CO-ACTIVATION PATTERNS FOR THE STUDY OF BRAIN CONNECTIVITY IN MULTIPLE SCLEROSIS PATIENTS

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Gent, August 31, 2018

The promoter,  The author,

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Foreword

This thesis was written by me, Samuel T. Wauthier, and was revised by prof. dr. Daniele Marinazzo. A portion of the disease description of multiple sclerosis was contributed through private communication with dr. N. Vanderlinden.

Throughout Chapter 1-4, I have summarized my findings from a literature study in the matter of co-activation patterns. The last chapters, i.e. Chapter 5 and 6, cover the research based on a given dataset.

The dataset in this thesis was provided by Matilde Inglese (Department of Neurology, Radiology and Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA). It consisted of functional MRI scans of 41 patients and 24 controls. Information on the subject’s age, sex, type of multiple sclerosis and disease duration was included. No other personal information was given; subjects received a code for reference.

I wish to thank prof. dr. Daniele Marinazzo for granting the opportunity to work on such an interesting topic, as well as for always being available for questions and for the swift replies to these questions. Some additional words of thanks go out to dr. N. Vanderlinden, for her contribution to the part on multiple sclerosis, to D. Van den Akker, A. Schotte and B. Vanhecke, for taking the time to help me obtain my results, and to dr. E. Amico, for the CAPsToolbox and his old files. I would also like to thank my family for their support, in particular those who took the time read this work. Lastly, I would like to thank María Guijorro González-Conde, without whom this thesis might have taken a lot more effort to complete.

August 31, 2018
Samuel Wauthier
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Abbreviations

*i.i.d.* independently and identically distributed

ANOVA analysis of variance
ATP adenosine triphosphate
BOLD blood-oxygen-level dependent
CAP co-activation pattern
CNS central nervous system
CSF cerebrospinal fluid
DMN default mode network
EEG electro-encephalography
FDR false discovery rate
FFX fixed effects
fMRI functional magnetic resonance imaging
FWER family-wise error rate
ICA independent component analysis
MEG magneto-encephalography
MFG medial frontal gyrus
ML maximum likelihood
MRI magnetic resonance imaging
MS multiple sclerosis
NMR nuclear magnetic resonance
OLS ordinary least squares
PCC  posterior cingulate cortex
PET  positron emission tomography
ReML  restricted maximum likelihood
RF  radio frequency
RFT  random field theory
RFX  random effects
rs-fMRI  resting state fMRI
RSN  resting state network
SD  standard deviation
SPECT  single photon emission computed tomography
SS  summary statistic
WLS  weighted least squares
Summary

Functional brain connectivity refers to the measure of temporal correlation of activity in the brain. This is usually measured using functional magnetic resonance imaging (fMRI). Magnetic resonance imaging (MRI) is an imaging technique based on the phenomenon of nuclear magnetic resonance (NMR). The technique measures the relaxation time of the magnetization of bodily tissue to form a 3D image of the brain. In the case of fMRI, the blood-oxygen-level dependent (BOLD) signal is measured instead. The BOLD signal is a measure for the difference between the amount of oxygenated and deoxygenated blood, which in turn is a measure for the activity of a brain region. The fMRI scan, then, yields a 3D image for every point in time, or similarly, a time series for every voxel. In resting state fMRI (rs-fMRI), the subject is asked to lie still for the duration of the scan, as opposed to task-based fMRI, where the subject is asked to perform a certain task.

Co-activation patterns (CAPs) are a way to summarize the patterns of correlation in the brain. This is done in two steps. The first step consists of reducing the data. A seed area is selected, for which time frames with peaks in the BOLD signal above a certain threshold are held on to, while the rest of the time frames are thrown away. After that, the second step is clustering the data. The time frames are placed into groups depending on their similarity and averaged per group.

Individual CAP time frames are entered into a second-level analysis in SPM12 with correction for non-sphericity. Hypothesis testing is done using the method of contrasts.

The CAP process was applied to a dataset of multiple sclerosis (MS) patients and controls. With the number of CAPs fixed to 8 and a seed region chosen in the medial frontal gyrus (MFG), results showed visually distinct CAPs. Many CAPs displayed co-activation of the default mode network (DMN) in both patients and controls. Results also showed significant (FWER = 0.05) reduction in co-activation from controls to patients.
1 | Introduction

1.1 Functional connectivity

When researching functionality of the brain, one can distinguish between two key properties: functional segregation and functional integration \([1]\). Investigation of the former is equivalent to attempting to identify which brain areas are specialized for which functions. In contrast, investigation of the latter is equivalent to finding out how specialized brain areas interact with each other. The latter is the purpose of connectivity analysis \([1]\).

We distinguish three types of connectivity: structural connectivity, functional connectivity and effective connectivity. The first type, as the name suggests, refers to the physical presence of connections between brain areas by axons. The second type measures statistical dependency between brain areas, i.e. temporal correlation of activity. The last type expresses the directed influence and causality between brain areas. In other words, it measures the influence one brain region has over another \([1]\). In this work, we focus on the second type, functional connectivity.

Generally, when employing fMRI for functional connectivity analysis, the objective is to compare different states of activity of the brain. Often one might want to compare the brain while performing a certain task with the brain at rest. For example, tapping a finger versus doing nothing. However, even when at rest, the brain is always active. This is reflected by the spontaneous BOLD activity seen on fMRI images. Imaging of the spontaneous BOLD signal is also called rs-fMRI \([1]\).

1.1.1 Resting State Networks

Different methods of functional connectivity analysis have given rise to a series of networks that are strongly connected while at rest \([2]\). These networks are called resting state networks (RSNs). One of most studied RSNs is the DMN, displayed in Fig. 1.1. This network is usually active when a person is awake and at rest, and it activates when a person is performing internal tasks, such as daydreaming or retrieving memories. Unsurprisingly, it is negatively correlated with brain regions that deal with external visual signals \([1]\). The central node of the DMN is widely known to be the posterior cingulate cortex (PCC) region. The frontal node is located in the MFG and the lateral nodes in the angular gyri \([2]\).

1.2 Multiple sclerosis

In 2015, about 2.3 million people world-wide were affected by MS \([4]\). Not much is known about the disease and it can cause a wide range of symptoms. Research is necessary for a better understanding.
1.2.1 Disease description

MS is a disease which affects the central nervous system (CNS) i.e. the brain and spinal cord. The central nervous system is a network of cells (neurons, glia, etc.), that is organized in centers of grey matter connected by white matter. Grey matter is responsible for most bodily functions like muscle control, sensory perception, memory, etc. White matter, instead, relays information between different grey matter areas. The color difference between grey and white matter is caused by the substance ‘myelin’ that surrounds the nerve cell, called a myelin sheath. Myelin appears white and gives white matter its color. The myelin sheath acts as an insulator for the nerve cell and is essential for correct transport of pulses.

Under the microscope, the nerve tissue of an MS patient with brain damage displays areas of inflammation. Inflammation is a part of a complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells or irritants, and is a protective response. MS is thought to be an autoimmune disorder. Myelin is a fatty tissue that surrounds and protects the nerve fibers. Myelin is lost in multiple areas with MS. This loss of myelin forms scar tissue called sclerosis. These areas are also called plaques or lesions. When damaged in this way, the nerves are unable to conduct electrical impulses to and from the brain. The size and location of inflammatory lesions may vary. The cause of inflammation is yet unknown. Fig. 1.2 shows an illustration of damaged myelin.

Lesions can cause loss of bodily function in two ways. First of all, through the lesion itself, much like a person with a sprained ankle cannot use their foot. Second of all, through permanent damage from the lesion. Some lesions lead to damaged myelin sheaths, while others do not. As a result, some lesions lead to complaints and symptoms, while others do not, and these can be temporary or permanent. Why some lesions lead to brain damage and others do not is also yet unknown. Although they occur in the white matter, research shows that lesions can also affect grey matter. It is thought that loss of grey matter is the cause of permanent neurological impairment. In fact, recent studies point out that pathological changes can also be detected in normal-looking white matter and grey matter.
In private communication, dr. N. Vanderlinden explained MS can occur anywhere in the white matter. Therefore, a wide range of symptoms can occur, for example, blurred or double vision, difficulty with walking, parasthesia (this refers to an abnormal sensation, or pain, such as numbness, prickling, or ’pins and needles’), muscle weakness in the extremities, difficulty with coordination, fatigue, tremor and dizziness. In addition, progression of the disease is not predictable. Most people with MS have a relapsing-remitting disease course. They experience periods of new symptoms or relapses that develop over days or weeks and usually improve partially or completely. These relapses are followed by quiet periods of disease remission that can last months or even years. About 60 to 70 percent of people with relapsing-remitting MS eventually develop steady progression of symptoms, with or without periods of remission, known as secondary-progressive MS. Some people with MS experience a gradual onset and steady progression of signs and symptoms without any relapses. This is known as primary-progressive MS.

Dr. N. Vanderlinden clarified that there is no definitive test available to diagnose MS. However, a probable diagnosis can be made by following a careful process which demonstrates findings that are consistent with MS, that also rule out other causes and diseases. There are two criteria used to diagnose MS:

1. There must have been two attacks at least one month apart. An attack is a sudden appearance of worsening of any MS symptom or symptoms that last at least 24 hours.

2. There must be more than one area of damage to the central nervous system myelin, which must have occurred at more than one point in time and was not caused by any other disease.

More commonly nowadays, a single attack together with certain patterns of changes in brain tissue seen on a MRI (see section 1.2.2) of the brain can strongly suggest the diagnosis of MS.

Treatment of MS has 2 aspects: immunomodulatory therapy (IMT) for the underlying immune disorder and therapies to relieve or modify symptoms. IMT is directed toward reducing the frequency of relapses and slowing progression, thus described by dr. N. Vanderlinden.

Altogether, MS can lead to both structural and functional disconnectivity within the CNS. A recent review paper, lists learning, memory, information processing speed, social cognition and executive function among the signs of functional disconnectivity, linked to brain areas including, but not limited to, the mesial temporal lobe (e.g. hippocampus), thalamus, frontal lobe (e.g. dorsomedial prefrontal cortex), tempo-parietal junction and cingulate cortex.

1.2.2 Importance of MRI

Conventional MRI, i.e. T1- and T2-weighted MRI, makes it possible to visualize lesions in the CNS. In a typical T1-weighted image, the lesions can show up as white spots of different sizes. If uncertainty exists over whether the spots are MS lesions (as opposed to a tumor, for example), a contrast fluid can be used to get a more accurate diagnosis.

The link between MS and conventional MRI is still unclear. Conventional MRI typically does not yield any information on the progression of the disease. For example, there is no direct association between the occurrence of MS symptoms and the tracing of new
abnormalities in the MRI image. The amount of MRI abnormalities could increase, while the symptoms remain the same and vice-versa.

Despite this disadvantage, conventional MRI remains the most important method for diagnosis of patients and for the assessment of treatment efficacy. However, to quantify pathological changes, other methods must be used. Considering it can be used to investigate connectivity in the brain and since MS mainly affects white matter, fMRI is an appropriate method.

1.3 Objectives

This work has two main objectives. The first objective is to provide an overview of the CAPs method for brain analysis. The second objective is to apply the CAP method to a novel dataset of MS patients.

To this end, this work is split up in a number of chapters:

- Chapter 2 illustrates the workings of MRI and fMRI. This used as a basis to understand what the data mean and what is being analyzed.

- Chapter 3 describes how CAPs are calculated from both an intuitive and mathematical perspective.

- Chapter 4 explains how the dataset was analyzed.

- In Chapter 5, we present the outcome of the analysis.

- Lastly, in Chapter 6, we discuss the implications of the analysis.
Functional MRI

Since the early 1990s, using the signal has been the main technique for brain mapping research. This chapter explains the underlying physics of MRI and how the blood-oxygen-level dependent (BOLD) signal is obtained. Lastly, some corrections for common inconsistencies of the functional magnetic resonance imaging (fMRI) images are described.

2.1 Physical background

MRI is based on the phenomenon of NMR. In order to understand this phenomenon, one must understand how nuclear spins behave in a magnetic field.

2.1.1 Nuclear spin and energy level splitting

In the nuclear shell model, similar to the electronic shell model, the protons and neutrons that make up the nucleus, i.e. the nucleons, are bound to orbitals orbital the nucleon find itself in, the rest is intrinsic to the particle and is called ‘spin’. Depending on the arrangement of nucleons in the orbitals, the nucleus has a specific amount of energy. Different arrangements have different energies. The collection of possible arrangements, or energies, is often called the ‘energy states’. The lowest of the energy states, i.e. the one with the lowest energy, is the ‘ground state’ and corresponds to the arrangement where all the nucleons are in their lowest possible orbitals. As a result of being bound to orbitals, each nucleon possesses some amount of angular momentum. A part of the angular momentum comes from the orbital the nucleon find itself in, the rest is intrinsic to the particle and is called ‘spin’.

Since the nucleus is composed of protons and neutrons, it also possesses a certain amount of angular momentum. This is usually referred to as nuclear spin and is denoted ‘I’. In quantum mechanics, when a nucleus with non-zero $I$ is placed inside a static magnetic field $B_0$, each of its energy states splits into multiple states with different energies. The new states will be labeled with the magnetic quantum number $m$, which takes on the values $-I, -I+1, \ldots, I-1, I$, i.e. $2I + 1$ possible values. Fig. 2.1 shows how the ground state of a nucleus with $I = \frac{1}{2}$ splits and the energy difference between the levels increases as the magnetic field strength increases. The splitting of the levels can be interpreted in classical mechanics: the spin of the nucleus takes on a specific orientation with respect to the magnetic field (and precess about the magnetic field lines). As an example, consider a nucleus with spin $I = 2$, which can take five different orientations, as shown in Fig. 2.2.

\[1\] The author speculates that the word ‘nuclear’ was left out of the name of the imaging technique, because of its connotation with nuclear power and radioactivity. He would like to reassure the reader that no ionizing radiation is used in this technique.
The energy of level \( m \) is given by the classical potential energy of a magnetic dipole in a magnetic field:

\[
E = -\mu_z B_0 = -m\hbar\gamma B_0,
\]

(2.1)

where \( \mu_z \) is the \( z \)-component of the magnetic dipole moment (the component parallel to the magnetic field), \( \gamma \) is the magnetogyric ratio of the nucleus, \( \hbar \) is the reduced Planck constant and \( B_0 = |B_0| \) is the magnetic field strength \( 12 \). Note that the most favorable energy state is the one with the largest magnetic quantum number \( m \).

The energy difference between level \( m \) and \( m' \) is given by

\[
\Delta E = (m' - m)\hbar\gamma B_0,
\]

(2.2)

or for adjacent levels,

\[
\Delta E = \hbar\gamma B_0.
\]

(2.3)

Adding or removing an amount of energy equal to \( \Delta E \) changes the orientation of the nucleus \( 12 \).

In MRI, the energy \( \Delta E \) is provided by applying a weak pulsed radio frequency (RF) magnetic field. The frequency \( \omega_0 = \frac{\Delta E}{\hbar} = \gamma B_0 \) required for this is called the Larmor frequency. The term ‘resonance’ in nuclear magnetic resonance and magnetic resonance imaging refers to the point at which the oscillation of the RF field matches the Larmor frequency. At this point, some of the energy of the RF field is absorbed and the nucleus undergoes a state transition. The former shows up as a peak in absorption spectra \( 12 \).

It is important to note that the magnetogyric ratio \( \gamma \) depends on the nucleus. As a result, the resonance frequency varies between nuclei of different proton and neutron numbers. This makes it possible to affect one type of nucleus in a sample, while leaving the others intact. In reality, the resonance frequency also depends on the compound the nucleus is located in. For example, the resonance frequency of \( ^{13}C \) in carbon dioxide and in ethanol differs due to differences in the local magnetic field. This is called the chemical shift \( 12 \).

2.1.2 Magnetization

If a large amount of the same nucleus is placed in a static magnetic field \( B_0 \), the system will eventually reach statistical equilibrium \( 13 \). In equilibrium, the orientation of the
spins follows the Boltzmann distribution, i.e. the probability density distribution of being in a state with energy $E$ follows

$$F(E, T) \propto e^{-\frac{E}{kT}},$$

where $k$ is the Boltzmann constant and $T$ is the thermodynamic temperature. In other words, the system of nuclear spins is a canonical ensemble \cite{13}. As a result, there is a slightly larger amount of nuclei in the lower energy state and, therefore, a net polarization parallel to the external magnetic field.

Since the system has a spatial distribution, i.e. the nuclei have different positions in space, we can define the magnetization

$$M = \frac{dm}{dV},$$

where $m$ is the magnetic dipole moment of the nuclei and $V$ is the volume filled by the nuclei. This quantity provides a measure of how the system reacts to the external magnetic field and varies in space \cite{14}.

### 2.1.3 Relaxation and imaging

The discussion in the previous section can be applied to the case where bodily tissue enters a magnetic field. In this case, the nuclei in the tissue are a canonical ensemble and the tissue acquires a certain amount of magnetization. When the RF pulse is applied, the tissue is excited and the equilibrium is disrupted \cite{15}. The time for the system to return to equilibrium can be measured. Two types of relaxation can be distinguished: spin-lattice and spin-spin, also known as $T_1$ and $T_2$. The first type refers to the mechanism where the component of the magnetization parallel to the static magnetic field reaches equilibrium. The decay constant $T_1$ determines the speed of the decay. In contrast, the second type refers to the mechanism where the transversal component of the magnetization reaches equilibrium. In this case, the decay constant $T_2$ determines the speed of the decay. After the RF pulse, the transversal magnetization produces an oscillating magnetic field, which also decays over time. The decay of this phenomenon is determined by the time constant $T_2^* \ [15]$.

In ideal cases, $T_2^*$ is the same as $T_2$. In general, however, $T_2^*$ is shorter. On the other hand, $T_1$ is usually longer than $T_2$. Typical values are: around 1 second for $T_1$ and a few tens of milliseconds for $T_2$ and $T_2^* \ [15]$.

Image contrast is obtained by differences in relaxation times between locations in the tissue. In general, $T_1, T_2$ and $T_2^*$ all vary between locations, however, in some cases the
difference in one of the relaxation times, say $T_1$, is larger than in the others. In that case, it would be more useful to measure this relaxation, $T_1$, since the difference is clearer \cite{15}.

\section*{2.2 Blood-oxygen-level dependent signal}

Up until this point, we have discussed the basic physical principles of NMR, which form the basis for MRI. In turn, fMRI, the most common way of studying brain activity, is performed by measuring the BOLD signal. The BOLD signal (sometimes called BOLD contrast) is best understood through the brain metabolism.

\subsection*{2.2.1 Brain metabolism}

Whenever the brain executes a function, a series of processes occurs, known as the hemodynamic response to the neural event \cite{9}. The area of the brain responsible for the execution of the task requires additional energy in the form of adenosine triphosphate (ATP). ATP is primarily produced by the mitochondria through aerobic respiration. A process in which glucose and oxygen are used to create water, carbon dioxide and ATP. As the local stores of oxygen get used up, a chemical signal causes the capillaries to dilate. Dilation of the vessels causes an increase in blood flow, which serves to restore the local oxygen levels. For yet unknown reasons, more oxygen is delivered than is necessary to replenish the oxygen deficit \cite{9}.

In terms of hemoglobin, the neural event initially causes a build-up deoxygenated hemoglobin (Hb) and a decrease in oxygenated hemoglobin (HbO2). Around a second later, this is followed by an increase in HbO2 and a decrease in Hb \cite{9}.

\subsection*{2.2.2 Measurement}

Oxygenated and deoxygenated hemoglobin have different magnetic properties \cite{9}. HbO2 is diamagnetic, whereas Hb is highly paramagnetic. The difference causes local gradients in magnetization, which, in turn, causes differences in $T_2$ and $T_2^*$ relaxation times. For magnetic field strengths of 1.5T and 3T, the difference in $T_2^*$ is most prominent, while for larger field strengths, $T_2$ becomes more important \cite{9}.

In order to form an fMRI image, the $T_2$ or $T_2^*$ relaxation time is then measured at different positions in the brain at different time points. This means that the whole brain is scanned at regular intervals, which creates a ‘4D’ image: the brain in three dimensions over time. Every point in 3-dimensional space, typically called a ‘voxel’, is a time-series that shows the BOLD signal.

\section*{2.3 Preprocessing}

In reality, the ideal experiment as defined by theory is generally not possible. For example, an MRI scanner cannot scan the whole volume of the brain at the same time. Therefore, the slices in a time frame are not obtained exactly at the same time. In addition, the human body is always in motion and cannot lie completely still. Consequently, time frames are not necessarily aligned. To account for these limitations, a number of corrections can be applied, which are generally referred to as preprocessing.

Some preprocessing steps are \cite{16}. 

• Time-slice correction: to correct for time of acquisition of each slice in a time frame.

• Motion coregistration: to correct for head motion. Each time frame is realigned with a reference time frame. This yields motion parameters for translation in three dimensions, pitch, roll and yaw.

• Coregistration: alignment of functional and structural images. Used to map functional information into anatomical space.

• Correction for physiological noise, such as breathing and cardiovascular function.

• Spatial smoothing: to improve signal-to-noise ratio. Remove high frequency information.

• Spatial normalization: transform the images to a template, an anatomical reference. This allows for coordinates to be expressed in a standard space and for subjects to easily be compared. Typically, images are transformed to MNI space\(^2\). For example, the PCC in MNI coordinates is located at \([0 \ - \ 5326]\).

• Lesion-filling: improvement of measurement by reducing intensity contrast within known lesions.

A detailed explanation on these preprocessing methods are beyond the scope of this work. However, an indication is important to understand the data and analysis.

\(^2\)Named for the Montreal Neurological Institute.
3 | Co-activation patterns

In this chapter, we justify the use of CAPs for the investigation of functional brain connectivity. Subsequently, we present the process for the calculation of CAPs. This consists of the ‘data reduction’ and ‘clustering’ steps. For both, we include a mathematical description.

3.1 Motivation

Traditional methods of connectivity analysis employ temporal correlations over the entire scan. Methods such as “seed”-based correlation analysis or independent component analysis (ICA), typically reveal core functional systems, but neglect time-varying information. That means, these methods tend to make assumptions about the BOLD signal, such as stationarity of interregional interactions \[17\]. Recent studies, however, suggest that spontaneous brain activity is nonstationary \[17,18\]. The brain goes through “avalanches” of activation, i.e. short instances of co-activation of brain regions \[17,19\].

The data coming out of an fMRI scan consist of many time series covering the brain \[20\]. As a result, datasets of this kind can be extremely large and methods have to be developed in order to deal with the size.

A method that does not suffer from the assumption of stationarity is that of CAPs. Research shows that CAPs reveal more fine-grained information about functional connectivity compared to seed-based correlation maps \[17,18,21\]. Additionally, the CAP method innately reduces the amount of the data.

3.2 Data reduction

Tagliazucchi et al. have shown that important information is enclosed in the large amplitude peaks of the BOLD signal \[22,23\]. As a result, the time series embody large time spans that contain information which is not necessarily useful for a specific analysis. A way to remove the excess information is to convert the time series into a series of time intervals \[24\]. In particular, Tagliazucchi et al. showed that the fMRI dataset can be reduced to a spatio-temporal point process \[19\]. The latter is defined as the sequence of time points at which the BOLD signal crosses a threshold from below. This is equivalent to taking a Poincaré section (see section 3.2.2).

Instead of dealing with the entirety of the data, only a few points are selected. This process not only helps speed up computations, but also inherently selects the data which contains the most important information. One type of selection is based on a threshold value (e.g. one standard deviation). If the BOLD signal crosses this value from the bottom, the time point is added to the selection (Fig. 3.1). Other types of selection
exist, however, all of them require the signal to be above (or below) some threshold. In addition, one typically chooses an area of the brain (a seed area) to be investigated for co-activation, for example, the PCC. That is, one investigates which areas are correlated to the seed area. In that case, the data reduction step is done on this seed area. This, so-called, point process can lead to reductions in data of over 90%.

After obtaining the time points at which the BOLD signal is above threshold value, it is possible to construct spatio-temporal mappings of correlated activity. To do this, all the time frames corresponding to the formerly obtained time points, must be clustered.

### 3.2.1 A few time points suffice

As explained in Chapter 3, the data reduction step (i.e. the point process) in the CAP calculation is beneficial for analysis. As it turns out, signals with long-range correlation are particularly suitable for the point process, as large amplitude peaks contain the most information. Cifre et al. pointed out that this also applied to signals of other origins.

It is very well known that brain activity is both temporally and spatially correlated. The BOLD signal, in particular, is convoluted by the hemodynamic response function, which in itself introduces temporal correlation. With these properties, it makes sense that the points which contain the most information are the ones where the derivative changes sign. The remaining time points become redundant, since the signal can more or less be reconstructed from the point process, as shown in Fig. 3.2.

### 3.2.2 Mathematical description

A Poincaré section $S$ of a dynamical system exhibiting a periodic orbit is a plane that is transversal to the flow of the system and intersects with the trajectory in phase space. The points on $S$ indicate the points through which the trajectory leaves the plane and subsequently returns, as displayed in Fig. 3.3. Such a plane reduces the trajectory in $n$-dimensional phase space to a number of points in $(n-1)$-dimensional space.

Now, let $y = g(x)$, with $g$ a scalar observable function, such as the BOLD signal. Selecting a Poincaré section, i.e. selecting the times at which $y$ crosses the threshold $y_c$ from below yields a sequence $\{t_i\}_{i=1,...,N}$ that defines the spatio-temporal point process.

It is not trivial, however, that this approach is valid. Indeed, even if the system could be reconstructed from the original time series, this does not ensure that the system can be reconstructed after the Poincaré section. Here, we show that this approach is valid.
3.2. DATA REDUCTION

Figure 3.2: Two minute recording of a BOLD signal. The horizontal dashed lines indicate thresholds for the point process. The points at the bottom indicate the points included in the point process. The dashed lines show a piece-wise linear time series constructed from the peaks and valleys (indicated with arrows). Image adapted from Cifre et al. (2017) [20].

Figure 3.3: Schematic representation of a Poincaré section with trajectory in phase space [30].

in the case of scalar signals, such as the BOLD signal.

Consider a nonlinear autonomous dynamical system defined by

\[ \dot{x} = f(x), \]  

(3.1)

where the dot indicates the time derivative, \( f \) is a nonlinear function and \( x \in \mathbb{R}^d \) with \( d \) the dimension of the phase space. Let \( \gamma(x_0, t) \) be a solution of Eq. (3.1) in the open interval \( I = (t_0, T) \), under the condition that the system is not in a fixed-point or equilibrium, i.e.

\[ |\gamma(x_0, t)| \neq 0 \quad \forall t \in I. \]  

(3.2)

Takens’ theorem [31] provides the conditions under which a chaotic dynamical system can be reconstructed from a sequence of observations of the state of the system [32]. The exact definition of this theorem is beyond the scope of this work, however, we will show that our point process can be used to reconstruct the dynamical system. Takens’ theorem
is more usually defined in terms of the attractor. This is the subset of phase space that corresponds to the typical behavior of the system, i.e. the subset of phase space towards which the system tends to evolve [33]. In this sense, Takens’ theorem gives the conditions under which a smooth attractor can be reconstructed from observations [32].

The arc-length,

$$s(x_0, t) = \int_{t_0}^{t} |\dot{\gamma}(x_0, \tau)| \, d\tau,$$

fulfills the conditions imposed by Takens’ theorem, since it is a smooth function in phase space. The attractor can be reconstructed using the line segments

$$\tilde{s}(t_{i+1}, t_i) = s(x_0, t_{i+1}) - s(x_0, t_i).$$

Taking the derivative of $s$ with respect to time $t$, we obtain

$$\frac{ds}{dt} = |\dot{\gamma}(x_0, t)| = |f(x)|.$$  (3.5)

Eq. 3.3 can then be rewritten as

$$\frac{dx}{ds} = \frac{f}{|f|}.$$  (3.6)

The dynamics of $y = (x, t)$ is then given by

$$\frac{dy}{ds} = g(y) \text{ with } g = \left( \frac{f}{|f|}, \frac{1}{|f|} \right).$$  (3.7)

With Eq. 3.7, the trajectory in $d$ dimensions has been reparametrized in $(d + 1)$-dimensional extended phase space. Time is now a dependent parameter and Takens’ theorem can, therefore, be applied to time, or time intervals. That is, the attractor can be reconstructed using from time intervals [24].

### 3.3 $k$-means clustering

The goal of data clustering is to discover the ‘natural’ groupings of a set of points, patterns or objects [25]. For the construction of CAPs, this means grouping for pattern. Indeed, the clustering algorithm will group together time frames with similar patterns of correlated activity. So that, subsequently, the means of these groups yield the CAPs.

#### 3.3.1 Mathematical description

One of the most popular clustering algorithms is the $k$-means algorithm. This algorithm attempts to solve a specific problem [34].

**Problem.** Let $\Omega = \{x_1, x_2, \ldots, x_n\}$ be a set of $n$ observations with $x_i \in \mathbb{R}^d$, a $d$-dimensional vector. The $k$-means problem is to partition the $n$ observations into $k(\leq n)$ sets $\Lambda = \{S_1, S_2, \ldots, S_k\}$, so as to minimize the within-cluster sum of squares, i.e.

$$\arg\min_{\Lambda} \sum_{i=1}^{k} \sum_{x \in S_i} \|x - \mu_i\|^2,$$  (3.8)

where $\mu_i = \frac{1}{|S_i|} \sum_{x \in S_i} x$, the mean of the observations in $S_i$. 
From a mathematical point of view, this is a combinatorial optimization problem. In fact, it is classified as an NP-hard problem in Euclidean space, even for dimensions as low as $d = 2$ (the plane) and for cluster numbers as low as $k = 2$. Therefore, iterative methods must be employed in order to solve the problem.

### 3.3.2 Algorithm

The $k$-means algorithm tries to solve the problem in an iterative way (Alg. 1). The process starts by choosing a set $M$ of $k$ centroids which will serve as the mean for each partition. There are various ways to make this choice. Often, $M$ will be chosen as a random subset of $\Omega$.

Subsequently, the algorithm iterates over two steps. The first step builds the partitions $S_i$, i.e. it subdivides the observations $x_1, x_2, \ldots, x_n$ into the different $S_i$ depending on their Euclidean distance to the centroids $\mu_i$. For example, an observation $x$ will be put into $S_1$ if the distance between $x$ and $\mu_1$ is smaller than the distance between $x$ and any other $\mu_i$ ($i \neq 1$). The second step adjusts the value of the $\mu_i$. The value of $\mu_i$ becomes the mean of the observations in $S_i$.

The new values become the centroids used in the first step, so that one obtains new partitions. The iterations stop when the values of $\mu_i$ do not change any more. At that point, the partitions, too, do not change any more and convergence has been reached.

**Algorithm 1 $k$-means algorithm**

1: procedure $k$-MEANS($\Omega, k$)
2: Initialize starting values of $M = (\mu_1, \ldots, \mu_k)$
3: repeat
4: $S_1, \ldots, S_k \leftarrow \emptyset$
5: for all $x \in \Omega$ do
6: $i \leftarrow \arg\min_{i=1,\ldots,k} \|x - \mu_i\|^2$
7: $S_i \leftarrow S_i \cup \{x\}$
8: end for
9: for $i \leftarrow 1, k$ do
10: if $S_i \neq \emptyset$ then
11: $\mu_i \leftarrow \frac{1}{|S_i|} \sum_{x \in S_i} x$
12: end if
13: end for
14: until $M$ remains unchanged
15: return $S_1, \ldots, S_k, M$
16: end procedure

In the CAP setting, the observations $x_1, x_2, \ldots, x_n$ are the time frames corresponding to the different time points. The time frames can easily be represented in $\mathbb{R}^d$-space. The $k$-means algorithm, then, yields clusters of similar time frames and the means of those clusters. The latter are the CAPs.

---

1 Problems in NP-hard are at least as hard as the hardest problem in NP. They are not necessarily elements of NP and need not be decidable.
3.4 A note on CAPs

In fact, a CAP is somewhat of a general object \cite{20,35}. One could, for example, have a set of time series of Gaussian noise, apply the CAP process to this set and obtain CAPs that are similar to the seed-based correlation map. Fig. 3.4 shows the similarity, i.e. the correlation, between a CAP produced using a percentage of a simulated dataset and the corresponding seed-based correlation map. One can see that for approximately 5\% of the data, the similarity is already around 0.5 and grows to just over 0.6 before dropping again when large portions of the data are used. When applied to real-world data, CAPs characterize the underlying structures and, thus, can be considered as a form of spatial clustering.
4  |  Statistical analysis

In this chapter, we introduce the statistics necessary for the analysis of functional connectivity. We first show how the data is modeled; how the data is decomposed into effects and error. Then we explain how to form statistics using the estimates.

4.1 SPM

The analyses of the CAPs were done in Statistical Parametric Mapping 12 (SPM12) [36]. SPM12 is software developed for the analysis of brain imaging data sequences and designed for fMRI, positron emission tomography (PET), single photon emission computed tomography (SPECT), electro-encephalography (EEG) and magneto-encephalography (MEG). SPM analyses are voxel-based, which means that parametric models are assumed at each voxel [36].

4.1.1 Estimation

Parameter estimation in SPM12 is done in MATLAB through the spm_spm.m file. For this, one first specifies a design matrix \( X \), so that a general linear model can be evaluated at each voxel:

\[
Y = X\beta + \varepsilon, \tag{4.1}
\]

with the data \( Y \), parameters \( \beta \) and residual errors \( \varepsilon \sim \mathcal{N}(0,\sigma^2) \) [37]. The model is explained in more detail in section 4.2.

For fMRI data, where the assumption of independently and identically distributed (i.i.d.) errors does not hold, there is the issue of non-sphericity [37]. This issue is explained in more detail in section 4.3. A short summary follows now. In this case, covariance components are first estimated through restricted maximum likelihood (ReML) parameters. The ReML estimates are then used to whiten the data. That means that the data are modeled a second time with an augmented model.

\[
WY = WX\beta + W\varepsilon, \tag{4.2}
\]

where \( WW^T = V^{-1} \) with \( V \) the non-sphericity matrix based on the parameter estimates, i.e. \( \text{Var}[\varepsilon] = \sigma^2V \). The covariance matrix of the parameter estimates is computed as

\[
\text{Var}[\beta] = (WX)^+ \text{Var}[W\varepsilon][(WX)^+]^T, \tag{4.3}
\]

where \( A^+ \) indicates the Moore-Penrose inverse of matrix \( A \) [37].
4.2 Second-level analysis

A two-level model is a hierarchical model with two levels\(^3\). Hierarchical models are a type of statistical models where, at each level, the distribution of parameters is given by the level above. In general, for \(n\) levels,

\[
Y = X^{(1)} \theta^{(1)} + \varepsilon^{(1)} \\
\theta^{(1)} = X^{(2)} \theta^{(2)} + \varepsilon^{(2)} \\
\theta^{(2)} = X^{(3)} \theta^{(3)} + \varepsilon^{(3)} \\
\vdots \\
\theta^{(n-1)} = X^{(n)} \theta^{(n)} + \varepsilon^{(n)},
\]

where \(Y\) is the response, \(X^{(i)}\) is the \(i\)-th level regressor matrix, \(\beta^{(i)}\) is the \(i\)-th level parameter vector and \(\varepsilon^{(i)}\) is the \(i\)-th level error vector\(^3\). This type of model is also called a random effects (RFX) model, for reasons that will soon become clear.

Second-level analysis refers to the analysis using a two-level model. In the context of brain imaging, this is usually employed for group analysis, i.e. the analysis of many subjects at the same time\(^3\). In other words, it attempts to deal with multiple sources of variance. For example, it can be used to investigate whether a certain co-activation is different between groups of subjects. In contrast, a first-level analysis examines subjects individually\(^3\).

Suppose we have a subject and we devise an experiment for which we obtain a response. Each time we repeat the experiment, the response may be slightly different. In this case, there is a source of variability within the subject. To draw inference on the response, we must treat it as a random variable\(^4\).

Suppose, now, that we have a number of subjects. The response will be different for each subject. As a result, there is a source of variability between the subjects. If we want to treat the subjects as a sample of a wider population and be able to draw inference about the response of the population, we must treat the subject as a random effect\(^4\).

More mathematically, the foregoing description could be written as follows. Assume the mean effect of the population is \(\mu_p\) and the variance of the effect between subjects is \(\sigma_b^2\). Subsequently, assume that the mean response for subject \(i\) is \(\mu_i\) where \(\mu_i\) is drawn from the distribution of the population and the variance of the response within subject \(i\) is \(\sigma_w^2\). If we assume that \(\sigma_w^2\) is the same for all subjects, we may write for the \(j\)th response \(Y_{ij}\) of subject \(i\):

\[
Y_{ij} \sim \mathcal{N}(\mu_i, \sigma_w^2), \quad \mu_i \sim \mathcal{N}(\mu_p, \sigma_b^2),
\]

where we have assumed a normal distribution for both the population and subject variability\(^4\).

Consequently, the response in a RFX analysis is modeled as

\[
Y_{ij} = \mu_i + W_{ij} \\
\mu_i = \mu_p + B_i,
\]

where \(W_{ij}\) is the within-subject error and \(B_i\) is the between-subject error, which both have zero mean and have variance \(\sigma_w^2\) and \(\sigma_b^2\), respectively\(^4\). With this, we can calculate\(^1\)

\(^1\)That is to say, the mean of the means of the responses of the subjects.
the maximum likelihood (ML) estimate of the population mean:

$$\hat{\mu}_p = \frac{1}{Nn} \sum_{i=1}^{N} \sum_{j=1}^{n} y_{ij},$$

(4.7)

with $N$ subjects and $n$ responses per subject. This estimate has expected value $E[\hat{\mu}_p] = \mu_p$ and variance

$$\text{Var}[\hat{\mu}_p] = \frac{\sigma_p^2}{N} + \frac{\sigma_w^2}{Nn}.$$  

(4.8)

Notice how the variance takes into account both the between- and within-subject variance.

For brain images, this model is usually adapted slightly. This is because brain images tend to be composed of hundreds of thousands of voxels. The previous model can be computationally intensive.

The summary statistic (SS) approach instead models the sample mean of subject $i$, $\bar{Y}_i$:

$$\bar{Y}_i = \mu_i + W_i$$

$$\mu_i = \mu_p + B_i.$$  

(4.9)

In essence, this brings the sample means forward from the 1st level, so that

$$\bar{Y}_i = \mu_p + B_i + W_i,$$

(4.10)

which can be seen as a general linear model with subject as factor. For this approach, the ML estimator is

$$\hat{\mu}_p = \frac{1}{N} \sum_{i=1}^{N} \bar{y}_i,$$

(4.11)

with expected value $E[\hat{\mu}_p] = \mu_p$ and variance

$$\text{Var}[\hat{\mu}_p] = \frac{\sigma_p^2}{N} + \frac{\sigma_w^2}{Nn}.$$  

(4.12)

Indeed, the mean and variance are identical to the ones obtained earlier for the regular RFX model, which confirms its validity.

It is important to stress that this approach assumes that the within-subject variance is approximately equal for every subject and that there are no extreme outliers. When this is not the case, a correction for non-sphericity is required (see section 4.3).

### 4.2.1 Application to CAPs

The individual CAP frames obtained after the data reduction step summarize information on co-activation of the seed region in the subject, but are not indicative of the population. To draw inference on the population, we need to consider the variation between subjects, i.e. we need to estimate the population variance. Therefore, individual CAP frames are entered as the 1st level in the SS approach.
Sphericity is the condition that the variances of differences between levels of a factor are equal. For example, consider three groups A, B and C. Denote the variance of the difference between two levels by $s_{X-Y}^2$. Then, we must have $s_{A-B}^2 = s_{B-C}^2 = s_{A-C}^2$.

In brain imaging, this concept is used in a broader sense and is, therefore, defined slightly differently. Foremost, consider the general linear model \[ Y = X\beta + \varepsilon. \] The parameters $\beta$ can be estimated using ordinary least squares (OLS):

\[
\hat{\beta} = (X^TX)^{-1}X^Ty.
\]

This is only valid, however, if the errors are $i.i.d.$ Mathematically, $\varepsilon \sim \mathcal{N}(0, \sigma^2 I)$, with $I$ the identity matrix [39].

For fMRI data, the assumption of $i.i.d.$ errors generally does not hold [37,39]. The concept of sphericity, in this context, can be most easily understood if one considers a bivariate distribution. Morcom [39] uses the example of the distribution of lengths and weights of men, which we shall adopt. Consider the univariate distributions of these variables in Fig. 4.1. If we construct the bivariate distribution and assume that the parameters are independent, we expect the contours of this distribution to be circular. If this assumption is untrue, the contours will not be circular, as seen in Fig. 4.2. Applying this to multiple independent parameters means that the contours will become hyperspheres, i.e. spheres in multidimensional space. Accordingly, for dependent parameters, the contours will not be hyperspheres, or non-spherical.

To see this from a mathematical perspective, sphericity implies $i.i.d.$ variables [43]. This can be understood through the covariance matrix. Sphericity, then, means that the covariance matrix is a multiple of the identity matrix:

\[
\text{Var}[\varepsilon] = \sigma^2 I.
\]

Examples of non-sphericity are non-identity: \[
\begin{pmatrix}
4 & 0 \\
0 & 1
\end{pmatrix},
\]

and non-independence: \[
\begin{pmatrix}
2 & 1 \\
1 & 2
\end{pmatrix}
\] [42,43].
**Figure 4.2:** Bivariate distributions of the lengths and weights of men, with mean \(\mu = \begin{pmatrix} 180 \\ 80 \end{pmatrix}\) and covariance \(\Sigma = \begin{pmatrix} 200 & 100 \\ 100 & 200 \end{pmatrix}\). Figure obtained from Morcom (2017) [39].

In order for our inference of repeated measures analysis of variance (ANOVA) to be correct, we need to ensure sphericity. To deal with non-sphericity, it is important to have a good model of the error structure [39]. One way to solve the issue is to use first-order autoregressive errors:

\[
\varepsilon_t = a\varepsilon_{t-1} + \zeta_t \quad \text{with} \quad \zeta_t \sim \mathcal{N}(0, \sigma^2),
\]

where \(\zeta_t\) is a new error term frequently referred to as ‘white noise’ [39]. This defines a matrix \(V\), so that \(\varepsilon \sim \mathcal{N}(0, \sigma^2 V)\) [43].

In a first pass through the data, this matrix \(V\) is estimated. In a second pass, a filter matrix \(W\) is defined, with \(WW^T = V^{-1}\). Subsequently, the model,

\[
WY = WX\beta + W\varepsilon,
\]

is estimated, which ‘undoes’ the correlations. The process is often called ‘whitening’ [43].

After whitening, the parameters can be estimated using OLS [39]. In this sense, the method is equivalent to weighted least squares (WLS). The covariance of the parameters is computed as

\[
\text{Var}[\hat{\beta}] = (WX)^+ \text{Var}[W\varepsilon](WX)^+^T,
\]

where \(A^+\) indicates the Moore-Penrose inverse of matrix \(A\) [37].

### 4.4 Restricted maximum likelihood

In order to compute the likelihood of a sample, it is first necessary to assume that the data follow some distribution. Such a distribution usually involves one or more unknown parameters, which must be estimated from the data. Estimation is done by finding the value that maximizes the likelihood, i.e. the ML estimate [44].

A well known issue with ML is that the estimate for the variance in, for example, linear regression is biased [15]. Indeed, consider \(Y = \beta_0 + \beta_1 x + \varepsilon\), with response \(Y\), predictor \(x\), parameters \(\beta_0\) and \(\beta_1\), and the error \(\varepsilon \sim \mathcal{N}(0, \sigma^2)\). In this case, for \(n\) observations,

\[
\hat{\sigma}^2 = \frac{1}{n} \sum_{i=1}^{n} \left( y_i - \left(\hat{\beta}_0 + \hat{\beta}_1 x_i\right) \right)^2,
\]

(4.19)
with \( \hat{\beta}_0 \) and \( \hat{\beta}_1 \) the ML estimators for the parameters. It can easily be shown that

\[
E[\sigma^2] = \frac{n - 2}{n} \sigma^2 < \sigma^2. \tag{4.20}
\]

In SPM12, ReML is used to estimate the variance bias-free \([37]\). In general, the bias in the variance can be attributed to a loss in degrees of freedom due to estimation of the mean components (in our example: \( \hat{\beta}_0 \) and \( \hat{\beta}_1 \)). Estimation with the true mean, instead of using the estimated mean, would result in an unbiased estimate for the variance. The idea behind ReML is to maximize a modified likelihood that is free of mean components \([45]\).

ReML consists of partitioning the likelihood into two terms \([44]\):

1. A likelihood that involves the mean components, for which its maximization is independent of the estimate of the variance.
2. A residual likelihood that involves only the variance parameter and, therefore, can be maximized independently of other parameters.

For a detailed elaboration on ReML, the reader is referred to \([44, 45]\).

### 4.5 Contrasts

Hypothesis testing in the general linear model is based on the general linear null hypothesis \([46]\):

\[
H_0 : L\beta = m, \tag{4.21}
\]

where \( L \) is a matrix of weights, \( \beta \) is a vector of model parameters and \( m \) is a vector of proposed values. For example, one can test \([46]\):

1. for zero mean: setting \( L = 1 \), \( \beta = \mu \) and \( m = 0 \), to form \( H_0 : \mu = 0 \).

2. if the difference between two parameters \( \beta_0 \) and \( \beta_1 \) is zero: \( H_0 : (1 \ -1) \begin{pmatrix} \beta_0 \\ \beta_1 \end{pmatrix} = 0 \).

The linear combination \( L\beta \) is called a contrast. The purpose of a contrast is to ask a question about the model in mathematical terms. They are linear combinations of the model parameters. Their interpretation, therefore, depends on the parameters. For a model with multiple predictors, any test on a parameter is interpreted after adjusting for all other predictors included in the model. Thus, even if a parameter is given weight zero, the other parameters are still adjusted for its presence. This makes sense, contrasts are used to ask questions about the model, they do not alter the model \([46]\).

The matrix \( L \) is an estimable function, if there exists a matrix \( T \), for which

\[
L = TX, \tag{4.22}
\]

where \( X \) is the design matrix. In words, this means \( L \) is only meaningful if it is formed from a linear combination of the rows of the design matrix. Then, an estimable contrast is as a linear combination of the model estimates, since

\[
L\hat{\beta} = TX\hat{\beta} = T\hat{Y}. \tag{4.23}
\]

Indeed, a question is only meaningful if it can be formulated in terms of the model estimates \([46]\).
Contrasts can be used to form test statistics [46]. For this, we need to first define \( m \) in Eq. (4.21). In SPM12, \( m = 0 \) by default [46].

A \( t \)-contrast is used to test hypotheses using a \( t \)-statistic:

\[
t = \frac{L\hat{\beta} - m}{\hat{\sigma}\sqrt{L(X^T X)^{-1}L^T}},
\]

(4.24)

In SPM12, p-values for \( t \)-contrasts are always upper-tailed values. That means, the program will only show results for positive \( t \)-statistics. Unsurprisingly, this is used for strong directional hypotheses [46].

An \( F \)-contrast is used to test hypotheses using an \( F \)-statistic:

\[
F = \frac{(L\hat{\beta} - m)^T (L(X^T X)^{-1}L^T)^{-1}(L\hat{\beta} - m)}{r\hat{\sigma}^2},
\]

(4.25)

where \( r \) is the number of restrictions, i.e. the number of rows in \( L \) (and \( m \)). An \( F \)-contrast with a single row is equivalent to a \( t^2 \)-contrast. Multiple rows can be interpreted as an ‘or’-question. In SPM12, p-values for \( F \)-contrasts are always two-tailed values [46].

In the literature, a contrast is defined so that the weights always sum to zero. In SPM12, the term contrast is used more liberally and is also used to simply mean ‘linear combination’. This means a contrast does not have to sum to zero and it can be used to test individual parameters, sums or averages [46].

It is important to note that testing is done at the voxel-level. This entails that many tests are done per image. Therefore, it is necessary to adjust for multiple testing. There are many ways to do this, for example, by fixing the false discovery rate (FDR) or family-wise error rate (FWER), or selecting a minimum cluster (of voxels) size. SPM12 allows us to set both the FWER and to select a minimum cluster size.

Brain voxels typically have a lot of correlation. Due to this, a Bonferroni correction would be too conservative. Instead, FWER correction in SPM12 is done through random field theory (RFT). Details on RFT can be found in [47].
5 | Application to a novel multiple sclerosis dataset

5.1 Dataset

The CAP calculation and analysis was done on a novel rs-fMRI dataset of MS patients obtained in the United States by M. Inglese et al. (Department of Neurology, Radiology and Neuroscience, Icahn School of Medicine at Mount Sinai, New York, USA). This dataset consisted of 65 subject: 41 patients (aged $54.4 \pm 7.6$, range $35 - 66$; 15 male, 26 female) and 24 controls (aged $50.5 \pm 7.6$, range $36 - 66$; 15 male, 9 female). Per subject, an fMRI scan consisted of $91 \times 109 \times 91$ voxels in 400 time points i.e. 26000 frames in total.

The data were preprocessed by our colleagues in the US, according to the following steps:

1. Time-slice correction;
2. Motion coregistration and creation of mean image;
3. Coregistration of T1 image to mean image space;
4. Lesion-filling of T1 image and segmentation;
5. Spatial normalization of realigned functional T1 data and T1 to MNI space;
6. Spatial smoothing;
7. Extraction of time-course white matter and cerebrospinal fluid (CSF) signal from functional data.

In addition, for every subject, they took the following steps:

1. Removed linear trend from the data sets;
2. Band-pass filtering;
3. Calculation of network of interest (NOI) following a seed-based approach while accounting for the 6 head-motion parameters as well as white matter and CSF signal time-course.
5.2 Co-activation patterns

The seed region for the CAPs was chosen to be a $6 \times 6 \times 6 \text{mm}^3$ cube centered at the MFG ([0, 53, 26] in MNI coordinates). All CAPs were calculated using a 1 standard deviation (SD) threshold. After the reduction, 3622 frames of the original dataset remained (2289 for patients, 1345 for controls).

Clustering was done around two different sets of centroids with the number of centroids fixed to 8. The first set of centroids was computed from the dataset. For both the patient and control groups, we obtained 8 time frames (16 in total) that were used as centroids for the $k$-means algorithm. This allowed us to see which CAPs were present in the dataset. These CAPs can be viewed in Fig. A.1 and A.2 in Appendix A.1.

The second set of centroids was obtained from the CAPs computed by Liu et al. [17]. In their work, Liu et al. calculated 30 CAPs based on a rs-fMRI dataset from the 1000 Functional Connectomes Project (FCP) [48], which includes data on 102 subjects from three independent studies. We selected 8 CAPs based on their similarity to the CAPs calculated from our controls. Similarity was measured by dividing the CAP by Liu et al. and control CAP by their respective SD and calculating the Euclidean distance between them. The purpose of this set of centroids was to form a robust benchmark, so that CAPs between the two groups could be compared. The CAPs by Liu et al. are shown in A.3 in Appendix A.2. The CAPs produced this way are shown in Fig. 5.1 for the patient group and in Fig. A.4 in Appendix A.3 for the control group. These last CAPs were used for all subsequent statistical analyses.

5.3 Statistical analysis

All statistical analyses were done in SPM12 and adjusted for age, sex and movement parameters (translation in $x$, $y$ and $z$, pitch, roll and yaw). The group variances were not assumed to be equal, therefore, a correction for non-sphericity was applied. All $p$-values were adjusted (through RFT in SPM12) so that the FWER remained fixed at 0.05.

A first $t$-contrast was used to search for areas significantly co-activated with the MFG. The results are shown in Fig. 5.2 for the patient group and in Fig. B.1 in Appendix B.1. CAP 1 shows co-activation between the area around the MFG that extends towards the superior frontal gyrus and middle frontal gyrus, the posterior cingulate, the angular gyrus extending towards the supramarginal gyrus, the middle temporal gyrus extending towards the inferior frontal gyrus, the lingual gyrus extending towards the fusiform gyrus, the parahippocampal gyrus, the precentral gyrus, the cerebellum posterior lobe and cerebellar tonsil. CAP 2 shows co-activation between the area around the MFG that extends towards the superior frontal gyrus, the precuneus extending towards the cingulate gyrus, the angular gyrus extending towards the supramarginal gyrus and middle temporal gyrus, the middle frontal gyrus extending towards the inferior frontal gyrus, the parahippocampal gyrus and the cerebellar tonsil. CAP 3 shows the co-activation in the area around the MFG that extends towards the superior frontal gyrus, a small part of the posterior cingulate, a small part of the angular gyrus and supramarginal gyrus, the superior temporal gyrus, the middle frontal gyrus extending towards the inferior frontal gyrus, the parahippocampal gyrus and a small part of the precentral gyrus. CAP 4 shows co-activation in the MFG extending towards the superior frontal gyrus, middle frontal gyrus and inferior frontal gyrus, and the middle temporal gyrus, the inferior parietal lobe, the poste-
rior cingulate and the cerbellum posterior lobe. CAP 5 shows co-activation between the MFG extending towards the middle frontal gyrus, and the parahippocampal gyrus extending toward the posterior cingulate, precuneus and angular gyrus. CAP 6 shows co-activation in the MFG, the middle frontal gyrus, the superior frontal gyrus and the parahippocampal gyrus extending toward the cingulate gyrus, precuneus and the inferior parietal lobule. CAP 7 shows co-activation in the MFG extending towards the superior frontal gyrus, precentral gyrus, postcentral gyrus and inferior frontal gyrus. CAP 8 shows co-activation of the MFG extending towards most of the prefrontal cortex and a small part of the inferior parietal lobule. CAPs for controls were visually similar.

Since MS causes functional disconnection within the CNS, we expect to see a decrease in co-activated areas. The question in this case is: where do MS patients show a significant decrease in co-activation compared to controls? This translates to a one-sided $t$-test. Therefore, a $t$-contrast was used to test if areas were significantly more co-activated in controls than in patients. To ensure we are comparing significantly co-activated areas, the control $t$-contrast was used as an inclusive mask. The results are shown in Fig. 5.3.

The largest significant clusters of voxels (more than 100 voxels) in CAP 1 appeared in the middle temporal gyrus near to the angular gyrus (both sides: cluster size 344/449), inferior temporal gyrus (474), middle temporal gyrus (172), superior temporal gyrus (323), sub-gyral matter in the temporal lobe (346), the medial frontal gyrus near the subcallosal gyrus (922), the cingulate gyrus near the medial frontal gyrus (512), middle frontal gyrus (222), sub-gyral matter in the frontal lobe (169), posterior cingulate (395), parahippocampal gyrus (323), cerbellum posterior lobe (both sides: 210/514) and cerebellar tonsil (cluster size 422). For CAP 2, this was an area near the thalamus (cluster size 132), cerebellum anterior lobe (both sides: 140/549) and middle frontal gyrus (153). For CAP 3, the largest clusters appeared in the inferior temporal gyrus (cluster size 277) and posterior cingulate (274). For CAP 4, the largest cluster appeared in the cerebellar tonsil (cluster size 120). CAP 5 did not show clusters larger than 100 voxels, smaller clusters were observed in, among others, the middle frontal gyrus, medial frontal gyrus and cerbellum. For CAP 6, the largest clusters appeared in the medial frontal gyrus (cluster size 386) and precuneus (233). For CAP 7, the largest clusters appeared in the postcentral gyrus (both sides: cluster size 734/359), fusiform gyrus (284), paracentral lobule (390), middle occipital gyrus (332), sub-gyral matter in the frontal lobe (multiple: 681/202), cerebellum posterior lobe (122), middle temporal gyrus (both sides: 499/114), cuneus (364) and lingual gyrus (296). Lastly, CAP 8 did not show clusters larger than 100 voxels, smaller clusters were observed in, among others, the superior frontal gyrus, precuneus, thalamus and cerebellum. CAPs with large clusters also showed smaller cluster. Clusters with cluster sizes equal or smaller to 5 were ignored. The reasoning behind this was that for these cluster sizes it was unsure if the clusters were true positives.

As a last analysis, we compared the occurrence rates of specific CAPs in patients and controls. Fig. 5.4 shows boxplots of the occurrence rates. The rates per CAP were compared between groups using a $t$-test with unequal variance. At the 5% level, no significant differences were found ($p > 0.35 > 0.05$ for all CAPs).
Figure 5.1: CAPs calculated with Liu et al. benchmark centroids for the patient group. Shown are slices in the z-plane at heights: -20, -8, 4, 16, 26, 34, 42 and 50. Color values indicate BOLD signal difference from baseline in arbitrary units.
Figure 5.2: $t$-contrasts of co-activated areas obtained from CAPs with Liu et al. benchmark centroids for the patient group. Shown are slices in the $z$-plane at heights: $-20, -8, 4, 16, 26, 34, 42$ and $50$. Color values indicate $t$-values.
Figure 5.3: One-sided \( t \)-contrasts obtained from CAPs with Liu et al. benchmark centroids to show areas of the brain where co-activation exists for controls but not for patients. Shown are slices in the \( z \)-plane: (a) \( z = -44 \) to 40 in increments of 4, (b) \( z = -22 \) to 34 in increments of 4. Color values indicate \( t \)-values.
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Figure 5.4: Boxplots showing the occurrence rates of CAPs in subjects.
6 | Discussion

In this work, we aimed to introduce the concepts of fMRI and CAPs. Firstly, the physical and neurological background of fMRI was explained. Afterwards, the data reduction and clustering steps of CAP calculation were touched on. We also introduced the statistical method used to analyze these CAPs, consisting of a second-level analysis. The CAP and statistical methods were subsequently applied to a novel MS dataset.

Recent studies [17, 18, 21] have pointed out that CAP methods yield more fine-grained information on functional connectivity than traditional seed-based methods. Our calculation, as well, yields visually distinct CAPs. This suggests that the brain goes through multiple patterns of co-activation when at rest and reinforces the theory that interaction between brain-regions is non-stationary, as pointed out by Liu, Chang and Duyn [17]. Indeed, if CAPs are regarded as spatial clustering of patterns of correlated brain regions, they express common co-activations that occur in the brain at different times.

A previous task-based study showed that both healthy controls and MS patients displayed a distributed pattern of activations in the DMN, fronto-parietal lobes, cerebellum and insula [49]. In line with this study, our results show patterns of co-activation with the MFG that include some of these regions. Many of the CAPs in both patients and controls show similarity to the DMN, as expected in rs-fMRI. In addition, co-activations of the cerebellum and areas of the fronto-parietal lobe can be found in our CAPs.

Other areas beside the DMN that are known to be used for cognitive functions can be retrieved in our CAPs. For example, the parahippocampal gyrus is known to be involved in memory encoding and retrieval. The superior frontal gyrus has been found to play a role in self-awareness. The lingual gyrus is thought to play a role in encoding visual memories.

Interestingly, the posterior lobe of the cerebellum, which is known to play an important role in the inhibition of involuntary movement, can also be found. We suspect that this is an effect of the subjects trying to move as little as possible while under the fMRI machine.

In the second t-contrasts, some of the areas containing significant clusters of voxels are a part of the default mode network. These are areas in the medial prefrontal cortex, such as the medial and superior medial frontal gyri, and the angular gyrus. Many regions often referred to as part of the subsystems of the DMN also contain clusters: gyri in the temporal lobe, the parahippocampal gyrus and the supramarginal gyrus.

One study reports decreased activation in the medial prefrontal cortex in African American patients with MS [50]. Similarly, our results show significant differences in co-activation in the MFG in CAPs 1, as well as several smaller clusters in the lower part of the MFG in CAPs 2, 3, 5, 6, 7 and 8.

These results suggest that there exists functional disconnectivity in MFG CAPs in MS patients in the wakeful rest state. The same previously mentioned task-based study
found significant deactivations in MS patients in areas known to be involved in cognitive functions when performing working memory tasks [49]. In other task-based studies, it has been demonstrated that for some neurological conditions, including MS, the ability to modulate the deactivation of the DMN during tasks is reduced. The fact that our results point towards functional disconnectivity in MS patients may indicate that the inverse is also true, i.e., reduced ability to modulate co-activation in resting state. However, the latter is speculation.

Lastly, no significant differences in CAP occurrences between groups were observed. Therefore, no comments can be made about reductions in certain patterns such as the DMN, or possible coping mechanisms.

6.1 Future prospects

The obtained CAPs depend on the choice of seed. In order to get more information on DMN co-activation, it would be useful to analyze co-activation with the PCC in particular. This area is another node in the DMN and centrally located.

In addition to varying the seed area, it may be instructive to vary the amount of clusters in the CAP calculation. The choice of eight in this work was arbitrary. Liu, Chang and Duyn mention that their choice of thirty CAPs was “a compromise between extracting too many and too few distinct CAPs based on what was afforded by the data” [17]. Considering this, researching the cluster number is likely an interesting endeavor.

Lastly, other tests could be done with the presented dataset. For instance, one might want to investigate whether there is more movement under the fMRI machine in patients than in controls. Results to these types of questions may give additional insight into diseases such as MS and make use of the information available in the data to the fullest.
A | CAPs of interest

A.1 No benchmark CAPs

Figure A.1: CAPs calculated without benchmark centroid for the patient group. Shown are slices in the z-plane at heights: −20, −8, 4, 16, 26, 34, 42 and 50. Color values indicate BOLD signal difference from baseline in arbitrary units.
Figure A.2: CAPs calculated without benchmark centroid for the control group. Shown are slices in the $z$-plane at heights: $-20$, $-8$, $4$, $16$, $26$, $34$, $42$ and $50$. Color values indicate BOLD signal difference from baseline in arbitrary units.
A.2 CAPs for benchmark centroid

Figure A.3: CAPs taken from Liu et al. used as centroids for the calculation of new our CAPs [17]. Shown are slices in the z-plane at heights: -20, -8, 4, 16, 26, 34, 42 and 50. Color values indicate BOLD signal difference from baseline in arbitrary units.
A.3 Benchmarked CAPs for control group

Figure A.4: CAPs calculated with Liu et al. benchmark centroids for the control group. Shown are slices in the \( z \)-plane at heights: \([-20, -8, 4, 16, 26, 34, 42 \text{ and } 50]\). Color values indicate BOLD signal difference from baseline in arbitrary units.
B | \(t\)-contrasts of interest

B.1 \(t\)-contrast of co-activated areas for control group

Figure B.1: \(t\)-contrasts of co-activated areas obtained from CAPs with Liu et al. benchmark centroids for the control group. Shown are slices in the \(z\)-plane at heights: \(-20, -8, 4, 16, 26, 34, 42\) and 50. Color values indicate \(t\)-values.
APPENDIX B. T-CONTRASTS OF INTEREST
Bibliography


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