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Intensifying sleep slow oscillations does not improve metabolic control in healthy men

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ABSTRACT

Impaired sleep quality and sleep loss compromise glucose homeostasis and metabolic function, but the mechanisms linking sleep and metabolic health are largely unclear. In order to gain insight into the relevance of specific electrophysiological sleep characteristics for metabolic control, we assessed the acute effect on glucose homeostasis as well as energy intake and expenditure of enhancing slow oscillatory activity, a hallmark of slow-wave sleep, by closed-loop auditory stimulation in healthy men. Twenty-two young, normal-weight men underwent an oral glucose tolerance test (oGTT), indirect calorimetry and the assessment of ad-libitum breakfast intake in the morning after nocturnal sleep with or without auditory stimulation in phase with the ongoing rhythmic occurrence of slow oscillation up-states during 210 min of slow-wave sleep in the first night-half. Stimulation vs. no stimulation strongly increased slow oscillatory activity without changing overall sleep structure, but did not alter fasting or oGTT-stimulated measures of glucose homeostasis. Food intake and energy expenditure were likewise comparable between conditions. Findings indicate that in healthy humans electrophysiological sleep quality is tuned to allow for optimal metabolic control. Future studies should investigate the potential of sleep stage-specific interventions to enhance metabolic control and well-being in patients with metabolic ailments.

1. Introduction

Sleep impairments affect metabolic function including glucoregulation and add to the impact of more traditional risk factors like obesity and sedentarism (Anothaisintawee et al., 2016; Kim et al., 2017). In healthy young men submitted to an intravenous glucose tolerance test (ivGTT), a reduction in nocturnal sleep duration to four hours for six consecutive days reduced glucose uptake by 40% and impaired the acute insulin response (Spiegel et al., 1999) as well as insulin sensitivity (Leproult and Van Cauter, 2010). Respective reductions in nocturnal sleep for intervals between two and 14 days were found to decrease glucose tolerance and insulin sensitivity in healthy men and women (Buxton et al., 2010; Schmid et al., 2011). Sleep curtailment can also increase food intake (Broussard et al., 2016) and reduce energy expenditure (Benedict et al., 2011). It is largely unclear however which electrophysiological properties of sleep contribute to metabolic control. Slow-wave sleep (SWS), which primarily occurs during the first half of nocturnal sleep, is characterized by slow EEG oscillations of < 1 Hz; it is tightly linked to nocturnal growth hormone release (Born et al., 1988). Notably, SWS has been reported to be reduced in obese subjects and patients with type 2 diabetes independent of sleep-related breathing disorders (Pallayova et al., 2010). In a groundbreaking experimental study in healthy men by Tasali et al. (2007), selectively suppressing SWS by disruptive acoustic signals for three nights (while keeping total sleep time unchanged) reduced ivGTT-measured insulin sensitivity by 25% and increased sympathovagal balance by 14%. These findings were replicated in experiments using an oral glucose-tolerance test (oGTT) that moreover showed that the suppression of rapid eye-movement (REM) sleep, which is primarily found in the second night-half, has no effect on insulin sensitivity

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(Herzog et al., 2013).

We examined the relevance of slow oscillatory activity, the electrophysiological hallmark of SWS, for glucose homeostasis and energy fluxes in healthy young men. We have previously demonstrated that closed-loop auditory stimulation of slow oscillatory activity during SWS not only improves declarative memory consolidation (Ngo et al., 2013) but also modulates sleep-associated endocrine and immune function (Besedovsky et al., 2017). Considering that both of these studies demonstrated positive effects of manipulating a highly specific sleep phenomenon in healthy young subjects, we hypothesized that in a corresponding sample of male subjects with normal glucoregulation and regular eating behavior, stimulating slow oscillatory activity during SWS would improve oGTT-assessed insulin sensitivity and reduce food intake in the subsequent morning.

2. Research design and methods

2.1. Subjects and procedure

Twenty-two young healthy male non-smokers (mean age ± SEM, 24.36 ± 0.80 years, range 19–34 years; mean BMI, 22.51 ± 0.35 kg/m^2, range 19.17–25.03 kg/m^2) participated in the within-subject, crossover-design study. All of them had a normal sleep-wake rhythm, i.e., did not engage in shift work in the preceding six weeks and did not do intercontinental travels in the four weeks preceding the experiment. Subjects and the experimenters performing the metabolic measurements were blinded to the experimental conditions. In anamnestic examinations, we ensured that participants did not display signs of current illness and were currently not using any medication. We also asked subjects about their exercise routines, weekly alcohol and caffeine intake and if they expected any stressors during the period of study participation (e.g., university exams). Subjects were only included if they indicated a low to medium exercise frequency (≤ three times/week of cardio training, but no weightlifting or competitive sports) and moderate alcohol and caffeine consumption (≤ five beers/week and two coffees/day) and did not expect major stressful events. Subjects gave written informed consent to the study that was approved by the local ethics committee and conformed to the Declaration of Helsinki.

Following an adaptation night that included the attachment of electrodes for polysomnographic recordings and wearing in-ear headphones, participants took part in two sessions (auditory stimulation and control; Fig. 1A) in balanced order. The adaptation and the first experimental night were spaced apart by at least 48 h (including one night of nocturnal sleep at home). In order to prevent any carry-over effects of the stimulation, there were at least ten days between the two experimental nights (the median interval was 21 days). On experimental days, participants abstained from napping, strenuous physical activities, caffeine and alcohol intake, and food intake after 1800 h. After their arrival at the sleep lab around 2030 h, we confirmed adherence to these instructions, additionally ruling out any strong psychological stressors during the preceding wake period. Subjects were served a light dinner consisting of a sandwich with cucumber, tomato slices and cervelat sausage, and unsweetened tea at 2000 h. Lights were turned off at 2300 h, when polysomnographic and EEG recordings started. Both experimental conditions (auditory stimulation and control) were performed in a completely identical fashion except for the omission of auditory stimulation in the control condition. Thus, both nights were spent in the same room, with comparable dinner, polysomnography, in-ear headphones, and lights-off and -on times (the latter scheduled as close as possible to 0700 h, but not in REM sleep nor SWS).

2.2. Polysomnography and auditory slow-oscillation stimulation

EEG was continuously recorded with a BrainAmp DC amplifier (Brain Products) from five Ag-AgCl cup electrodes (international 10–20 system: Fz, C3, Cz, C4 and Pz) referenced to linked mastoid electrodes. Electrode impedances were always below 5 kOhm. Electrooculogram (EOG) was obtained from two electrodes placed at the lower right and upper left canthi, and electromyogram (EMG) from electrodes attached to the chin. Signals were acquired with a sampling rate of 500 Hz and stored on a PC together with the stimulation triggers. For offline analysis, EEG recordings and the EOG were preprocessed with a band pass filter of 0.3–30 Hz and the EMG signal with a high-pass filter of 5 Hz. Total sleep time (TST), time spent in different sleep stages (wake after sleep-onset, WASO, sleep stages 1, 2, SWS and REM sleep), and arousal index (calculated as the number of movement epochs divided by the number of epochs) were determined for the whole night as well as for the 210 min stimulation period. Recordings were visually scored offline according to standard criteria (Iber, 2007) and jointly approved by three independent experts.

We stimulated slow oscillatory activity for 210 min according to the closed-loop paradigm described by Ngo and coworkers (2013), starting ~5 min after the participant displayed stable stage 2 (or deeper) sleep. For stimulation, an additional EEG recording system consisting of a D360 EEG amplifier (Digitimer) and a "Power1401 mk 2" high-performance data acquisition interface (Cambridge Electronic Design) connected to a separate PC was used. Prefrontal EEG was recorded from an electrode at AFz (located on the midline centered between Fpz and Fz) that was referenced to the mastoids. The EEG was filtered between 0.25 and 4 Hz and sampled at 200 Hz. A custom-made script running under Spike2 software Version 7 (Cambridge Electronic Design) enabled responding to the incoming EEG signal in real time. As illustrated in Fig. 1B, an auditory stimulus (50-ms "clicks" of pink 1/f noise with 5-ms rising and falling flanks) was triggered whenever a negative slow oscillation half-wave was identified, i.e., a signal crossing an adaptive threshold towards larger negative values with a default value of -80 μV, and presented binaurally via MDR-EX35 in-ear headphones (Sony Deutschland). Every 0.5 s it was updated to the minimal (i.e., largest negative) instantaneous EEG amplitude within the preceding 5-s interval, whenever this value exceeded (in negativity) default value. Upon the detection of a negative slow oscillation half-wave, a first stimulus was delivered after an individually adapted delay to ensure a temporal coincidence with the upcoming slow oscillation up-state. The delay was determined based on the average delay between the slow oscillation negative and positive peaks during the first SWS period of an individual's adaptation night, which across all subjects averaged 0.51 ± 0.01 ms. A second auditory stimulus was applied after a constant delay of 1.075 s followed by a pause of 2.5 s before the next endogenous slow oscillation could be detected. The detection algorithm halted whenever subjects showed arousals or REM sleep. Successful stimulation of slow oscillatory activity was assessed as previously described (Ngo et al., 2013, 2015). First, the immediate EEG response was determined by averaging the EEG signal time-locked to the first stimulus or a corresponding time-point during the control condition with a 4-s window and a 1-s pre-stimulus offset. Second, spectral power was calculated for artifact-free SWS epochs. Each power spectrum was normalized with reference to cumulative power up to 30 Hz to account for inter-individual variability, resulting in a unit of 1/Hz.

2.3. OGTT and blood measurements

We awakened the participants around 0700 h, whenever they entered light non-REM (stage 1 or 2) sleep, and collected a blood sample for the determination of cortisol and all oGTT-related parameters. From 0815 h on, participants underwent a 2-hour, 5-sample oGTT protocol including ingestion of a standardized 75 g glucose solution (Accu-Chek Dextrose, Roche, Switzerland). Blood samples were centrifuged, and samples were stored at −80 °C. Plasma or serum concentrations of glucose, insulin, C-peptide and non-esterified fatty acids (NEFA) were determined in samples taken before the glucose challenge at minute 0, and then at 30, 60, 90 and 120 min. Concentrations of glucose in fluoride plasma were determined with the ADVIA® Chemistry XPT

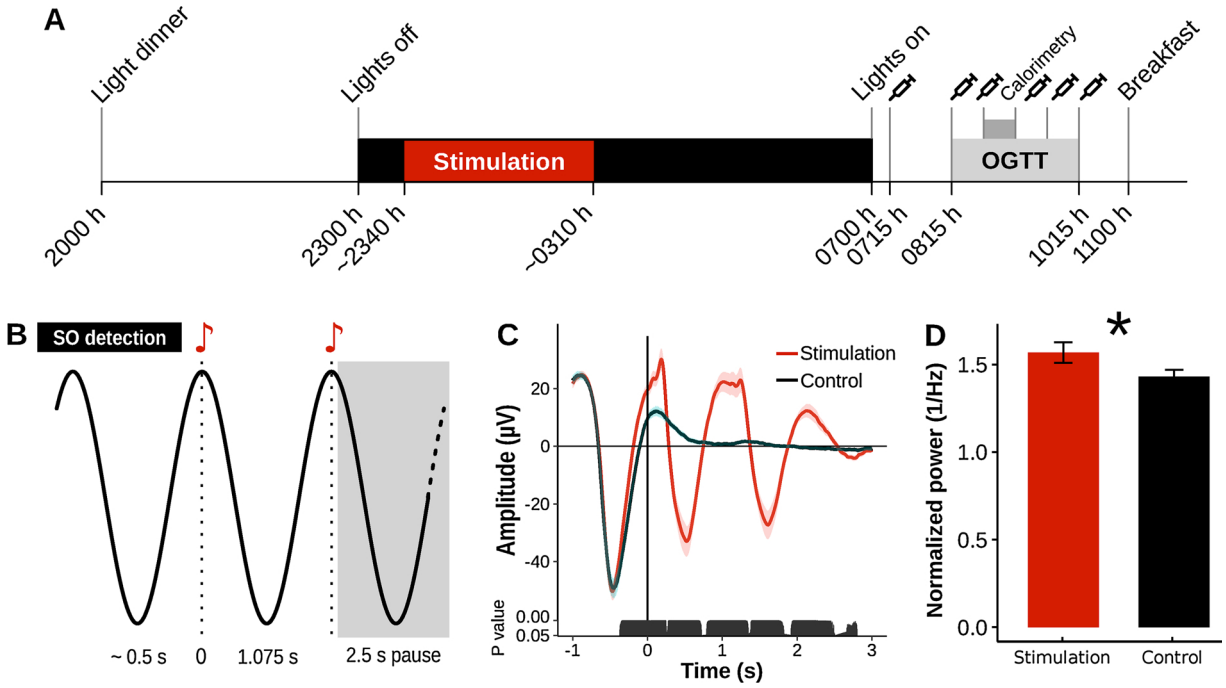

Fig. 1. Experimental procedure and electrophysiological effect of auditory stimulation. (A) After a standardized light meal at 2000 h, subjects went to bed at 2300 h. Stimulation started once stable slow-wave sleep was detected (i.e., around 2340 h) and continued for 210 min. (B) Pink-noise bursts (50 ms, 55 db SPL) were presented to the sleeping subject via in-ear headphones with an inter-stimulus interval of 1.075 s to coincide with two consecutive slow oscillation up-states. On control nights, respective time points were marked in the EEG, but participants did not receive stimulation. At 0715 h blood was collected to obtain fasted values. The oGTT was performed from 0815 to 1015 h, with indirect calorimetry included 30 min after onset to assess postprandial thermogenesis. At 1100 h, food intake from an *ad libitum* breakfast buffet was covertly assessed. (C) Mean ± SEM EEG signal recorded from the central electrode position (Cz) during the 210-min stimulation period, time-locked to the first of the two tones (t = 0). (D) Mean spectral power between 0.875–1.125 Hz, i.e. corresponding to the 1.075 s interstimulus interval, during SWS measured across the whole recording period (i.e., between "lights-off" and "lights-on"); n = 17.

clinical chemistry analyzer according to the hexokinase method. Serum insulin, C-peptide, and cortisol concentrations were measured with the ADVIA® Centaur XPT immunology analyzer. Plasma NEFA concentrations were determined according to the ACS-ACOD method (NEFA-HR (2), Wako Chemicals GmbH, Neuss, Germany) with the ADVIA® Chemistry XPT clinical chemistry analyzer (all instruments form Siemens Healthcare Diagnostics, Eschborn, Germany).

2.4. Energy expenditure and food intake

Between the 30-min and 60-min oGTT blood samplings, diet-induced thermogenesis was measured using indirect calorimetry (Deltatrac II, MBM-200 Metabolic Monitor; Datex-Engström Deutschland, Achim, Germany), yielding both carbohydrate and fat utilization calculated according to the non-protein respiratory quotient (Péronnet and Massicotte, 1991). Before each use, the device was calibrated with Quick Cale calibration gas to 5% CO_2 and 95% O_2. After the oGTT, at 1100 h, we assessed free-choice, ad-libitum food intake from a standardized breakfast buffet that comprised a vast collection of food items (see Table 1). Participants were left undisturbed during breakfast and were not aware that their food intake was measured by weighing buffet components before and after. For analyses, caloric and nutritional content was obtained either from the manufacturer or the USDA Food Composition Database (https://ndb.nal.usda.gov/ndb) whenever not available otherwise (e.g., for fruits). This paradigm has been repeatedly shown to enable the precise assessment of food intake (Hallschmid et al., 2010; Ott et al., 2013; Santiago and Hallschmid, 2017).

2.5. Statistical analyses

Power calculations based on previous experiments using auditory stimulation of slow oscillatory activity (Besedovsky et al., 2017; Ngo et al., 2013) indicated that a minimum group size of 15 subjects would enable us to detect significant intervention effects on metabolic parameters; therefore, 22 subjects were recruited to account for potential dropouts. Of these participants, we had to exclude five subjects because of short or impaired sleep, a Matsuda Index > 2 SD above the mean of all participants, technical failures or data loss, yielding complete sleep data sets from 17 participants; in these subjects, we obtained full data sets on oGTT, energy expenditure and food intake in, respectively, 15, 14, and 16 subjects.

Analyses were carried out with R 3.4.2. Two-tailed, paired t-tests were used to compare polysomnographic measures including power spectra and evoked potentials; correction for multiple comparisons was performed with a Monte-Carlo-based cluster correction (2000 repetitions, alpha level = 0.05). To account for differences between subjects, each spectra was normalized with reference to its cumulative power up to 30 Hz. oGTT-derived values were subjected to repeated-measures ANOVA including the factors condition and time-point and, in addition, t-tests comparing areas under the curve (AUCs) between 0815 h and 1015 h calculated according to the trapezoidal rule, and Matsuda Indices (Matsuda and DeFronzo, 1999). Analyses of food intake from the test buffet relied on ANOVA including the factors condition, macronutrient and taste category. In an exploratory fashion, we also calculated Pearson correlation coefficients between the intervention-associated changes (i.e., differences between conditions) in slow oscillatory activity (in terms of normalized EEG power calculated for the whole night) and those in the metabolic outcome parameters. Results are presented as means ± SEM. A P value < 0.05 was considered significant.

Table 1
Composition of the breakfast buffet.

	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Neutral					
Buns	300	798.00	148.26	9.99	26.55
Wholegrain bread	165	452.10	78.44	7.47	17.61
White bread	30	79.80	14.83	1.00	2.66
Butter	120	860.40	0.07	97.33	1.02
Milk	1000	640.00	46.50	36.60	32.80
Sweet					
Chocolate spread	40	218.80	22.76	12.72	2.64
Honey	40	121.60	32.96	0.00	0.12
Vanilla pudding	125	182.50	19.38	10.00	0.38
Curd with fruit	125	145.00	19.38	5.00	5.50
Fruit custard	50	100.00	23.34	0.00	0.00
Apple	105	54.60	14.50	0.18	0.27
Orange	240	112.80	28.20	0.29	2.26
Banana	150	133.50	34.26	0.50	1.64
Pear	150	85.50	22.85	0.21	0.54
Tangerine	130	68.90	17.34	0.40	1.05
Orange juice	220	94.60	19.36	1.10	1.54
Strawberry milk	200	122.00	17.60	2.80	6.80
Sugar	24	92.88	24.00	0.00	0.00
Hearty					
Poultry salami	40	42.40	0.88	1.51	5.92
Cervelat sausage	34	123.08	1.13	10.35	5.93
Cheese slices	100	381.00	0.00	28.57	23.81
Fresh cheese with herbs	40	28.00	1.20	2.00	0.80
Cheese spread	33	99.90	2.23	9.99	2.23
Total	3461	5037.36	589.45	238.00	142.06

3. Results

3.1. Auditory stimulation increases slow oscillatory activity during SWS

Closed-loop auditory stimulation compared to the control condition induced a marked increase in slow oscillatory activity in the averaged EEG time-locked to the first auditory stimulus (Fig. 1C). Spectral analyses performed on SWS epochs from the entire recording period showed stimulation-triggered power increases in the stimulation band (~0.9 Hz) that averaged 9.78 ± 3.11% (1.57 ± 0.06 vs. 1.43 ± 0.04 1/Hz, $t(16.00) = -0.14$, 95% CI [−0.23, −0.04], $P = 0.008$; Fig. 1D). Total sleep time and absolute (minutes) as well as relative (% of TST) amounts of time spent in different sleep stages were comparable between conditions (Table 2). Likewise, there was no difference between the stimulation and the control conditions in arousal index (14.35 ± 2.32 vs. 13.12 ± 1.49, $t(16) = -1.24$, $P = 0.50$). The average number of auditory clicks presented to the subjects in the stimulation night was 647.14 ± 59.77, ranging from 133 to 1110. 80 ± 0.05% of them were presented during SWS and 20 ± 0.05% during sleep stage 2.

3.2. Stimulating slow oscillatory activity does not acutely alter glucoregulation

Fasting levels of glucose, insulin, C-peptide, and NEFA did not differ between conditions (all $P > 0.53$). Responses to the oGTT were comparable between conditions with regard to AUC values of glucose ($t(14) = -0.10$, $P = 0.94$; Fig. 2A) and insulin ($t(14) = -156.73$, $P = 0.58$; Fig. 2B) and respective single time-point comparisons (all $P > 0.13$; $P > 0.4$ for ANOVA factor condition; Fig. 2C/D), as well as to the Matsuda Index (14.28 ± 1.08 in the stimulation compared with 14.20 ± 1.18 in the control condition; $t(14) = -0.08$, $P = 0.94$). C-peptide and NEFA concentrations measured during the oGTT were likewise unaltered by the intervention (all $P > 0.08$; Fig. 2E/F), as were fasting cortisol concentrations (stimulation, 507.07 ± 15.65 nmol/L, control, 506.00 ± 26.23 nmol/L; $t(13) = -1.07$, $P = 0.96$).

3.3. Comparable energy expenditure and food intake after stimulating slow oscillatory activity

Calorimetric measurements did not indicate a modulating influence of stimulating slow oscillatory activity on parameters of diet-induced thermogenesis (all $P > 0.83$; Table 3). Also, ad-libitum food intake from the breakfast buffet did not differ between conditions; trendwise increases in carbohydrate and protein intake after stimulation (Table 3) were not confirmed by overall analyses ($P > 0.57$ for condition, $P > 0.96$ for condition × macronutrient, $P > 0.90$ for condition × taste category).

3.4. Relationship between changes in slow oscillatory activity and in metabolic parameters

Exploratory correlational analyses indicated that the stimulation-

Table 2
Sleep architecture.

	Whole recording period			Stimulation period		
	Stimulation	Control	P	Stimulation	Control	P
TST (min)	479.29 ± 4.04	478.56 ± 3.94	0.87			
WASO (min)	9.53 ± 1.41	10.03 ± 1.84	0.72	4.0 ± 1.0	3.1 ± 0.9	0.50
WASO (%)	2.00 ± 0.30	2.11 ± 0.39	0.71	2.1 ± 0.5	1.7 ± 0.5	0.50
REM (min)	91.44 ± 4.43	98.62 ± 4.32	0.14	22.9 ± 2.7	21.9 ± 3.3	0.81
REM (%)	19.01 ± 0.82	20.58 ± 0.84	0.11	12.3 ± 1.4	12.2 ± 1.9	0.98
S1 (min)	18.56 ± 2.44	21.56 ± 2.93	0.26	5.4 ± 1.6	4.2 ± 1.1	0.29
S1 (%)	3.89 ± 0.52	4.51 ± 0.62	0.25	2.9 ± 0.8	2.4 ± 0.7	0.39
S2 (min)	267.53 ± 8.82	254.00 ± 7.24	0.15	89.9 ± 5.4	87.5 ± 5.9	0.65
S2 (%)	55.85 ± 1.83	53.08 ± 1.48	0.11	49.2 ± 3.1	48.9 ± 3.1	0.90
SWS (min)	90.82 ± 9.07	94.12 ± 7.38	0.66	61.0 ± 8.0	61.9 ± 6.2	0.86
SWS (%)	18.94 ± 1.85	19.67 ± 1.55	0.64	33.2 ± 4.2	34.7 ± 3.5	0.56

Data are means ± SEM. Percentage values refer to the whole recording period (from “lights-off” until “lights-on”; left columns) and the stimulation period of 210 min (following the start of stimulation ~5 min after the participant displayed stable stage 2 or deeper sleep; right columns). WASO, wake after sleep-onset, REM, rapid-eye movement sleep; S1, stage 1; S2, stage 2, SWS, slow-wave sleep. P values for comparisons between conditions as derived from paired, two-tailed t-tests; n = 17.

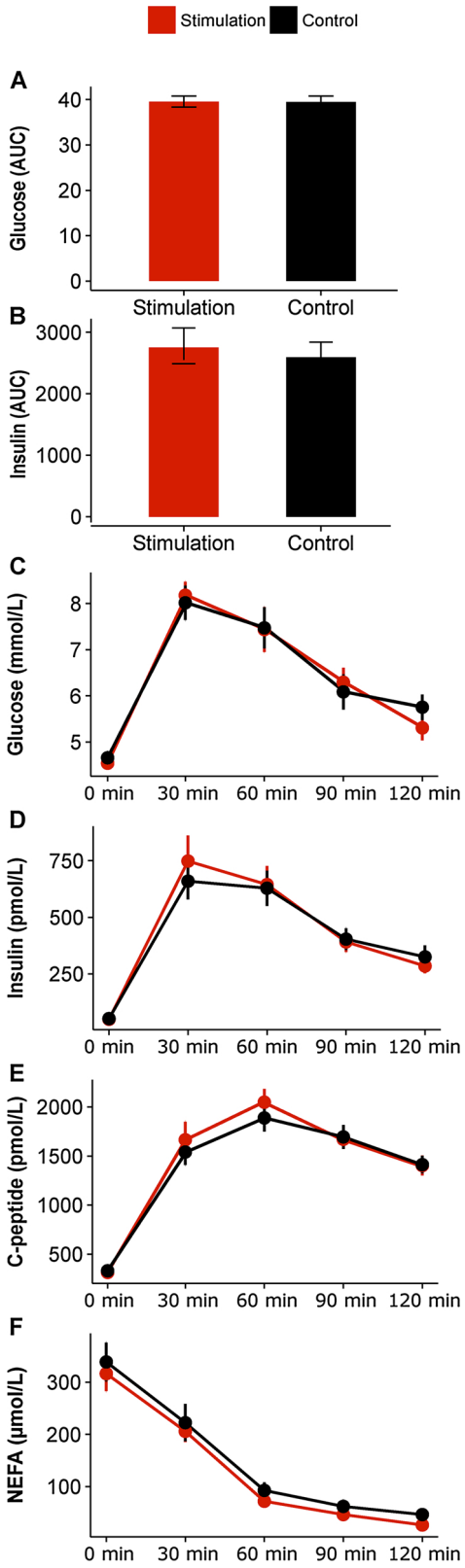

Fig. 2. Results of the 5-sample oral glucose tolerance test (oGTT) performed from 0815 to 1015 h. Mean ± SEM $AUC_{0815\text{-}1015\ h}$ of (A) plasma glucose and (B) serum insulin; mean ± SEM concentrations of (C) plasma glucose, (D) serum insulin, (E) serum C-peptide and (D) plasma NEFA. There were no significant differences between conditions; n = 15.

induced changes in normalized EEG power and those in metabolic parameters were not significantly related or, on a non-significant level, rather indicated respective negative relationships: fasting insulin, r = 0.47, P = 0.076; fasting glucose, r = 0.21, P = 0.45; Matsuda Index, r = −0.48, P = 0.072; insulin $AUC_{0815\text{-}1015h}$, r = 0.17, P = 0.54; glucose $AUC_{0815\text{-}1015h}$, r = 0.033, P = 0.91; total calorie intake, r = -0.12, P = 0.68; energy expenditure, r = 0.049, P = 0.87.

4. Discussion

We investigated the role of sleep slow oscillations for glucose homeostasis and energy turnover in healthy men. We found that stimulating slow oscillatory activity during early sleep, without changing total sleep time or sleep architecture, does not acutely improve oGTT-assessed insulin sensitivity. The intervention neither altered energy intake or expenditure, and we also did not detect solid indicators of a relationship between stimulation-induced changes in slow oscillatory activity and metabolic outcomes.

SWS, which predominantly occurs during the first night-half, is a hallmark of sleep quality and an important mediator of the beneficial cognitive effect of sleep (Rasch and Born, 2013). Our closed-loop auditory stimulation prolonged spontaneously detected slow oscillations and increased power in the ~0.9 Hz band. This outcome is in line with previous studies where enhancing slow oscillatory activity moreover improved sleep-dependent memory consolidation (Ngo et al., 2013) and immediately reduced circulating cortisol concentrations and, with a delay of three hours, T and B cell counts (Besedovsky et al., 2017). While the complete suppression of SWS impairs glucose homeostasis (Tasali et al., 2007; Herzog et al., 2013), our results indicate that in healthy humans selectively enhancing slow oscillatory activity as the key feature of SWS does not impact metabolic control on the subsequent day.

The interruptive acoustic intervention in the studies by van Cauter's group (Tasali et al., 2007) and Herzog et al. (2013) reduced SWS duration by as much as 88% and 79%, respectively. Increasing time spent in SWS to the same extent – which in a strict sense could be considered the reversed intervention – is difficult because of its apparent homeostatic nature and refractoriness in the underlying neuronal networks (Ngo et al., 2015). The anticonvulsant tiagabine increases SWS duration by 55% (Feld et al., 2013) but, in contrast to the auditory stimulation paradigm (Ngo et al., 2013), does not improve memory consolidation, indicating that the electrophysiological properties, rather than the length of SWS, mediate its beneficial effects; metabolic parameters were not assessed in the respective experiments (Feld et al., 2013). Interruptive SWS suppression (Tasali et al., 2007) was associated with power decreases in the 0.5–4 Hz band of around 50%, whereas our intervention induced narrowband increases in the same band of around 10%. It is also to note that the deterioration of insulin sensitivity due to SWS suppression appeared to be somewhat stronger after three nights (Tasali et al., 2007) than one night of the intervention (Herzog et al., 2013). It is therefore possible that prolonging our intervention beyond one night – a paradigm which has not yet been tested – modulates glucoregulatory pathways. Beneficial effects might in principle be expected to emerge from respective changes in hypothalamic-pituitary-adrenal axis activity: cortisol concentrations were unaltered in the morning after stimulation in the present experiments, but previously found to be decreased during the first hour of auditory stimulation (Besedovsky et al., 2017), and increased cortisol levels are assumed to mediate some of the deleterious effects of sleep deprivation on glucoregulation (Spiegel et al., 1999; Stamatakis and Punjabi, 2010; Schmid et al., 2015).

With regard to energy turnover, we hypothesized based on the existing literature (summarized in Schmid et al., 2015; Anothaisintawee et al., 2016) that stimulating slow oscillatory activity would induce catabolic shifts. We have previously shown that acute sleep deprivation in healthy young men decreases energy expenditure measured in the

Table 3
Energy expenditure and food intake.

	Stimulation	Control	P
Energy expenditure (kcal/day)	1998.33 ± 44.82	1990.42 ± 54.26	0.92
Carbohydrate utilization (g/min)	0.17 ± 0.02	0.16 ± 0.02	0.87
Fat utilization (g/min)	0.08 ± 0.01	0.08 ± 0.01	0.88
Respiratory quotient	0.83 ± 0.01	0.83 ± 0.01	0.83
Total breakfast intake (kcal)	1423.84 ± 132.27	1318.70 ± 160.71	0.24
Carbohydrate (kcal)	682.66 ± 55.67	629.48 ± 48.33	0.08
Fat (kcal)	546.35 ± 61.44	528.19 ± 98.43	0.76
Protein (kcal)	194.84 ± 24.36	161.03 ± 22.10	0.06
Neutral foods (kcal)	772.22 ± 93.50	709.23 ± 109.94	0.54
Sweet foods (kcal)	384.78 ± 45.00	383.23 ± 35.20	0.96
Savory (kcal)	266.84 ± 43.87 kcal	226.24 ± 49.28	0.27

Data are means ± SEM. Neutral, sweet and savory foods contained in the test buffet are listed in Table 1. P values for comparisons between conditions as derived from paired, two-tailed t-tests; parameters of energy expenditure, n = 14, food intake, n = 16.

morning (Benedict et al., 2011). However, in the same study core body temperature was increased during sleep deprivation, and other studies found stimulating effects of shortened sleep on energy expenditure (Jung et al., 2011; Markwald et al., 2013). We did not detect respective differences between conditions in the present experiments. Food intake has been found in the majority of previous studies to be stimulated by sleep deprivation (e.g., St-Onge et al., 2011; Calvin et al., 2013; Markwald et al., 2013; Nedeltcheva and Scheer, 2014). In the present study, stimulating slow oscillatory activity during SWS, a sleep stage that is malleable by dietary changes (Collet et al., 2016; St-Onge et al., 2016) but to our knowledge has not yet been directly demonstrated to regulate appetite, did not reduce (and on a non-significant level rather increased) energy intake.

Against the background of previous observations in young, healthy men that slow oscillation auditory stimulation modulates cognitive (Ngo et al., 2013) as well as endocrine parameters (Besedovsky et al., 2017), we expected this intervention to improve metabolic control in our healthy participants, although it might be argued that its highly selective nature and moderate impact may per se limit effects in the healthy organism. In contrast to our assumption, however, the observed results point to a degree of fine-tuning of the sleep-associated electrophysiological-metabolic balance in healthy men that cannot be easily enhanced. Although our sample was of moderate, but sufficient size, larger investigations might be needed to unravel more subtle metabolic effects of stimulating slow oscillatory activity, and in light of reported sex differences in slow wave activity (Latta et al., 2005), the results also need confirmation in women. Moreover, individuals with less than optimal metabolic control might be more sensitive to respective interventions. Seventy million US adults have reported sleeping 6 h or less in a recent National Health Interview Survey (Ford et al., 2015), and a 6-week experimental sleep extension in subjects with this relatively short sleep duration yielded improvements in insulin sensitivity (Leproult et al., 2015) which, in the clinical context, usually require weight loss (Matarese and Pories, 2014) and exercise (Mann et al., 2014) or medication. Our results therefore do not rule out that (prolonged) auditory sleep stimulation protocols may support therapeutic interventions to improve metabolic function, particularly in elderly subjects with deteriorating SWS quality (Van Cauter et al., 2000). Future studies should focus on subjects with metabolic disorders and/or impaired SWS to further test the potential of sleep stage-specific interventions to enhance metabolic control (Ogilvie and Patel, 2017; Reutrakul et al., 2016).

Declarations of interest

None.

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