**Methods**

**Study Design and Participants**

The current data were collected as part of a larger research project EEG Post-Covid (EPOC), which investigates neurophysiological parameters identified from neuropsychological paradigms using a high-resolution stationary laboratory EEG to reflect cognitive impairments and fatigue. The primary goal of the EPOC study is to find EEG parameters that can serve as neurophysiological markers for progression- and therapy-evaluation concerning cognitive functions in PCS.

Participants for the EPOC study were recruited from COVIDOM, a population-based, prospective multi-centre study to investigate PCS within the German National Pandemic Cohort Network (NAPKON). COVIDOM participants had been recruited through public health authorities in Kiel, Berlin, and Würzburg. Patients were assessed between November 15, 2020, and September 19, 2021, at University Medical Center Schleswig-Holstein, Campus Kiel, and University Hospital Würzburg in Germany (Bahmer et al., 2022; Horn et al., 2021; Schons et al., 2022).

The participants were included based on the following criteria: A polymerase chain reaction (PCR) confirmed SARS-CoV-2 infection at least 6 months before study visit, a primary residence in one of the three study regions, age ≥ 18 years at the time of recruitment (Berlin) or infection (Würzburg, Kiel). Participants with acute reinfection of SARS-CoV-2 at the time of the scheduled study visit were excluded (Horn et al., 2021).

In the EPOC study, a subset of individuals from Schleswig-Holstein who participated in COVIDOM was selected, constituting of those with PCS and a control group without PCS.

As EPOC is still ongoing at the time of writing, the analysis was conducted based on a preliminary subset of 79 participants (mean age 48.52, range 22–78, female 48, male 31, diverse 0, years of education mean 15.27 years min 9 to 24 years) with PCS (49 participants, age mean 50.29 years min 22–78, F=32 M=17, education: mean 15.04 9–23) and without PCS (30 participants, age mean 45.63 years min 22–77, f=16,m=14 d 0, education mean 15.63 min 10–24). The study was conducted at the University Medical Center Schleswig-Holstein (UKSH), Campus Kiel. Participants did not receive payment/financial compensation for their participation. Transportation and parking costs were reimbursed.

**Ethics statement**

The study was approved by the Ethics Committee of the medical faculty of the Christian-Albrechts-University of Kiel, Germany (record identification: D 446/23). In accordance with the Declaration of Helsinki, informed written consent was obtained from all participants.

**Procedure/Study Design**

In brief, the experiment consisted of neuropsychological tests, assessing cognitive domains such as working memory, attention, preprocessing speed, cognitive flexibility, executive functions, and multisensory integration, EEG recordings, and questionnaires assessing fatigue, sleep quality, depression, and anxiety.

Participants first filled out a questionnaire on demographic data (e.g., age, education) and psychological and neurological conditions, followed by neuropsychological testing to assess cognitive domains such as working memory, attention, preprocessing speed, cognitive flexibility, executive functions, and multisensory integration. The first test administered was the Trial Marking Test (TMT). Following this, the EEG cap was placed, and participants completed a series of other neuropsychological tests, starting with the redundant target effect (RTE), followed by an oddball paradigm, an n-back task, and lastly the psychomotor vigilance task (PVT). Electroencephalographic activity was recorded continuously throughout these tests. Finally, resting state was measured, 5 minutes with eyes open and 5 minutes with eyes closed. During EEG recordings, participants were seated comfortably and instructed to minimize movement, and to focus on a fixation cross displayed on the screen in front of them to reduce eye movements, while the light was turned off. After completing the resting state measurement, the EEG cap was removed, and participants filled out three questionnaires assessing fatigue (FACIT-F), sleep quality (PSQI), depression (HADS-D), and anxiety (HADS-A). The participants got then the chance to wash their scalp/hair. In all, the experiment took up to 3 hours.

Since this thesis focuses on behavioral data obtained from the TMT, n-back and PVT, as well as EEG resting state data, and data from the questionnaires, the RTE, and the oddball task will not be further explained. In addition, the MoCA score was measured in the previous COVIDOM study.

**Cognitive tasks**

**MoCA**

The Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005) is a widely used, validated screening tool originally designed to detect mild cognitive impairment (MCI) (Hauffe, 2024). It assesses several cognitive domains, including visuospatial skills/ability, executive function, naming, memory (short-term and delayed recall), working memory, attention and concentration, language, abstraction, and orientation (Freitas, Simões, Alves & Santana, 2013; Hobson, 2015; Kang et al., 2018; Nasreddine et al., 2005). The MoCA has a total possible score of 30 points, with a score of ≥ 26 considered normal (Nasreddine et al., 2005). Cognitive performance on the MoCA is influenced by sociodemographic factors such as age and education (Kang et al., 2018; Larouche et al., 2016). To account for educational background the MoCA test manual specifies that one additional point is added for individual with ≤ 12 years of formal education, allowing for a maximum score of 30 points (Nasreddine et al., 2005). Additionally, to address variations in performance related to age, normative data for the MoCA are available for precise interpretation of scores (Larouche et al., 2016).

**TMT Part A and B**

Originally, developed as part of the Army Individual Test Battery (AITB) in 1944, the Trail Marking Test (TMT) was later integrated into the Halstead-Reitan Battery (Reitan & Wolfson, 1985; Tombaugh, 2004). It is now one of the most popular and widely used neuropsychological assessments, included in most test batteries (Tombaugh, 2004). Its widespread use is supported by strong evidence of its validity (Arbuthnott & Frank, 2000; Sánchez-Cubillo et al., 2009). The TMT assesses cognitive processing speed and executive functioning (Lezak, 1995; Mitrushina et al., 2005; Reitan, 1992; Sánchez-Cubillo et al., 2009; Strauss et al., 2016; Tombaugh, 2004), as well as visual search/scanning, and mental flexibility (Sánchez-Cubillo et al., 2009; Tombaugh, 2004).

The TMT consists of two parts: Part A (TMT-A), a number-connection task, and Part B (TMT-B), a number-letter alternation task. Both parts were administered (in this study) according to the guidelines provided by Strauss et al. (2006).

In TMT-A, participants were instructed to connect consecutively numbered circles from 1 to 25 on an A4 page by drawing lines between them, aiming to complete the task as quickly and accurately as possible. In TMT-B, the task becomes more complex (Gaudino, Geisler & Squires, 1995). Participants were instructed to draw lines alternating between numbered circles from 1 to 13 and lettered circles from A to L in sequential order (e.g, 1 to A, to 2, to B, etc.) on an A4 page. The aim, again, was to complete the task as quickly and accurately as possible.

The administration of the TMT began with TMT-A, followed by TMT-B. For each part, participants were first given an example to familiarize themselves with the task. After completing the example, they proceeded to the actual test. If participants made a mistake, the experimenter immediately pointed it out, and the participant was required to correct it before continuing. The experimenter timed each part, with the time of completion for each part representing its direct score. In addition to the direct scores, the difference between TMT-B and TMT-A (TMT-B – TMT-A) was calculated.

**N-Back Part A and B**

The n-back task (Kirchner, 1958) has become a widely used tool in neuroscience for assessing working memory (Jaeggi, Buschkuehl, Perrig &Meier, 2010; Pelegrina et al., 2015). N-back tasks are continuous-recognition measures, that present sequences of stimuli (Kane, Conway, Miura & Colflesh, 2007). In these tasks, participants must determine whether a given stimulus matches one that was presented “n” trials before. Reliability of the n-back task varies across studies, with more complex levels (e.g., 2-back, 3-back) generally yielding higher reliability coefficients (Jaeggi et al., 2010; Pelegrina et al., 2015).

In this study, participants completed two blocks of the n-back task: A 1-back task followed by a 2-back task, with a pause between blocks during which the instructor provided additional instructions before participants proceeded to the second block. The task was programmed using … and presented on a 27-inch computer screen.

In both conditions, participants were shown a series of 60 linguistic stimuli, consisting of 16 different consonants (B, C, D, F, G, H, J, K, M, Q, R, S, T, V, X, Z) presented individually in the center of the screen. Each block contained 20 target trials and 40 non-target trials. A trial began with a 250 ms fixation period (a red dot was shown on screen, for the participant to fixate), followed by a 150 ms black screen. The stimulus letter then appeared for 500 ms, succeeded by a variable inter-trial interval of 180 to 220 ms (black screen). Total trial duration ranged from 1080 to 1120 ms.

For the 1-back task, participants were instructed to press the spacebar when the current letter matched the previous one. For example, in the sequence “B, C, C, D,” participants were supposed to respond to the second “C” as it matches the previous letter. In the 2-back task, they were instructed to press the spacebar when the current letter matched the letter presented two trials prior. For instance, in the sequence “B, B, D, F, D,” participants should press the spacebar when the second “D” occurred, as it matches the letter presented two trials before. The response window was limited to the 500 ms stimulus presentation period. Reaction time, hits, misses, and false alarms were recorded. In total, the experiment took around 5 minutes. The light was turned off during the experiment.

**PVT**

The Psychomotor Vigilance Task (PVT) is a widely used reaction time test developed in 1985 to assess sustained attention, particularly in contexts involving fatigue and sleep deprivation (Drummond et al., 2005). It has been shown to be sensitive to sleepiness in clinical and experimental settings (Molina, Sanabria, Jung & Correa, 2019).

The key feature of the PVT is its monotonous and unpredictable target presentation which makes participants highly prone to lapses of attention. This unpredictability minimizes learning effects, ensuring that performance remains largely independent of prior abilities and experience (Basner and Dinges, 2011) (stolen from Molina et al., 2019). Reaction time measured by the PVT has been linked to cognitive function in both healthy subjects and patients, supporting its validity as an assessment tool (Jakobsen, Sorensen, Rask, Jensen & Kondrup, 2011).

This study employed a 5-minute version of the PVT, which has been established as a valid alternative to the traditional 10-minute PVT-192 (for assessing fatigue) (Lamond et al., 2008).

The dynamic stimulus appeared as a red number, counting up in milliseconds, representing the participant’s reaction time. Participants were instructed to respond immediately, when the stimulus/red number occurred, by pressing the spacebar. Between trials, a white fixation cross was displayed on a black screen for a variable interval ranging from 2 to 10 seconds. Participants were required to maintain their gaze on this fixation cross. Each trial concluded when a response was made. Following each response, the participant’s reaction time was displayed on the screen for 50 ms as feedback before the next trial began.

After receiving instructions, participants underwent a training block of 8 trials to familiarize themselves with the task. Following the training, participants proceeded to the main experiment, which consisted of 50 stimulus presentations. The light was turned off during the experiment.

**Resting state**

Do I even need to write something here?

Maybe to investigate delta and beta frequency eyes closed condition and why?

**Questionnaires**

**PSQI**

The Pittsburgh Sleep Quality Index (PSQI), developed by Buysse, Reynolds, Monk, Berman, and Kupfer in 1988 is a self-rated questionnaire that assesses sleep quality and disturbance over the past month. The PSQI is the most commonly used sleep health assessment tool in both clinical and research settings. Its reliability and validity have been consistently demonstrated in multiple studies (e.g. Carpenter & Andrykowski, 1998; Manzar et al., 2018; Mollayeva et al., 2016). The questionnaire consists of 24 items in total, 19 of which are self-reported by the patient and 5 of which require input from a room or bed partner. Only the 19 self-reported items are used for the quantitative evaluation of sleep quality, as perceived by the patient (Buysse et al., 1989; Manzar et al., 2018). The response formats across the items vary, including the recording of usual bed and wake times, number of hours slept, minutes taken to fall asleep, as well as forced-choice Likert-type responses (Buysse et al., 1989). The items are categorized into seven components, which are sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications, and daytime dysfunction, for each component given a score. Together, these component scores generate a global sleep quality score ranging from 0 to 21, with scores >5 indicating poor sleep quality (Buysse et al., 1989; Hinz et al., 2017).

**HADS**

The self-assessment Hospital Anxiety and Depression Scale (HADS) was originally developed by Zigmond & Snaith in 1983 to identify the presence of anxiety and depression states among patients in non-psychiatric hospital clinics. HADS is an extensively used, reliable, and valid instrument to measure anxiety and depression, not only in psychiatric, and clinical patients (Herrmann, 1997) but in general populations (Bjelland, Dahl, Haug & Neckelmann, 2002; Herrero et al., 2003; Spinhoven et al., 1997). The questionnaire assesses anxiety and depression symptoms during the past week, excluding symptoms also related to physical disorders, e.g., headache, dizziness, or insomnia (Bjelland et al., 2002; Hinz & Braehler, 2011; Zigmond & Snaith, 1983). The scale consists of 14 items, divided into a 7-item anxiety (HADS-A), and a 7-item depression subscale (HADS-D). Both subscales are rated on a four-point Likert scale, giving subscale scores ranging from 0 to 21 (Zigmond & Snaith, 1983). There is no universally accepted cut-off score for the HADS (Herrero et al., 2003; Spinhoven et al., 1997). In this study, the cut-off point was set to eight, indicating elevated/caseness anxiety and depression for scores ≥8, following the recommendations by Zigmond and Snaith (1983), as well as Bjelland et al. (2002) and Herrero et al. (2003).

In this study the German Version (HADS-D) of the scale was used.

**FACIT-F**

The 13-item Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue Scale (FACIT-F; Version 4) was used to assess self-reported fatigue and its impact on daily activities and functions (Cella, Lai, Chang, Peterman & Slavin, 2002; Yellen et al., 1997) during the last 7 days. While it was originally developed for cancer-related fatigue (Cella et al., 2002; Yellen et al., 1997), it has been shown, that the FACIT-F is a reliable and valid measure of fatigue across various health conditions (Cella et al., 2002), making it a widely used tool for both clinical practice and research (Cella et al., 2022; Butt et al., 2013; Montan, Löwe, Cella, Mehnert & Hinz, 2018; Tinsley, Macklin, Korzenik & Sands, 2011). This questionnaire utilizes a five-point Likert scale, with total scores ranging from 0 (severe fatigue) to 52 (no fatigue). Based on general population data, scores ≤30 indicate clinically significant fatigue (Piper & Cella, 2010).

**Cluster Analysis**

To classify individuals into two distinct groups based on objective cognitive assessment, a cluster analysis was conducted. The cluster analysis is an exploratory statistical method that organizes objects, data points, or observations into homogeneous groups, known as clusters, based on similarities (Ketchen & Shook, 1996). The objective is to maximize intragroup homogeneity while ensuring high intergroup heterogeneity (Bacher, Pöge & Wenzig, 2010; Backhaus, Erichson, Gensler, Weiber & Weiber, 2011).

In this study, cognitive performance data from the MoCA, TMT, n-back task and PVT were used as cluster variables. The goal was to identify two distinct clusters with significantly different cognitive performance levels, suggesting that one group exhibits superior performance compared to the other.

**Data Preprocessing.** All participant data was imported from a TSV file (participants.tsv) into R (using read.delim()), where preprocessing and analysis of the data was conducted. Specific variables of interest were selected and stored as subset including demographic information, cognitive test scores and clinical/questionnaire measures. The MoCA variable was converted to a binary variable: Scores ≤25, indicating cognitive impairment, were coded as 1, while scores >25, indicating no impairment, were coded as 0. For the n-back task, missing values were assigned if a participant reported not understanding the task. This applied to five participants. Missing values in the relevant cognitive test variables (PVT reaction time, n-back miss 1, n-back miss 2, TMT-A time, TMT-B time) were identified using is.na(). To ensure complete cases for analysis, rows with missing values in those key cognitive variables were removed using drop\_na(). As a result nine rows were deleted, leaving a final dataset of 70 participants.

To ensure the reliability of the cluster analysis, it is essential to address outliers, as they can significantly impact the result by distorting the clustering process, obscuring underlying patterns, and introducing bias (Backhaus et al., 2011; Wentura & Pospeschill, 2015). To mitigate these effects, winsorizing was applied, to replace outliers by capping extreme values beyond 1.5 times the interquartile range (IQR) for the relevant cognitive variables. A custom function, winsorize\_variable(), was implemented to replace these outliers with the nearest non-outlier values (). Winsorized versions of variables were created with suffix. In total, four outliers were detected and winsorized for PVT reaction time, two for TMT-A time, and four for TMT-B time.

A new variable, TMT\_diff , was calculated as the difference between TMT B and A (TMT-B – TMT-A).

**Variable transformation**

To account for the influence of age on cognitive performance, participants were categorized into four distinct age groups, and z-scores were calculated within each group to adjust the data accordingly. The Age groups were adapted from the TMT norms (Tombaught, 2004; Strauss, Sherman & Spreen, 2006),which originally defined nine different age groups. However, due to limited number of participants, the groups were adjusted to ensure sufficient sample size while maintaining the assumption that cognitive performance gets worse with increasing age. For TMT\_diff, an alternative age grouping was applied, as it fit the data better. After the age groups were created, the mean and standard deviation of each cognitive variable (PVT and TMTa, TMTb and TMT difference) were computed within each group (Table 2). The function calculate\_z\_scores\_individual() was then applied to compute z-scores for each participant based on age group norms within this study, adjusting for age-related differences in cognitive performance. The resulting variables were labeled with the suffix.

Since no clear age-related trend was observed in the n-back miss scores (miss 1 and miss 2) (appendix table…), z-scores were not calculated for these variables. Instead, standardization was applied using the scale() function to ensure comparability across measures, creating new variables with suffix.

Table 2

*Age groups and*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | PVT (ms) | | TMT-A (s) | | TMT-B (s) | |
| Age group | *n* | *M* | *SD* | *M* | *SD* | *M* | *SD* |
| 18 – 34 years | 12 | .3166 | .0529 | 18.2 | 3.9 | 39.94 | 11.8 |
| 35 – 49 years | 19 | .3247 | .058 | 24.54 | 7.09 | 45.92 | 10.54 |
| 50 – 64 years | 33 | .3278 | .0543 | 27.14 | 7.48 | 59.02 | 16.16 |
| 65 – 80 years | 6 | .3337 | .0177 | 34.57 | 9.97 | 65.13 | 21.3 |

The final cleaned and preprocessed dataset was saved for further use as a file named “clean\_data.Rdata” in the cluster analysis.

**Hierarchical cluster analysis.**

As mentioned above, cluster analysis is primarily an exploratory method, meaning that it does not follow a straightforward, single-step procedure but rather involves a multi-stage process, with each step depends on the outcome of the previous one (Bacher & Wenzig, 2010). Consequently, the analysis and interpretation of results may require revisiting certain steps, particularly when the initial outcomes do not allow for a meaningful interpretation (Backhaus et al., 2011). The goal was to identify the best possible solution for the dataset.

Initially, both a hierarchical cluster analysis approach and k-means clustering were performed on the preprocessed data to identify clusters among participants. However, hierarchical clustering was chosen for reporting and further analysis as it provided a clearer and more interpretable classification of participants (see Appendix for k-means clustering results).

**Selection of variables**

It is generally impossible to predict in advance which combination of variables, similarity measures, and clustering techniques will result in measningful and informative classifications. Therefore, different combinations of variables were tested, to find the best possible solution. Initially, the the standardized variables PVT reaction time, TMT A, TMT B, n-back miss 1 and n-back miss 2 were used as cluster variables. TMT\_diff and MoCA were excluded from the clustering process, as cluster variables should meet specific criteria, including relevance for grouping, measurability (ensured by standardizing the test data), representativeness, and independence (Bacher & Wenzig, 2010; Backhaus et al., 2011; Everitt et al., 2011; Wentura & Pospeschill, 2015). TMT\_diff was excluded due to its lack of independence, as it is highly correlated with TMT-A and TMT-B. However TMT-A, TMT-B and PVT are also correlated. This raises the question of whether two of them should have been excluded as well. If varibales are highly correlated, they may introduce implicit weighting, leading to biased clustering results (cite). However, as all three variables are similarly correlated and all measures of cognition, the impact of their dependancy might be negligible. Cluster results with different variable combinations indicated that including all three produced the most promising one. The binary MoCA variable was not included as it does not provide enough information to be relevant for cluszering. Moreover, the goal of this study was to investigate different performance levels in objective cognitive task, whereas MoCA already categorically determines whether a person has cognitive impairment or not. Therefore, it is of more interest to compare the clusters based on MoCA scores to assess how well MoCA detects cognitive difficulties in this study population.

The inclusion of the two n-back variables resulted in poor clustering outcomes, as overall performance on these tasks (esspecially on the 2-back task) was generally low across participants. Consequently, these variables were excluded from further analysis.

**Clustering Methodology**

The next step (remember, that the is no strict order) was to determine an appropriate proximity measure and clustering algorithm.

**Choosing of Proximity measure**

The starting point of the cluster analysis is a raw data matrix containing *N* objects (in this case, 70 participants) described by *J* variables (PVT, TMT-A and TMT-B). To quantify the relationships between these objects, a statistical measure is required to transforme the data into a distance or similarity matrix, which provides a numerical representation of how similar or dissimilar the objects are. Similarity measures reflect the degree of resemblance between two objects, with higher values indicating greater similarity. In contrast, distance measures capture dissimilarity, where larger values signify greater differences between objects. If two objects are identical, the distance between them is zero.

In this study, a distance matrix, more specific, Euclidean distance was chosen. The resulting distance matrix served as the basis for the clustering algorithm.

**Cluster algorithm**

Ward’s method was selected for clustering, as it is widely used in practice and known for its effectiveness in identifying distinct clusters (Backhaus et al., 2011). It is considered a reliable algorithm, provided that the variables are on a metric scale, are uncorrelated, and do not contain outliers (Wentura & Pospeschill, 2015). Ward’s method is considered as an conservative approach, meaning it tends to form clusters of similar size.

The method follows an iterative merging process, where clusters are combined step by step in a way that minimizes the increase in overall variance. The process can be described as follows:

1. The mean values of all variables are calculated for each cluster. At the beginning, each object is its own cluster.
2. The squared Euclidean distance of each object in a cluster is calculated from the cluster mean.
3. The calculated distance of each object to its respective cluster mean is summed across all objects.
4. The two clusters that, when combined, result in the smallest increase in the total sum of the squared distances are merged into a new cluster.
5. Step 2 and 3 are repeated until all objects are merged into a single cluster. Note, in hierarchical clustering, once a cluster is formed, it cannot be split during the merging process, unlike in k-means clustering, where clusters can be reassigned in each iteration.

**Number of clusters**

In my case maybe more a “konfirmatorische Clusteranalyse” (Bacher & Wenzi, 2010) as the number of clusters ist bestimmt (von mir). "Korrekte" Bestimmung der Clustervariablen entscheidet darüber, wie gut die Ergebnisse einer Clusteranalyse später genutzt werden können.

Created desired number of clusters. Cut\_mean <- cutree (hclust\_median, k = 2) In consideration of the research question and the two groups, withPCS and withoutPCS, a two-cluster solution was chosen. K-means/elbow measure suggested 4 clusters. Therefore a 4-cluster solution was also looked at. As validation, the 2-cluster solution was compared to their self repoRTEd group assignments (withPCS or withoutPCS. Do I here need to mention, how many people are in which cluster? Or is that already result? To visualize cluster on dendrogram abline function used. Stability tested, different proximity measures have been used. But with euclidean best result. Also different algorithms have been tested. Also non-hierarchical clustering was compared to the hierarchical clustering (k-means). Why did I do the analysis with ward and not with k-means? What was my decision there? For the selected number of cluster, three additional analyses were performed using the complete, single, and weighted-average linkage methods. The agreement(Übereinstimmung) was assessed using the adjusted Rand index (Hubert and Arabie, 1985). Change of algorithm and alteration of number (which numbers where tested?) of clusters was variated. For adjusted rand index: library(fossil) adjusted rand index calculated with rand.index function.

PVT reaction time, TMT a, TMT b, TMT b-a, MoCA, and n-back scores were included. Euclidean distance was used as a distance matrix, and Ward’s method was selected for clustering, as it is widely used in practice and known for its effectiveness in identifying distinct clusters (Backhaus et al., 2011). It is considered a reliable algorithm, provided that the variables are on a metric scale, are uncorrelated, and do not contain outliers (Wentura & Pospeschill, 2015). As stated earlier, cluster analysis is an explorative method used to identify patterns in data. However, in this study, the approach is only semi-exploratory, as the number of clusters to be generated was predetermined based on prior knowledge. A two-cluster solution was explored, as that aligns with the self-reported groups (with PCS, and without PCS), thereby allowing for good comparisons between the cluster solution and the self-reported groups. One could also consider this as a confirmatory cluster analysis (Bacher et al., 2010). The stability of the clusters was tested by comparing different proximity measures and algorithms using the adjusted Rand index (Hubert & Arabie, 1985). **Verwendete Kriterien zur Bestimmung der Clusteranzahl**

Inhaltlich, da zwei verschiedene Gruppen. Later 4 because of k-means

**DurchgefühRTE Stabilitätsprüfung**

Für ausgewählze Clusteranzahl noch drei weitere Analysen mit dem Complete-, Single- und Weighted-Average-Linkage gerechnet. Die Übereinstimmung wurde mittels des adjustieRTEn Randindex (Hubert und Arabie 1985) beuRTEilt.

Auch: Wechsel des Algorithmus und Veränderung Gruppenzahl (Ein Cluster bleibt gleich)

**DurchgefühRTE Validitätsprüfung**

Zur Validitätsprüfung wurde auf Variablen Z1, Z2 usw. zurückgeriffen

**Statistical analysis.** The two clusters were compared in several aspects. First, the two clusters were compared in their cognitive performance levels to validate whether significant differences exist between clusters. Clusters were then compared across demographic variables and results in questionnaires. Of particular interest was to examine how those two clusters differ from or align with the self-reported perception of cognitive performance level. To investigate differences between objective and subjective cognitive performance levels, comparisons occurred not only between two clusters but also within the clusters between the subjective groups with PCS and without PCS. Additionally, to maximize the insights from the cluster analysis, the with PCS groups in cluster 1 was compared to the with PCS group in cluster 2, and similarly for the without PCS groups. A t-test was used for these comparisons. Effect size and cohens d were also compared (need to check why)

The clusters were compared in several expects with each other. In demographical variables (sex, age, and years of education), in the used variables for cluster analysis. But also in their other cognitive variables (PVT, TMT, n-back, MoCa). Also results in the scores from questionnaires were compared. Not only were the two groups compared between each other, but also within comparison took place. WithPCS and withoutPCS within one cluster were compared. Also withPCS and withoutPCS were compared between clusters (that means, withPCS in Cluster 1 was compared to withPCS in Cluster 2 to clarify). All comparisons were tested by t-test. T-test robust to….. Data is not normal distributed. That was tested by… cat function was used.

The two clusters where compared

Alongside the comparisons of demographic, cognitive data, and questionnaire results, the clusters were also examined for their EEG resting state patterns.

**EEG Recording and Analysis**

For each group (withPCS and withoutPCS), 5 minutes of resting state with eyes open and 5 minutes of resting state with eyes closed were recorded using high-density EEG. Since the eyes-closed condition represents a simple, standardized procedure (Babiloni et al., 2016), it is the most commonly used (Babiloni et al., 2022) and will therefore be analyzed in this study to ensure comparability. EEG signals were recorded using a 128-channel EEG cap (128Ch Standard Brain Cap for actiCHamp Plus, Easycap GmbH, Wörthsee, Germany) with electrodes positioned in an equidistant layout, connected to an actiCHamp Plus Amplifier (Brain Products GmbH, Gilching, Germany).

The sampling rate was 1000 Hz with an amplitude resolution of 0.1 µV. Electrolyte gel was applied to improve conductivity between skin and electrodes, ensuring impedances remained below 20 kΩ. Eye movements and changes in the resting potential of the retina (EOG activity) were monitored using two EOG electrodes placed below each eye, with impedances also maintained below 20 kΩ. In addition, a ground electrode was positioned on the forehead, and a reference electrode was positioned on the tip of the nose. Impedances for both the reference and ground electrode were kept below 5 kΩ.

**Preprocessing**

Data preprocessing/analysis was performed using the FieldTrip toolbox (Fieldtrip-20240504; Oostenveld, Fries, Maris & Schoffelen, 2011) and the EEGLab toolbox (v2024.0; Delorme & Makeig, 2004) in Matlab (v24.1.0.2578822 (R2024a) Mathworks Inc., 2024, MathWorks® https://de.mathworks.com) on Windows.

The participants' EEG data were organized in BIDS (Brain Imaging Data Structure) format (Gorgolewski et al., 2016; Pernet et al., 2019). BIDS is a community standard that ensures homogeneity in the organization and description of raw neurocognitive/brain-derived/neuroscientific data, enabling efficient data sharing, minimizing errors, and supporting completely automated analysis workflows (Gorgolewski et al., 2016; Pernet et al., 2019; Truong, Robbins, Delmore & Makeig, 2023). The rsEEG data, organized according to this standard, were identified and imported into MATLAB using the FieldTrip Toolbox. A trial defining function was built to select the data from the eyes-open condition for subsequent processing. This resulted in approximately 300 s per participant.

**Filtering and Resampling**

A finite impulse response (FIR) windowed-sinc (firws) filter, designed with a hamming windowed sinc function and implemented in the FieldTrip toolbox, was used for both high-pass and low-pass filtering of the continuous data. For high-pass filtering, a cut-off frequency of 0.1 Hz was applied to eliminate very low frequencies (drift) (Keil et al., 2013). This cut-off was based on the findings of Delorme (2023) and Winkler, Debener, Müller and Tangermann (2015), where filtering at 0.1 Hz or higher significantly improved data quality compared to no filtering. Filters above 0.1 were not used due to….

Prior to applying low-pass filtering, the data was downsampled from 1000 Hz to 250 Hz, to reduce computational load while preserving sufficient temporal resolution for subsequent analysis. A cut-off frequency of 45 Hz was then used to eliminate high-frequency noise and mitigate potential 50 Hz line noise (Delorme, 2023). Finally, the data underwent re-referencing using the Common Average Reference (CAR) technique to remove the influence of the reference and improve signal quality (Ludwig et al., 2009). As the name implies, an average of the recordings from all electrode sites was computed and used as the reference (Ludwig et al., 2009; Offner, 1950). The function reref() was used (to perform this step). The data was converted into the EEGLAB data structure for further processing.

Due to empty dataset from one participant, the participant was excluded, leaving the dataset with 69 participants (something like that. But where should I write that?)

**Artifact removal**

After the initial filtering and resampling, the preprocessing pipeline continued with detecting and removing artifacts. First, large artifacts, including the removal of flat-line channels, noisy channels, and short-time bursts of noise, were removed from the data using the EEGLAB pop\_clean\_rawdata() function with specific parameters. Channels with flat lines for more than 5 seconds were removed (FlatlineCriterion = 5), based on the default recommendation (for this parameter) by Pernet et al. (2021). This ensured the exclusion of “dead” or disconnected channels, thereby improving data quality. Channels were further excluded if their signal could not be predicted from a randomly selected subset of the remaining channels for at least 85% of the recording time (ChannelCriterion = 0.85), to remove those that were highly dissimilar from the rest of the channels (Gil Ávila et al., 2023; Pernet et al., 2021). The euclidean distance metric was used to calculate the similarity between channels. Data segments with abnormally high amplitude bursts, exceeding 100 SD compared to neighboring segments, were eliminated (BurstCriterion = 100), as such extreme bursts are considered unlikely to reflect brain signals (Chang et al., 2018). The default BurstCriterion is set to 20, but it may be adjusted if the default setting results in rejecting too many data segments. Some scientist recommend setting the threshold to 100 (EEGLAB, "Automated Pipeline Tutorial", 2024), which aligns with the optimal cut-off range of 10 to 100 suggested by Chang et al. (2018). Therefore, a mild threshold of 100 was chosen here, as it still effectively removes large-amplitude artifacts while retaining valuable data (Chang et al., 2018). Time windows where more than 40% of the channels were marked as noisy were removed (WindowCriterion = 0.4), to ensure the quality of the remaining data. A more lenient threshold of 0.4 was chosen over the default of 0.25 to retain more data (even if it is potentially noisier). How many “bad” (excessively noisy) channels were detected or removed in this process?

Again the data is re-referenced to the average reference (CAR), this time using the EEGLAB function pop\_reref() (Gil Ávila et al., 2023).

Secondly, Independent Component Analysis (ICA) (Bell and Sejnowski, 1995; Hyvärinen, 2013, Jung et al., 2000; Lee et al., 1999; Palmer et al., 2008) was performed on the data, to detect and reject further artifacts, such as eye or muscle movements (Makeig et al., 1995). ICA was performed with the “runica” algorithm and function pop\_runica() with the extended InfoMax method. The runica algorithm was employed with the extended InfoMax method. using the pop\_runica function implemented in EEGLAB. To avoid rank deficiency, the number of components was set to one less than the total number of channels (Kim, Luo, Chu, Cannard, Hoffman & Miyakoshi, 2023). This approach decomposes the EEG signal into independent components, potentially separating artifacts from neural activity. Due to the non-deterministic nature of the ICA algorithm, its results vary across repetitions. That is, every repetition of the ICA algorithm leads to small differences in the reconstructed time series after removing artifactual components (Gil Ávila et al., 2023). The resulting ICA weights, which represent the transformation matrix for this decomposition, were saved in a separate file.

Automatic component rejection was implemented using ICALabel (Pion-Tonachini, Kreutz-Delgado & Makeig, 2019), as automatic artifact rejection is preferred over the manual one to ensure standardization (Miljevic et al., 2022). Artifactual components are automatically classified by the ICLabel classifier (Pion-Tonachini et al., 2019). Thresholds were set at probabilities of 0.8 (80%) for muscle-related components (Pernet et al., 2021) and 0.5 (50%) for eye-related components. Components exceeding these thresholds were flagged and automatically removed using the EEGLAB function pop\_subcomp(). By default, only components whose probability of being “muscle” is higher than 80% were subtracted from the data (Pernet et al., 2021). The two EOG channels (31 and 32) were removed from the dataset. The cleaned dataset was then checked for consistency using eeg\_checkset().

After this steps, an average of 110.3 good channels remained in Cluster 1 (*SD* = 11.4, Range = 75 - 125) and 109 good channels in Cluster 2 (*SD* = 11, Range = 78 - 124) in the two-cluster solution. This corresponds to approximately 85-86% good channels in both groups.

In the four-cluster solution an average of **110.8 good channels remained** in **Cluster 1** (SD = 11.84, range = 75–125), **109 good channels** in **Cluster 2** (SD = 11.03, range = 78–124), **108.6 good channels** in **Cluster 3** (SD = 12.19, range = 77–122), and **113 good channels** in **Cluster 4** (SD = 6.64, range = 105–123) after ICA. This corresponds to approximately 85-88% good channels.

Thirdly and finally, an additional artifacts removal step was implemented to address any remaining problematic channels. This process involved a statistical approach to identify outlier channels based on their signal characteristics. The standard deviation and mean were calculated for each channel across all time points. Then, overall mean values for these standard deviations and means were computed across all channels. Thresholds were established at 2.5 standard deviations above and below the overall mean, creating an acceptable range for channel activity. Channels with standard deviations falling outside this range were identified as outliers. These outlier channels were then removed from the dataset using the EEGLAB function pop\_select(), further refining the EEG data quality. This step ensures that channels with unusually high or low variability, which might represent persistent artifacts or malfunctioning electrodes, are excluded from subsequent analyses. As a result, the dataset retained an average 108 good channels in Cluster 1 (*SD* = 11.2, Range = 73 - 121) and 106.6 good channels in Cluster 2 (*SD* = 10.9, Range = 77 - 121) in the two-cluster solution. This corresponds to approximately 83-84% good channels in both groups.

In the four-cluster solution an average of **108.4 good channels remained** in **Cluster 1** (SD = 11.46, range = 73–121), **106.6 good channels** in **Cluster 2** (SD = 10.92, range = 77–121), **106.3 good channels** in **Cluster 3** (SD = 12.17, range = 74–120), and **111.1 good channels** in **Cluster 4** (SD = 6.20, range = 104–121). This corresponds to approximately 83-87% good channels.

**Interpolate bad channels**

Channels removed in the previous step were interpolated using the EEGLAB function pop\_interp() with the default spherical splines method (Perrin, Pernier, Bertrand & Echallier, 1989), ensuring a consistent number of channels across participants (Gil Ávila et al., 2023). Interpolated channels were inserted into the original channel order. On average 16-17% of the channels in each group were interpolated.

**Epoch length and number**

Lastly, the continuous data were segmented into epochs using the eeg\_regepochs() function implemented in the EEGLAB toolbox, which defaults to 2-second epochs (Gil Ávila et al., 2023). However, longer epochs improve frequency resolution, making them preferable for slower frequencies like delta. To achieve higher resolution while maintaining an adequate trial count, EEG data for each participant were segmented into 5-second nonoverlapping epochs. This function then outputs the new epoch EEG as a dataset on EEGLAB (Bonello, Garg, Garg & Audu, 2018).

The preprocessing resulted in an average of 37.7 good epochs (SD = 14.7, Range = 4 – 60) in Cluster 1, 37.9 good epochs (SD = 16.6, Range = 3 – 59) in Cluster 2 in the two-cluster solution. A two-sided *t-*test did not indicate a significant differences in epoch number between groups, t = -0,0585, p = .95. In the four-cluster solution, the dataset retained an average of **37.6 good epochs** (SD = 16.2, range = 4–58) in **Cluster 1, 37.9 good epochs** (SD = 16.6, range = 3–59) in **Cluster 2, 35.9 good epochs** (SD = 14.5, range = 9–60) in **Cluster 3**, and **42.0 good epochs** (SD = 12.5, range = 24–60) in **Cluster 4**.

**Power Analysis**

Preprocessed EEG data were converted from EEGLAB format to FieldTrip format using the eeglab2fieldtrip function. A common method for characterizing rsEEG is to decompose oscillatory signal into spectral power across distinct frequency bands (Babiloni et al., 2016; Perez et al., 2024). Spectral power reflects the distribution of neural activity at specific frequencies and is associated with various cognitive processes (Babiloni et al., 2016; Perez et al., 2024; Ward, 2003). Spectral parameterization was performed using SpecParam (formerly FOOOF, Fitting Oscillations & One Over F; Donoghue et al., 2020), which is implemented in the Brainstorm Toolbox (Tadel et al., 2011) and available in FieldTrip. This approach separates the periodic and aperiodic components of the power spectrum.

Since changes in absolute power may not solely reflect true neural activity but could also result from shifts in the aperiodic exponent, broadband offset, or frequency center (Donoghue et al., 2020), this analysis focused on relative power while also examining the aperiodic exponent and offset to account for these cofounding factors.

Spectral analysis of relative power across the 128 scalp electrodes was conducted using FieldTrips’s multitaper spectral estimation with Hanning taper, analyzing frequencies between 0.3 and 30 Hz with a frequency resolution of 0.2 Hz. The fooof output was set to a fixed aperiodic mode. To obtain relative delta and beta power, the aperiodic components were substracted from the original power spectra. Delta power was defined as 0.6-4 Hz, and beta power as 14-30 Hz. The summed power across all frequencies within each band was used to compute the relative power per channel.

Once the relative power per channel was computed, the data were transferred to R Studio (…) for fruther analysis using R Statistical Software (….). To identify and remove extreme values, an initial outlier detection was performed. For each participant, channels exceeding ±3 SD from the mean relative power were excluded. This process was applied seperately for delta power, beta power, aperiodic exponent, and aperiodic offset. Starting with … channels per cluster, this step retained ..% of channels in Cluster 1 and …% of channels in Cluster 2 for delta power, …% for beta power, …% for the aperiodic exponent, and ….% (Cluster 1) and …% (Cluster 2) for the aperiodic offset. Further outlier removal was considered. André (2022) advocates for a hypothesis-blind approach, where outliers are removed across clusters rather than within them. However, Karch (2023) questions this method, suggesting that extreme values should either be corrected or removed and that statistical methods less sensitive to outliers, such as a sign-rank test, may be more appropriate. For delta and beta power, an outlier removal approach across clusters was used, retaining X% of channels in cluster 1 and X% in cluster 2 for delta power, and X% in both clusters for beta power. A comparison between within-cluster and across-cluster outlier removal showed that the choice of method did not influence the significance of the results.

EEG power values are inherently non-negative; however, when using the **FOOOF/SpecParam** method, negative power values can sometimes occur. To ensure meaningful relative power estimates, these negative values were set to zero. Specifically, **X%** of channels in **Cluster 1** and **X%** in **Cluster 2** had negative values for **delta power**, while for **beta power**, the proportion was considerably smaller, at **X%** and **X%**, respectively. Removing these values may reduce the overall variance in the data.

**Statistical Analysis**

T-test for two group comparing. For four groups different test.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Age group |  | PVT (ms) | | TMT-A (s) | | TMT-B (s) | | TMT\_diff (s) | | Nback\_miss1 | | Nback\_miss2 | |
| *n* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* |
| 18 – 34 | 12 | .3166 | .0529 | 18.2 | 3.9 | 39.94 | 11.8 | 21.73 | 9.91 | 6.75 | 4.41 | 11.25 | 4.45 |
| 35 – 49 | 19 | .3247 | .058 | 24.54 | 7.09 | 45.92 | 10.54 | 21.38 | 6.4 | 8.79 | 3.58 | 14.21 | 3.29 |
| 50 – 64 | 33 | .3278 | .0543 | 27.14 | 7.48 | 59.02 | 16.16 | 31.88 | 14.03 | 10.06 | 6.61 | 13.61 | 4.46 |
| 65 – 80 | 6 | .3337 | .0177 | 34.57 | 9.97 | 65.13 | 21.3 | 30.56 | 17.99 | 8.83 | 4.22 | 15.17 | 4.92 |