Extended Research Project

Detection of neuronal replay of parabolic flight experiences during sleep in humans
A feasibility study

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June 15, 2018

Abstract

Neuronal replay of recent wake experiences during sleep is thought to be an important concept in memory consolidation. Evidence for replay during human sleep is sparse, however, due to the high number of learning experiences that humans experience during a day. As the novelty of an experience is correlated with the chance of finding neuronal replay, a highly novel experience during wakefulness might be needed for the detection of this replay during sleep. The current project explored the possibility of finding evidence for the existence of neuronal replay by means of extreme vestibular learning events, specifically that of parabolic flights. Due to the limited research on parabolic flights and neuronal replay, this research is mainly conducted in an exploratory fashion. Results show indirect evidence for the existence of neuronal replay with significant differences in sleep characteristics after the experience of a parabolic flight. The use of parabolic flights is therefore a possible method in research towards neuronal replay.

The research conducted in this project consists of two main part. The first part is the in-depth analysis of the flightdata and sleepdata, this is done by first identifying gravity related changes in EEG signal during the flight and then comparing that to non-invasive EEG measurements during sleep before and after the event. The second part uses these results combined with machine learning techniques to find differences between preflight and postflight sleep. The first part is the main report, the second part is the additional Artificial Intelligence section of the project.

Contents

1 Abbreviations

BCI	Brain-Computer Interface is the system that controls software by means of brain signals.
ECoG	Electrocorticography measures the electrical activity from the exposed surface of the brain.
EEG	Electroencephalography measures the electrical activity of the brain from outside the scalp.
ESA	European Space Agency.
G	Gravity. Often in combinations with either 0 (micro-gravity), 1 (normal gravity) or 1.8G (hypergravity).
ICA	Independent Component Analysis is a computational method that divides a signal into multiple statistically independent subcomponents.
MEG	Magnetoencephalography measures the electrical activity by measuring the magnetic fields from outside the skull.
MRI	Magnetic Resonance Imaging uses magnetic fields to detect the anatomy of the brain. The functional MRI (fMRI) uses these fields to detect activity inside the brain.
N2	Sleep stage 2 that can be identified by its sleep spindles and some short periods of SOs. N2 is a part of NREM-sleep.
N3	Sleep stage 3 that can be identified by its SOs. N3 is a part of NREM-sleep.
ReLU	Rectified Linear Unit is a possible layer in a Neural Network that converts all negative numbers to 0, but leaves all positive numbers untouched.
NREM	NonREM sleep contains sleep stage N2 and N3. During these stages, the muscles are paralyzed and thus hardly any movement is made.
REM	Rapid Eye Movement sleep is the sleepstage that is mostly recognizable by the rapid movement of the eyes and the brain signals that are similar to signals during wake-state. Dreaming occurs mostly during this sleepstage.
SO	Slow oscillations are slow waves ($\leq$ 1Hz) mostly visible during the SWS-stage of sleep.
SWS	Slow wave sleep is a sleepstage, often referred to as N3 (and N4), that contains mostly SOs.

Main part of thesis.

2 Introduction

Why do we sleep? Scientists have tried to find the answer to this question for the past centuries, but there has been no definite answer yet. Where sleep was first assumed to be a total shut-down of the brain, it is now known that the brain is very active during sleep and is likely to be involved in many processes. This is also reflected in the amount of research papers published on sleep that nearly tripled in the past 3 years. Research is currently primarily focused on the essential role of sleep on learning, memory and neuroplasticity, ranging from cellular and molecular studies in animals to behavioral studies in humans (Bray, 2017; Schouten et al., 2016). Many studies point towards the consolidation of memory as an effect of sleep-dependent mechanisms of neuroplasticity (Hobson & Pace-Schott, 2002). How sleep would promote neuroplasticity, however, is largely unknown and results are often controversial.

Neuronal replay is the neuronal activity observed during sleep that is a reflection of the neuronal activity observed during wakefulness. This replay is mostly observed in animals after a spatial learning event (Wilson & McNaughton, 1994). It has been one of the main discoveries in neuroscience of the past 30 years and is often thought to be one of the main principles of learning. Due to the complexity of human daily life and the ethical standards of neuroimaging, this replay is yet to be discovered in humans.

3 Background

3.1 Neuronal replay

A human brain is complex and consists of billions of cells, including neurons, each with their own electrical activity. These cells are connected to each other through an efficient network to jointly carry out a bodily or cognitive function (Pletser & Quadens, 2002). Buszaki (1996) hypothesized that the experiences during wakefulness are transferred from the neocortex to the hippocampus, these experiences are then consolidated and stored into memory during the sleep period after the event. For consolidation to occur, however, these memory traces should have a specific neuronal representation. Evidence for such a representation is given by Wilson & McNaughton (1994), who discovered that neuronal firing patterns that were elicited during spatial navigation tasks were replayed during consecutive sleep in rats. However, this replay often occurs at a different timescale than during the learning event itself (Genzel & Robertson, 2015). These similar electrophysiological patterns of neuronal synaptic activity during wakefulness and sleep, is called 'neuronal replay' and is primarily shown in the hippocampal place cells in rats. These cells fire when a rat is within a certain area of the environment independent of the direction of movement (Wilson & McNaughton, 1994). Because of the refiring of neurons, along with evidence that neuronal activations can modify synaptic connections (Dickson, 2010), it is proposed that these replay processes promote memory and neuroplasticity by strengthening and weakening synaptic connections.

Even though evidence for neuronal replay has been repeatedly replicated in animals, evidence of neuronal replay in humans is sparse. This is due to a variety of reasons. One of the problems is the similarity between experimental learning and general information processing during the same day and the many daily experiences that humans have. These two aspects of human life probably lead to similar replay signals during sleep that are all mixed together. This is less of a problem in animals as animals can be brought up with limited stimuli and learning opportunities. Another main reason is the difference in recording possibilities and their spatial scales. Animals can be subjected to intracranial electrocorticography recordings (ECoG), where measurements can be taken from inside the skull, which is only ethically approved in humans when they have these implants for medical reasons. Brain research towards neuronal oscillations in humans is usually done by means of external electroencaphalography (EEG) or magnetoencephalography (MEG), which both measure the brain signals from outside the skull. As each electrode is quite far from the source and therefore records activity from many neurons at the same time, it is impossible to isolate the activity of only one neuron or even a very small number of neurons, such as the hippocampal place cells.

Although human neuronal replay is more difficult to detect in humans, there has been some progress in the field. Research with fMRI has shown that certain brain areas are reactivated in the same sequential order during sleep as during the activity (Peigneux et al, 2003, 2004; Macquet et al., 2000). The reactivation of specific brain areas is shown to be correlated with the time spent on learning and the performance on the tasks after sleep in animals and humans (Peigneux et al., 2003, 2004). Furthermore, human hippocampal cells, with similar behavior to the hippocampal place cells in rats, are shown to exist (Ekstrom et al., 2003). It is therefore hypothesized that neuronal replay also plays an essential role in the learning processes in humans. The reactivations of brain areas might thus be reflections of the neuronal replay on a larger spatial scale.

Jiang et al. (2017) were the first to find evidence for neuronal replay by ECoG recordings. They matched firing peaks across widespread cortical regions during wakefulness after a learning event to the ECoG recordings during the sleep and compared that to the recordings before the learning event. They have found more matches in the sleep after the event than in the sleep before the event. These matches occurred during sleep spindles and down-to-up-transitions of slow oscillations. Spindles are a distinctive feature in EEG recordings during the Non-REM phase of sleep, characterized by a quick oscillation between 10 and 16 Hz and their short duration of maximally 1 second (De Ganarro Ferrara, 2003). Gais et al. (2002) found that the density of spindles was larger after a learning event, especially in the first 90 minutes of sleep. This theory was provided with more evidence by Lustenberger et al. (2016), who have shown a functional relationship between spindle density and memory consolidation. Spindles can be divided into slow (around 12 Hz) and fast (around 14 Hz) spindle oscillations. Mölle et al. (2011) found that the coupling of fast spindles and up-state of slow oscillations is also correlated with memory consolidation. Furthermore, the number of fast spindles has also been correlated with dream recall (Nielsen et al., 2016). The remaining spindle characteristics (duration, frequency and amplitude) of both fast and slow spindles seem to change in relation to learning events and subsequent performance, but there is only limited research on the specific characteristics and its effects (Schabus et al., 2006). Based on these different researches, there seems to be a relationship between spindles, slow oscillations, replay and dreams. Sleep spindle characteristics, slow oscillations and their coupling will therefore be a good starting point to research the possible existence of neuronal replay.

3.2 Parabolic flight

Studies have shown that the higher the novelty of a stimulus, the more likely it is to be remembered (Tulving & Kroll, 1995). Together with evidence that the reactivations of brain areas correlate positively with performance (Peigneux et al., 2004), it is proposed that the neuronal replay, if it exists, will also become more visible as a result of extremely novel environments. Thus, to increase the chances of finding neuronal replay, one can increase the novelty of the experiences during the day. However, this is hard to do under normal circumstances as it is hard to control the novelty of a stimulus per participant.

One possible way to provide an extremely novel experience is the use of parabolic flights. Parabolic flights are the ultimate method of introducing extremely novel experiences, as it is quite easy to guarantee that none of the participants have experienced the different gravity levels. Parabolic flights simulate different gravity conditions between microgravity (close to 0G) and hypergravity (up to 1.8G) with a refitted aircraft that flies a parabolic shape at approximately 45°angles (ESA, 2015). At the top of the curve the passengers experience around 20 seconds of microgravity, with increased gravity of nearly 1.8G for 20 seconds each during the ascent and descent before and after this period. A typical parabolic flight goes through 31 of such parabolas within two hours of time. The time length, its repetition and the novelty increase the likelihood of making neuronal replay visible. Furthermore, as the periods of micro- and hypergravity are nearly constant with set periods of normal gravity in between the them, parabolic flights enable a perfect blocked design. This design makes it easier to identify the neural signatures of the different gravity levels during the flight that can later be identified in the sleep after the event.

3.3 Effect of different gravity conditions on the brain

Different gravity conditions induce multiple biological changes that are often complex and affect various systems and processes within a human. Since 1960s the effects of different gravity conditions on the brain during space flights, parabolic flights and other gravity stimulating studies have been studied (Pletser & Quadens, 2003). Many studies have shown that behavioral performance is changed, such as a decrease in vertical spatial representation performance and skewed mental image transformations (Grabherr & Mast, 2010). Research on changes of the actual brain signals during different gravity conditions is more limited and often with incongruent results. These incoherent results might be an effect of capshifts, the individual variability in physiological processes and the small sample size of subjects (Van Ombergen et al., 2017). Further complications are caused by the effect of the task on the resulting brain signals, which makes it harder to distinguish task-related activity and gravity changes (Van Ombergen et al., 2017). Even though final conclusions are often different, cortical sensory areas and vestibular-related pathways are often shown to be affected (Van Ombergen et al., 2017). These areas might be affected due to the possible affected hippocampal activity and its communication with the neocortex during wakefulness.

Both cortical sensory areas and vestibular-related pathways receive input from the vestibular system. The vestibular system is a sensory system that coordinates movement by processing the balance and spatial orientation of the human. Part of the vestibular system are the otolithic organs that process magnitude and direction, which is impaired during microgravity as it is abruptly deprived of a sense of gravity (Grabherr & Mast, 2010). This might have its effect on the vestibular nuclei of the brain and its projections to sensory integration areas, such as the thalamus or the temporoparietal region (Lopez & Blanke, 2011). Due to the slow changes in gravity, otolith afferent signals are mostly low frequency.

Evidence for distinctive neuronal representations of different gravity levels mainly comes from animal research. In rats, the hippocampal place cells and head direction cells in the thalamus use idiothetic cues, such as activity of the vestibular system, and external landmarks to derive the direction and location of the rat (Knierim et al., 2000). Knierim et al (2000) propose that three-dimensional navigation in microgravity might lead to inconsistent associations between head direction cells and landmarks, which leads to an inconsistency in the hippocampal place code. They have shown that the place cells in rats exhibit abnormal patterns of spatial selectivity when first placed in microgravity. Thus, different gravity conditions are likely to have a different effect on the neuronal activity of the hippocampal place cells and the neuronal activity of its projections. This change in activity provides the possibility of distinguishable neuronal activity during the different gravity conditions of the flights.

In conclusion, theoretically it should be possible to detect neuronal replay during sleep, especially with the use of parabolic flights as they produce the novelty and neocortical signature that is needed for detection. This thesis will examine the feasibility of this method and will explore the possible measurements to find direct and indirect evidence for the existence of neuronal replay. The feasibility will be examined in two parts: the first part aims to determine if there is a neuronal signature of the different gravity conditions on both a time and frequency scale, whereas the second part aims to find evidence for the existence of neuronal replay in humans. This evidence will be sought in the differences in sleep characteristics that have been correlated with neuronal replay between pre- and postflight sleep. If parabolic flights are a usable method in detecting neuronal replay, it will pave the way for further research on neuronal replay and memory related processes.

4 Method

This part of the project consists of two main parts. The first part of the study aims to find the neural correlates that indicate significant changes of brain signals under different gravitational conditions (0G, 1G and 1.8G) that can later be correlated with signals during sleep to detect neuronal replay. As parabolic flights change gravity levels, specific focus will be on changes around the vestibular system. To examine the possibility of detecting neuronal replay by means of parabolic flights, EEG measurements are made during parabolic flights. The second part of the study focuses on the effects of the different gravity conditions on sleep characteristics. Conclusions about a possible role for parabolic flights in neuronal replay research are based upon these results.

4.1 Participants

A total of nine participants were recruited within the age-range of 18-44 years with an average age of 28.4 years and a standard deviation of 5.9 years. In total, four women and five men were recruited. All of the participants had no neurological (sleep) disorders, no experience with parabolic flights and normal eyesight. Furthermore, none of the participants were on any medication.

4.2 Parabolic flight

All three parabolic flights were conducted at Merignac International Airport in Bordeaux, France. An Airbus A300 "ZeroG" is used especially for these parabolic flights. The flights were organized by NOVESPACE and the experiment was run with approval of the Radboud University Faculty of Social Sciences Nijmegen (The Netherlands) and approval of the medical ethical committee of the university of Caen (France). The flights were a part of the European Space Agency's (ESA) Fly Your Thesis! campaign of 2017. In the ESA Fly Your Thesis! Campaign students are given the opportunity to conduct research under different gravity conditions (microgravity, hypergravity and normal gravity). Within this campaign, I was a part of Team BrainFly. The team consisted of four women from different Dutch universities, all enrolled in a master programme within the field of Cognitive Neuroscience. As a team we examined the possibility of the use of continuous Brain Computer Interfaces (BCI) in space, but all of the members addressed the topic with a different research question.

The whole flight consisted of 31 parabola, of which 26 were used for the experiment. Each parabola consisted of three separate stages (1.8G, 0G and 1.8G), with each stage lasting approximately 20 seconds. These blocked changes in gravity provided a good trial design to find the neural correlates under these different gravitational conditions. Three participants participated in the experiment per flight. Between each parabola there was a two minute normal gravity phase before the next parabola started. After each 5 parabola, there was a 4-8 minute break, with a 30 minute break after 16 of the parabola. In total 8 minutes of microgravity and 16 minutes of hypergravity are recorded. The total flight lasted approximately 2 hours.

All participants were medically checked by a professional specifically for the parabolic flight and had to sign informed consent. Scopolamine, an anti-motion sickness drug, was professionally administered intravenously at a dose of 0.5-0.8mg to each participant before the flight.

During the flights, participants were strapped to an airplaneseat with a seatbelt around their waist. This provided a controlled and safe testing environment for the participant in all gravity conditions. Furthermore, this also limited changes in neuronal activity caused by motion or other (free-floating) interference. To limit the stimulus input, surroundings were covered by a black curtain. Participants did not have any device to block out noise due to safety restrictions set by NOVESPACE. Video recordings were made during the whole flight from three angles that were used for trial rejection in the data analysis.

4.3 Data recording

4.3.1 Flight

All EEG measurements were done with a 64-channel ANT Neuro EegoSports waveguard system and an additional gyrometer to timelock the EEG-signal to the gravity level in the data analysis. EEG was recorded at a samplingrate of 250 Hz. The EEG electrodes were attached to the participants before the flight outside of the airplane. The amplifier was stored in a backpack carried on the front of the participant and was connected to a laptop by USB cable. The laptop was attached to a table that was strapped to their lap. Due to safety reasons, the laptop and amplifier were not connected to an external powersource during the measurements.

During the first parabola no task was assigned. During two of the 26 parabola participants were instructed to keep their eyes closed, during another two they were instructed to keep their eyes open while focusing on a cross on the screen. A P300-task was carried out in three parabola. The rest of the parabola were dedicated to a BCI game. These different tasks were performed for the research questions of the rest of the teammembers. An additional benefit of these tasks was that it prevented motionsickness by requiring focus, which limited the movement of the participant further. The BCI game consisted of a canon that had one-directional (left-right) movement with the task to shoot aliens that were descending from the top of the screen. Participants had to move the canon by means of their brain signals, for which they were extensively trained two months in advance. The P3-task consisted of a square that randomly changed color with distractor colors and targetcolors, the number of target color changes had to be counted.

4.3.2 Sleep

All EEG measurements are done with the same EEG system as was used during the flight, but now recorded at a sampling-rate of 500Hz. The EEG system was detached and reattached between the flight and the postflight sleep. Data was recorded with Eego64 (ANT Neuro Software 1.8.0). Furthermore, an additional EOG channel, measuring eye-movement, and EMG channel, measuring muscle activity, were attached to the participant. Participants had no additional sleep between the flight and the sleep measurement. As a baseline condition, the same measurement was performed during a night of sleep two weeks prior to the actual flights with the same set-up.

4.3.3 Questionnaires

Sleep disorders
The Pittsburgh Sleep Quality questionnaire was conducted under the participants to verify that they did not suffer from any obvious sleep disorders.

Sleep evaluation
A general sleep questionnaire was conducted after the pre- and postflight sleep to see if any adverse events happened during the night. Participants were asked to write down any specific dreams they remembered during the night (if they were consciously awake) and in the morning after the night of sleep.

Dream occurrences in the general public of flyers
A general dream questionnaire was conducted under parabolic flyers (previous or this flight, frequent and non-frequent) about dreams and a possible flying/falling sensation during postflight sleep. This questionnaire was conducted to examine if this sensation occurs often and to find slight evidence for possible replay during the night after the flight.

4.4 Data analysis

All data analysis was done in MATLAB2017b with the Fieldtrip toolbox[1].

4.4.1 Flightdata

Datacleaning & Preparation
Inspection of the flightmovies identified parabola in which the participants were not focused on the screen or in which the software did not function properly (BCI did not react or the laptop displayed errors). These trials were removed from the data.

As the official measuring software referenced all electrodes to the CPz, all data

[1] Copyright (C) 2008-2016, Donders Institute for Brain, Cognition and Behaviour, Radboud University, The Netherlands (DCCN, DCC, DCN). Version 14-11-2017 is used.

is rereferenced to the common average over all electrodes.

Visual artifact rejection is performed to remove bad channels and bad trials by removing outliers in both the time and frequency domain. To further clean the data, ICA-component analysis is performed and noisy and irrelevant components (such as eyeblinks or task related activity) are regressed out of the data.

Time analysis

Gyrometer data

The different gravity levels were detected by setting a threshold to the first hypergravity phase. The parabola were then cut out based on this data (20 seconds before this threshold was passed, and 80 seconds after. These 80 seconds include the first and second hypergravity phase, the microgravity phase and 20 seconds of normal gravity after the parabola. These parabola were plotted on top of each other to see how much the parabola differ in their accelerations. These detected timepoints were used as trials for further analysis of the EEG data. As the gyrometers detect only relative acceleration, the timecourse is compared to the official G-measurements of the airplane.

EEG data

All (clean) trials per EEG channel were averaged and examined for a significant and consistent change per gravity level. First low frequencies (up to 5Hz) were examined as these reflect the slow changes in gravity. Frequency analysis was then done over the entire frequency spectrum to detect significant changes in the EEG-signal during the different gravity conditions. The frequency range of these changes was then used as a bandpassfilter on the data. The main focus was placed on the channels over the vestibular system of the brain, TP7 and TP8, as the biggest change is expected here. As changes in the signal can also result from consistent muscle movement (moving the neck or arms during changes in gravity), the variation in the signal was also calculated.

The mean and variation of the signal were also inspected to give an indication of voltage changes that could have resulted from the cap shifting.

Only time signal analysis was done in this part of the thesis as we do not expect the same frequencies of the brain signals during the night due to evidence of a faster replay of signals found in rats. However, additional frequency analyses were done to examine other effects of gravity on brain signals. Results are shown in the appendices.

4.4.2 Sleepdata

Questionnaires

Sleep disorders

The Pittsburg Sleep Quality Index is calculated to detect any sleep disorders and the general dream questionnaire is analyzed to see if lucid dreaming and falling and flying dreams are a normal occurrence during the sleep of the participants.

Sleep evaluation
The pre- and postflight sleep questionnaire is inspected to see if any adverse events happened during the night.

Dream occurrences in the general public of flyers
The general dream questionnaire, held under general (frequent and non-frequent) parabolic flyers, was analyzed on the occurrence of lucid dreams and dreams of flying and falling and the possible influence of flight frequencies and Scopolamine on these dreams.

Hypnogram
The sleep was mainly analyzed using the SpiSOP toolbox version 2.3.5.1 (Weber, 2013), which is an extension of the Fieldtrip toolbox. Sleep scoring of sleep stages is done by following the AASM manual of scoring sleep and associated events (Berry et al., 2015). Sleep data was preprocessed by rereferencing to the mastoids, M1 or M2, and scored based on the EOG, EMG and the rereferenced frontal, central and occipital electrodes from either the left or right hemisphere in 30 second time-windows (epochs). Furthermore, a bandpass filter was applied from 0.3 to 35 Hz to the EOG- and EEG-channels and a 10 to 100Hz filter was applied to the EMG-channel. Movement and other visual artifacts were detected per epoch during the scoring and removed before further processing of the data.

The hypnogram was examined to detect any changes in the duration and latency of the sleep and its stages.

Datacleaning
Any adverse events during the night indicated by the questionnaire were removed from the data or taken into account with the analysis.

As the official measuring software referenced all electrodes to the CPz, all data is rereferenced to the common average over all electrodes.

The movement and other artifacts detected in the sleep scoring were removed from the data as an entire epoch. To further clean the data, ICA-component analysis was performed and noisy and irrelevant components were regressed out of the data.

Spindle characteristics
As Jiang et al. (2017), among others, found that sleep spindles could be connected to replay and spindle characteristics could in their turn give an indication of memory consolidation (Schabus et al., 2006), spindle characteristics were examined and compared between pre- and postflight nights.

Spindles were detected using the SpiSOP software. Slow spindles were detected by their frequency which was expected to be between 10 and 13 Hz, while fast spindles were detected using a frequency range of 13 to 16 Hz. Both spindles had to have a duration between 0.5 and 2 seconds to be a valid spindle.

Sleep characteristics were compared between the stages and between the pre- and postflight sleep. Furthermore, comparisons of these characteristics are made between frontal (FPz, FP1, FP2, AF3, AF4, Fz), central (C3, C4, Cz, FC1, FC2) and vestibular locations (TP7, TP8, P7, CP5, CP6, P8 - only left or right side is used depending on available electrodes) and averaged over electrodes. The focus was on spindles for the NREM phase as spindles mostly exist in this stage.

The average value and the standard deviation were plotted in a bar graph. The value of the two-sample t-test between the two datasets was calculated per characteristic and site of electrodes to examine which differences are significant. The values of the first 30 minutes (60 epochs) were plotted in a graph, to detect any changes in variation between the epochs.

The following characteristics were examined:

Duration
The average duration of sleep spindles over all epochs was measured.

Amplitude
The average amplitude of sleep spindles per 30 second epoch was measured. The amplitude was calculated by calculating the distance between the maximum trough of the spindle and the maximum peak. As datasets can differ significantly in their signal amplitude, the amplitude is normalized such that comparison between datasets was possible.

Density
The average number of sleep spindles per 30 second epoch was calculated.

Frequency
The average frequency of sleep spindles over all epochs was calculated.

The frequency was further examined by performing a time-frequency analysis averaged over spindles to see if there are any changes in the main frequency or surrounding time points. To examine this further, the number of spindles per frequency range were counted for both fast and slow spindles per 0.2Hz bin. The final histogram will show this number normalized over the bins. The plots were then inspected for a change in distribution of the frequency of spindles between pre- and postflight nights.

Spindle and slow oscillation coupling
Mölle et al. (2011) found that fast spindles to slow oscillation upstate (depolarization) coupling was enhanced by prior learning, from which they have concluded that this coupling could play a role in sleepdependent memory processing. As this memory processing might be connected to replay, the coupling between the spindle and the up- or downstate (hyperpolarization) of the slow oscillation is examined.

First, as both spindles (fast and slow) and slow oscillations have been related to memory, the density of these sleep phenomenon are examined over the NREM phase over the night and plotted to compare their occurences during the night. This density is normalized to see where the highest density of all three characteristics is taking place during the night. As Gais et al. (2002) found that the spindle density is higher in the first 90 minutes of sleep after a big learning event, the densities of all three characteristics are then examine on a shorter time scale. The first 90 minutes of the unnormalized densities during sleep are then compared to examine the absolute density differences between pre- and postflight sleep.

To have a closer look at the spindle and slow oscillation coupling, the coupling of the spindle (slow or fast) to the state (up or down) of the slow oscillation is examined. This is done by comparing the ratio between coupled and non-coupled spindles and the position of the peak of the spindle to the position of the trough or max of the slow oscillation in seconds. This coupling is compared between frontal, central and occipital regions with a two-sample t-test. Specific focus will lay on the coupling of fast spindles and the up-state of slow oscillations, after Mölle er al. (2011).

Slow oscillations were detected using the SpiSOP software by their frequency between 0.5 and 1 Hz.

REM-sleep analysis
Frequency changes
Depending on their memory of the dream when they wake up (acquired from the sleep questionnaire) and if the sleep stage at waking up was REM-sleep (acquired from the hypnogram), the REM-sleep is analyzed on changes in frequency (0.5 - 100Hz) and topography in the last 8 seconds of their sleep (after Siclari et al, 2017). Specific focus was placed on the REM-sleep as this is the sleepstage where most dreams occur. The topography was compared to the baseline to 1 second before the last 8 seconds before waking up. The two nights were compared by subtracting the last 8 seconds of the first REM-sleep of the pre-night from the last 8 seconds of the post-night. The main focus was set on the changes in the TP7- and TP8-electrodes as these are the electrodes over the vestibular system.

5 Results

5.1 Participants

Only two participants had a successful preflight and postflight recording and will be considered in this report. Both also had a successful flight recording. Participant 1 was a 26 year old male that acquired 0.8mg Scopolamine before the flight, participant 2 was a 24 year old female that acquired 0.5mg Scopolamine before the flight.

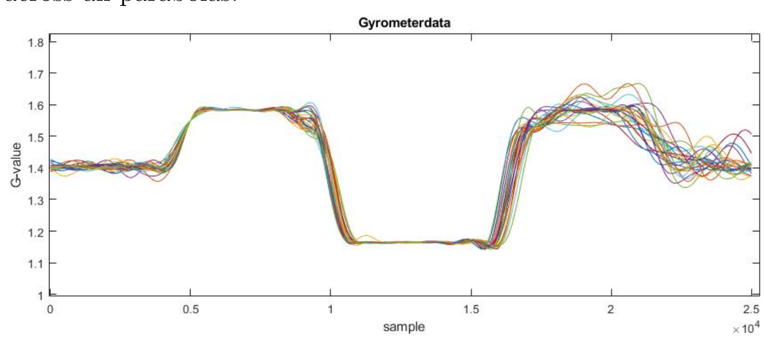
They will be indicated and participant 1 and 2 for the rest of this report.

5.2 Flight

5.2.1 Parabolas

For the results of this experiment, only 24 parabola were used. The first parabola was discarded due to the extra excitement of the novel event. The 26^{th} parabola was also discarded as EEG systems were taken off to quickly and did not record the whole effect of the changing gravity conditions.

Variability per parabola
To test if the parabolas had the same timecourse, the gyrometer data was compared across all parabolas.



The lines represent a parabola. The x-axis represents the values per sample, measured at 500Hz. A total of 25000 samples are acquired per parabola (100 seconds). The y-axis represent the relative acceleration of the gyrometer. The values of the gyrometer are relative and thus not go from 0 to 1.8G. However, they are checked with the timecourse of the official gyrometer data of the airplane. Value 1.58 on this graph corresponds to 1.85G in real gravity values. The value 1.16 on this graph corresponds to approximately 0.1G.

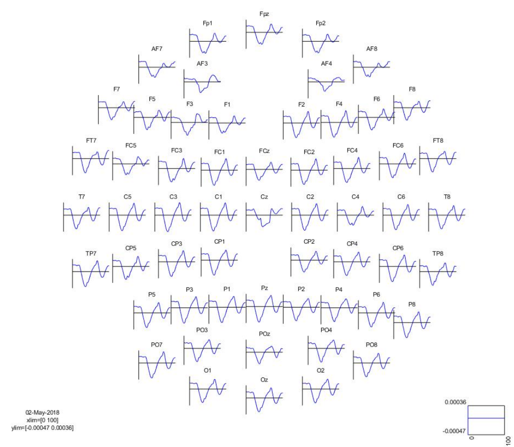
<u>Intermediate results</u>
Parabolic flights are thus quite consistent in their course of parabolas, with the second hypergravity phase showing the biggest variation. Parabolic flights are a good method to consistently change gravity levels with (roughly) equal time

lengths.

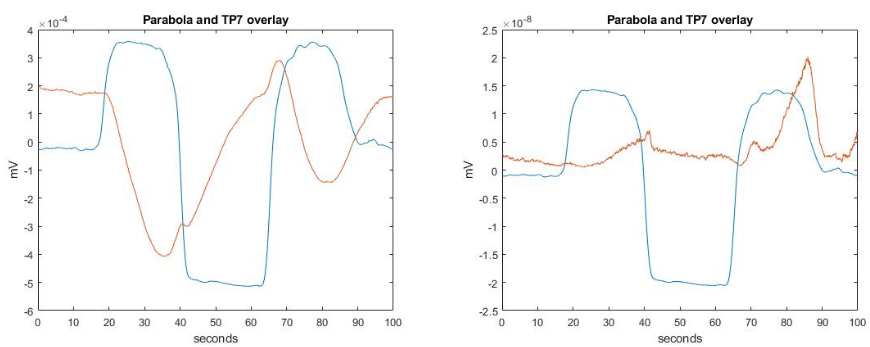
5.2.2 Neuronal signatures of gravity

Low frequency

Participant 1

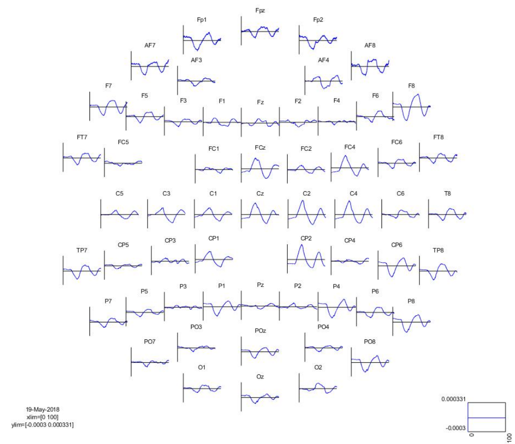


The mean signal per electrode bandpassed between 0.1 and 5 Hz.

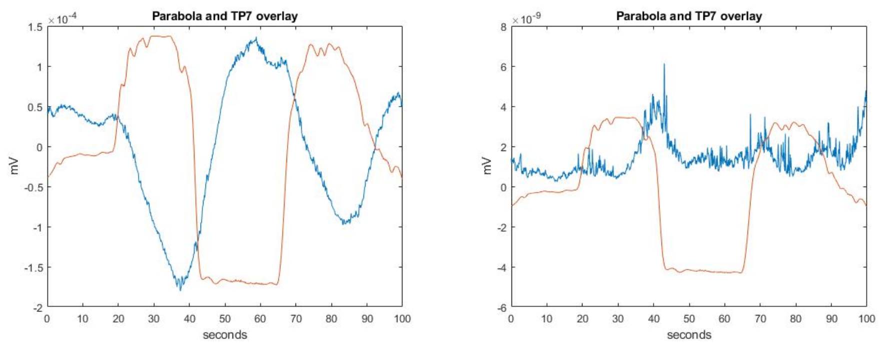


The red line shows the EEG signal of the TP7 channel with the parabola course scaled to its values (blue line). The left plot shows the signal, the right plot shows the variance of this signal over trials.

Participant 2



The mean signal per electrode bandpassed between 0.1 and 5 Hz.



The blue line shows the EEG signal of the TP7 channel with the parabola course scaled to its values (red line). The left plot shows the signal, the right plot shows the variance of this signal over trials.

Intermediate conclusions

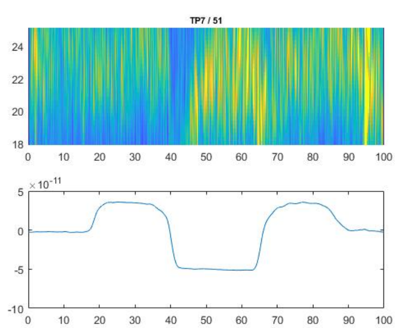
Both participants had similar effects in their EEG signal over the different gravity levels. The low frequency signal overlaps with the course of the parabola. It shows decreased activity in the hypergravity phases and increased activity in the microgravity phase. Variance is largest in the change to microgravity and the end of the second hypergravity, which overlaps with the largest variance in gyrometer data. If this change in signal, however, is brain-related, it shows that there is some identifiable signal for the different gravity levels. If there is replay of this neuronal activity in sleep, there should then also be some changes in sleep characteristics between pre- and postflight sleep according to previous researches.

The immediate change in activity, the large variation and the whole brain activity during changes in gravity, however, might be indications of muscle movement or capshifts. The first worry on performing EEG in parabolic flights is the cap shift that might be evoked by the change of gravity conditions. Expecting the possibility of bridges during hypergravity as the cap is pressed onto the skull and expecting a loss of signal during microgravity. Even if the signals in the low frequency time course are from the loosening and tightening of the cap due to gravity, no distorting bridges between electrodes are apparent. Even though there were slight trends visible over the parabolas, the shift had little effect on the consecutive measurements as the same effect was seen every parabola, with no added noise or different offset. EEG-measurements during parabolic flights are therefore possible despite the variation in levels of gravity. The muscle movements could be another great influence on the changing time signals. Especially since the largest variation in signal is around the changes in gravity, the muscle movements are a likely source of these signal changes. However, it is hard to distinguish neuronal activity from muscle activity, because it is hard to predict the movements and neuronal activity due to limited research on the topic. This will be a key issue in parabolic flight research that needs a lot of focus and controlled research before definite conclusions can be made on changes in brain signals.

High frequency

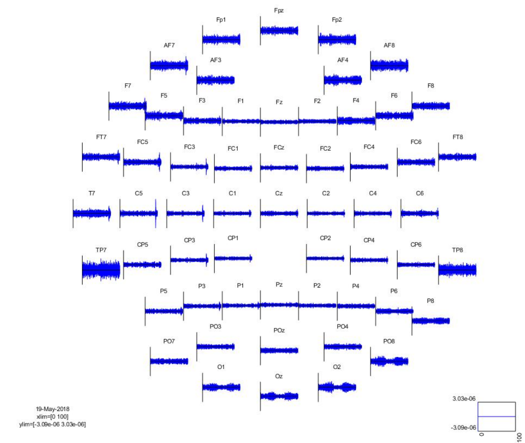
Frequency analysis of all frequencies was done over the vestibular channels (TP7 and TP8) and the interesting frequencies were analyzed.

Participant 1

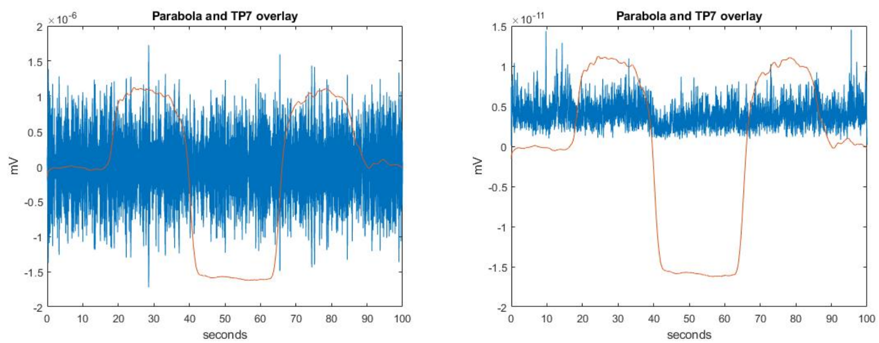


The top plot shows the time-frequency plot between 18 and 25 Hz. The bottom plot shows the parabola course on the same timescale as the topplot.

As 18 to 25Hz frequencies showed interesting characteristics over the different gravity levels, these frequencies were further analyzed.

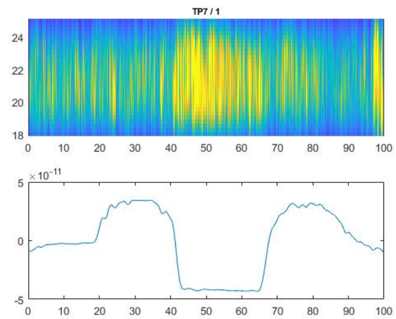


The mean signal per electrode bandpassed between 18 and 25 Hz.



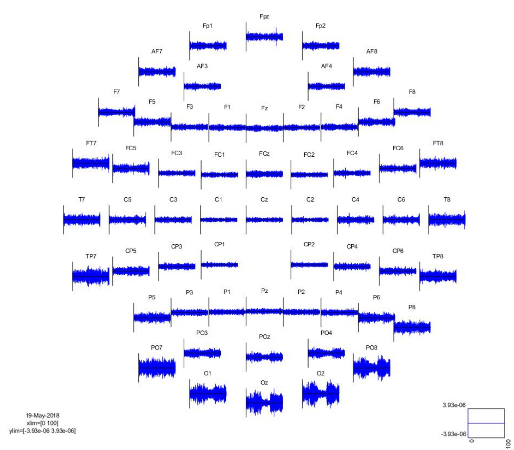
The blue line shows the EEG signal of the TP7 channel with the parabola course scaled to its values (red line). The left plot shows the signal, the right plot shows the variance of this signal over trials.

Participant 2

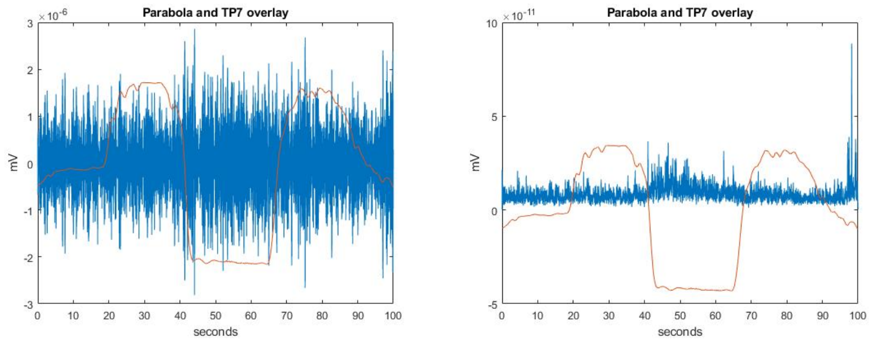


The top plot shows the time-frequency plot between 18 and 25 Hz. The bottom plot shows the parabola course on the same timescale as the topplot.

As with the first participant, 18 to 25Hz frequencies showed interesting characteristics over the different gravity levels, these frequencies were further analyzed.



The mean signal per electrode bandpassed between 18 and 25 Hz.



The blue line shows the EEG signal of the TP7 channel with the parabola course scaled to its values (red line). The left plot shows the signal, the right plot shows the variance of this signal over trials.

Intermediate conclusions

Both participants showed a strong increase in power in the vestibular area between 18 and 25 Hz during microgravity. The signal was therefore bandpass-filtered between these two frequencies. In the topoplot of the timecourses of both participants, it can be seen that the vestibular electrodes showed a strong signal during and around the parabola with no clear changes in signal (in the time domain) and variance over the timecourse of the parabola. There thus seem to be some neuronal differences around the vestibular system during the different gravity levels that can possibly be replayed and therefore detected in the neocortex during sleep. If replay of the different gravity levels exists during sleep, these vestibular areas should also be reactivated. The TP7 and TP8 electrodes are therefore a good set of electrodes for detecting neuronal signatures during the wake event and the activation in vestibular areas during sleep.

As with low frequency analysis, these changes in brain signals during the different gravity levels might also be an effect of muscle movements. Especially because the TP7 and TP8 are very close to neck and facial muscles. The effect of muscle movements seems less apparent than in the low frequency time signal though, as variance is roughly equal during the entire parabola and the effect of gravity seems to be mainly visible in the TP7 and TP8 electrodes. As the muscles are quite large around that area, the effect is expected to also be more prominently seen in the surrounding electrodes. Again, no definite signature of the different gravity levels is possible, as the other influences of the flight cannot be excluded as a possible influence.

Additional conclusions

Frequency analysis has also been done on the flightdata of which the method and results can be found in the appendices. Although these signals cannot be used in the detection of replay, it does show that there are some significant changes in brain activity during different gravity levels. Therefore, there must be some identifiable activity during changes in gravity that can later be detected in the sleep after the event.

5.3 Sleep

5.3.1 Questionnaires

Participant 1
Participant 1 scored 6 out of 21 on the Pittsburgh Sleep Quality Index, from which we can conclude that no obvious sleep disorders are present.

He dreamed less than once a month, and had a nightmare less than once a year. He did experience lucid dreaming about 2 to 4 times within a year, which were never dreams in which he was falling, but occasionally (less than once a year) a dream in which he was flying. This, however, was never a lucid dream.

During preflight sleep, he slept for 3 hours. His sleep started around 11:00 pm. He rated his rest 3 out of 5 (1 being not rested and 5 being well rested). He rated the influence of the EEG cap on his sleep a 2 out of 5 (1 being no influence, 5 being high influence). He woke up multiple times during the night and did not sleep differently than normal (by own report). He rated a 3 out of 5 on movement during sleep (1 being no movement and 5 being a lot of movement). He remembered waking up a couple of times during the night, but did not remember any of his dreams.

During the postflight sleep, he slept for 5 hours. His sleep started at 12:30 am, which was 14.5 hours after the first parabola. He rated his rested-state a 4 out of 5 and was not influenced in his sleep by the EEG cap at all. He woke up once during the night and did not sleep differently than normal. He rated 3 out of 5 on the movement during sleep. He remembered he was about to fall in his dream just moments prior before waking up.

Participant 2
Participant 2 scored 5 out of 21 on the Pittsburgh Sleep Quality Index, from which we can conclude that no obvious sleep disorders are present.

She had dreams about once a month, with a nightmare about 2 to 4 times a year. She never experienced lucid dreaming. Furthermore, her dreams were falling dreams about once a year, but never flying dreams.

During preflight sleep, she slept for about 3.5 hours. Her sleep started around 1 am. She rated her rested-state a 3 out of 5 and rated the influence of the EEG-cap on seep a 3 out of 5. She remembers waking up once during her sleep, but did not remember any dreams. She did not sleep differently than normal and did not think she moved at all during her sleep.

During the postflight sleep, she slept for approximately 2.5 hours. Her sleep started at 1:15 am, which was 16,5 hours after the first parabola. She rated his rested of 4 out of 5 and rated 2 out of 5 on the influence of the EEG cap on her sleep. She woke up once during the night and did sleep differently than normal, very short and restless this time. She rated 1 out of 5 on the movement during sleep. She did not remember any dreams.

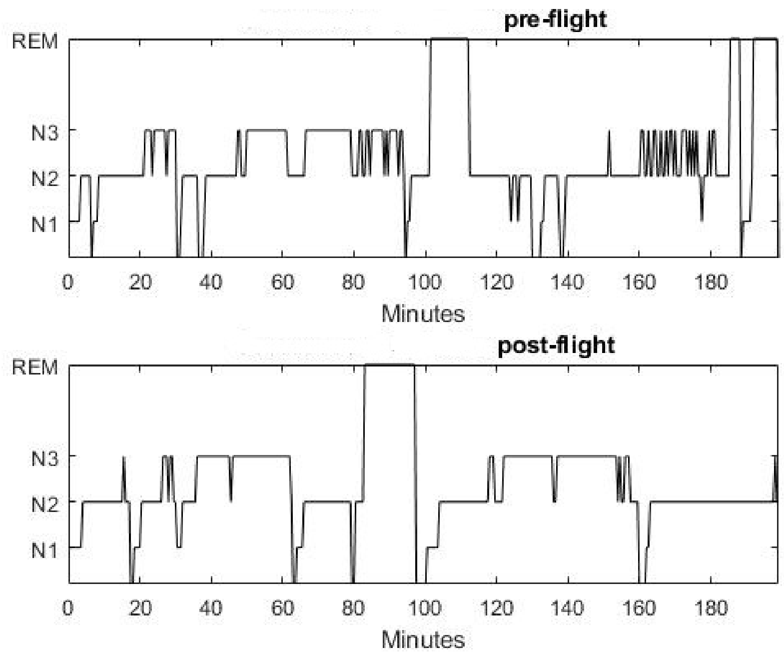
Intermediate conclusions
A lower Pittsburgh Sleep score is interpreted as a better sleep quality, on a rating scale of 0 to 21. Thus, both participants had fairly good quality of sleep in general.

Both participant had only a short period of sleep before and after the flight. However, the periods were long enough to have at least one sleep cycle to make analysis of the night possible. Participant 1 remembers a dream about falling, which could indicate an influence of vestibular experiences on sleep. This can be used in the analysis of the REM-sleep as that is the stage in which dreams occur most often. If the vestibular areas were activated during this stage, it could be that this falling sensation could be induced by the (re-)activations of these areas.

5.3.2 General sleep

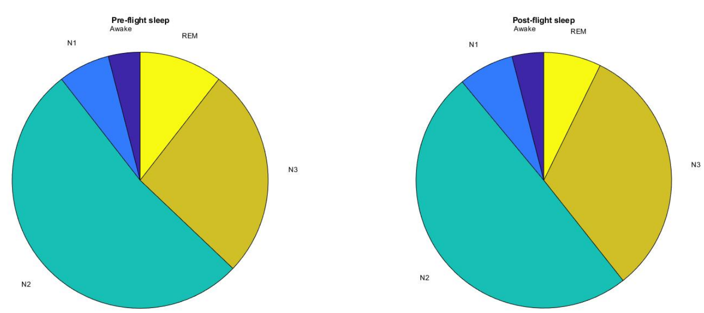
Participant 1
Sleep onset of the preflight sleep was after 46 minutes. Total sleep duration of preflight sleep was 199 minutes. The postflight sleep was 294 minutes in total. To equal the sleepnights in length, both nights were cut to 199 minutes of sleep duration.

The hypnograms of both sleepnights:



The topplot shows the hypnogram of the preflight sleep. The x-axis represents the minutes, the y-axis the sleep stage (with the 0/x-axis representing the wake-period). The bottomplot shows the hypnogram of the postflight sleep.

The division per sleep stage:



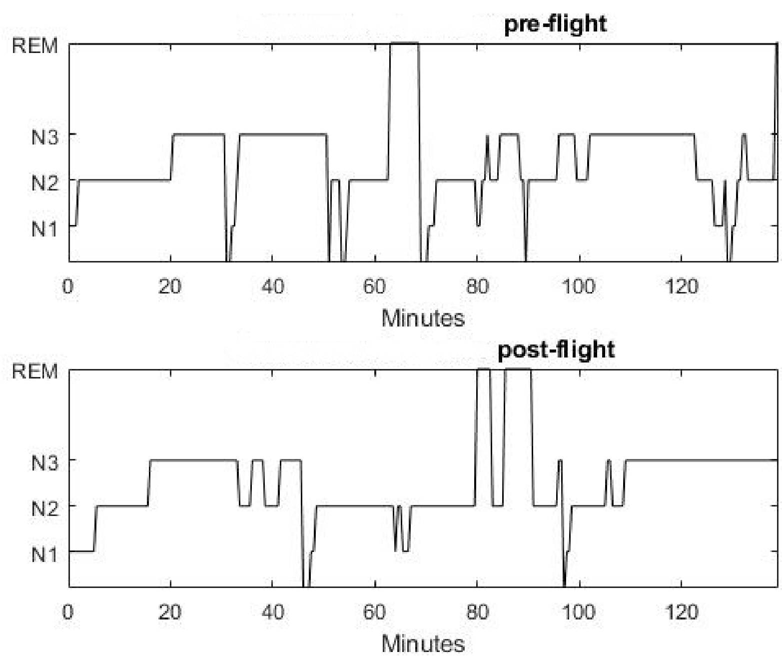
The left pie-chart represents the preflight sleep. The right pie-chart represents the postflight sleep. A different color is used per sleep stage.

	preflight sleep	postflight sleep
Awake	4.0100 %	4.0100 %
N1	6.5163 %	7.0175 %
N2	52.3810 %	49.6241 %
N3	26.5665 %	32.0802 %
REM	10.5263 %	7.2682 %

Participant 2

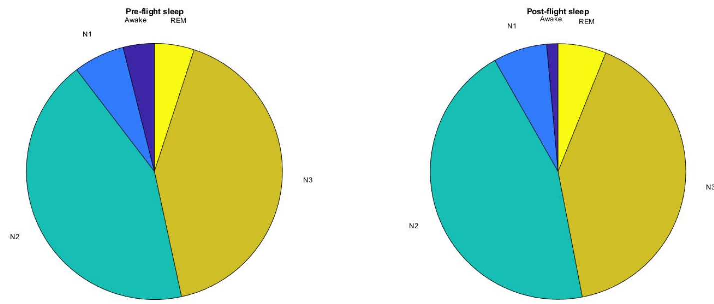
The preflight sleep lasted 210,5 minutes after sleep onset, which was after 18 minutes. Postflight sleep lasted 139 minutes after sleep onset, which was at 15.5 minutes. If we take the first 139 minutes after sleep onset, we can compare.

The hypnograms of both sleep nights:



The topplot shows the hypnogram of the preflight sleep. The x-axis represents the minutes, the y-axis the sleep stage (with the 0/x-axis representing the wake-period). The bottomplot shows the hypnogram of the postflight sleep.

The division per sleep stage:



The left pie-chart represents the preflight sleep. The right pie-chart represents the postflight sleep. A different color is used per sleep stage.

	preflight sleep	postflight sleep
Awake	3.9427 %	1.4337 %
N1	6.4516 %	6.8100 %
N2	43.0108 %	44.8029 %
N3	41.5771 %	40.8602 %
REM	5.0179 %	6.0932 %

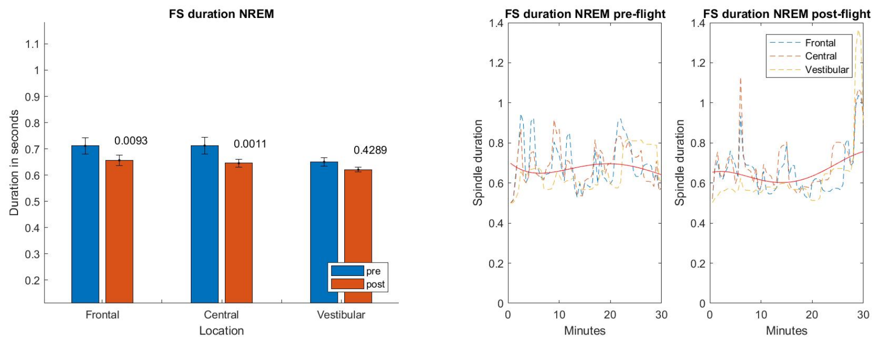
Intermediate conclusions

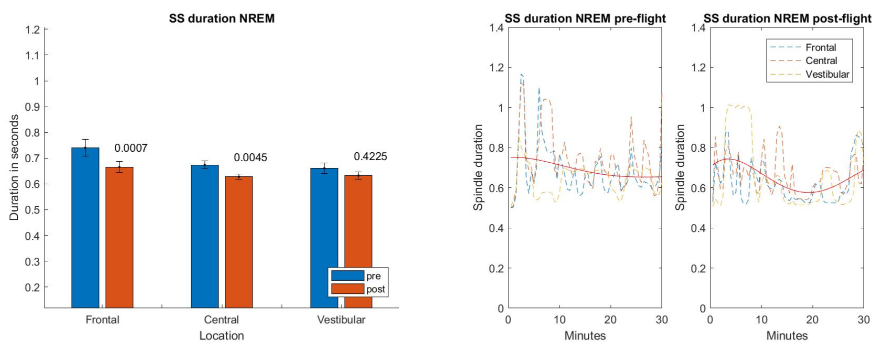
There is not much difference between the stages of preflight and postflight sleep and the duration of these cycli. Both participants spend most time in NREM sleep. Therefore, parabolic flights do not seem to affect the time spend in specific sleep stages.

5.3.3 Spindle characteristics

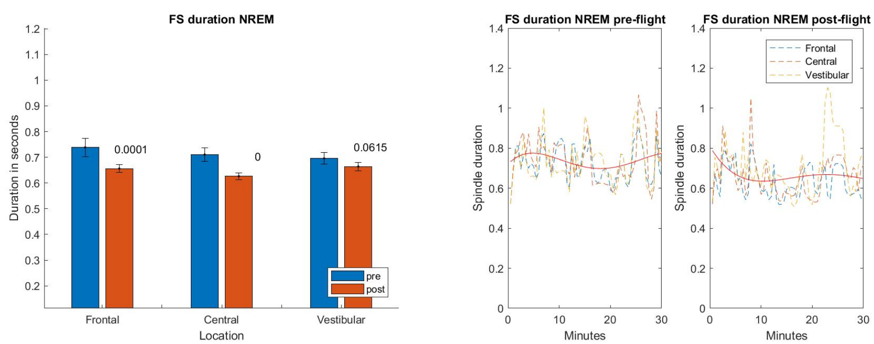
Spindle duration

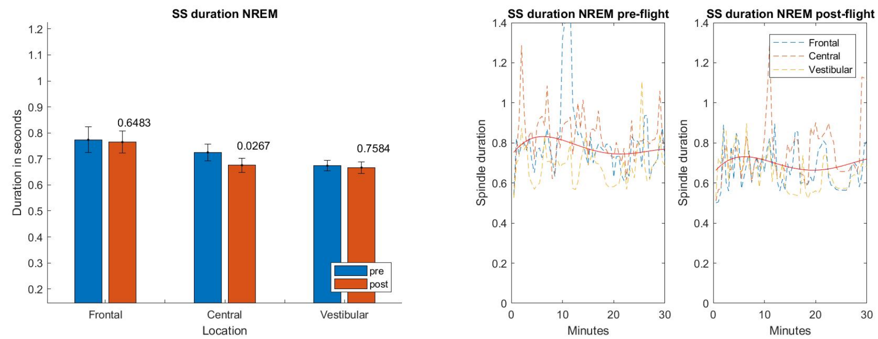
Participant 1





Participant 2



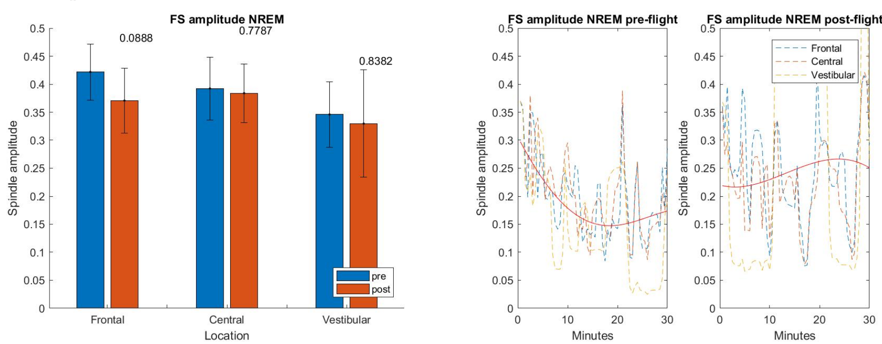


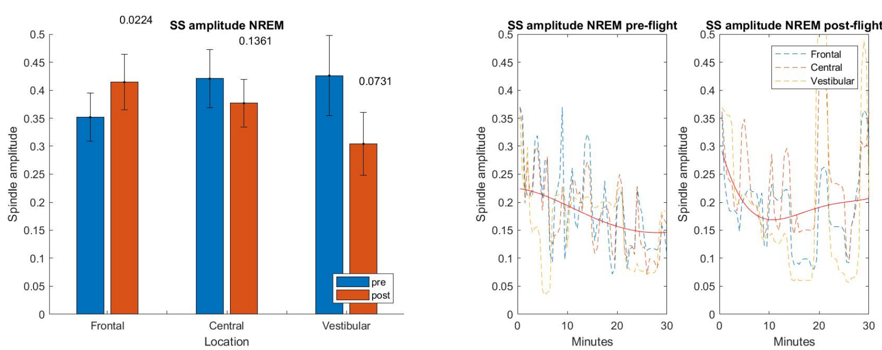
Intermediate conclusions
Fast spindle duration seems to significantly decrease in frontal and central areas, but not in vestibular areas when comparing pre- and postflight nights. Slow spindle duration is only significantly reduced in central areas.

Spindle duration in vestibular areas thus seems to be less affected by the parabolic flight experience than the other areas. Furthermore, slow spindles in frontal areas are also not significantly affected by the parabolic flight.

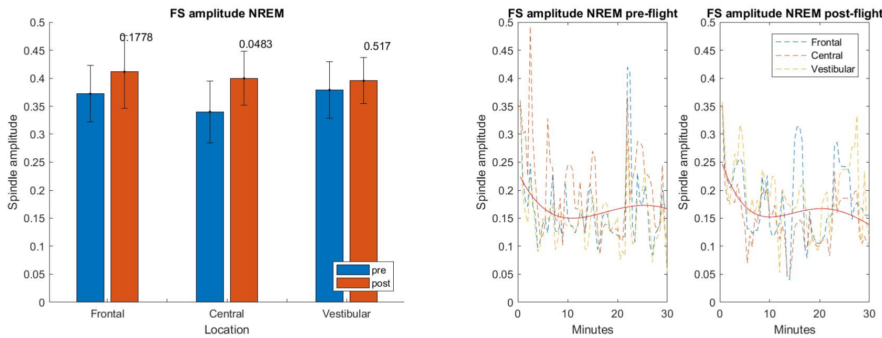
Spindle amplitude

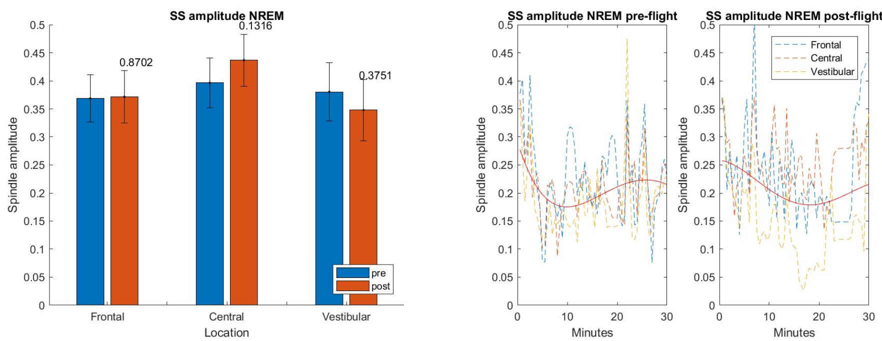
Participant 1





Participant 2



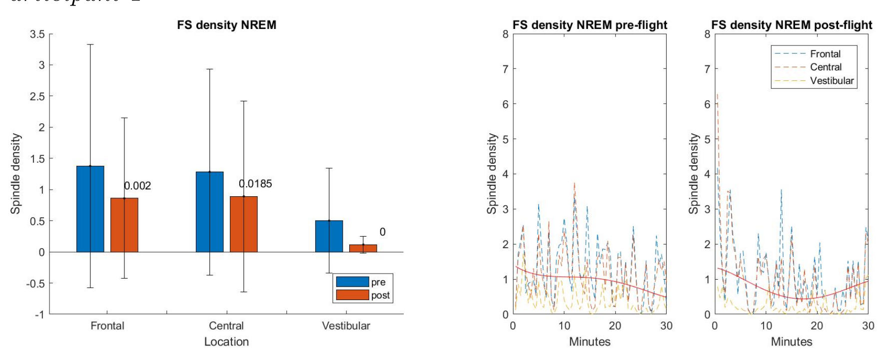


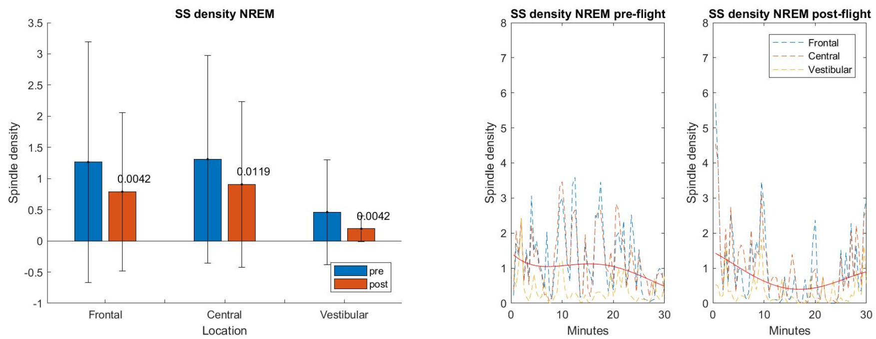
<u>Intermediate conclusions</u>

No consistent significant changes were noticeable in spindle amplitude in both participants between pre- and postflight as changes were in different directions over the three sites. Thus, the flight had no significant effect on spindle amplitude.

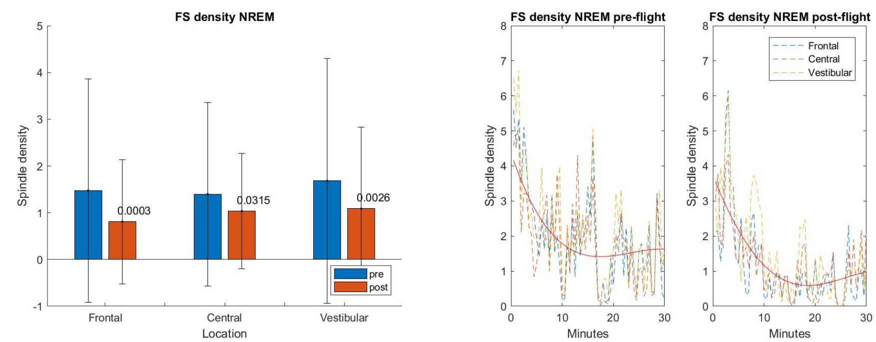
Spindle density

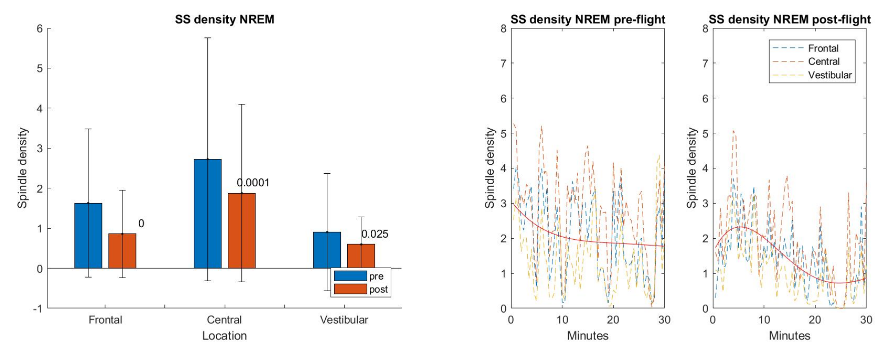
Participant 1





Participant 2



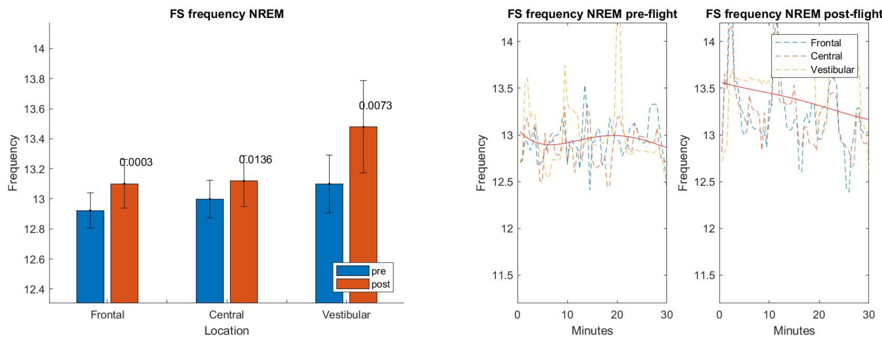


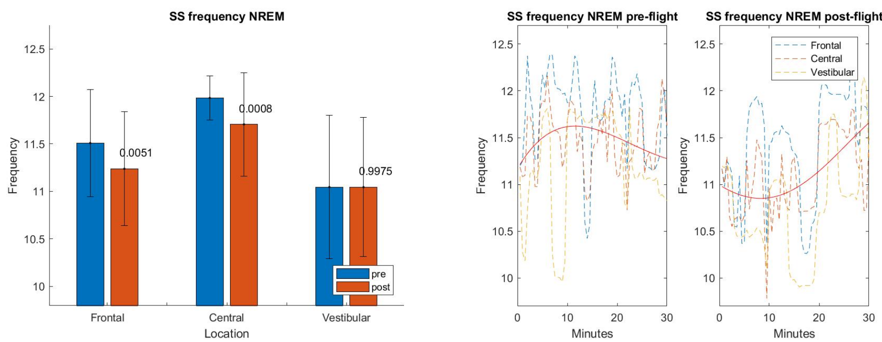
Intermediate conclusions

There is a significant decrease in average fast and slow spindle density in frontal, central and vestibular brain areas in both participants when comparing pre- and postflight sleep.

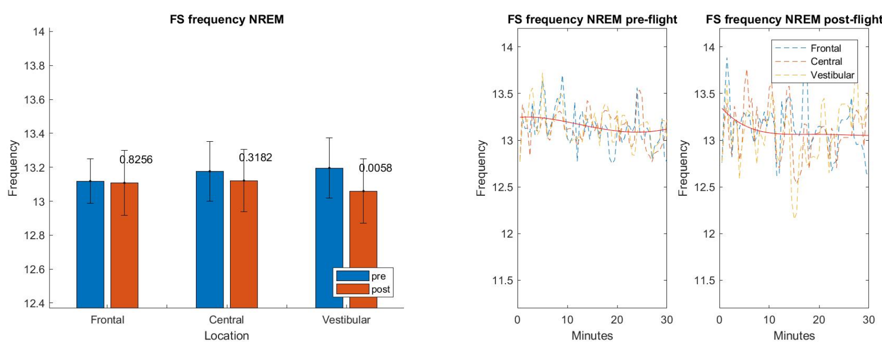
Spindle frequency

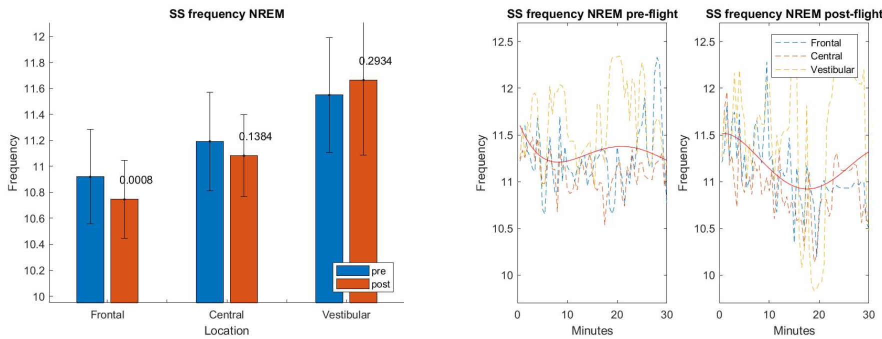
Participant 1





Participant 2





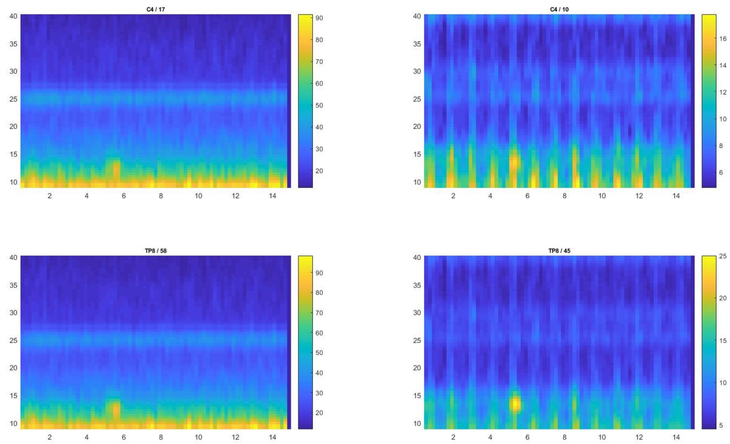
<u>Intermediate conclusions</u>
Only frontal slow spindle frequency was significantly reduced in both participants when comparing pre- and postflight sleep.

<u>Summary intermediate results of spindle characteristics</u>
The experience of parabolic flights seems to have some effect on sleep characteristics. Duration changes are only significantly reduced in frontal and central areas, whereas the density was reduced in all areas. However, this could be caused by the differences in density over time. If spindle density would be higher at the beginning, and lower at the end (as proposed by Gais et al., 2002), it could result in an overall lower average of sleep spindle density. Slow spindle frequency was only significantly reduced in frontal areas. Furthermore, the graphs of the spindles in the first 30 minutes of NREM-stage, do not show any clear differences over time.

Some clear differences are visible when looking at the significant differences in sites over the two flights. Spindle duration and density both significantly change in the vestibular area in both participants when comparing pre- and postflight. Indicating most change to be around the vestibular area. This could be a result of possible replay processes of the vestibular experience of the parabolic flight.

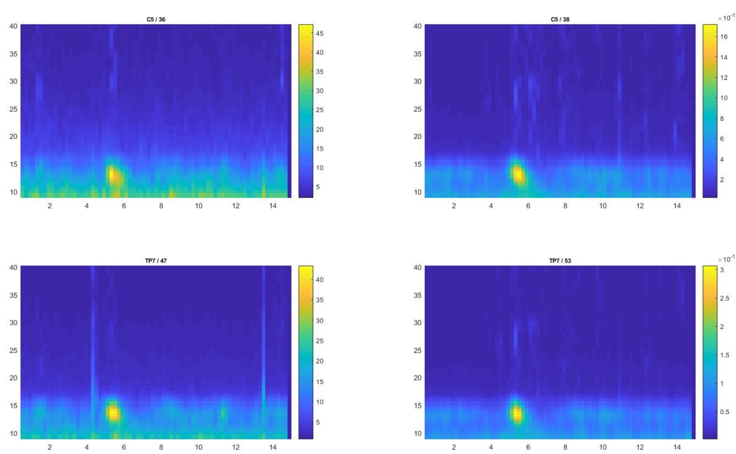
Frequency vs number of spindles
A frequency analysis of the fast spindles provided the following result:

Participant 1



Frequency analysis of a fast spindle averaged over all spindles between 10 and 40 Hz. The left two plots show the preflight analysis, the right two plots show the post-flight analysis with the central spindles on the top row and the vestibular spindles on the row below. Please note that the stripes in the right column are caused by a mismatch in window/taper and samplingrate.

Participant 2

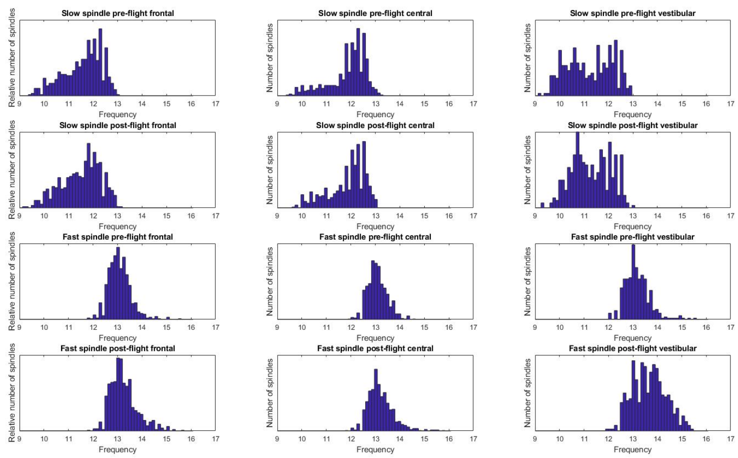


Frequency analysis of a fast spindle averaged over all spindles between 10 and 40 Hz. The left two plots show the preflight analysis, the right two plots show the post-flight analysis with the central spindles on the top row and the vestibular spindles on the row below.

At first sight it seemed as if the spindles were more focused around the same frequency, resulting in a smaller area of maximum power. To see if this is indeed the case, the distributions of the frequencies are plotted.

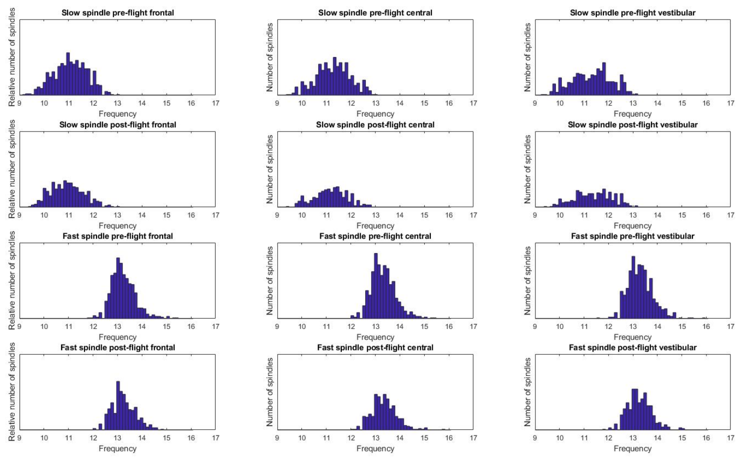
All spindles are divided over 0.2 Hz frequency bins to make their distribution visible and make any differences between pre- and postflight sleep apparent.

Participant 1



The histograms represent the normalized number of spindles per frequency in 0.2 Hz frequency bins. The left column of plots represents the slow (top two plots) and fast (bottom two plots) spindles in the frontal area. The middle plots represent these spindles in the central area and the right plots represent these spindles in the vestibular area. The first and third row of plots represent the preflight spindles, the second and the fourth row of plots represent the postflight spindles.

Participant 2



The histograms represent the normalized number of spindles per frequency in 0.2 Hz frequency bins. The left column of plots represents the slow (top two plots) and fast (bottom two plots) spindles in the frontal area. The middle plots represent these spindles in the central area and the right plots represent these spindles in the vestibular area. The first and third row of plots represent the preflight spindles, the second and the fourth row of plots represent the postflight spindles.

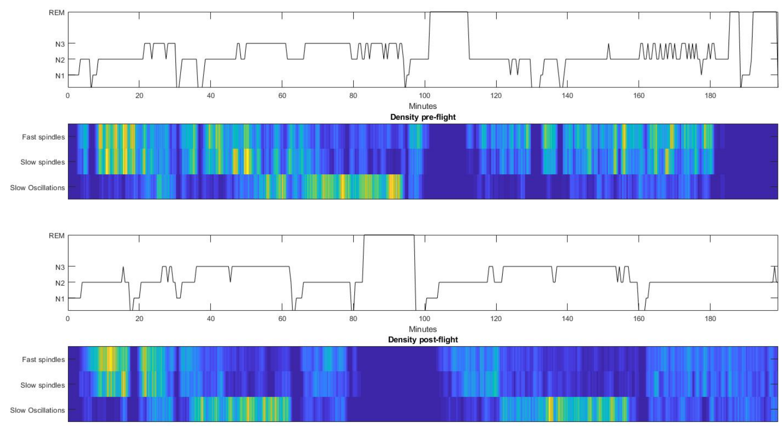
Intermediate conclusions

No apparent changes are seen in the distribution of slow or fast spindles over frequencies in any of the brain areas between pre- and postflight. This is a similar conclusion resulting from the t-tests in the previous analysis of the mean and variance of the frequencies.

Spindle and slow oscillation coupling

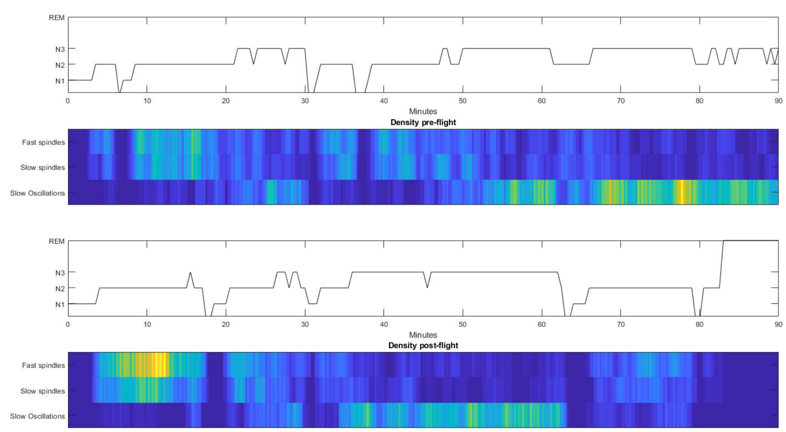
To check whether the spindle density changes over night as proposed in previous intermediate conclusions, the density per 30 second epoch of fast and slow spindles and slow oscillations is plotted in the following graphs. De densities are normalized over all characteristics to show the highest and lowest value over the period of a night.

Participant 1



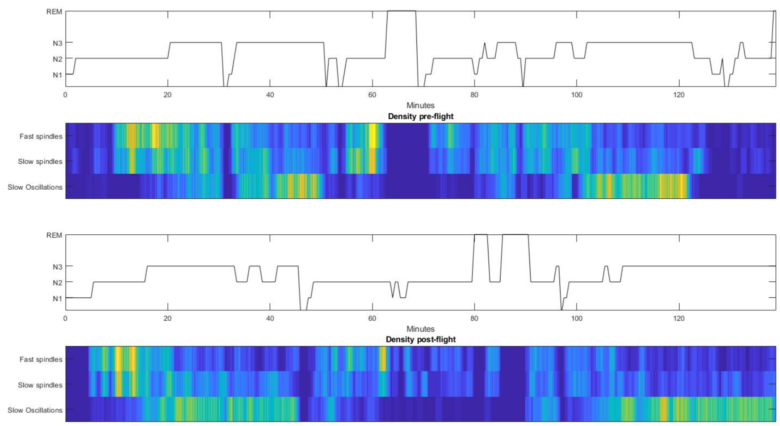
The normalized densities of the sleep characteristics during the whole period of sleep. The first two figures represent the preflight sleep, the bottom two figures represent the postflight sleep. The topplot of each two figures represent the sleep stages. The bottomplot of each two figures represents the normalized density of the slow and fast spindles and slow oscillations on the timescale of the topplot (x-axis).

If we zoom in on the first 90 minutes as the density of spindles in supposed to be higher in that period after a learning event, we get the following plot:



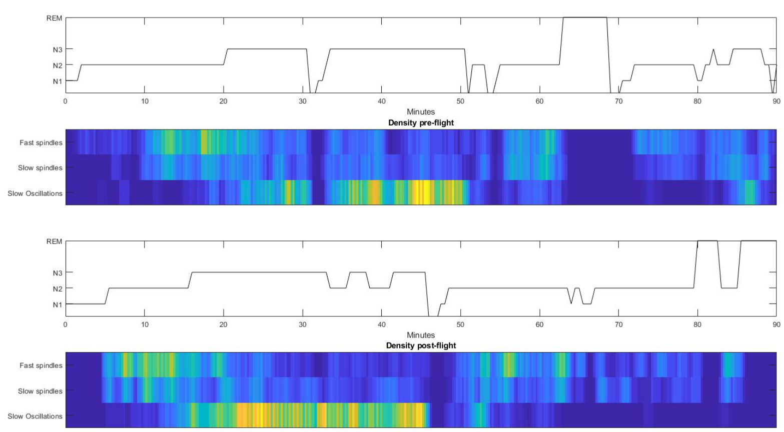
The unnormalized densities of the first 90 minutes of sleep. The first two figures represent the preflight sleep, the bottom two figures represent the postflight sleep. The topplot of each two figures represent the sleep stages. The bottomplot of each two figures represents the normalized density of the slow and fast spindles and slow oscillations on the timescale of the topplot (x-axis).

Participant 2



The normalized densities of the sleep characteristics during the whole period of sleep. The first two figures represent the preflight sleep, the bottom two figures represent the postflight sleep. The topplot of each two figures represent the sleep stages. The bottomplot of each two figures represents the normalized density of the slow and fast spindles and slow oscillations on the timescale of the topplot (x-axis).

If we zoom in on the first 90 minutes as the density of spindles in supposed to be higher in that period after a learning event, we get the following plot:



The unnormalized densities of the first 90 minutes of sleep. The first two figures represent the preflight sleep, the bottom two figures represent the postflight sleep. The topplot of each two figures represent the sleep stages. The bottomplot of each two figures represents the normalized density of the slow and fast spindles and slow oscillations on the timescale of the topplot (x-axis).

No differences in density over slow oscillations and slow and fast spindles was seen between frontal, central and vestibular areas.

Intermediate conclusions

It seems as if the slow and fast spindles have a higher density at the beginning of the N2 sleepstage and then relatively lower densities during the rest of the sleeping period when comparing pre- and postflight sleep. Furthermore, the slow oscillations have a higher density more towards the beginning of a N3-stage in postflight compared to preflight sleep.

When we zoomed in on the first 90 minutes, it is indeed visible in both participants that the fast spindle densities are higher during the first part of the night. This is according to the Gais et al.(2002) research, in which they found that spindle density is higher in the first 90 minutes of the sleep after a learning event. The learning event in this case could be the parabolic flight, indicating that the event is processed by the brain. This could be an indication for replay as spindles and replay are often thought to be connected.

A closer look was given to the spindle coupling to the state of the slow oscillations. Specifically, the timing of fast and slow spindles to that of the up- and downstate of a slow oscillation is compared.

Participant 1

Fast spindles (Up)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal	22.87	-0.0500	13.93	-0.1253
Central	18.97	0.0351	24.17	-0.1459
Vestibular*	15.22	-0.0171	16.45	-0.1750

Fast spindles (Dn)				
	preflight		postflight	
	% of spindles	mean sec. to tr	% of spindles	mean sec. to tr
Frontal	4.79	-0.2744	10.66	-0.0515
Central	7.18	-0.2064	7.50	-0.0100
Vestibular	4.35	-0.2000	12.90	0.0725

Slow spindles (Up)				
	preflight		postflight	
	% of spindles	mean sec. to tr	% of spindles	mean sec. to tr
Frontal	20.57	-0.0886	14.52	-0.1378
Central	14.79	-0.1038	24.51	-0.1820
Vestibular	10.87	-0.2480	7.14	-0.0600

Slow spindles (Dn)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal	4.57	0.0650	17.74	0.0500
Central	4.23	0.2017	13.73	-0.0007
Vestibular	6.52	0.1100	14.29	0.0425

Participant 2

Fast spindles (Up)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal*	17.36	0.0014	27.34	0.0792
Central	12.20	0.0080	23.64	-0.0104
Vestibular	12.59	-0.0049	18.18	-0.0073

Fast spindles (Dn)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal	7.85	-0.2884	14.39	0.0090
Central	9.76	0.0300	10.91	-0.0767
Vestibular	7.91	0.0195	11.19	-0.0125

Slow spindles (Up)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal*	12.13	-0.0396	22.22	-0.0478
Central	10.34	0.0146	20.39	-0.0119
Vestibular	11.35	-0.0988	16.46	0.0431

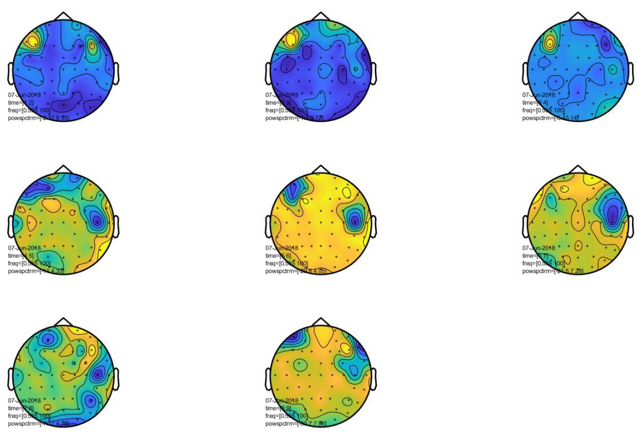
Slow spindles (Dn)				
	preflight		postflight	
	% of spindles	mean sec. to pk	% of spindles	mean sec. to pk
Frontal	11.39	0.0241	16.46	0.0622
Central	12.93	0.0297	10.68	-0.0564
Vestibular	7.80	0.0427	15.19	0.1242

$* = p \leq 0.05$

Intermediate conclusions
No consistent significant changes in coupling of the spindles and slow oscillations between pre- and postflight sleep were found in the two participants. Not even in between the fast spindles and the upstate of slow oscillations. Therefore, the coupling does not provide any evidence of the processing of vestibular experiences.

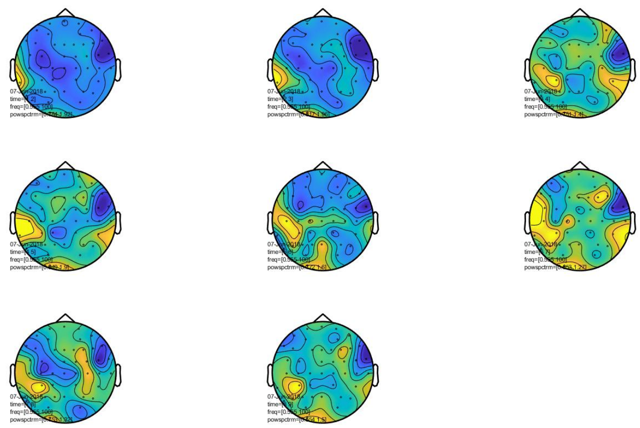
Frequency REM
As dreams are correlated with reactivations of specific areas during REM-sleep (Siclari et al., 2017), the REM-sleep is examined for activations of the vestibular system. If the falling dream of participant 1 was indeed caused by these re-activations, it could provide evidence for neuronal replay of the parabolic flight.

Participant 1
The following topoplots are created from the last 8 seconds of the first REM-sleep from both night subtracted from each other.



The first topoplot represent the 8^{th} second before waking up, the second topoplot represents the 7^{th} second etc. All topoplots are referenced to the 9^{th} second before waking up.

Participant 2
The following topoplots are created from the last 8 seconds of the first REM-sleep during preflight sleep:



The first topoplot represent the 8^{th} second before waking up, the second topoplot represents the 7^{th} second etc. All topoplots are referenced to the 9^{th} second before waking up.

Intermediate conclusions

The difference between the last 8 seconds of the pre- and postflight shows some activity around the vestibular area in participant 1 (the right hemisphere only) and participant 2 (strong activity in both left and right hemisphere). This could indicate possible reactivation of those areas that were active during the vestibular learning event, resembling replay processes during REM-sleep. These reactivations of the vestibular area could also be the basis for the falling dream before waking up that participant 1 had reported.

General sleep questionnaire

In a general questionnaire under a variety of parabolic flyers, including the participants, the other teams and previous flyers, the recurrences of the flying and falling sensation during sleep were examined.

In total, 27 people filled in the questionnaire. 5 people experienced the sensation of flying in a dream of which two were experienced flyers (≥ 150 parabolas in total over many flights). Only one flyer (first time) had a sensation of falling. It is worth noting that two (one first time and one frequent flyer) had the sensation of falling during wakefulness when lying down. No effect was found on the doses of Scopolamine injected before the flight.

Intermediate conclusions

It seems as if there is some possibility of replaying the sensations during sleep, however, it was not (remembered) in most of the flyers. This might indicate some replay processes during the night after the parabolic flights.

6 Conclusion & Discussion

Parabolic flights provide a consistent change in gravity to enable a perfect block design. This study shows that EEG-measurements are possible during the different gravity levels without too much distortion from a possible cap shift and that there could be significant changes in activity recorded by the electrodes during the different gravity phases. These changes were mostly detected by the TP7 and TP8 electrode, which are above the vestibular area. Even though the signals explored in this report could be from muscle or cap movements, changes in frequency of these signals (as shown in the appendix), give evidence that there must be a neuronal representation of different gravity levels. Parabolic flights could therefore provide the possibility to measure signals that identify different levels of gravity that could later be detected in during sleep to prove the existence of neuronal replay.

The experience of parabolic flights might also have caused some changes in sleep characteristics that could provide indirect evidence of this neuronal replay. Based on significant results in some of the spindle characteristics, such as spindle duration and density across the night, it could be possible that replay is present. Furthermore, when looking at a more general population of parabolic flyers, it seems to be as if there could be some replay of these wake-sensations during the following sleep. If these sensations or dreams have their neurological basis in replay processes, they could be an indication of the existence of this replay. This indication has already acquired some slight evidence during this research, as there were vestibular (re-)activations during the last 8 seconds of REM-sleep before waking up in both participants with one participant having had these falling sensations. Thus, there is evidence that parabolic flights could be a useful method to detect the possible existence of neuronal replay in humans.

Even though results seem promising, results should still be carefully interpreted. Due to the small sample size (n=2), most conclusions cannot be made on statistical analysis. Not only were the results based on a small sample size, there were also many other factors influencing the data. Some of these influences are stated below:

Flight
As parabolic flights are quite uncommon, little is known about the effects of these flights on data recordings. A major side note must be placed with the EEG-measurements concerning muscle activity. Both the low frequency and higher frequency (18 to 25Hz) components of the different gravity levels, could (partly) be explained by movement of the body. Although this influence seems less apparent in the 8 to 25 Hz frequency range, a signature of the gravity conditions was not possible as the exact influences of the flights were unknown. Furthermore, the EEG-cap could shift slightly during the different gravity phases and thus measure slightly different areas or have less or more impedence due to the loosening or tightening of the cap. These movements are hard to predict,

making it difficult to distinguish neuronal activity from cap movements. Large movement of the cap has most likely not occurred during the data acquisition used for this report as no apparent bridges were formed based on trial to trial variability.

There are also many brain processes that could have influenced the signal. The different gravity conditions might also have an effect on the contents of the blood and other physiological aspects, causing a change of neuronal activity. One example is the possible increase in arterial flow and the decrease in venous flow during microgravity, due to a decrease in oxygenated blood and an increase of this blood during microgravity (Schneider et al., 2013). Another chance might be that the redistribution of the blood volume and the increase in oxygenated blood causes changes in the central nervous system and anaemic processes (De Santo et al., 2005). Brummer et al. (2011) showed that there is an increase in frontal lobe activity and a decrease in temporal and occipital cortex in microgravity. On a smaller scale, Meissner & Hanke (2005) found that microgravity slows the propagation of action potentials, while the transmission speed of these potentials is increased in hypergravity. However, latencies between action potentials are decreased in microgravity and increased in hypergravity, possibly leading to an increase in these potentials in microgravity and a decrease in hypergravity. These changes could also result from a higher excitability, a shorter refractory period, or a change of properties of the membrane possibly due to the change in fluid pressure caused by the changes in gravity (Meissner & Hanke, 2005). Furthermore, Marusic et al. (2014) have found that emotional stressors are also a major influence during a parabolic flight. Being confined within the airplane and a negative perception of the environment all contribute to stress (Schneider et al., 2009). Research of Schneider et al. (2007, 2008) has shown that the stress response was correlated with stress hormone concentration and higher frequency spectra in the EEG signal. Not only stress can cause changes in EEG signal, a wide range of emotions go through a subject when subjected to a parabolic flight. Examples are anxiety, excitement and surprise, all leading to different neuronal responses. These emotions are normally mostly expressed in frontal areas (Coan & Allen, 2004). Furthermore, all participants had Scopolamine administered to them to avoid motion sickness. The effects of this drug on brainsignals and memory are unknown. It might thus be that this drug had some effect on brainsignals and thus possible replay could have been affected by this drug.

Further variability could have been caused by additional noise during a parabolic flight, either from the plane itself (in a frequency or auditory manner) or the other people and experiments on the plane. This experiment was not the only one being conducted, despite careful consideration of the placement of our experiment relative to the other, some interference might have occurred as sounds and vision could not be fully blocked.

Further research should be done on any of these influences with more participants and more parabolic flights, such that we can distinguish these influences from the actual brain signals resulting from the changes in gravity.

Sleep

The conclusions based on sleep characteristics are only preliminary, a lot could have influenced the differences in pre- and postflight sleep. Due to ethical approvals, postflight sleep could only be done in The Netherlands, which caused extra stress from travelling on the participant and could therefore have diminished the effect of the parabolic flight. An additional influence on postflight sleep could have been the sleep deprivation resulting from excitement of the flight or the process leading up to the flight and sleep measurements. Furthermore, both preflight and postflight sleep were done in a novel environment with preflight sleep conducted in a room where other participants were present. All of these influences could have caused changes in sleep characteristics between the nights that have not been direct consequences of the experience of a parabolic flight.

Even general changes in homeostasis between two nights could have resulted in the found changes. It is thus very important to interpret the results with care and only use them as a basis for further research. Further research should include more participants and more nights per participant with less stressful events leading up to the postflight sleep to make more definite conclusions on the possible existence of replay.

In conclusion, the use of parabolic flights seems like a promising method of detecting neuronal replay in humans. This detection could then shed light on memory related processes of humans during sleep. Results, however, should be carefully interpreted. More research needs to be done towards the different influences on EEG-signal during a parabolic flight, such that it is possible to isolate brain processes related to gravity from all other sources of activity. More sleep measurements should be done before and after the flight, such that parabolic flight related changes could be distinguished from random changes in sleep between nights.

A.I. part of thesis.

Introduction

Machine learning techniques can find differences in datasets that are sometimes not visible to the naked eye or basic analysis alone (Bishop, 2006). Therefore, this part of the thesis will be focused on applying machine learning techniques on the datasets of the previous part. With these techniques a more in-depth analysis can be done to see if any combination of values or even significant changes between sleepnights can be detected with these techniques that were not detected in previous analysis.

Method

Data

The same sleep EEG-recordings and spindle detection of the previous part was used for this part of the thesis. Spindles were extracted from the data with 3 seconds before and 3 seconds after the start of the spindle, as this is where replay is expected. As mostly fast spindles are connected to replay, only fast spindles are taken into account. As we expect the spindles and memory to be mostly influence in central and vestibular, only the spindles from these areas are taken into account.

Datacleaning

The spindles were bandpassfiltered between 0.1 and 100Hz. The dataset of the extracted spindles was further cleaned by visual trial and artifact rejection and removal of noisy ICA components.

Data analysis

Machine Learning Techniques
Machine learning techniques were used according to the book of Bishop (2006). The main machine learning techniques were applied and compared in accuracy.

The machine learning techniques chosen were:

- Support Vector Machine - Tries to find the best linear boundary between classes (Bishop, 2006).
- K-means - Tries to form K clusters out of the datapoints (in this case 2) (Bishop, 2006).
- Naive Bayes - Tries to classify instances based on prior beliefs and the likelihood of a point belonging to a certain class (Bishop, 2006).

The values for the characteristics of the EEG-signal with their associated class (preflight or postflight) were the input for machine learning algorithms to try to detect a significant difference between the two classes. The subjects were analyzed on an individual basis. To avoid overtraining on the dataset, the dataset was divided in 80% trainingdata and 20% validationdata, with the validation accuracy being used as the final accuracy.

Spindle characteristics
NREM sleep is compared by comparing sleep characteristics between the preflight and postflight night. The spindle characteristics and slow oscillation characteristics (duration, frequency, density, amplitude) that were used in the previous part are input to the different machine learning techniques.

Frequency domain
The extracted spindles were analyzed on frequency by the Fast Fourier Transform between 0.1 and 40 Hz in steps of 0.5Hz. The timecourse was analyzed in windows of 0.2 seconds with 0.05 second overlap. Each trial (frequency analysis of a spindle) was normalized in frequency, before serving as the input to the machine learning technique.

Waveletanalysis
Time domain
The significant time signals of the low frequency bandpassfiltered flightdata found are used as a wavelet in custom wavelet analysis and its values between preflight sleep and postflight sleep are compared. The timesignal from TP7 is applied to three random spindles in this electrode during pre- and postflight-sleep. The timesignal of Cz is used as a wavelet for three random spindles in the same electrode during pre- and postflightsleep. Its scaling was between 0.01 and 4 in steps of 0.05. As each wavelet is represented as 1 second, this will result in a range of 10Hz to 250Hz (with changes expected between 10 and 100Hz) of the first hypergravity and microgravity phase. The wavelet analysis will be applied to the extracted spindle in the time domain.

Results

Spindle characteristics

The following accuracies were reached per characteristic and per brain area

Participant 1

	SVM	K-means	Naive Bayes
Spindle Characteristics C	48%	48%	52%
Spindle Characteristics V	42%	49%	53%
Spindle Characteristics C & V	52%	54%	47%
Slow Oscillations C	51%	52%	54%
Slow Oscillations V	47%	48%	52%
Slow Oscillations C & V	50%	58%	54%

The accuracies reached per machine learning techniques and per characteristic used. C = characteristics from central brain areas, V = characteristics from vestibular brain areas. K-means was done with 2 means, each representing a class.

Participant 2

	SVM	K-means	Naive Bayes
Spindle Characteristics C	46%	52%	52%
Spindle Characteristics V	41%	47%	51%
Spindle Characteristics C & V	56%	51%	45%
Slow Oscillations C	41%	46%	54%
Slow Oscillations V	52%	45%	51%
Slow Oscillations C & V	48%	51%	56%

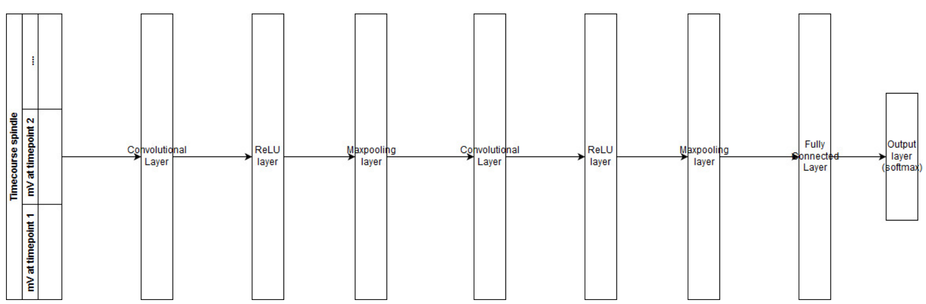
The accuracies reached per machine learning techniques and per characteristic used. C = characteristics from central brain areas, V = characteristics from vestibular brain areas. K-means was done with 2 means, each representing a class.

<u>Intermediate conclusions</u>

The spindle and slow oscillation characteristics are not distinct enough to be detected by either the Support Vector Machine, K-means or Naive Bayes. It therefore provides no evidence for neuronal replay during postflight sleep.

Frequency domain

The following neural network showed the best results when trained on the time-frequency representations of spindles with learningrate 0.001:



Input was a 80 by 41 image (80 frequencies and 41 time points), convolutional layer of 30x30, a rectifier linear unit, a max pooling layer of 4x4 with stride 1, a convolutional layer of 10x10 with stride 1, a a rectified linear unit, a max pooling layer of 2x2 with stride 1, a fully connected layer, a soft-max activation output layer.

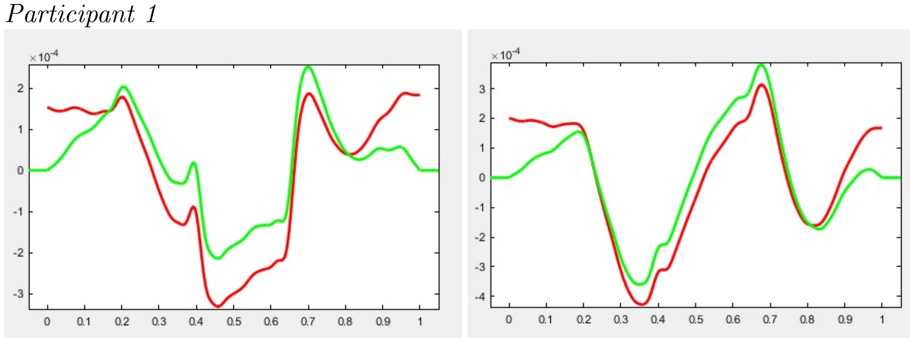
The following accuracies were reached:

	Participant 1		Participant 2	
	Central	Vestibular	Central	Vestibular
Accuracy	62%	61%	58%	59%

<u>Intermediate conclusions</u>
The spindle time-frequency representations of the preflight and postflight sleep are not distinct enough to be recognized by a neural network.

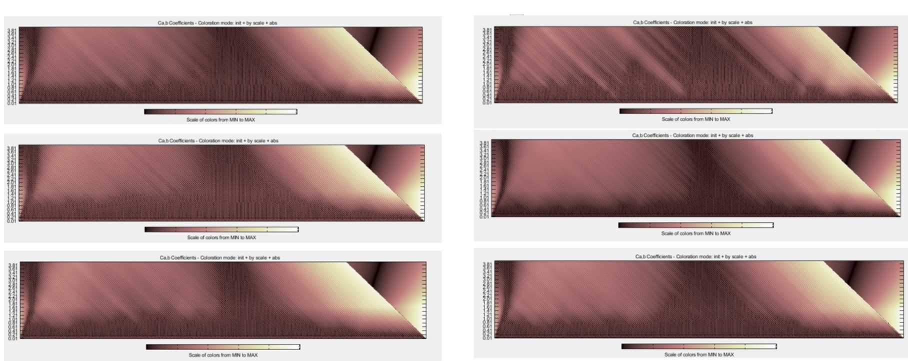
Waveletanalysis
The following wavelets were formed for participant 1 of the Cz and the TP7.

Participant 1



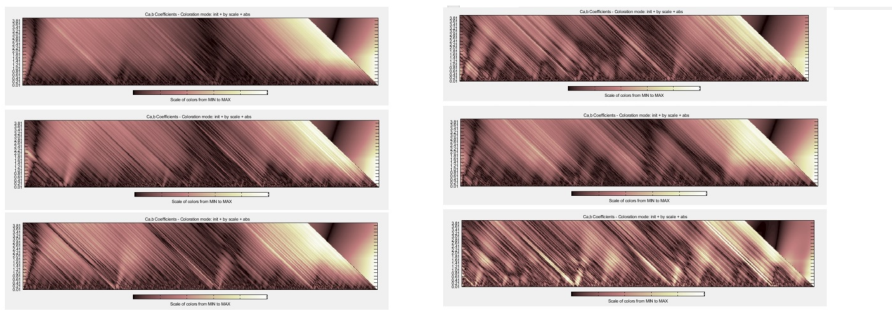
The red line in the left plot is the time signal averaged over trials from the flight in the Cz electrode. The green line in that plot is the formed wavelet. The red line in the right plot is the time signal averaged over trials from the flight in the TP7 electrode. The green line in that plot is the formed wavelet.

The following frequency analysis were the result from the waveletanalysis on the preflight dataset:



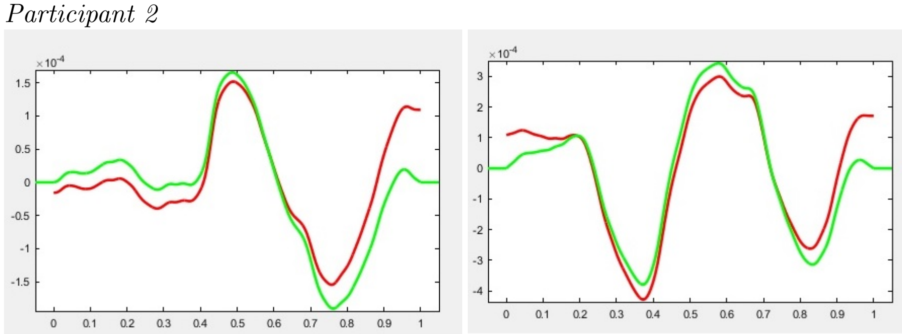
Preflight waveletanalysis - The left plots show the waveletanalysis (with the Cz-wavelet) over three random Cz spindles. The right plot shows the waveletanalysis (with the TP7-wavelet) over three random TP7 spindles. The x-axis represent the time, while the y-axis represent the scale of the wavelet.

The following frequency analysis were the result from the waveletanalysis on the postflight dataset:



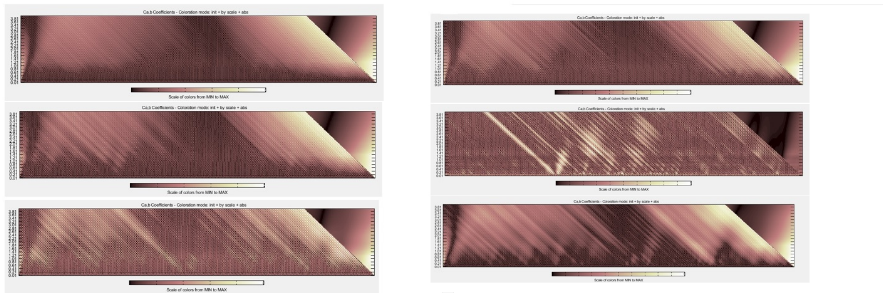
Postflight waveletanalysis - The left plots show the waveletanalysis (with the Cz-wavelet) over three random Cz spindles. The right plot shows the waveletanalysis (with the TP7-wavelet) over three random TP7 spindles. The x-axis represent the time, while the y-axis represent the scale of the wavelet.

Participant 2



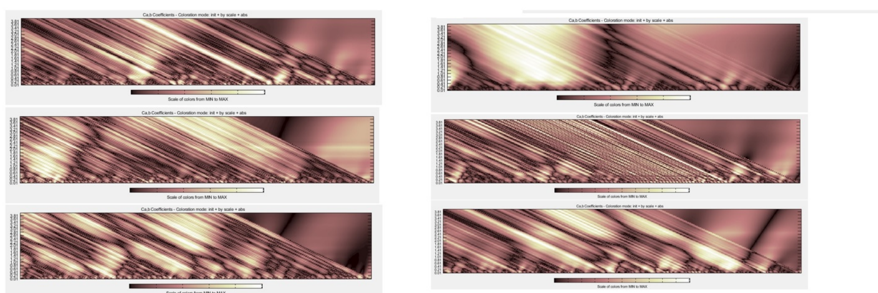
The red line in the left plot is the time signal averaged over trials from the flight in the Cz electrode. The green line in that plot is the formed wavelet. The red line in the right plot is the time signal averaged over trials from the flight in the TP7 electrode. The green line in that plot is the formed wavelet.

The following frequency analysis were the result from the waveletanalysis on the preflight dataset:



Preflight waveletanalysis - The left plots show the waveletanalysis (with the Cz-wavelet) over three random Cz spindles. The right plot shows the waveletanalysis (with the TP7-wavelet) over three random TP7 spindles. The x-axis represent the time, while the y-axis represent the scale of the wavelet.

The following frequency analysis were the result from the waveletanalysis on the postflight dataset:



Postflight waveletanalysis - The left plots show the waveletanalysis (with the Cz-wavelet) over three random Cz spindles. The right plot shows the waveletanalysis (with the TP7-wavelet) over three random TP7 spindles. The x-axis represent the time, while the y-axis represent the scale of the wavelet. Please note that the postflight dataset was measured with 250Hz, and thus results in a more irregular structure.

<u>Intermediate conclusions</u>

The postflight waveletanalysis plots seem more irregular than the preflight waveletanalysis plot in both Cz and TP7 areas, which could indicate a higher similarity with the used wavelets. However, no clear effect is seen around a specific scale.

Conclusion

The different machine learning techniques (Support Vector Machines, K-means, Naive Bayes and Neural Networks) could not detect a significant difference between the time course, frequencies, or the characteristics of the spindles between preflight and postflight night. Furthermore, only two persons were analyzed in this report. Therefore, no conclusions can be made on the existence of neuronal replay.

Discussion

More research is needed on neuronal replay. Better conclusions can be made based on data from more participants, who each have their sleep-EEG recorded on several nights before and after a learning event.

Furthermore, this waveletanalysis is only done on a few spindles, more concrete results could come from a more complete analysis on all spindles. Also, only the low frequency time course of the flightdata trials is used, from which is not even clear if they are brain signals or movement artifacts. More participants and more trials are needed to be able to detect a definite signature of different gravity levels.

7 Acknowledgements

This research was supported by the European Space Agency, Novespace and ANT Neuro for which I would specifically like to thank Nigel Savage (ESA Education), Thomas Neville (Novespace) and Jaap van der Spek (ANT), who provided the necessary equipment and guided the legal and safety aspects of the project. I would also like to thank my supervisors, Martin Dresler (DCCN), Alberto Llera Arenas (DCCN) and Jason Farquhar (DCN) for their guidance and insights during the duration of the project.

I would also like to show my gratitude to the Brainfly team, consisting of Evelien Lageweg, Rebecca Mourits and Anouck Schippers, who supported me during the project and helped me tremendously with conducting the research. Furthermore, I would like to express a great gratefulness to our six participants, who have come in to train for the BCI part of the study and did their best during the entire experiment.

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9 Appendices

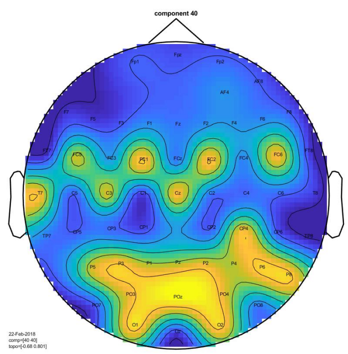
9.1 Additional results

9.1.1 Beta Rhythm

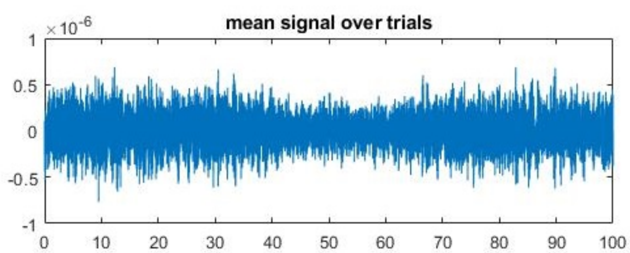
Method
Independent Component Analysis was performed on all trials from the cleaned data from the first part of this thesis to examine the main components in the data.

Results

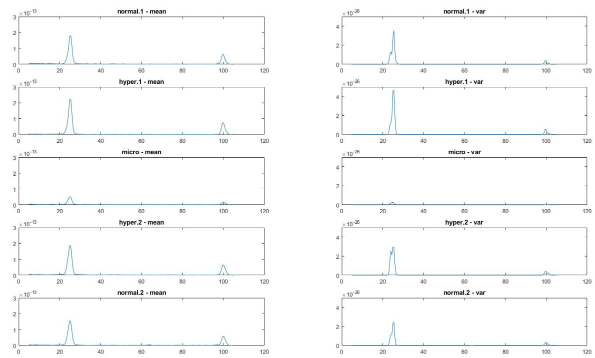
Participant 1
One of the clear ICA components was the following:



The source localization of the ICA component.



The mean time signal over trials of the component. The signal starts with 20 seconds of normal gravity. The hypergravity started at 20 seconds, the microgravity at 40 seconds and the hypergravity again at 60 seconds. The normal gravity ended the trial at 80 seconds.



The left plots shows the power of the components in the different gravity conditions. The right plot shows the variance of the power in the different gravity conditions.

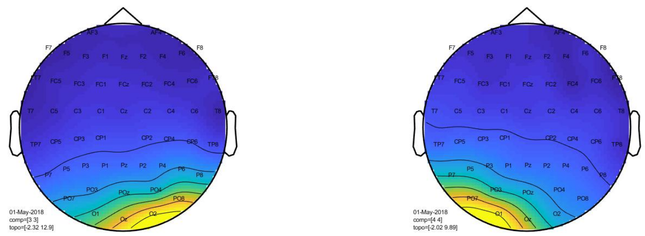
Participant 2
The other participant showed no such an effect when unmixing the data with this component.

Conclusion
A clear topological location is seen around the motorcortex and the parietal lobe. You can see a clear pattern of beta bursts of around 25 Hz that decreases in amplitude when microgravity hits.

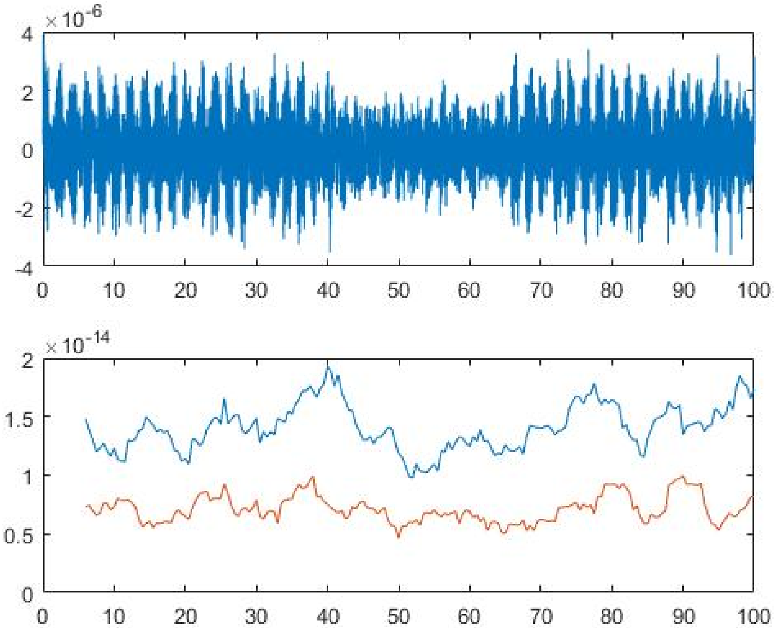
9.1.2 Alpha rhythm eyes closed

Method
Independent Component Analysis was performed on the eyes-closed trial from the cleaned data from the first part of this thesis to examine the main components in this data. The power of this component is determined by calculating the average power between 7.5 and 12.5 Hz.

Results
The following components of the ICA training was evident in both participants.



The source localization of the ICA components.



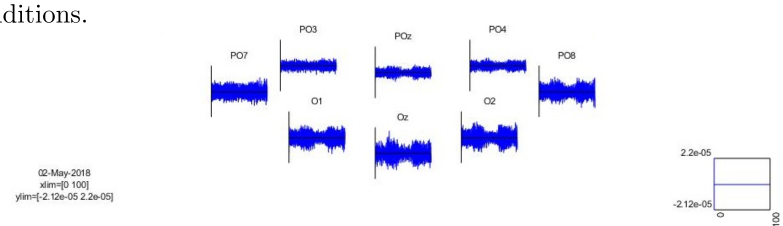
Topplot: the mean time signal over trials of the component. The signal starts with 20 seconds of normal gravity. The hypergravity started at 20 seconds, the microgravity at 40 seconds and the hypergravity again at 60 seconds. The normal gravity ended the trial at 80 seconds. Bottomplot: The power of the 10Hz signal at the different timepoints. The blue line represents participant 1, the red line represents participant 2.

Conclusion
A clear increase in alpha power in the occipital lobe is seen when microgravity begins, but them quickly returns back to the average level.

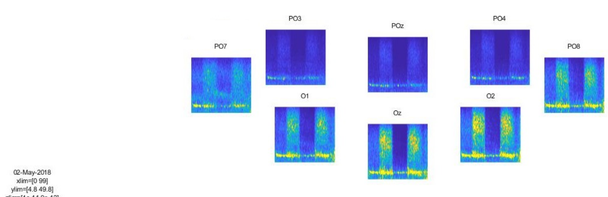
9.1.3 Alpha rhythm eyes open

Method
The average time signal over all trials with eyes open between 5 and 45 Hz (of the cleaned data of the first part of this thesis) is calculated and the signals around the occipital lobe are used in the analysis. These timesignals are then analyzed in the frequency domain and examined for significant changes in different gravity levels.

Results
Only in participant 2 did the alpha rhythm overall change in different gravity conditions.



The timesignals averaged over trials over the occipital lobe.



The frequency analysis of the timesignals averaged over trials over the occipital lobe.

Conclusion
The increase in power around the occipital lobe is around 10 Hz (alpha) and 35 Hz around hypergravity.