Methods for Human Sleep Deprivation Experiments

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Introduction

Experimentally imposed sleep loss is an important method used to determine the effects of sleep on multiple physiological processes. The two most common approaches, sleep deprivation and sleep restriction, both involve altering the amount of sleep obtained relative to the amount of wake. In sleep deprivation protocols, the goal is to maintain wakefulness continually throughout a period of time that would usually involve sleep. In sleep restriction protocols, sometimes termed partial sleep deprivation, a shortened sleep opportunity is imposed over multiple nights. Because the time course of sleep loss differs between the two approaches, their effects may differ on subjective and objective measures of performance and sleepiness and other physiological measures. Each technique has practical relevance in operational and clinical settings.

Since sleep exerts a strong influence on multiple physiological processes (e.g., endocrine, cardiovascular, immune) and since each of these processes is also affected by other factors, including circadian rhythms, pharmaceuticals, age, medical conditions, and general health, the experimental methods should consider those factors to allow appropriate interpretation of results. Here we review common techniques involved in human sleep loss experiments performed in the inpatient setting and considerations for interpreting their results.

Concept of Sleep Homeostasis

One purpose of sleep deprivation and sleep restriction protocols is to evaluate the effects of altered sleep homeostasis or increased sleep pressure on the variable(s) of interest. Therefore, a quantifiable measure of sleep homeostasis or pressure is required to document that the experimental manipulation has resulted in an altered state. The major assumption underlying the concept of sleep homeostasis is that the duration or stage composition of sleep is regulated by the duration of prior wakefulness: there is a positive relationship between amount of wake and the accumulation of sleep pressure or drive. This homeostatic mechanism reacts to both recent (e.g., length of prior wake immediately prior to the sleep episode) and longer term (e.g., amount of wake and sleep over preceding days or weeks) sleep-wake history relative to the individual's sleep "need" [1]. Any proposed marker of sleep homeostasis would be expected to increase with increasing wake duration and decrease during sleep. Markers of homeostasis have been proposed

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with respect to sleep deprivation, but there are no accepted markers for chronic sleep restriction, which has different time course of effects on performance than sleep deprivation [1].

Current metrics that can be quantified after the individual is asleep relative to immediate prior wake duration (i.e., sleep deprivation) are: Slow Wave Sleep (NREM Sleep Stages 3 and 4 in the Rechtschaffen and Kales scoring system and N3 in the current American Academy of Sleep Medicine system), delta (~0.5–4.5 Hz) power in the EEG during NREM sleep and sleep latency. Note that in the literature, sleep onset latency may have multiple definitions (e.g., first epoch of any stage, first epoch of a block of three or more consecutive epochs of any stage, etc.). During wake, theta/low frequency alpha (TLFA, 5.5–9 Hz) power in the EEG during the Karolinska Drowsiness Test (KDT) and slow eye movemetns (SEMs) during wake have been proposed a marker of homeostasis [2, 3]. Markers of sleep homeostasis/drive that can be obtained when the individual is awake are particularly useful in circumstances when there are problems with sleep (such as insomnia) and/or under conditions in which measuring homeostatic sleep pressure is required during times when sleep should not be occurring (e.g., when the individual is at work).

Methods

Participant Selection and Preparation

One of the most important considerations in human protocols involves inclusion and exclusion criteria. The participant population should be uniform in certain important characteristics, especially with regard to factors known to influence sleep and/or performance, including caffeine and alcohol use. This is particularly important given the presence of inter-individual differences as well as variance in physiological measurements and performance outcomes. Special consideration should be made to whether the participants have undiagnosed sleep disorders or other conditions (e.g., pain) or medications that may interfere with sleep. Undiagnosed sleep disorders can be particularly challenging, since occult presence of sleep disordered breathing or periodic limb movements of sleep may not cause symptoms of which the participant is aware, but nevertheless may affect the experimental results. A thorough medical history and exam is important, including all medical conditions and prescription drug dosage and use. Objective testing for sleep disorders should be strongly considered; for example, even rigorously screened asymptomatic low-risk individuals may have sleep apnea on formal testing [4].

The history should include documentation of over-the-counter medications and supplements, illegal drugs, caffeine, tobacco, and alcohol use. All of these may affect sleep and wake obtained at home and may cause withdrawal symptoms if not used or allowed during inpatient study procedures. The history may be supplemented by objective serological and/or urine testing for substances.

The amount and timing of sleep obtained at home in the 1–2 weeks before the study starts is important to at least record and potentially regulate. For example, it is critical to know if participants have traveled across time zones in the past few months, recently stayed awake late or all night, worked rotating or night shifts, or had an irregular sleep-wake schedule. These issues have practical implications for experimental design and interpretation: if participants have not obtained enough sleep at home before the experimentally imposed sleep loss begins, they may already be sleep-deprived in a manner that might impact both baseline measurements in the study and their response to the intervention. In addition, if individual participants enter the experimental portion of the study with undocumented and uncontrolled prior sleep patterns, this could confound their responses in the experimental portion. Pre-study conditions of a regular ~ 8 h nightly sleep schedule with no use of substances that may affect sleep (unless required for health/medical reasons, allowed under exclusion criteria, and included in the experimental documentation) for at least 2 weeks are recommended to mitigate the above concerns. Verification measures are strongly encouraged in addition to sleep-wake diaries. Examples include wrist actigraphy and/or call-ins to a time-stamped recorder at the time of going to bed and awakening.

A psychological screening should be performed and individuals with a history of psychiatric disorders or with immediate family members affected by these disorders should be identified. Specifically, a personal history of adverse reaction to sleep deprivation (e.g., mania or psychiatric instability) should have additional consideration before admission to an inpatient sleep protocol involving deprivation. Finally, the potential occurrence of recent events (e.g., death of a family member or friend) that might affect the individual's physical and/or psychological state should be queried.

Of extreme importance is thorough discussion with the potential participant of the expected events and conditions. If possible, the potential participant should visit the facility. Thorough familiarity with the protocol is expected to reduce stress from unknown and unexpected (and potentially unnatural) situations of the study. For example, experimental settings that prohibit use of internet or phone, or live TV/radio, for multiple days may be challenging to those accustomed to daily media availability. As part of the enrollment and consent process, members of our research team ask potential participants during the screening process to describe to us, after the protocol is explained to them, what they believe is involved in the study; this is an additional opportunity to ensure that the participant understands the study requirements and conditions, which is a critical aspect of required informed consent regulations.

Experimental Conditions

The experimental conditions should include appropriate equipment for monitoring the desired physiology and allow the study procedure of sleep loss to occur without undesired interference such as uncontrolled noise or extraneous light. A diversity of monitoring resources is preferred, as measurements may span bio-sampling (blood, saliva, urine), moderately invasive (e.g., rectal thermistor) and noninvasive (e.g., EEG) sleep-wake physiology, and cognitive performance or other psychological testing.

Although individual experimental protocols may require different degrees of specialization, we describe as an example our inpatient facility at the Brigham and Women's Hospital. This center, supported as a NIH Clinical Translational Science Center (CTSC), enables study of multiple physiological functions concurrently. A highly skilled nursing and technical staff with documented standard operating procedures and ongoing training is present. Related facilities, including a clinical centrifuge, a cold room for special handling of blood following collection, ultrasound and other imaging resources, and metabolic kitchens for preparation of required measured diets for the study volunteers, are nearby. Each experimental room is private, sound-proof, light-proof, and has an ante-room and bathroom with shower. The ante-room lowers the risk of outside noise or light affecting the room in which the participant is living. Private rooms decrease inter-participant interaction that may affect measures of interest (e.g., performance testing, mood, sleep timing and content). Almost all studies are conducted under time-free conditions, such that the participant does not know the current time or day, since knowledge of time of day or time elapsed may influence the subjective measures under study. The staff is trained to not speak of anything time-related or the timing of events during the protocol, as well as not to discuss potentially emotional issues (e.g., politics, certain sports teams). Watches, mobile phones, computers, and visitors are not allowed. The control room includes, for each experimental suite, video monitoring equipment, intercom, one computer for polysomnographic (PSG) recordings, one for monitoring computerized performance testing, and one to study schedule event prompting and monitoring. For all protocols, an investigator can have the following information available online during the study and for analysis after each experiment is complete: precise actual timing of events and of specimen collection; core body and skin temperature data; room temperature data; light levels in suites minute by minute; data from computerized performance and alertness tests; and continuous recording of EEG, EOG, EMG, ECG, heart rate, O_2 saturation, and respiratory effort.

The hardware and software for this facility form a single-source data collection system: all

experimental events (e.g., time of actual rather than scheduled time of blood samples, light levels, start/end of sleep episodes), temperature data, and results of performance tests are stored on a single computer system that meets data security and integrity guidelines. In addition, external equipment (e.g., PSG recorders) is synchronized with this system at the start of every recording, so that the timing of the recordings matches that of the system. Timing of events (e.g., specimen collection, meal presentation, other events) is entered by event marker equipment inside and outside each suite. For quality assurance, technicians must "sign off" with their personal identification on all procedures (e.g., blood sample, meal administration) that they have conducted. All data stored on this system are by de-identified study code, rather than participant name or other identifiers.

For neurocognitive testing, both simple and complex tasks that assess different functions are included and new neurobehavioral tasks can be programmed and added as needed. After every sleep episode, participants complete a questionnaire in which they indicate their estimates of time to fall asleep (sleep latency), number of awakenings during the sleep episode, total amount of wake before the end of each sleep episode, and total amount of sleep obtained in each sleep opportunity. These subjective estimates, which are frequently used in clinical PSG assessments as well as in outpatient research field studies, can be compared with objective measures of sleep obtained from scored PSG data. Note that numerous studies have documented mismatch between objective and subjective measurements of these sleep-wake variables [5, 6]. Self-assessment of performance questionnaires may also be useful for comparison with objective performance measures. Both subjective and objective measures are monitored because they assess different aspects of vulnerability to circadian and sleep homeostatic influences [7–9], including sleep inertia.

Sleep PSG recordings should be conducted and scored using standardized accepted measures, such as those approved by the American Academy of Sleep Medicine [10] by trained scorers blind to study interventions. Waking PSGs may also be useful; the waking EEG has been shown to have homeostatic sleep and circadian components [11]. For example, waking EEG activity can be assessed using the KDT. In the KDT, participants sit quietly and fix their gaze for 5 consecutive minutes (4 min eyes open followed by 1 min of eyes closed) allowing for the intermittent recording of short bouts of uncontaminated EEG at specified times within longer periods of sustained wakefulness during a study. Spectral analyses or other signal processing of the EEG can then be conducted. The waking PSG recordings are also used to monitor whether any unintentional sleep occurred during scheduled wake episodes. The EOG can be recorded for the assessment of slow eye movements (SEMs) [3], another measure of sleep homeostasis.

Actigraphy

Activity and light-measuring devices (i.e., "actigraphy") should be worn by all participants during at least the week before inpatient portion of the protocols begin to confirm compliance with outpatient protocol instructions. Actigraphy data should be downloaded and reviewed at least weekly with the participant during screening to improve compliance. Actigraphy can also be worn during the inpatient portion of the protocol for comparison of data under known activity and light conditions. Light data and either raw activity data or activity data scored into Sleep or Wake from validated algorithms [12–16] also can be used for further analyses.

Temperature Recordings, Blood Samples, Urine Samples, and Saliva Samples: For Circadian-based Analyses

Core body temperature (CBT) is commonly measured as a surrogate for circadian phase under specific inpatient conditions and can be continuously monitored by means of a rectal thermistor; ambulatory outpatient or inpatient CBT is not an appropriate circadian phase marker [17]. Participants typically have no trouble tolerating this approach to CBT. Skin temperature, by contrast, has not been proven to be an accurate measure of circadian phase.

In protocols that require frequent blood sampling, placement of an indwelling intravenous (IV) line can facilitate sampling during wake and sleep episodes. The IV setup allows for blood sampling from within or outside the room, so that blood sampling during sleep episodes does not disturb the participant. The exact time the blood sample is collected (rather than the scheduled time) should be recorded. This is extremely important for studies involving hormones whose secretion varies in a circadian and/or pulsatile manner.

Urine samples can be collected approximately every 2–3 h while the participant is awake, before and after sleep episodes, and after spontaneous voids. Saliva can be collected by having the participant spit into a test tube. The exact time the sample is collected (rather than the scheduled time) should be noted.

Sleep Deprivation and Sleep Restriction Protocols

Sleep deprivation and sleep restriction can be conducted in different ways, each of which may affect both experimental results and inferences made about the impact of sleep on functional outcomes. For example, the choice of timing of the habitual sleep and sleep deprivation/restriction are important. The timing of baseline (presleep loss intervention) sleep episodes should be relative to the individuals' habitual sleep time. If an individual's habitual sleep times (as established during the 1–2 weeks prior to the inpatient study, as recommended above) are 2–10 am, then an experimentally imposed sleep episode beginning at 10 pm will likely have a marked sleep latency simply because it differs so greatly from the habitual sleep pattern and therefore may not be appropriate as "baseline" for comparison conditions. For sleep restriction methods, consider whether to start the sleep episode late, end the sleep episode early or both, depending on the importance of the pattern of light exposure, sleep latency, and other factors affected.

During the "extra" wake time associated with sleep deprivation/restriction protocols, the environment and activities should be considered. The light levels in the room and from electronic devices should be relatively low, so as to not shift the circadian pacemaker [18, 19]. Interaction with other individuals or entertainment media should be relatively stress-free. For example, we recommend no intense discussions and only G-, PG-, or PG13-rated movies, TV shows, or games, since those with other ratings are designed to elicit strong emotions that may impact sleep and/or physiological measurements.

Protocols involving manipulation of selective sleep stages can provide insights into the relative importance of specific aspects of sleep. These require trained personnel to score the sleep stage in "real time" and then intervene when criteria are met (e.g., 1 epoch of N3 sleep). The possibility of false positives (i.e., intervention when the stage did not occur) or false negatives (i.e., lack of intervention when the stage did occur) should be accounted for when the sleep recording is rescored later. The choice of intervention (e.g., spoken participant name or gradual increase in noise level) should be considered, as the response to generic stimuli may differ from responses to "high-valence" stimuli (such as the participant's name or the sound of a baby crying). Also note that indirect effects of sleep perturbations may occur. For example, NREM sleep deprivation is often associated with substantial REM sleep deprivation since REM sleep tends to occur only after a NREM sleep. In addition, slow wave sleep (N3) deprivation may be accompanied by increases in stage N1, N2, and arousal index. Thus, it should consider that indirect effects may plausibly be causally related to conclusions attributed solely to the primary intervention.

Analysis Considerations

Consultation with a statistician is crucial given the diversity of measurements, longitudinal aspects, correlated data streams, and other considerations that impact study design and analysis. Careful consideration of these issues will ensure appropriate statistical power with the number of participants to be enrolled and the metrics to be collected. Immediately after each

data set is collected, checking for errors, inconsistencies, missing data, and other potential questions should be performed to identify problems rapidly and implement changes if necessary. Performing such checks during the study, rather than waiting until the end of the study (after all participants have been enrolled), allows the opportunity to resolve any discovered issues and quickly implement necessary changes. Statistician involvement throughout the study, and after all primary data are collected and analyzed, is crucial to ensure that planned statistical tests are appropriate. Common errors are to apply statistics (e.g., mean and standard deviation) that assume a statistically normal (e.g., Gaussian) distribution inappropriately, such as to data (1) that do not fit that distribution or (2) with too few data points, or (3) that are not independent (e.g. multiple observations from each individual).

Conclusion

This chapter outlines suggestions for conducting sleep deprivation and restriction experiments in an inpatient experimental setting. Given the amount of time and resources required to conduct inpatient sleep experiments, it is critical to control for the many factors that may affect results to ensure that the obtained data are robust and reliable.

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