and a session with a regular night's sleep as a control condition. Except for SWS suppression, the design of both sessions was identical, and the participants received the same instructions. We did not tell them about subsequent SWS suppression, but prior to sleep in each session, we informed them that sounds would be presented at various times during the night. Sessions were separated by an interval of 1–3 weeks.

The participants arrived at the research unit at 19:45 h for each experimental session in a fasting state (4 h of fasting). After the first saliva sample was obtained (ie, at 20:00 h) and until bedtime (ie, at 23:00 h), they were kept in dim-light (<10 lux) conditions. At 20:05 h, the participants ate a meal; thereafter, each participant's sleepiness level was measured using the Visual Analog Sleepiness Scale (VASS) and the Stanford Sleepiness Scale (SSS). At 22:40 h, the participants were prepared for nocturnal PSG. Electrodes were attached to register electroencephalography (EEG), electrooculography, electromyography, and electrocardiography. At 23:00 h, the participants went to bed, and the lights were turned off. At 07:00 h, the participants were awakened. After awakening, they completed a subjective sleep assessment questionnaire, followed by an oral glucose tolerance test (OGTT). At 08:20 h, they completed the SSS and VASS. After the last glucose measurement, at 09:20 h, the participants ate breakfast. Meals were identical in composition during each session of the study. Salivary samples were collected seven times: three times in the evening (20:00, 21:30, and 23:00 h), twice during the night (01:30 and 4:00 h), and twice in the morning, at 07:00 (ie, immediately after awakening) and 07:40 h. Night samples and the first morning sample were collected in the dark (0 lx). The study's protocol is presented in detail in Fig. 1. SWS suppression was achieved by presenting an acoustic tone with a gradually increasing decibel level. A detailed description of the stimulation scheme and EEG criteria for starting and stopping the stimulus presentation have been reported previously [15].

2.3. Measures

2.3.1. Oral glucose tolerance test

Blood glucose was measured after an overnight fast before and 1 and 2 h after consuming 75-g glucose (ie, glucose dissolved in 300 ml water within 5 min) in finger-prick capillary samples using the FreeStyle Precision Neo (Abbott) system. To achieve greater accuracy, each glucose measurement was performed twice, and the results were averaged. FreeStyle Precision Neo was chosen because it is one of the most accurate glucose monitoring systems [28]. The area under the blood glucose curve (AUC) during the OGTT was calculated using the trapezoidal rule.

2.3.2. Polysomnographic data acquisition and scoring

PSG recordings were performed using a digital EEG amplifier Encephalan-EEGR-19/26 (Medicom MTD, Taganrog, Russia) with a sampling rate of 250 Hz. PSG recordings included an EEG (F3, F4, C3, C4, O1, and O2, placed in accordance with the International 10–20 System), electrooculogram, electromyogram, and an electrocardiogram (ECG) in lead II. PSGs were scored offline by two scorers who were blinded to the experimental conditions. Visual scoring of each 30 s epoch of PSG recording as awake, NREM stage 1, 2, or 3 (SWS), or REM sleep was performed according to standard AASM criteria [29]. Inter-scorer reliability was >94%. The PSG variables analyzed, included sleep onset latency, total sleep time (TST), wakefulness after sleep onset (WASO), sleep period time (SPT) and sleep efficiency. SPT was measured as the period beginning when the participant fell asleep and ending at the last wake-up, including the duration of awakenings if they occurred. Sleep efficiency was calculated as a percent value of TST, referred to SPT. The arousal index (AI) was defined as the number of arousals per hour of sleep. Spectral analysis was used to analyze slow wave activity (SWA) in the 0.5–4 Hz range during SWS using BrainVision Analyzer 2 (Brain Products GmbH, Hamburg, Germany). Artifacts were removed manually. Thereafter, for C3 EEG channel, we calculated absolute power spectral density (PSD) using the Fast Fourier Transform with Hanning window in 10-s epochs.

2.3.3. Heart rate variability analysis

For the assessment of heart rate variability (HRV), we recorded standard lead-II ECGs during sleep and in the waking state before and after sleep. HRV indices were measured using free HRV analysis software (https://anslabtools.univ-st-etienne.fr/en/index.html) in five 5-min fragments: in the evening at bedtime, during SWS in each of the first three sleep cycles, and shortly after awakening in the morning. During the first three sleep cycles, all the participants experienced SWS in both experimental sessions. However, during the fourth sleep cycle, in both sessions this stage of sleep was only

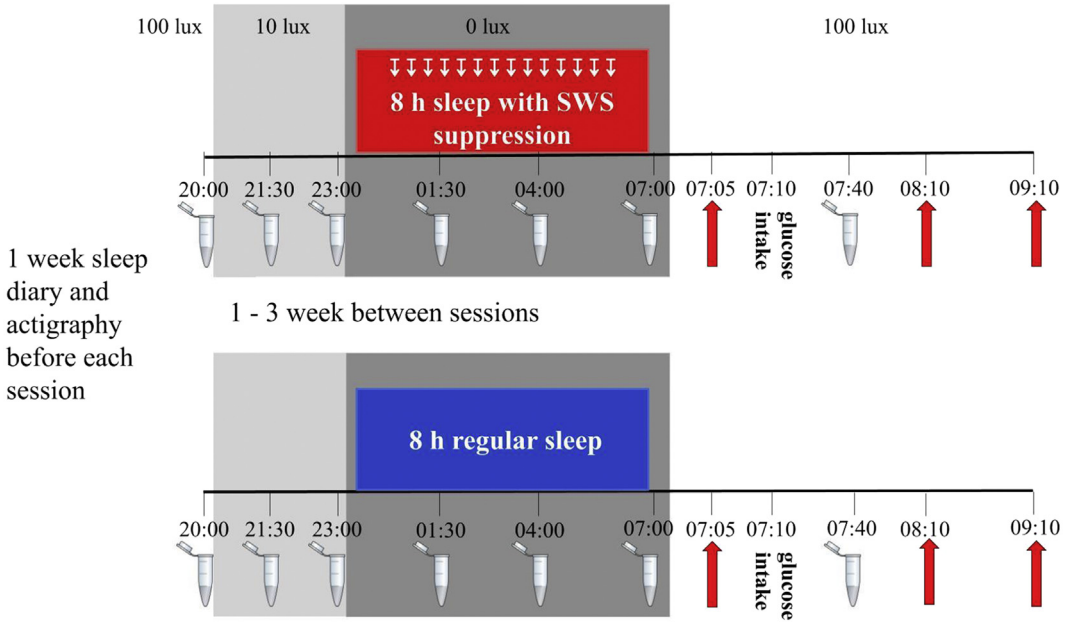

Fig. 1. Study Design. The schema illustrates the two experimental conditions: one session with selective slow-wave sleep (SWS) suppression during 8 h night time sleep and one session with 8 h regular night time sleep (control). – saliva collection, ↑ – blood glucose measurement. Other explanations are in the text.

observed in nine participants, therefore the fourth sleep cycle was excluded from the analysis.

The R waves of the ECG were detected by an automatic algorithm; thereafter, each QRS complex was visually validated before being implemented in the analysis. Artifact signals due to movement and ectopic waveforms were removed from the analysis. Heart rate data, as well as time and frequency domain indices, were extracted. Time-domain indices were calculated as SDNN (ms, standard deviation of normal R–R intervals), rMSSD (ms, square root of the mean squared differences of successive R–R intervals), and pNN50 (percentage of differences between adjacent normal R–R intervals more than 50 ms). Fourier indices were calculated as very-low-frequency (VLF; ms2, between 0.0033 and 0.04 Hz), low frequency (LF; ms2, from 0.04 to 0.15 Hz), high frequency (HF; ms2, from 0.15 to 0.40 Hz), and the LF/HF ratio [30]. HF power is modulated by parasympathetic activity, whereas LF power is controlled by both the sympathetic and parasympathetic branches. Thus, the LF/HF ratio is used to estimate sympathetic modulation [31].

2.3.4. Salivary samples

Saliva sampling was performed using special sampling devices SaliCap (IBL International GmbH, Hamburg) equipped with a special straw. Saliva was collected by passive drool; it usually took approximately 2 min to obtain the required 1–1.5 ml of saliva. Samples were collected more than 30 min after drinking, eating, and teeth brushing and stored at −60 °C. Samples with even minimal coloration due to blood contamination were excluded from the analysis. In all samples, melatonin and cortisol concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.3.5. LC-MS/MS analysis of melatonin and cortisol

The LC-MS/MS analysis was performed with the AB SCIEX QTRAP 6500 tandem mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) source (AB SCIEX, Concord, ON, Canada) using the Waters® UPLC® ACQUITY System for chromatographic separation. Commercially available cortisol and melatonin were purchased from Sigma Aldrich (St. Louis, MO, USA). The deuterated internal standards (ISs), cortisol-d4 and melatonin-d7, were obtained from Cambridge Isotope Laboratories (MA, USA). MS-grade methanol and water, as well as HPLC-grade acetone, MTBE, and formic acid, were purchased from PanReac AppliChem.

Stock solutions of hormones and ISs were prepared in methanol at concentrations of 1 mg/ml and stored at −20 °C until use. The substock solution contained cortisol and melatonin at a concentration 20 times higher than the highest working calibrator. Working calibrators were prepared before the analysis by serially diluting the standard substock in methanol and then adding 10 μl of each dilution to 190 μl of deionized water. The working calibrators covered the ranges 0.1–100 ng/ml for cortisol and 5–5000 pg/ml for melatonin. Six levels of calibrators were used for all the analytes. The working IS solution was prepared by diluting each steroid IS stock solution with methanol. Quality controls (QCs) were prepared in deionized water at three levels (low, medium, and high) by spiking with the substock solution at concentrations corresponding to 1%, 10%, and 50% of the highest working standard. Consequently, the achieved precision and coefficient of variation (CV) were better than 9% and 9% for melatonin, and 3% and 15% for cortisol. All the solutions of standards, calibrators, and QCs were stored in 2.0 ml glass vials with screw caps at −20 °C.

For the current study, 10 μl of the working IS solution and 0.2 ml of acetone were added to 0.2 ml of patient saliva/calibrator solution in 10 ml borosilicate glass vials with disposable caps, and the mixture was vortexed for 1 min. Next, it was extracted with 2 ml MTBE for 1 min with vigorous stirring. The vials were allowed to freeze for 10 min at −40 °C, and then the organic layers were transferred to 10-ml borosilicate glass test tubes and evaporated to dryness in a stream of nitrogen at 35 °C. The residues were reconstituted in 0.2 μl of a methanol-water mixture (1:1) and, after centrifugation, transferred to 2.0 ml autosampler vials with 0.25 ml glass inserts.

Following extraction, 10 μl of the reconstituted sample was injected into a reverse-phase column (Acquity UPLC BEH C18, 1.7 μ, 2.1 × 100 mm, and 0.2 μ in-line pre-column filter). LC separation was performed using a gradient mobile phase: phase A (water+0.1% formic acid) and phase B (methanol +0.1% formic acid) at a flow rate of 0.3 ml/min. The column temperature was maintained at 35 °C throughout the separation. The following optimized instrument parameters were applied for the detection of analytes and internal standards: the nebulizer current was set at 3 mA with a source temperature of 500 °C and nebulizer gas set at 30 psi. Nitrogen and dry air were produced by a PEAK Scientific generator (Parker Balston, Haverhill, MA, USA) GENIUS 3031. The hormones were monitored in a positive-ion mode using multiple reaction monitoring (MRM) in a positive-ion mode (Table 1).

2.3.6. BMI estimation

BMI was computed using objectively measured height and body weight data using the following formula:

$$\mathrm{BMI} = \frac{\text{weight in kilograms}}{(\text{height in meters})^2}$$

2.3.7. Sleepiness assessment instruments

Subjective sleepiness was assessed using the SSS and VASS. The SSS [32] is a single-item 7-point self-report questionnaire that measures an individual's subjective level of sleepiness. The VASS consists of two statements with opposite meanings (sleepy and alert) located at the ends of a 100-mm line. The participants were asked to put a vertical mark on the line between these statements at a point that best reflected their perceived alertness.

2.3.8. The subjective sleep assessment questionnaire

A self-report questionnaire used for the subjective assessment of sleep consists of three 10-point scales that measure participants' subjective perception of how deeply they slept, the number of times they woke during the night, and sleep quality.

2.3.9. Munich Chronotype Questionnaire

The Munich ChronoType Questionnaire (MCTQ) [33] is a questionnaire designed to determine chronotypes and quantitatively estimate the timing of sleep within a 24-h day separately for working days and days off. Participants were asked about their sleep habits during normal conditions (eg, without parties): what time they go to bed and wake up, as well as how much time they need to fall asleep and how long they stay in bed after waking up. In addition, we asked how much time they spend outside during the day. Additionally, latency time, sleep onset, and the midpoint of sleep were counted.

2.3.10. Anxiety and depression assessment instruments

The State-Trait Anxiety Inventory (STAI) and the Beck Depression Inventory-II (BDI) were used to assess anxiety and depression, respectively.

STAI is a 40-item self-reported scale questionnaire that is divided into two parts to differentiate state (S-Anxiety) and trait (T-anxiety) anxiety. While answering S-Anxiety questions, a

Table 1
Multiple reaction monitoring transitions and the optimum LC-MS/MS conditions for the analytes.

Analyte	Q1 Mass (Da)	Declusteringpotential, Volts	Quantitative MRM		Qualitative MRM		Retention time, min
			Q3 Mass (Da)	CE, Volts	Q3 Mass (Da)	CE, Volts	
Melatonin	233.1	35	174.1	20			3.1
Melatonin-d7	240.1	35	178.1	20			3.1
Cortisol	363.2	49	121.0	31	345.5	31	3.7
Cortisol-d4	367.2	49	121.0	21	349.2	21	3.7

Note. Q1, first quadrupole; Q3, third quadrupole; Rt, retention time; DP, declustering potential; CE, collision energy.

participant evaluates each item on a 4-point scale ranging from 'not at all' to 'very much so'. These items describe the intensity of anxiety at the current moment. While answering T-Anxiety questions, a participant evaluates each item on a 4-point scale ranging from 'almost never' to 'almost always.' These items describe how often, in general, a participant experiences an anxiety state [34].

The BDI is a 21-item self-reported scale questionnaire that is used to evaluate the severity of depression. Each item is evaluated on a 4-point scale ranging from 0 to 3. Based on the summed scores, four levels of depression are differentiated: minimal depression, mild depression, moderate depression, and severe depression [35].

2.3.11. Statistical analysis

The data analyses were performed using Statistica 10 software (Stat Soft. Inc., Tulsa, OK, USA). The data was evaluated for a normal distribution using the Shapiro–Wilks W test. The homogeneity of individual blood glucose responses to the OGTT, was investigated by hierarchical cluster analysis [36]. Each subject was characterized by two values of AUC: in control and in suppression conditions. During the hierarchical cluster analysis, the Euclidean distance was used as a measure of the proximity of the sets of characteristics of two participants in the two-dimensional feature space, while the Ward method was used as a 'rule of amalgamation' clusters. As a result, a cluster dendrogram was constructed, where subjects characterized by a similar set of parameters were located on the same branch and where the distance to the node uniting them is small. K-means cluster analysis was carried out to clarify the cluster members.

A repeated-measures analysis of variance (ANOVA) was conducted to examine differences in glucose and night melatonin concentrations. For post hoc analyses, the Newman–Keuls test was performed. Independent t-test was performed to compare anxiety and depression scores. Mann–Whitney and Wilcoxon tests were used to compare data that were not normally distributed. Fisher's exact test was done to assess seasonal differences in the schedule of experimental sessions. Pearson correlation analysis was conducted to assess the correlations between blood glucose concentrations and sleep variables. Statistical significance was set at $p < 0.05$.

After dividing the entire sample into two groups according to the cluster analysis results, small sample size was a potential concern. Therefore using free software G*Power 3.1 we calculated the appropriate effect size estimate for all tests performed and interpreted these estimates as follows: d value of 0.20 indicates a small effect, of 0.50 indicates a medium-sized effect, and of 0.80 indicates a large effect; f value of 0.1, 0.25, and 0.4 indicates a small, medium, and large effects, respectively [37].

3. Results

3.1. Blood glucose

Morning baseline concentrations of blood glucose did not differ between experimental conditions. Following glucose intake, the postprandial rise in blood glucose was increased after SWS suppression compared with the control session. There was a significant difference in blood glucose AUC [$F(1,19) = 4.7604$, $p = 0.042$, $f = 0.501$; Fig. 2a]. However, in both the control and suppression conditions, individual data showed wide variations in blood glucose levels during the OGTT. Therefore, we conducted a hierarchical cluster analysis to identify statistically significant types of individual reactions to the carbohydrate load and its changes after SWS suppression. The cluster analysis was based on blood glucose AUC values in control and suppression conditions. The results are shown as a dendrogram (Fig. 2b). They suggest that the study sample was heterogeneous and consisted of at least two groups in which the glucose tolerance was significantly different. According to the hierarchical cluster analysis results, the participants were divided into two groups with different blood glucose responses to the OGTT [ANOVA main effect for the groups X condition X blood glucose $F(2,36) = 3.8671$, $p = 0.030$, $f = 0.464$; Fig. 2c]. A post hoc analysis of between and within-group differences, indicated that the first group, who we called 'non-responders' to the OGTT ($N = 9$), had low blood glucose levels on the OGTT in both the control and suppression conditions. After the night with SWS suppression, they showed only modest and insignificant increase in blood glucose concentrations 2 h after an oral glucose load ($p = 0.127$). In contrast, the second group ($N = 11$), 'responders', even in a control condition, had an evident rise in blood glucose on the OGTT (for 1 h, $p = 0.002$, and for 2 h, $p = 0.006$, relative to the first group). After the night with SWS suppression, they showed a further increase in postprandial blood glucose 1 h after the glucose load ($p = 0.007$ when compared to the control condition, and $p < 0.001$ when compared to the non-responders group). Responders and non-responders did not differ in their BMI (Supplementary Table S1), but there was a marginally significant ($p = 0.076$) difference in age: mean age of responders was 23.09 ± 1.87 years and mean age of non-responders was 21.67 ± 1.41 years.

3.2. Sleep architecture

Table 2 summarizes the measures of sleep quality and quantity for the SWS suppression and control conditions in the overall sample, and in two groups with different blood glucose responses to the OGTT. In the entire group, there were significant differences in the amount of SWS, during stage 1 and stage 2, in the two experimental conditions. As expected, according to the experimental protocol, SWS suppression reduced the time spent in SWS by 53.1 min ($p < 0.0001$), which was 50% of the amount of SWS in the control condition. Suppression also led to a decrease in SWA ($p = 0.011$). Additionally, we observed a 12.3 min increase in time spent in stage 1 sleep ($p = 0.001$) and a 27.8 min increase in the duration of stage 2 ($p = 0.006$). Although TST, SPT, and WASO were comparable between both conditions, SWS suppression led to a decline in sleep efficiency ($p = 0.040$) and to an increase in AI ($p < 0.001$).

An analysis of group differences in the control conditions indicated, in responders, increased sleep latency ($p = 0.002$) and lower

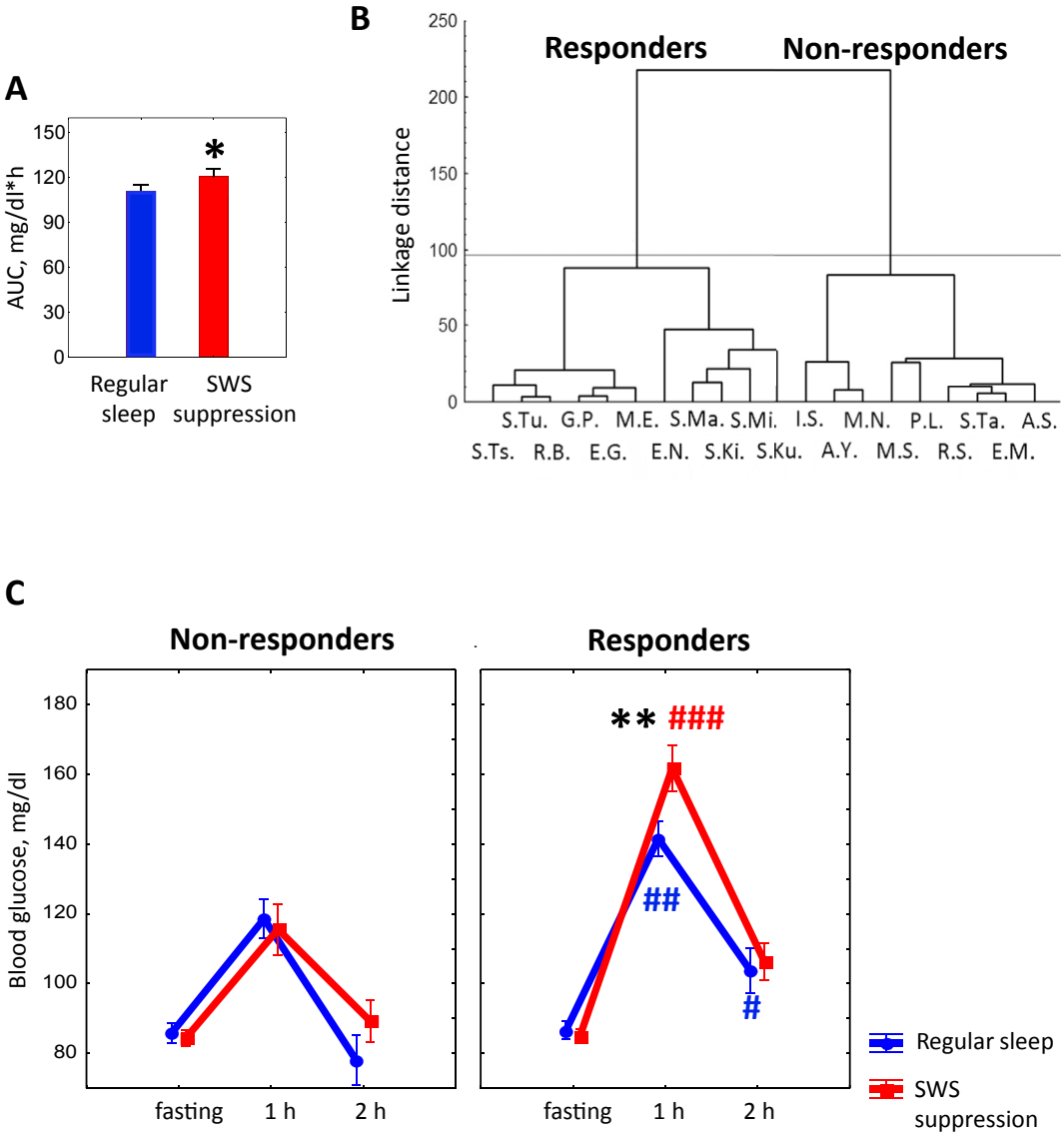

Fig. 2. Changes in blood glucose response to an oral glucose tolerance test. (A) Area under the blood glucose curve (AUC) in the entire sample in control (blue bar) and slow-wave sleep (SWS) suppression (red bar) conditions. (B) The dendrogram representing hierarchical cluster analysis results. (C) Blood glucose response to the oral glucose tolerance test in responders and non-responders in control (blue line) and SWS suppression (red line) conditions. The data shown in A and C are means ± SEM. * indicates a significant between-condition difference. # indicates a significant between-group difference: blue #, in control condition; red #, in SWS suppression condition (ANOVA). #, **/##, ### represent significance $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

TST (p = 0.007), SPT (p = 0.004), and REM sleep (p = 0.001) duration when compared with non-responders. During the night with SWS suppression, in addition to the reduced TST (p = 0.007) and REM sleep (p = 0.020), responders showed increased REM sleep latency (p = 0.003), a greater amount of WASO (p = 0.016) and stage 1 (p = 0.025) and lower sleep efficiency (p = 0.017), indicating more disturbed sleep than in non-responders. Notably, in both groups, SWS suppression led to a similar reduction in SWS. Moreover, despite substantially different levels of glucose tolerance, there were no differences between responders and non-responders in SWS duration and SWA, in either the control or suppression conditions.

Furthermore, we compared polysomnographic data of responders and non-responders, calculated separately for the three parts of the night: early night (from 23:00 to 01:30 h), middle night (from 01:30 to 04:00 h), and late night (from 04:00 to 07:00 h), which included the time periods preceding saliva sampling at 01:30, 04:00, and 07:00 h. In responders and non-responders, SWS and REM sleep were distributed differently during the night (Fig. 3). In the control condition, responders had more SWS during the middle night phase (p = 0.027, d = 1.000), and less REM during middle (p = 0.028, d = 1.003) and late (p = 0.006, d = 1.188) night compared to non-responders. In the suppression condition, responders showed an increased duration of WASO during the middle night phase (p = 0.044, d = 1.000) and a greater amount of SWS (p = 0.047, d = 0.900) and shorter duration of REM (p = 0.009, d = 1.200) during the late night phase. In general, responders were characterized by a more uniform distribution of SWS and REM sleep during the night, especially in the suppression condition. In contrast, the non-responders data showed crucial differences in their distribution, with SWS concentrated primarily during early night and REM during late night.

Table 2
Mean values of polysomnographic data and subjective scores of sleep in control sessions and in sessions with SWS suppression.

	Entire sample (N = 20)			Non-responders (N = 9)				Responders (N = 11)				Intergroup differences			
	Regular Sleep	SWS suppression	P	Regular Sleep	SWS suppression	P	d	Regular Sleep	SWS suppression	P	d	Regular Sleep		SWS suppr.	
												P	d	P	d
Total sleep time (min)	422.80 ± 30.44	412.80 ± 42.83	0.502	442.61 ± 17.69	435.56 ± 23.98	0.767	0.318	406.59 ± 29.46	394.18 ± 46.67	0.657	0.332	**0.010**	1.183	**0.009**	0.966
Sleep period time (min)	460.28 ± 18.11	459.15 ± 22.49	0.629	472.00 ± 13.14	466.78 ± 9.66	0.483	0.371	450.68 ± 16.12	452.91 ± 28.12	0.328	0.070	**0.006**	1.177	0.287	0.617
WASO (min)	37.23 ± 23.59	46.18 ± 24.64	0.052	28.94 ± 11.20	32.39 ± 16.71	0.767	0.433	44.00 ± 29.06	57.46 ± 24.87	**0.045**	0.623	0.621	0.638	**0.018**	1.017
Sleep efficiency %	0.92 ± 0.05	0.90 ± 0.06	**0.044**	0.94 ± 0.02	0.93 ± 0.04	0.859	0.401	0.90 ± 0.06	0.87 ± 0.06	**0.026**	0.627	0.732	0.800	0.053	1.000
Sleep onset latency (min)	18.73 ± 15.12	17.83 ± 20.64	0.913	9.11 ± 5.19	11.83 ± 9.57	0.776	0.433	26.59 ± 16.18	22.73 ± 26.03	0.477	0.081	**0.004**	1.090	0.328	0.528
Latency of REM (min)	93.10 ± 41.94	110.98 ± 57.41	0.211	78.72 ± 35.28	72.72 ± 27.51	0.314	0.273	104.86 ± 44.80	142.27 ± 57.13	**0.033**	0.757	0.080	0.623	**0.004**	1.211
Stage 1 (min)	16.75 ± 9.20	29.05 ± 16.46	**0.001**	13.78 ± 8.05	19.94 ± 12.26	0.051	0.785	19.18 ± 9.73	36.50 ± 16.09	**0.009**	1.055	0.196	0.587	**0.025**	1.006
Stage 2 (min)	203.73 ± 30.39	231.53 ± 29.42	**0.006**	211.56 ± 30.81	241.94 ± 22.83	**0.011**	1.171	197.32 ± 29.93	223.00 ± 32.42	0.091	0.608	0.287	0.469	0.149	0.644
SWS (min)	106.40 ± 26.38	53.30 ± 20.93	<**0.001**	102.61 ± 29.56	55.39 ± 25.66	**0.008**	2.098	109.50 ± 24.49	51.59 ± 17.27	**0.003**	2.726	0.382	0.261	0.621	0.182
REM (min)	89.73 ± 21.95	89.58 ± 31.30	0.985	105.83 ± 19.78	108.11 ± 26.96	0.722	0.124	76.55 ± 13.33	74.41 ± 26.79	0.722	0.095	**0.002**	1.333	**0.023**	1.077
AI	11.25 ± 7.80	17.10 ± 9.79	<**0.001**	10.40 ± 9.44	14.43 ± 8.15	**0.008**	0,462	11.95 ± 6.56	19.29 ± 10.83	**0.003**	1178	0.149	0,199	0.095	0,496
SWA (μV^2)	151.28 ± 129.13	116.56 ± 91.91	**0.011**	173.47 ± 182.10	137.17 ± 122.32	0.138	0.293	133.13 ± 65.91	99.71 ± 58.21	0.062	0.607	0.323	0.312	0.939	0.408
N Sounds early	–	420.60 ± 302.01	–	–	528.56 ± 359.29	–	–	–	332.27 ± 225.74	-	–	–	–	0.153	0.649
N Sounds middle	–	431.75 ± 357.97	–	–	496.44 ± 281.25	–	–	–	378.82 ± 416.34	-	–	–	–	0.480	0.329
N Sounds late	–	313.05 ± 249.02	–	–	299.89 ± 287.66	–	–	–	323.82 ± 226.58	-	–	–	–	0.837	0.096
Subjective deepness of sleep	7.53 ± 1.84	6.60 ± 2.06	0.063	7.56 ± 1.94	7.56 ± 1.21	0.800	<0.001	7.50 ± 1.84	5.82 ± 2.32	**0.021**	0.909	0.899	0.032	0.113	0.845
Subjective N of awakenings	2.18 ± 1.26	3.28 ± 2.30	0.211	2.11 ± 1.27	2.94 ± 2.04	0.310	0.369	2.25 ± 1.32	3.55 ± 2.55	0.401	0.440	0.777	0.111	0.443	0.265
Subjective sleep quality	7.89 ± 1.37	6.60 ± 1.96	**0.028**	8.33 ± 1.12	7.00 ± 2.12	0.059	0.698	7.50 ± 1.51	6.27 ± 1.85	0.208	0.518	0.228	0.605	0.419	0.372

Note. Data are mean values ± standard deviation. WASO, wakefulness after sleep onset; SWS, slow-wave sleep; REM, rapid eye movement sleep; AI, arousal index (per total sleep time hour); SWA, slow wave activity; N Sounds early, N Sounds middle, N Sounds late, number of sounds presented to suppress SWS during the early, middle and late parts of the night. P-values for within-group and intergroup differences are derived from the Wilcoxon's matched pairs test and from the Mann–Whitney U test, respectively. d, effect size estimate Cohen's d. Significant differences are highlighted in bold.

Actigraphy-estimated sleep duration in the nights before each experimental session was similar in responders and non-responders (Supplementary Table S1), thus, their differences in sleep architecture were not caused by sleep debts.

We also compared responders and non-responders on a number of sounds, which were presented to suppress SWS (Table 2). The number of sounds was counted separately for early night, middle night, and late night. The number of sounds in each part of the night was comparable in both groups. These data confirm that the degree and timing of acoustic sleep disturbances were similar between responders and non-responders.

Among subjective assessments of sleep characteristics (Table 2), in the entire sample, there were significantly lower scores of sleep quality after the night with SWS suppression when compared to the control night ($p = 0.028$). Responders in the suppression condition rated their sleep as more shallow than in the control condition ($p = 0.020$). There were no significant differences between responders and non-responders in their subjective assessments of sleep.

In order to identify more clearly the links between morning glucose tolerance and sleep quality Pearson correlation analysis was performed. Correlations between blood glucose and sleep parameters are summarized in Table 3. The blood glucose AUC and blood glucose 1 h after the glucose load showed similar correlations with polysomnographic data. Positive correlations were found with WASO (0.417, $p = 0.007$ and 0.313, $p = 0.049$ for glucose AUC and glucose 1 h, respectively) and time spent in stage 1 sleep (0.345, $p = 0.029$ and 0.448, $p = 0.004$), as well as with sleep onset latency (0.410, $p = 0.009$ and 0.393, $p = 0.012$) and REM latency (0.498, $p = 0.001$ and 0.535, $p < 0,001$). Negative associations were found with TST (−0.496, $p = 0.001$ and −0.456, $p = 0.003$), SPT (−0.382, $p = 0.015$ and −0.421, $p = 0.007$), sleep efficiency (−0.450, $p = 0.004$ and −0.355, $p = 0.024$) and REM duration (−0.500, $p = 0.001$ and −0.513, $p = 0.001$). Blood glucose 2 h after the glucose load was positively associated with WASO (0.390, $p = 0.013$), and negatively – with TST (−0.357, $p = 0.024$) and sleep efficiency (−0.399, $p = 0.011$). There were no significant correlations between time spent in SWS and glucose data.

3.3. Melatonin data

In the entire group, in the morning sample collected at 07:00 h, immediately after wakening, melatonin was significantly higher in the session with SWS suppression compared to the control session ($p = 0.011$, data not shown). A comparison of the two groups with different blood glucose responses showed significant differences between them at 07:00 h ($p = 0.013$, $d = 1.114$) and 07:40 h ($p = 0.038$, $d = 0.802$) in the suppression condition: responders had higher melatonin levels than non-responders (Fig. 4a). An analysis of within-group differences showed only in responders in the suppression condition, was there a significant increase in melatonin in the morning (ie, at 07:00 h, $p = 0.010$, $d = 1.238$), while in non-responders, SWS suppression led to an increase in early night melatonin (ie, at 01:30 h, $p = 0.012$, $d = 2.231$). Furthermore, in both conditions, the two groups had different dynamics of salivary melatonin during the night: non-responders had higher melatonin levels at 01:30 h than at 04:00 h; in contrast, responders had higher melatonin levels at 04:00 h than at 01:30 [$F(1,18) = 11.185$, $p = 0.004$, $f = 0.788$ for the ANOVA group X time effect]. In both sessions, in responders and non-responders evening salivary melatonin concentrations were similar, indicating comparable dim-light melatonin onset. There were no significant seasonal differences in the schedule of their experimental sessions (Supplementary Table S1). This suggests that between-group differences were not influenced by day length.

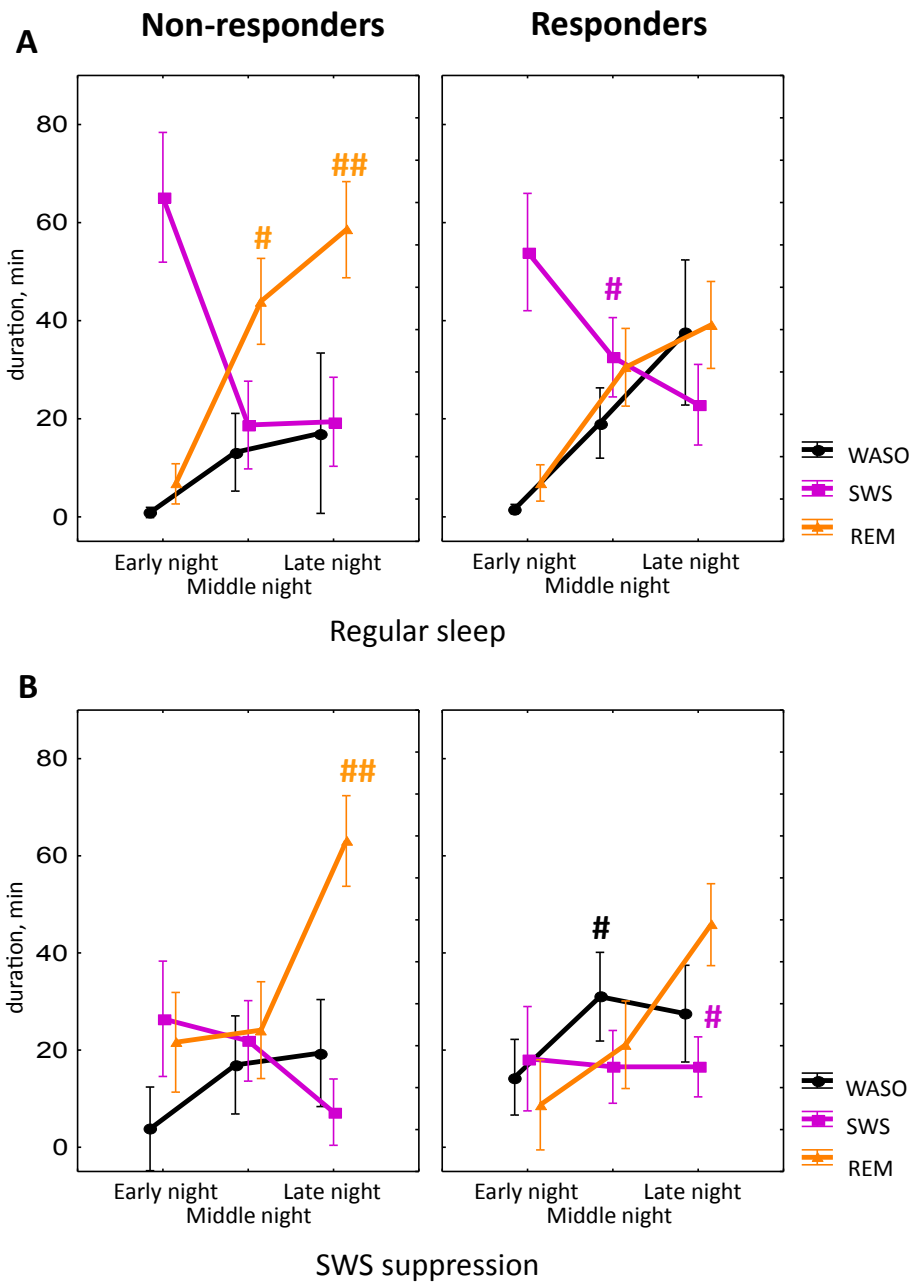

Fig. 3. Durations of REM sleep, SWS and WASO in responders and non-responders in control (A) and SWS suppression (B) condition. The data are mean duration ± SEM of slow-wave sleep (SWS, purple line), rapid eye movement sleep (REM, orange line) and wake after sleep onset (WASO, black line) during the three parts of the night: early night (from 23:00 to 01:30 h), middle night (from 01:30 to 04:00 h), and late night (from 04:00 to 07:00 h). # indicates a significant between-group difference (independent t-test): purple #, in duration of SWS, orange #, REM and black #, WASO. #, ##, represent significance $p < 0.05$, $p < 0.01$, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Cortisol data

SWS suppression did not have any effect on salivary cortisol in the responders group (Fig. 4b). In non-responders, it resulted in a decreased cortisol concentration at 07:40 h ($p = 0.017$, $d = 1.024$). However, a comparison of the two groups in the suppression condition showed higher cortisol levels at 07:00 h in non-responders ($p = 0.019$, $d = 1.155$). In general Fig. 4b suggests, that morning rise in cortisol in non-responders begins earlier than in responders.

3.5. Munich Chronotype Questionnaire

Considering the different melatonin and cortisol profiles in responders and non-responders, we compared chronotypes and sleep habits in these two groups using the Munich Chronotype Questionnaire. We analyzed the following MCTQ variables: bedtime, sleep preparation time, sleep latency, sleep end, sleep inertia, sleep duration, sleep onset, the midpoint of sleep, and light exposure. The results of the Mann–Whitney U test are summarized in Table 4. The only significant differences between responders and

Table 3
Correlations between the oral glucose tolerance test response and sleep parameters.

	AUC	Fasting glucose	Glucose 1 h	Glucose 2 h
Total sleep time (min)	**−0.496**	−0.033	**−0.456**	**−0.357**
	p = .001	p = 0.839	**p = .003**	**p = .024**
Sleep period time (min)	**−0.382**	−0.049	**−0.421**	−0.185
	p = .015	p = 0.763	**p = ,007**	p = 0.253
WASO (min)	**0.417**	0.024	**0.313**	**0.390**
	p = .007	p = 0.883	**p = .049**	**p = .013**
Sleep efficiency %	**−0.450**	−0.017	**−0.355**	**−0.399**
	p = .004	p = 0.915	**p = .024**	**p = .011**
Sleep onset latency (min)	**0.410**	0.064	**0.393**	0.274
	p = .009	p = 0.693	**p = .012**	p = 0.088
Latency of REM (min)	**0.498**	−0.002	**0.535**	0.260
	p = .001	p = 0.991	**p = .000**	p = 0.106
Stage 1 (min)	**0.345**	−0.039	**0.448**	0.082
	p = .029	p = 0.811	**p = .004**	p = 0.614
Stage 2 (min)	−0.150	−0.311	−0.141	−0.105
	p = 0.355	p = 0.051	p = 0.386	p = 0.521
SWS (min)	−0.163	0.243	−0.160	−0.104
	p = 0.316	p = 0.131	p = 0.325	p = 0.524
REM (min)	**−0.500**	0.102	**−0.513**	−0.291
	p = .001	p = 0.532	**p = .001**	p = 0.069

Note. Data are Pearson correlation coefficients and P-values. N = 40 (data of 20 subjects in two sessions). AUC, area under the blood glucose curve. Fasting glucose, blood glucose measured after an overnight fast; Glucose 1hr, blood glucose measured 1 h after consuming 75-g glucose; Glucose 2 h, blood glucose measured 2 h after consuming 75-g glucose. WASO, wakefulness after sleep onset; SWS, slow-wave sleep; REM, rapid eye movement sleep. Significant correlations are highlighted in bold.

non-responders were in sleep latency during working days ($p = 0.046$, $d = 1.058$) and in sleep duration during days off ($p = 0.043$, $d = 0.948$). In comparison to non-responders, it took more time for responders to fall asleep during working days, and they slept more during days off.

3.6. Heart rate data

An analysis of HRV parameters showed (Fig. 4c) that in the responders group SWS suppression led to rise in LF/HF; increased LF/HF was found during SWS in all the analyzed sleep cycles ($p = 0.026$, $d = 1.271$; $p = 0.013$, $d = 1.019$; and $p = 0.016$, $d = 0.957$ for the first, second, and third sleep cycles, respectively). In the non-responders group, this effect was not observed. Moreover, in the morning after SWS suppression, non-responders showed a decrease in LF/HF ($p = 0.008$, $d = 3.868$). Compared with responders, in SWS suppression condition they had significantly lower LF/HF during the second sleep cycle ($p = 0.028$, $d = 1.839$) and after awakening ($p = 0.008$, $d = 1.919$). There were no significant effects of SWS suppression on other analyzed HRV parameters: heart rate, SDNN, rMSSD, pNN50, VLF, LF, and HF.

3.7. Subjective assessments of sleep and sleepiness data

Selective SWS suppression had no significant effect on sleepiness according to data from the VASS and SSS. The data are shown in Table 5.

3.8. Anxiety and depression assessment

The independent t-test was used to compare the data of the STAI and BDI in both groups. The results showed no significant differences in symptoms of anxiety and depression between responders and non-responders (Supplementary Table S2).

4. Discussion

Suppression reduced SWS duration by 50%. At the entire sample level, SWS suppression was followed by a significant decrease in morning glucose tolerance, according to the glucose AUC data. However, we found different effects of SWS suppression depending on the individual participant's blood glucose response to the OGTT. Responders already in control condition, had low glucose tolerance compared with non-responders, and after SWS suppression, it declined further. Non-responders had high glucose tolerance in both the control and suppression conditions.

These two groups differed significantly in their sleep architecture characteristics. Responders, even in control condition, showed a lower TST, SPT, and duration of REM sleep compared to non-responders. During SWS suppression, they had a further decrease in sleep quality and quantity, showing a rise in the amount of WASO and NREM stage 1, and a decline in sleep efficiency. Hence, we may assume that the responders' sleep was more vulnerable to the disturbances than that of non-responders. Notably, responders and non-responders had a comparable amount of SWS in both the control and suppression conditions. At this point, our data conflicts with a number of previous investigations showing that, SWS plays an important role in the regulation of insulin sensitivity and glucose tolerance [6,7]. Apparently, glucose tolerance is more closely associated with the total duration of sleep and its continuity, than with the amount of SWS. A somewhat unexpected result is that responders and non-responders differed significantly in REM sleep duration and latency.

In non-responders, SWS suppression resulted in decreased cortisol concentrations at 07:40 h. On the other hand, at 7:00 their cortisol level was significantly higher than those of responders. In the responders group, SWS suppression did not have any effect on salivary cortisol. Therefore, we may conclude that cortisol was not responsible for impaired glucose tolerance in responders.

An analysis of HRV parameters, showed that in the responders group, SWS suppression prevented the physiological nighttime decrease of sympathetic activity and led to increased LF/HF in all analyzed sleep cycles. In non-responders, this effect was not pronounced. Furthermore, after awakening, they had a significant decrease in LF/HF compared to the control session. Sustained sympathetic activation could lead to a significant decrease in glucose tolerance due to such effects as arteriolar vasoconstriction [38] and lipolysis induction [39], both of which are accompanied by a decrease in glucose disposal. However, it is unlikely that sympathetic activation was the only cause of impaired glucose tolerance in responders, considering that in the morning after SWS suppression, their level of LF/HF was comparable with those after regular sleep.

We show that SWS suppression leads to an increase in salivary melatonin. At this point, our data is in line with previous observations of Salin-Pascual et al. [40], Ackermann et al. [24], Davies et al. [41], and Zeitzer et al. [23], who found an increase in melatonin levels during sleep deprivation and during extended wakefulness. However, an analysis of group differences revealed that responders and non-responders differed in melatonin secretion timing. In both conditions, the two groups had different dynamics of salivary melatonin during the night: non-responders had higher melatonin levels at 01:30 h than at 04:00 h; in contrast, responders had higher melatonin levels at 04:00 h than at 01:30 h. Moreover, in the suppression condition melatonin levels increased at different time points in responders and non-responders. Non-responders showed increased melatonin at its peak time (at 01:30) and responders had a significant rise in melatonin in the morning upon waking up, after its peak time, which was at 04:00. Unfortunately, the small number of saliva samples we collected at night were not

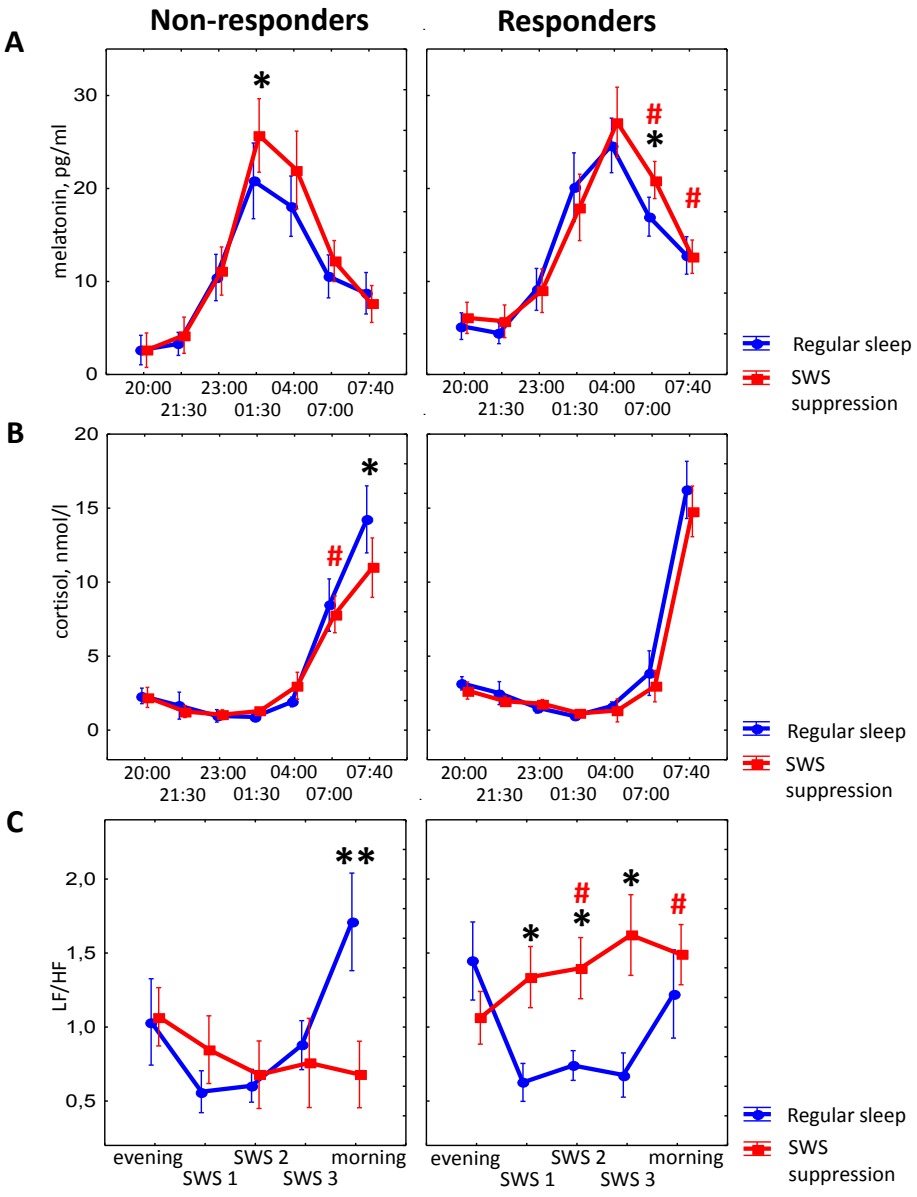

Fig. 4. Melatonin (A) and cortisol (B) secretion and sympathovagal balance (C) in responders and non-responders in control and slow-wave sleep suppression condition. The data are means ± SEM in control (blue line) and slow-wave sleep (SWS) suppression (red line) conditions. LF/HF, ratio of low frequency and high frequency of heart rate variability. SWS1, SWS2, SWS3, periods of slow-wave sleep in the first, second and third sleep cycles, respectively. * indicates a significant between-condition difference (Wilcoxon's matched pairs test). # indicates a significant between-group difference (Mann–Whitney U test): blue #, in control condition; red #, in SWS suppression condition. */#, **, represent significance $p < 0.05$, $p < 0.01$, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Mean Values of the Munich Chronotype Questionnaire data.

	Working days				Days off			
	Non-responders	Responders	P	d	Non-responders	Responders	P	d
Bedtime	24.29 ± 0.33	23.93 ± 0.39	0.083	0.900	24.69 ± 0.8	1 ± 0.71	0.594	0.419
Sleep preparation time	24.53 ± 0.29	24.3 ± 0.32	0.120	0.625	24.93 ± 0.76	1.34 ± 0.66	0.399	0.569
Sleep latency	0.18 ± 0.1	0.36 ± 0.2	**0.046**	1.058	0.16 ± 0.1	0.32 ± 0.21	0.110	0.889
Sleep onset	24.71 ± 0.27	24.65 ± 0.42	0.824	0.171	1.1 ± 0.74	1.66 ± 0.65	0.307	0.767
Midpoint of sleep	4.33 ± 0.53	4.49 ± 0.35	0.168	0.372	5.12 ± 1	5.97 ± 0.8	0.198	0.876
Sleep end	8 ± 0.8	8.32 ± 0.55	0.267	0.353	9.16 ± 1.29	10.3 ± 1.03	0.143	0.904
Sleep inertia	0.21 ± 0.24	0.24 ± 0.21	0.594	0.136	0.23 ± 0.12	0.38 ± 0.33	0.657	0.556
Sleep duration	7.25 ± 0.59	7.67 ± 0.7	0.198	0.627	8.07 ± 0.61	8.62 ± 0.45	**0.043**	0.948
Light exposure	1.34 ± 0.79	2.08 ± 1.16	0.168	0.705	2.18 ± 1.18	2.88 ± 1.32	0.267	0.551

Note. Data are mean values ± standard deviation. Decimal time is used. P-values are derived from the Mann–Whitney U test. d, effect size estimate Cohen's d. Significant differences are highlighted in bold.

Table 5
Mean values of the SSS and VASS.

	Scale	Regular Sleep				SWS suppression			
		Evening	Morning	P	d	Evening	Morning	P	d
Entire sample	SSS	3.06 ± 0.87	2.88 ± 1.23	0.529		3.00 ± 1.00	2.84 ± 1.07	0.572	
	VAS	4.57 ± 2.10	3.59 ± 2.08	0.081		4.86 ± 1.69	3.74 ± 1.87	0.115	
Non-responders	SSS	3.00 ± 0.76	3.00 ± 1.20	0.933	<0.001	3.25 ± 1.04	3.13 ± 1.25	0.735	0.086
	VAS	4.52 ± 1.82	3.13 ± 1.99	0.116	0.813	4.67 ± 2.06	3.83 ± 1.88	0.463	0.306
Responders	SSS	3.10 ± 0.99	2.80 ± 1.32	0.345	0.277	2.82 ± 0.98	2.64 ± 0.92	0.673	0.174
	VAS	4.61 ± 2.37	3.90 ± 2.20	0.398	0.270	4.98 ± 1.53	3.68 ± 1.97	0.114	0.638

Note. Data are mean values ± standard deviation. P-values are derived from the Wilcoxon's matched pairs test. d, effect size estimate Cohen's d. SSS, the Stanford Sleepiness Scale; VASS, the Visual Analog Sleepiness Scale; SWS, slow-wave sleep.

enough to clearly identify the melatonin rhythm's acrophase. Nevertheless we can assume that responders had a delayed melatonin rhythm when compared to non-responders, and after SWS suppression, it was delayed even more. In this regard, changes in melatonin release in the responders group are in line with the data of Cajochen et al. [42], who reported a phase delay in the melatonin rhythm after one night of sleep deprivation.

Apparently, the features of melatonin secretion in the two groups contributed to different effects of SWS suppression on glucose tolerance. The data on the role of melatonin in the control of carbohydrate metabolism is controversial [43]. Both inhibitory [44] and stimulatory [45,46] effects of melatonin on insulin secretion have been reported, but in light of recent data, different effects of this hormone on glucose tolerance can be attributed to immediate and prolonged actions of melatonin [47]. First, melatonin, like any other hormone, acts through its receptors. There are two types of membrane melatonin receptors: 1A and 1B. Both these receptors act primarily by interfering with the formation of cAMP through inhibitory Gi proteins [43]; thus, the immediate consequence of melatonin interacting with molecular effectors is an inhibited insulin release via the reduced formation of cAMP. Second, in addition to this classical hormonal way of action, melatonin also has prospective effects. The latter are not seen during the night when melatonin is being released; instead, they are evident during the day, triggered by the cessation of melatonin production. One example is cAMP/PKA/CREB pathway hypersensitization [48]. After nocturnal sustained adenylyl cyclase inhibition induced by melatonin through its Gi-protein coupled receptors, the cessation of the inhibitory signal leads to an increased response to any agonists that activate adenylyl cyclase, and consequently to increased insulin secretion. Another example of melatonin's prospective effects, is its action on the transcription or translation of the clock genes and the clock-controlled genes [49,50], including those involved in the regulation of carbohydrate metabolism.

Hence, we may assume that impaired glucose tolerance after SWS suppression in responders, was a result of elevated morning melatonin and its immediate inhibitory effect on insulin release. Although melatonin decreased 40 min after waking up, it was still higher than in non-responders and could negatively impact glucose tolerance. In contrast, in non-responders, SWS suppression led to an early-night melatonin increase, so its level declined before awakening and did not adversely affect glucose tolerance. Moreover, given the early melatonin peak time, we can suppose that in non-responders in the morning, prevail the prospective effects of this hormone, leading to an increase in insulin secretion and action, and providing a consistently high glucose tolerance in both conditions.

Why did SWS suppression lead to a rise in melatonin level? Unfortunately, how sleep quality affects melatonin secretion remains largely unexplored. Therefore, we can only refer to the data obtained in total sleep deprivation studies. Metabolic profiling during total sleep deprivation, conducted by Davies and colleagues [41], showed a significant increase not only in melatonin, but also in tryptophan, serotonin, and taurine levels compared with those during undisturbed sleep. Tryptophan is vital to the formation of serotonin and melatonin via the indoleamine pathway, and taurine has been shown to increase pineal melatonin by stimulating the activity of its rate-limiting biosynthetic enzyme, N-acetyltransferase [51]. Therefore, increased tryptophan, serotonin, and taurine could explain elevated melatonin production during sleep deprivation. Since SWS suppression led to an increase in the duration of wakefulness and stage 1 sleep in our study, consequent growth in the amount of melatonin's precursors, tryptophan and serotonin, and its synthesis catalyst, taurine, could cause the rise in melatonin levels.

One wonders why, in both non-responders and responders in the SWS suppression condition, circulating melatonin increased at different time points. Possibly, low melatonin in the latter part of the night in non-responders could be explained by a feature of their sleep architecture, such as long-duration REM sleep. REM sleep which usually predominates in the latter part of the night, is a brain state characterized by very low serotonin levels – even lower than in SWS [52]. Hence, a decline in late night melatonin in non-responders might be interpreted as the consequence of a lack of its immediate precursor. Responders had less REM sleep than non-responders, particularly during the latter part of the night, and during SWS suppression in the middle part of the night, they had more WASO. In combination, the shorter duration of REM sleep and the longer duration of wakefulness in the middle and late night, may contribute to the shift of peak melatonin secretion towards the morning hours. Therefore, we may conclude that individual differences in melatonin release are associated with features of sleep architecture, such as the distribution of periods of NREM and REM sleep throughout the night, and the duration of REM sleep and awakenings.

What determines the sleep architecture differences in non-responders and responders? Considering the different melatonin and cortisol profiles in responders and non-responders, we compared chronotypes and sleep habits in these two groups. However according to the MCTQ data, both groups had comparable chronotype characteristics, so circadian rhythms cannot explain their differences in sleep patterns. Nor can we attribute variations in sleep structure to individual types of stress response caused by SWS suppression. Because cortisol and sleepiness scale data indicate that our participants did not experience stress during SWS disruption, nor did they report increased sleepiness the next morning. Furthermore, we did not find any significant differences between non-responders and responders on anxiety and

depression scores. Hence, the exact mechanisms underlying the observed individual variance in sleep architecture requires further study, but since differences between non-responders and responders were observed in both experiments, we assume that they are related to some constant features of initiating sleep and sequencing its architecture. In this regard our work has potential clinical implications in the assessment of risk factors for Type 2 diabetes.

Some limitations of the present study should be mentioned. Although the findings clearly demonstrate the importance of sleep architecture for melatonin secretion, the sleep manipulations we applied in the current study only included SWS suppression. SWS plays a crucial role in the secretion of many hormones, so its disruption may have affected several endocrine and physiological processes and exerted an additional unpredictable effect on our results. Therefore, to confirm the associations between sleep architecture and melatonin secretion, further investigations involving REM sleep suppression are needed. A second limitation of our study is the small number of saliva samples we collected, which was not sufficient to clearly identify the melatonin rhythm's acrophase. Third, the sample size was relatively small, especially after dividing the entire sample into two groups of responders and non-responders. Finally, since we did not measure blood insulin concentrations, we were unable to assess the effects of SWS suppression and elevated melatonin on insulin sensitivity, and explore more fully the links between sleep architecture and carbohydrate metabolism. So our findings lay the foundation for a more complete study in the clinic.

5. Conclusions

Our data suggest that one night of SWS suppression led to a significant increase in melatonin levels, and elevated melatonin appears to be a key player in glucose tolerance impairment after disturbed sleep. Depending on the individual secretion timing, melatonin has either immediate or delayed effects on morning glucose tolerance, leading to its decrease or increase, respectively. Furthermore, our data points to an important role in melatonin release of characteristics of sleep architecture, such as the duration of REM sleep and nocturnal awakenings. Collectively, the results indicate that glucose tolerance is associated with the total duration of undisturbed sleep and the length of REM sleep rather than the amount of SWS.

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Author's contributions

Conceived and designed the experiments: U.Yu.V., L.K.M., S.K.A., B.D.A., N.A.N.

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Conflict of interest

The authors declare that the research was conducted in the absence of any financial or non-financial relationships that could be construed as a potential conflict of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2019.11.1254.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2019.11.1254.

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