



Master thesis submitted in order to be awarded the Master's Degree in
Biomedical Engineering

Characterising neurophysiological processes behind Information Processing Speed in Multiple Sclerosis

Olivier Daniel Burta

Academic year: 2023-2024

Promotor: Prof. Dr. Ir. Jeroen Van Schependom

Supervisor: Ir. Fahimeh Akbarian

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Remark on the master's dissertation and the oral presentation

This master's dissertation is part of an exam. Any comments formulated by the assessment committee during the oral presentation of the master's dissertation are not included in this text.

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Abstract

People with multiple sclerosis (PwMS) often suffer from cognitive impairment. The most widely affected cognitive domain, and one of the first deficits to emerge, is information processing speed (IPS). The Symbol Digit Modalities Test (SDMT), a standard test to capture IPS, has previously been analysed in MS using fMRI. Although this approach enables the analysis of brain network activations, its limited temporal resolution makes capturing highly dynamic brain activity impossible. This thesis uses a magnetoencephalography (MEG) dataset, with much higher temporal resolution, recorded during the SDMT in healthy controls and PwMS. The research goal is to provide the first description of the neurophysiological processes underlying an IPS task. To achieve this, both traditional static (event-related field, functional connectivity) and novel dynamic Time-Delay Embedded Hidden Markov Model (TDE-HMM) analysis techniques were employed. The TDE-HMM approach enables the extraction of data-driven functional brain networks that activate during the task. Static task-MEG analyses revealed significantly reduced spatial attention and visual function during the SDMT in PwMS compared to healthy controls. The TDE-HMM provided a 3-dimensional (temporal, spectral, and spatial) characterisation of different brain networks forming several components contributing to IPS: sensory speed (occipital state), cognitive speed (prefrontal executive state and visuospatial processing state), and motor speed (sensorimotor state). PwMS were found to have altered activation of the occipital and prefrontal executive states. This work forms the first step towards a more objective assessment of the cognitive speed component of IPS.

Keywords

Multiple Sclerosis, Information Processing Speed, Symbol Digit Modalities Test, MEG, TDE-HMM, dynamic functional networks

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Abstract – People with multiple sclerosis (PwMS) often suffer from cognitive impairment. The most widely affected cognitive domain, and one of the first deficits to emerge, is information processing speed (IPS). The Symbol Digit Modalities Test (SDMT), a standard test to capture IPS, has previously been analysed in MS using fMRI. Although this approach enables the analysis of brain network activations, its limited temporal resolution makes capturing highly dynamic brain activity impossible. This thesis uses a magnetoencephalography (MEG) dataset, with much higher temporal resolution, recorded during the SDMT in healthy controls and PwMS. The research goal is to provide the first description of the neurophysiological processes underlying an IPS task. To achieve this, both traditional static (event-related field, functional connectivity) and novel dynamic Time-Delay Embedded Hidden Markov Model (TDE-HMM) analysis techniques were employed. The TDE-HMM approach enables the extraction of data-driven functional brain networks that activate during the task. Static task-MEG analyses revealed significantly reduced spatial attention and visual function during the SDMT in PwMS compared to healthy controls. The TDE-HMM provided a 3-dimensional (temporal, spectral, and spatial) characterisation of different brain networks forming several components contributing to IPS: sensory speed (occipital state), cognitive speed (prefrontal executive state and visuospatial processing state), and motor speed (sensorimotor state). PwMS were found to have altered activation of the occipital and prefrontal executive states. This work forms the first step towards a more objective assessment of the cognitive speed component of IPS.

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I. INTRODUCTION

Multiple sclerosis (MS) is the most common neurodegenerative and -inflammatory disease of the central nervous system (CNS) [1], with up to 70% of people with MS (PwMS) being affected by cognitive impairment [2]. Among the cognitive domains that were shown to be affected in PwMS are: complex attention, efficiency and speed of information processing, and working memory [3]. However, information processing speed (IPS) stands out as a core cognitive deficit in PwMS [4]. IPS is the cognitive domain most widely affected by MS [5] and is one of the first deficits to emerge [6].

The standard cognitive test used to capture IPS deficits is the Symbol Digit Modalities Test (SDMT) [7],[8]. While in the past, the original test on paper was predominating,

nowadays the computerised version of the SDMT has become more widespread [9].

Over the last decade, researchers have turned to using a computerised SDMT as a task paradigm in functional neuroimaging studies. Only previously studied in functional MR imaging (fMRI), PwMS showed larger recruitment and more connections to frontal areas [10], and a decreased activation of the cingulate cortex during execution of the SDMT [11]. However, fMRI is not the optimal functional neuroimaging modality to study cognitive processes required during the SDMT. For instance, due to the nature of the haemodynamic response that is measured, fMRI captures information on a timescale of a few seconds. As a consequence, the exact neurophysiological processes underlying SDMT execution are currently unknown.

In this thesis, the goal is to provide the first description of the neurophysiological processes underlying an IPS task. By using a magnetoencephalography (MEG) dataset, the dynamic brain activity can be measured and studied at a much higher temporal resolution. Further, we hypothesise that this will enable us to uncover altered and likely slowed brain dynamics in PwMS as compared to healthy controls (HCs).

II. SDMT DATASET

A. Participants and data acquisition

The SDMT dataset contains neuroimaging data from HCs (N=37) and PwMS (N=73). This data was collected by the hosting research group between 2015-2018, with ethical approval from the National MS Center Melsbroek and the University Hospital Brussels. The demographic and clinical characteristics of the SDMT dataset are reported in Table 1.

As part of the data acquisition, first, an MEG was recorded while the subject performed a computerised SDMT paradigm. The MEG data were collected using a 306-channel Elekta Neuromag Vectorview system in the Laboratoire de Cartographie Fonctionnelle du Cerveau in Erasme Hospital (prof. Xavier de Tière, ULB) at a sampling rate of 1000 Hz. MEG recordings took place in a magnetically shielded room, and the headshape was saved during subject preparation (Polhemus digitiser).

Secondly, structural brain information for all subjects was collected through MRI (T1-weighted) using a 3T Philips MR system at 1x1x1 mm³ resolution.

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TABLE 1

Demographic and clinical characteristics of the entire SDMT dataset (mean $\pm$ std). Significant group differences (*p-value < 0.05) using a χ^2 test (dichotomous outcome variables) or a Mann-Whitney U-test (continuous outcome variables).

	Controls	PwMS	p-value
N	37	73	N/A
% Female	59.46	73.97	0.18
Age (years)	47.38 $\pm$ 11.82	48.17 $\pm$ 9.86	0.84
Education (years)	15.16 $\pm$ 2.22	13.79 $\pm$ 2.73	0.005*
SDMT score	54.51 $\pm$ 9.4	48.6 $\pm$ 10.74	0.007*
Disease dur. (years)	N/A	16.66 $\pm$ 9.78	N/A
EDSS	N/A	3.22 $\pm$ 1.49	N/A

B. Task design

The SDMT was employed as a task paradigm during MEG data collection. A projector is used to permanently display a reference table containing nine symbol-digit matches. Then, a first candidate symbol-digit combo is shown, which corresponds to a single trial. The subject then has at most 6 seconds to provide a response by pressing one of two buttons (correct/incorrect), indicating whether the shown combo is the same as in the reference table. This is repeated for a large amount of trials per subject (N=128).

In contrast to the original on-paper SDMT [12], this SDMT adaptation shows one stimulus at a time. The advantage of this adaptation is that each trial can be precisely isolated in time, facilitating the study of task-related brain activity.

C. Data preprocessing and source reconstruction

The raw MEG data was preprocessed using the OHBA Software Library (OSL) package written in Python by the OHBA Analysis Group, University of Oxford, UK [13]. The processing pipeline consists of the following steps: preprocessing, co-registration to structural MRI, source reconstruction and parcellation, orthogonalisation, and lastly sign-flipping.

As part of preprocessing, the MEG data was first downsampled from 1000 Hz to 250 Hz. A bandpass filter (0.01-70 Hz) and notch filter (centered at 50 Hz, width of 2 Hz) were used to reduce noise in specific frequency ranges. This was followed by an automated bad-segment and bad-channel detection, where data segments and sensor channels of poor quality are identified. Next, an independent component analysis (ICA) was performed to remove ocular and cardiac artefacts. Lastly, after the dimensionality reduction from 306 sensors to 64 independent components, the channels previously labelled as bad were reconstructed through interpolation.

Before source reconstruction, it is important for the Polhemus headshape coordinates (acquired before the MEG recording) and MRI coordinates to be co-registered as accurately as possible. This co-registration was performed using the Registration of Headshapes including Nose in OSL (RHINO) framework.

Next, the data was transformed from sensor space to source space by applying a Linearly Constrained Minimum Variance (LCMV) Beamformer using a single-shell forward model [14]. The time series of all brain sources were parcellled into 38 parcels by using an fMRI-based parcellation atlas.

To reduce the effect of magnetic field spread, the parcellated data was orthogonalised through a multivariate

leakage correction algorithm based on singular value decomposition (SVD).

Lastly, the parcellated data was sign-flipped to partially solve the dipole ambiguity problem caused by the ill-posed nature of the source reconstruction algorithm.

III. BEHAVIORAL STATISTICS

The subjects' task performance during the SDMT was assessed. This was investigated by looking at two metrics: success rate and reaction time. The success rate is the proportion of correct subject responses throughout the entire 128 trials. The reaction time is the time between the stimulus presentation and the first recorded button press. Due to skewed outcome variable distributions, the non-parametric Mann-Whitney U-test was used for group comparisons.

Additionally, the effect of benzodiazepines (BZDs) on task performance was investigated. Group comparisons for BZD positive (BZDp, N=18) and BZD negative (BZDn, N=55) PwMS were also performed.

IV. STATIC ANALYSIS

The first major type of MEG analyses conducted in this thesis are inherently static. Static analysis methods such as event-related field (ERF) or functional connectivity (FC) analysis aim to capture task-evoked neurophysiological activity inside a fixed time window around the stimulus onset ([-500,1000] ms). To mimic traditional ERF and functional connectivity analyses, the input MEG data to these analysis methods was not orthogonalised nor sign-flipped.

A. Event-related field

Event-related field (ERF) analysis is based on the principle of trial-averaging. The higher the number of trials, the higher the signal-to-noise ratio of the trial-average time signal. For each subject, the continuous MEG signal was epoched between 500 ms before and 1000 ms post-stimulus onset. Next, all epochs of the same paradigm condition (correct/incorrect) were averaged together, per subject. To observe the global ERF trend, the subject-specific ERFs were averaged across all 38 parcels and all subjects. Before averaging, each epoch was baseline-corrected using the signal in the pre-stimulus time window.

To study differences between healthy controls and PwMS, the ERFs were averaged separately for those two cohorts. To test for statistically significant group differences, a permutation test (N=10 000) with max statistic was used. Permutation tests are non-parametric, and using the maximum statistic allows to correct for multiple comparisons across each time point where the ERF magnitude is compared between the two groups.

B. Functional Connectivity

The brain can be seen as a network of regions communicating with one another. To study the functional connectivity (FC) during the SDMT in this thesis, the coherence metric was used. Coherence reflects the strength of oscillatory synchronicity between the signal of 2 parcels at a certain frequency. The 38x38 coherence matrices were computed using each subject's ERF grand average and the `spectral_connectivity_epochs()` function from MNE Python [15]. The coherence analysis was performed in 5

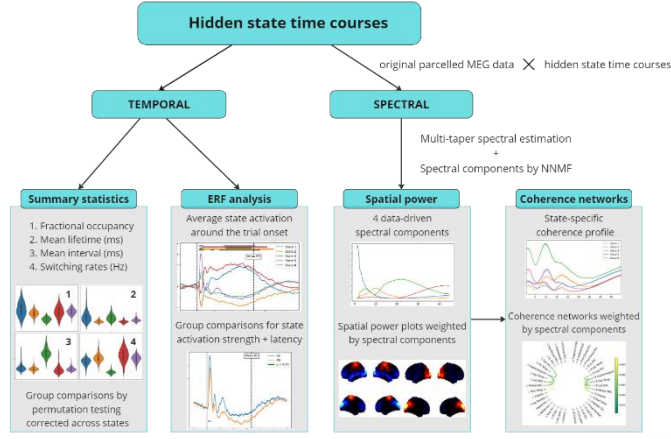


Figure 1. Overview of the different analysis methods after inference of the hidden state time course.

classical frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha1 (8-10 Hz), alpha2 (10-13 Hz), and beta (13-30 Hz).

To perform group comparisons on the subject's coherence matrices, a separate statistical test is needed per unique connection. 38 parcels lead to 703 unique connections. The multiple comparison problem can be avoided by reducing the dimensionality of the coherence matrices. Collapsing multiple original parcels into 5 brain regions (occipital, parietal, temporal, frontal, and medial) was accomplished by averaging the subject grand average's parcels (forming 1 epoch per brain region) before computing the coherence. Group comparisons were performed using permutation tests ($N=10\,000$), with p-values corrected by a false discovery rate (FDR) procedure.

V. TDE-HMM ANALYSIS

To explore and characterise the dynamic functional brain networks occurring during the SDMT, a Hidden Markov Model (HMM) approach was used. This approach is dynamic by itself, as no fixed time window around the task onset needs to be specified for this type of data analysis. To ensure the use of state-of-the-art algorithms that have proven to be successful in describing brain dynamic patterns in other task paradigms [16],[17], this thesis used the HMM implementation from the OSL Dynamics Toolbox [18]. More specifically, the Time-Delay Embedded (TDE-HMM) was selected, as this model enables an additional spectral description of the extracted functional brain networks.

A. Model specification

Before the hidden state time courses could be inferred from the observed MEG data, a few data preparation steps were required. The input MEG data should be orthogonalised and sign-flipped in order to optimise the TDE-HMM inference. Next, the MEG time series of all 38 parcels was time-delay embedded over a certain amount of time lags ($N=15$). Then, a principal components analysis (PCA) was applied to reduce the data dimensionality, both lowering the risk of model parameter overfitting and reducing the computational cost.

Afterwards, the different HMM model parameters (hidden states time course, transition probability matrix, and observation model parameters) can be learned from the prepared MEG data. The TDE-HMM is trained by using a stochastic variational Bayesian inference scheme [19], where the different model parameters are repeatedly updated for small batches of training data.

The main output after TDE-HMM model training consists of the inferred hidden states time course. Before this output can be used for further analyses, mutual exclusivity of the states is enforced (i.e. taking the Viterbi path). In essence, instead of each time point having a probability (between 0 and 1) for each state being active, only the state with the highest activation probability becomes the only state assigned to that time point.

B. Post-inference analyses

Figure 1 provides an overview of the different analysis methods applied to the inferred hidden state time courses. Overall, the extracted states can be studied based on temporal and spectral properties. For the temporal domain, summary statistics can be extracted, and an ERF-like analysis can be conducted. On the one hand, summary statistics (e.g., fractional occupancy, mean lifetime, mean interval, switching rate) describe the general dynamics of the states' activation throughout the entire MEG recording. On the other hand, an ERF analysis of hidden state time courses allows us to identify how and which states significantly activate during the SDMT.

For the spectral domain, state-specific spatial power maps and functional connectivity networks can be generated. First, the power spectral densities (PSDs) are estimated for a wideband frequency range (1-45 Hz). Instead of using the conventional frequency bands, the frequency bands can be estimated in a data-driven manner by using the non-negative matrix factorisation (NNMF) approach proposed by Vidaurre et al. [20]. The NNMF is applied to the wideband coherence spectra and returns a pre-specified amount of spectral components ($N=4$). This allows studying a state's spectral and spatial characteristics only within a frequency band that is representative of that state's activity. Combining the temporal, spectral, and spatial characteristics allows us to identify a state's functional role during the SDMT.

VI. RESULTS

A. Behavioral Statistics

HCs have significantly higher success rates compared to PwMS (Mann-Whitney U-test, p-value = 0.00696). Regarding the reaction times (RTs), subjects have a slower RT when presented with incorrect stimuli (mean RT = 1.814 s) compared to correct stimuli (mean RT = 1.668 s). The RT differs significantly between the HCs and PwMS groups for both paradigm conditions

(correct/incorrect), with slowed RTs in PwMS (Mann-Whitney U-test, p -values < 0.001). Furthermore, comparing BZDp with BZDn showed significantly slower RTs in BZDp PwMS (Mann-Whitney U-test, p -value = 0.003).

B. ERF analysis

Group comparisons on the parcel-specific ERFs (i.e. without averaging across all 38 parcels) revealed that the right inferior occipital, left superior occipital, posterior cingulate cortex, and right orbitofrontal brain parcels contain time points with significant differences (permutation test with max statistic, p -value < 0.05) between HCs and PwMS. The most important findings are shown in Figure 2. The right inferior occipital parcel (left) shows a significantly lower peak amplitude between 100 and 120 ms post-stimulus in PwMS. Additionally, this peak appears to be delayed in PwMS. In the posterior cingulate region (right), the ERF peak at 150 ms post-stimulus has a significantly larger magnitude in HCs compared to PwMS.

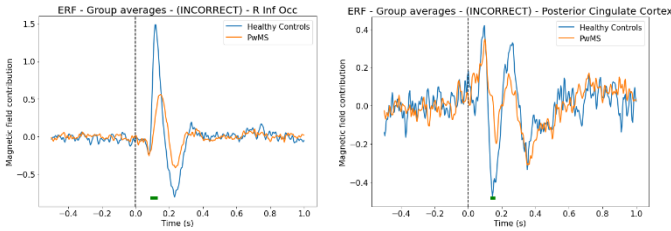


Figure 2. Group differences plots (HCs vs. PwMS) of ERFs for all brain parcels and trial types. Time points showing statistically significant differences after permutation testing ($N = 10\,000$) using the max statistic ($p < 0.05$) are marked as green horizontal lines.

C. Functional Connectivity

The connection pairs (parcels collapsed into brain regions) with significant differences (i.e. $p < 0.05$ from permutation tests corrected for multiple comparisons via FDR) in coherence between HCs and PwMS are reported in Table 2. In the theta band (4-8 Hz), the coherence between the parietal and frontal brain regions (p -value = 0.020) as well as between the temporal and frontal brain regions (p -value = 0.020) is significantly reduced for PwMS compared to HCs. The results found in the theta band only hold for the incorrect trials.

In the alpha2 band (10-13 Hz), the coherence between the occipital and parietal regions showed a significant difference (p -value = 0.037) between the two groups, only for correct trials.

TABLE 2

Results from the coherence group comparisons on the collapsed brain parcels (mean $\pm$ std). p -values are obtained from permutation testing ($N=10\,000$) and false discovery rate (FDR) correction for multiple comparisons.

Regions	Trials	f	HC	PwMS	p-value
Parietal/ Frontal	Incorrect	θ	0.65 ± 0.17	0.53 ± 0.20	0.020
Temporal/ Frontal	Incorrect	θ	0.56 ± 0.17	0.47 ± 0.17	0.020
Occipital/ Parietal	Correct	$\alpha 2$	0.52 ± 0.17	0.42 ± 0.18	0.037

D. HMM states' description

The TDE-HMM model was run for a pre-specified number of states equal to 6. Out of the 6 inferred states, 5 were found to be task-related (i.e. states that significantly activate during the task). However, as state 4 shares very similar characteristics to state 6, this state was omitted in the Results and Discussion sections.

Three relevant spectral components were obtained through data-driven spectral decomposition using NNMF. The first component contains the delta (1-4 Hz) and theta (4-8 Hz) bands. Spectral component 2 represents the alpha band (8-13 Hz), and spectral component 3 mainly captures the beta band (13-30 Hz).

State 1 – This state is characterised by a significant peak of activation between 450 and 1500 ms after stimulus onset. On the spectral level, state 1 shows a high peak in mean coherence around the beta range while the mean PSD is rather flat across all frequencies. Therefore, the spectral content of state 1 is mainly focused on spectral component 3. The phase-coupling shows a rather broad activity pattern, with the strongest connections occurring between the temporal, occipital, and cuneus regions of the right hemisphere. Additionally, the medial prefrontal cortex also shows strong coherence with the right superior parietal and cuneus regions.

State 2 – This state significantly deactivates between 150 and 1300 ms post-stimulus, after which reactivation builds up. This reactivation occurs at about 450 ms before the mean RT to the stimuli. Based on a peak in the PSD and the strongest coherence peak both occurring in the beta range, the main spectral content of state 2 is summarised by spectral component 3. The spatial PSD map displays high beta activity in the supramarginal and sensorimotor cortex, present in both hemispheres. On top of the regions identified through the mean PSD, the coherence networks show strong connections between the left and right superior prefrontal, and the left and right angular gyri. Notably, the lateral and medial sensorimotor cortices show inter-hemisphere connections.

State 5 – State 5 shows a task-related response significantly activated between 250 ms and 1250 ms post-stimulus. For both paradigm conditions (correct/incorrect), the probability of state 5 activating at early response to the stimulus is significantly decreased in PwMS compared to HCs. The activity of state 5 is mostly contained within spectral component 1. From the mean PSD map, mostly frontal regions show the highest activity. The coherence networks reveal the involvement of prefrontal (e.g. superior, ventrolateral, orbitofrontal), medial sensorimotor, posterior cingulate cortex, and anterior temporal regions.

State 6 – This state is characterised by the strongest significant activation between 50 and 100 ms post-stimulus. Additionally, both paradigm conditions show consecutive time points at 50 ms post-stimulus with significantly lower state activation for PwMS than for HCs. Very distinct alpha peaks in both mean PSD and coherence plots across all subjects lead to state 6 being associated with spectral component 2. The PSD map highlights the occipital lobe. This is confirmed by the coherence networks showing high synchronisation between several regions in the occipital lobe. More specifically, this concerns the inferior occipital, middle occipital, occipital pole, and cuneus regions, with multiple connections spanning across the two hemispheres.

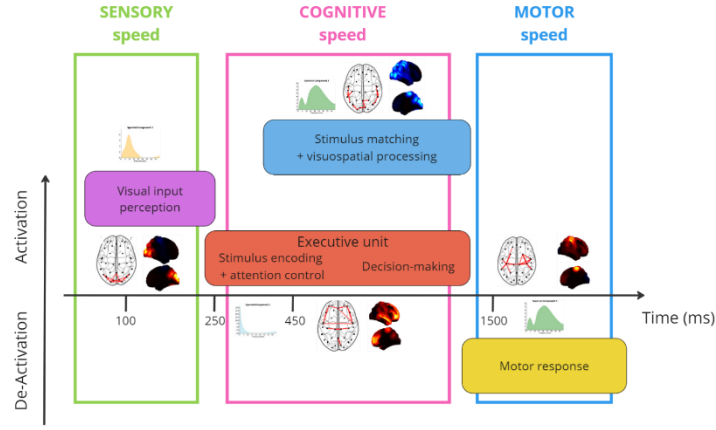


Figure 3. Summary of the proposed pathway of neurophysiological processes during the computerised SDMT. The rectangles with filled colors represent the states: state 6 (purple), state 5 (red), state 1 (blue), and state 2 (yellow). Using the tri-factor model of IPS in MS [31], the pathway is divided into 3 parts: sensory, cognitive, and motor speed.

VII. DISCUSSION

A. Performance during the computerised SDMT

PwMS were found to have significantly reduced task performance and reaction times compared to HCs during the computerised SDMT. This has previously been reported in the original on-paper SDMT [21], as well as in certain SDMT adaptations for fMRI studies [11],[22]. Interestingly, other neuroimaging studies reported no significant differences in SDMT performance between both groups [23]. Whether significant differences in task performance between both groups are observed, could depend on the complexity and imposed time constraints during the employed computerised SDMT adaptation. As such, the main disadvantage of the task paradigm used in this thesis is the pair-matching nature of the task, which causes IPS not to be examined exactly in the same way as during the original on-paper SDMT [11].

B. Static analysis

From the ERF analysis, the posterior cingulate cortex showed significantly reduced peak magnitude in PwMS. Grothe et al. reported a similar result in an fMRI study during the SDMT, where the cingulate cortex was activated in HCs but not in PwMS [11]. Related to information processing, an N2 response in EEG, generated in the midcingulate cortex, is believed to indicate difficulties in cognitive information processing [24]. The cingulate cortex is believed to be involved in attention mechanisms related to cognitive functioning [25].

Another significant group difference relates to the ERFs in the occipital cortex. More specifically, the inferior occipital (M100) and superior occipital (M300) regions show significantly reduced magnitude and latency in PwMS. Visual function is often reported to be impaired in PwMS [26],[27]. The differences in latency at this early visual input processing response could be a first and important contributor to the reduced IPS in PwMS.

Reduced frontoparietal connectivity observed in the FC analysis has been shown to correlate with working memory capacity in PwMS [28]. Further, several studies reported that intact connectivity between the parietal and frontal regions is crucial for good SDMT performance [10],[29]. This suggests that a non-negligible working memory subprocess could be involved during the SDMT.

The reduced occipitoparietal coherence in the alpha2 band has previously been related to spatial attention functions [30], which represents an important component of the SDMT [31].

C. Dynamic TDE-HMM analysis

The TDE-HMM was used to extract data-driven brain states with distinct spectral profiles, involved during the execution of the SDMT. In the following, the extracted states are discussed in their order of activation throughout the task.

State 6 – Occipital – The visual stimuli presented during the SDMT are first captured in the occipital cortex. This significant activation at 100 ms post-stimulus is similar to the visual P100 response reported in visual task EEG studies [32]. This network is impaired in PwMS, which aligns with previous reports of MS-induced visual impairments that can be picked up in M/EEG [33].

State 5 – Prefrontal executive unit – The prefrontal theta activity characterising this state could be involved with stimulus encoding during the SDMT [34]. The need to remember the presented symbol-digit combo before matching it with the reference table justifies the presence of a working memory component during the SDMT. Further, the phase-synchronisation between the orbitofrontal cortices and posterior cingulate cortex has previously been associated with attentional control during cognitive efforts [35]. Considering state 5 remains activated until about 300 ms before the mean reaction time, this could capture the response button selection near the end of an SDMT trial. This decision-making process is often associated with frontal regions [36]. This prefrontal network was found to have impaired activation in PwMS, also reported by Rossi et al [37].

State 1 – Visuospatial processing – The distributed occipital, parietal, and temporal brain network with important beta activity represents visuospatial processing [38]. Although this thesis did not reveal this network to be impaired in PwMS, literature has repeatedly mentioned visual scanning deficits in PwMS [39].

State 2 – Sensorimotor – The spatial and spectral layout of this state correspond to a typical sensorimotor network, involved in the subject response submission during the SDMT trial. This state is characterised by beta activity in the somatosensory cortices, and similar states were previously identified in MEG task paradigm requiring a motor response using the TDE-HMM approach [16],[17].

Overview – To relate this work to IPS, the tri-factor model of IPS in MS is used [31]. This recent theoretical model states that IPS should be seen as the summation of several contributions: a sensory, cognitive, and motor component. Figure 3 provides a novel description of the neurophysiological processes underlying the SDMT task.

VIII. LIMITATIONS AND FUTURE WORK

From a methodological point of view, a different metric than coherence could be chosen to study static FC. Although the data preprocessing in this thesis includes two approaches to reduce the effect of electromagnetic field spread (transformation to source space, and orthogonalisation) coherence remains sensitive to this effect. Future studies could apply a measure that discards all zero-phase interactions (e.g. phase lag index) [40].

The TDE-HMM inference can be validated by assessing two properties: robustness and stability. A second inference was run for 8 states (instead of 6), where no additional task-related states were found. Future studies should also assess the stability of the HMM inference by training the same model several times and comparing the final cost function of each inference.

The TDE-HMM implementation used in this thesis has 2 intrinsic limitations: the use of the Markovian assumption, and the enforcement of mutually exclusive states. To overcome these limitations, the Dynamic Network Modes (DyNeMo) approach has recently been proposed by Gohil et al. [41]. Future studies should use this new technique to assess whether this leads to significant improvements and new insights for the description of brain dynamics during the execution of the SDMT.

IX. CONCLUSION

In this master’s thesis, an MEG dataset recorded during the SDMT task was used to uncover the neurophysiological processes behind IPS. Standard task-MEG analyses revealed significantly reduced spatial attention and visual function during the SDMT in PwMS.

The TDE-HMM provided a 3-dimensional (temporal, spectral, and spatial) characterisation of different brain networks forming several components contributing to IPS. PwMS were found to have altered activation of the occipital and prefrontal executive states. This new description considerably improves our understanding of IPS assessed by the SDMT, and is a first step towards a more objective assessment of IPS in PwMS.

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Glossary

AP	Action Potential
BOLD	Blood Oxygenation Level Dependent
BZD	Benzodiazepines
BZDn	BZD negative
BZDp	BZD positive
CNS	Central Nervous System
conpes	Contrast of Parameter Estimates
CT	Computed Tomography
dFC	Dynamic FC
DyNeMo	Dynamic Network Modes
ECG	Electrocardiography
EDSS	Expanded Disability Status Scale
EEG	Electroencephalography
EOG	Electro-oculography
EP	Evoked Potential
ERF	Event-Related Field
ERP	Event-Related Potential
FC	Functional Connectivity
FDR	False Discovery Rate
FID	Free Induction Decay
FLAIR	Fluid-Attenuated Inversion Recovery
fMRI	Functional MRI
FO	Fractional Occupancy
GLM	Generalized Linear Model
Hb	Hemoglobin
HC	Healthy Control
HMM	Hidden Markov Model
ICA	Independent Component Analysis
ICP	Iterative Closest Point
IPS	Information Processing Speed
ISI	Interstimulus Interval

LCMV	Linearly Constrained Minimum Variance
M/EEG	Magnetoencephalography or Electroencephalography
MAR	Multivariate Autoregressive
MEG	Magnetoencephalography
MI	Mean Interval
MLT	Mean Lifetime
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NNMF	Non-negative Matrix Factorisation
OHBA	Oxford Centre for Human Brain Activity
OSL	OHBA Software Library
PC	Principal Component
PCA	Principal Component Analysis
PSD	Power Spectral Density
PwMS	People with MS
RF	Radiofrequency
RHINO	Registration of Headshapes including Nose in OSL
RMS	Root Mean Square
RT	Reaction Time
SC	Spectral Component
SDMT	Symbol Digit Modalities Test
SE	Spin Echo
SNR	Signal-to-Noise Ratio
SQUID	Superconducting Quantum Interference Device
SR	Switching rate
TDE	Time-Delay Embedding
TDE-HMM	Time-Delay Embedded HMM
WM	Working Memory

Chapter 1

Introduction

The brain is undoubtedly one of the most complex organs in the human body. Various microscopic electrochemical processes contribute to macroscopic brain activity. Research has always strived to elucidate the precise mechanisms behind the brain's functioning. As a consequence, several scientific methodologies and analysis tools have emerged over the last decades to achieve this.

In this section, all the main techniques to study the brain's properties and functioning, relevant to this thesis, are introduced. First, the different available neuroimaging techniques to study the anatomy and neurophysiology of the brain are explained. Then, the types of experimental conditions used to study functional brain properties are mentioned. Finally, several neurophysiological data analysis methods are introduced.

1.1 Neuroimaging techniques

A large family of neuroimaging techniques is currently used to study the brain's properties. To give an overview, they can be classified based on the type of information they provide (structural or functional). Additionally, the temporal and spatial precision of these techniques often define important advantages or limitations. Figure 1.1 shows these classifications.

In the following, the working principles of the two neuroimaging techniques employed in this thesis are explained.

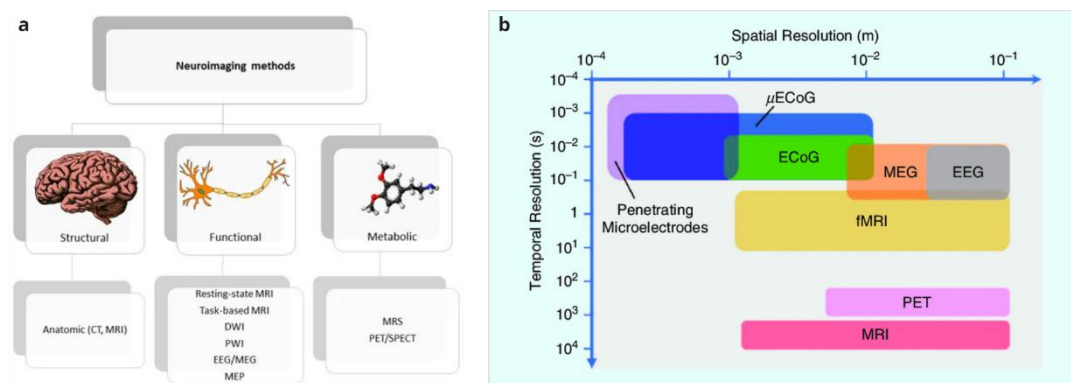


Figure 1.1: Classification of neuroimaging techniques. a) structural vs. functional imaging [42]. b) spatial vs. temporal resolution [43].

1.1.1 Structural imaging - Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is an important neuroimaging technique that allows the study of structural properties and anatomy of the brain. Compared to Computed Tomography (CT), MRI offers excellent soft tissue contrasts, enabling precise distinction between white and grey matter regions.

In the following, the signal generation and image acquisition for MRI are explained. This is relevant considering an MRI dataset is used in this thesis.

How is the signal generated?

The source of the activity picked up by MRI goes back to an important quantum mechanical feature of atom nuclei: the magnetic moment μ . Only the nuclei with non-zero spin and with sufficient presence in the body will contribute to MRI. Therefore, the Hydrogen atom is by far the most relevant.

A nuclei's magnetic moment rises from the nucleus spinning around its own axis. When a large number of hydrogen atoms are considered, there is zero net magnetisation, meaning no signal can be measured. If a strong external B_0 field is applied, however, the atom's spin axes align with the B_0 field and create a net magnetisation. This is shown in Figure 1.2. The rotation frequency for the precession of μ around B_0 is known as the Larmor frequency ω_L . As shown in equation 1.1, the Larmor frequency is proportional to the strength of B_0 and depends on the chemical element, as the gyromagnetic ratio γ is element-specific.

$$\omega_L = \gamma \cdot B_0 \quad (1.1)$$

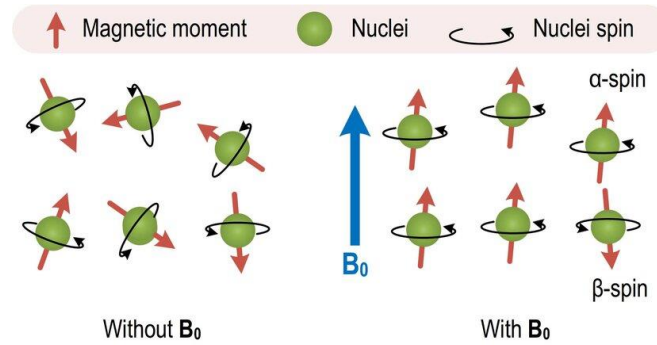


Figure 1.2: The net magnetic moment aligns with the strong external B_0 field [44].

In practice, further increasing the strength of B_0 is difficult to achieve. A more efficient energy transfer method consists in creating resonance. This requires applying short radiofrequency (RF) pulses (= rotating B_1 field) perpendicular to B_0 . Additionally, resonance is only possible when the frequency of the RF pulses matches the Larmor frequency ω_L . The consequence is that the longitudinal magnetisation (M_L) is flipped to become phase coherent in the transverse direction (M_T). This is shown in Figure 1.3.

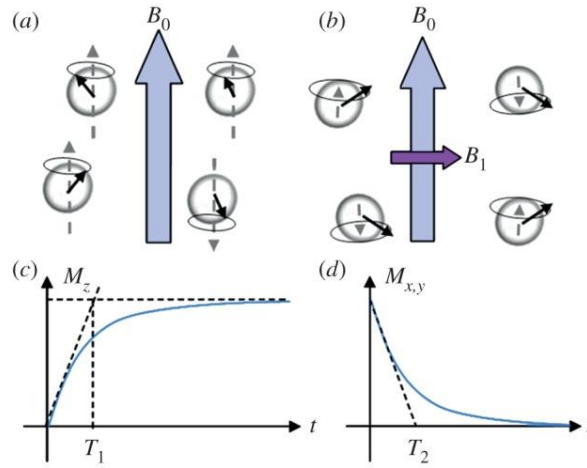


Figure 1.3: (a) Spins align with B_0 creating a net longitudinal magnetisation M_L . (b) RF pulse flips the net magnetisation from longitudinal to transverse (M_T). (c) After RF is turned off, M_L recovers and (d) M_T decays exponentially [45].

Afterwards, the transmission of radio waves is turned off. This is known as relaxation, where the M_L recovers, while the M_T gets out of phase and decays. This is also shown in Figure 1.3. Based on tissue characteristics, this exponential recovery or decay happens at different time constants. As such, T_1 and T_2 are the time constants for the tissue-specific M_L recovery and M_T decay, respectively. During decay, particles will emit a signal called Free Induction Decay (FID). It is the FID that forms the signal of interest to measure in MR. To measure the FID, a receiver RF coil is placed perpendicular to the orientation of M_T .

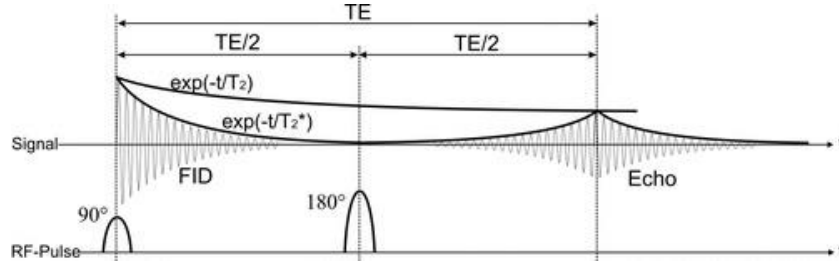


Figure 1.4: Conventional spin echo sequence [46].

The FID signal is very small in amplitude and quickly disappears. To improve the signal-to-noise ratio (SNR) and accurately measure the FID, the signal should be sampled multiple times. This can be achieved by applying specific MRI sequences where several RF pulses are applied in sequence. An example of such an MRI sequence is spin echo (SE), shown in Figure 1.4. After a first 90° excitation RF pulse, a second 180° inversion RF pulse is applied. The inversion pulse causes the fast spins to get behind the slower ones. Over time, the fast spins will catch up and become in phase with the slower spins. This leads to an echo, which is equivalent to the superposition of two FIDs. Using such MRI sequences allows to speed up and increase the sampling of the FID.

How are MR images formed?

Even though the main MR signal can be measured, each small signal contribution has the same ω_L . This makes it impossible to discern which part of the signal comes from which part of the anatomy. To solve this problem, gradient coils are used to add extra information to the measured signals. Gradient coils apply space and -time-varying magnetic fields to encode 3-dimensional information about the signal source in the body. In practice, a slice, phase, and -frequency encoding gradient are applied. An example of an image acquisition sequence is shown in Figure 1.5. By varying the amplitude of the phase encoding step, the measured echo signal fills the K-space (spatial frequency domain). Afterwards, a 2-dimensional inverse Fourier transform is applied on the filled K-space, leading to a single reconstructed image slice (spatial dimension domain). Repeating these steps multiple times allows for scanning and imaging of the entire head anatomy.

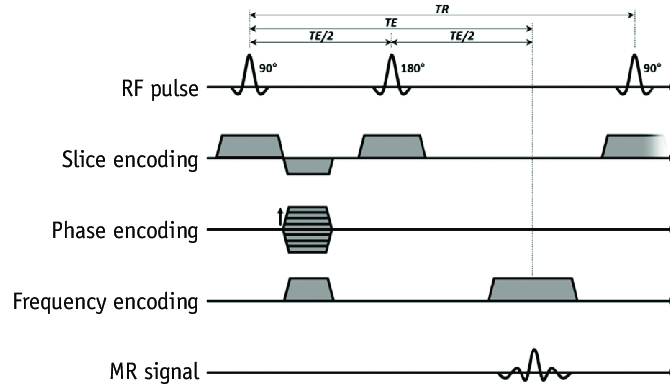


Figure 1.5: MRI acquisition using gradient fields and spin echo sequence [47].

1.1.2 Functional imaging - Magnetoencephalography (MEG)

The most important neurophysiological data of this thesis was collected using magnetoencephalography (MEG). Therefore, this section contains a detailed explanation of the main principles behind this technique. Furthermore, MEG is compared with other functional imaging techniques to identify its strengths and weaknesses.

What is measured with MEG?

MEG is used to pick up magnetic brain activity. The very origin of magnetic brain fields can be traced back to the most fundamental unit of the brain: the neuron. When an action potential (AP) is fired and travels along a neuron's axon, two types of currents are generated: primary and secondary currents. Primary currents rise from the inward and outward ionic currents that flow through the axon membrane during the different phases of an AP (depolarisation, repolarisation, hyperpolarisation, and back to rest). Due to changes in the membrane potential caused by an AP, voltage-gated ion channel dynamics generate transmembrane currents. These are known as secondary currents. Figure 1.6 shows both current types in a neuron.

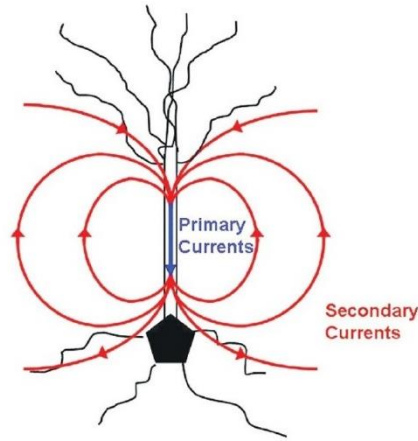


Figure 1.6: Primary and secondary currents generated by a neuron [48].

The electrical signals produced by individual neurons have a very small amplitude and rapidly decay as they move further away from the neuron. The signal measured using MEG needs to be recordable at the scalp, meaning that some level of signal summation is required. Spatial summation over a group of neighbouring neurons, which implies co-localisation, comparable orientation, and equal signal polarity, is a crucial requirement. Moreover, to amplify the signal, these neurons should be active synchronously. As such, large pyramidal neurons located in the cortex meet these criteria [49]. These neurons' main longitudinal axes are parallel to each other, resulting in a summed contribution of the longitudinal current components. MEG is also known to be more sensitive to primary currents [50].

Following Ampère's law, the electrical current induces a magnetic field perpendicular to the current direction. Even though pyramidal neurons located in the cortex are perpendicular to the cortical surface, only the currents produced by pyramidal neurons tangential to the surface of the skull (i.e. located in the sulci) will contribute to the MEG measurement. This is because magnetic fields induced by currents from radial pyramidal neurons are not likely to reach outside the head. This is also shown in Figure 1.7. In conclusion, MEG mainly measures the primary currents generated by tangential pyramidal neurons from the cortex.

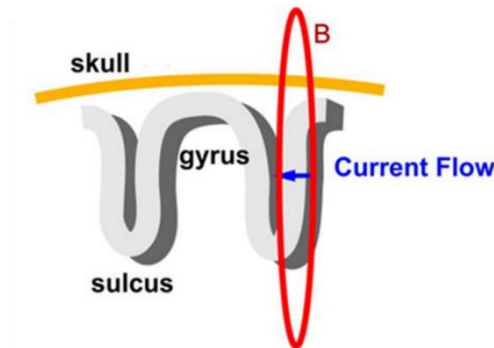


Figure 1.7: MEG measures magnetic fields induced by tangential current flow [51].

How is the signal measured?

With the signal of interest being very small, even below the order of pT, there is an important influence from internal and external sources of noise [50]. Internal noises arise from physiological activity such as eye movements, heartbeats, and muscle activity. Meanwhile, external noises include the earth's magnetic field, and urban magnetic noise from common electronic devices [52]. Considering these noises are always present, it is crucial to reduce their interference with the MEG recording. This is achieved by placing the MEG scanner in a magnetically shielded room. Making the room's walls out of materials with high magnetic permeability (e.g. Aluminium) allows bending the external magnetic fields, which deviates them from the MEG scanner [52].

Additionally, sensors with high sensitivity to small magnetic field changes are required. Sophisticated sensors, i.e. the superconducting quantum interference devices (SQUIDs), are used in MEG for this purpose. Each unit on the MEG helmet sensor array contains a SQUID sensor, which requires to be cooled in liquid helium to benefit from superconductivity. The full MEG setup containing the magnetically shielded room, SQUIDs, and cryogenic storage dewar, is shown in Figure 1.8.

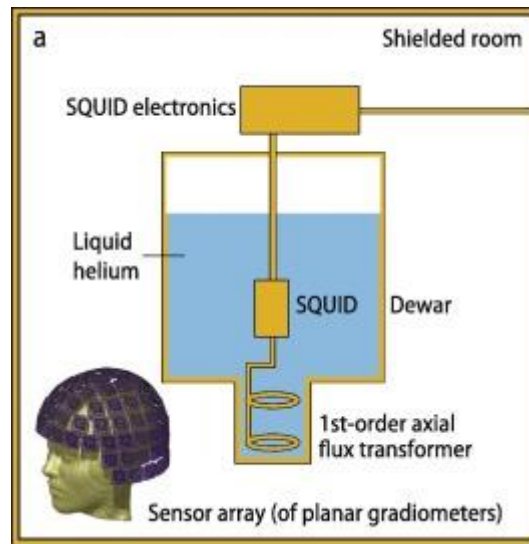


Figure 1.8: Components of an MEG measurement system [53].

The principles of SQUIDs involve conversions between several physical units, for which the steps are explained in [54]. First, a detection coil picks up a magnetic field and converts it to a current that runs through the flux transformer circuit (i.e. the combination of a detection and -input coil). The detection coil can be either a magnetometer or a gradiometer. A magnetometer consists of a single coil, while a gradiometer measures gradients between magnetic fields using two coils.

Secondly, the current running through the flux transformer leads to a change in the input coil's magnetic flux. Because the input coil is magnetically coupled to the SQUID sensor, this in turn leads to a change in the magnetic field that is felt by the SQUID sensor. The detection coil, input coil, and SQUID are all shown in Figure 1.9.

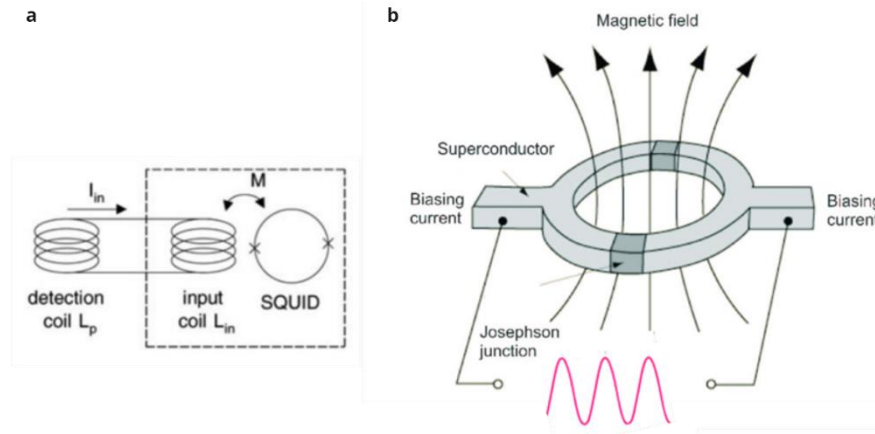


Figure 1.9: (a) Coupling between the detection coil, input coil, and SQUID. (b) SQUID electronics [55].

Thirdly, SQUIDS convert a change in magnetic flux into a sinusoidal output voltage. This is possible due to SQUIDS' superconductive properties and their components: two superconductors separated by an insulating layer called the Josephson junction.

Finally, the oscillating output voltage is linearised by using a negative feedback circuit called the flux-locked loop (FLL). Overall, SQUIDS convert very small magnetic field changes into a measurable output voltage, which becomes the final MEG signal.

How does MEG compare to other non-invasive functional neuroimaging modalities?

Besides MEG, two other non-invasive functional neuroimaging modalities are popular: functional MRI (fMRI) and electroencephalography (EEG). Each has its strengths and weaknesses, which can be found in Table 1.1 based on a comparison of several important properties.

	fMRI	EEG	MEG
Temporal resolution	Low	High	High
Spatial resolution	High	Low	Medium
Cost	High	Low	High
Attenuation by skull	Yes	Yes	No
Direct measure	No	Yes	Yes

Table 1.1: Comparison between non-invasive functional neuroimaging techniques: fMRI, EEG, and MEG.

fMRI measures the neural activity indirectly through the blood oxygenation level dependent (BOLD) contrast. When the neural activity increases, the provided energy is supplied by an increase in oxygenated blood flow. The oxygenated hemoglobin (Hb) causes a stronger contrast between the diamagnetic (oxygenated Hb) and paramagnetic (deoxygenated Hb) effect, which gives rise to a stronger BOLD signal [52]. Most notably, fMRI has a lower temporal resolution and a much higher spatial resolution compared to MEG.

EEG is closely related to MEG, where both imaging modalities are direct measures of neural activity. Instead of picking up the electric fields generated from primary currents, EEG is more sensitive to secondary extracellular currents. EEG measures voltage differences at the scalp using electrodes. The main advantage of MEG compared to EEG is the higher spatial resolution. This is because electric fields are attenuated by the skull, while magnetic waves are not [49]. However, MEG is a more expensive neuroimaging modality than EEG.

1.2 Task-based analysis

This section introduces the concepts of task-based paradigms and resting-state conditions, which represent the two major conditions for conducting functional imaging measurements in subjects. A comparison between the characteristics of both approaches will reveal which types of neuroscientific investigations can benefit from one approach over the other.

In the context of neuroimaging experiments, a task refers to an imposed paradigm that manipulates the subject's brain functions. More specifically, a task-based paradigm where either stimuli are presented, or a cognitive or motor task is performed, will lead to the activation of task-related brain functions [54]. This event-related activity can be measured in the brain as Evoked potentials (EPs). These potentials, which are characterised by the performed task type and the subject's current wakefulness and brain health, can be measured using EEG [56]. An example of a classical EP generated by presenting a stimulus is shown in Figure 1.10. In practice however, individual EPs as measured using EEG have a very low amplitude ($0.1 - 10 \mu\text{V}$) and are embedded in background activity which has an amplitude of a higher order of magnitude ($10 - 100 \mu\text{V}$) [56],[57],[58]. This means that, when presenting a single stimulus to a subject, distinguishing the generated event-related waveform from the background activity is often impossible.

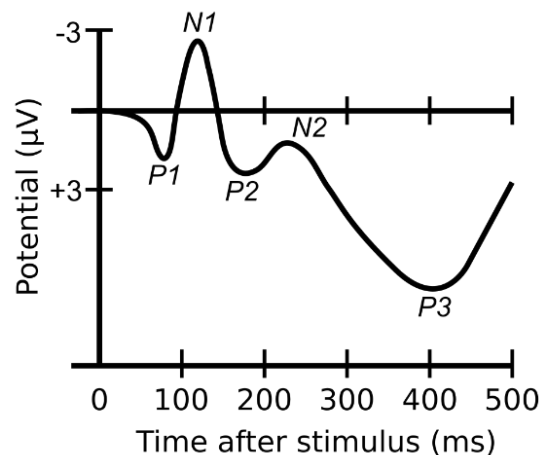


Figure 1.10: Example of an Evoked Potential [59].

Although this type of experiment design allows us to increase our knowledge about brain functions involving sensory or cognitive processes, setting it up requires extensive preparation. Typically, a task-based experiment requires a computer responsible for presenting the stimuli at precise timings, and a second computer to record and store the acquired neurophysiological data, at the very least. Figure 1.11 shows a setup for providing visual stimuli and acquiring the measured MEG signal, together with several timing parameters that determine how the sequence of stimuli is presented. Some parameters, such as the interstimulus interval (ISI) can be very complex to determine correctly. A well-determined ISI will provide sufficient time for neural activity to reach baseline levels between the offset of the previous and onset of the following stimulus, but at the same time avoid subject fatigue by reducing the total recording time [54]. Studies have also shown that applying some variability to the ISI across trials helps to eliminate anticipatory and habituation responses, which could otherwise lead to incorrect conclusions [60].

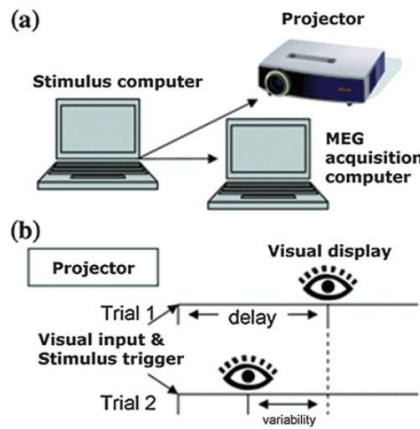


Figure 1.11: Example of a setup for task-based experiments. (a) Basic visual setup, with a stimulus and acquisition unit. (b) Different timing parameters to be determined [54].

In contrast to task paradigms, resting-state refers to a condition in which a participant is not subjected to any form of stimuli, or is not performing any cognitive or motor task. This approach is interesting if the goal is to study and characterise brain activity that is always present, regardless if any behaviour is absent or present [61]. A comparison between several characteristics of task-based vs. resting-state functional neuroimaging recordings can be found in Table 1.2.

	Task-based paradigm	Resting-state
Signal amplitude EEG	Low ($0.1 - 10 \mu\text{V}$) – from a single stimulus	High ($10 - 100 \mu\text{V}$)
Experimental setup	Difficult – Stimulus encoding, presentation	Easy – Only recording setup
Application	Study functional brain organisation	Study resting-state networks
Interpretability	High – Controlled experimental conditions	Low
Subject compliance	High – Unsuitable in people unable to perform task	Low – Subject can relax

Table 1.2: Comparison between task-based paradigms and resting-state measurements [56].

1.3 Static Approaches

Once neurophysiological MEG data during task performance is recorded, the next step is to extract meaningful information from the collected data. To analyse task-based M/EEG data, researchers in the field of neuroscience have applied a large variety of methods and algorithms. They can be classified into two major groups: Static and Dynamic analysis methods. Static approaches use a fixed time window that is placed around each task-related stimulus (= epoching). Dynamic approaches represent efforts to avoid the use of a window around the trials, enabling to capture more context than only the time-locked brain response to the task [54]. First, an overview of traditional Static approaches used to study task-based neurophysiological data is given. The concepts of trial-averaging, Event-related field (ERF), and Functional Connectivity (FC) analysis will be illustrated.

1.3.1 Event-Related Field (ERF)

As mentioned in section 1.2, an EP caused by a single stimulus cannot be distinguished from the ongoing background activity. Despite this limitation, trial-averaging is known to allow a clear and reliable identification of the task-evoked activity. By presenting the same stimulus for a large number of trials, and averaging the brain response within a small time window around all stimuli, only the time-locked task-specific waveform remains, also called the Event-related potential (ERP) [62]. Figure 1.12 shows an example of a standard ERP analysis, where the ERP can be described as a waveform constituted of several waves, each with a certain amplitude and latency with respect to the stimulus onset.

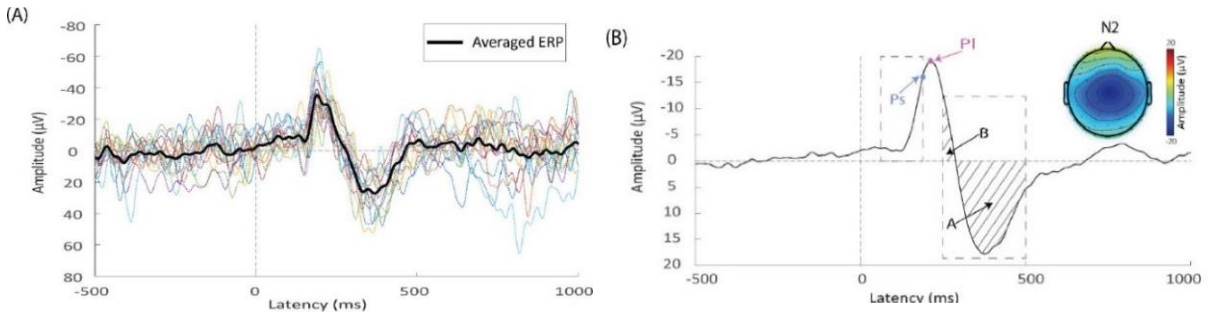


Figure 1.12: The ERP waveform, obtained through trial-averaging. (A) Colored lines represent single-trial evoked potentials and the black bold line shows the averaged within-participant ERP waveform (B) Group-level ERP (average of all within-participant responses) and topography map [63].

The reason why averaging is successful in extracting ERP waveforms lies in its ability to increase the SNR as the number of trials N increases. Assuming that the background activity (= noise n) can be approximated by a zero-mean Gaussian random process, the SNR can be increased by a factor N when using N^2 trials [54]. This is also shown through equation 1.2:

$$\text{Var}[\bar{x}(t)] = E[(\bar{x}(t) - E[\bar{x}(t)])^2] = \frac{1}{N^2} E\left[\left(\sum_{k=1}^N n(t,k)\right)^2\right] = \frac{1}{N^2} \sum_{k=1}^N E[n(t,k)^2] = \frac{\sigma^2}{N} \quad (1.2)$$

While ERPs can be obtained from EEG measurements, the equivalent measure for MEG is called Event-related field (ERF). Once this waveform is extracted, researchers usually study some of the waveform's properties. An example is shown in Figure 1.13, where both the peak's amplitude and latency with respect to the event onset are extracted from the ERFs for different types of visual stimuli [64]. While here this analysis is only performed for one brain region's time series, in practice, this can be repeated for each brain region (= parcel) in whole-brain data.

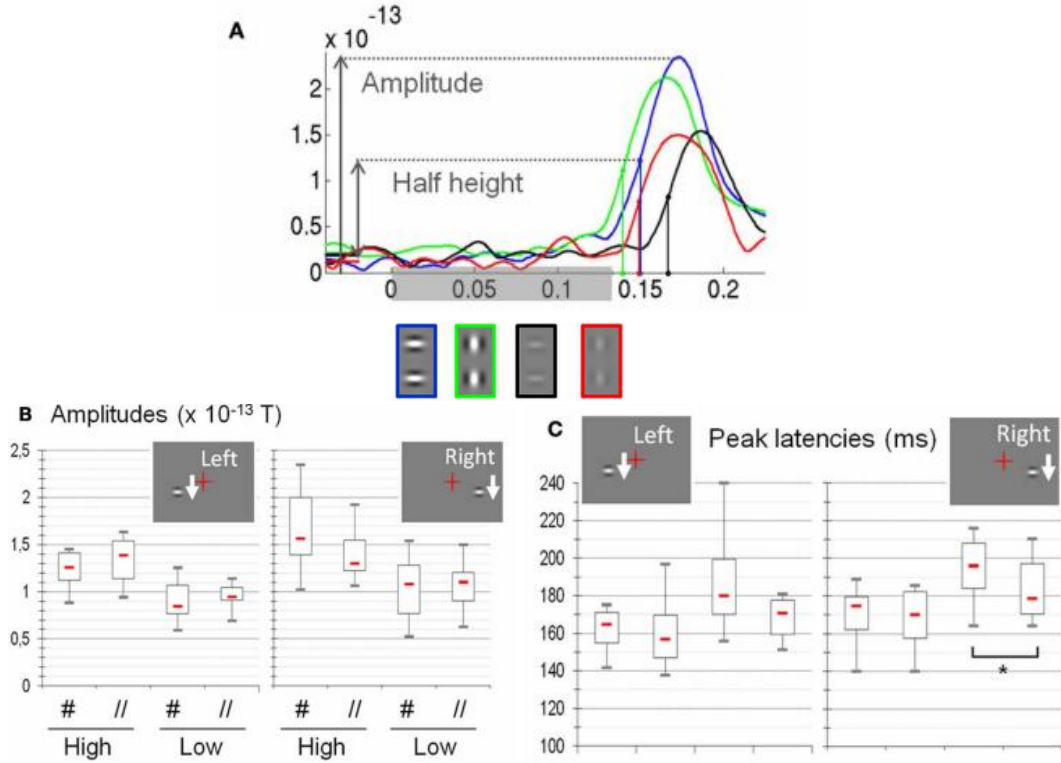


Figure 1.13: Amplitude and Latency measured on the ERF waveforms as a function of different experimental conditions [64].

1.3.2 Functional Connectivity

Another way to study neuroimaging data from a static perspective is through Functional Connectivity (FC). Instead of analysing each brain region's associated time series separately (e.g. through ERF analysis), in FC analysis the brain is seen as a network of brain regions that communicate with one another. During a task, FC is used to capture relations between the activity of several brain regions, reflecting their contribution in carrying out the task. To measure FC strength between two (or more) nodes, a large variety of connectivity metrics with different properties is available. The most used connectivity metrics are shown in Table 1.3, where each metric is characterised by the following four properties: directionality, dimensionality, signal domain (time or frequency), and linearity [52].

	Directionality	Dimensionality	Signal Domain	Linearity
Correlation	No	Bi-variate	Time	Yes
Cross-correlation	Yes	Bi-variate	Time	Yes
Coherence	No	Bi-variate	Frequency	Yes
Granger Causality Index	Yes	Bi-variate	Time	Yes
Directed Transfer Function	Yes	Multi-variate	Time	Yes
Partial Directed Coherence	Yes	Multi-variate	Frequency	Yes
Mutual Information	No	Bi-variate	Time	No
Phase Lag Index	No	Bi-variate	Frequency	No
Phase Locking Value	Yes	Bi-variate	Frequency	No
Phase Slope Index	Yes	Bi-variate	Frequency	No

Table 1.3: Overview of connectivity metrics and their properties [52],[65].

In terms of directionality, a connectivity metric can be undirected or directed. A directed metric will not only measure the connection strength but also the direction of information flow between the involved brain regions.

Although it is easier to compute and interpret connectivity between pairs of regions (= bivariate analysis), some connectivity metrics take into consideration the influence of more than 2 brain regions (= multivariate analysis). Investigating higher-dimensional relations could lead to the discovery of more complex relations underlying brain activity [66].

Such FC measures can be computed both in time or -frequency domain. On the one hand, time domain measures are used to study temporal dynamics and synchronisation of activity between regions. On the other hand, converting the signal to frequency domain allows the study of both the synchronisation of brain rhythms, and phase information, between regions.

A last property refers to how the relation between the different variables is modelled. Non-linear metrics, typically involving estimates of probability distributions (e.g. Mutual Information) assume non-linear interactions between brain regions, which often comes at the cost of computational expense.

When studying FC in non-invasive recordings such as M/EEG data, it is important to pay attention to the problem of volume conduction (EEG) or field spread (MEG) [65],[67]. These phenomena cause the generated activity from a single neuronal source to be picked up by multiple recording channels. If not taken into account, this can lead to biased estimates of FC. There exist several ways to reduce this undesired effect. A first strategy is to move from sensor space to source space data by using source reconstruction algorithms [68]. Another option is to use connectivity metrics that are phase-lag-based (e.g. Phase Lag Index). This works because those metrics discard contributions of 0° phase differences, which is exactly how volume conduction is otherwise picked up in sensor space [65].

1.4 Dynamic Approaches

The analysis approaches described in section 1.3 assume a certain stationarity in time, namely that the evoked activity or FC remains the same over the entire duration of the task. In recent years, however, many studies have shown that functional networks activated by several tasks are dynamic in nature [69],[70]. In this section, several analysis approaches that do not use a time window related specifically to the task are described. First, dynamic Functional Connectivity (dFC) and its challenges in task-based paradigms are discussed. This is followed by a more elaborate explanation of what Hidden Markov Models (HMMs) are, and what makes them interesting to be applied to functional neuroimaging data.

1.4.1 Dynamic Functional Connectivity

As mentioned above, FC is known to evolve over time. Looking at the literature related to Dynamic Functional Connectivity (dFC), most studies have applied this concept to resting-state data. The general idea is to compute connectivity matrices over several time windows, spread along the entire recording duration. This sliding window approach introduces the sought non-stationarity. For instance, once each time window is characterised by a FC matrix, the evolution of dFC matrices can be expressed by looking at the similarity of those FC matrices at each time point within the window [71]. This principle is illustrated in Figure 1.14.

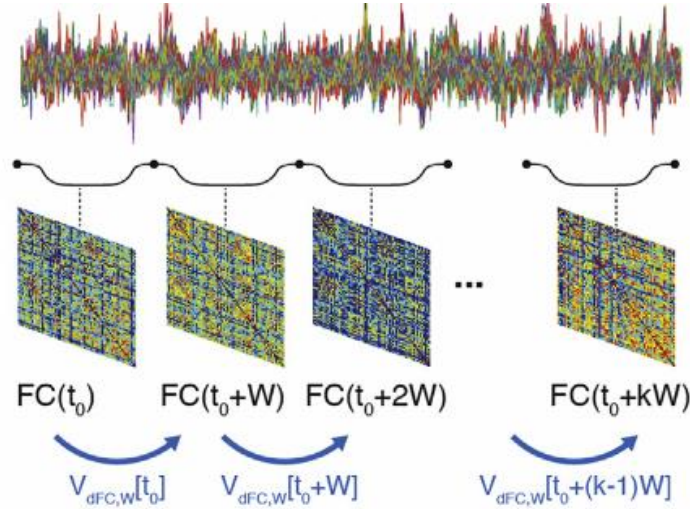


Figure 1.14: Sliding window estimation of time-resolved correlations [71].

In the last few years, only a few attempts to capture dFC during tasks were made. Liu et al. proposed a framework that detects change points of brain networks based on high-order singular value decomposition of ERP time series [72]. Although this approach seems promising, it has some important limitations. First, the input data to the pipeline consists of already epoched time signals, meaning that the brain signals between each trial are not utilised. Secondly, prior knowledge about the task-evoked activity is required in order to correctly choose the number of change points. These limitations make it difficult to reliably interpret the results of such analysis.

There are several possible explanations as to why no major and reliable pipelines, to study dFC in functional neuroimaging recordings specifically during tasks, have emerged yet. Firstly, task-induced activity can make it difficult to determine whether the observed connectivity patterns are driven by the task itself or by ongoing brain fluctuations [73]. This translates to a major confounding factor in the stability and interpretation of results. Secondly, additional research is required to find the most optimal data preprocessing scheme for the intended use of extracting task-based dFC. Without this, several methodological choices (which can vary between research institutes) will affect the strength of observed FC [74]. Finally, in contrast to resting-state data, exploration of task data is often limited to the specific cognitive domains relevant to the task. Therefore, in order to fully understand task-concurrent dFC evolution, additional and much broader datasets on different task paradigms would have to be collected [70].

1.4.2 Hidden Markov Models

A distinct approach to analyse neurophysiological data, without the explicit use of event-related time windows, is through the use of Hidden Markov Models (HMMs). The general idea behind HMMs is illustrated through a simple example, which will help understand this method's main principles and components. One of the problems that can be solved using an HMM creates room for applications in neuroscience, more specifically, the extraction of transient large-scale functional brain networks from M/EEG data [19].

To better understand the concepts and purpose of an HMM, let's start with a practical and straightforward example (see Figure 1.15). Imagine that a person's mood on a given day is determined by only the weather. The weather can be either Sunny (S) or Rainy (R), with only one of those weather types occurring on a given day. For each following day, how the weather will change from one state to another is determined by a set of transition probabilities (arrows going from and towards the S, and R nodes). A person can feel either Happy (H) or Grumpy (G), and how a person feels on a certain day is determined by the emission probabilities given the current weather type (arrows going from weather type to mood type).

Now, assuming that the weather knowledge is hidden, and the knowledge about a person's mood can be observed, the following question could be asked: "What is the most likely weather sequence for a given sequence of observed moods?" For example, if a person's mood sequence for the 5 previous days = [H, G, G, H, G], what is the most likely sequence of weather types explaining the person's observed mood? This problem can perfectly be solved using an HMM together with its associated algorithms.

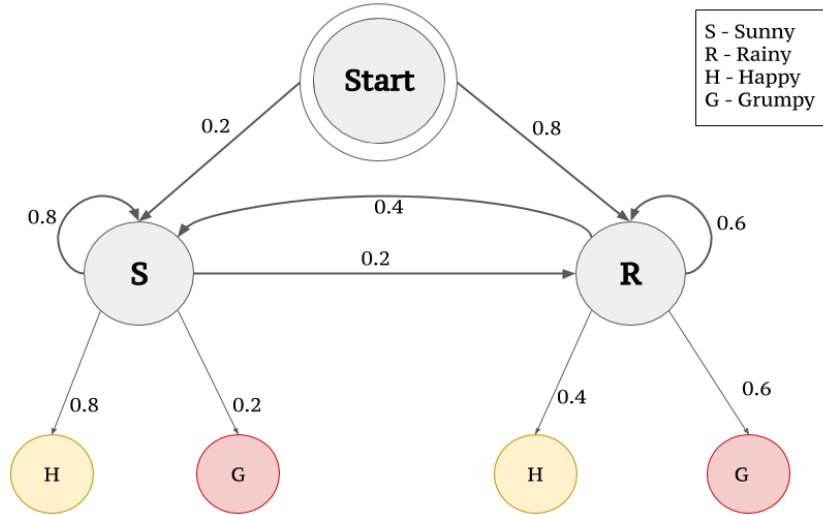


Figure 1.15: Simple Hidden Markov Model example [75].

Components and Assumptions of HMMs

Using the previously described example, the components of a basic HMM are as follows [76]:

$S = \{S_1, S_2, \dots, S_N\}$	set of hidden states	$\{S, R\}$
$a_{ij} = P(q_{t+1} = S_j q_t = S_i)$	transition probabilities	e.g. $P(q_{t+1} = S q_t = R) = 0.4$
$O = \{O_1, O_2, \dots, O_M\}$	observation sequence	$[H, G, G, H, G]$
$b_{ij} = P(o_t = O_j q_t = S_i)$	emission probabilities	e.g. $P(o_t = H q_t = S) = 0.8$

The transition probabilities a_{ij} represent the probability of moving from state i to state j at the next time point. Emission probabilities b_{ij} express the probability of an observation o_t being generated from state i . Typically, to initialise the model at the first time point, an initial state distribution is specified [75]. The set of hidden states, transition probabilities, and emission probabilities altogether form a Markov Chain. An HMM is often defined as a Markov Chain accompanied by a sequence of observed data.

Every Markov model is developed based on a set of crucial assumptions [75]. The first assumption is the Markovian assumption, stating that the probability of a particular state at a certain point in time only depends on the state at the previous time point. Secondly, the Output Independence assures that the probability of an output observation only depends on the current hidden state. Finally, the Stationarity assumption ensures that transition probabilities are time-invariant. These three assumptions allow for easier factorisation of the computed joint distributions, facilitating the computation of probabilities [77].

How can HMMs be applied to neuroscience?

Not long after HMMs were introduced, their first major application was in speech recognition systems. This was possible because speech has a temporal structure that fits nicely in the natural framework of an HMM [78]. The applications of HHMs in sequential signal analysis are countless, with growing importance in several fields: natural language processing, computational biology, protein sequencing and bioinformatics [79]. Part of the reason why HMMs are successful in many different domains is their ability to solve 3 large categories of problems [76]:

1. **Evaluation.** Given an observation sequence and an HMM, what is the probability that a particular observation sequence is produced by that model?
2. **Decoding.** Given an observation sequence and an HMM, what is the most likely sequence of states that provide the best explanation for the observed data?
3. **Learning.** Given an observation sequence, how can we learn the most optimal HMM parameters?

Relating this to the field of neuroscience, one of the major questions that can be investigated using HMMs is a combination of Learning and Decoding from neuroimaging data. The recorded M/EEG data represents the observed time sequence, and the goal is to decode the most likely sequence of hidden states that explain the recorded brain signals. But to do so, the HMM model parameters need to be learned prior to finding the most likely sequence of hidden states. For this specific application, it is believed that the HMM approach can be used to reduce measured cortical brain activity into sequences of fast, transient and reoccurring events, referred to as brain states [16],[19],[80],[81],[82]. Figure 1.16 shows an example of extracting meaningful brain states with their probability of activation during resting-state MEG in healthy subjects [80].

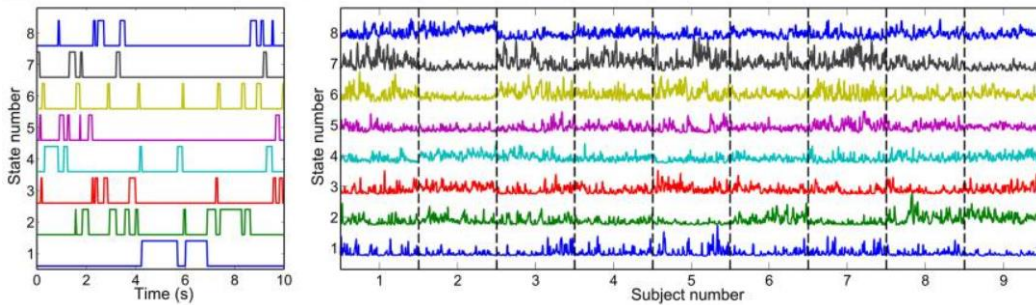


Figure 1.16: Example of an HMM brain states sequence along the time course, each state represented by its probability of activation [80].

The HMM approach adapted for neurophysiological signals can be used to conduct two types of studies, both used to investigate activity at millisecond timescale [18]:

1. Detecting bursts of oscillatory activity [18],[83].

Instead of interpreting neural oscillations as smooth brain rhythm fluctuations, whose amplitude can be modulated during sensory and cognitive tasks, several studies suggest that oscillatory activity could be the result of recurrent bursts of short activity [58],[59],[60]. More specifically, recent findings suggest that populations of neurons show bursts of oscillatory activity at a 100ms timescale [85],[86]. Knowing that M/EEG has excellent temporal resolution, together with the HMM's capability to extract brain states that activate at fast timescales, feeding M/EEG recordings (= observation sequence) to an HMM allows to detect bursts of oscillatory activity.

Figure 1.17 shows an example of burst detection in single-region resting-state data, revealing that the HMM is able to identify specific periods of high beta activity. The advantage of using an HMM is that bursts can be detected using richer spectral content over a broad frequency range, instead of simply applying amplitude thresholding over a small frequency band [83].

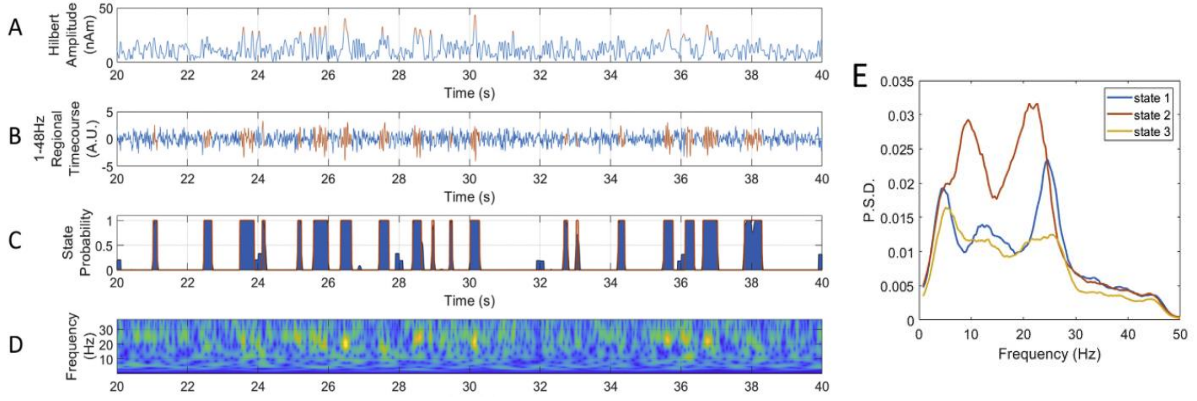


Figure 1.17: Burst detection using the Time-Delay Embedded HMM (TDE-HMM) in single-region resting-state data. (A) Beta amplitude with bursts (in red) identified using a simple thresholding technique. (B) Broadband data with annotated bursts (red) using the HMM. (C) Probability time course for the burst state (blue). (D) Time-frequency decomposition of the broadband data. (E) Power Spectral Density profiles for the 3 states identified by the HMM [83].

2. Identifying dynamic functional brain networks.

Traditional FC analyses are limited in the sense that they often assume one or two of the following: temporal stationarity, frequency-independence, or spatial inhomogeneity [69]. Besides the multi-dimensional complexity involving FC, a second challenge is the small timescale at which some functional brain networks operate. Physical movement and several cognitive processes (e.g. perception, working memory) involve brain networks that are known to operate at millisecond timescale [87]. However, not every neuroimaging technique allows to capture information at such high temporal resolution (e.g. fMRI). Using M/EEG data in combination with the HMM approach allows for capturing the multi-dimensional complexity of brain activity at high temporal resolution. Certain HMM implementations for whole-brain neuroimaging data have proven to be successful in extracting functional brain networks showing unique temporal, spectral, and spatial characteristics [16].

Chapter 2

Multiple Sclerosis

Multiple Sclerosis (MS) is the most common neurodegenerative and -inflammatory disease of the central nervous system (CNS) [1], affecting up to 2.8 million people worldwide [88]. Up to 70% of people with multiple sclerosis (PwMS) are affected by cognitive impairment, significantly reducing their quality of life [2]. Although the impairment type and severity strongly vary across people, several standardised ways to assess cognitive impairment in PwMS have emerged. These subjective assessments, however, can be confounded by other symptoms (e.g. depression) or may fail to detect early cognitive deficits as they emerge.

This resulted in several studies researching new and more objective approaches to assess cognitive impairment, one of which will be explored in this thesis.

2.1 Pathology

It is known that multiple sclerosis (MS) is an autoimmune disease of the CNS, where a person's immune system attacks the myelin sheaths around neuronal axons. The autoimmune response arises from activated and autoreactive T-cells, which attack and cause inflammation at the myeline [89]. Damaged myeline causes disruptions in the transmission of electrical signals between neurons. This damage results in the formation of lesions, which can be detected using T2-weighted or Fluid-attenuated inversion recovery (FLAIR) MRI sequences [90]. MS lesions can occur in multiple locations in the CNS, most commonly at the optic nerve, spinal cord, corpus callosum and deep periventricular areas, and can strongly differ from patient to patient [89],[91].

In reality, however, MS should be seen as a set of complex interactions between several mechanisms, which to this day are not completely understood. This makes MS a very diverse and not so well-understood disease. Recent research has found not only white matter but also cortical gray matter to be involved in MS [92],[93]. Additionally, it was discovered that neurodegenerative processes, unresponsive to immunosuppression therapies, were partially responsible for neurological impairment in MS [91].

There are two possible hypotheses to explain the interactions between each mechanism involved in forming an MS lesion, as shown in Figure 2.1. The most advanced hypothesis (pathway B) puts forward the idea of the immune system activating as a response to damage already present in the CNS. This ongoing damage, caused by e.g. toxic substances or infections, could lead to the release of substances from nerve cells, ultimately triggering a response from the immune system in the form of inflammation [91].

To conclude, it is important to mention that the specific causes of developing MS are not yet known. Nevertheless, evidence from research shows a non-negligible influence of genetic and environmental factors [94]. As such, MS disease may develop when certain genetic changes are activated by environmental influences, meaning that both factors interact with each other.

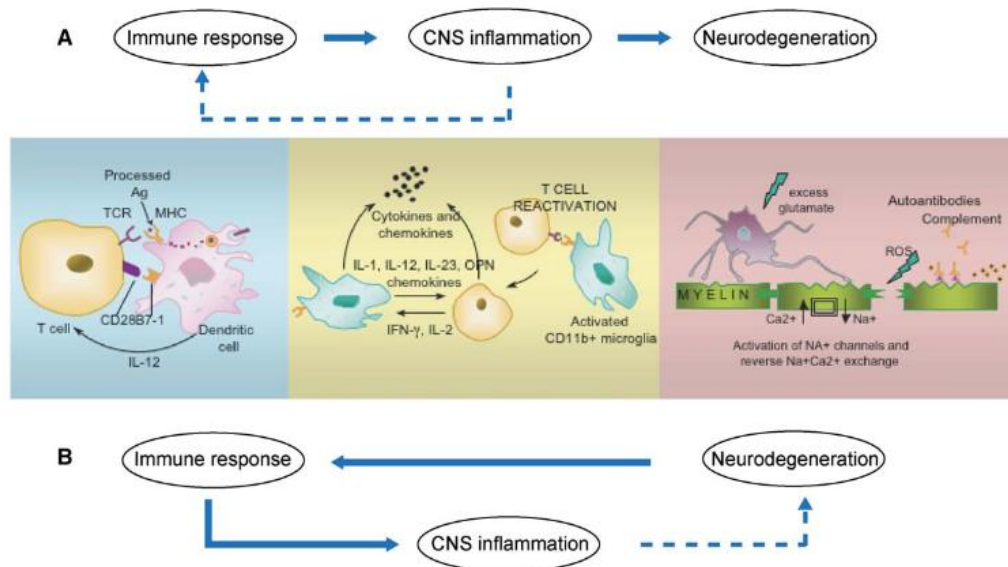


Figure 2.1: Three pathological mechanisms of MS are shown. The immune response (blue), inflammatory process (yellow) and neurodegeneration (pink). Two models of disease pathogenesis in MS based on these mechanisms: (A) CNS injury is a consequence of an autoimmune response, (B) Immune system is activated as response to damage already present in the CNS, where said damage could be caused by e.g. infections [91].

2.2 Cognitive Impairment

Cognition refers to the mental actions or processes involved in acquiring, understanding, remembering, and using information. It includes many aspects of high-level intellectual functions, such as perception, attention, memory, decision-making, and processing speed, among many others [95]. Cognitive deficit or impairment is therefore used to describe the impairment of one or different domains of cognition.

PwMS can show several symptoms, including motor, cognitive, and neuropsychiatric deficits, with all of them occurring independently of each other [3]. This widespread spectrum of affected domains in MS makes sense, considering MS lesions can appear at various locations in the CNS. Most notably, up to 70% of PwMS are affected by cognitive impairment [2]. Among the cognitive domains that were shown to be affected in PwMS are: complex attention, efficiency and speed of information processing, and working memory [3].

2.3 Assessing cognitive impairment

Since signs of cognitive impairment can already be detected in the early disease stages, it can help ensure an early diagnosis and effective treatment options. This leads to better patient

outcomes and potentially prevents the MS disease from evolving from relapse-remitting to a progressive stage [91].

In clinical practice, cognitive impairment in patients can be assessed either through so-called rating questionnaires, or neuropsychological examinations. Rating questionnaires are meant to be completed by the patients as well as their families. The MS Neuropsychological Screening Questionnaire (MSNQ) is an example of such a tool, designed to detect cognitive impairment specifically in PwMS [96].

Because self-reporting tends to be unreliable, a better approach consists of letting the patient perform a test involving cognitive efforts. In literature, these tests are referred to as Neuropsychological (NP) batteries, and they are used to assess a patient's cognitive abilities [97]. In the MS community, an international expert committee proposed the Brief International Cognitive Assessment for MS (BICAMS) battery, aimed to be standardised and easily applicable even in smaller clinical facilities [98]. This test battery consists of the Symbol Digit Modalities Test (SDMT), the Brief Visuospatial Memory test, and the California Verbal Memory test. These tests aim to assess information processing speed, visuospatial and -verbal memory, respectively.

Even though these tests allow to assess a subject's functioning without too much effort, they are far from perfect. First, they can become quite time-consuming, especially if several tests are conducted sequentially. Further, the evaluation is subjective as it depends on the operator. Lastly, neuropsychological assessments are prone to practice-effects, i.e. the subjects' test performance can increase because they get more familiar after repeated exposure to the test [99].

2.4 Potential biomarkers of cognitive impairment

To solve the limitations of standard cognitive assessment tests, researchers started applying neuroimaging techniques to discover new and reliable biomarkers of cognitive impairment. This section will first highlight some examples of potential neuroimaging biomarkers of cognitive deficits in PwMS. Afterwards, the cognitive domain of information processing speed (IPS) and a means of assessing it in PwMS will be explained. This will form a core aspect of this thesis.

2.4.1 Neuroimaging biomarkers

The first neuroimaging modality that was applied to find correlations with cognitive impairment in MS is MRI. In clinical practice, MRI is used to assess the extent of T1-hypointense, T2-hyperintense and Gadolinium-enhanced lesions in PwMS [99]. Even for the diagnosis of MS according to the McDonald criteria, several MRI parameters were included [100]. According to those diagnostic criteria, MRI should provide evidence of CNS lesions occurring across both space (at different CNS parts) and time (at different time points).

The aforementioned inclusion of MRI in clinical practice, as well as advancements in computational power, have improved the quantification of MRI scans aiming to detect cognitive impairment in PwMS. Figure 2.2 gives an overview of the different quantitative features related

to MS that can be extracted from MR images. However, measures of structural damage provide poor correlation with cognitive assessment scores. This phenomenon is known as the clinico-radiological paradox [99], and currently remains a challenge in the quest for an objective, sensitive, and specific biomarker of cognitive impairment in PwMS.

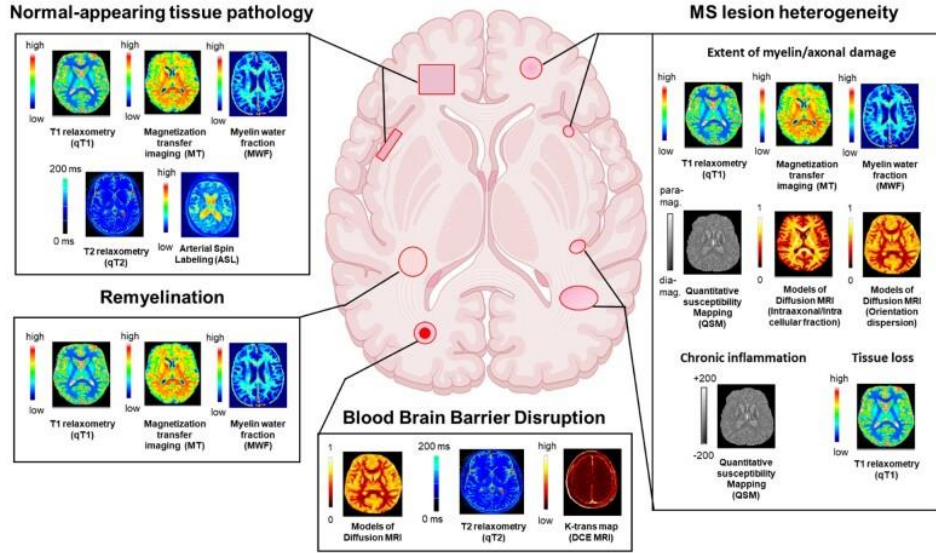


Figure 2.2: Overview of information provided by quantitative MRI features related to key aspects of MS [101].

More recently, researchers went beyond structural damage and started exploring changes in neurophysiological functioning relating to cognition. The non-invasive functional neuroimaging modalities fMRI, EEG, and MEG have previously been used in research to assess cognition in MS [99]. Using fMRI, Colorado et al. showed an increase in activation of the right dorsolateral prefrontal and anterior cingulate cortex while MS subjects performed a working memory (WM) task (N-back), compared to healthy controls (HCs) [102].

As explained in chapter 1, fMRI has high spatial and low temporal resolutions. However, it is known that cognitive processes involve brain dynamics occurring at millisecond timescale [87]. M/EEG can solve this limitation with its high temporal resolution. As such, resting-state MEG studies have shown an increase in FC in the beta band, which correlates with overall cognitive scores [103],[104].

Overall, substantial progress still needs to be made before an excellent neuroimaging biomarker of cognitive impairment in MS is identified. Features extracted from neurophysiological data often have a limited ability to detect cognitive impairment in MS and are not always easy to interpret [99]. Another obstacle is the often incomplete understanding of the exact brain functioning involved during certain cognitive processes. Understanding how and which brain regions communicate during an action involving e.g. WM could drastically facilitate the identification of a robust biomarker of cognitive impairment. On top of that, MS is a very diverse disease, making this an only more difficult challenge.

2.4.2 Symbol Digit Modalities Test (SDMT)

Among the cognitive domains that are affected in PwMS, information processing speed (IPS) stands out as a core cognitive deficit [4]. IPS is the cognitive domain that is most widely affected by MS [5] and is one of the first deficits to emerge [6]. As this cognitive function is essential for any task that involves reacting and deciding upon various stimuli, the execution of everyday tasks is affected in people with slowed IPS.

The standard cognitive test used to quantify IPS in clinical practice is the Symbol Digit Modalities Test (SDMT). The SDMT, published in 1968 by A. Smith, was a rather simple screening test meant to be sensitive to brain damage in children [12]. An example of an SDMT on paper is shown in Figure 2.3. At the very top, a reference table shows matches between the digits 1 through 9, and nine different symbols. Next, the subject has a limited time duration (90 seconds) to fill the empty spaces with the digit that matches the shown symbol. Test performance is quantified by the SDMT score, which simply corresponds to the number of correct given answers within the test’s time limit.

[illegible]

Figure 2.3: Example of a Symbol Digit Modalities Test on paper [12].

As seen in section 2.3, standard neuropsychological tests alone have a limited ability to assess cognitive function. Along with the fact that nowadays the computerised version of the SDMT has become more widespread [9], researchers have turned to using this test as a task paradigm in functional neuroimaging studies. This was previously applied mainly in fMRI studies, where, for instance, Grothe et al. discovered decreased cingulate activation during the SDMT in PwMS compared to HCs [11].

Chapter 3

Research Goal

Cognitive impairment affects a significant proportion of people with multiple sclerosis (PwMS) [2]. Most importantly, information processing speed (IPS) stands out as a core cognitive deficit in PwMS. Over the past decades, there have been extensive efforts to find objective ways of assessing IPS deficits. Although the Symbol Digit Modalities Test (SDMT) has proven to be a sensitive measure of IPS in PwMS [4], this classic neuropsychological assessment remains subjective. What makes things even more concerning is that to this day, the neurophysiological processes underlying the SDMT are still unknown.

In current literature, several researchers have turned to using a computerised version of the SDMT as a task paradigm, both with fMRI [11],[22] and EEG [105]. These studies were limited to purely static analysis methods for comparing healthy controls (HCs) with PwMS cohorts, and using these functional neuroimaging modalities did not advance our understanding of the cognitive processes underlying the SDMT.

This thesis explored a unique MEG-SDMT dataset consisting of MEG recordings while large cohorts of HCs and PwMS perform a computerised SDMT. This combination is also shown in Figure 3.1. MEG has the advantage of capturing fast brain dynamics at higher temporal resolution compared to fMRI. Considering that the SDMT paradigm has not yet been analysed during MEG, we strongly believe it is valuable to use both static & dynamic neurophysiological analysis approaches.

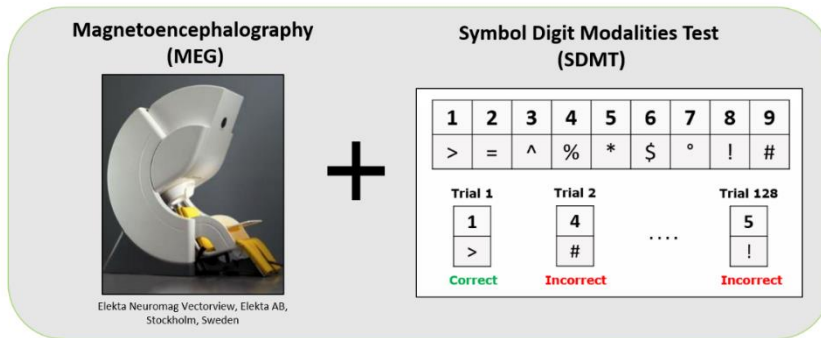


Figure 3.1: Combination of MEG and a computerised SDMT. Adapted from [106].

In this thesis, our goal is to provide the first description of the neurophysiological processes underlying an IPS task. Further, we hypothesise that this will enable us to uncover altered and likely slowed brain dynamics in PwMS as compared to HCs.

Chapter 4

Methodology

This chapter serves to explain and provide details about the analysis methods employed to reach the research goal of this thesis. The MEG-SDMT dataset is presented, together with how the computerised SDMT paradigm was implemented as part of the data collection. This is followed by an overview of the applied data preprocessing and source reconstruction steps. Next, the different types of used analyses are explained: Behavioral Statistics, Static analyses, and Dynamic analyses. Furthermore, several statistical comparison methods will be introduced to compare features between HCs and PwMS.

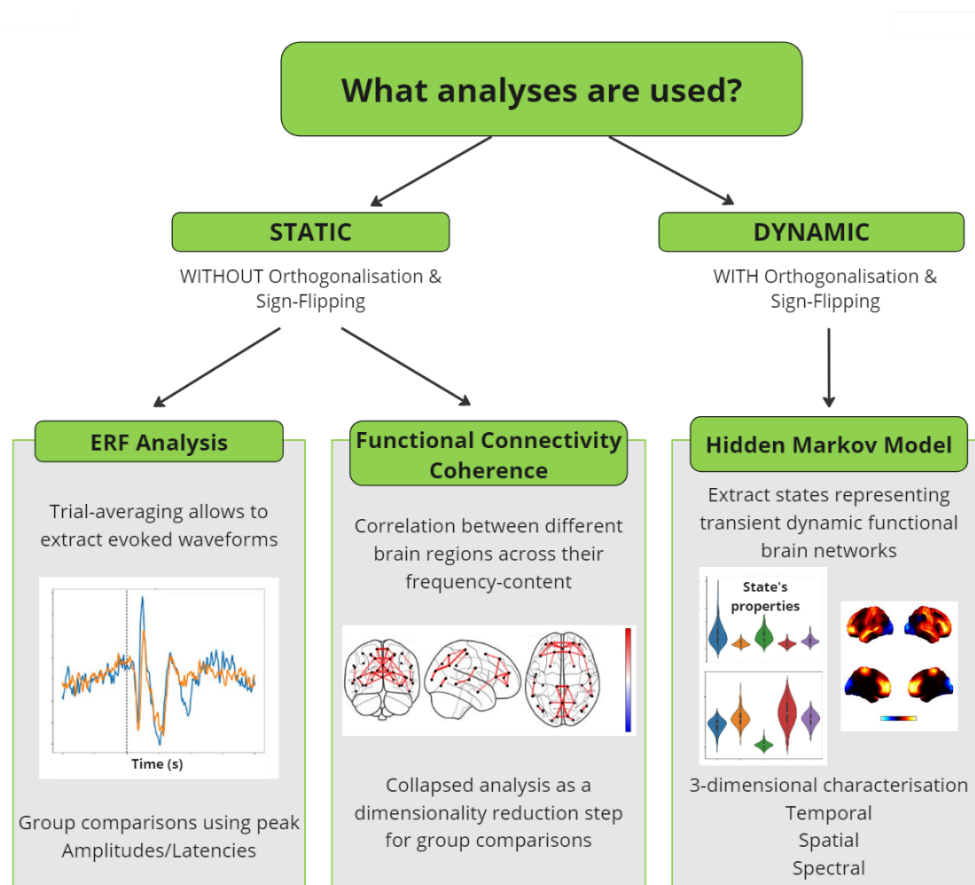


Figure 4.1: Main overview of the conducted analyses during this master's thesis.

4.1 SDMT dataset in healthy controls and people with MS

The SDMT dataset used in this thesis is a subset of a larger dataset collected by the Artificial Intelligence supported Modelling in clinical Sciences (AIMS) research group between 2015 and 2018. The original dataset consists of 150 subjects, 50 HCs and 100 PwMS, who have been carefully matched on age, gender and educational level. The SDMT subset includes all subjects who had an MEG recording while performing the computerised SDMT, bringing the total amount of subjects down to 139 (46 HCs and 93 PwMS).

Because it was the first time this SDMT dataset had been analysed, there were several instances throughout the work where subjects had to be excluded from the dataset. A detailed overview of the reasons and amount of excluded subjects can be found in Appendix C. The final SDMT dataset, as used for each of the further analyses, consists of 110 subjects (37 HCs and 73 PwMS).

In the following, all aspects of the multimodal SDMT dataset are explained. This contains structural (MRI), functional (MEG), and SDMT-related information.

4.1.1 MEG dataset

The MEG data were collected using a 306-channel Elekta Neuromag Vectorview system in the Laboratoire de Cartographie Fonctionnelle du Cerveau in Erasme Hospital (prof. Xavier de Tièrè, ULB) at a sampling rate of 1000 Hz. The 306 sensors form 102 triplet sensors (1 triplet sensor = 1 magnetometer, 1 planar and 1 axial gradiometer). During subject preparation, the Head Position Indicator (HPI) coil locations, the position of three fiducial points and the head shape were saved using a three-dimensional digitiser (Polhemus). MEG recordings took place in a magnetically shielded room (Elekta, MaxShield), during which the subjects performed a computerised SDMT paradigm. Additionally, dedicated electro-oculography (EOG) and electrocardiography (ECG) channels were used to measure ocular and -cardiac-related activity, respectively.

Before the recorded continuous MEG data was stored, the MaxFilter software (Elekta, MaxFilter) transformed the data by compensating for close-by-artefacts and head movement using a temporal extension of the Signal Space Separation method (tSSS) [107]. Afterwards, the data was stored in .fif format (Fractal Image Format), which is the native file format for Elekta MEG devices.

Finally, it is important to mention that the MEG scanner received an upgrade throughout the data collection. Considering this upgrade could lead to a change in MEG sensor sensitivity, the importance of this factor was investigated in section 4.4.1.

4.1.2 Stimulus presentation

While the subjects' brain magnetic waves were recorded, they participated in a computerised SDMT. The main workflow is shown in Figure 4.2. Inside the recording room, a projector is used

to permanently display the reference table with the correct nine symbol-digit matches. Then, a first candidate symbol-digit combo is shown, which corresponds to a single trial. The subject then has at most 6 seconds to give a response by pressing one of two buttons (correct/incorrect), indicating whether the shown combo is the same as in the reference table. This is repeated for a large amount of trials per subject.

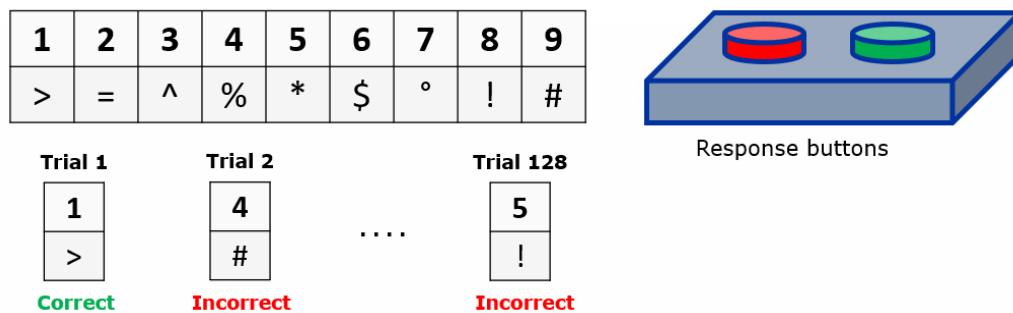


Figure 4.2: Graphical representation of the workflow during a computerised SDMT: a reference table, response buttons, and examples for a few trials are shown.

Initially, the task paradigm consisted of 256 trials for each subject. However, multiple subjects complained about the long duration of the full MEG recording. Therefore, at some point throughout the data collection, it was decided to cut the total amount of trials in half. Considering the full dataset, this leads to an imbalance in the total amount of trials per subject. This is why for all of the analyses conducted in this thesis, it was chosen to only retain the information from the first 128 trials in each subject's recording. The reason for this choice is to avoid any differences in fatigue-induced effects, reducing inter-subject variability in terms of the available data. It is known that task paradigms with long time durations can induce fatigue effects, which translate to altered neurophysiological signals [108],[109].

4.1.3 Event encoding and extraction

An important requirement for static analyses of neurophysiological data is an accurate extraction of the stimulus timings throughout the task. For the SDMT dataset, several Arduino channels were used to encode events as annotations in the .fif files. The order of events that occur within a single task trial is shown in Figure 4.3. A trial always starts with a stimulus event (STIM). About 40 ms later, a DIODE signal is sent. This is the exact moment at which a new digit-symbol combo is shown to the subject. The next event is when the subject presses one of two buttons to submit their response (BUTTON). Finally, the current trial ends when a new STIM event occurs.

One challenge observed during the event extraction step is that the Arduino ports used to record the DIODE and the two BUTTON response channels appear to be inconsistent across different recording sessions. By observing the channel layout for each subject, a functioning methodology to automatically extract the correct channel layout for each separate subject was found (see Pseudo-code 1).

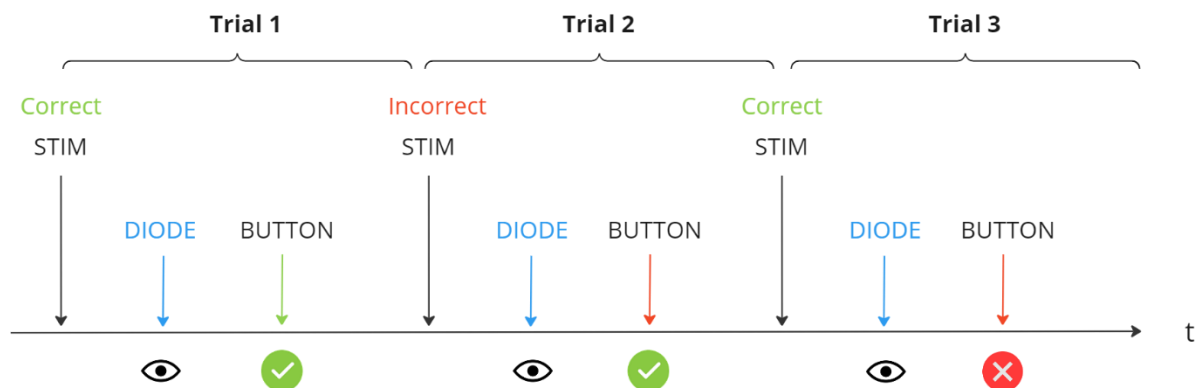


Figure 4.3: Overview of the order of encoded events during the computerised SDMT. The subject gave the right response for the first two trials but made a mistake for the third trial.

After the correct channel layouts were successfully identified, the event responses and other derived parameters were extracted. The entire thought process is summarised in Pseudo-code 2. If multiple BUTTON presses are recorded within a single trial, only the first one is considered. The reaction time (RT) to the stimulus is found as the difference between the DIODE time and the BUTTON response time.

4.1.4 MRI dataset

Structural brain information for all subjects was collected using MRI. This step was performed as the last, to ensure that the induced magnetisation from the MRI would not interfere with the MEG recording. In this dataset, MRI scans were taken using a 3T Philips MR system at 1x1x1 mm³ resolution (T1-weighted and FLAIR). The MRI scans are available in the original Digital Imaging and Communications in Medicine (DICOM) and the converted .nifti format. This data allows for co-registration between the head anatomy and the spatial location of the MEG sensors as part of source reconstruction. More details on this will follow in section 4.2.

Pseudo-code 1: Deciphering the STIM and RESPONSE channels layout

Problem → Inconsistent connection of input/output ports across different recording sessions

Step 1. Extract all unique event IDs + how many times they occur during the recording

IF ID 128 in unique event IDs:

DIODE channel = index

BUTTON channel = index + 1 # 1st candidate for the first button press channel

ELSE:

Exclude subject from dataset

Step 2. Find the exact index of the 1st button press channel

WHILE event count at current index < 20:

BUTTON index += 1 # Assumption: The first channel coming after DIODE
and with a sufficient count of events (> 20), is the
first BUTTON response channel (out of two)

Step 3. Identify the pattern (2 possibilities) for the second BUTTON response channel index

IF index modulo 256 = 0:

INCORRECT BUTTON response channel = index

CORRECT BUTTON response channel = index · 2

ELIF index modulo 256 = 128:

INCORRECT BUTTON response channel = index

CORRECT BUTTON response channel = (index - 128) · 2 + 128

Result → Correct identification of DIODE + two BUTTON response channel port IDs

Pseudo-code 2: Extracting stimuli timings and subject response types

Step 1. Merge correct & incorrect trials chronologically + keep the first 128 trials

Step 2. Find exact time DIODE of current stimulus (= when subject sees symbol-digit combo)

IF current trial != last trial:

Stimulus duration → $t_{\text{CURRENT trial}} < t_{\text{DIODE}} < t_{\text{NEXT trial}}$

IF current trial = last trial:

Stimulus duration → $t_{\text{CURRENT trial}} < t_{\text{DIODE}} < t_{\text{CURRENT trial}} + 6 \text{ seconds}$

Step 3. Find which button + at what time it was first pressed

IF at least 1 BUTTON response recorded in $[t_{\text{DIODE}} ; t_{\text{NEXT trial}}]$:

Select the first button response

Step 4. Determine response type (ok, mistake, missed) & which of the two buttons was pressed

Result → Pandas DataFrame containing: trial type, epoch time, trial duration, response type, reaction time, and button press time

4.2 Data Preprocessing and Source Reconstruction

In this thesis, the starting point is the raw MEG data that was acquired as explained in section 4.1.1. Raw MEG data typically contains a large amount of artefacts and noise, meaning that the data needs to be cleaned before it can be used for further analyses [110]. To perform this processing, the Oxford Centre for Human Brain Activity (OHBA) Software Library (OSL) package written in Python by the OHBA Analysis Group, University of Oxford, UK [13], was applied. The OSL package provides state-of-the-art algorithms that allow running the entire processing pipeline on the raw MEG data for each subject in the SDMT dataset. Figure 4.4 shows the entire processing pipeline with all the steps that were implemented in OSL Python. In the following, each step is explained in more detail.

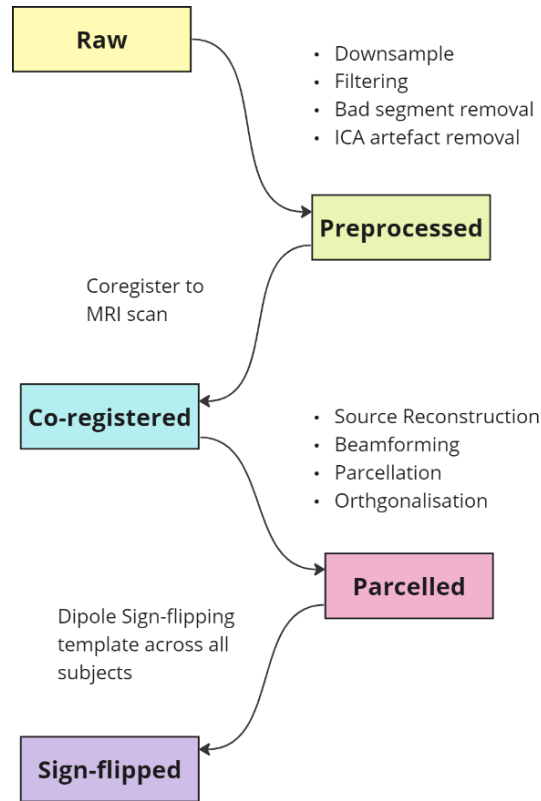


Figure 4.4: Overview of preprocessing and source reconstruction steps. First, the sensor-space data is cleaned through several preprocessing steps (downsampling, filtering, bad-segment/channel removal, and artefact removal). After co-registration of the MEG sensor’s spatial location to the head anatomy (MRI), the source-space data is estimated using a beamformer. Finally, the source data is parcellated, orthogonalised, and sign-flipped for dimensionality reduction and additional corrections.

Step 1: Preprocessing

The raw data was first downsampled from 1000 Hz to 250 Hz. Next, two filters were applied to reduce noise in specific frequency ranges: a 5th-order Butterworth bandpass filter from 0.01 – 70 Hz, and a notch filter centered at 50 Hz (width of 2 Hz). The bandpass filter removes non-neurophysiological signals, while the notch filter suppresses artefactual power line interference.

The small highpass cutoff (0.01 Hz) of the bandpass filter is different from 0 Hz, allowing to reduce the well-known baseline drift in MEG scanners [54].

To detect data segments or recording channels with poor quality, an automated bad-segment and bad-channel detection was performed. Both procedures are implemented using a generalized extreme Studentised deviate (ESD) test [111], where each window of 500 samples (= 2 seconds) is analysed separately. Bad segments, on the one hand, receive annotations such that they can automatically be discarded during, for example, the epoching of continuous MEG data (section 4.4). On the other hand, bad channels are first removed, and afterwards (as the very last preprocessing step) reconstructed. To further remove the remaining artefactual components, an automated independent component analysis (ICA) was performed. The goal is to remove ocular (e.g. eye blinking, eye movements) and cardiac artefacts, both of physiological nature, by correlating the time course of dedicated EOG/ECG channels with the extracted independent components. The components with the highest correlation are then automatically rejected. For a total of 306 sensors, the dimensionality is reduced to 64 independent components. It is only after the ICA is completed that the channels previously labelled as bad are reconstructed through interpolation.

As a small note, all the previously mentioned steps are automated and were successful in all but one subject from the SDMT dataset. The ICA failed in 1 subject due to missing ECG/EOG channels in the data. To work around this, artefactual components were removed by applying a manual ICA for that subject, for which extra details can be found in Appendix C.

Step 2: Co-registration to structural MRI

Before source reconstruction (step 3) can take place, it is crucial to ensure that the Polhemus headshape coordinates (acquired during MEG) and MRI coordinates are co-registered as accurately as possible. Figure 4.5 shows this co-registration process. The co-registration algorithm used in OSL Python is named Registration of Headshapes including Nose in OSL (RHINO). Compared to older co-registration algorithms, RHINO enables using a large amount of Polhemus points, and also includes rigid parts of the face (headshape points) and the nose (from the MRI surface) in the computations [112]. Altogether, this improves the alignment and prevents the obtained solution from getting stuck in local minima.

The mathematical implementation of RHINO is based on an iterative process called the Iterative Closest Point (ICP) method [113]. At each new iteration, a nearest-neighbour search using a K-D Tree algorithm will find the closest neighbour for each scalp surface point. This is followed by a least-squares best-fit, which computes the most optimal affine transformation from one space to the other [13]. The algorithm terminates either when it converges (see equation 4.1), or when all iterations have been executed. In the latter case, the optimal affine transformation is the one with the lowest Root Mean Square (RMS) value.

$$\text{abs}(\text{RMS}_{\text{previous}} - \text{RMS}_{\text{current}}) < \text{tolerance} \quad (4.1)$$

$$\begin{aligned} \text{RMS} &= \text{Room mean square} \\ \text{tolerance} &= 0.0001 \end{aligned}$$

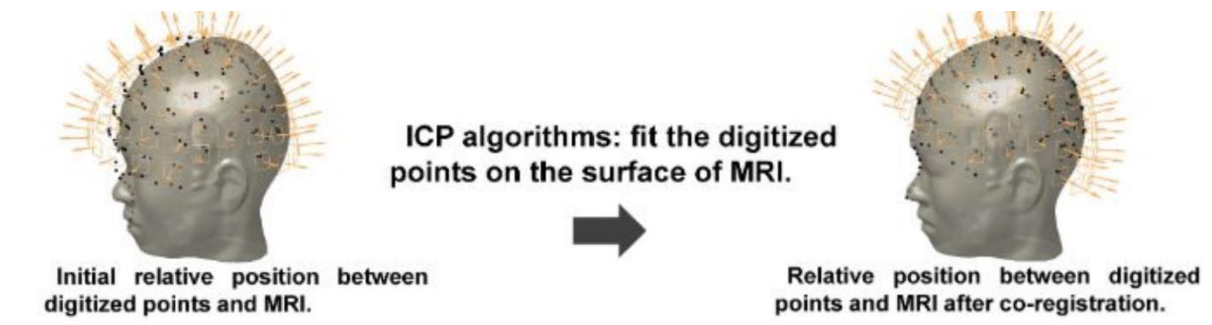


Figure 4.5: Co-registration using Polhemus. The digitized Polhemus points and fiducials are fit onto the outer surface of the scalp (MRI) using Iterative Closest Point (ICP) algorithms [114].

Step 3: Source reconstruction and parcellation

Source reconstruction consists of converting the neurophysiological data from sensor level to source level. The measured MEG signal is recorded from outside the brain, and the goal is to determine which brain area generated the detected brain activity. As previously mentioned in section 1.3.2, source reconstruction also helps to reduce the effect of volume conduction.

To implement source reconstruction, two types of problems need to be solved: the forward problem, and the inverse problem. Both problems and what space transition they form are shown in Figure 4.6. On the one hand, to solve the forward problem, a set of potential source locations, and a head model that captures both electromagnetic properties (e.g. conduction) and head anatomy, are required. On the other hand, the inverse problem is ill-posed, as any observed sensor signal can be caused by an infinite amount of different linear combinations of source contributions. Finding a solution to the inverse problem therefore requires an algorithm that provides estimates for the source data based on observed sensor data.

In OSL Python, the algorithm that solves the inverse problem is the Linearly Constrained Minimum Variance (LCMV) Beamformer [14]. Beamformers use the principle of spatial filtering, where the general idea is to maximise the signal power from a few specific sources, all the while minimising the signal interference from the remaining sources.

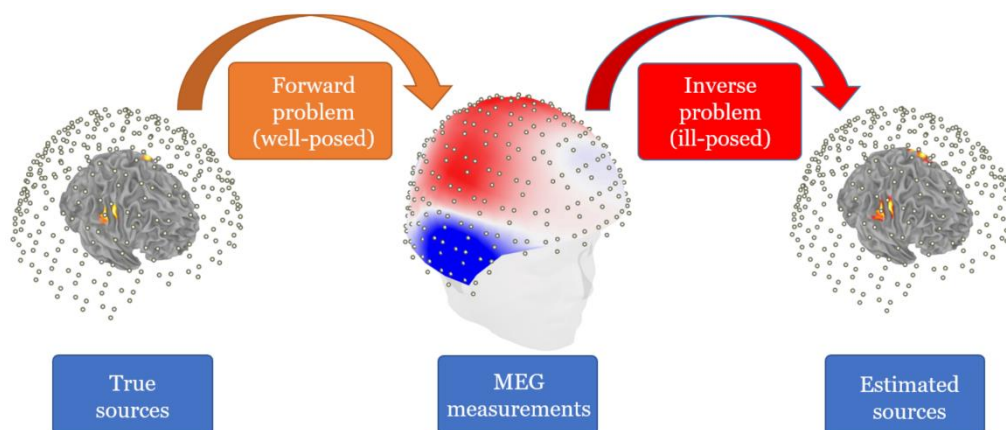


Figure 4.6: Forward and inverse problem in MEG [115].

One major consequence when applying source reconstruction is the increase in dimensionality (leading to >1000 sources). To facilitate further analyses while retaining the majority of the relevant information, the brain is parcellated using a parcellation atlas. The atlas used in this thesis is “fmri_d100_parcellation_with_PCC_reduced_2mm_ss5mm_ds8mm.nii.gz” (can be found in [13]), and it contains 38 parcels derived from an ICA of fMRI data from the Human Connectome Project [116]. Using the spatial basis method, each parcel’s time series becomes the first principal component (PC) from all voxels’ time series within that parcel, weighted by the spatial map’s coefficients.

Step 4: Orthogonalisation

Knowing that the inverse problem as part of source reconstruction is ill-posed, the reconstructed source data is likely to show artefactual temporal correlation between neighbouring voxels. This problem is known as source leakage. To correct for source leakage, OSL Python uses a symmetric multivariate leakage correction algorithm proposed by Colclough et al. [117]. This algorithm is applied to the parcelled data and uses an orthogonalisation procedure through singular value decomposition (SVD). The goal is to remove correlations at zero phase lag, while remaining as similar as possible to the original parceled time courses [117].

Step 5: Sign-flipping

So far, all the previous steps have been applied to single-subject data. However, this poses a problem for analyses where data from all subjects is concatenated to study FC (e.g. HMM, see section 4.5.1). This is because source reconstruction is characterised by an inherent dipole ambiguity problem. Indeed, the source reconstruction step is carried out separately for each subject (in LCMV Beamforming) which implies a risk of inconsistency in dipole polarity across subjects [20]. To solve this, Vidaurre et al. proposed a mathematical framework called sign-flipping.

First, the optimal template subject is determined. This template will be used to align the dipoles for all remaining subjects. The optimal template subject is determined by taking the median subject after calculating the similarity (using Pearson correlation) of each subject’s covariance matrix.

Secondly, all remaining subjects, one by one, go through a sign-flipping procedure to closer match the dipole polarities of the template subject. This is performed by flipping the time series’ sign over a limited amount of randomly selected parcels (= 20 selected parcels out of 38 total parcels) and comparing the similarity between the template’s and the current subject’s new covariance matrix. This is repeated for several iterations (= 500), after which the best flipping configuration (i.e. maximising the similarity) is applied to produce the final MEG time series per subject. The values for the number of iterations and maximum number of channels to flip during one iteration were taken from recommended values from example codes in the GitHub repository of OSL Python [13].

4.3 Behavioral Statistics

At first, several clinical characteristics of the study population in the SDMT dataset were investigated. An overview of the features of interest is shown in Table 4.1. Statistical group comparisons between HCs and PwMS were performed for these features. For continuous outcome variables, a non-parametric Mann-Whitney U-test was used [118]. For dichotomous outcome variables, a chi-squared (χ^2) test was employed [119]. The χ^2 test assumes that at least 80% of the expected contingency table's cells have a value equal to or larger than 5. In case this assumption was not fulfilled, the Fisher exact test was used [120].

Field name	Description
code	Subject code (to match the patient with MEG/MRI files)
age	Age at the moment of MEG recording (in years)
edu	Years of education (in years)
SDMT	Performance score from a prior SDMT on paper
EDSS	Expanded Disability Status Scale, a scale for the degree of disability in PwMS [121]
Isms	0 = HC / 1 = PwMS
gender_isfemale	0 = male / 1 = female
benzos	0 = no Benzodiazepines / 1 = yes Benzodiazepines
disdur	Disease duration (in years)
type	MS classification [122]: • NaN = Healthy Control • RRMS = Relapsing Remitting • SPMS = Secondary Progressive • PPMS = Primary Progressive • CIS = Clinically Isolated Syndrome

Table 4.1: Study population characteristics used for statistical analysis.

Additionally, several studies have reported that PwMS treated with benzodiazepines (BZDs) can show altered brain functioning [116],[123]. Considering that the knowledge of whether a certain MS subject has been treated with BZDs or not is available, group comparisons between BZD positive (BZDp) and BZD negative (BZDn) PwMS were also performed.

Further, it is relevant to assess whether certain patient groups show different task performance during the computerised SDMT. This was investigated by looking at two derived metrics: the success rate and reaction time (RT). A subject's success rate is calculated as the number of correct responses given by the subject divided by the total number of trials. The RT is the time between the stimulus presentation and the first recorded button press. Because plotting both outcome variables as histograms revealed skewed distributions, the non-parametric Mann-Whitney U-test was used for group comparisons. For the RTs, group comparisons were conducted separately for both the different trial types (correct/incorrect) and response types (ok/mistake).

4.4 Static analysis

The first major type of MEG analyses conducted in this thesis are static. As seen in section 1.3, static analysis methods aim to capture task-evoked neurophysiological activity inside a fixed time window around the stimulus onset. As this was the first time that an MEG dataset with the SDMT paradigm was analysed, the data analysis of this thesis started with a classic Event-related field (ERF) analysis. This was followed by an examination of the functional brain connectivity during the SDMT. The starting point for both analyses is the parcelled MEG data without orthogonalisation or sign-flipping. It was decided not to apply those last 2 processing steps, specifically for static analyses, considering their currently unknown impact on the results. As such, the impact of the sign-flipping algorithm on source-reconstructed MEG data was only recently investigated by Rossi et al. [124]. This way, traditional ERF and functional connectivity (FC) analyses are mimicked.

4.4.1 ERF

To perform MEG signal averaging across trials, a certain epoch window around the stimulus onset is considered. Since this dataset is unique, no existing literature has reported a suitable time window for conducting static analyses of MEG data recorded during an SDMT task. For this thesis, an epoch window of [-500,1000] ms w.r.t. to the stimulus onset was chosen. This interval is long enough to capture all stimulus-related brain responses, as well as provide sufficient pre-stimulus duration for applying baseline correction. The different ERF analyses conducted in this thesis can be divided into smaller 4 parts and are shown in Figure 4.7.

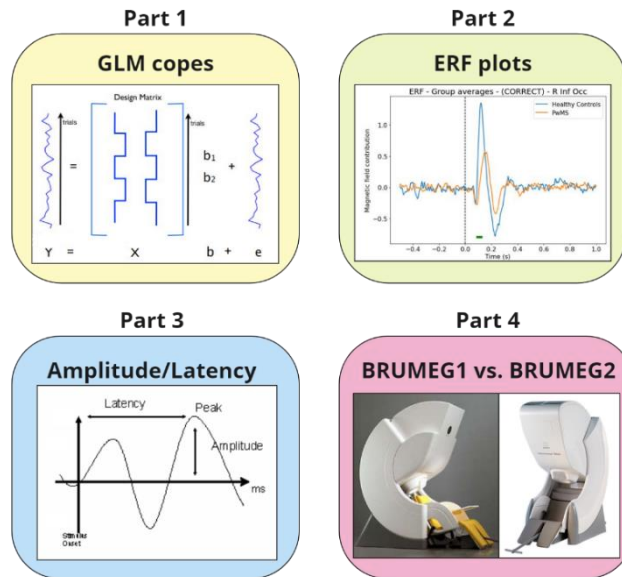


Figure 4.7: Overview of performed ERF analyses. Adapted from [106],[125],[126],[127].

Part 1: Generalized Linear Model (GLM) Contrast of Parameter Estimates (copes)

As a first exploratory step, the MEG data was used to fit a Generalized Linear Model (GLM). The GLM is a general statistical framework that is used to model the relation between an outcome variable and one or several determinants [128]. The linear equation of a GLM is shown in Figure 4.8. Taking into account the computerised SDMT design, the GLM design matrix consists of 3 regressors: 2 categorical regressors for the paradigm conditions (correct/incorrect stimulus), and 1 constant regressor (mean activity over the 2 task conditions) that serves as a baseline. For each regressor, the GLM optimises β weights through a maximum likelihood algorithm. To compare different experimental conditions in a 1st-level analysis, contrasts of parameter estimates (copes) were computed. The idea is to compare the β weights of different regressors (e.g. baseline versus correct stimuli) at each signal time point within the epoch window.

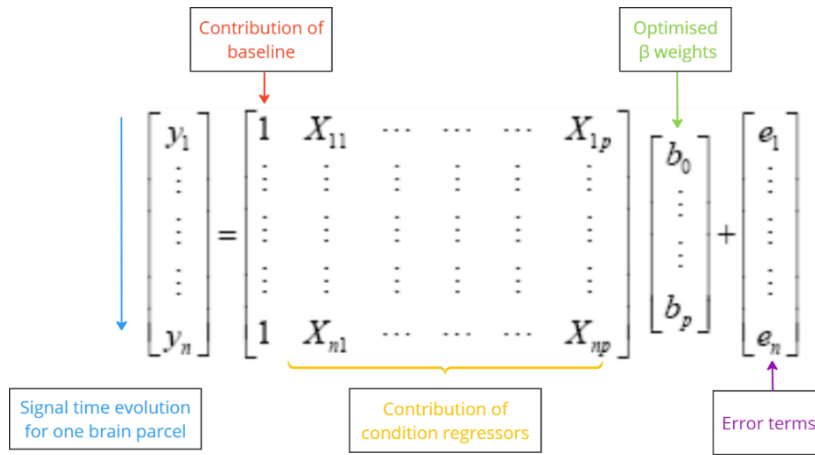


Figure 4.8: Matrix representation of a GLM.

This approach is useful for a first observation of the task-evoked waveform trends across all 38 parcels. To implement the GLM analysis, the OSL Python package was used. Visual inspection as well as a principal component analysis (PCA) on the GLM time courses allowed us to determine suitable time windows for an Amplitude/Latency analysis of the ERFs (see section 4.4.1 Part 3).

Part 2: ERF plots

Next, a standard ERF analysis was performed. For each subject, all epochs of the same paradigm condition (correct/incorrect) were averaged together. Each subject now has a grand average for all 38 parcels separately, for both correct and incorrect trials. To observe the global ERF trend, the time series across all parcels and all subjects were then averaged. Before averaging, each epoch was baseline-corrected using the signal in the pre-stimulus window.

Further, instead of taking the average across all subjects, the ERFs were averaged separately for the two cohorts of interest: HCs and PwMS. This allowed us to observe the difference in ERFs between both groups. To test for statistically significant group differences, a permutation test with max statistic was used [129]. In a non-parametric permutation test, a null-distribution generated by randomly dividing the subjects without replacement into groups is compared

against the observed statistic in the original groups (HCs and PwMS). The generated null-distribution and the observed statistic are shown in Figure 4.9. To evaluate the significance of the permutation test, the p-value was computed as the sum of permutations with an associated statistic that is larger than the observed statistic, divided by the total amount of permutations. For each group comparison of the ERFs, the t-statistic and 10 000 permutations were used.

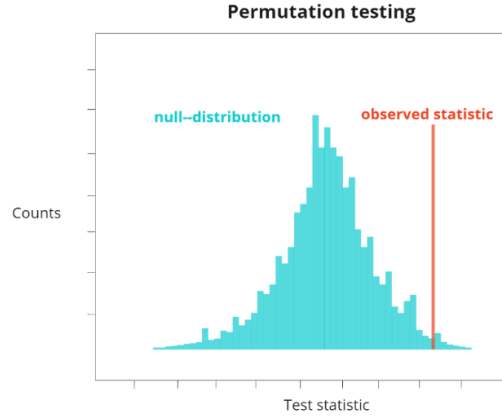


Figure 4.9: Example of a standard permutation test.

Because the group comparison was applied to each time point individually, an epoch window of $[-500, 1000]$ ms at a sampling rate of 250 Hz leads to 375 permutation tests. If the chosen significance threshold is 5% and the amount of conducted statistical tests increases, on average 5% of the tests will show false positives (i.e. rejection of the null hypothesis when in reality there is no effect) [130]. This is known as the multiple comparison problem. To work around this problem, the maximal t-statistic across time was considered [16], which has been found to control the familywise error in functional neuroimaging studies [131]. These group comparisons were performed for both: ERFs averaged across all parcels and parcel-specific ERFs.

Part 3: Amplitude/Latency

A common strategy to identify differences in evoked brain responses between several subject groups is through an Amplitude/Latency study of the ERFs. After several peaks in the ERF waveforms were identified (using the GLM copes analysis), the peak's absolute amplitude and latency w.r.t. to the stimulus onset were extracted for each subject's 38 parcel grand averages. The studied peaks can be found in Table 4.2. To investigate whether HCs and PwMS significantly differ in certain peak features, a permutation testing approach ($N = 10\,000$) was employed.

Peak name	Interval (ms)
P100	[70 – 215]
N100	[70 – 215]
P300	[220 – 360]
N300	[220 – 360]

Table 4.2: Peak names and intervals for the Amplitude/Latency study.

Part 4: BRUMEG1 vs. BRUMEG2

As mentioned in section 4.1.1, the MEG recordings for the SDMT dataset started with the Elekta Neuromag Vectorview scanner (BRUMEG1, 33 subjects) before transitioning to the upgraded Elekta Neuromag Triux scanner (BRUMEG2, 77 subjects). To conclude the ERF analysis, the grand averages for BRUMEG1 and BRUMEG2 were compared for HCs and PwMS separately. Additionally, to investigate whether the scanner upgrade causes a frequency-specific change in sensor sensitivity, the Power Spectral Density (PSD) for BRUMEG1 and BRUMEG2 subjects was compared.

4.4.2 Functional Connectivity

One way to measure how different brain regions communicate during the execution of a task is through functional connectivity (FC) analysis. More specifically, synchronous firing of a large group of neurons at certain frequencies is known to indicate FC within brain networks.

In this thesis, the Coherence metric was employed to study the FC during the SDMT. An overview of the two analysis branches is shown in Figure 4.10. First, the global coherence networks for all 38 parcels averaged across subjects were computed (upper branch). As neurological disorders such as MS can disrupt brain functions [132], a statistical group comparison between the coherence networks of HCs versus PwMS was performed to identify connections with disrupted oscillatory communication in MS (lower branch).

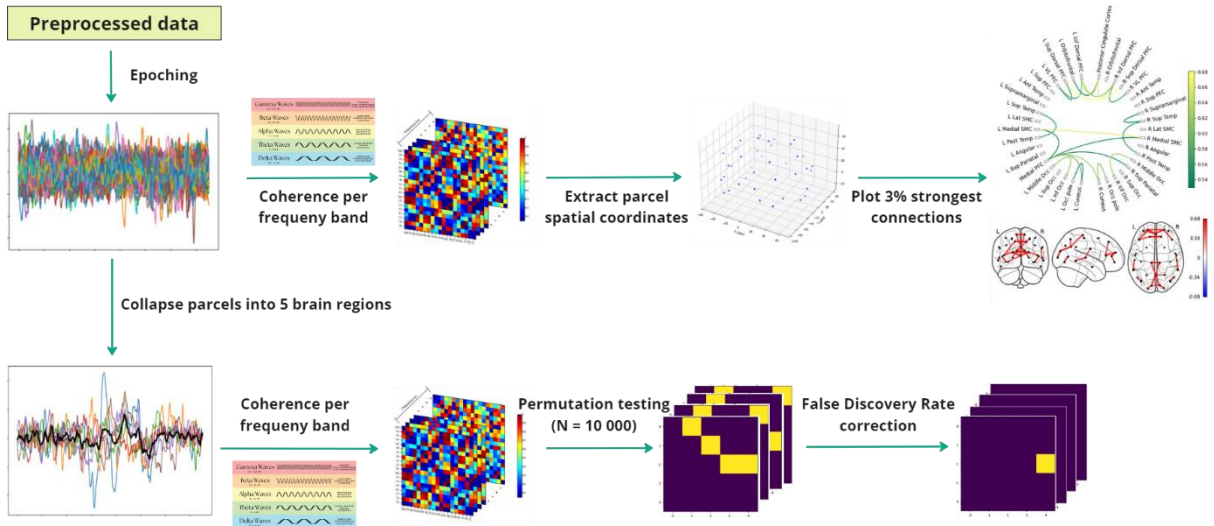


Figure 4.10: Overview of the FC analysis. Adapted from [133],[134].

Part 1: Global Coherence Networks

Coherence is an FC metric that summarises the strength of oscillatory synchronicity between the signal of 2 parcels at a certain frequency. The formula for computing the coherence is shown in equation 4.2. To calculate the coherence matrices (38x38 with 38 as the number of brain parcels) for each subject using their ERF grand averages, the *spectral_connectivity_epochs()* function from the MNE Python package was used [15].

$$\text{Coh}_{xy}(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f) \cdot S_{yy}(f)}} \quad (4.2)$$

$S_{xy}(f)$ = Cross spectrum between parcel x and parcel y at frequency f
 $S_{xx}(f)$ = Power spectrum magnitude of parcel x at frequency f

In M/EEG literature, coherence is often computed over the traditional frequency bands of brain waves [132]. Computing the coherence across a specific frequency range is achieved by averaging out the coherence values of each frequency bin within the considered frequency range. The 5 traditional frequency bands used in this thesis can be found in Table 4.3.

Brain wave	Frequency band (Hz)
Delta	1 – 4
Theta	4 – 8
Alpha1	8 – 10
Alpha2	10 – 13
Beta	13 – 30

Table 4.3: Brain wave frequency bands for the FC analysis.

To report the coherence networks for each of the SDMT paradigm conditions (correct/incorrect) and frequency bands (delta/theta/alpha1/alpha2/beta), two visualisation methods were used: circular plots and brain masks. Both are shown in Figure 4.11. For the circular plot, all parcel names are placed on the circle in an order that is determined by the parcels' spatial coordinates in the parcellation atlas (see section 4.2). More specifically, the y-coordinate of each parcel's central voxel is ordered from low to high. Additionally, the left and right sides of the circle correspond to the separation between both brain hemispheres. The resulting circular plot shows an axial view of the brain where the parcels are ordered from anterior (circle top) to posterior (circle bottom).

Using the brain mask approach, the coherence connection strengths can be visualised in the coronal, sagittal and axial planes. For both visualisation methods, only the 3% strongest connections were plotted.

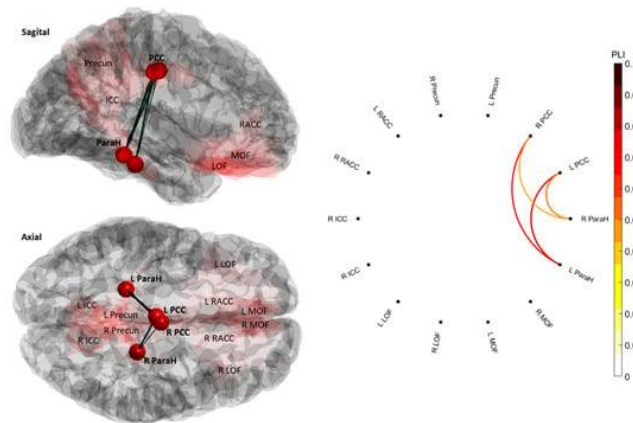


Figure 4.11: Visualisation of FC using a brain mask (left) or circular plot (right) [135].

Part 2: Group comparisons

To perform group comparisons on the subject’s coherence matrices, a separate statistical test is needed per unique connection. According to equation 4.3, data with $N = 38$ parcels has 703 unique connections between two nodes. This again leads to a multiple comparison problem, where any observed statistically significant difference doesn’t survive after the multiple comparison correction step. Another issue is that multiple comparison correction algorithms assume each statistical test to be independent, which is not exactly true in this scenario. Therefore, the 38x38 coherence matrices were not suited to perform group comparisons between HCs and PwMS.

$$\# \text{ unique connections} = \frac{N \cdot (N - 1)}{2} \quad (4.3)$$

An alternative approach consisted of reducing the dimensionality of the coherence matrices. This was achieved by using a mathematical operation to collapse the ERF time series from multiple original parcels into a single brain region. Table 4.4 shows which brain parcels were combined to form new occipital, parietal, temporal, frontal, and medial brain regions. To collapse the ERF time series of parcels, the average of the subject grand average across parcels was taken. Since the new amount of brain regions is 5, the amount of unique connections is reduced to 10 (see equation 4.3).

To perform the actual group comparisons, a permutation testing approach was applied to each unique connection. The p-values were corrected for multiple comparisons by the False Discovery Rate (FDR) [136]. The main idea is to limit the amount of false positives by adjusting the p-values through the Benjamini-Hochberg procedure. This procedure involves ordering and ranking all the raw p-values, and adjusting them one-by-one using a set of simple mathematical conditions. The group comparisons were repeated for the different paradigm conditions and frequency bands.

4.5 Dynamic analysis

Going beyond standard static analyses, it was chosen to use an HMM approach to learn more about the spontaneously occurring networks underlying the measured MEG data from the SDMT dataset. This is a dynamic approach, meaning no fixed time window around the task onset needs to be specified. This powerful HMM approach allows us to extract recurrent patterns of network activity in a fully data-driven way [20].

In this section, the HMM implementation tailored to be compatible with the study of whole-brain neurophysiological data is explained. First, the principles of the Time-Delay Embedded HMM (TDE-HMM) approach are summarised. Secondly, the analysis methods used to characterise the extracted hidden states are described in detail. Additionally, the latter includes explanations on how group comparisons between HCs versus PwMS were carried out.

Collapsed brain region	Original parcels
Occipital	L Inferior Occipital
	R Inferior Occipital
	L Superior Occipital
	R Superior Occipital
Parietal	L Superior Parietal
	R Superior Parietal
	L Angular
	R Angular
Temporal	L Superior Temporal
	R Superior Temporal
	L Anterior Temporal
	R Anterior Temporal
Frontal	L Superior Prefrontal
	R Superior Prefrontal
	L Superior Dorsal Prefrontal
	R Superior Dorsal Prefrontal
	L Orbitofrontal
	R Orbitofrontal
	L Inferior Dorsal Prefrontal
	R Inferior Dorsal Prefrontal
Medial	Medial Prefrontal
	L Cuneus
	R Cuneus
	Posterior Cingulate

Table 4.4: Original parcels collapsed into 5 brain regions by an averaging operation.

4.5.1 Model specification

To ensure the use of state-of-the-art algorithms that have proven to be successful in describing brain dynamics patterns during other tasks [16],[17], the HMM implementation from the OSL Dynamics Toolbox was selected. This Python package is written by the OHBA Analysis Group, University of Oxford, UK [18]. OSL Dynamics has two HMM implementations: Amplitude Envelope (AE) and Time-Delay Embedding (TDE) HMM, which refer to how the data is prepared prior to HMM inference.

For this thesis, it was decided to use the TDE data preparation, as it allows for a more complete 3-dimensional characterisation of transient functional brain networks on the temporal, spatial, and spectral levels [20]. By considering time-lagged versions of the original 38 parcels' time series, the autocorrelation function (ACF) of this original data is included in the data's covariance matrix [18]. The advantage is that the covariance matrix now becomes sensitive to oscillatory activity from the original data, allowing us to extract brain states with distinct spectral phase-locking characteristics [20].

How is the data prepared?

Before the processed continuous MEG data (see section 4.2) could be given as input to the HMM for inference of the hidden state time courses, a few data preparation steps were required. Figure 4.12 gives an overview of those preparation steps. A major difference between static analyses (see section 4.4) and this dynamic analysis, is that the MEG data should now be orthogonalised and sign-flipped. As described in section 4.2, orthogonalisation is used to reduce source leakage in the source-reconstructed MEG time series. Although this approach reduces zero-lag (or small-lag) interactions between several parcels, it only does so on average across the entire time series [20]. This means that after orthogonalisation, there will still be transient periods of small-lag synchronisation that can be captured by calculating the coherence [20]. On the other hand, sign-flipping is essential because the MEG data used for HMM inference were concatenated across all subjects (see section 4.2).

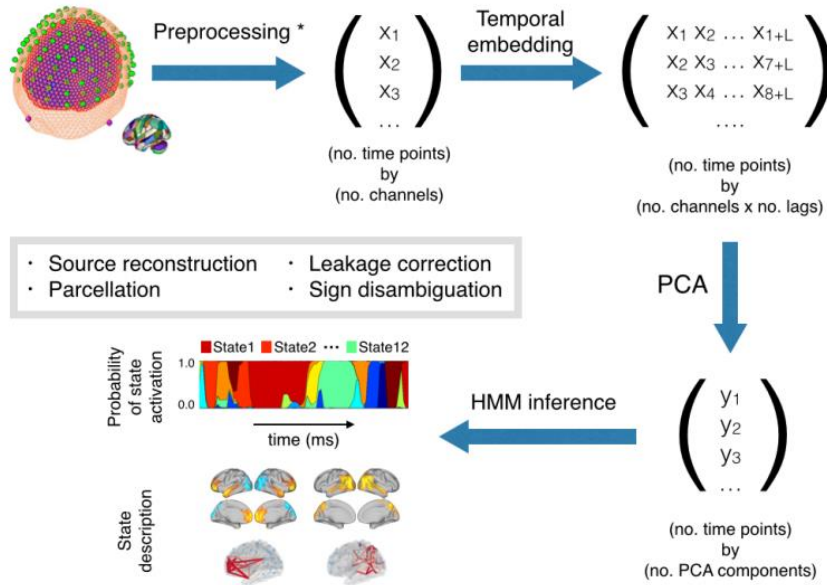


Figure 4.12: Overview of the TDE-HMM method [20].

In the next step, all 38 parcel time series were time-delay embedded (TDE) over a certain amount of time lags. For example, a time-delay embedding at time lag +1 means that the entire original time course is shifted by 1 time point to the right. In this thesis, 15 embeddings $[-7, -6, \dots, +6, +7]$ were used, which has been found to work well for several datasets [17],[20],[116]. After TDE, the data consists of 38 parcels x 15 embeddings = 570 channels.

To reduce both the risk of model parameter overfitting and the computational cost, the dimensionality of the TDE data was reduced by applying PCA. The appropriate number of principal components (PCs) is at least twice that of the number of parcels in the original data (e.g. $38 \times 2 = 76$) [20]. The number of PCs is an additional hyperparameter, as it can determine the HMM to be more sensitive towards lower or higher frequencies. As such, a lower number of PCs can bias the HMM to lower frequencies, because low frequencies provide a higher explainability for variance in the data [16]. Finally, the data is standardised (i.e. normalised to zero mean and unit variance). After these preparation steps, this formed the observed data that was given as input to the HMM for inference.

How are the hidden states inferred?

The inference scheme (i.e. learning the HMM model parameters) is summarised in Figure 4.13.

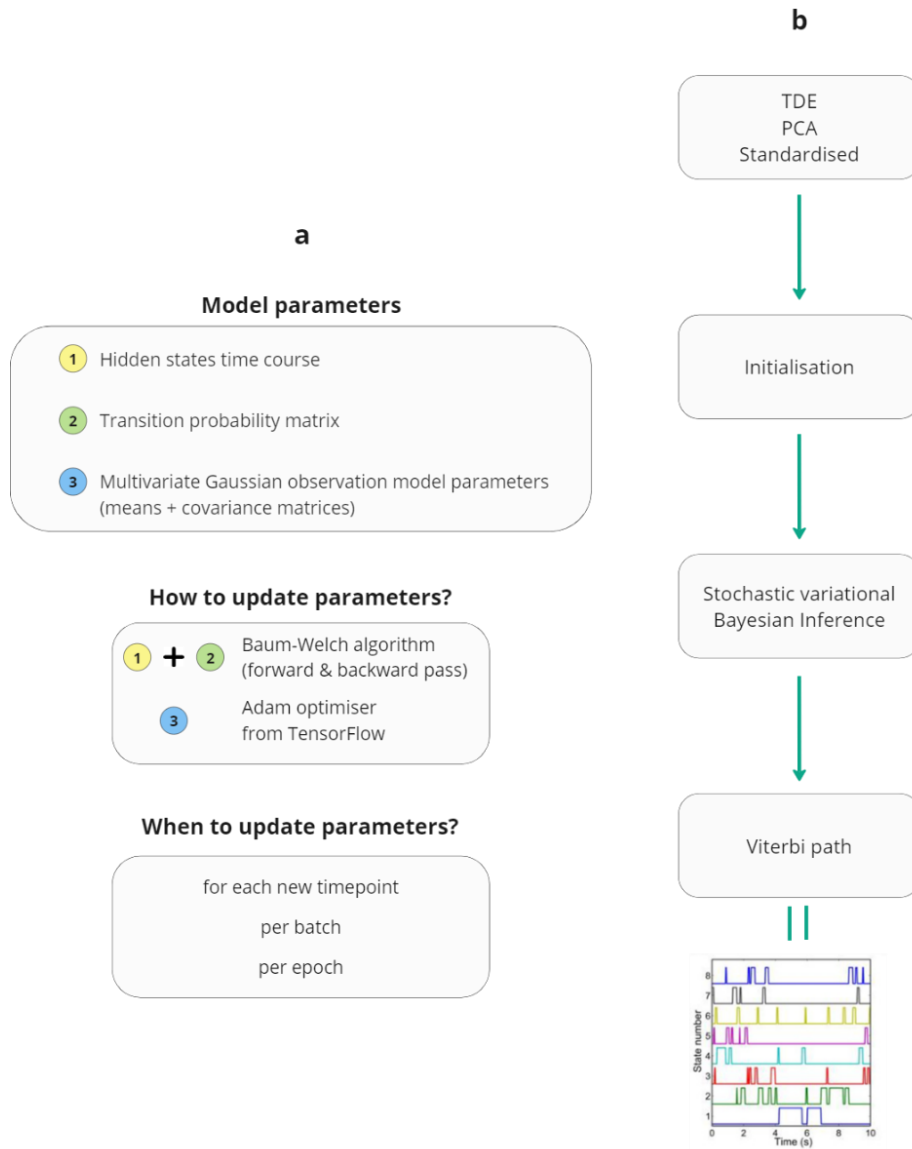


Figure 4.13: TDE-HMM inference (i.e. learning the model parameters). (a) The three types of model parameters, update algorithms, and update iterations. (b) Overview of the steps during model training. Adapted from [80].

The HMM model parameters that are learned from the observed data (TDE + PCA + standardised MEG data) are the following:

1. **Hidden states time course.** Each time point in the observed data sequence is assigned a probability for a certain state being active, and this for each state. The Markovian assumption, the fact that a specific state at time point t only depends on the state at time point $t-1$, is of high importance. The states' time course after HMM inference is the only important output required for further analyses (see section 4.5.2).
2. **Transition probability matrix.** Describes the probabilities of transitioning from one state at time point $t-1$ to the state at the current time point t .
3. **Observation model parameters.** An observation model defines the link between the hidden states and the observed data. In the past decade, the multivariate autoregressive (MAR) observation model was quite popular [81]. A major limitation of the HMM-MAR is that the amount of model parameters scales very strongly with the number of brain parcels, which makes it inefficient for whole-brain data. The TDE-HMM implementation used in this thesis works with an alternative observation model: a multivariate Gaussian. Figure 4.14 shows the principle of an observation model using state-specific univariate Gaussian distributions. Each state has a separate observation model, which is the probability distribution that the observed data at a given time point does indeed come from that hidden state. The dimensionality of the multivariate Gaussian will be the same as that of the observed data space (= number of PCs). Using the state-specific observation models, each state can be described as state means and covariances. Importantly, when using the TDE-HMM, only the dynamics from the covariance matrices are modelled (i.e. the state means are set to zero) [18]. This is because the differences in FC patterns between different states are not contained within the mean signal amplitude.

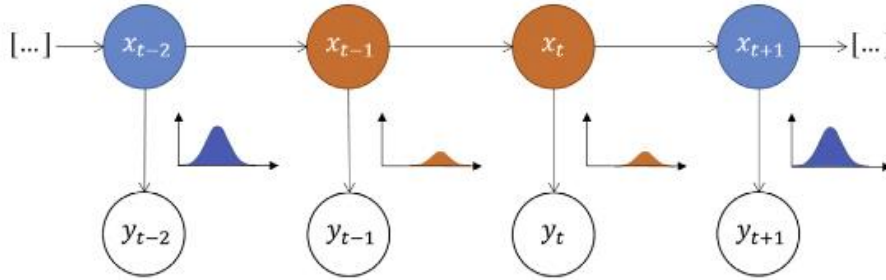


Figure 4.14: Diagram of a simple HMM. At each new time point, an observation y_t (e.g. a simple time point from MEG data) and an associated hidden state x_t . Each observation is assumed to be drawn from a state-specific Gaussian distribution, characterised by a mean and standard deviation. This model only uses 2 states (blue and orange) with univariate Gaussian observation models [83].

Before the HMM model parameters can be learned, an initial distribution of those parameters at time point 0 is required. To obtain a good starting point for the full training, a small amount of random initialisations are performed. At each initialisation, a random transition probability matrix is created. This is followed by an iterative update of the three types of HMM model parameters for 1 epoch (i.e. a single iteration over the entire observed data). Next, the initialisation (and its corresponding HMM model parameters) with the lowest Free Energy is retained. The Free Energy is the cost function that is minimised during HMM inference [137].

With a good initial model parameter distribution, the HMM can be trained by using stochastic variational Bayesian inference [19]. In simple terms, the entire data is divided into small batches of a certain time length, which are used to train and update the model parameters iteratively. By using the Baum-Welch algorithm [138] and Adam optimiser [139], the HMM model parameters are tweaked to minimise the Free Energy cost function. Over time, the Free Energy loss will converge, resulting in the HMM model parameters that can best explain the observed data.

At the end of training, the hidden states' time course corresponds to the probability of each state activating at each separate time point. To ensure that the mutual exclusivity of the hidden states in HMMs is respected [16], each time point is assigned only one state. This conversion from probabilistic to categorical association is known as the Viterbi path [140], where the state with the highest activation probability becomes the only state assigned to that time point. Those categorised hidden state time courses are the starting point for further analyses (see section 4.5.2).

Both in the TDE data preparation process as well as HMM inference, several hyperparameters have to be specified. An overview of those hyperparameters and the values for which the TDE-HMM was run in this thesis are shown in Table 4.5. This work used the same parametrisation as proposed by Vidaurre et al. [20]. Additionally, to verify the robustness of the HMM inference (i.e. whether running the model for a different amount of states provides states with a similar description), the inference was run twice, for 6 and 8 states.

HMM process	Hyperparameter	Value
TDE data preparation	embeddings	15
	PCs for PCA	80
Inference	hidden states	6
	random initialisations	3
	sequence length	2000
	learning rate	5e-4
	batch size	64
	epochs	200

Table 4.5: Chosen hyperparameters for the data preparation and inference process [20].

4.5.2 Functional brain network characterisation

Starting from the categorised hidden state time courses inferred by the TDE-HMM, there exist several ways to compute and study the properties of the HMM states [16]. Figure 4.15 shows an overview of the analysis methods used to characterise the state's properties on 3 levels: temporal, spatial, and spectral. In the following, each post hoc analysis method that helps towards describing the properties of the inferred dynamic functional networks is explained in detail.

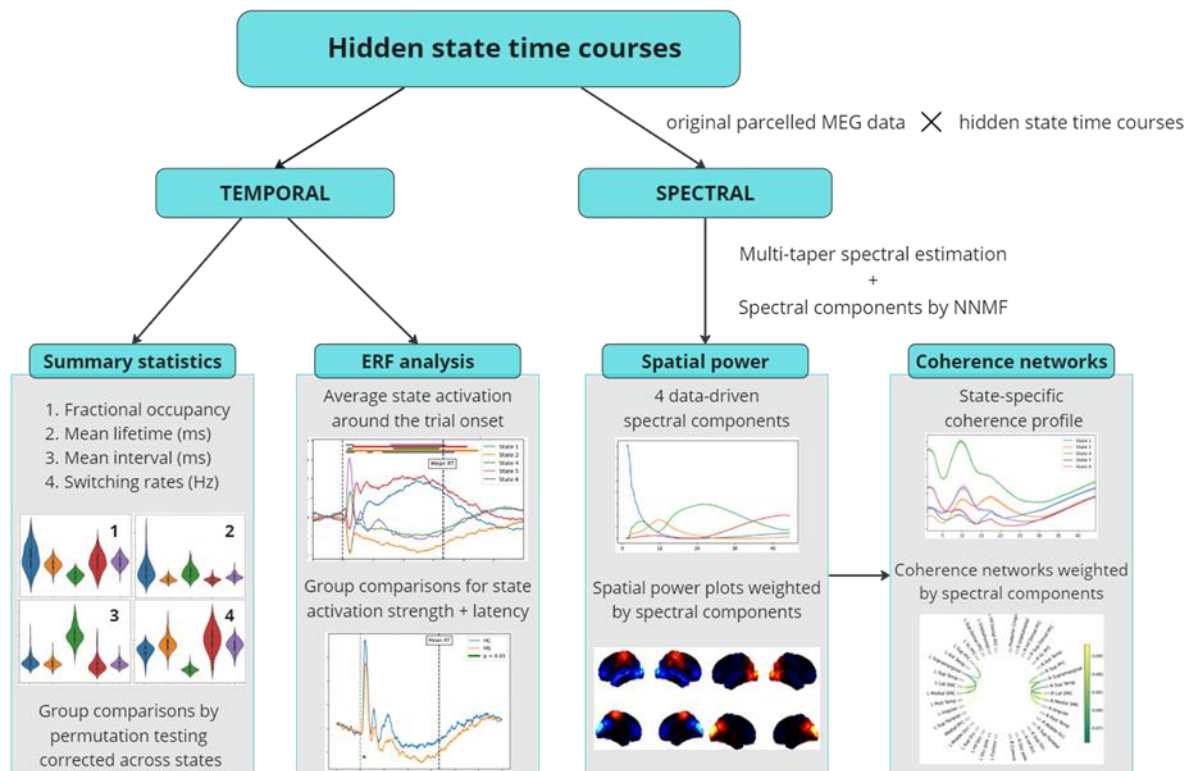


Figure 4.15: Overview of the different analysis methods after inference of the hidden state time courses.

Part 1: Summary statistics

A first analysis of the hidden state time courses consisted of computing summary statistics that describe the general dynamics of the states' activation. Four metrics were computed: fractional occupancy (FO), mean lifetime (MLT), mean interval (MI), and switching rate (SR) of each state [18]. Table 4.6 provides an explanation of what each metric represents. These metrics were computed on subject-level, which allowed us to perform group comparisons in these metrics between HCs and PwMS. For each of the four summary statistics, a permutation test with maximal statistic ($N = 10\,000$) was used. The maximal t-statistic was taken across the different states.

Metric	Description
Fractional occupancy	Fraction of total recording time spent in a particular state.
Mean lifetime	Average time duration of a state visit.
Mean interval	Average time duration between successive state visits.
Switching rate	Average number of visits to a state per second.

Table 4.6: Description of summary statistics [18].

Part 2: Temporal ERF analysis

Working with task-MEG data allows us to investigate how the different states (de-)activate while the subject is performing the SDMT. To do so, an ERF analysis of the hidden state time courses was performed. For the epoching of the hidden state time courses, the guidelines from OSL Dynamics example tutorials were followed [18]:

- Start from the epoch timings in the original MEG data.
- Reject epochs with a bad-segment annotation (see section 4.2).
- Keep the same total number of epochs across all subjects ($N = 128$).
- Pad-lock the hidden state time courses (i.e. align them to the original MEG time course). This is required as the TDE preparation causes a small misalignment due to the introduced time lags.
- Epoch the hidden state time courses between $[-500, 3000]$ ms. The choice for a larger epoch window is justified considering the mean reaction time (RT) for the SDMT stimuli lies at around 1.75 s post-stimulus (see Table 5.3 in chapter 5 Results). Given this mean RT, it was anticipated that certain brain states would remain activated at least up until that point during the trial.
- Average all trials separately for the different paradigm conditions (correct/incorrect).
- Baseline-correct using the pre-stimulus $[-500, 0]$ ms data.

To determine if the activation of states is significant at individual time points within the epoch window, a max stat permutation test ($N = 1000$) was used. Here again, multiple comparisons were corrected across states. Additionally, the subject-level grand averages were used to perform group comparisons between HCs and PwMS for each state separately. Similarly as described previously, a max stat permutation test ($N = 1000$) was used. Lastly, the same type of test was used to verify whether there are time points with statistically significant differences in state activations between correct and incorrect trials across all subjects.

Part 3: Spatial power

As mentioned in section 4.5.1, the TDE-HMM enables the extraction of states based on the data's transient spectral characteristics [20]. However, the PCA in the data preparation step can bias the HMM inference to lower frequencies. To solve this problem, a non-parametric multitaper spectral estimation was used for each state separately [81]. This method takes the original parcelled MEG data multiplied by the hidden state time course as input, and produces Power spectral densities (PSDs) as output. The spectra are estimated for the wideband frequency range $[1-45]$ Hz. To quantify phase-coupling between all brain parcels, the PSDs were used to compute the coherence at each frequency bin. According to Vidaurre et al., the coherence metric showed higher robustness compared to other phase-coupling metrics (e.g., phase-locking value) in this application [20].

Similar to the static FC analysis (see section 4.4.2), dividing the entire frequency range into smaller bands leads to easier interpretation of results. To estimate the relevant frequency bands in a data-driven manner, the non-negative matrix factorisation (NNMF) approach proposed by Vidaurre et al. was used [20]. The NNMF is applied to the coherence spectra and returns a pre-specified amount of spectral components (SCs), i.e. common frequency bands of coherent brain activity [18]. In this work, this spectral decomposition was performed for four SCs, as suggested in [20]. To decide which SC best describes the spectral properties of a certain state, first the mean PSD and mean coherence (averaged across all subjects and brain parcels) as a function of frequency for that state were plotted. Next, similarity was sought in the frequency ranges between each data-driven SC and the frequency range of the strongest peak in both the mean PSD and mean coherence plots. For example, if state 1 showed a strong peak in mean PSD and mean

coherence between 8-12 Hz, the SC with a spectral profile contained around that same frequency range was chosen as the most representative SC for state 1.

To plot spatial maps of the spectral power, each state's PSD was weighted with the most relevant SC for that state. Next, the weighted power was averaged across all subjects, and subtracted by the mean power to remove the baseline power present in all states. Further, group differences between the PSDs of HCs and PwMS were conducted using the max stat permutation test ($N = 1000$).

Part 4: Coherence networks

As previously mentioned, the coherence networks are computed using the state-specific PSD, weighted by the SC most representative of that state. The coherence networks were shown through two visualisation methods: circular plots and brain masks (see section 4.4.2). Only the 2% strongest connections were shown.

After all the previously described analyses were conducted, the 3-dimensional characterisation of the states allowed us to investigate the following questions:

- When and to what extent do the different states activate?
- Where in the brain do these states activate?
- Which brain regions form dynamic functional networks involved in the activation of these states?

Chapter 5

Results

This chapter presents the results obtained through applying the previously described analysis methods from the Methodology chapter. Starting from the raw MEG data, preprocessing was applied, followed by the analysis methods outlined in chapter 4: Behavioral Statistics, ERF analysis, FC analysis, and TDE-HMM analysis. The entire work was conducted in the programming language Python. Appendix D provides more details about the written Python code.

5.1 Behavioral Statistics

The demographic and clinical characteristics of different sub-groups in the SDMT dataset are shown in Table 5.1 and Table 5.2. Regarding the comparison between HCs and PwMS, these groups are matched on gender (χ^2 test, p-value = 0.18) and age (Mann-Whitney U-test, p-value = 0.84). The mean education level (Mann-Whitney U-test, p-value = 0.005) and SDMT score (Mann-Whitney U-test, p-value = 0.007) are significantly higher for HCs compared to PwMS in this dataset.

	Controls	PwMS	p-value
N	37	73	N/A
% Female	59.46	73.97	0.18
Age (years)	47.38 $\pm$ 11.82	48.17 $\pm$ 9.86	0.84
Education (years)	15.16 $\pm$ 2.22	13.79 $\pm$ 2.73	0.005*
SDMT score	54.51 $\pm$ 9.4	48.6 $\pm$ 10.74	0.007*
Disease dur. (years)	N/A	16.66 $\pm$ 9.78	N/A
EDSS	N/A	3.22 $\pm$ 1.49	N/A

Table 5.1: Demographic and clinical characteristics of the entire SDMT dataset (mean $\pm$ std).

The BZDp and BZDn groups in PwMS significantly differ in terms of gender (Fisher exact test, p-value = 0.004) and EDSS score (Mann-Whitney U-test, p-value = 0.040). The Fisher exact test had to be used for the gender variable because all BZDp subjects in the final SDMT dataset are females. Considering the significant difference in EDSS, this means that PwMS who are already treated with BZDs have a higher average degree of disability.

	BZDp	BZDn	p-value
N	18	55	N/A
% Female	100.0	65.45	0.004*
Age (years)	48.07 $\pm$ 7.97	48.2 $\pm$ 10.47	0.94
Education (years)	13.06 $\pm$ 2.58	14.04 $\pm$ 2.76	0.26
SDMT score	47.11 $\pm$ 7.51	49.09 $\pm$ 11.62	0.49
Disease dur. (years)	13.39 $\pm$ 6.77	17.73 $\pm$ 10.41	0.11
EDSS	3.75 $\pm$ 1.13	3.05 $\pm$ 1.56	0.040*

Table 5.2: Demographic and clinical characteristics of PwMS based on administered benzodiazepines (BZDs) (mean $\pm$ std).

The distribution of success rates during the computerised SDMT (i.e. the accuracy of the given response) for different sub-groups is shown in Figure 5.1. HCs have significantly higher success rates compared to PwMS (Mann-Whitney U-test, p-value = 0.00696). Overall, the majority of subjects only make a few or no mistakes throughout the total 128 trials.

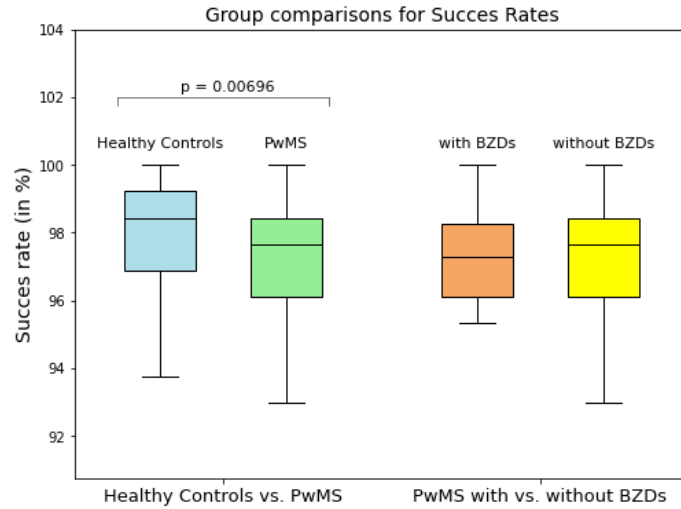


Figure 5.1: Group comparisons of success rates from the subjects' responses during the computerised SDMT.

Regarding the reaction times (RTs) to the presented stimuli, Table 5.3 shows the mean RTs per paradigm condition (correct/incorrect). When subjects are presented with an incorrect stimulus (mean RT = 1.814 s), their average RT is slower compared to when correct stimuli are presented (mean RT = 1.668 s). The results of group comparisons for the different paradigm conditions (correct/incorrect) and response types (ok/mistake) are shown in Table 5.4. The RT differs significantly between the HCs and PwMS groups for both correct stimuli (Mann-Whitney U-test, p-value = $4.9 \cdot 10^{-51}$) and incorrect stimuli (Mann-Whitney U-test, p-value = $1.7 \cdot 10^{-56}$), specifically for correct subject responses (response type = ok). Although the RTs for mistake responses also lead to p-values < 0.05 , these results were not used to draw any conclusions due to very small sample sizes. Considering the BZDp and BZDn groups, only the RT for correct stimuli significantly differ between those two groups (Mann-Whitney U-test, p-value = 0.003), again only considering the correct subject responses (response type = ok). The mean RTs for mistake responses are higher compared to correct responses, indicating it was indeed important to separate the analysis based on the response type.

MEAN Reaction time (s)	
Correct trials	1.668
Incorrect trials	1.814

Table 5.3: Mean RT for the two paradigm conditions (correct/incorrect) averaged across all trials and all subjects.

Trial type	Response	HC	PwMS	p-value	BZDp	BZDn	p-value
Correct	ok	1.52 ± 0.46	1.72 ± 0.52	$4.9 \cdot 10^{-51*}$	1.77 ± 0.55	1.72 ± 0.52	$0.003*$
	mistake	1.61 ± 0.48	1.91 ± 0.57	$0.002*$	1.62 ± 0.51	1.92 ± 0.57	$0.006*$
Incorrect	ok	1.65 ± 0.55	1.89 ± 0.66	$1.7 \cdot 10^{-56*}$	1.92 ± 0.67	1.89 ± 0.66	0.24
	mistake	1.71 ± 0.60	1.99 ± 0.71	$0.029*$	1.85 ± 0.57	1.99 ± 0.71	0.47

Table 5.4: Group comparisons of RTs for the two paradigm conditions (correct/incorrect) and separated based on the subject's response (ok/mistake) to the stimuli (mean $\pm$ std).

5.2 ERF analysis

The GLM copecs of paradigm conditions (correct/incorrect) versus baseline (i.e. mean across both paradigm conditions) at population-level are shown in Figure 5.2. Strong cope values can be observed in several time ranges for both paradigm conditions. More specifically, 100, 150, and 300 ms post-stimulus show the strongest cope values. Additionally, one of the parcels shows quite high cope values around 600 ms post-stimulus.

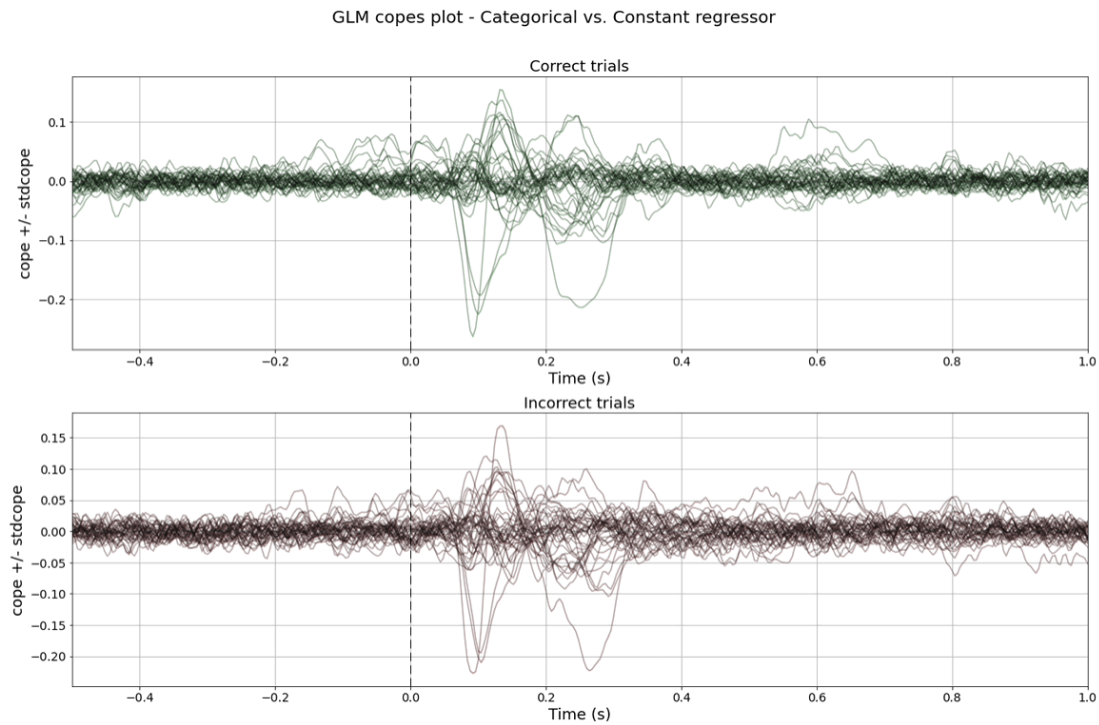


Figure 5.2: GLM copecs plots of the categorical (correct/incorrect) versus constant (baseline) regressor for correct trials (top) and incorrect trials (bottom) averaged across all subjects. Per paradigm condition, the cope time series for all 38 parcels are superimposed in the plot.

Further, a PCA was applied to the GLM time courses to obtain the average trend across all parcels. Figure 5.3 shows the results for the correct trials as categorical regressor, where only the 3 principal components (PCs) with the highest variance explainability are plotted. PC1 shows large peaks at approximately 100, 150, and 250 ms following the stimulus onset. PC2 displays peaks at around 100 and 200 ms post-stimulus, while PC3 shows a comparatively lesser peak amplitude around 150 ms post-stimulus. These preliminary results clearly show early post-stimulus neural responses to the task, which validate the data preprocessing and stimulus extraction steps.

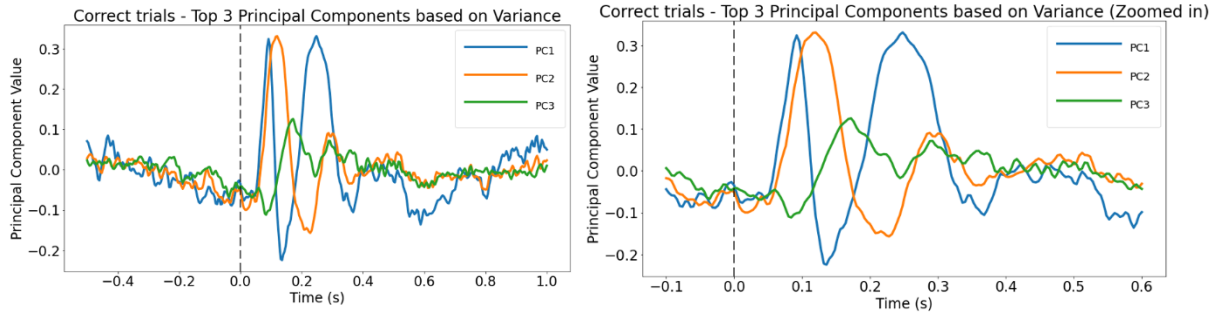


Figure 5.3: Time series of the 3 principal components (PCs) with the highest variance explainability resulting from a PCA on the GLM copes (correct trials). Full [-500,1000] ms epoch window (left), and zoomed in [-100, 600] ms window (right).

Figure 5.4 shows the global ERF waveforms (i.e. averaged across all trials of the same type, subjects, and parcels) obtained through trial-averaging of the parcelled MEG data, separated based on the paradigm condition. A clear waveform consisting of successive peaks and troughs can be observed: negative peak at 100 ms, positive peak at 150 ms, negative peak at 250 ms, positive peak at 350 ms, and a negative peak at 500 ms post-stimulus. A visual comparison between the global ERFs of both paradigm conditions did not reveal any major differences. Figure 5.5 shows the same global ERFs but now separated for the HCs and PwMS groups. The peaks at 150 ms and 250 ms post-stimulus show a smaller magnitude for PwMS in comparison to HCs. In contrast to HCs, PwMS do not display the peak at 500 ms post-stimulus for the incorrect trials. A permutation test with max statistic ($N = 10\,000$) did not reveal any time points with significantly different amplitudes between both groups.

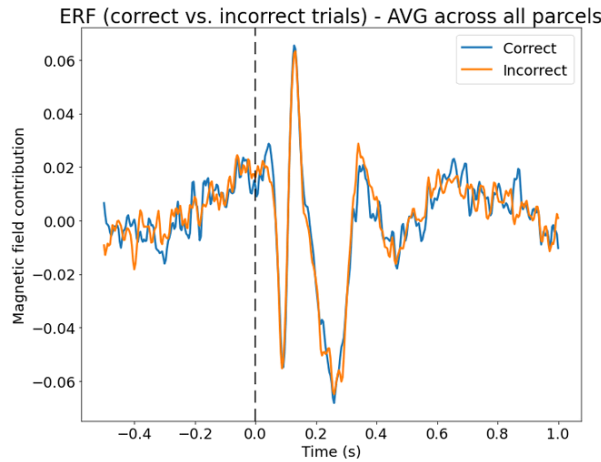


Figure 5.4: Global ERFs (i.e. averaged across all trials, subjects, and parcels) for correct (blue) and incorrect (orange) trials separately.

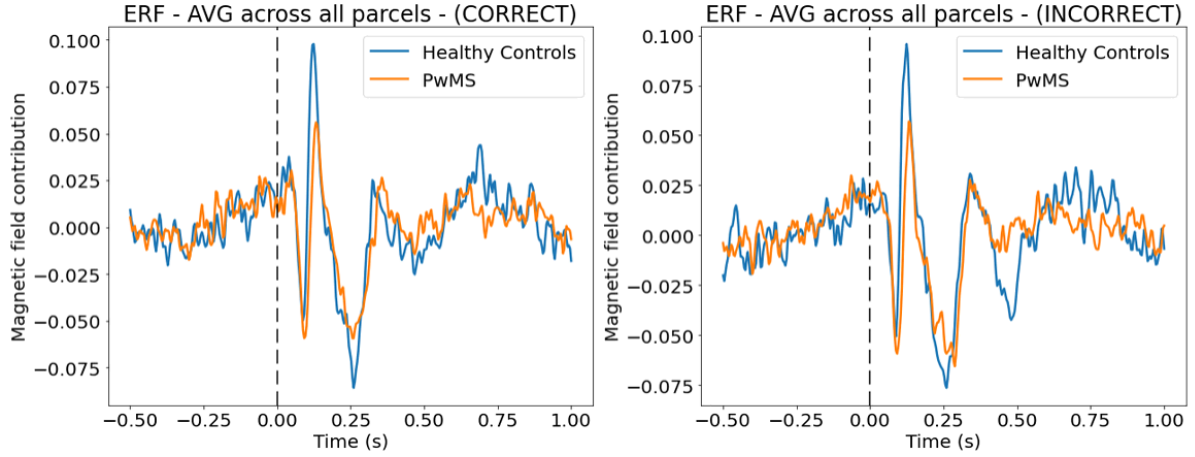


Figure 5.5: Group comparisons between the global ERFs (averaged across all 38 parcels) of HCs and PwMS for correct (left) and incorrect (right) trials separately.

Another group comparison was performed on the parcel-specific ERFs (i.e. without averaging across all 38 parcels) using the same statistical approach (i.e. permutation test with max statistic). The parcels and respective paradigm conditions containing time points with significant differences in signal amplitude between HCs and PwMS are shown in Figure 5.6. The exact time points are reported in Table 5.5. The right inferior occipital, left superior occipital, posterior cingulate cortex, and right orbitofrontal brain parcels show time points with significant differences (p -value < 0.05) between the two groups. The right inferior occipital parcel shows a significantly lower peak amplitude between 100 and 120 ms post-stimulus in PwMS compared to HCs, across both paradigm conditions. Additionally, this peak appears to be delayed in PwMS. In the left superior occipital parcel, the significant difference around 350 ms post-stimulus is due to a faster recovery to baseline after the peak at 200 ms post-stimulus in PwMS compared to HCs. Although this result manifests in both correct and incorrect trials, caution must be exercised when drawing conclusions from the results in the correct trials, as only a single time point shows a significant difference between groups. In the posterior cingulate region, the peak at 150 ms post-stimulus has a significantly larger magnitude in HCs compared to PwMS. Lastly, the right orbitofrontal parcel shows again a larger peak at 150 ms post-stimulus in HCs. However, significant difference between both groups was only occurs at a single time point in the epoch window, making this result unreliable.

Brain parcel	Trial type	Time-intervals post-stimulus (ms)
R Inferior Occipital	Correct	[96 – 120]
R Inferior Occipital	Incorrect	[100 – 120]
L Superior Occipital	Correct	336
L Superior Occipital	Incorrect	[320 – 328] $\cup$ [380 – 384]
Posterior Cingulate	Correct	144
Posterior Cingulate	Incorrect	[144 – 148]
R Orbitofrontal	Incorrect	128

Table 5.5: Group differences (HCs vs. PwMS) of ERFs for all brain parcels and trial types. Time points showing statistically significant differences after permutation testing ($N = 10\,000$) using the max statistic ($p < 0.05$).

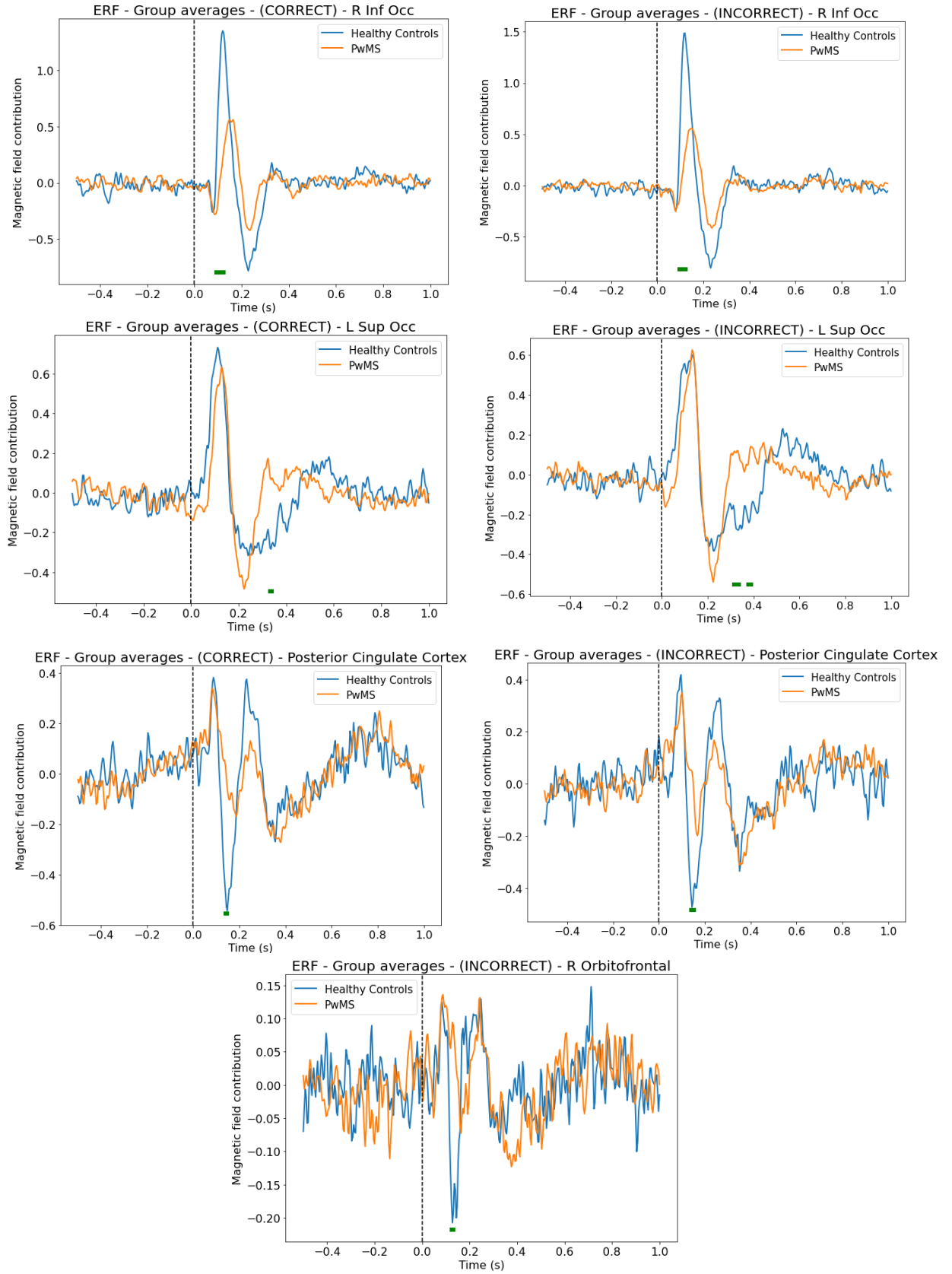


Figure 5.6: Group differences plots (HCs vs. PwMS) of ERFs for all brain parcels and trial types. Time points showing statistically significant differences after permutation testing ($N = 10\,000$) using the max statistic ($p < 0.05$) are marked as green horizontal lines.

Using the insights obtained from the previous ERF analyses, a peak amplitude/latency analysis, as outlined in section 4.4.1, was conducted. Figure 5.7 through Figure 5.10 show the amplitude or latency distributions for the parcels, corresponding task paradigm, and peak type for significant differences (permutation test, $N = 10\,000$) between HCs and PwMS. Due to the large number of comparisons, correcting for multiple comparisons via false discovery rate (FDR) leads to no surviving p-values. The reported p-values (see Figure 5.7 through Figure 5.10) are not corrected for multiple comparisons, implying that this amplitude/latency analysis is purely exploratory. However, the previously mentioned right inferior occipital (P100 amplitude – both correct and incorrect trials), left superior occipital (N300 latency – both correct and incorrect trials), and posterior cingulate cortex (P300 amplitude – both correct and incorrect trials) all show altered peak amplitudes and latencies between both groups.

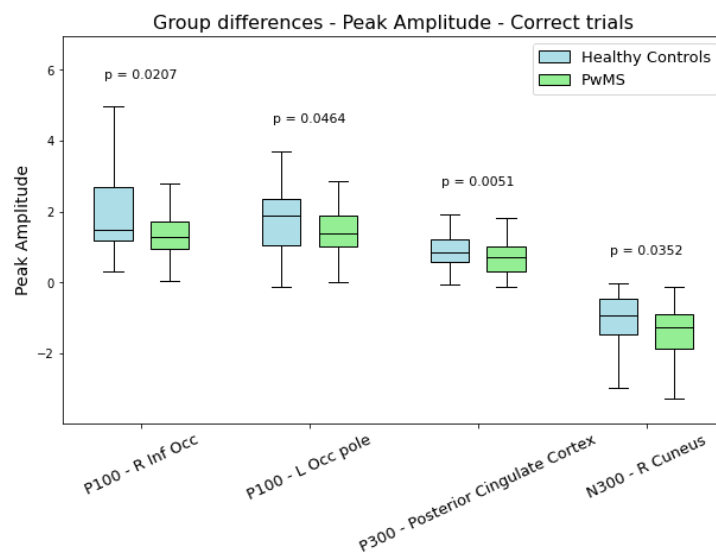


Figure 5.7: Statistically significant group differences in peak amplitude for all peak types and all parcels (correct trials). Note: the p-values were not corrected for multiple comparisons.

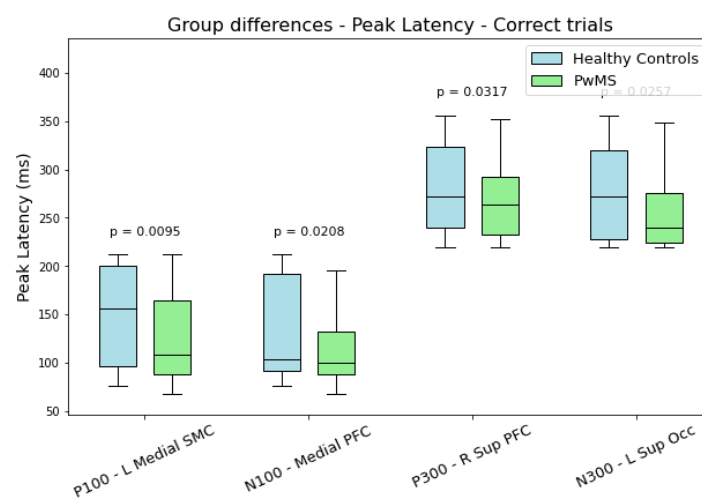


Figure 5.8: Statistically significant group differences in peak latencies for all peak types and all parcels (correct trials). Note: the p-values were not corrected for multiple comparisons.

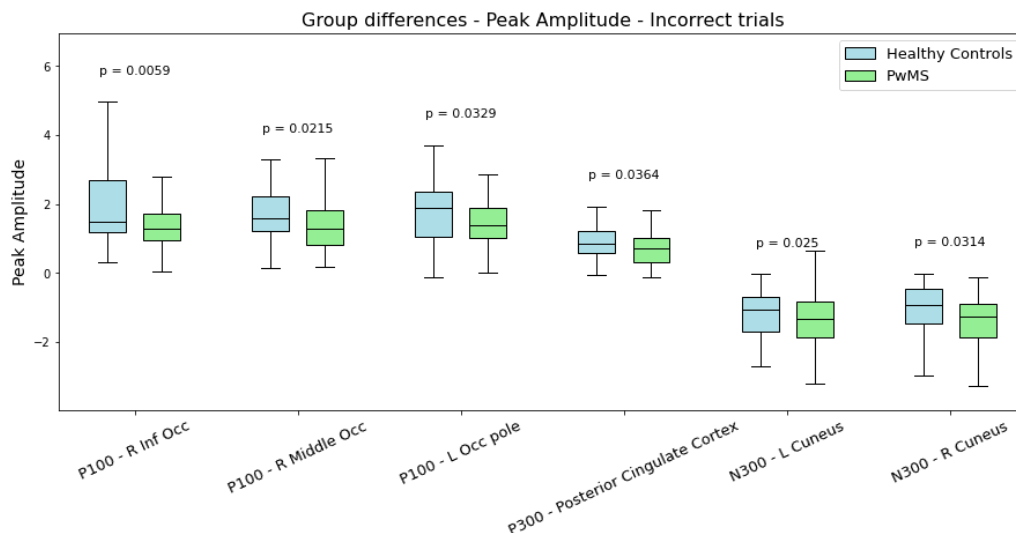


Figure 5.9: Statistically significant group differences in peak amplitude for all peak types and all parcels (incorrect trials). Note: the p-values were not corrected for multiple comparisons.

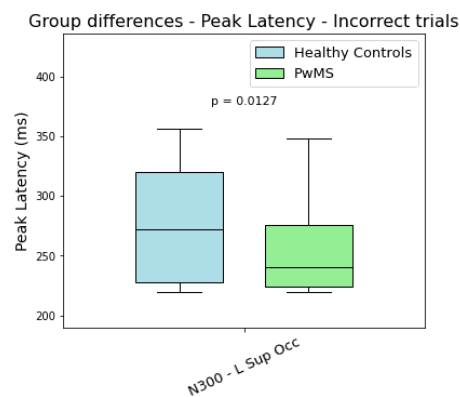


Figure 5.10: Statistically significant group differences in peak latencies for all peak types and all parcels (incorrect trials). Note: the p-values were not corrected for multiple comparisons.

Finally, the influence of the scanner type (BRUMEG1 vs. BRUMEG2) on the ERFs was investigated. Figure 5.11 shows the comparisons of the global ERFs between scanner types per paradigm condition (correct/incorrect) and subject group (HCs/PwMS). Based on visual inspection only, it seems that the peak amplitude for subjects whose MEG data was recorded with the upgraded scanner (BRUMEG2) is larger, both in HCs and PwMS. Using a permutation test with max statistic ($N = 10\,000$) for each of the four cases, however, did not reveal time points with significantly different MEG signal amplitude. By comparing the Power spectral density (PSD) of the ERFs between BRUMEG1 and BRUMEG2 (see Figure 5.12), the most striking difference appears as a peak near 16 Hz for BRUMEG2. From previous work of the hosting research group [141], this artefact is believed to have resulted from mechanical vibrations caused by construction works at the Erasme hospital site during data acquisition in BRUMEG2.

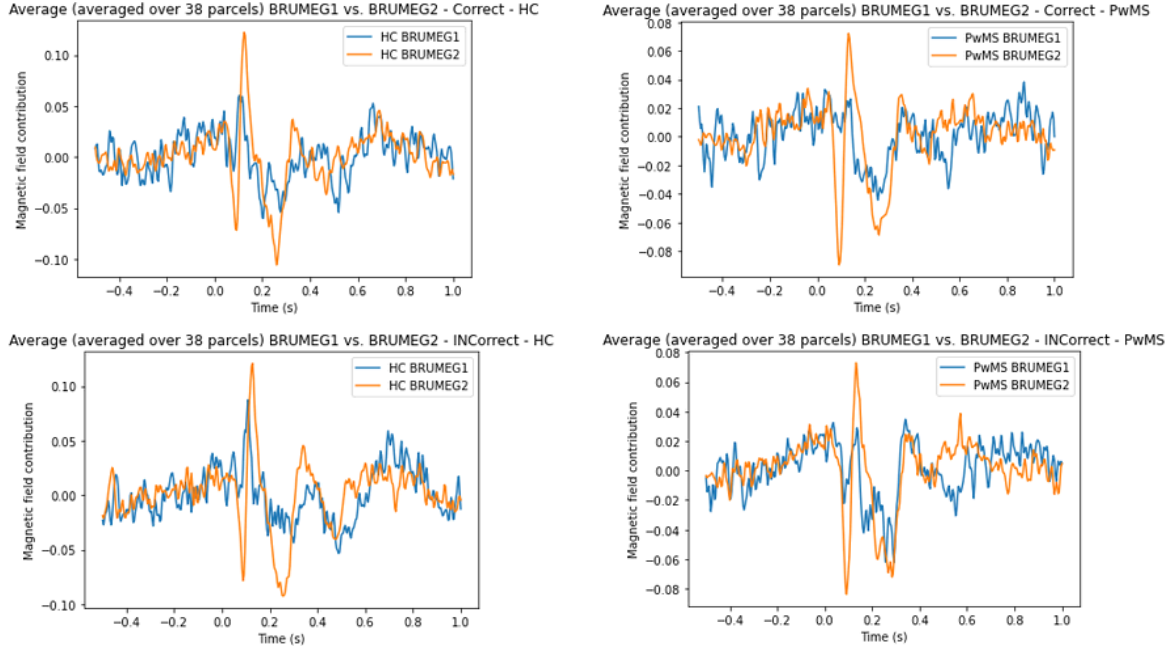


Figure 5.11: Comparison of global ERFs between the different scanner types (BRUMEG1 vs. BRUMEG2) for HCs and PwMS separately.

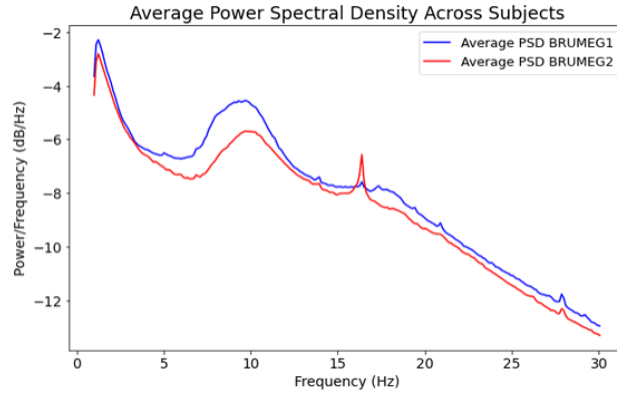


Figure 5.12: Difference in the average (taken across parcels) Power Spectral Density (PSD) between BRUMEG1 and BRUMEG2.

5.3 Functional Connectivity

The coherence metric was used to determine the whole-brain FC during the SDMT. The population-level coherence networks for 5 classical frequency bands (see Table 4.3) can be found in Figure 5.13 (correct trials) and Figure 5.14 (incorrect trials). The connection between the left and right medial supplementary cortex shows the highest coherence across all frequency bands, except in the beta band. In the delta band, the frontal regions (e.g., posterior cingulate cortex, right orbitofrontal cortex, and right ventrolateral prefrontal cortex) seem to show stronger coherence compared to other frequency bands. The coherence networks in the theta band display stronger coherence in occipital brain regions (e.g., superior and -inferior occipital cortex). In the entire alpha band (alpha1 + alpha2) the temporal regions can be seen to have stronger coherence. Lastly, the coherence in the beta band is more widespread, with high coherence in parietal, temporal, and mostly frontal brain regions (e.g., inferior and superior dorsal prefrontal cortex, in both hemispheres).

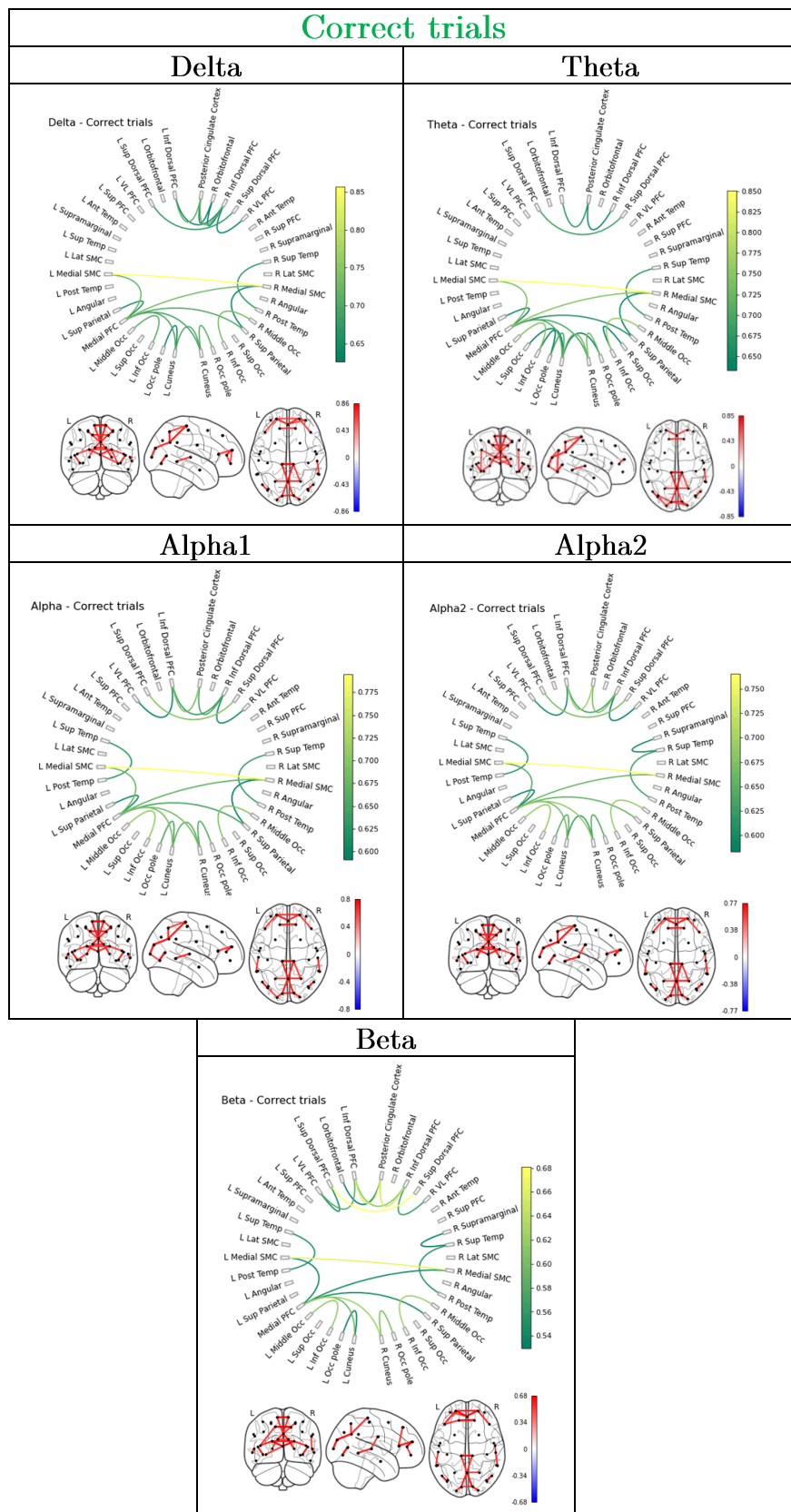


Figure 5.13: Circular plots and brain mask coherence networks for the 5 classical frequency bands (correct trials).

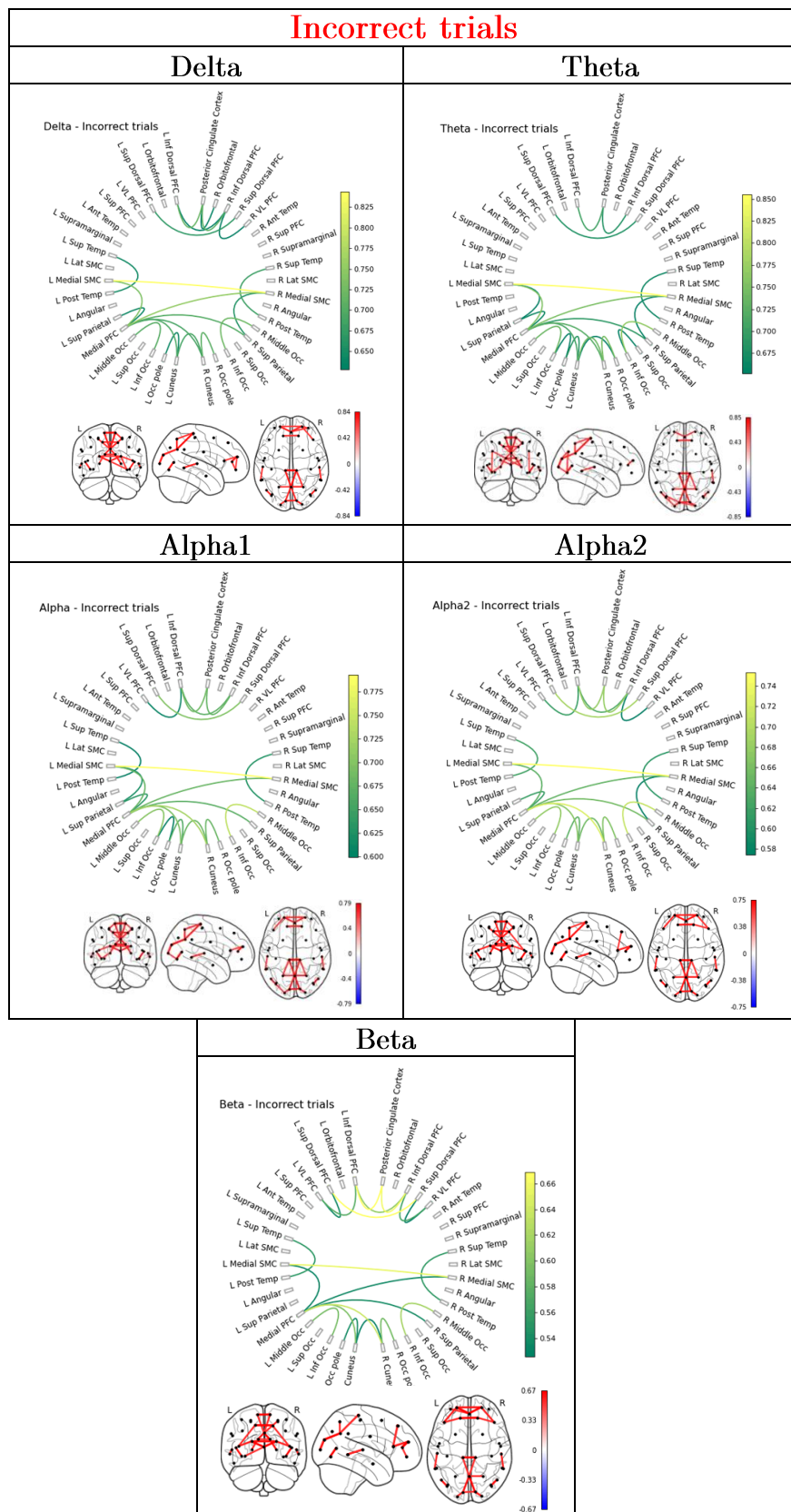


Figure 5.14: Circular plots and brain mask coherence networks for the 5 classical frequency bands (incorrect trials).

Instead of performing group comparisons on the upper triangular part of the 38x38 coherence matrices, the ERFs from multiple brain parcels were collapsed to a single brain region following the procedure outlined in section 4.4.2. The connection pairs with significant differences (permutation tests corrected for multiple comparisons via FDR) in coherence between HCs and PwMS are reported in Table 5.6. In the theta band (4-8 Hz), the coherence between the parietal and frontal brain regions (p-value = 0.020) as well as the temporal and frontal brain regions (p-value = 0.020) is significantly reduced for PwMS compared to HCs. The results found in the theta band only hold for the incorrect trials. In the alpha2 band (10-13 Hz), the coherence between the occipital and parietal regions showed a significant difference (p-value = 0.037) between the two groups, only for correct trials. The remaining frequency bands (delta, alpha1, and beta) did not reveal significant group differences in coherence between brain regions.

Connection	Trial type	Frequency band	HC	PwMS	p-value
Parietal / Frontal	Incorrect	Theta	0.65 ± 0.17	0.53 ± 0.20	0.020
Temporal / Frontal	Incorrect	Theta	0.56 ± 0.17	0.47 ± 0.17	0.020
Occipital / Parietal	Correct	Alpha2	0.52 ± 0.17	0.42 ± 0.18	0.037

Table 5.6: Results from the coherence group comparisons on the collapsed brain parcels (mean $\pm$ std). p-values are obtained from permutation testing ($N = 10\,000$) and false discovery rate (FDR) correction for multiple comparisons.

5.4 HMM states' description

The results presented in this section are for the TDE-HMM ran for a pre-specified number of states equal to 6. Although models were run for both 6 and 8 states, the latter provided some states with overlapping temporal and spatial characteristics. The results of the 8 states inference can be consulted in Appendix E. Furthermore, during the post hoc analyses of the inferred states, State 3 showed a flat temporal activation pattern and almost fully coherent brain networks across all parcels. It was discovered that this meaningless state was extracted due to the bad-segment annotation of the MEG data during preprocessing. Bad-segment annotation refers to the labelling of data segments with poor quality, so that if these segments are epoched, they will not be used for trial-averaging (see section 4.2, step 1 Preprocessing). Evidence for this finding is provided in Appendix E. Therefore, state 3 was not carried along for the following analyses.

First, temporal characteristics representative of the states' temporal behaviour during the entire model inference were computed. The distributions of the 4 metrics (fractional occupancy, mean lifetime, mean interval, and switching rate) across all subjects per state are shown in Figure 5.15. The exact values of the population-level mean and standard deviation per metric and state are reported in Table 5.7. Considering the fractional occupancy (FO), the average FO per state is around 19%. State 1 occurs the most (23% of the time), while state 2, 5, and 6 are activated for about 20% of the time. State 4 activates considerably less compared to the remaining states, at 11% of the time. The mean lifetime (MLT) across states goes from a shorter 70 ms (states 2, 5, and 6) to a longer 100 ms (states 1, and 4). The mean interval (MI) for states 1, 2, 5, and 6 are comparable and approximately 345, 305, 288, and 302 ms, respectively. State 4 has the longest

average time duration between successive state visits, at 802 ms. The switching rate (SR) lies between 2-3 Hz for states 1, 2, 5, and 6. As state 4 has the highest MI, it makes sense that this state would also have the lowest SR ($= 1.13$ Hz). Lastly, possible significant differences in summary statistics between HCs and PwMS were determined using a permutation test ($N = 10\,000$) with the maximal statistic taken across the states. No significant differences ($p < 0.05$) were observed between both groups.

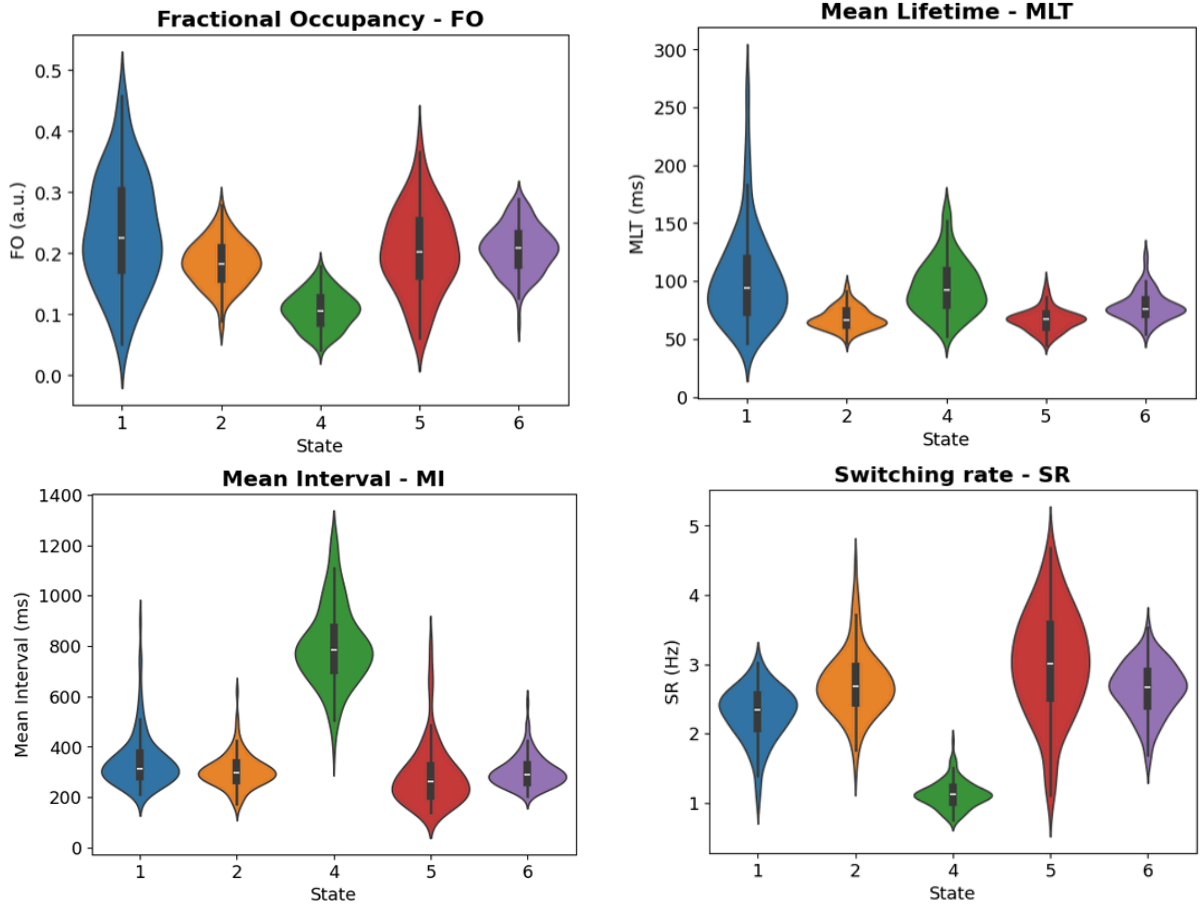


Figure 5.15: Summary statistics for the extracted HMM states: Fractional Occupancy (FO), Mean Lifetime (MLT), Mean Interval (MI), and Switching rate (SR).

	FO	MLT (ms)	MI (ms)	SR (Hz)
State 1	0.23 ± 0.09	103.61 ± 41.52	345.25 ± 108.87	2.28 ± 0.39
State 2	0.18 ± 0.04	67.93 ± 9.4	304.9 ± 67.17	2.73 ± 0.47
State 4	0.11 ± 0.03	95.44 ± 23.3	802.45 ± 155.32	1.13 ± 0.19
State 5	0.21 ± 0.07	66.7 ± 9.83	288.16 ± 125.63	3.02 ± 0.75
State 6	0.21 ± 0.04	78.85 ± 12.84	302.71 ± 31.45	2.65 ± 0.38

Table 5.7: Summary statistics values for the extracted HMM states (mean $\pm$ std).

As the subjects performed a task during the MEG recording, the states could likely have captured task-related dynamics. This was verified with an ERF analysis, for which the probability of state activations in an epoch around the stimulus onset ($[-500, 3000]$ ms) are shown in Figure 5.16 (correct trials) and Figure 5.17 (incorrect trials). The task-relevant states are those that are significantly activated or deactivated throughout the epoch window. For the hidden states inference from the MEG-SDMT data, all states 1, 2, 4, 5, and 6 appear to be task-related. The details of each state's activation or deactivation over time are explained in the individual state's full description. Additionally, Figure 5.18 and Figure 5.19 display the states containing time points with significant group differences between HCs and PwMS for correct and incorrect trials, respectively. For both paradigm conditions, the probability of state 5 activating at around 200 ms post-stimulus is significantly higher for HCs compared to PwMS. The same observation holds true for state 6 occurring at around 100 ms post-stimulus.

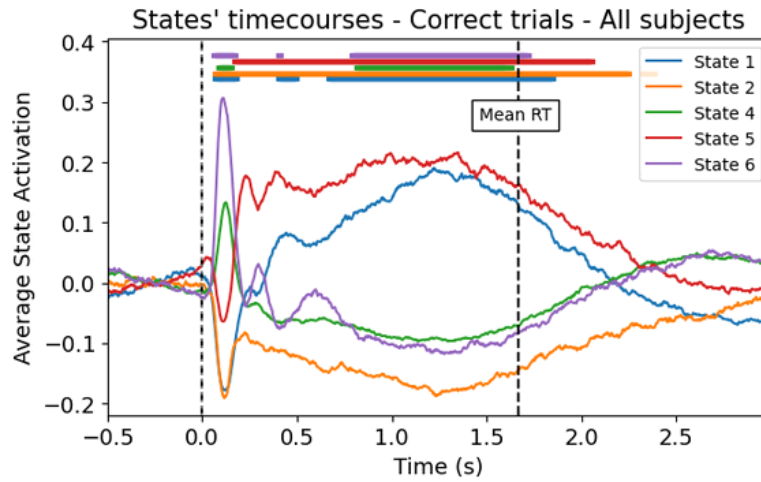


Figure 5.16: Average state activation in a time window $[-500, 3000]$ ms around the stimulus onset for all HMM states (correct trials). Horizontal lines at the top indicate time points of significant state activation or deactivation; determined by a permutation test ($N = 1000$) using the max statistic at a significance level of 5%.

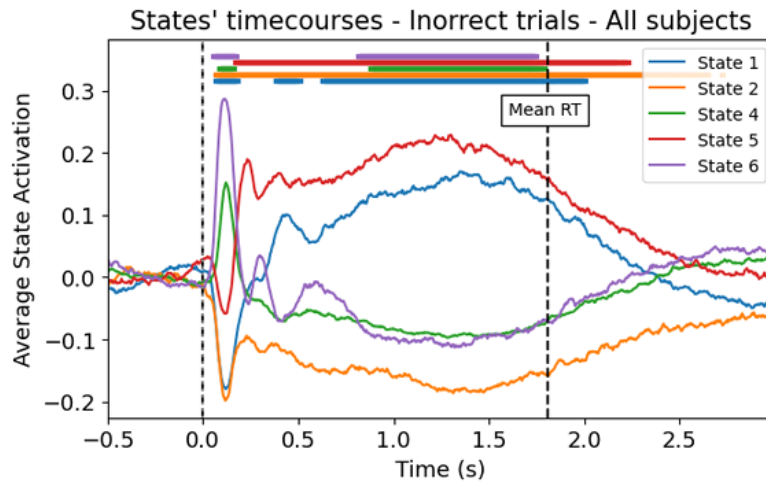


Figure 5.17: Average state activation in a time window $[-500, 3000]$ ms around the stimulus onset for all HMM states (incorrect trials). Horizontal lines at the top indicate time points of significant state activation or deactivation; determined by a permutation test ($N = 1000$) using the max statistic at a significance level of 5%.

Correct trials

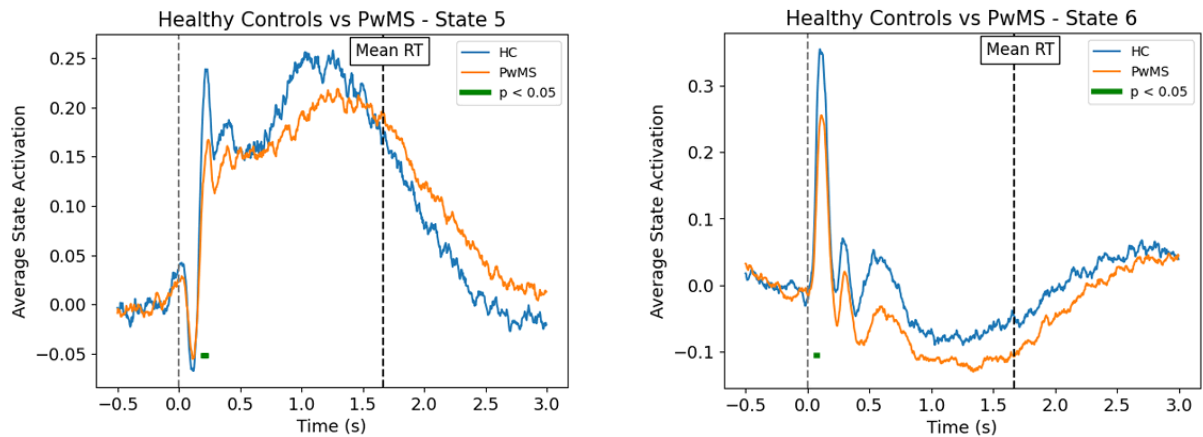


Figure 5.18: Group differences in average state activation or deactivation between HCs and PwMS (correct trials). Time points showing statistically significant differences after permutation testing ($N = 1000$) using the max statistic ($p < 0.05$) are marked as green horizontal lines.

Incorrect trials

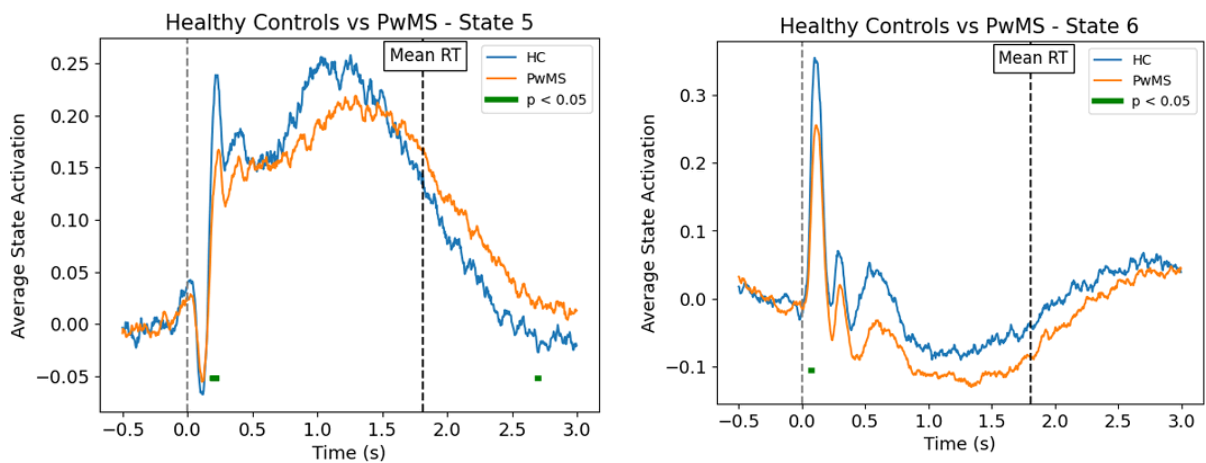


Figure 5.19: Group differences in average state activation or deactivation between HCs and PwMS (incorrect trials). Time points showing statistically significant differences after permutation testing ($N = 1000$) using the max statistic ($p < 0.05$) are marked as green horizontal lines.

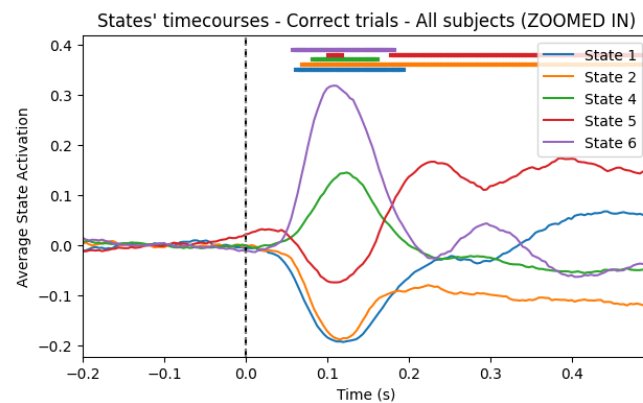


Figure 5.20: Average state activation in a zoomed-in time window $[-200, 500]$ ms around the stimulus onset for all HMM states (correct trials). Horizontal lines at the top indicate time points of significant state activation or deactivation; determined by a permutation test ($N = 1000$) using the max statistic at a significance level of 5%.

Figure 5.21 shows four spectral components (SCs) obtained through data-driven spectral decomposition using non-negative matrix factorisation (NNMF). Spectral component 1 (blue) contains the delta (1-4 Hz) and theta (4-8 Hz) bands, forming the classical low-frequency bands. Spectral component 2 (yellow) represents the alpha band (8-13 Hz), and spectral component 3 (green) mainly captures the beta band (13-30 Hz). The fourth spectral component (red) has been reported to capture high-frequency noise and is usually omitted during the states' description [142]. The three relevant spectral components (SC1, SC2, and SC3) were used to extract the state-specific spatial maps and spectral coherence networks. The state-specific mean PSD (Figure 5.22, left) and mean coherence (Figure 5.22, right) across all subjects are used to determine which spectral component best describes the properties of a particular state. This thought process is also reported in the states' description in the following paragraphs.

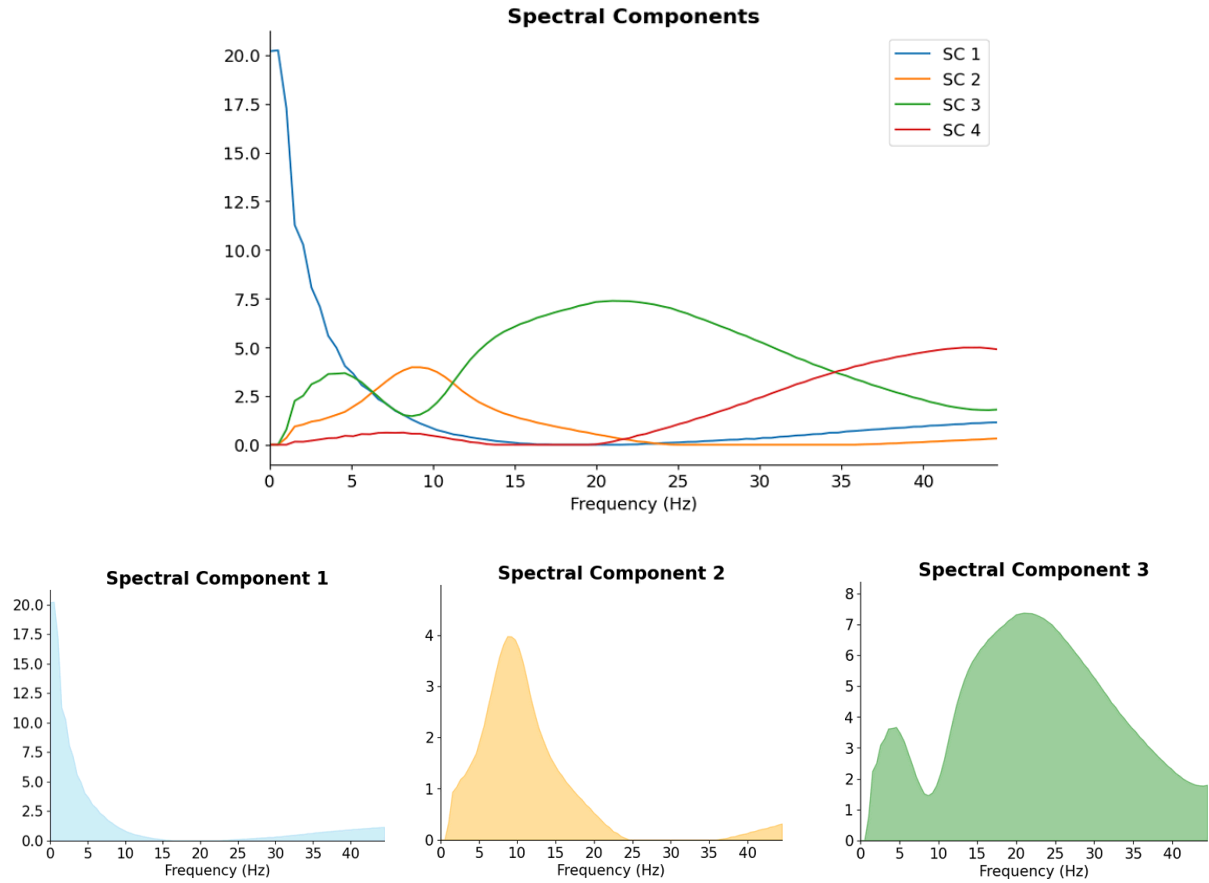


Figure 5.21: Data-driven frequency bands ($N = 4$) obtained by spectral decomposition using non-negative matrix factorisation (NNMF). The last spectral component (SC 4) filters high-frequency noise and is omitted during the description of the states' frequency content.

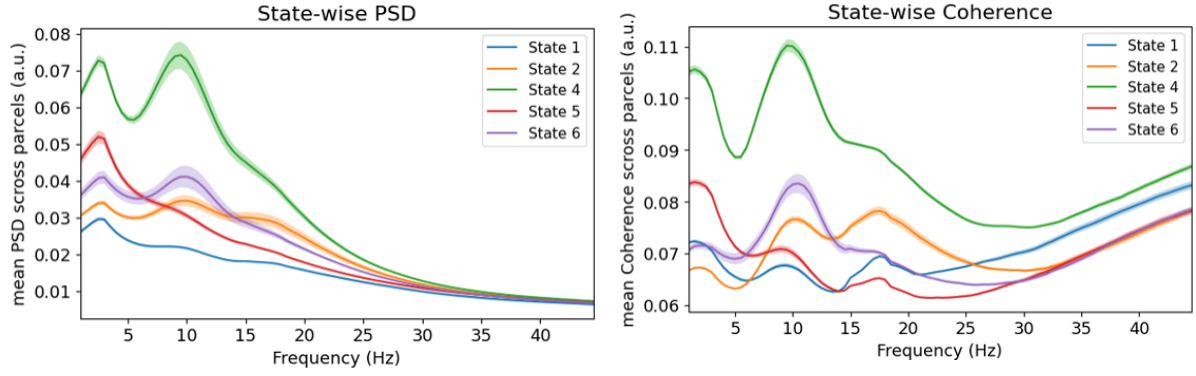


Figure 5.22: State-wise mean power spectral density (PSD) (left) and mean coherence (right). PSD and coherence are averaged across subjects, and across all regions (PSD) or pairs of regions (coherence). Shaded areas at each frequency bin correspond to the standard deviation across subjects.

States' description

State 1

State 1 is characterised by a significant peak of activation between 450 and 1500 ms after stimulus onset (Figure 5.23, left column). Afterwards, the task-related response decreases non-significantly. Additionally, this deactivation occurs at a higher latency for the incorrect than for the correct trials. The significant state deactivation around 100 ms post-stimulus is likely due to the mutual exclusivity assumption enforced by taking the Viterbi path (i.e. other states, state 4 and 6 in the presented results, significantly activate, causing the remaining states to deactivate w.r.t. baseline). Therefore, this specific peak is not task-related to state 1. On the spectral level, state 1 shows a high peak in mean coherence around the beta range (Figure 5.22, right) while the mean PSD (Figure 5.22, left) is rather flat across all frequencies. Therefore it was decided that the spectral content of state 1 is mainly focused on spectral component 3. The phase-coupling shows a rather broad activity pattern, with the strongest connections occurring between the temporal, occipital, and cuneus regions of the right hemisphere. Additionally, the medial prefrontal cortex also shows strong coherence with the right superior parietal and cuneus regions.

State 2

State 2 significantly deactivates between 150 and 1300 ms post-stimulus, after which reactivation builds up (Figure 5.23, right column). This reactivation occurs about 450 ms before the mean RT to the stimuli (1750 ms post-stimulus). Additionally, this reactivation starts earlier for the correct trials compared to the incorrect ones (Figure 5.23, right column). Based on a peak in the PSD (Figure 5.22, left) and the strongest coherence peak (Figure 5.22, right) both in the beta range, the main spectral content of state 2 is summarised by spectral component 3. The spatial PSD map displays high beta activity in the supramarginal and sensorimotor cortex, present in both hemispheres. On top of the regions identified through the mean PSD, the coherence networks show strong connections between the left and right superior prefrontal, and the left and right angular gyri. Notably, the lateral and medial sensorimotor cortices show inter-hemisphere connections.

State 4

State 4 significantly activates around 100 to 150 ms post-stimulus (Figure 5.24, left column). Afterwards, this state remains deactivated (significantly between 750 and 1600 ms post-stimulus). State 4 presents the highest peaks in PSD and coherence at the alpha frequency range, and is therefore associated with spectral component 2. The spatial power map shows the highest activation in the left and right cuneus, and left and right superior occipital cortex. Moreover, the PSD shows rather small alpha power in the frontal regions. In the same spectral component, the phase-coupling quantified by the coherence is mainly focused between the temporal and lateral occipital regions. More specifically, connections span between the superior temporal, middle occipital, and inferior occipital regions, observed in both hemispheres. Considering the strong similarities in state 4's description compared state 6 (see below), state 4 is not carried along in the Discussion chapter.

State 5

State 5 shows a task-related response significantly activated between 250 ms and 1250 ms post-stimulus (Figure 5.24, right column). After that, this state significantly deactivates until 2250 ms post-stimulus. For both paradigm conditions, the probability of state 5 activating at early response to the stimulus is significantly decreased in PwMS compared to HCs (Figure 5.18 left, and Figure 5.19 left). This significant difference is more pronounced in the incorrect trials, as the amount of time points showing significant differences between both groups is larger than for correct trials (Figure 5.19, left). The activity of state 5 is mostly contained within spectral component 1, as can be seen from the low-frequency peak in both PSD (Figure 5.22, left) and coherence (Figure 5.22, right). From the mean PSD map, mostly frontal regions show the highest activity. The coherence networks reveal the involvement of prefrontal (e.g. superior, ventrolateral, orbitofrontal), medial sensorimotor, posterior cingulate cortex, and anterior temporal regions. This state represents a prefrontal network.

State 6

State 6 is characterised by the strongest significant activation between 50 and 100 ms post-stimulus across all states, followed by several oscillations in activation, and ultimately leading to significant deactivation between 750 ms and 1750 ms post-stimulus (Figure 5.25). Additionally, both paradigm conditions show consecutive time points at 50 ms post-stimulus with significantly lower state activation for PwMS than for HCs (Figure 5.18 left, and Figure 5.19 left). Very distinct alpha peaks in both mean PSD and coherence plots across all subjects lead to state 6 being associated with spectral component 2. The mean PSD map highlights the occipital lobe. This is confirmed by the coherence networks showing high synchronisation between several regions in the occipital lobe. More specifically, this concerns the inferior occipital, middle occipital, occipital pole, and cuneus regions, with multiple connections spanning across the two hemispheres. This state represents occipital alpha activity at stimulus presentation.

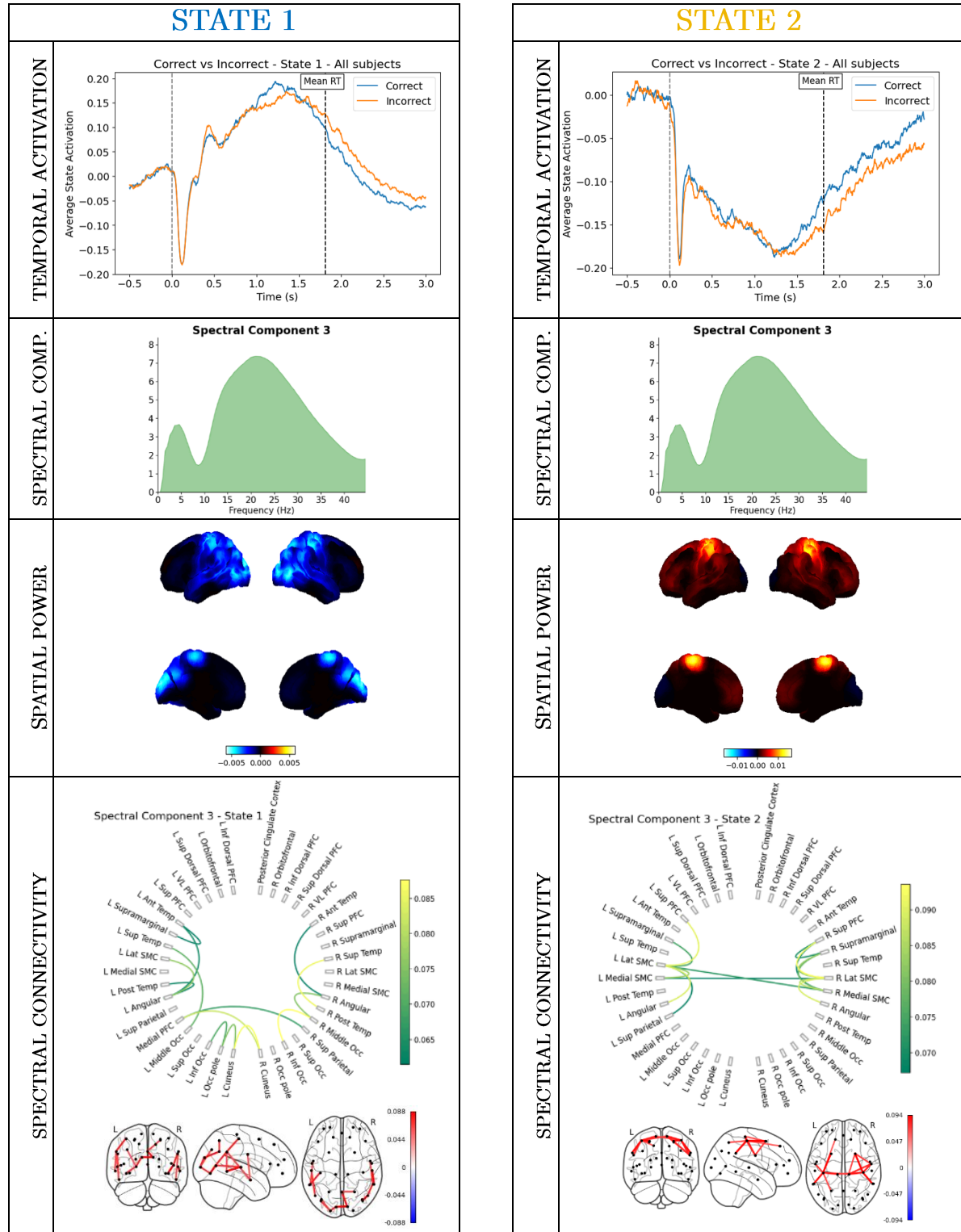


Figure 5.23: Complete description of State 1 and State 2. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted.

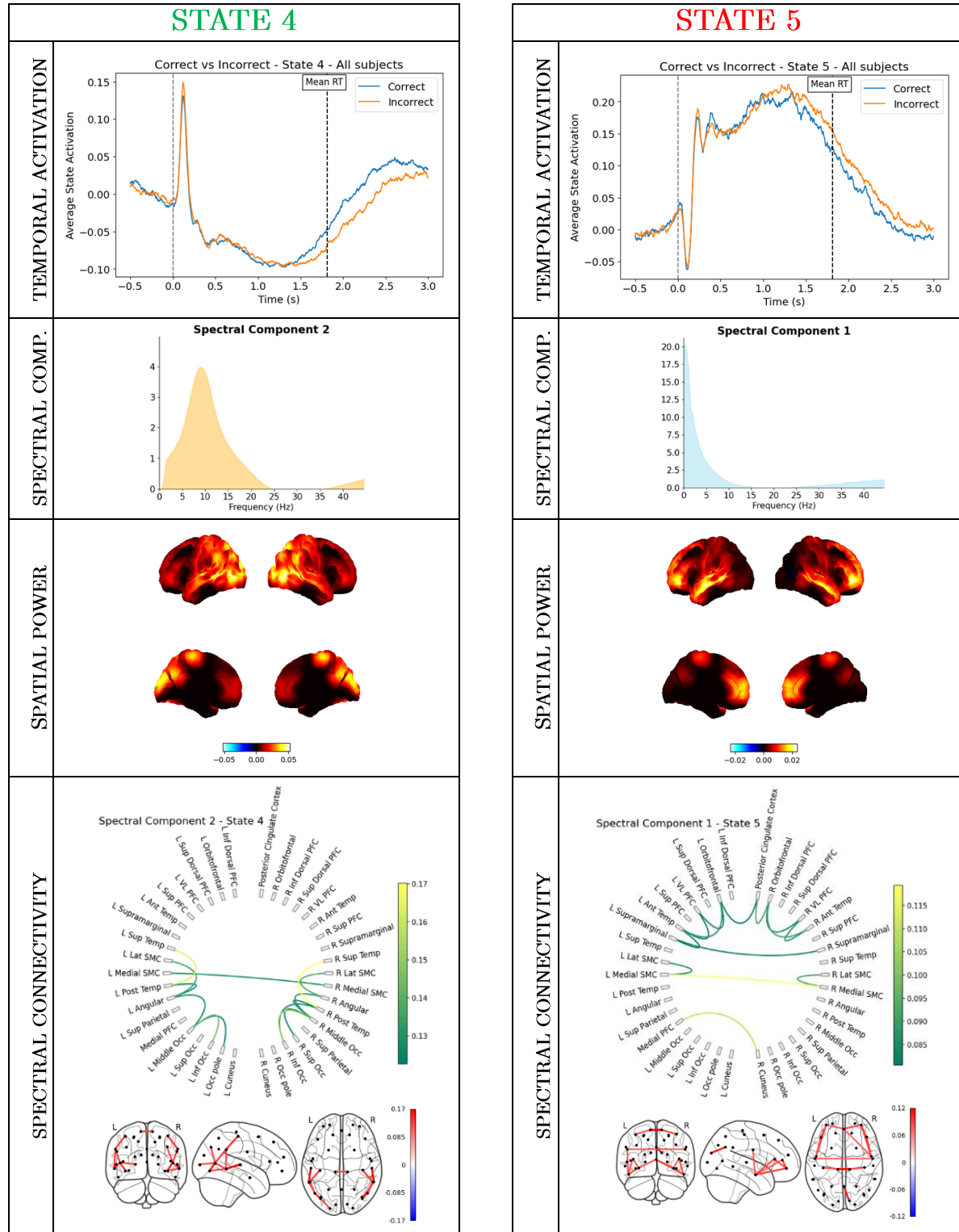


Figure 5.24: Complete description of State 4 and State 5. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted.

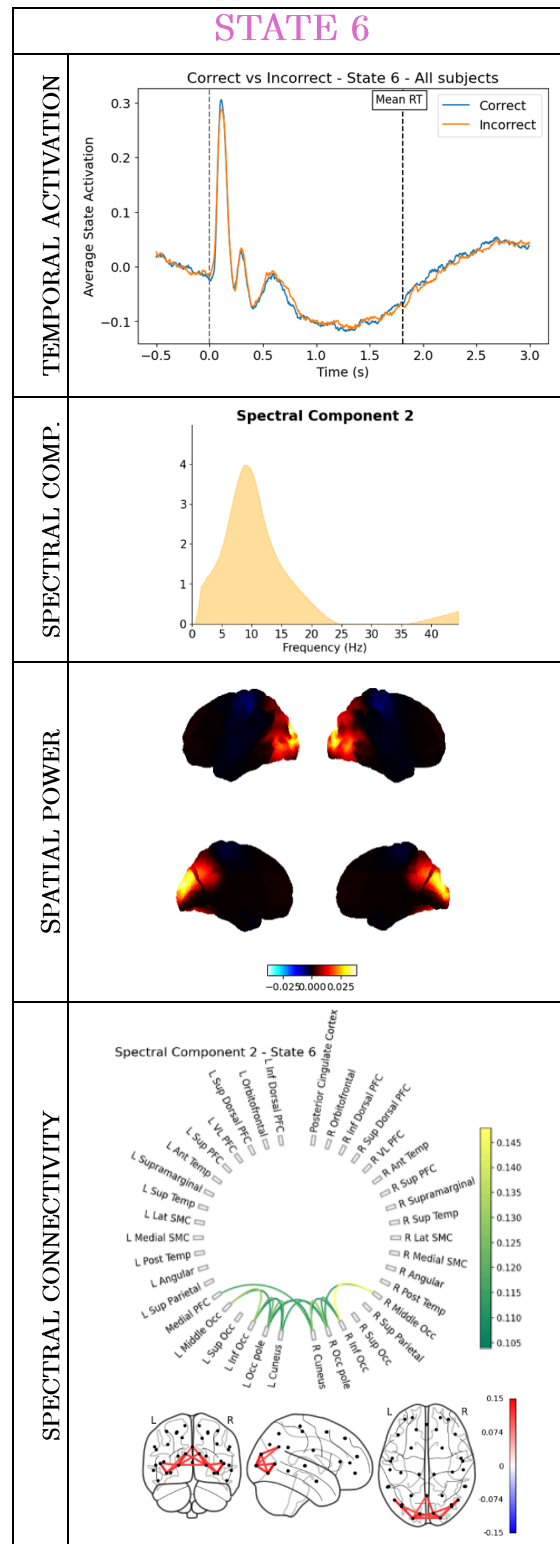


Figure 5.25: Complete description of State 6. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted.

Chapter 6

Discussion

This thesis used traditional static and novel dynamic approaches to study the neurophysiological processes recorded through task-MEG during a computerised SDMT. Using a dataset consisting of HCs and PwMS allowed us to examine which networks are altered in PwMS, leading to impaired information processing speed (IPS).

In this chapter, the results obtained in this thesis are discussed, and how they relate to the current knowledge gathered by previous research. First, the overall performance during the computerised SDMT is discussed. Secondly, the outcomes of the static analyses (ERF, FC) are explored. Lastly, a novel description of the neurophysiological brain networks underlying IPS, extracted by the TDE-HMM, is presented.

6.1 Performance during the computerised SDMT

PwMS were found to have significantly reduced task performance (i.e. success rate) during the computerised SDMT compared to HCs. This observation has previously been reported in the original on-paper SDMT [21],[143], as well as in certain SDMT adaptations for functional neuroimaging studies [11]. Interestingly, other neuroimaging studies reported no significant differences in SDMT performance between both groups [22],[23]. Whether significant differences in task performance between both groups are observed, could depend on the complexity and imposed time constraints during the computerised SDMT adaptation that is employed as task paradigm.

Reaction times (RTs) to the presented stimuli, submitted in the form of button presses, are significantly slower for PwMS compared to HCs. This result aligns with literature in which fMRI was used to measure functional brain activity during a computerised SDMT [22]. Our result shows that the SDMT adaptation employed in this thesis is sensitive to capture slowed IPS in PwMS. Furthermore, a significantly slower RT for the BZDp compared to the BZDn group was observed. Benzodiazepines (BZDs) are a type of medication prescribed to PwMS to treat anxiety, insomnia, or muscle spasms [144]. However, BZDs are known to cause long-term alterations in cognitive functions, including speed of processing [145]. Taking together the effect of BZDs on the RT to SDMT stimuli, and the fact that several neurophysiological studies have reported altered brain networks in both rest [116] and during a working memory (WM) task [37], the uptake of BZDs should be taken as an additional covariate in future analyses.

Caution should be exerted when comparing task performance and RTs across previous computerised SDMT functional neuroimaging studies. Over the last decades, the original on-paper SDMT has known several adaptations to become compatible with functional neuroimaging scanners, each adaptation having slightly different characteristics [146]. For instance, Forn et al. employed an SDMT adaptation where a row of symbols is presented, after which the subject has to pronounce out loud (i.e. oral response) which digit is associated with the presented symbol [147]. Meanwhile, the SDMT paradigm used in this thesis (described in section 4.1.2), originally adapted by Genova et al. [23], shows a single symbol-digit match at a time. Afterwards, the subject is asked to press a button (i.e. motor response) to indicate whether the combo matches one in the continuously displayed reference table. The SDMT adaptation used in this thesis facilitates the study of task-related neurophysiological activity, as each trial can be precisely isolated in time. The sufficient interstimulus interval (ISI) avoids the overlap of neural responses in succeeding trials. However, the main disadvantage is the pair-matching nature of the task used in this thesis, which causes IPS not to be examined exactly in the same way as during the original on-paper SDMT [11]. This should be taken into consideration when interpreting the results of the static and dynamic analyses conducted in this thesis.

6.2 Static analysis

An extensive ERF analysis of the MEG-SDMT data revealed early post-stimulus neural responses to the task around 100, 200, and 300 ms post-stimulus onset. Those responses are comparable to the typical ERPs observed in EEG studies with visual stimuli [148]. To the best of our knowledge and based on a recent review article of ERP analyses during cognitive tasks in PwMS [149], the task-evoked activity during the SDMT paradigm has not been previously studied using M/EEG. However, several studies reported distinct EPs in M/EEG studies during other cognitive paradigms that contain a visual component. As such, the observed M100 peak has been reported to occur as a response to visual stimuli [150]. Furthermore, Costers et al. identified an M300 stimulus-matching response in the inferior temporal brain region during a WM n-back MEG study in healthy participants [151]. Related to information processing, an N2 response in EEG, generated in the midcingulate cortex, is believed to indicate difficulties in cognitive information processing [24].

This is interesting considering our result of a significantly reduced peak magnitude in the posterior cingulate cortex at 150 ms post-stimulus in PwMS compared to HCs, most prominent in the incorrect paradigm condition. Grothe et al. reported a similar result in an fMRI study during an SDMT paradigm, where the cingulate cortex was activated in HCs but not in PwMS [11]. The cingulate cortex is believed to be involved in attention mechanisms related to cognitive functioning [25].

Another significant group difference relates to the ERFs in the occipital cortex. More specifically, the inferior occipital (M100) and superior occipital (M300) regions show significantly reduced magnitude and latency in PwMS. Visual function is often reported to be impaired in PwMS [26],[27]. The differences in latency at this early visual input processing response could be a first and important contributor to the reduced IPS in PwMS.

The functional connectivity (FC) analysis revealed a significantly reduced coherence between several brain regions in the theta and alpha frequency bands. In the theta band, the coherence strength between the parietal and frontal regions was significantly reduced in PwMS. Several studies previously reported that intact connectivity between parietal and frontal regions is crucial for good SDMT performance [10],[29]. Altered frontoparietal connectivity has been shown to correlate with WM capacity in PwMS [28]. This suggests that a non-negligible WM subprocess could be involved during the execution of a computerised SDMT task. In the same oscillatory band, coherence between the frontal and temporal regions is significantly reduced in PwMS during the SDMT. A study of structural brain damage found strong correlates between processing speed and the involvement of the frontal, parietal and temporal regions [152]. In the alpha2 band (10-12 Hz), PwMS showed significantly lower spectral synchronisation between the occipital and parietal brain regions. Previous work has related alpha activity between these brain regions to spatial attention [30],[153], which represents an important component of the SDMT [31]. Altered spatial attention could partially explain slowed IPS during the SDMT.

From a methodological point of view, the coherence metric was chosen to compute and compare FC between HCs and PwMS. Coherence is one of the most commonly used FC metrics, as it is a rather simple measure to implement and interpret results. However, a major drawback of this measure is its sensitivity to volume conduction, or in the case of MEG, electromagnetic field spread [65]. Although the data preprocessing in this thesis includes two approaches to reduce the effect of field spread: data transformation from sensor to source space, and orthogonalisation, the coherence measure will always remain sensitive to this effect. Future research could perform an FC analysis by applying a measure that discards all zero-phase interactions, such as the phase lag index [40].

6.3 Dynamic TDE-HMM analysis

The TDE-HMM was used to extract data-driven brain states with distinct spectral profiles, involved during the execution of an SDMT task. Although only a limited amount of fMRI studies have provided theories for the functional description of the processes involved during an SDMT task [11], the temporal dimension could not be explored due to the poor temporal resolution of this neuroimaging modality. In the following, the extracted TDE-HMM states are discussed in their order of activation throughout the task.

State 6 – Occipital

The visual stimuli presented during the SDMT are first captured in the occipital cortex. This significant activation at 100 ms post-stimulus is similar to the visual P100 response reported in visual task-EEG studies [32],[154]. State 6 is mainly characterised by alpha rhythms, which have previously been associated with the perception of visual stimuli [155],[156]. During the remainder of the task, state 6 remains significantly deactivated, which could reflect a mechanism to redirect attention and switch off the sensory occipital network once the stimulus of interest has been perceived [157].

This occipital alpha activity shows significantly reduced temporal activation in PwMS compared to HCs. Previous research has reported MS-induced early visual impairments, also observable using M/EEG [33]. Furthermore, this aligns with the previously discussed group differences in static ERFs observed in this thesis. In conclusion, visual impairments could be a first contributor to reduced IPS in PwMS.

State 5 – Prefrontal executive unit

Following the initial stimulus perception, a prefrontal network activates at 250 ms post-stimulus and persists until 1500 ms post-stimulus. State 5 arises predominantly in the prefrontal cortex, a brain region repeatedly reported to activate during the SDMT in fMRI studies [11],[146],[147]. Most of the spectral content is described in the low-frequency spectral component, further indicating that prefrontal theta activity could be involved with stimulus encoding during the SDMT [34]. The need to remember the presented symbol-digit combo before matching it with the reference table justifies the presence of a WM component during the SDMT.

State 5 is further characterised by phase-synchronisation between the left and right orbitofrontal cortices and the posterior cingulate cortex, which has been associated with attentional control during cognitive efforts [35]. In order to shift their sight from the presented symbol-digit combo to the relevant part of the reference table, the subject needs to maintain a certain level of attention throughout the task. Considering this state's overall description, Rossi et al. reported a TDE-HMM state with highly similar characteristics, inferred during an n-back WM task in healthy subjects [17].

Another cognitive process often associated with the executive frontal regions is that of decision-making [36],[158]. Considering state 5 remains activated until about 300 ms before the mean RT, this could capture the response button selection near the end of an SDMT trial. Further, the prefrontal network remains activated longer in incorrect than for correct trials, reflecting additional cognitive effort when the presented stimulus does not match an entry from the reference table.

PwMS also showed significantly reduced activation of this prefrontal network, only at early state activation (at 250 ms post-stimulus). Similar findings were reported using fMRI [23], and more recently in a follow-up study by Rossi et al. using the same TDE-HMM approach after the inclusion of PwMS [37]. Impaired functional activation of state 5 could be caused by structural brain damage [159]. As such, Grothe et al. have shown an association between IPS performance and structural white matter damage in frontoparietal regions in PwMS [160]. Altogether, the integrity of the executive control unit in the prefrontal cortex could be a second contributor to slower IPS in PwMS.

State 1 – Visuospatial processing

The next phase during the SDMT consists of comparing the presented stimulus with the reference table contents. Based on the distributed occipital, parietal, and temporal brain network with important beta activity, state 1 represents visuospatial processing [38],[161]. Both the original on-paper SDMT [12] and the SDMT adaptation used in this thesis require visuospatial processing to engage during stimulus matching.

Although this thesis did not reveal significant differences in this state's activation between HCs and PwMS, literature has repeatedly mentioned impairments in PwMS relevant to the regions involved in state 1. On the one hand, SDMT performance could be strongly influenced by visual scanning deficits in PwMS [39],[162]. On the other hand, MEG studies revealed associations between partial functional disconnection of the temporal brain regions and cognitive dysfunction in MS [163]. A possible explanation for the lack of effect on this seemingly crucial aspect of cognitive processing is the heterogeneity of the PwMS cohort included in the SDMT dataset. This cohort includes relapsing-remitting MS ($N = 63$), primary progressive MS ($N = 6$), secondary progressive MS ($N = 3$), and clinically isolated syndrome ($N = 1$) subjects. As it is advised to conduct analyses for the different MS subcategories separately [164], future studies should take this into consideration.

State 2 – Sensorimotor

The last step of this SDMT task consists of reporting whether the presented combo matches one in the reference table by pressing a button. The spatial and spectral layout of state 2 corresponds to a typical sensorimotor network, characterised by spectral beta activity in the left and right somatosensory cortices [165]. Previous TDE-HMM studies reported similar states in MEG task paradigms requiring a motor response [16],[17].

Summary and considerations

The extracted brain states represent dynamic functional brain networks with specific functions involved during the execution of the SDMT task. Four task-relevant states with distinct temporal, spectral, and spatial characteristics were identified. To relate this work to IPS, the tri-factor model of IPS in MS is used [31]. This recent theoretical model states that information processing should be seen as the summation of several contributions: a sensory, cognitive, and motor component. Using the previously discussed results and this framework, a novel description of the neurophysiological processes underlying the SDMT task is proposed in Figure 6.1.

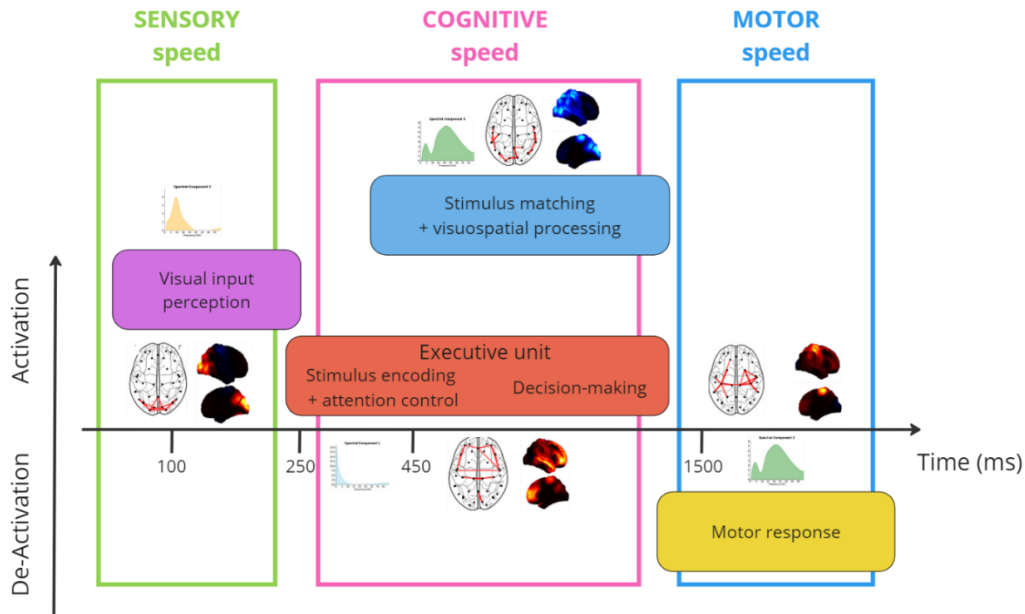


Figure 6.1: Summary of the proposed pathway of neurophysiological processes during the computerised SDMT. The rectangles with filled colors correspond to the respective states with the same colors (see Results chapter). Using the tri-factor model of IPS in MS [31], the pathway is divided into 3 parts: sensory, cognitive, and motor speed.

The SDMT is sensitive to capture slowed information processing speed (IPS) in several patient populations, including PwMS. However, it should be acknowledged that the SDMT is not a pure measure of IPS [166]. Aspects such as visual scanning or motor dysfunctions influence the assessment of IPS [8]. This is further supported by the results in this thesis, where the occipital state was found to have significantly weaker activation in PwMS. In theory, one could extract the pure cognitive speed components by isolating the sensory and motor components contributing to IPS. On the one hand, the sensory speed can be measured through the latency of visual evoked potentials (VEPs) [32]. On the other hand, the motor speed can be determined by means of transcranial magnetic stimulation (TMS) [167]. Future studies could explore this deeper by including additional experiments with the aim of isolating the targeted cognitive processing speed.

Although this is the first time an IPS task is studied using the TDE-HMM, Rossi et al. previously described the brain dynamics involved during a working memory (WM) task, the n-back task [17]. Comparing the state descriptions between the n-back (WM task) and the SDMT (IPS task) reveals both similar and certain task-specific brain states. Both tasks share a prefrontal theta, sensorimotor beta, and occipital alpha state. Rossi et al. identified an M300 state important for memory recall and response selection during the n-back task [17]. The work in this thesis revealed a visuospatial processing network unique to the SDMT. The fact that different tasks can capture different cognitive processes is an important statement.

Furthermore, Rossi et al. identified an altered prefrontal theta network during WM processing in PwMS [37]. This prefrontal executive unit was also found to be altered during the SDMT, indicating that different cognitive tasks can capture similar dysfunctions in PwMS.

Until now, fMRI studies have not been able to relate their findings to neuropsychological models of IPS. Although the extracted brain states' spatial maps in this thesis show strong similarities with activated regions identified using fMRI [11],[31], the TDE-HMM approach enables a more complete description with additional temporal and spectral information. This thesis serves as a first step towards an urgently required update of the neuropsychological model of IPS, as assessed through the SDMT.

Limitations

The main limitation of the HMM approach is the pre-specified choice for the number of states. This number is seemingly arbitrary, and the amount of task-relevant states usually varies depending on the task. Therefore, it is important to assess the robustness of the inference (i.e. whether the same states are consistently retrieved, even when the number of states the model is asked to extract is changed). Although only the results for the 6 states inference were discussed, an additional inference for 8 states was run. Regarding the latter, the same task-relevant states with closely resembling temporal, spectral, and spatial profiles were extracted. Furthermore, no additional task-relevant states were extracted in the 8 states inference (see Appendix E).

A second validation step consists of assessing the stability of the HMM inference for a fixed number of states. This can be done by comparing the Free Energy cost at the end of the model training for each run. Due to time constraints, the primary goal during this thesis was to optimise the OSL Dynamics pipeline for the full MEG dataset, leading to a single run for the 6 states inference. Future studies should verify the stability of the HMM inference.

The TDE-HMM implementation used in this thesis contains two intrinsic limitations. The first limitation is the Markovian assumption, which specifies that the state at a certain time point only depends on the state at the previous time point. Secondly, enforcing mutually exclusive states (i.e. taking the Viterbi path) can be interpreted as different brain states not co-existing simultaneously, which is likely to be an inaccurate representation of the real brain's functioning. Dynamic Network Modes (DyNeMo) is a novel technique that overcomes these limitations, recently proposed by Gohil et al. [41]. Instead of the Markovian assumption, a recurrent neural network (RNN) is used to model long-range temporal dependencies (LSTM) in the hidden states [168]. Additionally, using a mixture of modes results in a linear combination of different states coexisting simultaneously. Despite being introduced recently, this technique has already been applied to task-MEG data in HCs [41]. Future studies should use the DyNeMo approach to assess whether this leads to significant improvements and new insights for the description of brain dynamics during the execution of the SDMT.

Chapter 7

Conclusion

In this master's thesis, an MEG dataset recorded during a Symbol Digit Modalities Test (SDMT) task was used to uncover the neurophysiological processes behind information processing speed (IPS). The dataset consists of HCs (N=37) and PwMS (N=73), allowing us to investigate which brain networks are impaired in PwMS, causing slower IPS.

Static task-MEG data analyses revealed a reduced spatial attention ability in PwMS based on significantly smaller event-related field (ERF) amplitude in the posterior cingulate cortex. This finding was further supported by a significantly reduced parieto-occipital coherence in the alpha2 band (10-12 Hz) for PwMS as compared to HCs.

By applying the TDE-HMM technique to the task-MEG data, four task-relevant and data-driven dynamic functional brain networks were identified. The 3-dimensional (temporal, spectral, and spatial) state descriptions provide a new and more complete overview of the neurophysiological processes involved during the SDMT. IPS can be seen as the contribution of several components: sensory speed (occipital state), cognitive speed (prefrontal executive state and visuospatial processing state), and motor speed (sensorimotor state). Furthermore, the activation of the occipital and prefrontal executive states was found to be impaired by MS. This new description considerably improves the understanding of IPS assessed by the SDMT, and is a first step towards a more objective assessment of IPS in PwMS.

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Appendix A: Ethics and Ethical Implications

This work was conducted in full compliance with research ethical norms. Ethical approval for data collection and analysis was provided by the ethical committees of the National MS Center Melsbroek (2015-02-12) and the University Hospital Brussels (Commissie Medische Ethiek UZ Brussel, B.U.N. 143201423263, 2015/11). The data collection started in 2015 and was completed in 2018. All research participants signed an informed consent, and the appropriate institutional forms have been archived by the hosting research group.

To protect the participants' privacy, a random code was assigned to identify each subject. Therefore, the personal information used is not traceable to any individual. No data (MEG/MRI/demographics) were downloaded or stored on a personal computer. No ethical concerns have been identified regarding the impact of the research results of this thesis.

Appendix B: Reflection on sustainability

This work was carried out using the Python programming language (Python Software Foundation, <https://www.python.org/>). As with every programming language, computations lead to energy consumption. According to a study where the main programming languages were ranked in terms of energy efficiency, Python ranked the lowest (15th) [169]. To increase energy efficiency and therefore reduce energy consumption, other programming languages suited for data analysis such as C++ could be used. However, the state-of-the-art analysis tools used in this thesis (OLS Python [13] and OLS dynamics [18]) are currently only available in Python.

The usage and storage of data contribute significantly to pollution. During this thesis, previously collected data was used and new intermediate data was generated. Table B.1 shows an overview of the data storage in this thesis, expressed in gigabytes (GB). Out of the total 133 GB of data storage, 6.09% of this storage contribution was generated during this thesis. Because the data is stored and accessed via a remote desktop connection, the energy usage comes down to about $5 \cdot 10^{-6}$ kWh/GB (as opposed to 3-7 kWh/GB for cloud storage) [170]. In reality, the majority of the power consumption arises from the necessity of keeping the remotely accessible computer continuously powered on.

Data origin	Description	Storage (GB)
Provided at the start of this thesis	MEG recordings	123.8
	MRI scans	1.0
Generated during this thesis	Processed MEG	4.0
	Processed MEG (orthogonalised + sign-flipped)	4.0
	Dataframes (event extraction)	0.001
	TDE-HMM model parameters and outputs	0.0004
	Python Notebooks (written code)	0.1

Table B.1: Overview of data storage used throughout this master's thesis.

A last sustainability aspect relevant to this thesis relates the use of Graphics Processing Units (GPUs). As the TDE-HMM implementation used in this thesis is built as a machine learning framework using TensorFlow [171], there is the possibility to enable a GPU for a drastic decrease in model training duration. The trade-off between the higher power consumption, on the one hand, and more energy-efficient computations of a GPU, on the other hand, remains a challenge and requires more future research [172]. This thesis did not use a power-intensive GPU to speed up computations.

Appendix C: Exclusion of subjects

Throughout the work, several subjects (and their corresponding data) had to be excluded from the SDMT dataset. An overview is provided in Figure C.1, with a contribution from before (Dierickx Sara, ir. Fahimeh Akbarian) and during this thesis. Sara Dierickx (with prof. dr. Van de Steen F. as promotor, and prof. dr. ir. Nagels G. and prof. dr. ir. Van Schependom J. as co-promotors) identified several patients to exclude based on issues in the MRI scans and head-modelling step required for source reconstruction of MEG data [173]. Additionally, PhD student ir. Fahimeh Akbarian identified nine subjects with missing stimulus encoding for the DIODE channel, which is crucial for obtaining an accurate stimulus onset time. The remaining subjects in the overview were excluded for several reasons: missing STIM channels, missing BUTTON response channels, a lower amount of DIODE events than STIM events, MEG data with a flat Power Spectral Density (PSD) across all frequencies, failed parcellation step during data processing, and missing T1_MNI.nii file required for coregistration.

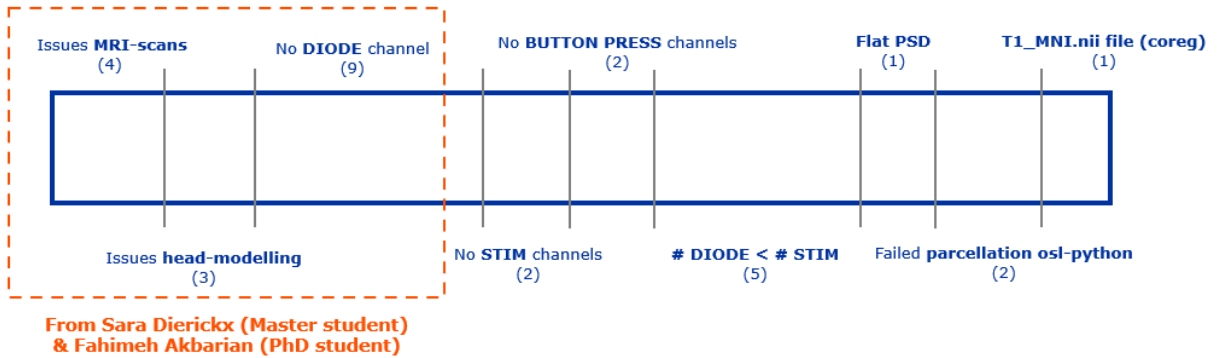


Figure C.1: Overview of subjects excluded from the final SDMT dataset. The number between brackets indicates how many subjects were excluded for the given reason.

As mentioned in section 4.2, the automated ICA could not be executed for 1 subject due to missing ECG/EOG channels in the MEG data. Instead of simply excluding that subject from the dataset, a manual ICA procedure was applied. 64 independent components were extracted from the entire time series, after which artefactual components were identified by visual inspection. Both topographic maps (Figure C.2) and additional spectral properties (Figure C.3) were taken into consideration. Four independent components (nr. 0, 1, 3, 4), believed to capture eye movements and eye blinks, were removed.

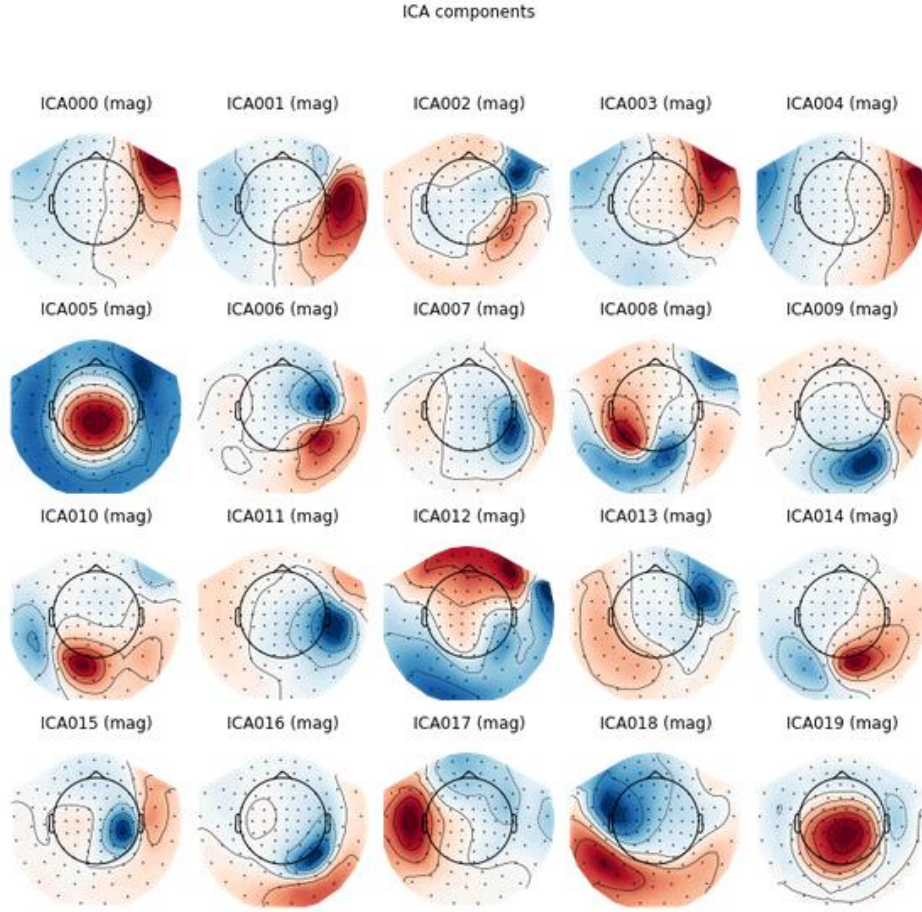


Figure C.2: Topographic maps for the 20 first (out of 64) independent components.

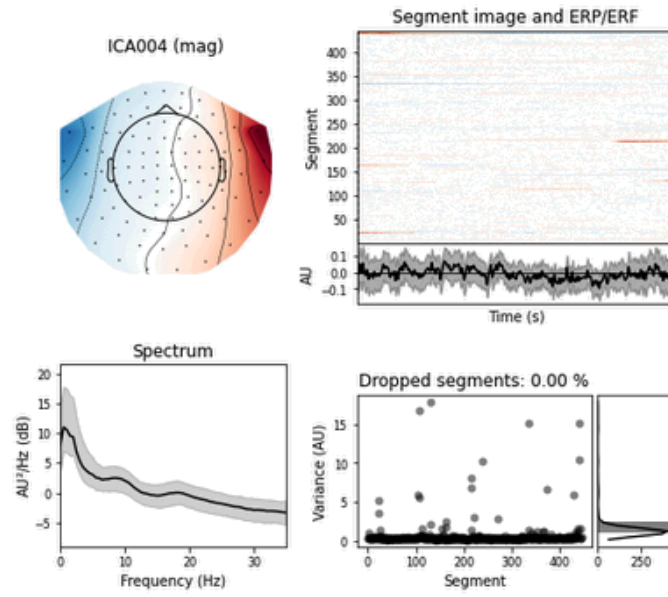


Figure C.3: Example of additional properties for ICA004.

Appendix D: Python code

This appendix contains additional information and provides access to the code written during this thesis. All the analyses were implemented using Python Jupyter Notebooks (.ipynb file format). The Python code was run exclusively on a remote computer of the AIMS research group, CrayZ 1. The code is divided into 5 major parts, each forming a separate notebook (except the TDE-HMM Analysis has 2 notebooks, one for the 6 states and another for the 8 states inference):

1. Preprocessing MEG data
2. Behavioral Statistics
3. ERF Analysis
4. Static Coherence Analysis
5. TDE-HMM Analysis

A GitHub repository was created to share access to the code that generated the results presented in this thesis. This public repository contains three folders: one with the raw .ipynb files, a second with the notebooks in .html format, and a third for the converted pdf format. Before downloading the code locally, all print statements containing patient data were removed. Access is provided via the following link: https://github.com/OlBurta/Thesis_2324

Appendix E: Supplementary Materials

HMM Analysis

Part 1. Flat HMM State

After HMM inference, state 3 was found to display a flat temporal activation near the stimulus onset, and to have almost fully coherent brain networks across all parcels. This was very unusual, and it was later found that the time points at which state 3 activates perfectly correspond to the time points of bad-segment annotations assigned during data preprocessing (see Figure E.1). Further, it was discovered that state 3 picks up recurrent data segments of zero MEG signal, as can be seen on the right plot of Figure E.2. Interestingly, it was found that bad-segment data is flattened out only after the source reconstruction step. Because of this meaningless state 3, it is as if the model is asked to extract 5 states instead of the pre-specified 6 states. This was an additional reason for running a second inference with 8 states (instead of 6 states), to ensure that no task-relevant states were omitted in the analysis.

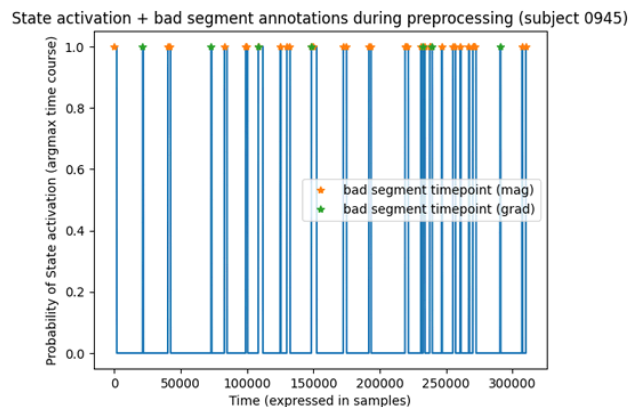


Figure E.1: Activation of state 3 aligns with time points of bad-segment annotation.

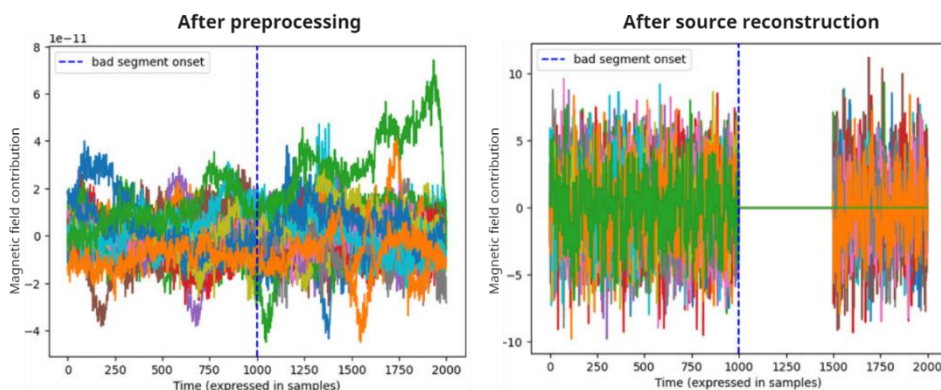


Figure E.2: Data segments labeled as bad-segments after the preprocessing steps (left) and after source reconstruction (right).

Part 2. Results of the 8 states HMM inference

In the 8 states HMM inference, state 5 was found to have flat activation around the stimulus onset and to have almost fully coherent functional connectivity networks (similar to state 3 in the 6 states HMM inference). Therefore, state 5 was not carried along for the display of results in this Appendix. The results are presented similarly as in chapter 5 of this thesis.

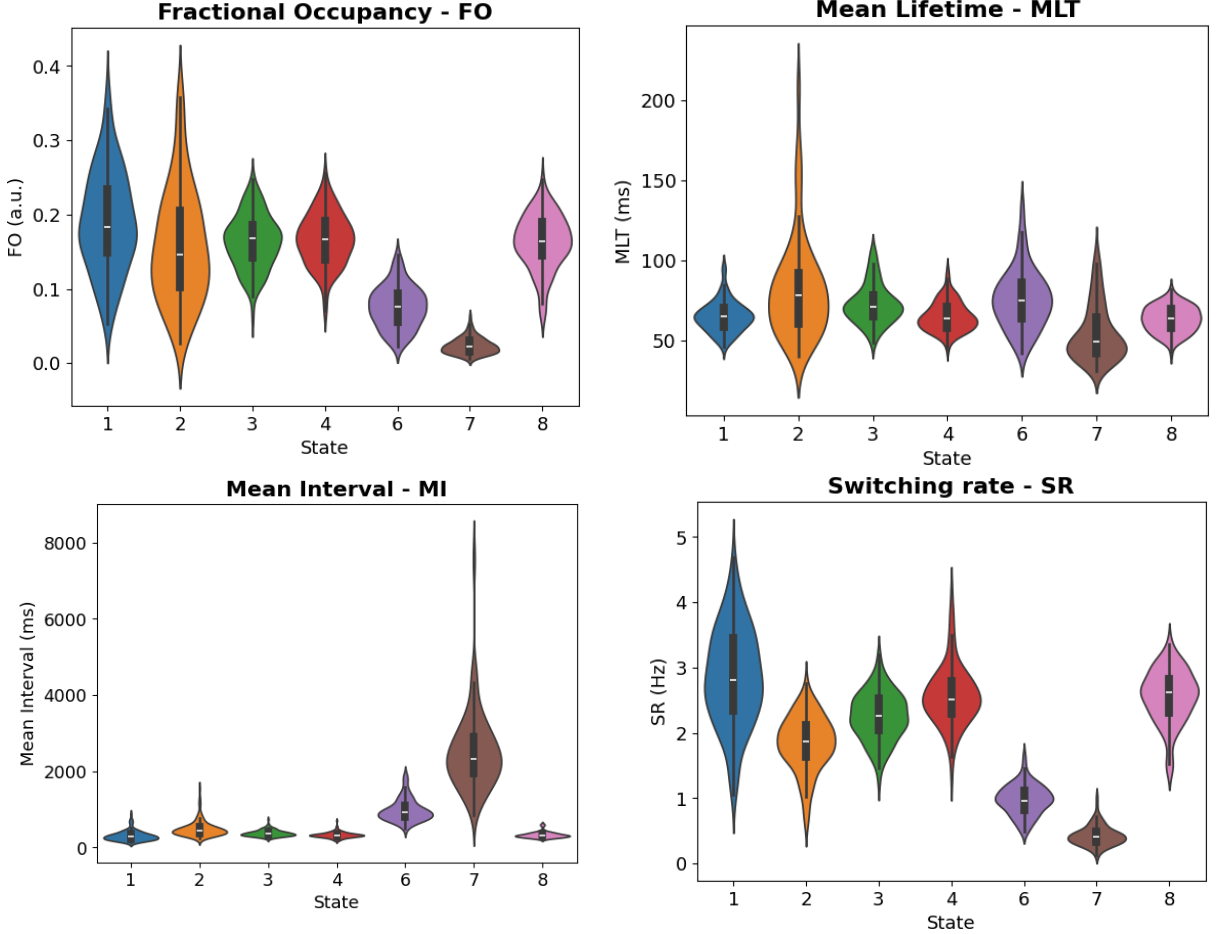


Figure E.3: Summary statistics for the extracted HMM states: Fractional Occupancy (FO), Mean Lifetime (MLT), Mean Interval (MI), and Switching rate (SR) – 8 states.

	FO	MLT (ms)	MI (ms)	SR (Hz)
State 1	0.19 ± 0.07	65.03 ± 9.46	309.44 ± 129.26	2.86 ± 0.74
State 2	0.16 ± 0.08	82.69 ± 32.37	493.53 ± 207.00	1.84 ± 0.42
State 3	0.16 ± 0.04	72.87 ± 12.33	376.97 ± 83.29	2.26 ± 0.37
State 4	0.17 ± 0.03	65.09 ± 9.07	330.83 ± 73.93	2.57 ± 0.45
State 6	0.08 ± 0.03	75.92 ± 18.35	984.97 ± 289.55	0.98 ± 0.23
State 7	0.02 ± 0.01	$54.79 \pm 17.50^*$	2533.48 ± 1039.50	0.43 ± 0.15
State 8	0.16 ± 0.04	63.37 ± 8.05	332.71 ± 77.10	2.57 ± 0.40

Table E.1: Summary statistics values for the extracted HMM states (mean $\pm$ std). Statistically significant group differences ($p < 0.05$)* between HCs and PwMS using a permutation test ($N = 10\,000$) with maximal statistic across states – 8 states.

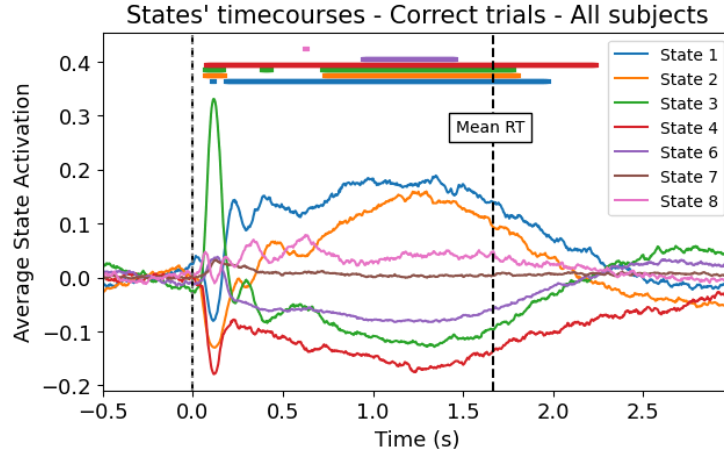


Figure E.4: Average state activation in a time window $[-500, 3000]$ ms around the stimulus onset for all HMM states (correct trials). Horizontal lines at the top indicate time points of significant state activation or deactivation; determined by a permutation test ($N = 1000$) using the max statistic at a significance level of 5% - 8 states.

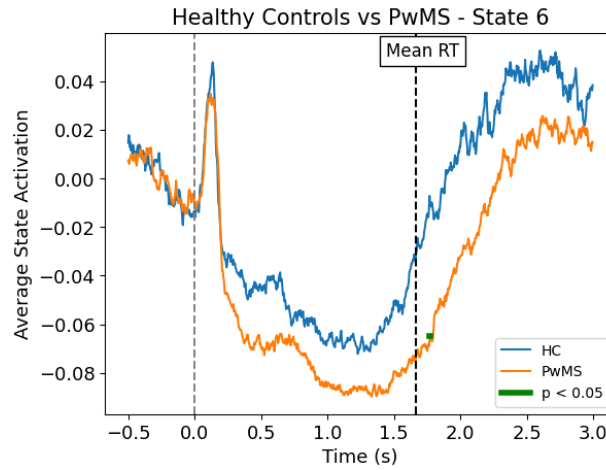


Figure E.5: Group differences in average state activation or deactivation between HCs and PwMS (correct trials). Time points showing statistically significant differences after permutation testing ($N = 1000$) using the max statistic ($p < 0.05$) are marked as green horizontal lines - 8 states.

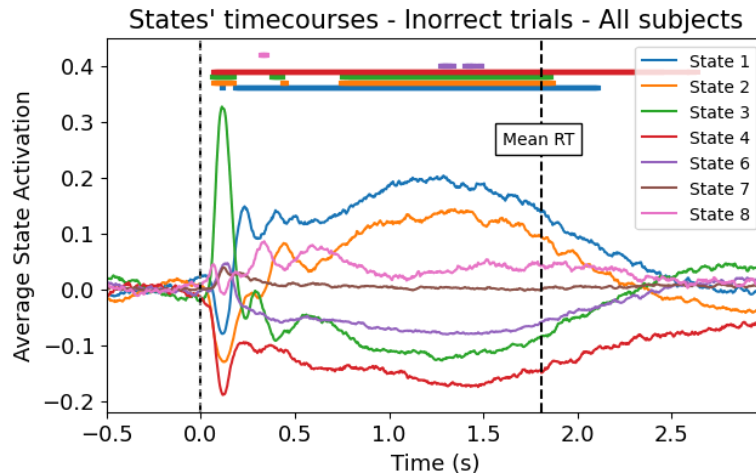


Figure E.6: Average state activation in a time window $[-500, 3000]$ ms around the stimulus onset for all HMM states (incorrect trials). Horizontal lines at the top indicate time points of significant state activation or deactivation; determined by a permutation test ($N = 1000$) using the max statistic at a significance level of 5% - 8 states.

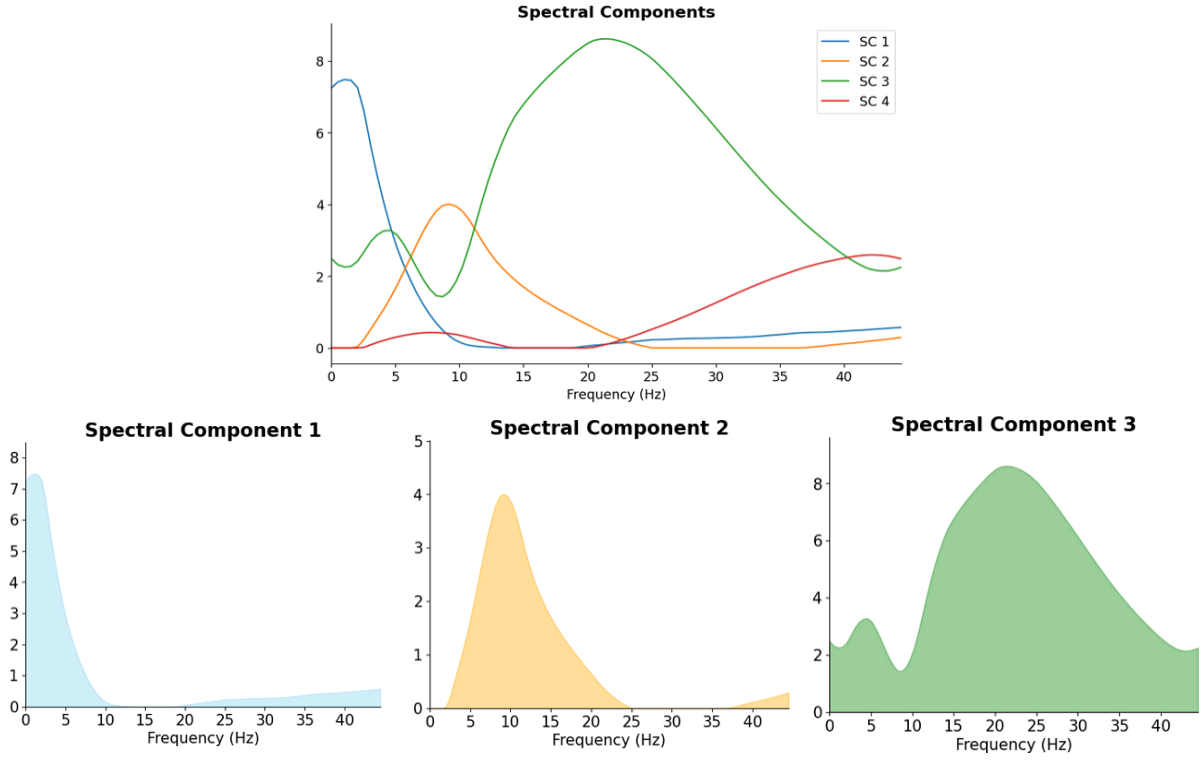


Figure E.7: Data-driven frequency bands ($N = 4$) obtained by spectral decomposition using non-negative matrix factorisation (NNMF). The last spectral component (SC 4) filters high-frequency noise and is omitted during the description of the states' frequency content – 8 states.

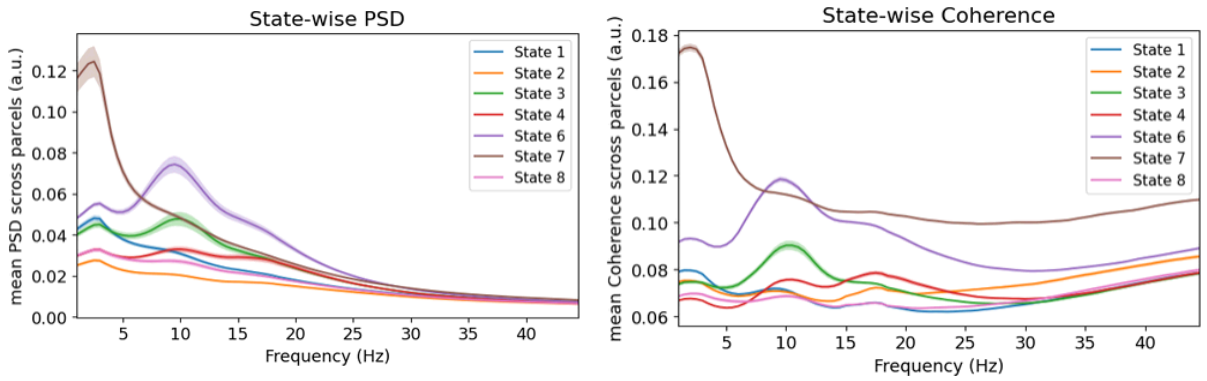


Figure E.8: State-wise mean power spectral density (PSD) (left) and mean coherence (right). PSD and coherence are averaged across subjects, and across all regions (PSD) or pairs of regions (coherence). Shaded areas at each frequency bin correspond to the standard deviation across subjects – 8 states.

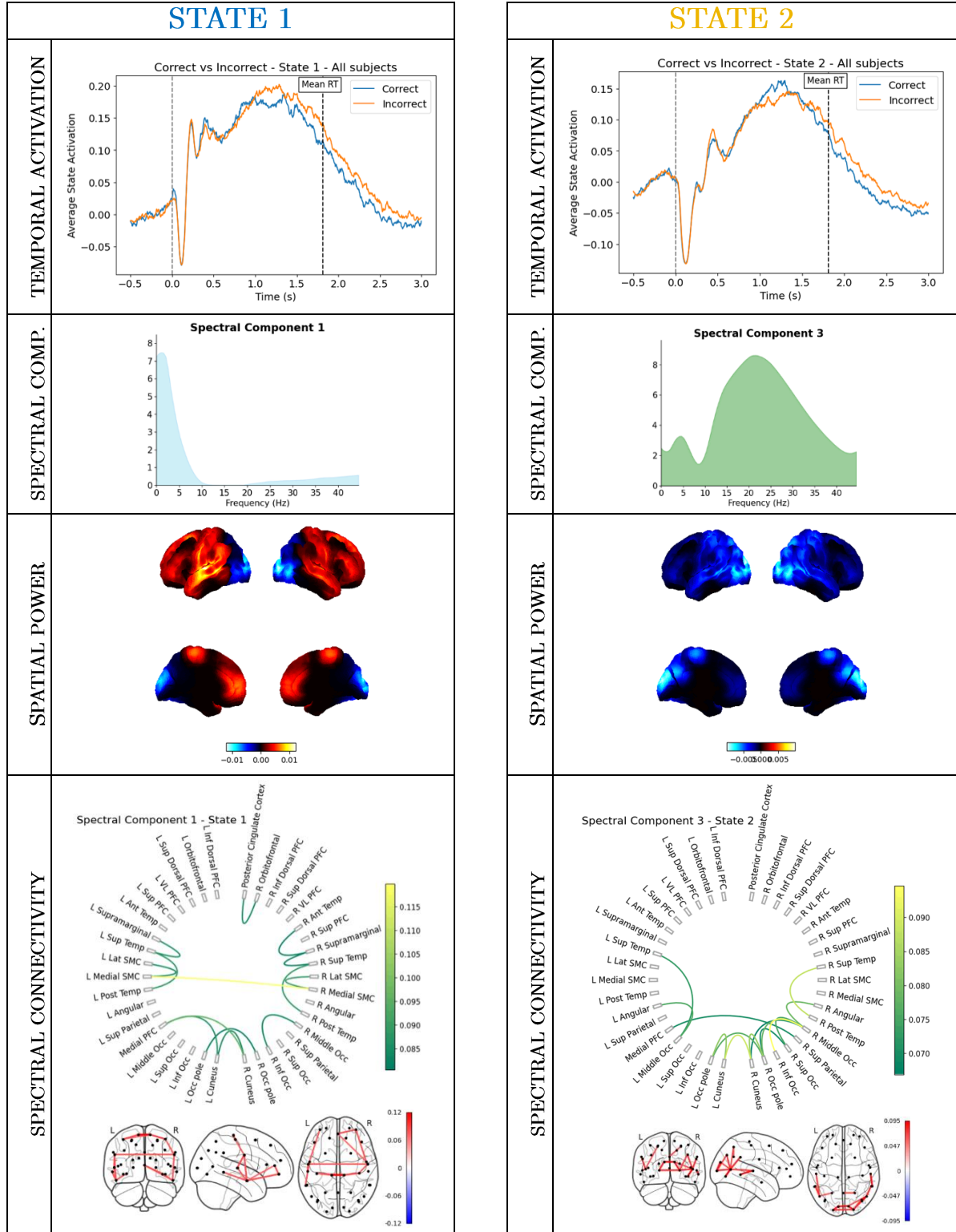


Figure E.9: Complete description of State 1 and State 2. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted – 8 states.

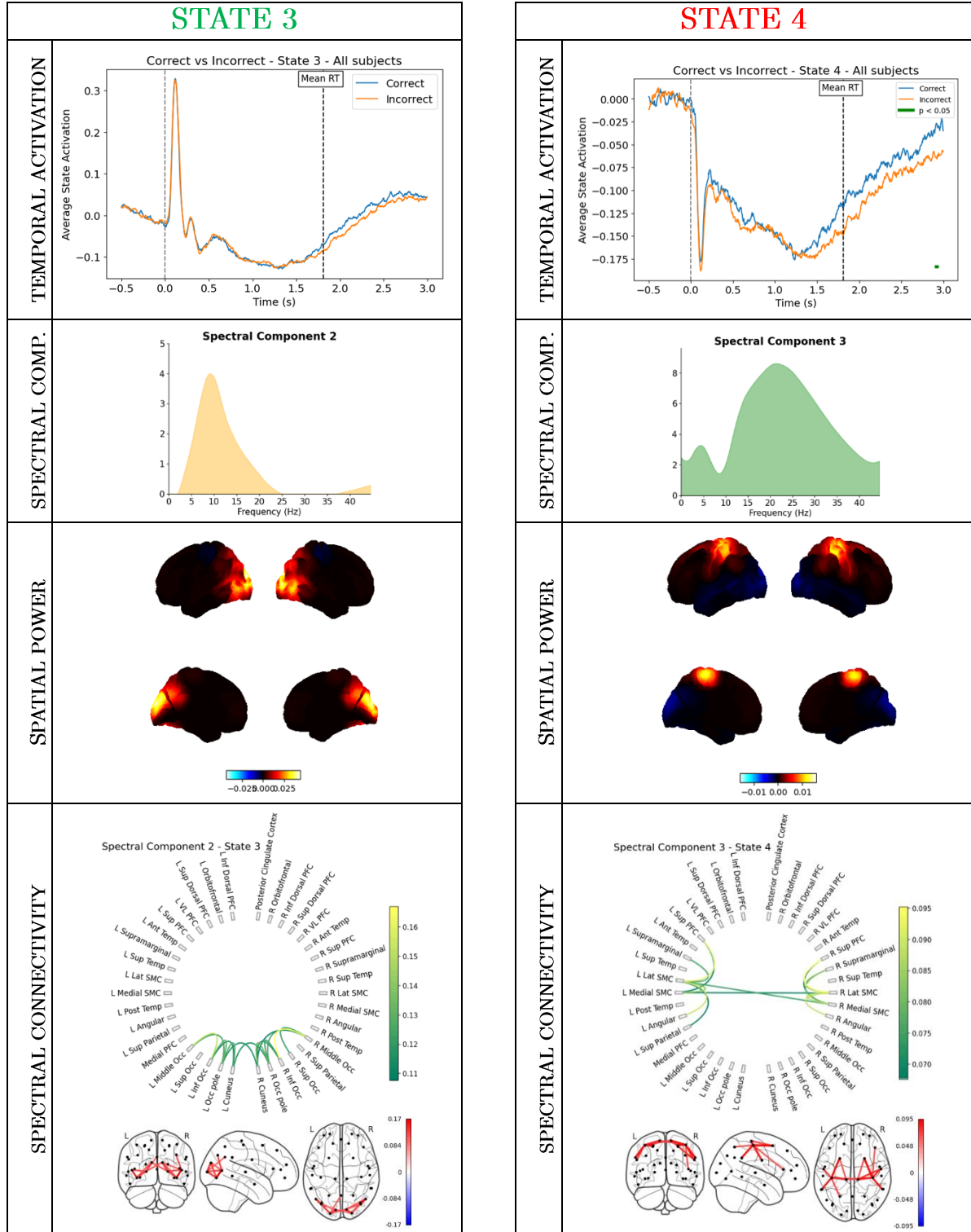


Figure E.10: Complete description of State 3 and State 4. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted – 8 states.

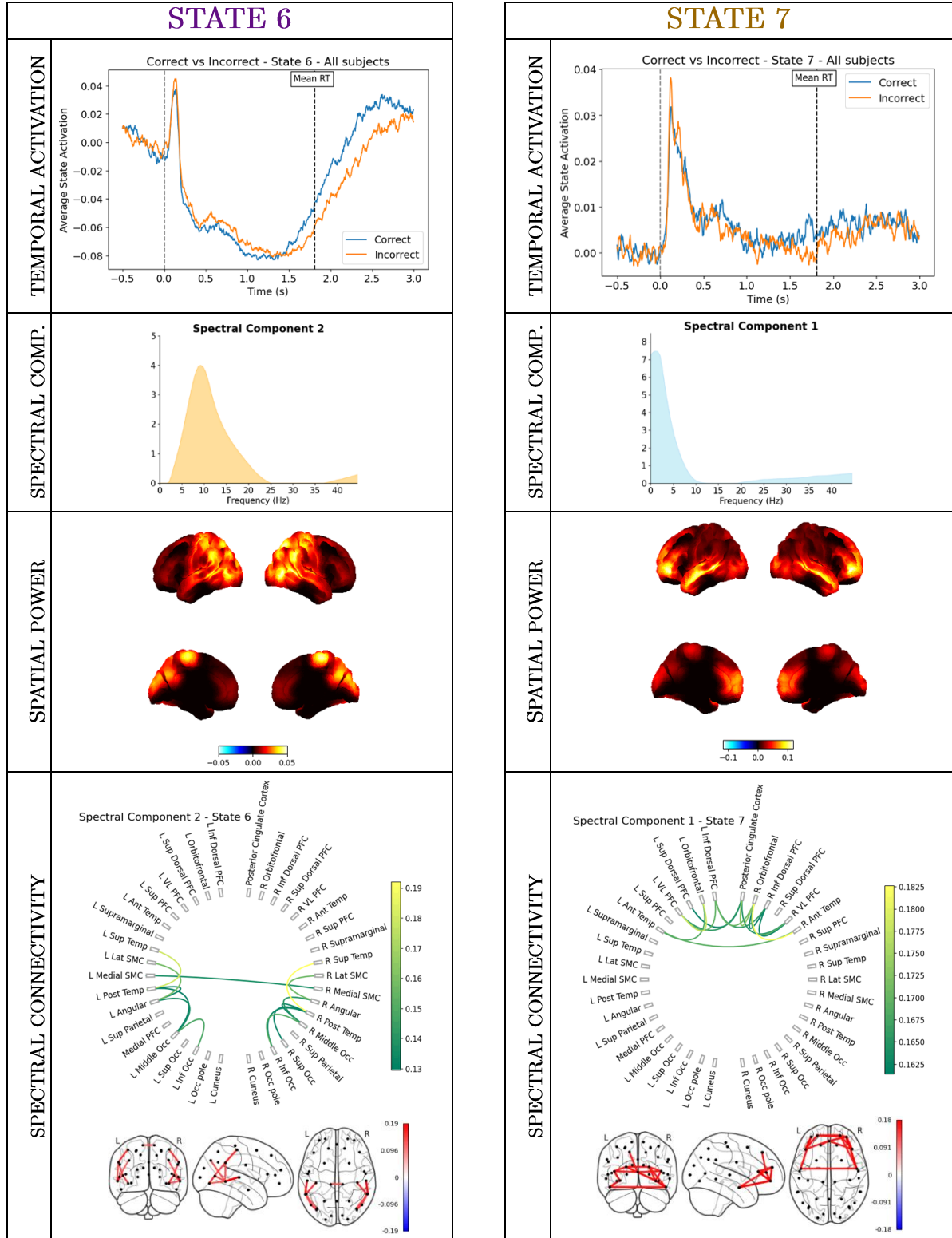


Figure E.11: Complete description of State 6 and State 7. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted – 8 states.

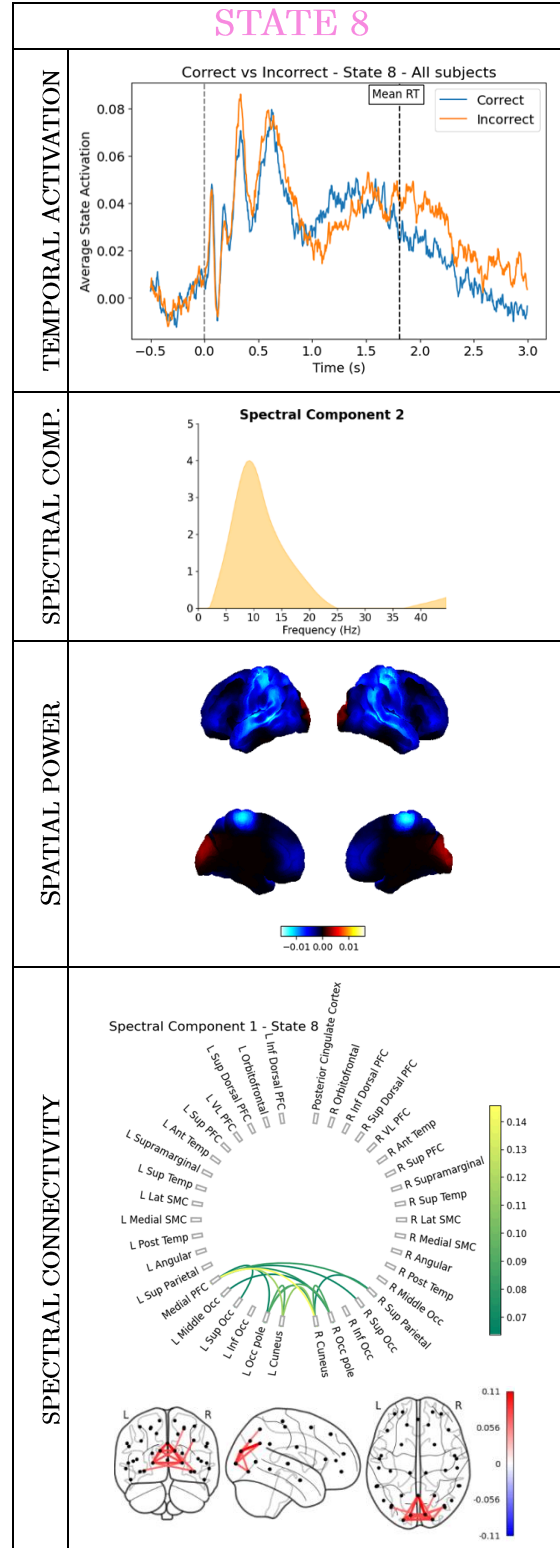


Figure E.12: Complete description of State 8. The top plot shows the task-evoked temporal activation of the states for the two paradigm conditions (correct/incorrect). Below, the spectral component best associated with the state is shown. Further, the average PSD map subtracted by the mean is shown. The power was computed for the weighted frequency range of the chosen spectral component. Lastly, the coherence networks are plotted in circular plots and through brain masks. Only the top 2% strongest connections are plotted – 8 states.

To verify the robustness of the HMM inference, the states' characteristics are compared between both inferences (6 states vs. 8 states). The comparison was performed using the temporal, spectral, and spatial characteristics of each state, and is shown in Table E.2. The model was able to identify highly similar states between both inferences. Furthermore, the 8 states inference did not reveal any additional task-relevant states (i.e. ones that have significant temporal activation around the stimulus onset), meaning no states were omitted in the general description provided in chapter 6 of this dissertation.

6 states		8 states	
State 1	SC3 – Visuospatial processing	State 2	SC3 – Visuospatial processing
State 2	SC3 – Sensorimotor	State 4	SC3 – Sensorimotor
State 3	FLAT	State 5	FLAT
State 4	SC2 – Temporal/Occipital	State 6	SC2 – Temporal/Occipital
State 5	SC1 – Prefrontal	State 1	SC1 – Prefrontal
State 6	SC2 – Occipital	State 3	SC2 – Occipital
		State 7	SC1 – Frontal
		State 8	SC2 – Occipital

Table E.2: Comparison of the states identified by the TDE-HMM across two inferences (6 states vs. 8 states). Each row represents a match between a state from one inference (6 states) with the corresponding state from the other inference (8 states). The colors (blue, yellow, and green) refer to the data-driven spectral components (see Figure E.5).