The Influence of Microgravity on the P3 Event-Related Potential: Visual Oddball Paradigm under Simulated Weightlessness Parabolic Flight Condition

Mourits, Rebecca

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The Influence of Microgravity on the P3 Event-Related Potential: Visual Oddball Paradigm under Simulated Weightlessness Parabolic Flight Condition

Rebecca F.E. Mourits

MSc in Psychology (research)
Specialization Cognitive Neuroscience
Institute of Psychology
Faculty of Social and Behavioural Sciences – Leiden University
External supervisor: Jason Farquhar, Radboud University
Internal supervisor: Femke Nijboer, Leiden University

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Abstract

Prior studies have shown ambiguous results concerning the question if and how cognitive performance and neurophysiological markers change under the influence of weightlessness. Additional research is necessary to confirm effects and shed light on experimental and interindividual differences and those related to various mechanisms underlying cognitive processes. This study aimed to assess the influence of microgravity on the neurocognitive marker P3 during parabolic flight using a visual-based oddball paradigm. **METHODS**: Participants were trained to perform a brain-computer interface (BCI) task, which included a visual oddball task. Nine participants performed this task during parabolic flight. Statistical analyses assessed the amplitude and latency of the P3 event-related potential (ERP). **RESULTS:** Results revealed no significant differences for P3 amplitude nor latency in the 0G condition versus the 1G condition. The latency of oddball stimuli did not differ from the latency of standard stimuli in 0G or 1G. However, the amplitude of oddball stimuli was significantly higher than the amplitude of standard stimuli in both 1G and 0G. **CONCLUSION:** There needs to be greater consensus and understanding concerning the effects of microgravity on cognitive performance and ERPs. The data presented here does not verify that short bouts of microgravity could enhance nor diminish neuro-behavioral performance. It does confirm that the visual-based oddball paradigm is feasible in microgravity conditions.

Acknowledgments

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1. Introduction

Astronauts typically encounter physical, psychological and neurophysiological challenges both during and after prolonged exposure to microgravity, such as muscular deterioration, decreased fine motor control, mental fatigue, altered time perception, and difficulty concentrating, orienting and estimating distances (Navarro Morales et al., 2023). These deteriorations can lead to life-threatening situations for the astronauts in space, and decreased quality of life once they have returned to Earth. As space travels are becoming longer and more frequent, understanding the potential risks and benefits of microgravitational environment on brain and cognition is becoming increasingly important. Moreover, a better understanding of how brain and cognition are affected by exposure to microgravity not only supports safe and successful space exploration, but also has potential benefits for humans on Earth who – as a result of brain trauma or neurodegenerative disease – experience challenges similar to those of astronauts. However, the results of previous studies evaluating cognitive and neurophysiological processes under the influence of microgravity have been inconsistent. Some studies report no direct or conclusive effect (Fowler & Manzey, 2000; Manzey, 2000; Brümmer et al., 2011); others, a potentially detrimental effect (Takácz et al., 2021; Navarro Morales et al., 2023), and yet others a potentially positive effect (Wollseiffen et al., 2016; Wollseiffen et al., 2019).

Thus, it is essential to investigate and describe the behavioral parameters of cognitive performance (e.g., reaction time) as well as the underlying neurophysiological effects that occur under the influence of weightlessness. Earlier studies have suggested that continued research should include classical tasks such as the flanker or oddball paradigm, specifically because event-related potentials (ERPs) related to these tasks have been clearly described (Wollseiffen et al., 2016).

Because the feasibility and affordability of long-term microgravity exposure research (e.g., on board the International Space Station) is very limited, this study was conducted in the simulated weightlessness environment that occurs during parabolic flight. This kind of microgravity research poses a challenge because the efficacy of brain imaging techniques is limited in extreme environments such as parabolic flight. Environmental effects – e.g., noise and the inescapable movements induced by the parabolas – influence the subject and the ability to measure data. Fortunately, electroencephalography (EEG) allows for assessing ERPs with a high temporal resolution, and suitable EEG systems can be used to study brain activity in extreme environments.

This study aimed to increase our understanding of how the brain adapts as a response to changes in gravitational force, specifically by focusing on an explicit signal of high-level cognitive processing: the P3 ERP, also called the P300. The P3, with its various components, is generally understood to be a measure of conscious perception and controlled attention (Linden, 2005; Polich, 2007; Patel & Azzam, 2005; Rutiki et al., 2015). To elicit the P3 signal, a visual oddball paradigm task was performed in parabolic flight.

Section 1.1 *P3/P300 event-related potential* gives an overview of the existing knowledge about this brain signal to date. In section 1.2, *Gravity and Cognition*, I discuss gravity and its impact on cognition and neurophysiology. Section 1.3 *Importance and Implications* explains the value of this research for not only space-related matters but humanity in general. In section 1.4, *Hypotheses*, I introduce the specific hypotheses that this study explored.

1.1 P3/P300 Event-Related Potential

> **Definition:** The P3 component, also referred to as ‘P300’ or ‘P3b’, is a positive wave that peaks roughly between 250-500 milliseconds after stimulus presentation and has a maximum amplitude over the central posterior region of the brain (Polich, 2007).

P3 characteristics and theory

Oscillations or electrocortical waves characterize brain states and underlie complex and integrative brain function (Cebolla et al., 2016). ERPs are specific brain oscillations that can be linked to specific cognitive or measurable psychological processes. EEG waves represent signals that arise from global electrical neural activity. Visual characterization of the EEG signal includes identifying or classifying certain waveform components based on a subjective characterization – e.g., negative or positive peak polarity – or the location within the measured brain areas (Ramele et al., 2018). The amplitude difference between different waveforms can be established, from which a relation can be inferred and categorizations indicated. Latency can be defined as the time interval between stimulus onset and the peak – i.e., maximum amplitude – of the specific ERP wave.

The P3 ERP is a type of brain oscillation that can be understood as a measure of attention or conscious perception. The P3 is one of the most studied ERPs, discovered in 1965 by Sutton, Barren, Zubin, and John (Ramele et al., 2018). Specifically, the P3 is a central-parietal positivity and a component of the ERP that is evoked roughly 300 milliseconds after stimulus presentation.

Its amplitude increases when a subject encounters an infrequent, i.e., deviant or unexpected, stimulus in a stream of frequent, similar stimuli (Somani & Shukla, 2014; Ramele et al., 2018). In other words, the P3 response arises when a person's attention is caught by a rare event that arises within a random series of stimulus events, also known as an oddball paradigm (Fabiani et al., 1987). It has been associated with various aspects of cognitive information processing, e.g., attention, working memory, and executive function (Linden, 2005; Polich, 2007; Van Dinteren et al., 2014) and is thought to be a neural signature of the mechanisms required to change the mental model of the environment to make an appropriate response (Linden, 2005; Nieuwenhuis et al., 2011). Along with the N200 negativity, the P3 is associated with conscious perception, selective attention, stimulus evaluation, and conscious discrimination or categorization (Patel & Azzam, 2005; Rutiki et al., 2015).

Subcomponents P3a and P3b, and the oddball paradigm

Since the initial discovery of the P3, research has shown that it is not a unitary phenomenon. Instead, we can distinguish between two subcomponents: the *novelty* P3, or P3a, and the *classic* P3, or P3b (Polich, 2007; Ramele et al., 2018). An infrequent stimulus that is not task-related can produce a positive potential with maximum amplitude in the central/parietal areas and short latency. This positive potential has been named the 'P3a', to be distinguished from the task-relevant 'P3b' potential.

Two classic paradigms used to obtain the P3 component are the two- or three-stimulus (visual or auditory) oddball task, of which the latter is used to examine both the P3a and P3b. Stimuli can be presented visually or audibly. In an oddball paradigm, the participant responds, overtly or covertly, to one of two different stimuli that are randomly presented: the deviant or oddball stimulus occurring less frequently than the standard stimulus. In the classic two-stimulus oddball task, a sequence of stimuli is presented to the participant consistently and steadily. For example, a grey circle is presented every second on a computer screen. A different, less frequent target or 'oddball' stimulus is presented during the standard sequence; for example, a blue circle is presented randomly.

As motivation to catch the infrequent stimulus increases, the amplitude of the P3 also tends to increase (Ramele et al., 2018; Rutiki et al., 2015; Van Dinteren et al., 2014). Therefore, subjects are typically instructed to actively engage with the oddball stimuli, e.g., by counting the oddball or pressing a button. The P3b is typically observed around 300 milliseconds after presentation of the target stimulus. In the three-stimulus oddball paradigm, an infrequent nontarget stimulus is randomly presented in the stimulus sequence in addition to the standard and target/oddball stimulus. To stick with our example: in a sequence of consistently presented grey circles, a subject is instructed to count the randomly appearing blue circles, while a red circle – the third, 'distractor stimulus' – also appears randomly but should be ignored.

The P3b has been shown to respond specifically to task-relevant stimuli. This means the distractor stimulus will not elicit a strong P3b because it is irrelevant to the task. The distractor stimulus will, however, still elicit an earlier positive potential – the P3a. When perceptually novel or unexpected distractors occur in a sequence of standard stimuli, a novelty P3a component can be seen with maximum amplitude in the frontal/central parts of the brain, short peak latency, and relatively quick habituation (Ramele et al., 2018; Polich, 2007; Patel & Azzam, 2005). Contrarily, the P3b will not habituate due to repeated presentation and has a slightly longer peak latency.

The scalp distribution of the P3b is generally larger over parietal areas. Its latency is usually around 300ms after stimulus onset. However, this can vary within a time window of roughly 250–450ms, depending on factors such as task conditions, fatigue, motivation, and age of the subject (Van Dinteren et al., 2014). The P3, specifically the task-relevant P3b component, is also widely utilized in the brain-computer interface (BCI) field because it is a distinct brain signal that can, for example, be harnessed for the application of spelling devices. When the intended target letter is presented, the BCI can signal this through the presence of the P3 (Nijboer et al., 2008; Ramele et al., 2018).

Presence, timing, topography, and amplitude of P3 oscillation can thus be used as measures of attention. Changes in amplitude, topography or latency may indicate underlying functional or structural brain changes. For example, decreased amplitude and increased latency seem to indicate a general slowing of cognitive processes (Linden, 2005; Takácz et al., 2021). Amplitude is also affected by stimulus meaning (i.e., stimulus or task complexity and motivational salience), subjective probability of occurrence, and task relevance regarding the distribution of attentional resources (Patel & Azzam, 2005; Polich, 2007). Furthermore, Polich (2007) found that P3 amplitude generally decreases as primary task difficulty increases when performing a primary task while simultaneously engaged in an oddball task. He proposed a model system in which the P3 results from the operation of inhibitory mechanisms engaged by incoming stimulus events to facilitate memory processing.

1.2 Gravity and Cognition

The human brain, cognition, and other bodily functions and parts developed partially due to dealing with the specific constrictions that gravity enforces. Changes in the gravitational environment have been found to directly and indirectly affect human psychology, physiology, and neurophysiology. Astronauts encounter these – often adverse – effects both during spaceflight and upon their return. Gravitational changes encountered in space flight affect both sensorimotor mechanisms, such as spatial disorientation (Navarro Morales et al., 2023; Clément & Ngo-Anh, 2013; Fowler et al., 2000; Grigoriev et al., 1993), motor speed (Fowler et al., 2000; Berger et al., 1997) and simple repetitive motor timing (Semjen et al., 1998), and (underlying) cognitive capacities such as memory, mental representation of space, and concentration (Navarro Morales et al., 2023; Manzey et al., 1995; White et al., 2016). Although most adverse effects gradually diminish as the body and brain adjust to the altered gravitational environment, they often reappear post-flight as the systems need to readapt to Earth (Wollseiffen et al., 2016; Bock et al., 2015; Clément & Ngo-Anh, 2013).

A change in gravitational force requires the brain to reshape the dynamic integration of neural information acquired in this newfound environment (Cebolla et al., 2016; Cheron et al., 2006). This reshaping can briefly and rapidly occur in the case of sudden and brief gravitational changes; reshaping can also occur more fundamentally, when exposed to a microgravitational environment for extended periods. Thus, on the one hand, it is necessary to explore how bodily systems adapt to long-term microgravity exposure, e.g., aboard the International Space Station. On the other hand, as space missions include several transitions between gravitational levels, short-duration effects must also be studied to understand better the acute effects on the various aspects of the brain and cognition (Clement & Ngo-Anh, 2013).

Previous microgravity ERP research

Microgravity research thus far has not only been scarce, but its findings are also inconsistent. Declines in cognitive function during and after gravitational shifts have been hypothesized to occur due to stress-related factors, and many studies were not able to distinguish between the direct influence of gravity and that of stress (Wollseiffen et al., 2016; De la Torre, 2014; Clément & Ngo-Anh, 2013). However, at least one parabolic flight study has argued that it could distinguish between the effects of stress and gravity (Wollseiffen et al., 2016). Participants performed a mental arithmetic task with increasing levels of difficulty. Reaction time and the amplitude of event-related potentials N1 and P2 – associated with increased involvement of superior frontal and medial frontal gyrus – were found to be reduced during microgravity as opposed to standard (1G) gravity. The decrease in reaction time was found to be more prominent with increasing task complexity, which, according to the researchers, could indicate that fewer cortical resources appear to be necessary to perform a task in 0G compared to 1G conditions. These findings were observed in both experienced flyers and novices. The researchers also suggested that previous findings of impaired cognitive performance due to weightlessness may have been due to stress-related factors. As the experimental data compared in-flight data 1G to 0G, the researchers argued that effects due to stress could be ruled out as they thought it unlikely that stress levels changed significantly in the short duration between 0G and 1G phases.

Although Wollseiffen et al. (2016) found P3 amplitude to be reduced during 0G versus 1G during parabolic flight, Chen et al. (2017) investigated various BCI varieties, among which a P3-Speller BCI, and did not find significant differences in BCI accuracy, P3 amplitude, nor latency between normal experiment condition and in-orbit measures. The small sample size and microgravity duration may have impacted these results. In comparison, this study contained a mere sample size of two, whereas Wollseiffen, Klein, and Schneider (2016) had a sample size of 17. Moreover, the two participants were two astronauts in orbit: i.e., they were in a microgravitational environment for a prolonged time, as opposed to experiencing short bouts of microgravity.

Various other studies on microgravity-related ERPs provide us with the notion that central cognitive processes change in (simulated) weightlessness (Cheron et al., 2014; Koppelmans et al., 2013; Messerotti Benvenuti et al., 2011). Changes in gravity conditions have been reported to influence neuronal activity and blood distribution and flow in the brain. A study conducted by Wollseiffen et al. (2019) compared behavioral performance (reaction time) and neuronal performance (ERP analysis: N2 and P3) using a complex arithmetic task in combination with an auditory oddball task during the 1G and 0G phases in a parabolic flight. No difference was found in reaction time between 1G and 0G for the oddball paradigm. The amplitude of the neurocognitive markers N2 and P3 was found to be significantly reduced during the 0G phase. Latency remained unaffected in both gravity conditions for the P3; however, it was lower in 0G for the N2. The researchers suggested that microgravity is likely to enhance neuro-behavioral performance and assumed that this has to do with brain hemodynamics, i.e., they theorized that the weightlessness-induced shift of fluid to the brain could positively affect cognitive performance.

Takácz et al. (2021) investigated whether prolonged exposure to space environment and stress-related factors impact visuospatial functioning during spaceflight. Results showed that astronauts’ reaction time and accuracy decreased during prolonged 1G exposure. P3a amplitudes decreased considerably in-flight compared to pre-flight. P3b amplitudes were also significantly lower in-flight and late post-flight compared to pre-flight. Upon return to Earth, task performance gradually reversed to pre-flight values, while ERP amplitudes reflected a slow re-adaptation to a typical (1G) Earth environment. Moreover, according to Takácz et al. (2021), the ERP measurements do not reflect a positive effect on cognitive function – as suggested by Wollseiffen et al. (2016) and Wollseiffen et al. (2019) – but instead, they could reflect diminished attentional resources. In contrast with weightlessness studies that found no cognitive impairments, their results indicated that prolonged spaceflight could impair cognitive performance.

Given the possible adverse effects of space exploration, the complexity of cognitive processes, and the inconsistency of previous studies, both behavioral responses and the neurophysiological underpinnings of cognitive processes still require thorough investigation.

1.3 Importance and Implications

Investigating exactly how the brain adapts during gravitational shifts contributes to our knowledge of how the constraints of a gravitational 1G environment have shaped neural systems and processes for the coupling of perception and action (McIntyre et al., 2001). It may also help develop countermeasures or training to counter any deteriorative effects of being in altered gravitational environments for a prolonged time, to rehabilitate or even prepare astronauts before their return to Earth. Moreover, knowledge about the impact of a change in gravitational environment on human cognition and neurophysiology could benefit non-astronauts who, for example, suffer from paralysis or are otherwise long-term bedridden. Microgravity-related physiological adaptations, as seen during and after space flight, are known to reflect the physiological adaptations that occur with prolonged inactivity on Earth. This similarity is shown in the effects of long-duration bed rest on various physiological systems, such as loss of bone density, muscular atrophy, and effects on the cardiovascular system (Zhang et al., 2014; Hargens & Vico, 1985; Scott et al., 2021).

This understanding of comparability provides valuable opportunities and insights into gravity-related cognitive and neurophysiological changes. For example, the procedure of head-down tilt bed rest (HDBR) is frequently used as an Earth-based analog for space flight to study effects related to gravitational changes and also to develop and test countermeasures to microgravity-related adaptations to Earth gravity (Zhang et al., 2014).

To this end, the P3 signal is multifunctional: both on Earth and in space, the P3 can be used to measure neurophysiological health and performance and to control BCIs. A BCI translates neurophysiological signals into computing commands, using brain activity to operate a technological device. As a result of the neurofeedback offered to a person operating such a device, creating a functional feedback loop, neurological networks can be strengthened, and self-awareness about one's brain state increased, and with that, the self-regulative ability of brain frequencies (Menon et al., 2009; Lebedev & Nicolelis, 2006).

On Earth, BCI technologies are put to use in helping to restore lost motor function, for example, in people who suffered from stroke, and for the enhancement of certain neural functions. Moreover, brain-computer interfaces can replace lost physical functionality, for example, in people with ALS (Nijboer et al., 2008), ranging from operating a robotic arm to a communication device. The potential for BCIs in the astronautic field is increasingly recognized (Lebedev & Nicolelis, 2006; Menon et al., 2009; Milàn et al., 2009; Chen et al., 2017). The development of qualitative and user-friendly P3-controlled BCIs could help to monitor and train astronauts.

Another possible application is the intercepting of adverse side effects of altered gravitational force on the human body, for example, as a functional replacement for decreased muscle functionality. BCIs could keep necessary activities during space flight available when these activities become more difficult or even impossible to execute physically. The fact that the P3 signal can be used to operate BCIs (Chen et al., 2017) is another crucial reason to investigate the signal during varying gravitational forces. This way, in the future, the functionality of these interfaces may be seamlessly adjusted to our capabilities.

1.4 Hypotheses

Existing research concerning the amplitude change of ERPs during microgravity has provided ambiguous results (Wollseiffen et al., 2019; Chen et al., 2017; Klein et al., 2019; Takácz et al., 2021). This ambiguity might be partially due to the difference between prolonged and short periods of microgravity, sample size, or inter-individual differences (e.g., fatigue, stress, motivation). The general lack of consensus emphasizes the need to investigate specific neurophysiological markers and cognitive performance. Prior studies have suggested that future research should expand on investigating classical tasks such as the oddball paradigm because the related P3 ERP component is a well-described, distinctive measure of neurophysiological performance (Wollseiffen et al., 2016). When the current experiment was conducted, insofar as I know, an experiment exploring short bouts of microgravity using a visual oddball paradigm had not yet been published. Therefore, the current study investigated the influence of short bouts of microgravity on the P3 event-related potential employing a visual oddball paradigm during parabolic flight.

The research questions addressed are as follows: under the influence of short periods of microgravity induced by parabolic flight, 1) how does the amplitude of the P3 change, and 2) how does the latency of the P3 change?

Earlier long-term weightlessness studies found a decreased P3 amplitude in 0G (Tacákz et al., 2021; Cebolla et al., 2016), as did earlier short-term weightlessness research using the auditory oddball paradigm (Wollseiffen et al., 2019). Following this line of research, I expected to find a decrease in the visually elicited P3 amplitude in 0G compared to 1G; I did not expect to find a significant difference in P3 latency in 0G compared to 1G.

Hypotheses

1. P3 amplitude is significantly lower in 0G compared to 1G
2. P3 latency is relatively the same in 0G compared to 1G

2. Materials and Methods

2.1 Experimental Design

Within-subjects design

This experiment had a 2 x 2 factorial within-subjects design. Independent variables are ‘gravity’ (1G, 0G) and the visual oddball stimulus presentation, ‘stimulus condition’ (standard, oddball). The dependent variables are the amplitude and latency of the P300 signal. The reason for choosing a within-subjects design, as opposed to a between-subjects design, is that there was no difference in treatment or allocation of participants to specific groups. All participants underwent all three gravity phases (1G, 0G, 1.8G) during the parabolic flight maneuvers. These phases were repeated multiple times, uninterrupted and equal for all participants.

Independent variable *‘gravity’*: 0G & 1G

Gravity is the first independent variable. The gravitational force changes as a result of the parabolas flown by the aircraft. The statistical analyses included two levels of gravity: 0G, or microgravity, and 1G, or standard/Earth’s gravity.

The aircraft’s acceleration fluctuates by nature. Therefore, these gravitational forces are approximations. Actual g-force slightly oscillates around these numbers. For example, when speaking of 0G, the actual range may have fluctuated between 0G and 0.05G.

Independent variable *‘stimulus condition’*: standard & oddball

The second independent variable is stimulus presentation with two factors: 1) standard stimuli and 2) deviant/oddball stimuli.

Dependent variable ‘P3’: amplitude & latency

The dependent variable is the ERP that results from the visual oddball paradigm, specifically the amplitude and latency of the P3 signal.

2.2 Participants

The experiment was conducted during three parabolic flights and included three subjects per flight (N=9). Recruitment took place via social media and posters at Radboud University. Eleven participants were initially recruited and screened, ensuring backup subjects in case of dropouts.

Nine healthy volunteers (mean age 28.8 years ± 5.8, four female, five male) with normal to corrected-to-normal vision (corrected only if lenses were possible) and hearing and without neurological, psychological, or physiological impairments were selected to partake in the experiment. Subjects had to be 18 years or older, Dutch- or English-speaking, ad have no prior experience with microgravity or history of motion sickness. Before the study, all subjects provided written informed consent. The study was approved by The Medical Ethics Committee of the Radboud University and the French Medical Ethical Committee of the University of Caen.

2.3 Measures

Parabolic flight

The experiment was conducted during three parabolic flights under the sponsorship of the ESA Education Office (Fly Your Thesis! 2017) and NOVESPACE during the 68th Parabolic Flight Campaign. The campaign used a ZERO-G Airbus A-300 aircraft and took place from December 3-7, 2017.

In parabolic flight studies, the airplane repeatedly performs parabolic maneuvers – also called parabolas – resulting in 0G, 1G, and 1.8G cycles. Figure 1 illustrates the general flight pattern of a parabola.

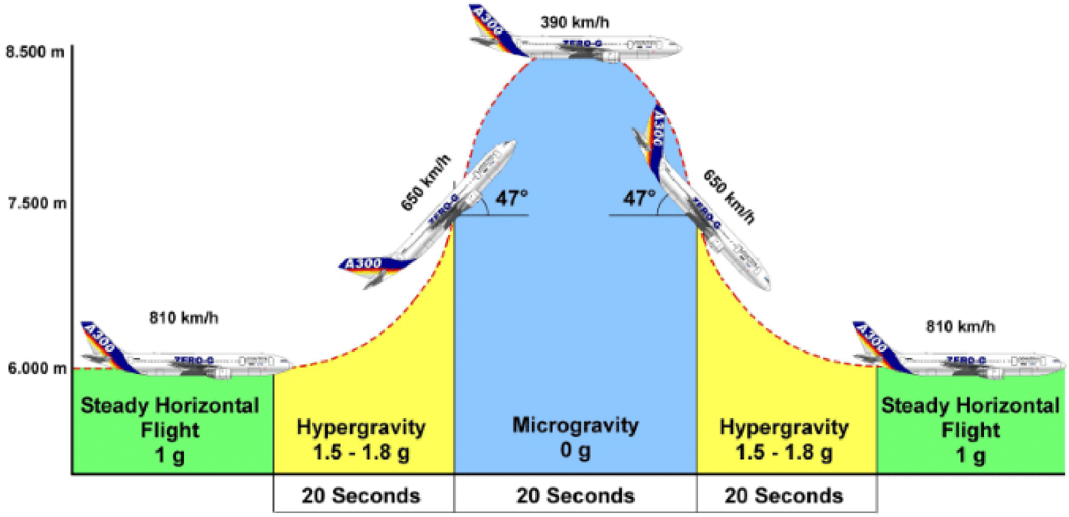

Figure 1. *Example of the parabolic flight pattern performed by the ZERO-G aircraft during a single parabola. Illustration: courtesy of Novespace, Paris, France.*

At point zero, the airplane starts to climb from ~6100m to ∼9000m (approximately 20 seconds) at an increasing angle, resulting in ∼1.8G (‘hypergravity’) in the cabin. Once an angle of 47° inclinations is reached, the engines are brought to idle, and the plane floats for ~22 seconds – performing its parabola – resulting in 0G (‘microgravity’) in the cabin. During its following descent, when the plane reaches a 42° angle, it accelerates and pulls out of the parabola, returning to a horizontal plane. During its pull-out, forces of ~1.8G occur for approximately 20 seconds.

Equipment

EEG signals were acquired with 64 Ag/AgCl electrodes of a high-density 64-channel eego Waveguards cap (ANT Neuro, Enschede, Netherlands) and an EEGoPro amplifier (ANT Neuro, Enschede, Netherlands). OneStep EEG-Gel was used to optimize electrode contact. The EEG signals were recorded at a sampling rate of 250 Hz. The experiment used Panasonic Toughbook laptops.

Questionnaires/visual analogue scales

- Demographics
- Checklist (caffeine, sleep, etc.)
- Visual analogue scales (e.g., motivation, focus, rest)
- Post-flight questionnaire

Subjects completed a post-flight questionnaire and VAS scales to assess their experienced arousal, stress/anxiety, fatigue, motivation, and focus. These questions were assessed to be considered covariates to investigate interindividual variability in an exploratory manner in later analyses. They were not included to test the primary hypotheses.

2.4 Experimental Procedures

Intake and Training Protocol

From the initial screening, eleven people were selected. One subject dropped out at the beginning of the selection procedure due to time and travel constraints. The remaining ten subjects participated in a series of 16 training sessions that took place at Radboud University, the Netherlands, over 8 weeks (see 2.5 Experimental procedures – *training protocol*). Informed consent was agreed upon and signed by each subject before general participation and each training session.

During the training protocol, the subjects all received the same motor imagery-based BCI training for a different experiment conducted simultaneously with this one, and for each subject, each week contained two training sessions of approximately 3 hours.

Because the visual oddball paradigm task does not require training, it was only added in the last two weeks. Each training session contained six runs of the task. During the final training sessions, subjects were also trained in the experimental flight set-up because they were required to operate the experiment themselves during the actual parabolic flight experiment.

Of the ten participants, nine were chosen to partake in the final experiment based on their success in performing the motor imagery-based BCI task.

Experiment

For the official parabolic flight experiment, participants were flown to the flight site of Novespace in Bordeaux, France. Each participant participated in one parabolic flight, with three subjects per flight. To be allowed on board the parabolic flight, subjects underwent a medical check-up to ensure they were in perfect health. Additionally, an anti-motion sickness drug, scopolamine, was administered intravenously to each subject with doses between 0.5 and 0.8mg before the flight.

Subjects were seated next to each other in airplane chairs and loosely strapped with a seatbelt around their waist. The straps ensured floating and thus the experience of weightlessness while improving safety and limiting changes in neuronal activity caused by motion or other interference. Subjects' hands and feet were also loosely strapped to the chair and the ground with Velcro to prevent movement, especially during microgravity phases (see Fig. 2).

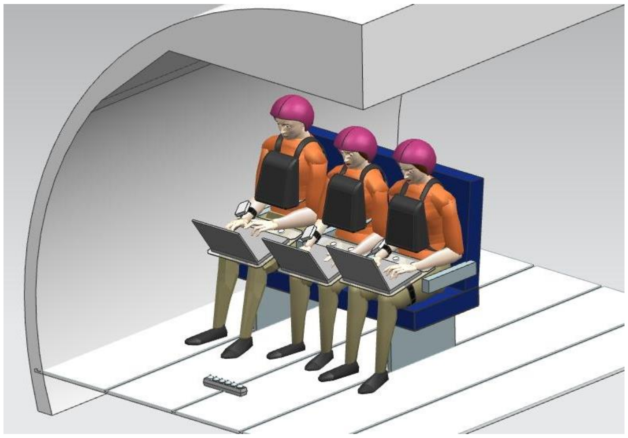

Figure 2. *Representation of the experimental set-up inside the aircraft.*

Each subject had a small table with a 13-inch laptop attached to their legs with Velcro. EEG caps were connected to the EEG amplifiers placed in backpacks, which were carried on the stomach and connected to the laptops. Chords ran through the backpacks to ensure minimal movement. The experimental set-up was shielded with a black curtain to limit visual distractions from the environment. There were no additional devices to block out auditory distractions due to safety restrictions set by Novespace.

Each flight went through 31 parabolas. There was 1 minute of rest in between each parabola. Upon the advice of the European Space Agency and Novespace, the first parabola did not contain any task instructions because of the novelty of the parabolic experience. The last five parabolas were not used for the experiment, which left 25 experiment parabolas. Participants performed the visual oddball paradigm task during 4 parabolas. The remaining parabolas were used for other experiments that were not relevant to the current study.

Visual oddball paradigm task

Participants perform the visual oddball paradigm task while wearing an electrode cap with EEG recording electrodes measuring their brain activity (see Fig. 3). The stimulus is displayed at the bottom of the screen. It varies between two stimulus types:

a) **Standard**: flashes at a constant rate every 0.5 seconds. This type of stimulus does not come with task instructions, i.e., it is not motivationally salient.
b) **Deviant/oddball**: flashes blue randomly twice every second. Participants are instructed to count these flashes to make the oddball stimuli motivationally salient as well as unexpected.

The neurophysiological responses to the different types of stimuli, i.e., the event-related potentials elicited and the P3 specifically, were compared. Expected was that the moment an oddball stimulus appears, a P3 signal is elicited because these stimuli are a) irregular, and thus to an extent unexpected and attention-grabbing, and b) motivationally relevant.

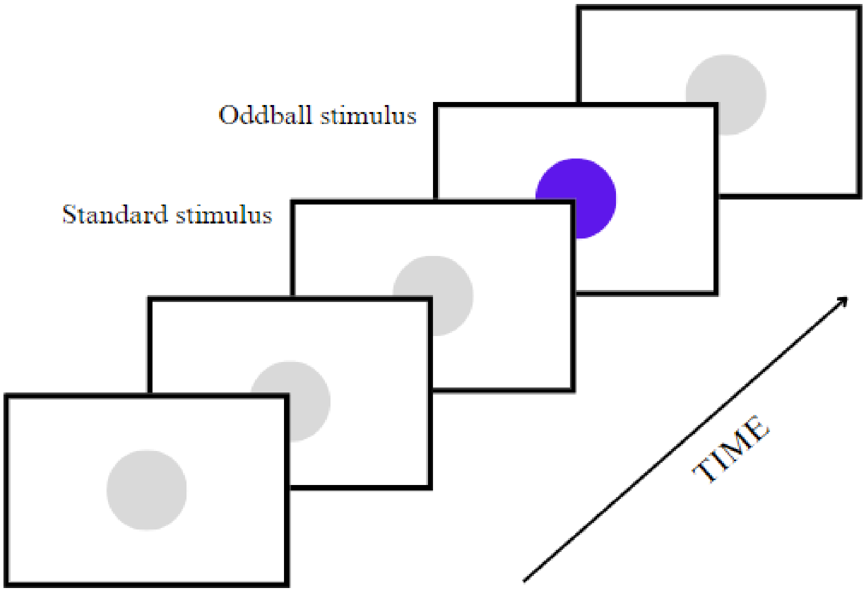

Figure 3. *Example of a visual oddball paradigm. Visual stimuli are presented in a temporal sequence. The grey circle stimulus is the 'standard' stimulus. The blue circle is randomly and rarely presented and called the 'oddball,' 'deviant,' or 'target' stimulus.*

2.5 EEG Analysis

Equipment and data acquisition

EEG data was recorded using an ANT Neuro electrode cap, Eego sportswear. A total of 64 Ag-AgCl electrodes were used. Each electrode was referenced to the combined potential measured by adhesive electrodes applied to the mastoids. Blinks and horizontal eye movements were monitored with electrodes placed at the lateral canthi of the eyes (horizontal) electrooculogram (EOG). Electrodes were filled with Electro-Gel™ (Electro-Cap International, USA) for optimal signal transduction. The signal was recorded with a 250 Hz sampling rate. Each task was saved under a separate recording file.

EEG processing

The data was processed using MATLAB B2020b. The EEG signals were filtered with an analogue bandpass of 0.5-30 Hz. All data was re-referenced to averaged mastoid recordings. Any channels exceeding 2.5 standard deviations more power were excluded from further analysis. The data was epoched into periods of 750ms: an interval of 750ms starting at the onset of each stimulus was cut out as an epoch. The epochs were baseline-corrected for the 100ms pre-stimulus interval. Epochs containing EEG fluctuation exceeding ~100 millivolts were rejected. The remaining epochs were averaged into six categories: standard and deviant/oddball stimuli over 0G, 1G, and 1.8G of which the 0G and 1G data were used for the analyses.

The P3 is detectable in centro-frontal and centro-parietal cortical regions: therefore, electrodes Fz, Cz, and Pz were chosen for further analysis. The most prominent positive peak between 250-450ms after stimulus onset was defined as the P3. Each participant's latency and amplitude components were defined individually. The oddball ERP was compared to the standard ERP.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 29.0.1.0. From the total of 9 participants, the EEG data of 8 participants was eligible for further ERP analysis. The data from subject 3 was excluded from processing and further analysis because their laptop experienced technical issues early on in the experiment. Subject 1 had to drop out after 15 parabolas because of motion sickness. Their data was included to the extent that it was present.

The remaining sample included eight healthy participants, with an age range of 24 to 42 (*Mage* = 32.3, *Sdage* = 4.8), including four males (*Mage* = 33,7, *Sdage* = 4,56) and four females (*Mage* = 27,8, *Sdage* = 1.24). There was no missing data to be excluded from the analysis. A two-way multivariate repeated measures ANOVA was conducted to compare the effect of the within-subjects independent factors 'gravity' (0G, 1G) and 'stimulus condition' on the dependent factors P3 'amplitude' and 'latency' for the Pz, Fz, and Cz electrodes. Descriptive statistics, including averaged means and standard deviations, are displayed in Table 2 (see Appendix II).

Relevant assumptions conducive to performing the two-way ANOVA were examined. Because this was a 2-by-2-factorial repeated measures design, sphericity was assumed (Field, 1998; Field, 2018) and confirmed by Mauchly's test of sphericity (Greenhouse Geisser epsilon ε = 1, lower-bound = 1). Normality plots and boxplots were used to detect outliers. The existing outliers fell within the expected bounds of normal distribution and were therefore not removed (Field, 2018). The significance level was set to α = .05.

3. Results

Pz electrode

Results showed that the interaction effect for Gravity*Stimulus was not significant (*F*(2) = 0.43, *p* = .67, *n*p2 = 0.13). There was also no significant main effect for Gravity (*F*(2) = 2.05, *p* = .21, *n*p2 = 0.41). However, there was a large significant main effect for Stimulus (*F*(2) = 0.43, *p* < .05, *n*p2 = 0.82). Pairwise comparisons using the Bonferroni correction showed that the amplitude of deviant stimuli was significantly higher than that of standard stimuli (MD = 19.6, SD = 5.47, *p* < 0.05, 95% CI [6.67, 32.55], indicating successful execution of the oddball task. No significant difference was found for Latency (MD = 12.25, SD = 21.58, *p* = .59).

Out of the three chosen electrodes, the Pz electrode had the largest effect size with regard to the main Stimulus effect (*n*p2 = 0.82 (Pz electrode) versus *n*p2 = 0.76 (Fz electrode) and *n*p2 = 0.71 (Fz electrode)). Therefore, averaged and subtracted waveforms of the Pz electrode were used for illustration. Upon visual inspection, subject 6 appeared to have the best visible P3 out of all subjects, which was also located at the Pz electrode (see Figures 4, 5 & 6). Figures 7, 8 & 9 show the grand averages and subtracted waveforms of all participants for the Pz electrode.

Figure 4. *Averaged standard & oddball ERP and subtracted wave: subject 6, electrode Pz in 0G*

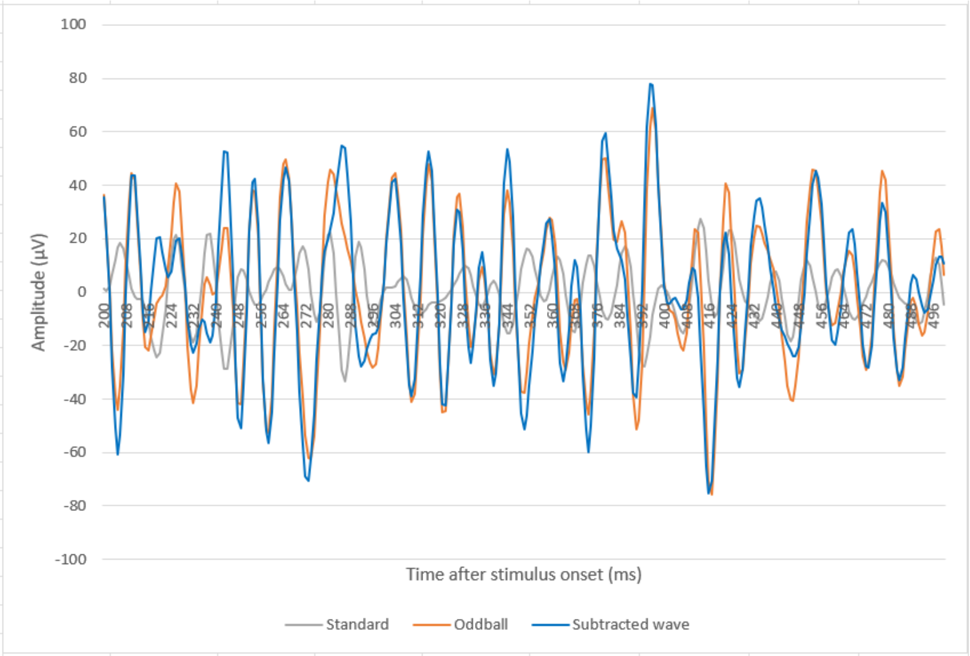

Figure 5. *Averaged standard & oddball ERP and subtracted wave: subject 6, electrode Pz in 1G*

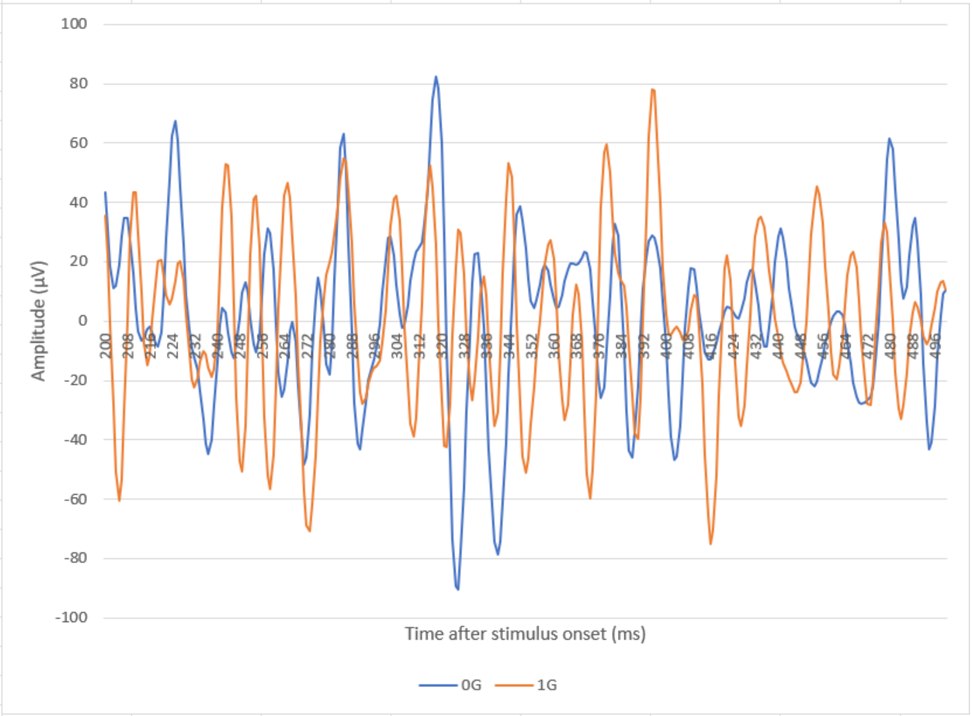

Figure 6. *Subtracted wave of subject 6, electrode Pz in 0G and 1G*

Fz electrode

Results showed that the interaction effect for Gravity*Stimulus was not significant ($F(2) = 1.17$, $p = .37$, np2 = 0.28). There was also no significant main effect for Gravity ($F(2) = 0.2$, $p = .98$, np2 = .01). However, there was a large significant main effect for Stimulus ($F(2) = 0.96$, $p < .05$, np2 = 0.76). Pairwise comparisons using the Bonferroni correction showed that the amplitude of deviant stimuli was significantly higher than that of standard stimuli (MD = 15.76, SD = 3.33, $p < 0.05$, 95% CI [7.88, 23.64], indicating successful execution of the oddball task. No significant difference was found for Latency (MD = -5.88, SD = 19.03, $p = .77$).

Cz electrode

Results showed that the interaction effect for Gravity*Stimulus was not significant ($F(2) = 4.71$, $p = .06$, np2 = 0.61). There was also no significant main effect for Gravity ($F(2) = 1.68$, $p = .62$, np2 = .36). However, there was a large significant main effect for Stimulus ($F(2) = 7.2$, $p < .05$, np2 = 0.71). Pairwise comparisons using the Bonferroni correction showed that the amplitude of deviant stimuli was significantly higher than that of standard stimuli (MD = 11.85, SD = 2.95, $p < 0.05$, 95% CI [4.88, 18.82], indicating successful execution of the oddball task. No significant difference was found for Latency (MD = -3.75, SD = 12.87, $p = .78$).

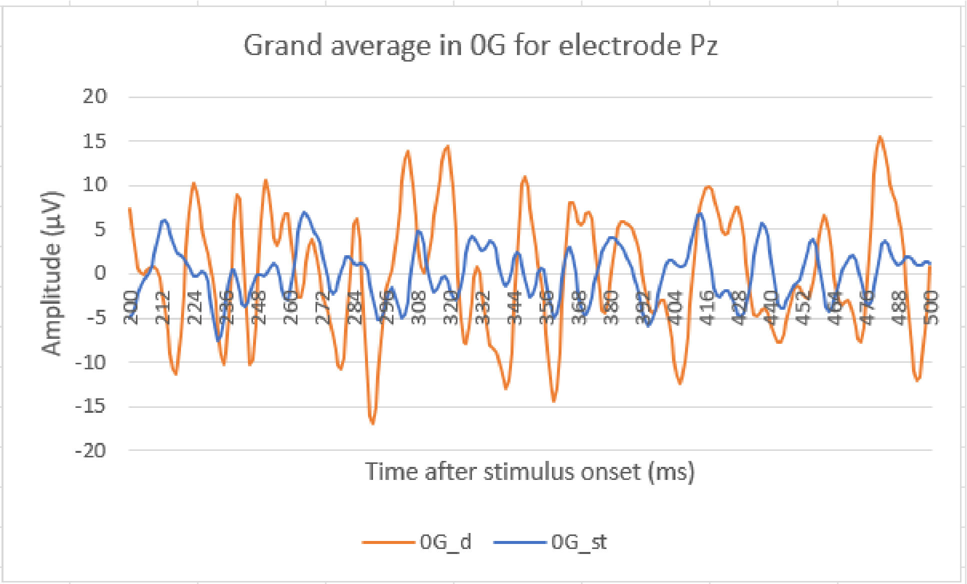

Figure 7. *Grand average of all participants in 0G for electrode Pz.*

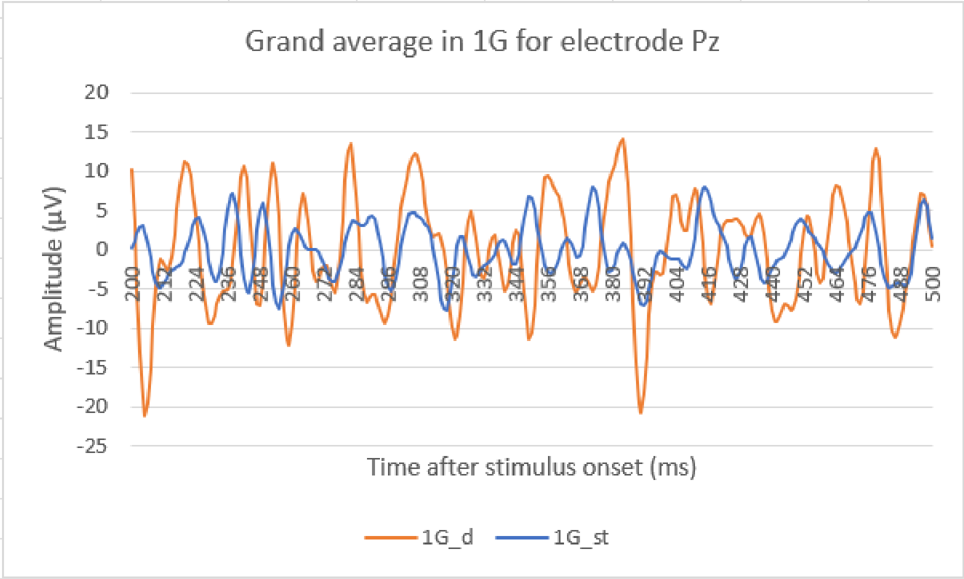

Figure 8. *Grand average of all participants in 1G for electrode Pz.*

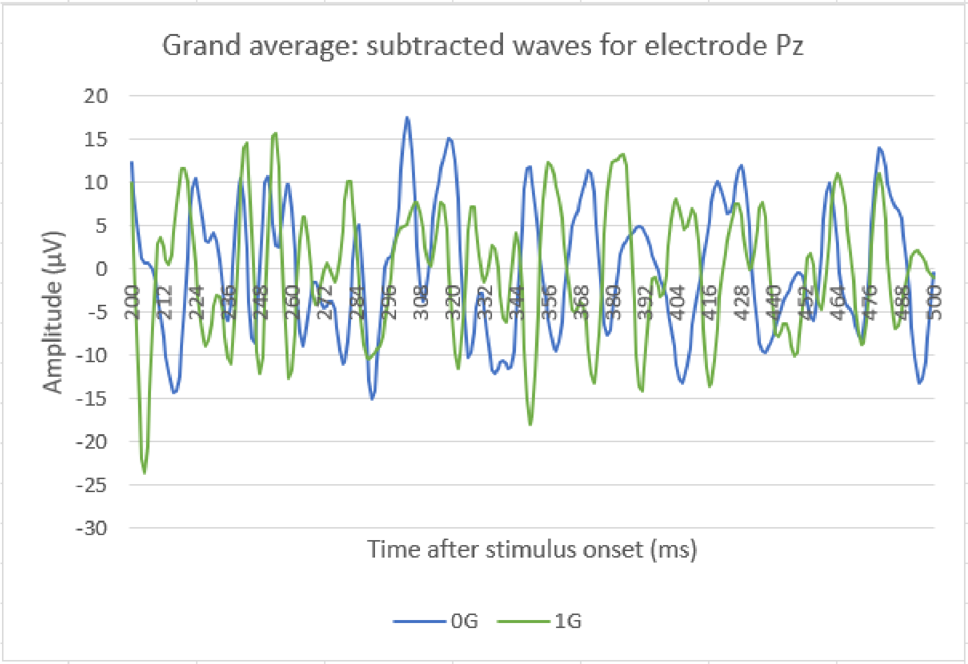

Figure 9. *Grand average (all participants) of the subtracted wave for electrode Pz.*

4. Discussion

This study aimed to explore the influence of short bouts of microgravity on the P3 event-related potential. As expected, the visual oddball task was successfully conducted during parabolic flight, affirming the potential for using visual-based P3 BCIs in space. Also meeting expectations, the findings suggest no difference in the latencies (Pz, Fz, Cz) between 1G and 0G. However, contrary to expectations, no difference was found in the amplitudes (Pz, Fz, Cz) between 1G and 0G, implying that short bouts of microgravity have no substantial effect on the P3 signal.

These results do not confirm studies that have found that short bouts of microgravity have an effect on P3 amplitude. The results do verify that short bouts of microgravity appear to not significantly change P3 latency. Moreover, this study confirmed that a visually-based oddball paradigm is feasible in a microgravity environment: even when external and internal distractions are plentiful. This offers promise for the viability of BCIs in space and for the neurophysiological monitoring of the health and well-being of astronauts in microgravity environments.

It should be noted that within space research, compared to rarer, more expensive, and more time-consuming space flight missions, parabolic flights are a relatively affordable and accessible option to investigate the influence of the changing gravitational environment on cognitive and neurophysiological processes. However, parabolic flight only provides us with brief periods of micro- and hypergravity, while prolonged periods of microgravity occur only during space flight missions. Given the relative scarcity of microgravitational research, comparative conclusions about cognitive and neurophysiological effects between prolonged microgravity environments and short bouts of microgravity should be made with caution.

Limitations and suggestions for future research

The limitations of this study were predominantly inherent to the inevitable experimental environment and limitations of parabolic flight. While a small sample size is common in space-related research, it remains a confining factor. Only three subjects per flight were allowed in the experiment, leading to a sample size of nine. Given the relatively low number of subjects, this study can be considered a pilot study, and results should be interpreted cautiously.

Another limitation of this study was that the time spent in different gravitational phases during each parabola only allowed for a limited amount of P3 measurements. Due to the limited amount of parabolas and time, irrelevant stimuli – additionally to standard and oddball stimuli – were not included in the oddball paradigm task. Incorporating irrelevant stimuli into the oddball task would have allowed us to investigate the difference between P3a and P3b. Future research could incorporate this 3-stimuli oddball paradigm to explore this fuller spectrum of the P300.

Moreover, exposure to more extended periods of microgravity, or familiarity with it – diminishing the experience of newness and stress – could elicit different results. As Schneider and colleagues (2007) described, parabolic flight can evoke stress and other emotional responses, especially in first-time flyers. Notably, all the subjects in this study were first-time flyers on a parabolic flight and the experience of weightlessness was new to all. Furthermore, although measures were taken to minimize noise and distractions, reducing them was challenging. Even with our best efforts, the subjects experienced visual, auditory, and bodily distractions, added to the interference of movements with the EEG signal. Table 1 shows the subjects' answers to the post-experiment question of whether or not they experienced distraction(s) during the flight. Mentioned are, e.g., light headaches, sickness, sounds, lack of comfort, and the specific bodily experience weightlessness brings with it. Motion sickness specifically was found to increase stress levels and affect neurophysiological parameters (Schneider et al., 2007).

Only subject 6 mentioned no distractions at all. Interestingly, this is the same subject whose P3 was most notable and thus used for illustrations (see Figures 4, 5 & 6). This supports the hypothesis that the physical discomforts and visual and audible distractions that the participants experienced may have influenced the P3 markers; especially taking into consideration that the P3 is a signal of attention and that distractions were higher during microgravity phases. Thus, the measurements and results cannot be expected to be of equal quality compared to results produced in a laboratory environment where noise and distractions can be better controlled. To better account for this, future research should include comparing baseline (1G) pre-flight measurements to (1G) in-flight measurements and also investigate interindividual differences (e.g., include measures such as focus, sleep, and motivation as covariates).

Aside from the experience of the subjects and the influence of distractions on the measurements, other external factors could have also added noise to the data. Even though sportswear EEG caps were used, the inevitable movements of the aircraft and the subjects themselves may have interfered with the electrical input the EEG caps received. My recommendation to minimize the interference that this causes is the same, which is that future research should include comparing baseline (1G) pre-flight measurements to (1G) in-flight measurements.

Table 1

Answers to the post-flight question about the experience of distractions.

Subject	**Distractions**
01	‘People talking, cheering. Increased touch and motion sickness due to which I had to quit after parabola 15.’
02	‘Screams of other people and touch of the participants next to me.’
04	‘The curtain in front of us came loose at one point.’
06	‘No distractions.’
07	‘People walking around me. The seat was too small, I had not enough space for my arms’
08	‘Little bit of motion sickness during the flight.’
09	‘Sitting next to each other made me look at the other screens and there were items floating during 0G. People in the other experiments made sounds or noises. The straps around my wrists were a bit rough. I also felt a light headache during the last 10 parabolas’
11	‘No distractions other than the bodily experience during weightlessness.’

As a final note, I want to highlight this study’s twofold relevance and importance. Successful elicitation of the P3 signal could provide astronauts with a means to support on-board technology in space. Cognitive tests incorporating tasks such as the oddball paradigm could also be a way to monitor and test astronauts’ cognitive and neurophysiological health and well-being. To ensure optimal efficacy, I advise future research to quantify better the scope and magnitude of interindividual variability and its underlying factors.

Beyond the utility for the realm of space flight, better understanding the effects of gravitational force on the P3 could improve the feasibility and use of P3-controlled BCIs for humans who have paralysis due to brain trauma (e.g., brain hemorrhage) or neurodegenerative disease (e.g., multiple sclerosis (MS) or amyotrophic lateral sclerosis (ALS)). For the last couple of decades, various BCIs have been examined and found to be successful in supporting people who are affected by such trauma or disease in their efforts to communicate or rehabilitate (Bock et al., 2015; Nijboer et al., 2008).

The continued discussion about potential causes and individual differences concerning reported changes, or lack thereof, of cognition and neurophysiological markers in relation to gravity shows an inadequate yet growing understanding of how the brain adapts to changing gravitational environments. Expanding upon neuroscientific studies conducted in gravitational environments helps to broaden our knowledge of cognitive and neurophysiological adaptation and BCI feasibility and improvement: as above so below, both in space and on Earth.

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Appendix I: The ESA Education Office Fly Your Thesis! 2017 Campaign

This study was part of the European Space Agency (ESA) Education Office Fly Your Thesis! 2017 Campaign. This is an annual student parabolic flight campaign that aims to foster cross-border collaborations, education, and knowledge-sharing among European countries. Specifically, it is a microgravity research campaign that provides students, usually as part of their master's or Ph.D. thesis, with the opportunity to design and execute a scientific experiment that is to be performed onboard an aircraft flying parabolic trajectories, also known as parabolas. The *Fly Your Thesis!* campaign is part of the ESA Education Office's broader initiative to promote space education and outreach, and to engage young people in the field of space science and technology (Duveaux-Béchon & Messina, 2002).

ESA and the ESA Education Office aim to advance a culture of collaboration, innovation, scientific interest, and accomplishment among young scientists and to provide them with the necessary experience in space research and technology. The selection process of their student research campaigns is very strict, and only a few teams per campaign are selected to participate each year. Team BrainFly was fortunate enough to be elected to participate in the 68th parabolic flight campaign (September 2016 – December 2017). The team consisted of four women from different Dutch universities (Evelien Lageweg, Danielle Trump, Anouk Schippers, and myself), at the time all enrolled in a master's program in the field of Neuroscience or Neuropsychology. We collaboratively conducted a neuroscientific parabolic flight study while simultaneously running our own individual experiments. The official two-week-long campaign took place in December 2017 in Bordeaux, France.

Appendix II: Descriptive Statistics

Table 2

Descriptive Statistics

Variable	**Mean**	**SD**	**N**
amp_0G_d.Pz	40,36	20	8
amp_0G_st.Pz	19,37	8,31	8
amp_1G_d.Pz	42,35	18,63	8
amp_1G_st.Pz	24,13	10,50	8
lat_0G_d.Pz	336,13	66,05	8
lat_0G_st.Pz	330,62	51,26	8
lat_1G_d.Pz	348,38	73,38	8
lat_1G_st.Pz	329,37	47,11	8
amp_0G_d.Fz	33,38	13,68	8
amp_0G_st.Fz	15,70	6,72	8
amp_1G_d.Fz	32,08	17,56	8
amp_1G_st.Fz	18,25	8,86	8
lat_0G_d.Fz	328,25	73,13	8
lat_0G_st.Fz	378,13	65,35	8
lat_1G_d.Fz	368,50	43,15	8
lat_1G_st.Fz	330,38	68,86	8
amp_0G_d.Cz	21,17	8,40	8
amp_0G_st.Cz	10,67	4,82	8
amp_1G_d.Cz	25,36	12,38	8
amp_1G_st.Cz	12,16	7,10	8

Grand averages (mean) and standard deviations (SD) of the P3 amplitude (amp) and latency (lat) in 1G and 0G for the deviant (d) and standard (st) stimuli over the Pz, Fz, and Cz electrodes.

Appendix III: Sponsor Overview

- European Space Agency
- The ESA Education Office / ESA Academy
- Novespace
- Universiteit Leiden
- Radboud Universiteit Nijmegen – Donders Instituut
- Universiteit Utrecht
- ANT Neuro
- Triple
- HE Space Operations
- Mat-Tech
- Panasonic
- WIA