Clinical Trial Registration

Study Protocol and Data Analysis Plan

Official Title: The Cognitive and Metabolic Effects of Sleep Restriction in Adolescents

Date: 2 November 2017

NCT ID: not yet assigned

Study Protocol
The present study investigates whether a continuous stretch of night time sleep (6.5 hours) will lead to better neurobehavioral outcomes relative to nocturnal sleep restriction (5 hours) and daytime nap (1.5 hours) of the same total duration, and how sleep restriction affects glucose metabolism. The 15-day protocol is conducted in a dormitory on 60 high school students, aged 15 to 19 years old. Participants are assigned to a nap or no-nap group. Both groups start with two 9-hour adaptation and baseline nights, followed by two successive cycles of sleep restriction (5-h time-in-bed [TIB]; 01:00-06:00 or 6.5-h TIB; 00:15-06:45) and recovery (9-h TIB; 23:00-08:00). Following each sleep-restricted night, the nap group receives a 1.5-h nap opportunity, while participants in the no nap group watch a documentary. Throughout the protocol, sleep-wake patterns are assessed with actigraphy and polysomnography. Sleepiness levels, mood, vigilance, working memory / executive functions, and speed of processing are assessed 3 times daily. Other cognitive functions such as memory and mindfulness levels are investigated through computer-based tasks. Glucose metabolism is measured using oral glucose tolerance tests in the mornings after the second baseline night, the third night of sleep restriction and second recovery night in the first cycle, as well as the last sleep restriction night in the second cycle. The study protocol can be found in Figure 1.

Statistical Analysis Plan
Primary outcome measures acquired in the current study are planned to be analyzed using SPSS Statistics (IBM) and MATLAB (MathWorks). Statistical analyzes that will be conducted include Mixed Models, Analyses of Variance (ANOVAs) and *t*-tests.

Primary Outcome Measure:
1.Change in sustained attention assessed with the Psychomotor Vigilance Task from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Number of attention lapses (>500ms) will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

2.Change in working memory assessed with the 1-back task from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Number of correct responses in the 1-back task will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

3.Change in executive functions assessed with the 3-back task from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Number of correct responses in the 3-back task will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

4.Change in the level of subjective sleepiness assessed with the Karolinska Sleepiness Scale from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Score on the Karolinska Sleepiness Scale (1-9 points) will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

5.Change in positive mood assessed with the Positive and Negative Affect Scale (PANAS) from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Total score on the positive subscale of the PANAS will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

6.Change in negative mood assessed with the Positive and Negative Affect Scale (PANAS) from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Total score on the negative subscale of the PANAS will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

7.Change in speed of processing assessed with the Mental Arithmetic Task from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Number of correct responses in the task will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol

between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

8.Change in speed of processing assessed with the Symbol Digit Modalities Task from morning to afternoon and then evening from baseline days to the first and second cycles of sleep restriction and recovery

Number of correct responses in the task will be measured. Mixed effect modelling will be used to compare the trajectory in this variable throughout the protocol between the two groups. The independent variables are group, day, and their interaction. Analyses will be performed on the daily average across the three time points per day, as well as for each individual time point (morning, afternoon, and evening).

9.Change in blood glucose level, measured using a blood glucose meter, from baseline following the first period sleep restriction, first period of recovery sleep, and second period of sleep restriction

Fasting glucose and 2-h glucose will be analyzed separately using ANOVA with group (nap vs no-nap) as a between-subject factor and session (baseline, sleep restriction 1, recovery, sleep restriction 2) as a repeated factor.

10.Change in meal and snack consumption from baseline following the first period sleep restriction, first period of recovery sleep, and second period of sleep restriction

Amount of meals and snacks consumed will be measured on an ad libitum basis. Calorie consumption (calories by meals and/or snacks) will be analyzed using ANOVA with group (nap vs no-nap) as a between-subject factor and session (baseline, sleep restriction 1, recovery, sleep restriction 2) as a repeated factor.

11.Change in memory performance in picture-word association task over sleep restriction nights and recovery night

Score on picture-word association task will be measured. Recall accuracy will be analyzed using a mixed ANOVA with group (nap vs. no-nap) as the between group factor and time of day (morning vs. evening) as the repeated factor.

12.Mind-wandering, assessed using a breath-counting task

Breath-counting data are broken down into count cycles that terminated with either the right arrow key press or spacebar key press. A count cycle containing 8 left arrow key presses followed by 1 right arrow key press was considered a correct cycle. Any count cycle that ends with a right arrow key press without 8 left-arrow key presses preceding it is considered a miscount cycle while cycles that ends with a spacebar key press is considered a reset cycle. Accuracy, the main dependent measure, is computed as correct cycles/total cycles * 100%.

Pearson's product moment correlations will be used to assess the mutual relationships between breath counting accuracy and 1) connectivity variables obtained from fMRI resting-state data, and 2) vulnerability to sleep deprivation.

13.Effect of sleep versus wake during delay on the implementation of an intention in the future, where the intention will be encoded either before a 12-hr interval including a night of sleep or a day of wakefulness

Memory score of correctly remembering to perform the intention following the wake or sleep interval will be measured. Analysis of variance (ANOVA) will be employed to determine the statistical significance of the group x session interaction on the semantic categorization task performance. Pairwise contrasts will be tested with independent- and paired-samples *t*-tests. Independent-sample *t*-tests will also be performed to test whether the two groups will differ in the percentage of target words correctly responded to in the prospective memory task.

14.Effect of sleep versus wake during delay on memory for rewarded pictures, where the pictures will be encoded either before a 12-hr interval including a night of sleep or a day of wakefulness

Memory score of correctly remembered encoded pictures following the wake or sleep interval will be measured. The primary dependent variable will be memory accuracy during the memory test, which will be performed following a wake or sleep interval. A 2x2x2 mixed ANOVA will be used with group (sleep/wake) as between-subjects variable, and phase (reward/prereward) and category (high reward/low reward) as within-subjects variables. Post-hoc tests will compare performance for each group and between groups.

15.Effect of sleep restriction on learning of novel material (about different animal species) across separate sessions

Memory score on test of learned material will be measured.

Conceptual knowledge task (1)
The primary dependent variable will be memory accuracy during 4 memory tests at different delays, which will be broken down into certain, somewhat certain, guess and overall responses. A 2x4 mixed ANOVA to compare group (Nap/No-Nap) and delay (1/2/3/4) will be used for each. Post-hoc tests will be used to compare group performance at each test. We will also record subjective responses for fatigue, motivation, focus and ability during the 4 learning blocks, which will be analyzed in a 2x4 ANOVA with group (Nap/No-Nap) and block (1/2/3/4). Spearman's or Pearson's correlations will be used to correlate performance on different measures.

Conceptual knowledge task (2)
Memory accuracy will be measured at 2 delays, broken down into certain, somewhat certain, guess and overall responses. ANOVA to compare group (Nap/No-Nap) and delay (1/2) will be used for each. Post-hoc tests will be used to compare group performance at each test. We will also record subjective responses for fatigue, motivation, focus and ability during the 6 learning blocks, which will be analyzed in a

2x6 ANOVA with group (Nap/No-Nap) and block (1/2/3/4). Spearman's or Pearson's correlations will be used to correlate performance on different measures.

16.Effect of sleep restriction on memory of spatial locations

Performance in four mountains task will be measured. The main outcome variable will be accuracy, which will be compared between groups via an independent samples t-test. The number of errors made will also be compared in a 2x3 mixed ANOVA with group (Nap/No-Nap) and error type (spatial/elemental/configural).

17.Effect of sleep restriction on problem-solving

Number of correctly solved equations in matchstick arithmetic task will be measured. Solution accuracy and response time will be recorded for the 4 types of arithmetic problems (1/2/3/4) of varying difficulty. A 2x4 mixed ANOVA to compare group (Nap/No-Nap) and test (1/2/3/4) will be used for each. Post-hoc tests will be used to compare group performance at each test.

18.Effect of sleep restriction on picture encoding

Memory score of correctly remembered encoded pictures of non-famous people, landscapes, scenes and objects will be measured. Outcome measures will be accuracy and reaction time during encoding, and the memory measure A' during retrieval, which is a measure that corrects for response bias. These will be compared between groups in separate independent samples *t*-tests.

19.Effect of sleep restriction on effort/temporal discounting

Choice preference (perform a longer duration task for a higher reward, or to take a break for a lower/no reward) in effort/temporal discounting task is measured. Area under the discounting Curve (AUC) will be the main outcome metric. A repeated-measures ANOVA will be performed with DAY (baseline, SR, recovery) as within-subjects factor. Furthermore, correlation analyses (Spearman rho) will be performed with AUC and PVT lapses as variables (baseline, SR, recovery).

20.Effect of prior knowledge about the position of items in a previously learned hierarchy on learning of the position of novel items in a new hierarchy

Percentage of correct responses on the test. The main analysis will be comparing memory performance and reaction times for familiar and novel hierarchies, using repeated-measures ANOVA with Schema vs. non-Schema and item distance (close inference, far inference and neighboring) as within-subjects factors.

21.Effect of sleep restriction on task switching performance

Difference in reaction time between switch trial and repetition trial in task-switching task will be measured. The switch task cost for response times and accuracy will be analyzed using an ANOVA with group (nap vs no-nap) as a between-subject factor and session (baseline, sleep restriction 1, recovery, sleep restriction 2) as a repeated factor.

22.Effect of sleep restriction on attentional bias to threat assessed using visual cues that are coupled either to an aversive sound (threat cue) or a neutral sound (neutral cue)

Correct responses to a target that is presented at the same (valid) or opposite (invalid) location of stimulus will be measured. Response times will be analyzed using ANOVA with group (nap, no-nap) as a between-subject factor and session (baseline, sleep restriction 1, recovery), validity (valid, invalid), and cue type (threat, safe) as repeated factors. Correlational analyses will also be performed to examine the relationship between trait anxiety (STAI score) and attentional bias.

23. Effect of verbal reward on procedural memory assessed using a finger tapping task

Accuracy of performance on the finger-tapping task will be measured. Performance (correct responses, accuracy) will be analyzed using a linear mixed model with group (verbal reward vs no-reward), sex, and score on the competence based self-assessment scale (CBSE) as between subject factors and session (learning and recall) as a fixed repeated factor.

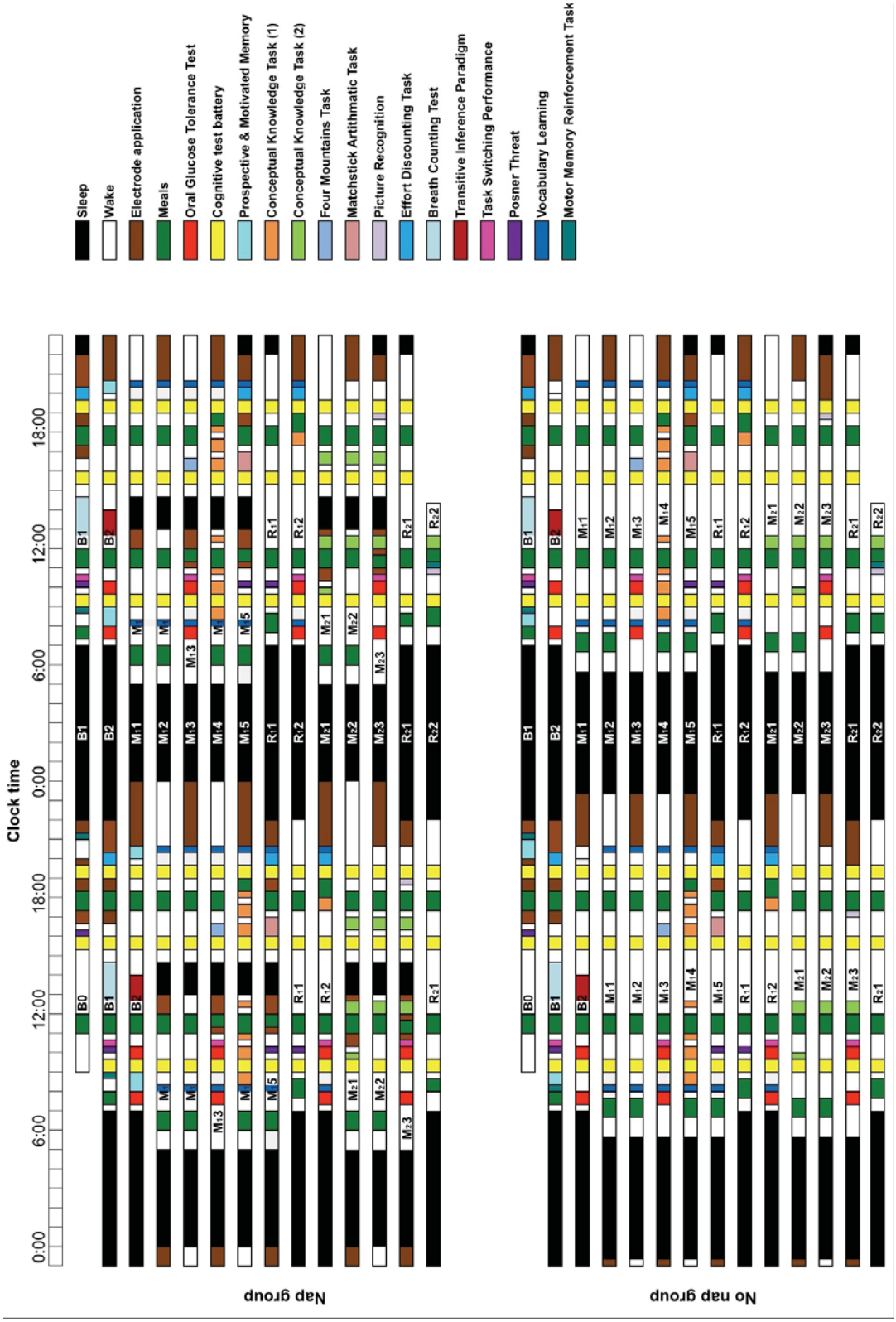

Figure 1. Study protocol. In this double raster plot, consecutive 24-h periods are plotted both next to and below each other. The occurrence of sleep periods, wake periods, meal times, test batteries and other cognitive tasks are represented by their corresponding colored bars as reflected in the legend. B1-B2: Baseline phase, $M_1$1-$M_1$5: Manipulation period 1, $R_1$1-$R_1$2: Recovery period 1, $M_2$1-$M_2$3: Manipulation period 2, $R_2$1-$R_2$2: Recovery period 2.