SUPPLEMENTARY MATERIALS FOR "A LABORATORY STUDY ON THE EFFECTS OF WIND TURBINE NOISE ON SLEEP: RESULTS OF THE POLYSOMNOGRAPHIC WITNES STUDY"

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Supplemental methods

Participant recruitment

Two study groups were recruited. One group lived close to wind turbines, and the other group did not live close to wind turbines. The group that did not live close to wind turbines is hereafter termed *Reference*. The group living close to wind turbines was potentially exposed to WTN at home, and is hereafter termed *Exposed*.

We used two approaches to obtain addresses for the recruitment pool of the *Exposed* group. First, we contacted local municipalities for records of complaints made about wind turbines (n=324 addresses). Second, we manually looked up areas throughout Sweden with several wind turbines using a public database maintained by the Swedish Energy Agency (vindlov.se), and obtained the addresses close to wind turbines in these areas from a public population register (n=151 addresses). We mailed a leaflet describing the study and a list of available study periods to all 475 addresses. This mailing included our email, telephone and postal information with which a recipient could contact us for more information and/or if they were interested in taking part. If we did not receive a response, we attempted to contact the recipient by telephone, if a number was available. In this way we successfully contacted 229 individuals (48.2% of all addresses) expressed an interest in taking part in the study. Interested respondents were asked a series of questions over the telephone to determine their eligibility for the study. Of those interested, 24 met the inclusion criteria and subsequently completed the study (30% of interested respondents; 10.5% of successfully contacted individuals; 5.1% of all mailed addresses).

We recruited the *Reference* group by two separate advertising strategies. The first was via a physical presence and posters at a large public science festival (n=22 participants recruited). The second was using a digital platform (studentkaninen.se) that has the specific purpose of recruiting research subjects from among the public (n=4 participants recruited). The exclusion criteria were identical to the *Exposed* group, with the exceptions that they should not live close to a wind turbine and were not required to have lived at their current address for at least year.

Morning questionnaire

Below is the questionnaire that was completed each morning during the study. Note that the original questionnaire was administered in Swedish, and is presented here as an English language translation, the validity of which has not been tested.

Questions to answer in the morning

Answer the questions within 15 minutes of awakening at 7:00 in the morning

Note that this form is two-sided

1. How would you rate your sleep quality during the night? Circle the appropriate number.

Very good 0 1 2 3 4 5 6 7 8 9 10 Very bad

Also give your answer on the following verbal scale

2. How would you rate your sleep quality during the night?

- [] Very good
- [] Rather good
- [] Not particularly good
- [] Bad
- [] Very bad

How are you feeling right now?								Circle the appropriate number					
3.	Very rested	0	1	2	3	4	5	6	7	8	9	10	Very tired
4.	Very relaxed	0	1	2	3	4	5	6	7	8	9	10	Very tense
5.	Very irritated	0	1	2	3	4	5	6	7	8	9	10	Very glad

6.	How long did it take you to fall asleep last night?		Minutes:
7.	How many times do you estimate that you woke up during the night before the morning alarm?	[] Did not wake	Woke times
8.	Did you have difficulty falling back to sleep after an awakening?	[] Did not wake	[]No []Yes

How was your experience of the night and your sleep?

Circle the appropriate number

9.	Easy to fall asleep	0	1	2	3	4	5	6	7	8	9	10	Difficult to fall asleep
10.	Better sleep than usual	0	1	2	3	4	5	6	7	8	9	10	Worse sleep than usual
11.	Deep sleep	0	1	2	3	4	5	6	7	8	9	10	Shallow sleep
12.	Never woke up	0	1	2	3	4	5	6	7	8	9	10	Woke up often

13. How disturbed was your sleep by *noise* from wind turbines during the night? *Circle the appropriate number*

Not at all 0 1 2 3 4 5 6 7 8 9 10 Extremely

Do you think that *noise* during the night disturbed your sleep so that you:

	Not at all	Slightly	Moderately	Very	Extremely
14. slept badly?	[]	[]	[]	[]	[]
15. were awoken?	[]	[]	[]	[]	[]
16. had difficulty falling asleep?	[]	[]	[]	[]	[]
17. felt tired in the morning?	[]	[]	[]	[]	[]

Hei Circ Woi	Here are two columns of words describing different emotional states. Circle the answer that best describes how you feel <u>right now</u> . Work quickly and respond with the option you first come upon! Respond for every word												
Key	Key: ++ Definitely + Somewhat - Not Definitely not												
	Agree	Def	Some	Not	Def not		Agree	Def	Some	Not	Def not		
	Sullen	++	+	-			Generous	++	+	-			
	Pleased	++	+	-			Optimistic	++	+	-			
	Helpful	++	+	-			Pessimistic	++	+	-			
	Unreasonable	++	+	-			Uneasy	++	+	-			
	Accommodating	++	+	-			Unhappy	++	+	-			
	Glad	++	+	-			In good humour	++	+	-			
	Friendly	++	+	-			Co-operative	++	+	-			
	Cross	++	+	-			Worried	++	+	-			
	Dejected	++	+	-			Irritated	++	+	-			
	Good natured	++	+	-			Resigned	++	+	-			
	Harmonic	++	+	-			Angry	++	+	-			
							Unconcerned	++	+	-			

Now you are done with the morning's questions!

Model checking

To ensure conformity with the regression model assumptions of normality, data were visually inspected. If appropriate, data were transformed prior to statistical analysis. Sleep onset latency (SOL), N3 latency, wakefulness after sleep onset (WASO) and total sleep time (TST) data were substantially positively skewed and were therefore log-transformed. REM latency, number of awakenings during the night and awakening frequency per hour were slightly positively skewed and so were square-root transformed.

Sound character period statistical analysis

The following PSG data were calculated for each of the 2-hour sound character periods: sleep time (minutes); amount of each sleep stage during the sound character period as a proportion of time asleep in the sounds character period (%); frequency of SSCs, awakenings, arousals and combined EEG reactions (n/h). Each of these outcomes was analyzed separately in a multilevel mixed regression model (SPSS MIXED procedure) with a random subject intercept. The models included Window (Closed/Ajar) and AM depth as the treatment variables of interest. Furthermore, candidate variables to include in these models were the covariates included in analysis of sleep macrostructure (study group, sex, noise sensitivity, age) and the window*AM interaction. The presentation number of the sound character period (ordinal: 1, 2, 3, 4) was also considered as a candidate variable, since sleep structure changes over the course of the night. Because of the rather limited sample size (n=48 participants with PSG data), to minimize the risk of overfitting we aimed to limit the number of candidate variables in each model. We therefore used a purposeful stepwise selection of covariates using the following approach:

1. In addition to Window and AM depth, candidate variables were included one at a time.

- If candidate variable $p \le 0.250$ then include in multiple regression in step 2.
- If candidate variable p>0.250 then put on a list for later double check (*)
- 2. Run a multiple regression with the variables from step *1*.

3. Delete candidate variables from the model that seem "unimportant" (p>0.250).

4. Compare the smaller and the larger model using the Akaike Information Criterion (AIC) values.¹ We used the model that most plausibly fit the data according to difference in the AIC following recommendations by Burnham and Anderson.² If the difference in AIC was >2, we used the model with the lowest AIC. If the difference in AIC values was <2, we used the most parsimonious model.

5. Repeat step 3 and 4 until the model consists of only "important" variables (no difference between larger and smaller models).

6. Reinsert, one at the time, of the variables rejected at step 1 (*) into the resulting multiple model from the previous step. The variable is checked for statistical significance (p<0.05).

7. Include all the extra variables statistically significant in step 6 one-at-the-time in the multiple model from step 5.

Any variable that then loses its statistical significance (p>0.05) are again excluded and the resulting model is compared via the AIC with the larger model from step 7.

8. Insert the Window*AM depth interaction into the resulting multiple model from step 7. We retained the interaction in the model if the F-test was statistically significant ($p \le 0.10$).

Supplemental results

Participants

On the first evening of the study, participants were asked to rate the noise environment in their bedroom at home, and their disturbance at home by different noise sources. The number of responses for each response level of each question is given in Table S1. We used Fisher's exact test of independence to test if the distribution of responses for each question was different between the *Reference* and *Exposed* study groups. Data are missing completely for n=1 participant who did not complete the questionnaire and n=3 participants who completed only the first side of the questionnaire.

		Study	group	1	
Question	Response level	Reference	Exposed	p-value	
Self-reported bedroom noise	Very quiet	6	6	n=49, p=1.0	
environment	Rather quiet	17	16		
	Not very quiet	2	1		
	Rather noisy	0	1		
	Very noisy	0	0		
Disturbed at home by road	Not at all	16	9	n=46, p=0.174	
noise	Not very much	7	12	, r	
	Quite a lot	1	1		
	A lot	0	0		
	Incredibly so	0	0		
Disturbed at home by rail	Not at all	22	21	n=46, p=1.0	
noise	Not very much	2	1		
	Quite a lot	0	0		
	A lot	0	0		
	Incredibly so	0	0		
Disturbed at home by	Not at all	10	11	n=46, p=0.543	
fridge/fan noise	Not very much	12	10		
	Quite a lot	2	0		
	A lot	0	1		
	Incredibly so	0	0		
Disturbed at home by	Not at all	8	13	n=46, p=0.223	
neighbor noise	Not very much	14	7		
	Quite a lot	2	2		
	A lot	0	0		
	Incredibly so	0	0		
Disturbed at home by noise	Not at all	4	4	n=24, p=0.661	
from other sources	Not very much	3	2		
	Quite a lot ^a	6	3		
	A lot ^b	0	1		
	Incredibly so ^c	0	1		

 Table S1. Self-evaluation of bedroom noise environment at home and disturbance by noise.

^a Sources of disturbance given: Pets (×2), plumbing system, beeping, children (×3), gardening equipment, bird song (×2), heaters; ^b Source of disturbance given: Radiator; ^c Source of disturbance given: Snoring partner

Seventeen participants (34%) were using medications during the study (9 in the *Reference* group, 8 in the *Exposed* Group). The different medications used and the number of participants using each type of medication is given in Table S2. Nine participants used only one medication, three participants used two different medications, two participants used three different medications, and two participants used four different medications.

	Generic/brand name		Users (n)		Frequency of side effects
Medication type	(if reported)	Total	<i>Reference</i> group	<i>Exposed</i> group	on sleep ^a
ACE inhibitor	Enalapril	1	1	0	Uncommon (up to 1/100)
Aminosalicylate	Asacol	1	1	0	-
Angiotensin II receptor antagonist	Losartan	1	1	0	Uncommon (up to 1/100)
Anticoagulant	Eliquis, Waran	2	1	1	-
Antidepressant (SSRI)	Brintellix, Sertralin	3	1	2	Very common (more than 1/10)
Antihistamine	Loratadine	1	1	0	Frequency not reported
Beta-agonist	Bricanyl	1	1	0	Occurring with unknown frequency
Beta-blocker	Metoprolol, Bisoprolol	2	1	1	Uncommon (up to 1/100)
Bronchodilator	Spiriva	1	0	1	Rare (up to 1/1000)
Calcium channel blocker	Amlodipin	1	1	0	-
Corticosteroid	Mometason evolan	1	1	0	-
Dietary supplements	-	1	0	1	-
Estrogen/progestin (Female HRT)	-	1	0	1	-
Ferrus sulphate	Duroferon	1	1	0	-
Insulin	Lantus, Novorapid	1	1	0	-
Nonsteroidal anti-inflammatory drug (NSAID)	Trombyl	2	0	2	-
Proton pump inhibitor	Omeprazol	2	1	1	Uncommon (up to 1/100)
Retinoid	Neotigason	1	0	1	-
Statin	Simvastatin	1	0	1	Very rare
Thyroid hormone	Levaxin	3	3	0	-
Vitamin D supplement	-	1	1	0	-

Table S2. Medications used by sleep study participants.

ACE: Angiotensin-converting enzyme; SSRI: selective serotonin reuptake inhibitors; HRT: Hormone replacement therapy. ^a Data from the Swedish Association of the Pharmaceutical Industry ³

Correlation analysis of regression model covariates

To confirm there were no potential issues with multicollinearity, all covariates included in the regression models were examined in a correlation analysis. Correlation coefficients were calculated appropriately to the type of variables (dichotomous, ordinal or continuous) being correlated. Results are given in Table S3. All correlations were weak (r<0.4) and therefore no covariates were omitted from regression analyses.

	Age	Sex	Study group	Noise sensitivity	Tiredness	Difficulty falling asleep
Age	1	$r_{\rm pb} = -0.0856$	$r_{\rm pb} = 0.055$	<i>r</i> = 0.291	$\rho = 0.107$	$\rho = 0.076$
Sex	-	1	$\phi = 0.077$	$r_{\rm pb} = 0.209$	$r_{\rm rb} = 0.0661$	$r_{\rm rb} = 0.111$
Study group	-	-	1	$r_{\rm pb} = 0.150$	$r_{\rm rb} = 0.355^*$	$r_{\rm rb} = 0.215$
Noise sensitivity	-	-	-	1	$\rho = 0.340^*$	$\rho = 0.135$
Tiredness	-	-	-	-	1	$\rho = 0.149$
Difficulty falling asleep	-	-	-	-	-	1

 Table S3. Correlation coefficients for covariates included in regression models.

r Pearson correlation; ρ Spearman rank correlation; r_{pb} point-biserial correlation; r_{rb} rank-biserial correlation; ϕ phi correlation. Statistically significant correlations are indicated with asterisks: * p<0.05

Sleep macrostructure regression model results

P-values for all variables included in the mixed effects regression model for each PSG outcome variables are given in Table S4. There was a significant interaction between study night (Control/WTN-night) and study group (Reference/Exposed) for the percentage of sleep time in N3 sleep (Figure S1).

Table S4. P-values for all treatment and covariate variables included in regression models of PSG macrostructure. P-values are from analysis where variables were transformed if appropriate.

				P-value:	S		
Variable category	Variable	Study night	Study group	Noise sensitivity	Sex	Age	Night × Group
Sleep times	TIB (min)	0.354	0.213	0.175	0.142	0.514	0.354
	TST (min) *	0.126	0.172	0.232	0.041	0.378	0.543
	Sleep period time (min)	0.061	0.290	0.149	0.134	0.358	0.179
	Sleep efficiency (%)	0.195	0.115	0.236	0.040	0.244	0.483
	Sleep onset latency (min) *	0.211	0.147	0.197	0.497	0.312	0.165
	REM latency (min) †	0.039	0.643	0.091	0.288	0.963	0.594
	N3 latency (min) *	0.195	0.812	0.409	0.298	0.284	0.136
	WASO (min) *	0.222	0.643	0.801	0.049	0.008	0.500
Sleep architecture	N1 (min)	0.769	0.176	0.699	0.014	0.248	0.392
	N2 (min)	0.859	0.063	0.961	0.195	0.481	0.082
	N3 (min)	0.910	0.700	0.069	0.251	0.523	0.062
	REM (min)	0.013	0.605	0.958	0.011	0.390	0.607
	NREM (min)	0.947	0.332	0.207	0.495	0.624	0.232
	N1 (% of TST)	0.846	0.107	0.947	0.003	0.179	0.476
	N2 (% of TST)	0.225	0.090	0.490	0.635	0.809	0.083
	N3 (% of TST)	0.280	0.387	0.210	0.744	0.681	0.034
	REM (% of TST)	0.010	0.894	0.673	0.047	0.613	0.354
	NREM (% of TST)	0.010	0.894	0.673	0.047	0.613	0.354
Sleep fragmentation	Arousals (n)	0.733	0.852	0.938	0.063	0.167	0.283
	Arousal index (n/h)	0.945	0.997	0.704	0.020	0.133	0.268
	Awakenings (n) †	1.000	0.256	0.688	0.311	0.026	0.207
	Awakening index (n/h) †	0.622	0.211	0.545	0.197	0.043	0.288
	Combined arousals+awakenings (n)	0.769	0.991	0.897	0.048	0.286	0.230
	Sleep fragmentation index (n/h)	0.850	0.844	0.638	0.014	0.237	0.230
	SSCs (n)	0.959	0.760	0.912	0.002	0.216	0.079
	SSC index (n/h)	0.414	0.883	0.646	< 0.001	0.156	0.196
Sleep continuity	First awakening (min)	0.309	0.937	0.019	0.023	0.859	0.540
	Final awakening (min)	0.214	0.531	0.255	0.114	0.902	0.595
	Max uninterrupted time in W (min)	0.768	0.144	0.103	0.117	0.972	0.435
	Max uninterrupted time in REM (min)	0.106	0.858	0.957	0.023	0.209	0.590
	Max uninterrupted time in N1 (min)	0.183	0.052	0.741	0.697	0.988	0.839
	Max uninterrupted time in N2 (min)	0.448	0.950	0.297	0.117	0.163	0.419
	(min)	0.587	0.037	0.353	0.074	0.739	0.974

* Log-transformed before analysis. † Square-root transformed before analysis.



Figure S1. Interaction between study night and study group for percentage of total sleep time (TST) in N3 sleep. Data from N=24 participants per night per study group. Error bars indicate 95% confidence intervals.

Effect of habitual sleep timing on sleep latency

We ran a linear mixed model with random intercept to check if there was an effect of habitual bedtime at home on the PSG sleep timing variables measured in the study (sleep onset latency SOL, N3 latency, REM latency). The residuals for SOL and N3 latency were positively skewed, so these variables were log-transformed before analysis. The model included self-reported habitual bedtime (hour) as a continuous covariate, and was adjusted for study night (*WTN-night/Control*), study group (*Reference/Exposed*), and sex. We initially included the study night×study group interaction term, but this did not contribute significantly to the model (SOL: night×study p=0.361; N3 latency: night×study p=0.582; REM latency: night×study p=0.888) and was therefore omitted. Person nights where participants were already asleep at the 23:00 scheduled lights out time (n=11 nights from 9 participants) were excluded, thus sleep timing data of 85 nights from 46 participants were analyzed. There was no statistically significant effect of habitual bed time on SOL (p=0.092), N3 latency (p=0.892) or REM latency (p=0.811).

Cortisol awakening response

The mean cortisol concentration at 0, 30 and 45 minutes after awakening is given in Figure S2. Results of regression models of the cortisol awakening response (CAR) are given in Table S5. There were no significant study group × study night interactions for any CAR measures. There were no significant effects of study night, study group or noise sensitivity for any CAR measures. In the model for ACOR, there was a significant effect of measurement time (p<0.0001, Figure S2). Post-hoc tests with Bonferroni adjustments for multiple testing indicated ACOR was higher than the awakening value after 30 minutes (estimated marginal mean (EMM) +0.20 μ g/dL, p<0.0001). Fifteen minutes later (45 minutes after awakening), had decreased to a level below the 30 minute measurement (EMM -0.09 μ g/dL, p=0.0003), yet remained higher than the awakening value (EMM +0.11 μ g/dL, p<0.0001).



Figure S2. Cortisol awakening response. Left pane: mean cortisol concentrations for all study participants in each morning. Right pane: mean cortisol concentrations stratified by participant study group. Error bars indicate standard deviation. There were no statistically significant effects of study night or study group for any cortisol measures.

Cortisol measure	Participants (n)		Study night		Study group		Sex		Noise sensitivity		Age		Night×group
	Control	WTN- night	F-stat	p- value	F-stat	p- value	F- stat	p- value	F- stat	p- value	F- stat	p- value	p-value
ACOR *	50 †	50 †	0.278	0.599	0.305	0.583	8.190	0.006	0.237	0.629	0.472	0.496	0.339
CARauc	50	50	0.053	0.819	0.176	0.677	9.342	0.004	0.339	0.563	0.105	0.747	0.635
CARi	50	50	0.042	0.839	0.720	0.401	5.863	0.020	0.166	0.686	0.032	0.859	0.152
AINC	50	50	0.000	0.996	0.717	0.402	7.261	0.010	0.234	0.631	0.000	0.987	0.181
MINC	50	50	0.019	0.892	0.772	0.385	5.161	0.029	0.076	0.785	0.031	0.862	0.153

Table S5. Regression model results for effect of study night and study group on cortisol awakening response.

ACOR: absolute cortisol concentration at 0, 30 and 45 minutes after awakening; CARauc: overall volume of cortisol released given by the total area under the CAR curve; CARi: change in overall volume of cortisol released relative to the waking value; AINC: absolute increase in cortisol, defined as the difference between the maximal value of post-awakening cortisol relative to the awakening value; MINC: difference between the mean values of post-awakening cortisol relative to the awakening value.

* Measurement time (0, 30 and 45 minutes after awakening) also included as a covariate (F=47.387, p<0.0001). ACOR was positively skewed and square-root transformed before analysis.

+ Three samples per participant

Self-reported

Figure S3. Graphical representation of unadjusted means (bars) and standard deviations (error bars) in *Control* and *WTN-night* for each self-reported outcome. Figures on the left present data for all study participants, figure on the right present data stratified by participant study group. The ordinate is scaled from the minimum to the maximum of the response scale for each questionnaire item, except for "Probability of reporting difficulty falling back to sleep after an awakening" which is extended to encompass the standard deviation error bars. Effect of Study Night: * p<0.05; ** p<0.01; *** p<0.001. Effect of Study Group: † p<0.05; † p<0.01; † p<0.001.









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