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QUANTIFICATION OF BRAIN NETWORKS IN HEALTHY AND DISEASED ADOLESCENTS USING NEUROIMAGING PROCESSING TECHNIQUES

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Internal Supervisor: Javier Pascau González Garzón

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ABSTRACT

In the last decades, neuroimaging, and particularly functional magnetic resonance, have lead to a high number of findings on brain organization that have helped the scientific community to better understand brain function. Despite these advances, the foundations of neural activity and connectivity are not yet understood, and little is known about its role in the etiology of psychiatric disorders. A vast amount of literature on neuroimaging demonstrates the presence of abnormal and dysfunctional brain patterns of activity, revealed by a wide number of different analytical approaches. However, the establishment of consistent relations between abnormal connectivity patterns and symptoms or diagnoses is still not confirmed. For this reason, the present project studied these relations in young individuals suffering from First Episode Psychosis (FEP) and healthy control participants by implementing a model-dependent atlas-based approach using the publicly available Seven Network Atlas. The statistical analysis of preprocessed images revealed various tendencies consistent with previous results from literature as well as new findings. Youth in psychosis presented hyperconnectivity in most resting-state networks when compared to controls, being significantly enhanced in Fronto-Parietal network (FPN) and Default Mode network (DMN). Also, within patients hyperconnectivity was negatively related to general cognitive performance and positively linked to clinical symptom severity. We estimate that the results of this work will eventually contribute to a better understanding of the early stages of psychosis in children and adolescents, critical for optimizing current interventions and treatments.

RESUMEN

En los últimos años, la comunidad científica ha hecho grandes avances para alcanzar una mejor comprensión del funcionamiento del cerebro gracias al gran número de descubrimientos relativos a la organización del mismo, llevados a cabo mediante neuroimagen y en particular por medio de resonancia magnética funcional. A pesar de estos avances, todavía se desconocen las bases de la actividad y conectividad neuronal así como su papel en la etiología de los trastornos psicóticos. Una gran cantidad de literatura sobre análisis de neuroimagen mediante diversos métodos demuestra de manera consistente la presencia de patrones de actividad cerebral alterados y disfuncionales; sin embargo, es imposible establecer de manera sólida, a través de diferentes estudios, la relación entre patrones específicos de conectividad funcional alterada con los diferentes síntomas y condiciones de estos trastornos. Por este motivo, el presente proyecto ha estudiado estos vínculos en niños y adolescentes que padecen un primer episodio de psicosis así como en controles sanos mediante un enfoque basado en un atlas público de 7 redes cerebrales. El análisis estadístico de las imágenes preprocesadas reveló varias tendencias coherentes con resultados previos extraídos de la literatura así como nuevos hallazgos. Los jóvenes con psicosis mostraron tener hiperconectividad en la mayoría de las redes en reposo en comparación con los controles, siendo dicha diferencia especialmente significativa en las redes Fronto-Parietal y por Defecto. Además, se observaron patrones de correlación negativa entre la conectividad y los procesos cognitivos, mientas que la relación de la conectividad con la gravedad de los síntomas mostró ser positiva. Los resultados de este trabajo contribuyen a una mejor comprensión de las etapas iniciales de la psicosis en niños y adolescentes, fundamentales para la optimización de los actuales tratamientos e intervenciones.

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Acronyms

AFNI – Analysis of Functional NeuroImages

ANCOVA – Analysis of COVAriance

BOLD – Blood Oxygen Level Dependent

DMN – Default Mode Network

EEG - ElectroEncephaloGraphy

FEP – First Episode Psychosis

FPN – Fronto-Parietal Network

FSL – FMRIB Software Library

GLM – General Linear Model

HDR – HemoDynamic Response

ICA – Independent Component Analysis

IQ – Intelligence Quotient

LIM – Laboratorio de Imagen Médica

MPFC – Medial PreFrontal Cortex

PANSS – Positive And Negative Syndrome Scale

PCC – Posterior Cingulate Cortex

PCu – PreCuneus

PET – Positron Emission Tomography

ROI – Region Of Interest

Rs-fMRI – Resting state functional Magnetic Resonance Imaging

SPM – Statistical Parametric Mapping

VM – Virtual Machine

This study has been done at the *Hospital General Universitario Gregorio Marañón* in Madrid in collaboration with the Department of Child and Adolescent Psychiatry.

1. INTRODUCTION

1.1 *MOTIVATION*

In the 20th century, the development of brain imaging techniques has made huge leaps forward, allowing the scientific community to understand the organization and topology of the human brain beyond previously established limits. Functional Magnetic Resonance Imaging (fMRI) is one of the most common brain imaging modalities to record brain activity. The analysis of the data provided by fMRI reveals how specific brain regions activate when implicated in certain behavioral tasks. Fairly recently an innovative alternative to fMRI called resting state fMRI (rs-fMRI), has made use of the low frequency fluctuations of neural activity (that were previously considered as random noise) enabling the discovery of new brain connectivity concepts (Biswal et al., 1995).

The unexpected interest in these spontaneous low frequency signals relies on theoretical and empirical arguments. The theoretical argument is based on brain energy consumption, which represents 20% of total body's metabolism. Despite this, the recorded activation in response to external stimuli or demanding tasks, that represent the fundamental of most brain studies, has shown to represent just 5% of this amount. At this point, it is reasonable to think that in order to better understand the functioning of the human brain one has to analyze the element that consumes most of its energy sources and this is characterized by the low-frequency neural fluctuations. The empirical arguments to study this signal rely on the multiple studies that proof that these oscillations are not random but highly organized. The identification of distinct regions showing strong temporal correlation in the low-frequency fluctuations has given rise to the discovery of so called resting-state networks, different groups of regions that show a high degree of co-activation (Biswal et al., 1995). Research has shown that these networks become highly active when the

subject transits from rest to task engagement. For example, it has been proven that in the transition from rest to a motor task a specific resting-state network becomes highly active, termed motor network. This line of research has led to the discovery of multiple resting-state networks associated with various cognitive functions. The degree of temporal co-activation between regions during rest is measured by means of correlation and is named functional connectivity.

Based on these insights, the interest in rs-fMRI and its relationship with human cognitive, emotional and motor behaviors has increased considerably over the last years. Furthermore, the application of rs-fMRI presents great advantages. Traditional task-based fMRI requires the subject to perform a task while data is acquired, but this is not necessary for rs-fMRI. This fact has led some researchers to use rs-fMRI in subjects with severe cognitive, emotional, or motor pathology such as autism spectrum disorders, major depression or Parkinson's disease. Studies using this modality have produced promising results showing that these disorders are strongly associated with abnormalities in single or multiple resting-state networks (Castellanos et al., 2013). An example of this is the lowered or enhanced functional connectivity when comparing diseased subjects with healthy controls. Therefore, studies using rs-fMRI may provide new biomarkers required in the clinical field and new knowledge about the possible underlying mechanisms that are associated with these disorders.

Psychotic disorders are common, severely disabling, and associated with enormous clinical and socioeconomic impact. However, at present, it is not possible to predict their onset, nor their subsequent course: some patients may show a good recovery, others may recover and then relapse in further episodes, and a third subgroup may follow a chronic, unremitting course. The inability to forecast what will happen to a patient who has just become psychotic makes it impossible to tailor psychiatric treatments that fit the particular needs of each patient.

Diagnosis of psychotic disorders needs to be based on objective, quantitative and clinically meaningful measures. However, despite substantial advances in our understanding of the neurobiological basis of psychotic disorders, the assessment of

patients with psychosis is still based on clinical interviews. Moreover, the overwhelming evidence of the pathophysiological heterogeneity of psychotic disorders (Kapur et al., 2012), that hinders the quest for biomarkers to aid early diagnosis, stratification and measurement of disease progression. Indeed, this lack of biomarkers is a key barrier for the industry to develop novel treatments and therapies. Stratifying patients in an objective and quantitative way according to clinical and functional outcomes may generate patient clusters that are more clinically meaningful (van Os and Kapur, 2009). This stratification is beneficial for clinical trials, as a new drug may work well just in a subgroup of patients. The absence of this patient classification may hide the true therapeutic effect of the medication as it is diluted across a heterogeneous sample. Neuroimaging techniques such as rs-fMRI may be able to provide such objective and quantitative measures. Research on this field has substantially advanced our understanding of the biological basis of psychotic disorders and the overall goal should be to translate these findings into clinical practice.

Therefore, the motivation behind this TFG is driven by the need for new objective criteria for stratifying young patients who have just developed psychosis together with the promising results of previous studies using rs-fMRI in psychiatric disorders and the advantages of the technique for data acquisition in a complicated group of patients.

1.2 *HYPOTHESES AND OBJECTIVES*

The present project attempts to expand current knowledge about the mechanisms that characterize psychotic disorders by analyzing the aberrant patterns of brain functional connectivity observed in First Episode Psychosis (FEP) patients compared to healthy controls. For this purpose, and considering previous literature on this subject, three hypotheses were examined:

- Hypothesis 1: FEP patients have increased functional connectivity (hyperconnectivity) with respect to healthy controls.

- Hypothesis 2: Functional connectivity is differentially related to overall cognitive performance (Intelligence Quotient, IQ) in FEP and controls.
- Hypothesis 3: Hyperconnectivity is positively related to clinical symptom severity

In order to test these hypotheses, the following objectives were set:

- Effective preprocessing of fMRI images using various software packages implemented using a single pipeline script. This will allow reducing noise effects and registration to a 7-network atlas.
- Extraction of functional connectivity scores of interest for the study.
- Implementation of statistical analyses to test the hypotheses using ANCOVA models specified in MATLAB scripts.
- Interpretation of results to identify significant functional connectivity differences between patients and controls and relate it to intellectual performance and clinical symptom severity.

2. STATE OF THE ART

2.1 *INTRODUCTION TO CONVENTIONAL fMRI*

2.1.1 Magnetic Resonance Imaging

Magnetic resonance is a radiologic imaging tool that exploits the nuclear magnetic resonance (NMR) of the nucleus of hydrogen atoms, quite abundant in human body, to generate a signal. This signal, which comes mainly from the water molecules in our body, is mapped to obtain high resolution images in a non invasive, safe manner. Due to the different properties and water content of the tissues in our organism, this technique provides also good contrast. The basics of the technique were developed in the 1940s by Bloch and Purcell, although it was in the 1970s when the application of gradient coils was introduced, enabling the acquisition of three-dimensional images.

The phenomena responsible for the acquisition of the signal starts by applying a strong, static, homogeneous magnetic field that tends to align the hydrogen nuclei in our body, whose spins (intrinsic physical property of atoms) begin to precess at a specific frequency (called Larmor frequency) around the direction of the field. Later, a radio frequency (RF) pulse at Larmor frequency is used to make these atoms resonate (rotate in phase) and orientate the magnetization from Z plane to XY plane. At this stage, nuclei absorb energy that is released when the pulse is removed and nuclei return to their original state of magnetization (relaxation). This energy is detected by coils to finally obtain the image representing the map distribution of the magnetic signal. The lineup of the nuclei due to the strong static field allows their extremely small signals to add up coherently so that it can be measured by the system. The received (raw) data is encoded in k-space (Fourier), as shown in figure 1, and is transformed to image space by Inverse Fourier Transform. The images obtained are three-dimensional volumes composed of several slices representing body anatomy.

This depiction can provide information about shape, volume, surface area or tissue integrity among other parameters. Furthermore, other conditions like inflammation or edema can be inferred from the image (Mark A. Brown, 2010).

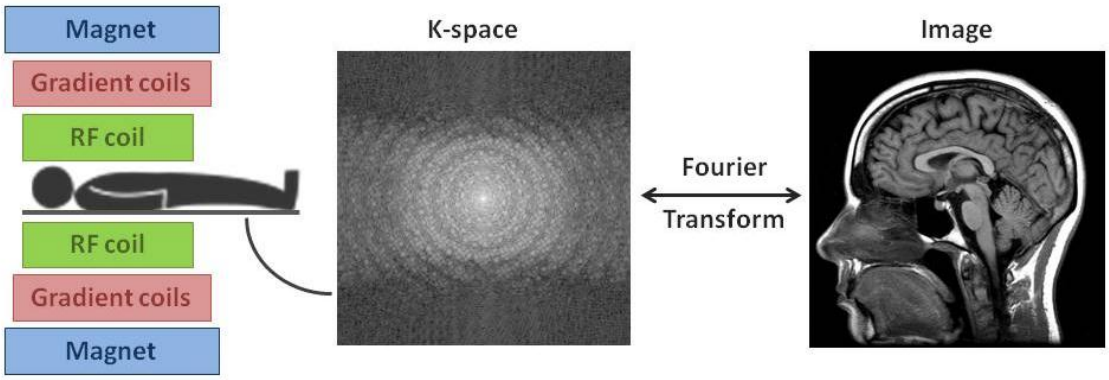


Figure 1. Schematic representation of MRI acquisition showing the main components of MR scanner, the raw data acquired in k-space and the Fourier transformation into the final image of interest.

The variation in the strength of the signal coming from hydrogen nuclei according to its surroundings, and thus tissues, is the key of MRI to obtain high resolution, static structural images of the human body. The RF pulse timing parameters can be manipulated to obtain different contrasts according to the properties of the tissues in order to meet various needs. Depending on what we want to discriminate or emphasize, repetition time (TR), the time between two excitation pulses, and echo time (TE), the period between pulse and data acquisition of atom relaxation, are modified. In order to distinguish clearly gray and white matter, short TR and TE are used; and to discriminate brain tissue and cerebrospinal fluid, long TR and TE are employed.

2.1.2 fMRI - Blood Oxygen Level Dependent

In the 1990s, Seiji Ogawa observed that the different magnetic behavior of blood between its oxygenated and deoxygenated state (previously discovered by Faraday, Pauling and Coryell in 1936) could be used as a contrast, with which he

established the basis of fMRI (Ogawa et al., 1992). The effect, called Blood Oxygen Level Dependent (BOLD), allowed physicians to indirectly measure neuronal activity with MRI during cognitive task performance by detecting the changes in blood flow due to oxygen metabolism derived from glucose consumption, the energy source of neurons.

This innovation has significantly contributed to neurological and psychiatric insights and findings in the past decades due to its good spatial and temporal resolution while being a non-invasive, non-radiative technique that allows in vivo recordings. It is also being used for clinical and commercial purposes.

fMRI relies on the same phenomena of MRI but taking advantage of the changes of magnetization of blood. The difference comes from hemoglobin (Hb) molecules in red blood cells, the ones responsible for carrying oxygen, which are more magnetic (paramagnetic) if deoxygenated and practically resistant to magnetism (diamagnetic) if oxygenated. Considering this, particular neural activity is indirectly measured due to the local response it generates. As neural activity increases, so does blood flow in the region (increased rate and blood vessel dilation) due to the oxygen demand (Jueptner and Weiller, 1995, Heeger and Ress, 2002). The hemodynamic response (HDR) (see figure 2), the change in MR signal due to neuronal activity, takes 1 or 2 seconds to start as vascular system's response to glucose need is not immediate. At this initial point, there is a momentary decrease in oxygenation and so does HDR. Then, blood flow increases to meet (and even overcompensate) oxygen demand during about 5 seconds (Fox and Raichle, 1986). Finally, if neuron activity stops, the HDR falls below original state, what is called post-stimulus undershoot, before recovering baseline level. Instead, if neuron firing remains, HDR experiences a flat plateau until activity ceases. Although blood flow changes are extremely sensitive to neural activity, oxygen metabolism is not, and this is the reason why the MR signal received from BOLD contrast is quite slight.

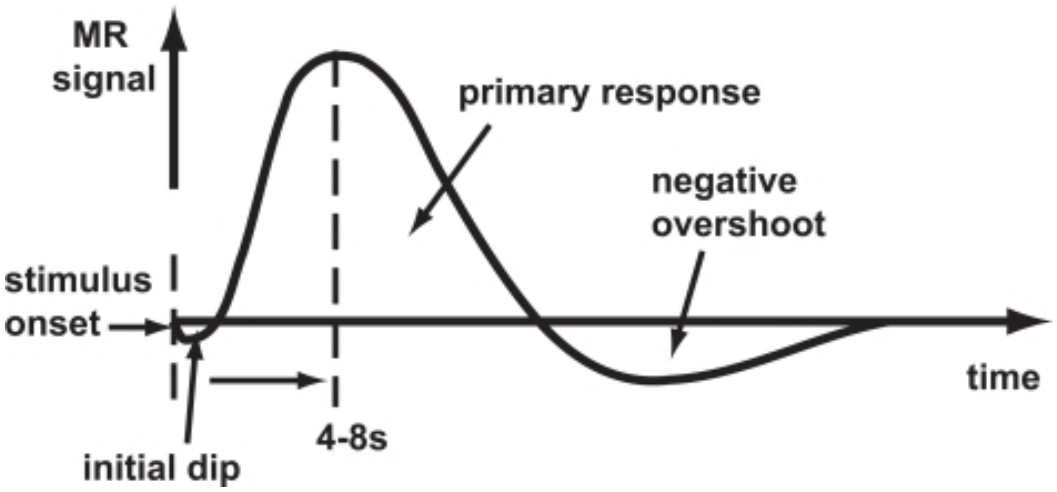


Figure 2. Representation of the profile of the HDR of brain neurons to a single stimulus.

Furthermore, due to the lag in vascular response, the signal obtained over a region is not quite specific but a sum, as in the case of excitatory and inhibitory activity, which can be cancelled out. In addition, there is evidence of differential glucose consumption (and thus oxygen) with respect to incoming blood flow across brain regions in accordance with the quickness of the response it deals with. All this adds up to several unwanted sources of noise that distort the signal under study, as it is shown in figure 3. These accidental signals, which can be even bigger than the one of interest, can come from the scanner itself but also from external factors like subject movement. Aiming to elucidate the signal of interest, several recordings can be done in order to be able to discard random noise of each trial by comparing it with the rest or specific processing steps can be applied to remove known artifacts.

The spatial resolution of the technique depends on the size of the voxels, three-dimensional box-shaped representations of the activation, which depend on slice thickness and area. Smaller voxels contain less signal, as fewer neurons are included per voxel, and take longer to be scanned. This implies longer acquisition times, that can be reduced by increasing the strength of the magnetic field (measured in Tesla). The temporal resolution of the technique is based on TR parameter although it is constrained to the HDR: sampling at a faster rate provides more points of the HDR profile, which can be obtained by interpolation.

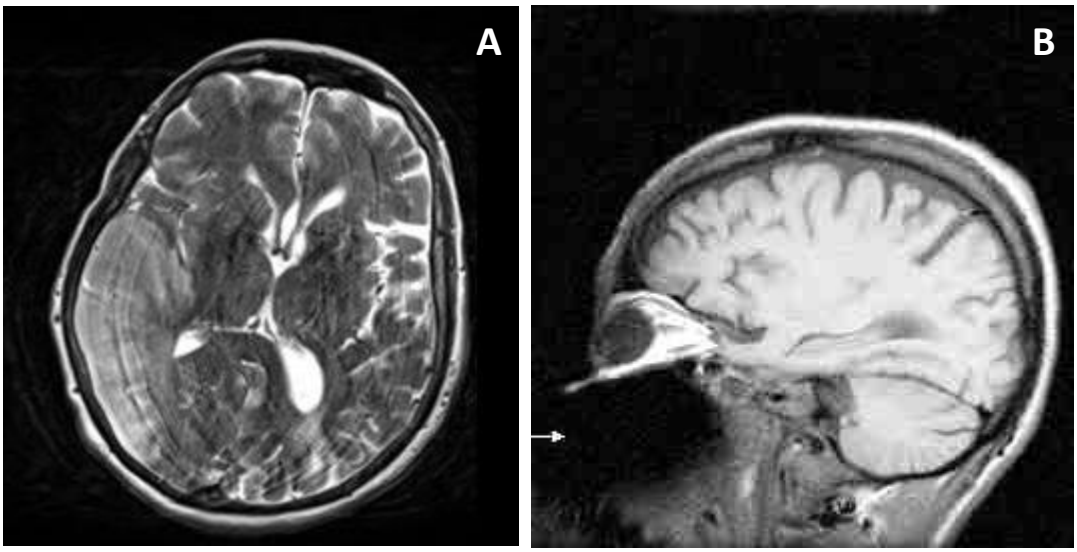


Figure 3. Examples of sources of noise: (A) head motion and (B) field inhomogeneity indicated with white arrow.

The reliability of the technique has been checked by comparing the obtained BOLD signal with the one coming from other methods providing direct electrophysiological measurements like electroencephalograms (EEG) and magnetoencephalograms (MEG). The similarity with MEG signal, that records the local field potential of the interior of neurons and post-synapses, suggests that BOLD signal does not represent the output firing of these cells but the integrative routine.

2.1.3 Task-based fMRI

Task-based fMRI experiments measure brain activity when a specific assignment is being performed. The aim is to identify which areas of the brain are used (active) when the task is being executed. The typical fMRI experiment uses block design, where the subject is instructed to perform a specific task during an experimental period and alternate it with a baseline period in which a different task is executed, usually staying at rest. This way, the HDR related to the cognitive process of interest is allowed to return to baseline level, what makes it easier to infer which brain regions are involved just in the performance of that specific task. Once the experiment is performed, the timecourse of the activity of image voxels, taking into account the

lag of the vascular response, is compared with the model of the experiment. If both patterns fit a positive activation score is assigned, if there is no correlation low or zero statistical value is given, and voxels with opposite activation obtain negative scores. The fit comparison in every voxel results in an activation map, where the areas with high scores are assumed to be activated by the stimulus.

However, this procedure implies some drawbacks. There is an increased possibility of head motion while performing the task, which leads to signal artifacts. Also, the baseline condition may introduce high activation signals that complicate isolation of signal associated with the experimental condition. In addition to this, the predictable character of the task performance in the block design can induce subject learning or boredom and alteration of the cognitive processes it generates, probably decreasing the amplitude of the signal. There is another type of design, called event-related, in which stimuli are presented in a random order with different timings, avoiding this last problem, although the statistical power of this complex method is lower. A schematic representation of these two possible designs is shown in figure 4.

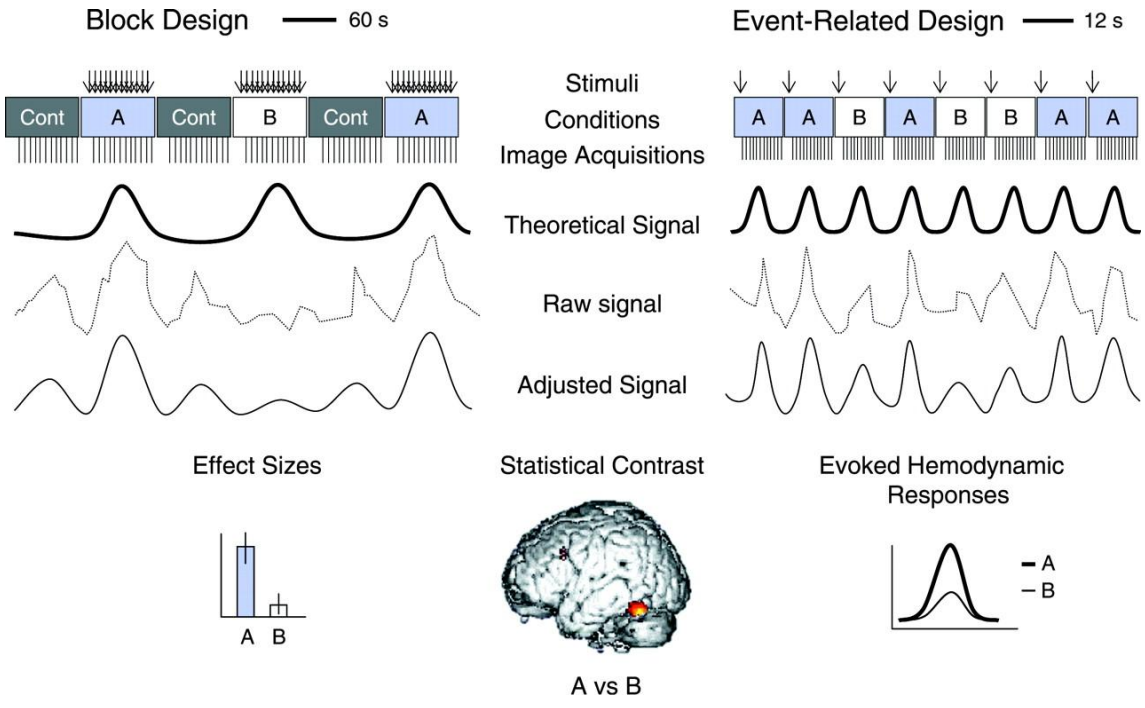


Figure 4. Schematic representation of fMRI block and event-related designs. (Demonet et al., 2005).

Both designs, block and event-related, can be applied due to the fundamental assumption that states that the BOLD response of two simultaneous tasks can be added linearly. Therefore, the difference between both conditions of the design is supposed to represent the differing cognitive process. However, these models assume that the combination of both cognitive processes does not affect the response of each one separately, that in some cases may not be true. Therefore, and considering that any task can generate additional cognitive processes than the ones of interest, it is important to carefully select the activation and control tasks that will define the paradigm.

fMRI is usually combined with other methods, apart from physiological measurements, in other to supplement the advantages and disadvantages of each method. A clear example of this is to merge the high temporal resolution of EEG with the high spatial resolution of fMRI. Also, structural MRI is often used together with fMRI as a reference for visualization, feature extraction or tissue integrity evaluation.

2.2 *INTRODUCTION TO RS-FMRI*

Most of our current knowledge on brain function comes from task-based fMRI studies providing insights about the specific function of individual regions of the brain in response to certain tasks or stimuli. However, the neuronal activity observed during these experiments has proven to use only 5% of the overall brain energy consumption so there is a need to understand the underlying mechanisms in which brain devotes most of its metabolic consumption.

In 1992 Bharat Biswal discovered that the brain is significantly active even when the subject is at rest, without any external stimuli or demanding task. He observed in an fMRI experiment that when individuals were awake but not performing any active goal-directed task, there were spontaneous low frequency fluctuations of the BOLD signal that revealed patterns of brain connectivity and organization (Biswal et al., 1995). Biswal observed that the activated areas in primary motor networks during a finger tapping task correlated within and across hemispheres with the patterns of

connection of these areas during the resting state. These results were later consistently reproduced by other researchers that used this technique, called resting state fMRI (rs-fMRI), to identify different functional networks; such as primary visual, auditory and higher order cognitive networks.

Before Biswal's revolutionary discovery, it was thought that the little increase in BOLD signal in task-based experiments with respect to the baseline condition did not reflect the total amount of energy consumed by the brain, and the characteristic low frequency fluctuations (from 0.01 to 0.08 Hz) on the signal were discarded as noisy components. Today, the foundations of these oscillations are not yet fully understood but they have proved to be of critical importance for the understanding of brain organization, supported by the high number of findings it has facilitated while mapping brain at rest.

2.2.1 Functional connectivity

Functional connectivity can be defined as the temporal dependency of the BOLD signal in spatially distinct anatomical brain regions. Therefore, what rs-fMRI measures is the degree of co-activation of the neuronal activity time-series during rest between regions. These correlations have shown to appear between distinct regions responsible for a common function, and are consistent across subjects, studies and scanners. Such a group of distinct but co-active regions is called a resting-state network. In figure 5, Posterior Cingulate Cortex (PCC) and Medial Prefrontal (MPF) cortex are assumed to belong to the same resting state network due to their high functional connectivity while intraparietal sulcus (IPS), showing a negative correlation with respect to this region, displays low functional connectivity and does not belong to the same network.

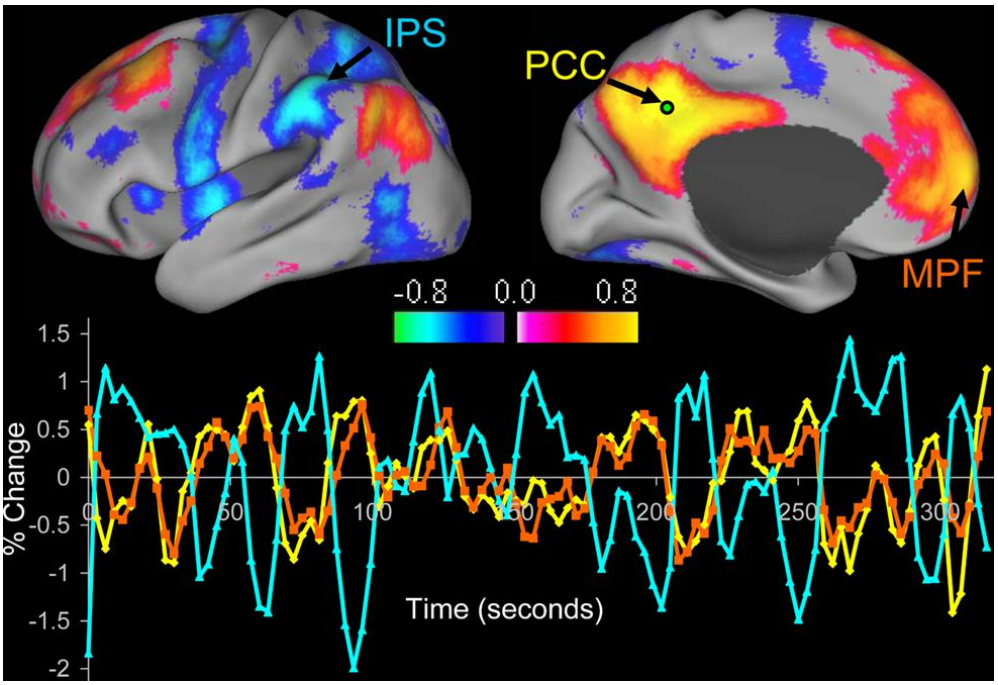


Figure 5. Correlation of activity of MPF (orange) and PCC (yellow) and anti-correlation of IPS (blue). PCC and MPF show high functional connectivity and are thus assumed to correspond to the same network, while IPS displays a negative correlation and thus low functional connectivity. (Fox et al., 2005).

2.2.2 Resting state networks

Most of these resting state networks are consistent with those inferred from task-based experiments, which show selectively increased activity during either motor, perceptive or cognitive tasks. The most consistently detected resting state networks are: the sensorimotor networks, the visual and auditory networks; the language network; two networks (dorsal ad ventral), involved in cognitive control; the Fronto-Parietal network (FPN), responsible for decision-making processes; and the cingulo-opercular network, which is supposed to influence in goal-directed tasks. Figure 6 shows a comparative evaluation of the networks obtained from MEG and fMRI independent analyses using Independent Component Analysis (ICA).

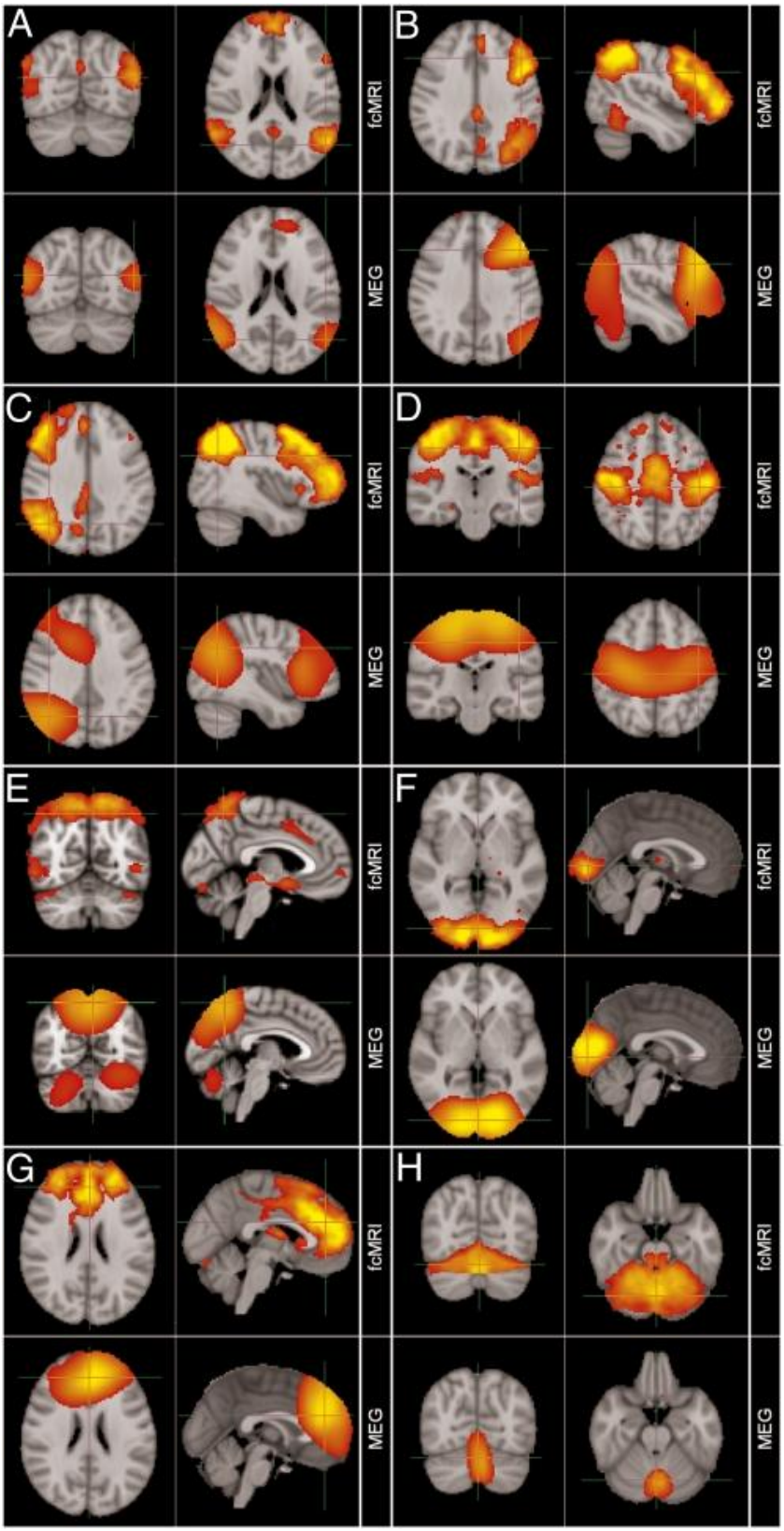


Figure 6. Comparison of brain networks obtained using ICA on independent MEG and fMRI images. (A) Default Mode network; (B) left FPN; (C) right FPN; (D) sensorimotor networks; (E) medial parietal regions; (F) visual network; (G) frontal lobes including anterior cingulate cortex; (H) cerebellum. (Brookes et al., 2011).

In 2011, Thomas Yeo and colleagues published a paper, “The organization of the human cerebral cortex estimated by intrinsic functional connectivity”, where they used rs-fMRI to divide brain in 7 resting state networks depicted in figure 7(Yeo et al., 2011). The goal of their investigation was to understand the relation of patterns of functional connectivity with the organization of brain, and to provide reference maps representing the organization of cerebral cortex.

With this purpose, they assessed the functional connectivity in 1000 young healthy adults using rs-fMRI. This sample was evenly divided in two subgroups of 500 individuals (discovery and repetition samples) in order to test the reliability and reproducibility of the method and results. After preprocessing the images, they were registered to a common coordinate system by implementing surface-based alignment from FreeSurfer. Finally, a clustering approach was applied in order to parcellate the brain cortex, giving rise to 7 networks (named in figure 7) that converge with previous descriptions and reported seed-based analyses.

The findings of Yeo et al. can be summed in two types of networks; local networks on topographically adjacent areas devoted to sensory and motor regions, and widely distributed networks corresponding to association areas following more abrupt boundary transitions. Yeo and colleagues have made this resting state 7-network map publicly available to the scientific community (see https://surfer.nmr.mgh.harvard.edu/fswiki/CorticalParcellation_Yeo2011) and this is the one that have been used in this study analyses (see *Areas analyzed*).

The reason of the continuous activity of resting state networks during rest is hypothesized to be a more efficient organization and coordination of brain processing in order to provide successful neural responses. The resting activity of the brain is assumed to be organized as a “predictive dynamic system”, an optimized association based on previous activation responses to certain stimuli. This way, the discovered resting functional networks have emerged due to repeated implication of distinct regions in a certain task and remain active in case they are not involved in any task. This theory agrees with the subject variability of the baseline signal coming from these

networks, which differs depending on experience or efficiency of task performance. Thus, most resting-state networks increase in functional connectivity when the brain transits from resting-state to active task engagement.

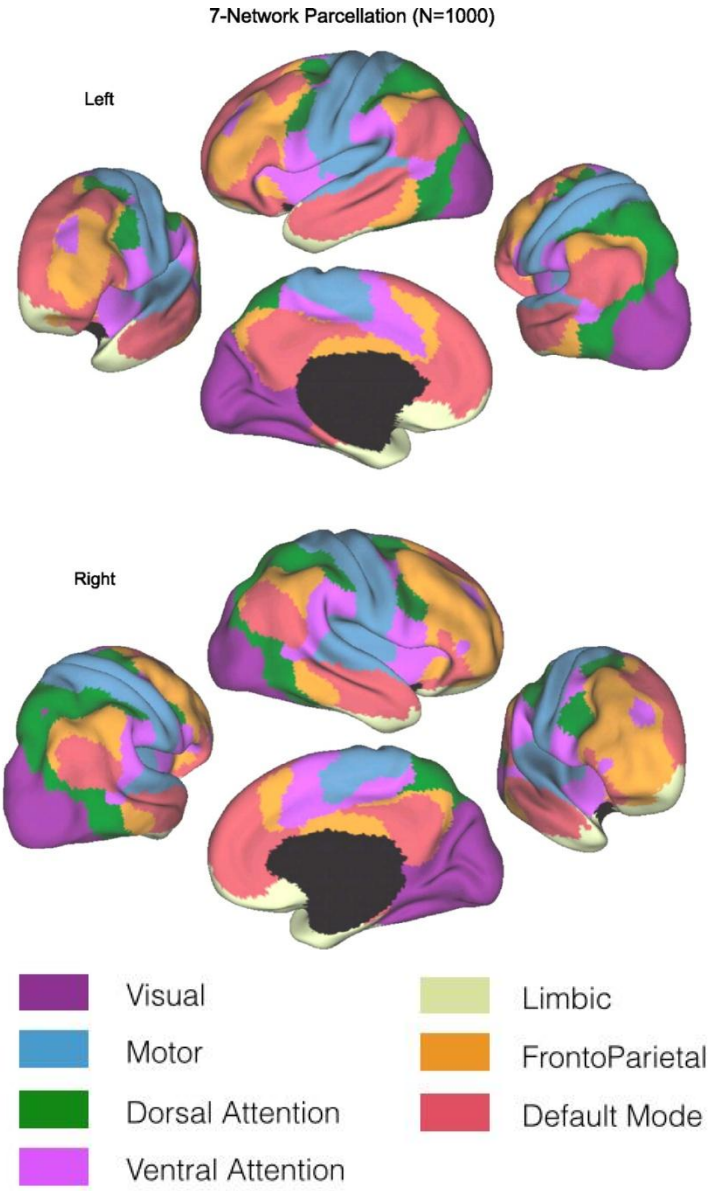


Figure 7. Yeo et al. 7 resting state networks. Adapted (Yeo et al., 2011).

2.2.3 Default Mode network

There is one resting-state network that does not increase its activity in response to task or stimuli but instead decreases it progressively as the external cognitive demand increases. This is the Default Mode network (DMN), which was first coined by Raichle (Raichle et al., 2001). This network is associated with internal self-referential modes of cognition including remembering, thinking about the future, mind wandering and even judging a moral situation or others perspectives. The negative correlation of this network with the others (Fox et al., 2005) suggests a competing organization of internal and external brain processing. The DMN, probably the most investigated resting state network, is defined anatomically by the following subsystems: PCC and precuneus (PCu), medial prefrontal cortex (MPFC) and inferior parietal cortex.

Almost 70% of total brain energy consumption is due to the cited resting state networks that can be classified according to their response to stimuli. DMN is the only one that deactivates due to external task while the others activate, and that is why they can be defined as task positive networks. These arrangements are consistent across different techniques, analytic procedures, subjects and resting and cognitive states. However, the relationship between the functional connectivity of resting-state networks and the structural connections is not yet defined nor understood. White matter tracts are considered as the paths for functional connection between remote regions; however, not all functionally connected networks have been proved to have direct anatomical link. One must note that, although functional connectivity implies the existence of some degree of structural connectivity, it does not mean that both areas have direct anatomical connection (de la Iglesia-Vaya et al., 2011).

2.2.4 Strengths

As stated at the beginning of this section, task-based fMRI has provided the scientific community knowledge about brain function. However, fMRI has been criticized due to the low power of the statistical inferences carried on studies with low number of individuals. Rs-fMRI has the benefit of smaller acquisition times (around 5

to 10 minutes) compared to task-based experiments as there is no specific paradigm to follow. This allows not only sampling larger populations but also sharing information across other resting studies as expected patterns of activation do not differ among resting subjects (Vanderwal et al., 2013). The absence of a paradigm also eliminates the limitations and considerations involved in its design and analysis.

2.2.5 Limitations

On the contrary, there are also issues about rs-fMRI that have concerned the scientific community since the technique was introduced. There is not yet any proof to ensure that the resting signal analyzed is not corrupted by non-neural fluctuations that can lead to confounds. Rs-fMRI and fMRI in general include several sources of ambiguities like noise, giving rise to false connectivity patterns (Satterthwaite et al., 2012) or the lack of complete understanding of the physiological basis underlying BOLD signal. Also, the wide number of possible analytical approaches increases the risk of inferring different results from the same study, what presses researchers to explicitly state the exact preprocessing pipelines and findings, aiming to be able to develop universal standard methods. Finally, it is important to note that the lack of constraint derived from the resting state is often considered an advantage; however, one needs to be aware of behavioral, clinical and cognitive condition of each subject, circumstances that are often not taken into account.

2.2.6 Reliability

As a result of the previously cited limitations, there have been multiple studies merely focused on proving the reliability and validity of resting state experiments (Castellanos et al., 2013). The reproducibility of the measures has been tested by repeating the trials over time and locations as well as across scanners. However, there are still concerns that can affect the consistency of the results; these are mainly dependant on subject circumstances such as eyes closing or opening. The validity is mainly checked by comparing the resulting images with the brain information provided

via other techniques like task-based fMRI, intracranial recordings and even histological studies or dissections. Specificity and sensitivity are also critical values of resting state measures for the recognition of biomarkers of specific mental disorders, a common application of rs-fMRI. Rs-fMRI, just as task-based fMRI, can be combined with other imaging techniques such as EEG or positron emission tomography (PET) to prove the reliability of the method.

The development of rs-fMRI has allowed the scientific community to achieve new insights about higher order cognitive processes in brain that could not be addressed in task-based experiments or other imaging modalities. However, there is still a lot of research needed to elucidate the complete organization of brain in a consistent manner. In order to attain this goal, reliable processing and analytical methods to determine the network connectivity of rs-fMRI signals must be developed.

2.2.7 Preprocessing

Before data analysis, a series of preprocessing steps must be performed in order to remove any source of noise or artifact (e.g. heat, physiology, arbitrary neural activity, scanning hardware or differences across subjects) distorting the weak BOLD signal of interest. These confounding factors must be carefully considered by investigators in order to avoid wrong inferences. There exist several programs and software packages that provide multiple tools to preprocess and analyze fMRI data, common examples are FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), SPM (http://www.fil.ion.ucl.ac.uk/spm/) or AFNI (http://afni.nimh.nih.gov/afni/). Most preprocessing steps carried out on resting state images are common to task-based fMRI.

Motion correction

Probably the most frequent problem distorting data analysis is subject movements, especially head motion. If this occurs, the timecourse in a voxel will not be representing the same neuron activity as in previous scanned slices. These movements can be erroneously correlated to the task or stimulus and give serious

confounds, increasing the variance and reducing the sensitivity of the analysis. Motion correction applies a rigid-body transformation including six parameters (3 translations and 3 rotations) to account for all the possible directions of movement with respect to a reference volume (usually the first or an average).

Despiking

There is a method that is said to likely improve motion correction by affecting its parameter estimates: despiking. It consists of adjusting intensity spikes on each voxel, which are mainly derived from sudden scanner noise.

Slice timing correction

Another commonly implemented step is slice timing correction. The fMRI scanning system acquires brain volume in the form of slices that are usually sampled one by one sequentially or interleaved. This means that the complete set of slices representing a 3D volume does not contain the signal from a single time point. In order to solve this issue, a single slice is taken as a reference and the phases of the rest are shifted and interpolated for it to represent the same time point as the fixed slice. Slice timing is more critical in task-based studies due to the alternating conditions affecting brain activity.

Field map correction and detrending

There are other problems derived from the scanning system such as field inhomogeneities and drifts of fMRI data. The most used method to correct field distortion is to acquire a field map in order to account for the non-uniformities in the analyzed volumes. On the other side, detrending on each voxel is used to correct trends originated by the scanner by applying different order polynomials (linear, quadratic or cubic).

Registration

Rs-fMRI can be combined and related to other modalities like T1-weighted images, but a registration step is needed in order to correctly align both image volumes. It is common to register functional images to structural ones, which usually

have higher spatial resolution, with two purposes: optimizing spatial normalization (see next paragraph) and identification of regions of interest (ROI) for network analyses. Both processes are more effectively performed if high resolution T1-weigthed images are used, which are spacially related to functional images thanks to registration. Again, a linear transformation is performed to align both images but generally using 12 parameters to account 3 translations, 3 rotations, 3 scalings and 3 shears. As intensity values of different techniques cannot be straightforwardly compared, the algorithm seeks for shared information like entropy distribution to select the best alignment.

Registration is also necessary when comparing different subjects in a study. With this aim, all the individuals' images are normalized to a common coordinate space. There are several widely used templates available to use as standard space, the most common ones are Tailarach and Montreal National Institute (MNI). The second brain atlas cited is the most representative of human population as it was designed by averaging the brain of 152 individuals, while Tailarach, the first created, took the brain of a single elderly woman as a reference. Normalization also uses affine transformation with 12 parameters, although non-linear transformations may be also required in order to account for varying head shapes.

Image segmentation may be required prior to registration in order to remove unrelated tissue like bone. Segmentation can also be used to separate gray and white matter by iteratively addressing the probability of each voxel to belong to certain tissue.

Temporal filtering

Temporal filtering is a critical step of data preprocessing designed to remove the frequencies that do not represent the signal of interest. The methodology is the following: Fourier transform is applied to generate the power spectrum of the timecourse of the voxels, the undesired frequencies are removed and inverse Fourier transform is applied again to generate the modified timecourses. The removal of the frequencies is done via a band pass filter in order to keep just the frequencies inside the range of signal significance. Due to the low-frequency character of resting state

BOLD signal, the cut of frequency of the high pass filter must be carefully considered. Temporal filter aims to remove physiological signals like respiratory or cardiac in order to improve the quality of the data obtained from the scanner.

Spatial filtering

Filtering can also be done in the spatial domain, and it is commonly called smoothing. The idea is to average the signal intensities of neighboring voxels in order to have an even spatial map and reduce variability across subjects. It is generally done by convolving the data with a 3D Gaussian (low pass) kernel. For the filtering process to be effective, the size of the kernel must be at least twice the one of the voxels (full width half maximum, FWHM). The filter works on each voxel by assigning to it a weighted average considering the intensity of adjacent voxels covered by the kernel. Smoothing is considered to improve signal-to-noise ratio, blur registration errors and increase normal distribution.

Grand mean scaling

Finally, another important procedure to preprocess Rs-fMRI data is grand mean scaling (GMS), which divides voxel values by the average mean intensity of the 4D volume consisting on all the 3D volumes of the session. GMS can be done at first (within-subject) or second (between-subject) level, and it is aimed to facilitate interpretation of the data by having a common scale.

The order of implementation and the parameters used in these steps differ across studies and researchers. The decisions about these issues taken in this study as well as a more detailed description of the steps applied will be presented in “Materials and methods”.

2.2.8 Post-processing

Several methods have been developed to analyze fMRI data and resting state specifically. Although they rely on different assumptions, they can be classified in the following groups: model-based methods, model-free methods and graph theory.

Model-based methods: seed-based

Model-based methods rely on hypotheses to determine the functional connectivity. They are based on *a priori* selection of ROIs that are called seeds. The signal from these seeds (depicted in red in figure 8), which can be formed by one or several voxels, is used then as a model to compute the degree of connectivity of it with the other regions of the brain. This bivariate measurement between time-series, called seed-based analysis, has the drawback of strong dependence on the initial selection of ROIs. There are algorithms that predict the optimal number of groups to start with, although there is no single optimal solution to this issue. Meta-analysis can also be used to determine the seeds by considering the results of several neuroimaging studies. The method provides a more consistent way to locate the regions of activation that will define functional connectivity. A threshold value selected by the researcher is always used to determine the necessary significance of a voxel correlation with the seed for the small region to be considered as functionally connected.

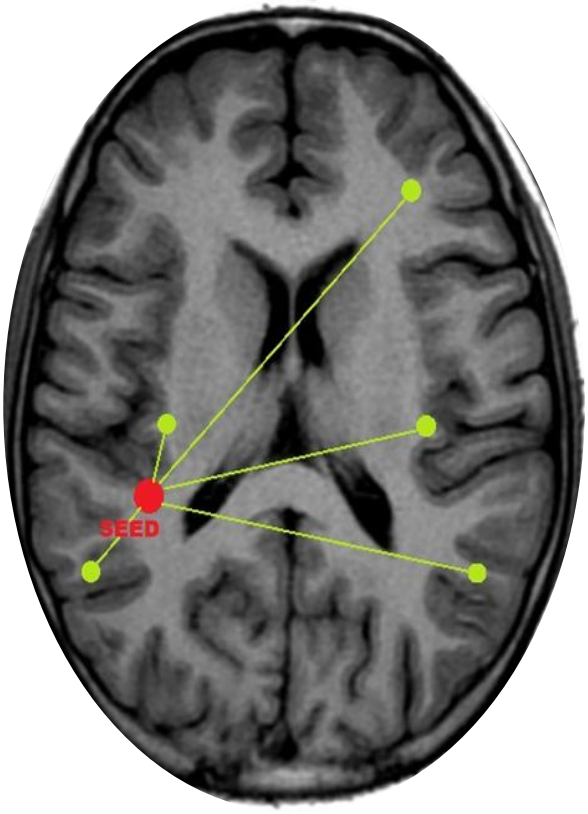


Figure 8. Seed-based analysis of brain functional connectivity. The points in light green present co-activation with the established seed (in red).

Model-free analysis: Independent Component Analysis

Model-free methods do not need such *a priori* assumptions, what makes them especially useful if the regions involved in the study are unknown. The most used technique in this field is ICA, a data-driven mathematical method that decomposes the fMRI data into a set of statistically non-overlapping spatial or temporal sets (as depicted in figure 9). There are multiple possible algorithms implemented on ICA, all of them allowing simultaneous grouping of the regions of the brain that follow common patterns of activation and even identifying and extracting noisy components such as motion or scanner drifts. The iterative nature of ICA algorithm potentially increases the variability of the results, which can be reduced if strict criteria of convergence are applied. Nevertheless, studies in similar populations using ICA have reported different number of components from resting state data across investigators and algorithms. Regardless of the differences found between seed-based analysis and ICA, there are studies that have obtained similar results from both analyses.

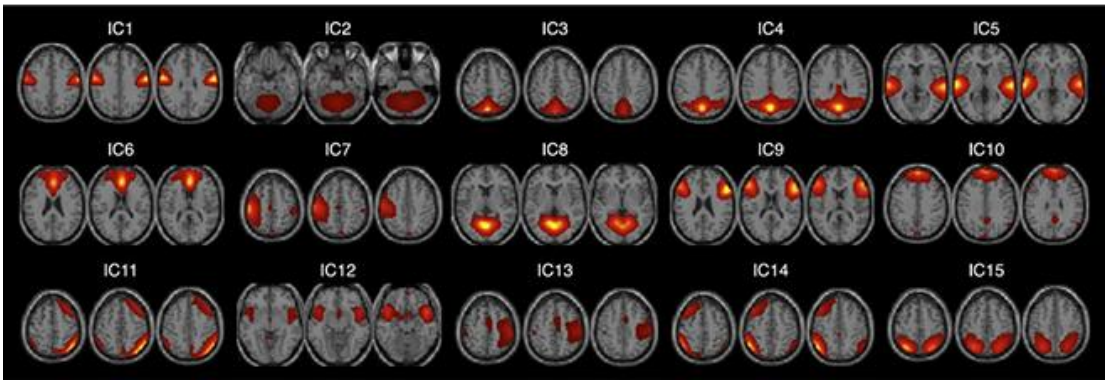


Figure 9. 15 independent components estimated with ICA. (Ma et al., 2011)

Graph theoretical methods

Finally, graph theory is used to infer the topological organization of the brain at local and global level. The model is defined by nodes graphically represented as dots and edges plotted as lines, that represent brain regions and connections between them, respectively. The obtained networks are characterized by several parameters

describing different features that help classifying the networks in regular, random or small-world, which is the one that best describes brain connectivity (Sporns, 2006). Brain connections are organized in regions that are highly linked as hubs and other distant connections among these central clusters that work as highways for information. This model, that resembles social networks, enables the efficient functional communication between brain networks responsible for different functions. These networks are characterized by a high clustering coefficient and small path lengths. Other parameters that help classifying networks are: modularity, efficiency, centrality, degree distribution or hierarchy. Furthermore, the graphs can be identified by the order, defined by the number of nodes it has, or by the labeling of their links, being weighted if present or binary otherwise (see figure 10).

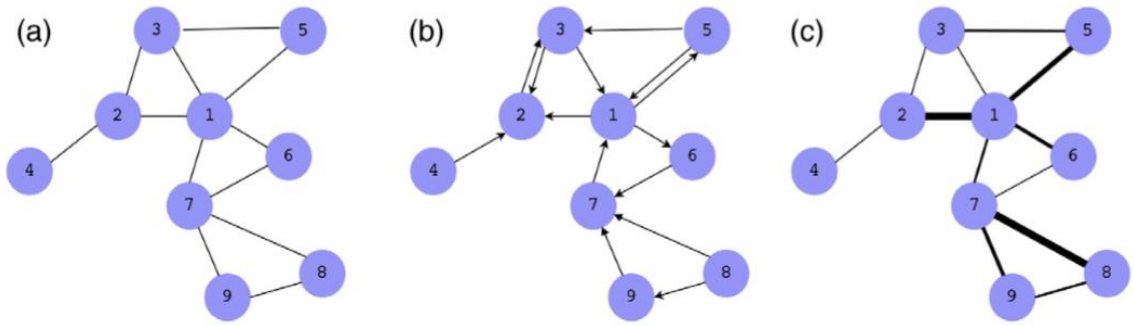


Figure 10. Examples of undirected(a), directed(b), and weighted(c)networks computed with graph analysis. (Onias et al., 2014).

2.3 *PSYCHOTIC DISORDERS*

Psychosis is neurodevelopmental disorder characterized by a set of symptoms involving abnormal perception and interpretation of reality that affects behavior and emotions. FEP appears before the age of 18 years and it can derive into several relapses as shown in figure 11. Some people only experience a psychotic episode while others can develop into various disorders such as schizophrenia, schizophreniform disorder, brief psychotic disorder and bipolar disorder or major depression with

psychotic symptoms. These are serious mental disabling health problems associated with a poorer quality of life (Saarni et al., 2010) and significantly decreased life expectancy (up to 20 years) (Suvisaari et al., 2013). In fact, schizophrenia, the most common type of psychosis, is said to be the third most disabling condition after quadriplegia or dementia, implying high human and economic costs. This brain disorder develops in 2-3 percent of the population (Perala et al., 2007) although its consequences are devastating not only for patients but also for those around them, like family members (Millier et al., 2014). Anyhow, there exist several possible treatments, including drugs and therapy, to help managing the different symptoms and improve quality of life.

Psychosis is characterized by the following symptoms that can appear in different rates and severities:

- Positive symptoms: These are conditions that are not commonly experienced by the general population and are characterized by delusions (false, irrational, personal beliefs that are not based in evidence or reality), hallucinations (false sensory perceptions that have no real source and only exist on person's mind) or abnormal, disorganized movements.
- Negative symptoms: These differ from healthy population in the absence of normal (emotional) behaviors. Lack of motivation or avolition are common manifestations.
- Affective dysregulation: Some examples are depression or mania.
- Severe cognitive impairments: These cognitive deficits negatively affect executive functioning, concentration and working memory.

Particularly in subjects between 13-18 years old, positive symptoms are present in around 7% of them (Kelleher et al., 2012) while 10% manifest sub-threshold symptoms like abnormal thinking or reality confusion (Calkins et al., 2014). The presence of these symptoms in teenagers indicates their susceptibility of developing another type of mental disorders in the future (Carrion et al., 2013) even when psychotic disorder does not develop in the mid-term.

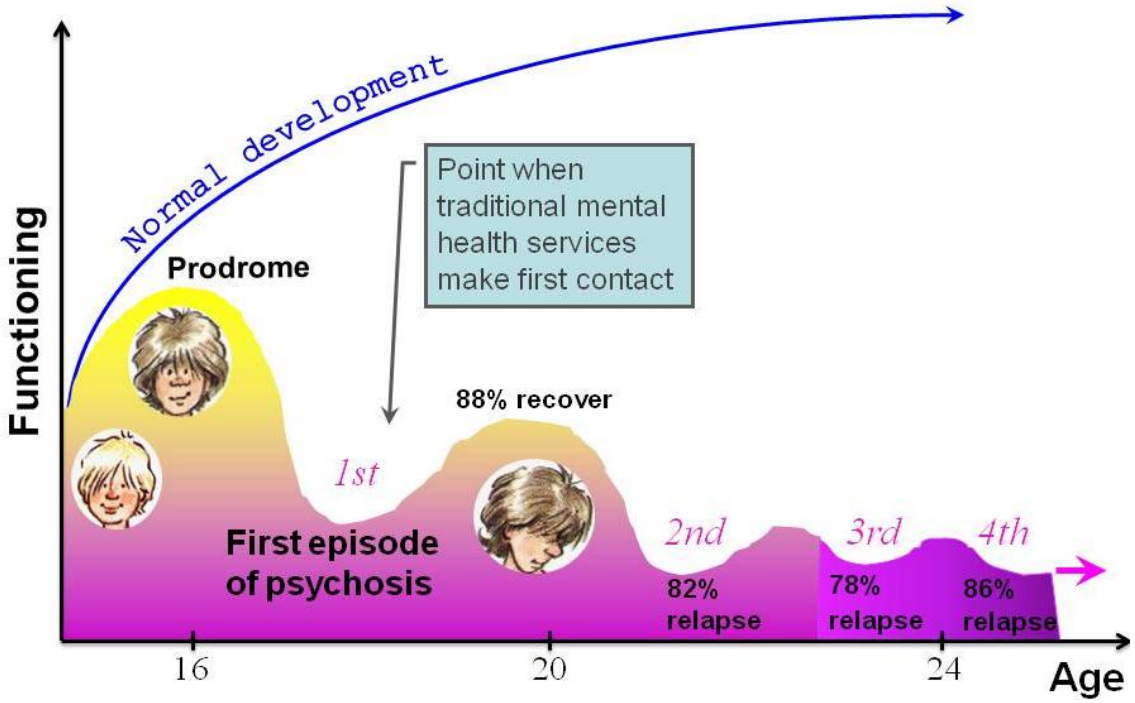


Figure 11. Usual course of FEP. Adapted. (Robinson et al., 1999).

2.4 *USEFULNESS OF RS-FMRI IN PSYCHOTIC DISORDERS*

2.4.1 Why doing neuroimaging in psychiatry?

The unequivocal identification of a psychiatric disorder is complicated due to several factors such as overlapping symptoms between different diseases, the reliance and objectivity of self-reported abnormalities and the lack of a direct relationship found between genetic biomarkers and a disease. Characterization of the underlying pathology of FEP is likely to require an integrative platform that enables researchers to combine findings across various scientific disciplines. For example, observations from human neuroimaging studies could be linked with findings obtained using experimental techniques (such as cellular assays, histology or animal models) that can characterize pathological processes at a high level of information, specificity and resolution. Neuroimaging remains one of the few techniques that can be used to investigate brain pathology in vivo, offering a unique opportunity to provide information that could facilitate the diagnosis and treatment of FEP in the clinical

setting. This technique is thus a promising tool to shed light on the relation of genetic alterations with structural and functional brain abnormalities assessing disease diagnosis. For this reason, growing efforts are now devoted to developing neuroimaging into a translational tool—that is, a tool that forms part of a chain of multidisciplinary inputs and outputs that provides results that can be translated into medical practice and can be used as a measure of health outcomes. In the future, integration of neuroimaging into the translational research cycle (see figure 12) will be crucial in order to facilitate translation of findings from bench to bedside.

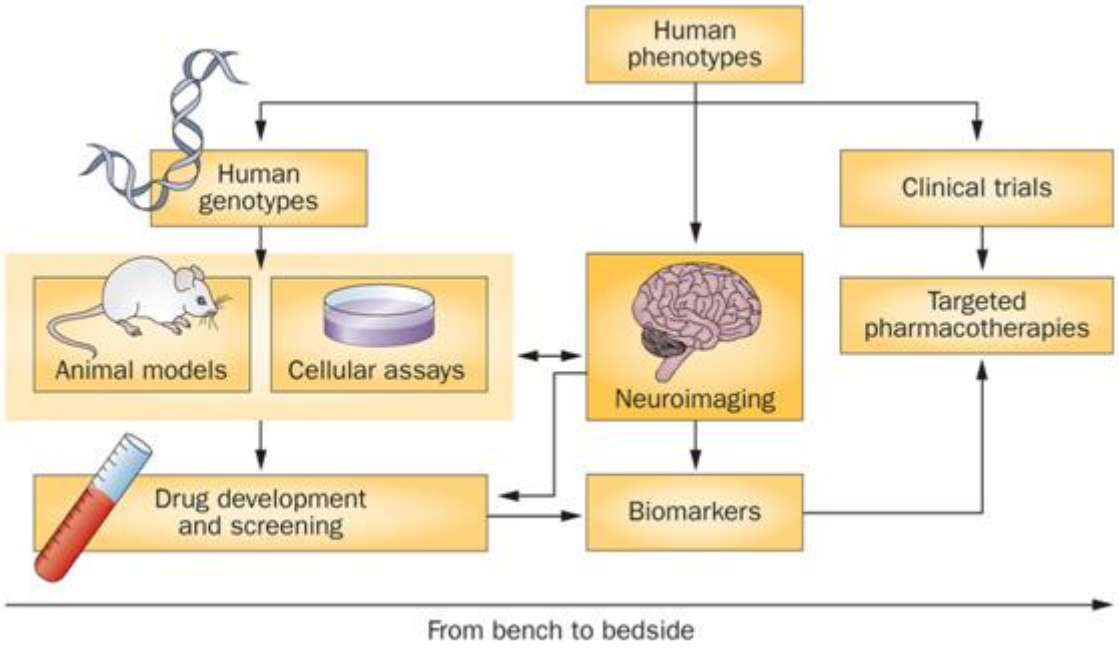


Figure 12. Integration of neuroimaging in the translational research cycle. (Ecker and Murphy, 2014).

Thus far, no psychiatric disorder has yet been diagnosed by using neuroimaging alone, and the uses at clinical level are limited mainly to structural modalities such as computed tomography (CT) or MRI. Otherwise, functional imaging modalities like PET, single-photon emission tomography (SPECT), fMRI or diffusion tensor tractography (DTI) characterize the foundations of most research studies on psychiatry. However, functional modalities are proving to be a very useful tool not only to assess disease diagnosis and progression by studying brain circuits but also to observe the effects of treatments on the brain.

FEP comprises a group of conditions with a large degree of etiological and phenotypic heterogeneity. Probably for this reason, the diagnosis of psychosis continues to be based on symptoms rather than etiology, and individuals with suspected psychosis are conventionally assessed via behavioral observations and/or clinical interviews. Although the behavioral diagnosis of psychosis has clear advantages in the clinical setting, it is less beneficial for the classification of disorders and the development of new treatments. This is significantly evident in clinical trial cohorts, which typically exhibit a high degree of clinical and/or phenotypic heterogeneity. These studies can potentially include individuals belonging to different biological subgroups within FEP, who are unlikely to be treatable using a 'one size fits all' approach. Consequently, strong effects of a given treatment within a biologically homogeneous subgroup of patients with FEP might be masked by a small effect of the treatment across the whole cohort. Neuroimaging techniques might enable stratification of patients into homogeneous subgroups of individuals who are more likely to respond to a given treatment (See figure 13).

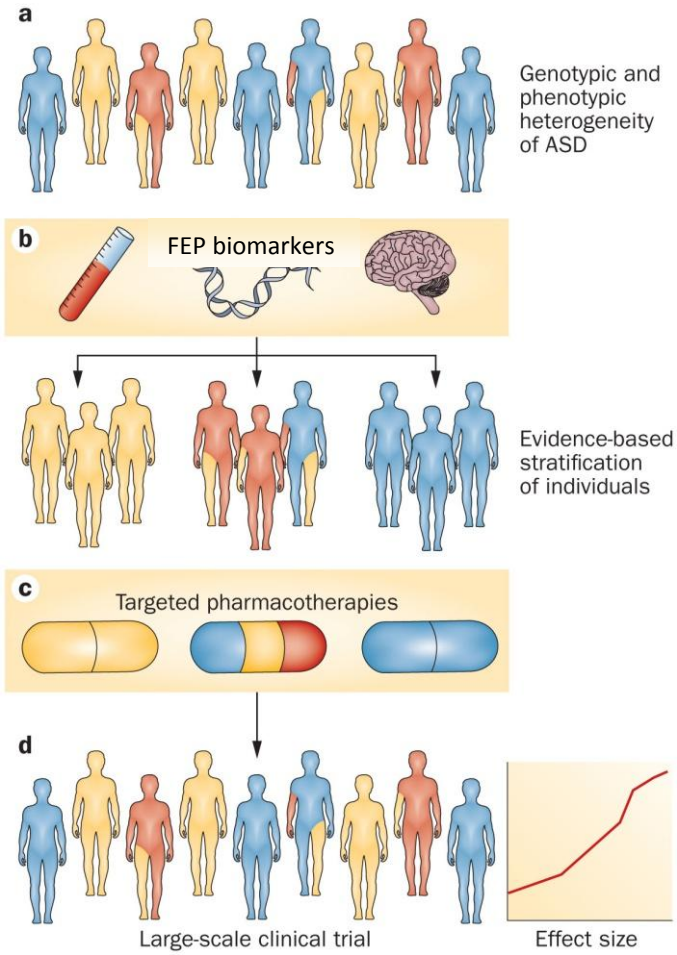


Figure 13. Stratification of FEP patients according to biomarkers enables more efficient treatment development and planning. Adapted. . (Ecker and Murphy, 2014)

2.4.2 Advantages of rs-fMRI for the study of psychosis

Rs-fMRI is one of the imaging modalities that has provided great valuable information about brain connectivity alterations in psychotic disorders. This technique has certain properties that make it particularly advantageous for the study of psychosis. The individuals suffering this kind of disorders often present symptoms that hamper the performance of certain tasks and thus, the limited behavioral demand of the resting conditions make it easier to perform optimal recordings. Second, rs-MRI provides extremely rich data regarding functional brain networks, which may be a particularly informative unit of analysis for psychotic disorders, and are amenable to many complementary analytic strategies. The task independence also favors the sharing of data across different studies, as cited previously. Despite these advantages, the psychotic condition is also particularly compromising in rs-fMRI. This technique is quite sensitive to motion, which is significantly more likely to occur in the presence of psychotic symptoms and can give meaningful group differences when analyzing the data of patients and controls.

Numerous findings suggest the low-frequency BOLD signal from rs-fMRI as a potential biomarker for neuropsychiatric diseases, its prognosis and therapy evaluation (Castellanos et al., 2013, Satterthwaite et al., 2015) These biological indicators have helped to improve the differential diagnosis of these disorders, what has always been a critical challenge for psychiatrists and clinicians. Despite all these promising findings, work is still needed to enable the use of rs-fMRI in clinical context.

2.4.3 State of the art of rs-fMRI in psychotic disorders

Over the last decades, research has been devoted to the study of functional connectivity of the brain as a potential biomarker for neuropsychiatric diseases. People suffering psychotic disorders have shown alterations in brain patterns of connectivity when performing resting state studies that have shown to be correlated to cognitive impairments.

The majority of these disruptions have been found in the DMN, the one responsible for the integration of cognitive and emotional processing. This is

consistent with the disturbed thoughts and emotional flatness characteristic of psychotic disorders. Studies on first episodes of psychosis have revealed hyperconnectivity of this network not only during resting state (Satterthwaite et al., 2015) but also during working memory tasks (Fryer et al., 2013), what agrees with the idea of a deficient suppression of DMN activity during task performance at the early overt stages of the disease. This proposal is supported by the loss of anti-correlation found by Wotruba et al. (Wotruba et al., 2014) between task positive networks and DMN.

FEP is however a non-specific disorder that can be precursor of various clinical conditions and prognosis. For this reason, research is needed in order to find a robust relation between symptom severity or cognitive impairment and network disruptions. This characterization of the psychosis continuum will allow the dissection of the disease in different stages. FEP often derives into schizophrenia, a dysconnectivity disease showing increased activity and connectivity on its first stages, as in FEP. Schizophrenia is said to be derived from aberrant maturation of brain specially affecting association cortex. The hyperactivity and connectivity on the DMN has been related to positive symptoms of early-onset schizophrenia by Whitfield-Gabrieli (Whitfield-Gabrieli et al., 2009), although Bluhm et al. (Bluhm et al., 2007) associate them to hypoconnectivity. Lui (Lui et al., 2010) has also negatively related temporo-PCu connectivity to Positive and Negative Syndrome Scale (PANSS) scores, although there are other researchers as Alonso-Solís and colleagues (Alonso-Solis et al., 2012) that have not found any relation between DMN functional connectivity and symptoms. This group has instead detected decreased connectivity of the MPFC with PCu and PCC in the DMN, consistent with other investigators' findings (Bluhm et al., 2007, Whitfield-Gabrieli et al., 2009).

The connection between frontal regions and the DMN has also been assessed. Dysfunctional interactions are present between FPN and DMN in FEP (Buckner, 2013, Satterthwaite et al., 2015). FPN anatomically overlaps with DMN and is related to initiation and modulation of cognitive control tasks and external attention. The decoupling of this network with DMN is thus consistent with the disordered thoughts in psychosis, as executive control systems and cognitive processing are disrupted. FPN shows also less modular organization in psychotic disorders, what decreases the

effective connectivity among external control systems. This is consistent with outcomes reporting impaired FPN in cognitive control tasks (Fornito et al., 2011). Furthermore, there have been different findings about activity of the network itself; while Lui and colleagues (Lui et al., 2010) have found increased overall activity, others have reported hypoactivity (Zhou et al., 2007).

Association between structural abnormalities and aberrant functional connectivity has also been found across several studies that mostly report decreased gray matter (GM) in frontal and parietal regions of FEP (Arango et al., 2012, Schmidt et al., 2014). However there is no clear evidence linking reduced gray matter and psychotic symptoms.

Cognitive impairment is a hallmark characteristic of FEP (van Os and Kapur, 2009), with prior literature demonstrating that rs-fMRI may be associated with cognitive function/dysfunction. Previous studies have found that higher IQ scores are associated with greater functional connectivity within a FPN in healthy individuals, suggesting that the coordination of these regions is an important neural basis of individual intelligence (Song et al., 2008). A region-specific analysis of the lateral prefrontal cortex, part of the FPN, found that its global connectivity predicted cognitive performance (Cole et al., 2012). Two studies have also reported an association between efficiency of global communication and intellectual performance, suggesting that individuals with higher intelligence have a more organized brain network overall (van den Heuvel et al., 2008).

At this point, it has been described the usefulness of rs-fMRI as a powerful tool to understand and analyze brain organization in healthy and diseased individuals. Currently, it is widely used as a promising technique to identify biomarkers of psychiatric diseases based on patterns of brain connectivity. For this reason, this project analyzes fMRI images of FEP individuals looking for quantifying abnormalities in the brain functional connectivity of these patients compared to healthy individuals and relate them with cognitive performance and symptom severity. According to the previously cited literature, we expect to find hyperconnectivity in FEP with respect to controls, and a negative relation of this connectivity with intellectual performance.

Also, it is expected that symptom severity is higher as connectivity increases in patients.

At the beginning of this document, the motivation, objectives and hypotheses of the project have been specified. Next, the state of the art of the technique and its relation to psychotic disorders have been detailed such that the reader can understand the context of the study. Hereafter, the materials and methods used in this study will be described, starting from the patient recruitment and subsequent image acquisition and finishing with image statistical analysis after preprocessing tools and steps have been specified. Further on, the results of these analyses are presented and a final discussion will compare our findings to other studies, determine the limitations of our approach and suggest future lines of work. Every source of information used to develop this project is alphabetically compiled at the end of the current document.

3. MATERIALS AND METHODS

3.1 *SAMPLE (SUBJECT SELECTION CRITERIA)*

For this study, developed in the Child and Adolescent Psychiatry Department at the *Hospital General Universitario Gregorio Marañón* (Madrid, Spain), 30 patients with FEP and 18 age-matched healthy controls were recruited. Patients were recruited at the inpatient or outpatient clinic at the time of their first episode of psychosis, and healthy controls were recruited from the community, at publicly-funded schools with characteristics similar to those attended by patients and located in the same geographic area.

The inclusion criteria for all patients were being aged 7 to 18 years at the time of first assessment, speaking Spanish correctly, and having a DSM-IV-TR diagnosis of either a first episode of psychotic disorder. The inclusion criteria for healthy controls were the same as for patients, except for no current or previous psychiatric disorder. Exclusion criteria included mental retardation per DSM-IV-TR criteria, neurological disorders, history of head trauma with loss of consciousness and pregnancy.

The study protocol and informed consent form were approved by the Institutional Review Board of *Hospital General Universitario Gregorio Marañón* in Madrid. All parents or legal guardians gave written informed consent after receiving complete information about the study, and patients and controls agreed to participate.

3.1.1 Diagnostic assessment

All diagnostic assessments were conducted by child and adolescent psychiatrists with extensive experience in the diagnosis of psychosis, after directly assessing the young patient and the family and after reviewing all available medical

and educational reports. The Spanish adaptation of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) (Kaufman et al., 1997) was administered to both patients and healthy controls to obtain diagnosis in patients with FEP and to rule out concomitant psychiatric disorders in both groups. Also parents were administered K-SADS-PL in individually separated interviews, being the final diagnosis based on best clinical judgment taking into account all the available information. Patients were included in the FEP group if they fulfilled any DSM-IV-TR diagnosis of psychotic disorder (other than drug-induced psychosis) after the assessment described.

3.1.2 Clinical and cognitive assessment

Socioeconomic status was estimated as parental years of education, and handedness was assessed with item 5 of the Neurological Evaluation Scale (NES) for both groups. Estimated intelligence quotient (IQ) was computed using vocabulary and block-design tests of Wechsler Intelligence Scale for Children (WISC-R) in subjects under 16 years of age, and the Wechsler Adult Intelligence Scale (WAIS-III) in subjects 16 years of age or older.

PANSS scores were also administered to patients by child psychiatrist trained in the use of this scale, and PANSS positive, negative, general and total subscores were computed. Intraclass correlation coefficients for PANSS inter-rater reliability were above 0.8.

In table 1, the sample is concisely described.

	FEP	Controls	Statistics
Age [Mean (sd)]	15.17 (2.276)	14.06 (2.940)	t=1.466, p=0.161
IQ [Mean (sd)]	88.96 (23.289)	108.00 (16.400)	t=-2.760, p=0.096
Gender [%males]	43.3%	22.2%	X^2=2.192, p=0.214
Panss [Mean (sd)]			
Positive	19.80		
Negative	16.80		
General	36.80		
Total	73.40		

Table 1. 48-subjects sample description.

3.2 *IMAGE ACQUISITION*

The image acquisition followed a specific MRI protocol performed in a 1.5 Tesla Philips Intera with software version 11.1.4.6 2006-03-10. The protocol included the following anatomical sequences, that are depicted in figures 14, 15 and 16:

- Sagittal anatomical sequence with T1 enhancement:

  Echo gradient (fast field echo, FFE) 3D

  175 slices

  Voxel size: 1x0.94x0.94 mm^3

  Matrix size: 256x256

  TR=25 ms

  TE=9.2 ms

  Acquisition time: 6 minutes

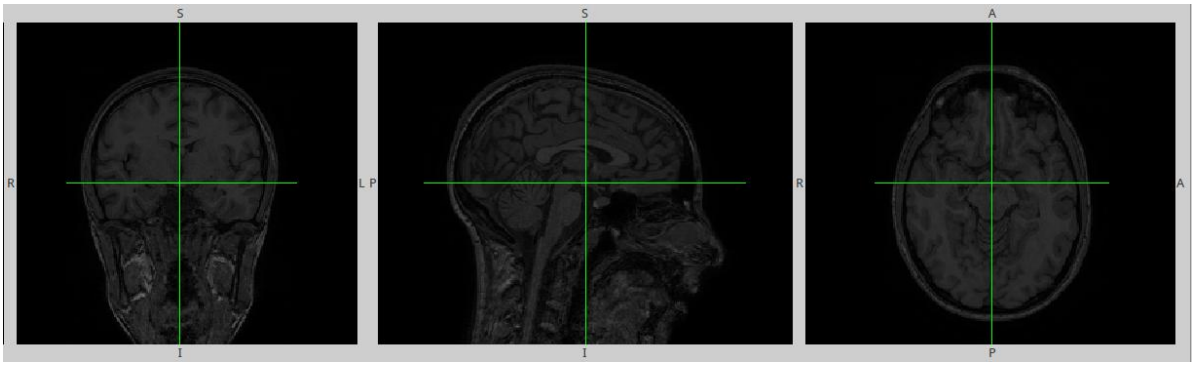


Figure 14. Coronal, sagittal and axial planes of T1-weithed image of a subject of the study.

- Axial anatomical sequence with T2 enhancement:

  Turbo spin-echo (TSE) sequence

  45 slices

  Voxel size: 1x1x3 mm^3

  Matrix size: 256x256

  TR=5809 ms

  TE=120 ms

  Acquisition time: 8 minutes

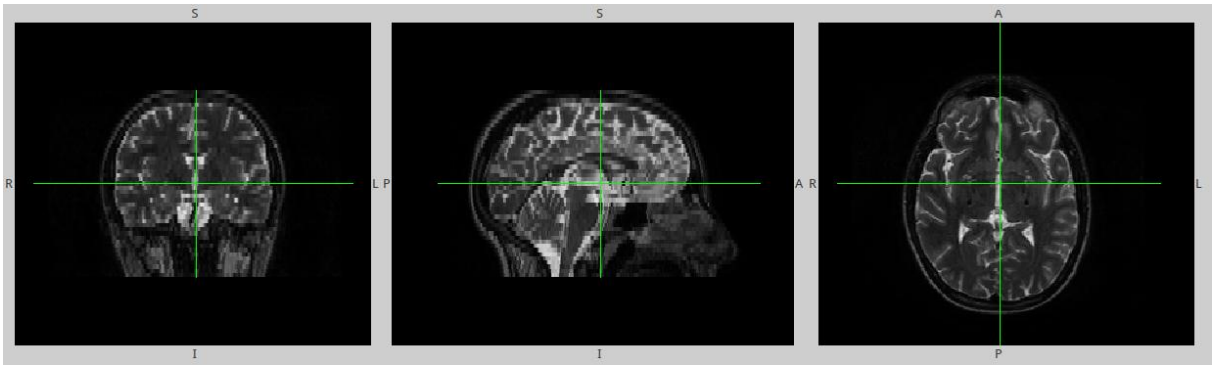


Figure 15. Coronal, sagittal and axial planes of T2-weithed image of a subject of the study.

- Resting state functional image (rs-fMRI):

  Echo-planar sequence (EPI)

  16 slices

  256 volumes

  Voxel size: 4x4x7 mm^3

  Matrix size: 64x64

  TR=2000 ms

  TE=20 ms

  Acquisition time: 10 minutes

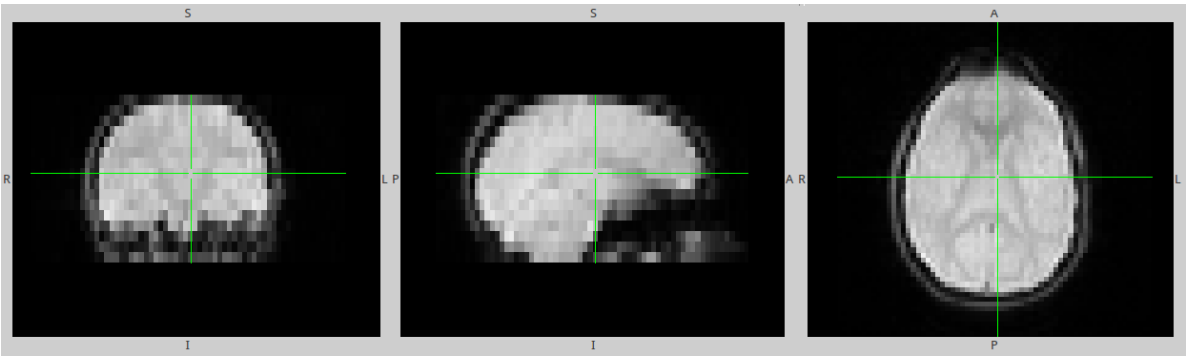


Figure 16. Coronal, sagittal and axial planes of rs-fMRI image of a subject of the study.

3.3 *AREAS ANALYZED*

The purpose of this study relies on the functional connectivity between brain regions during resting state. A model-driven approach assuming the existence of the 7

networks postulated by Yeo et al. was used in the dataset. From the networks and the regions that make them up, a number of networks and ROIs were selected. Due to the rather coarse resolution of the rs-fMRI scans of this study, some of the smaller regions postulated by Yeo et al. could not be assessed. The size of the voxels of the images from the experiment (see *Image acquisition*) is relatively large, limiting the possibility to select quite small regions in order to perform a reliable analysis.

In table 2 and figure 17, the networks selected, as well as the ROIs inside them are exposed.

Network name	Number assigned	**ROIs**
Dorsal Attention	3	1, 2 and 3
Ventral Attention	4	1, 2, 3 and 4
Limbic	5	1 and 2
Fronto-Parietal	6	1, 2, 3, 4, 5 and 6
Default Mode	7	1, 2, 3, 4 and 5

Table 2. Enumeration of the networks used and the ROIs inside them.

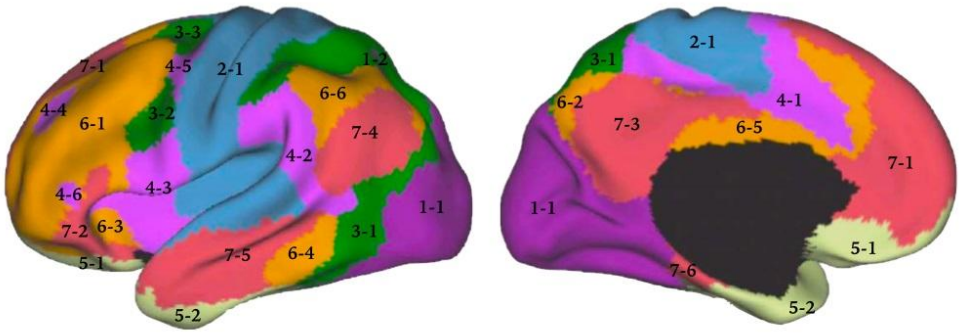


Figure 17. Location of ROIs described by Yeo. (Yeo et al., 2011).

3.4 *IMAGE ANALYSIS (PROCESSING PIPELINE)*

In order to obtain the connectivity between ROIs, that is represented in correlation matrices, previous processing must be applied to the images. The steps required to preprocess the volumes and analyze the correlation among regions are

detailed in configuration files. These files are loaded on a tool developed in MATLAB which is currently used to perform neuroimage functional analysis at LIM. This tool reads the configuration files and implements the processes indicated on them, considering the packages and parameters specified by the user. This way, the user needs to launch the processes using the files in order to obtain the matrices of correlation for every subject. The software packages versions used in this study are: Freesurfer 5.3.0, FSL 5.0, AFNI_2011_12_21_1014, SPM8 and MATLAB 7.12.0.635 (2011a). The preprocessing steps described in these files, represented in figure 18, can be divided in two parts: structural and functional.

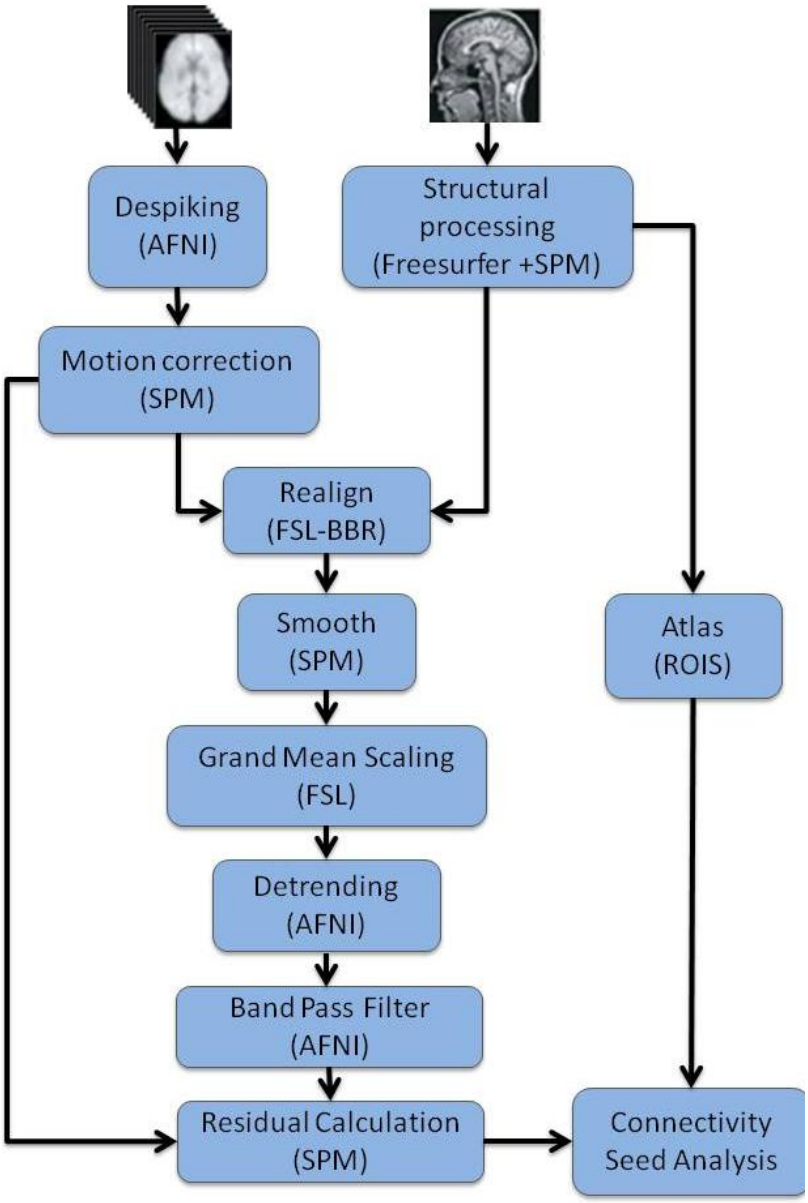


Figure 18. Schematic representation of the steps implemented in the configuration files for preprocessing and analysis of functional connectivity.

3.4.1 Structural preprocessing

- FreeSurfer: FreeSurfer is a multifunctional tool that is mainly used to identify functional regions in brain cortex. The first step implemented in structural preprocessing was to register the T1-weigthed structural image into Freesurfer space, resulting in a particular 265x265x265 image with isometric voxels of $1mm^3$.
- Segmentation: The next step is to segment this cubic image using SPM. The aim is to separate (in FreeSurfer space) gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) components of the image, as depicted in figure 19.

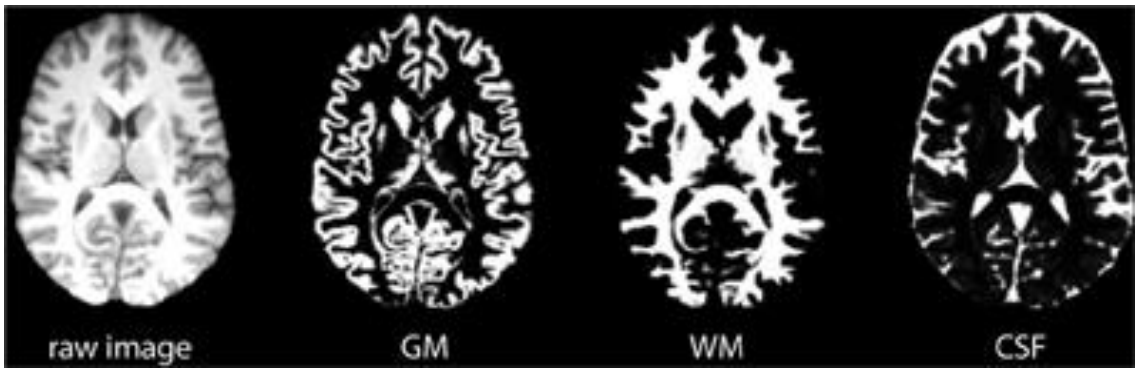


Figure 19. Segmentation of GM, WM and CSF of a raw brain image. Taken from Chang et al.2012.

- Atlas: Finally, FreeSurfer is again used to register the cortical ROIs to the segmented image. Identified gray matter tissue is aligned with the atlas of 7 Yeo regions (available in FreeSurfer). An example of the atlas registration to an image of the study is shown in figure 20.

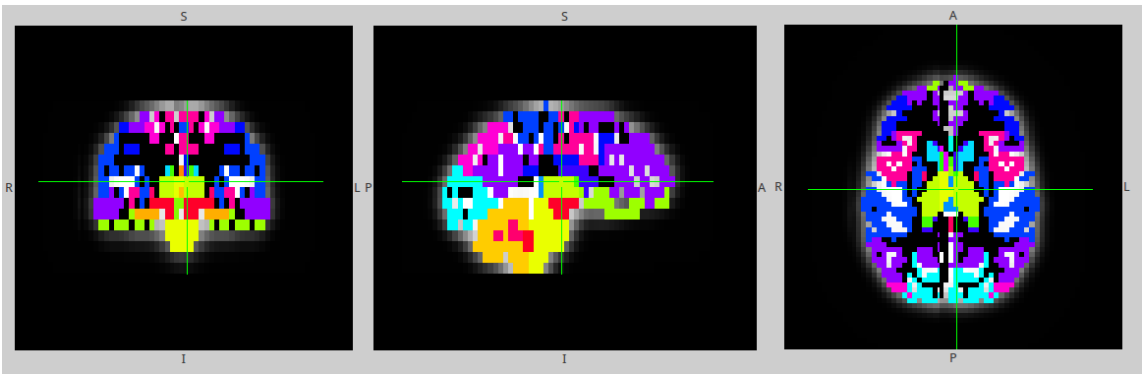
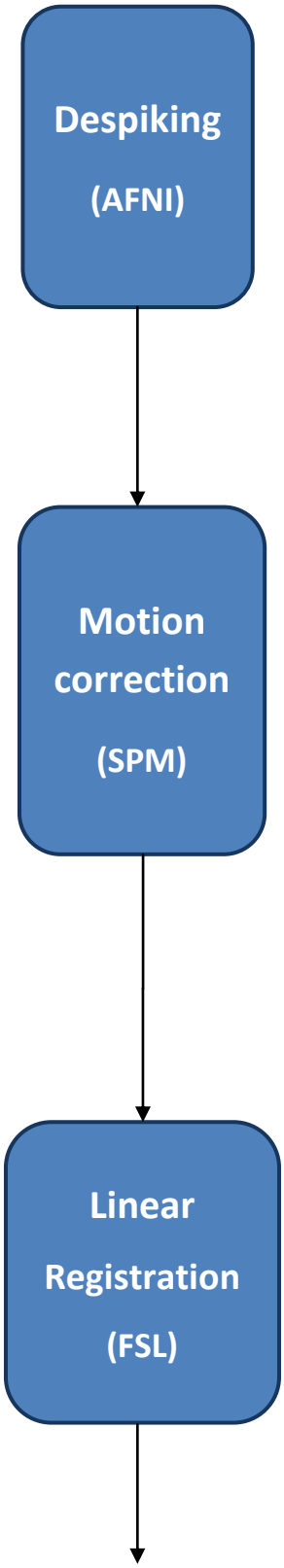


Figure 20. Registered Yeo 7-network atlas to a segmented T1-wegthed image of the study.

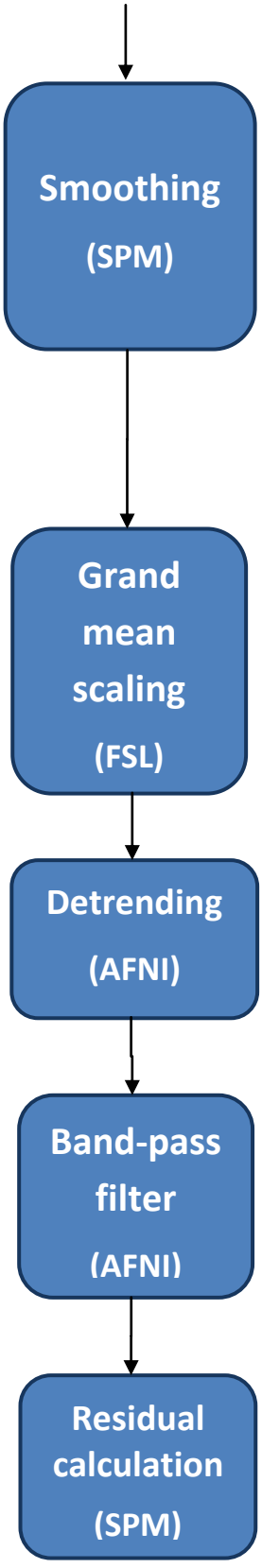
3.4.2 Functional preprocessing



Despiking: This tool was implemented in AFNI and the aim is to detect and eliminate short-term outliers in voxel time series replacing it by reasonable values fitting a smooth curve whose order can be selected by the user. It is also possible to select the threshold value defining the spikes. There are multiple studies proving that despiking improves alignment as well as motion correction due to less variable motion parameters.

Motion correction: Implemented in SPM, this method allows fixing artifacts derived from (preferably small) subject movement within a session. With this purpose, a reference volume is used as benchmark to compare it with the small differences in head position during the sequence. In this study a mean of the whole 4D acquisition of the session was used, that is preferred to the employment of the first 3D volume of the session as it contains more information about the performance although it must be noted that it is built from unaligned volumes. For this study, a linear rigid-body transformation of 6 degrees of freedom (DOF) was used.

Linear registration: It is used to merge T1 structural image to the functional one (EPI) of each subject. This step was implemented on FSL using Boundary-Based Registration (BBR) cost function, which exploits intensity differences in white matter boundaries of both images to realign them. This technique, that includes built in fieldmap-based distortion-correction even in the absence of fieldmaps, provides more accurate results and robustness to artifacts in EPI.



Smoothing: This tool is implemented in order minimize spatial differences across subjects. For this purpose, SPM was used to filter the images with an isotropic kernel of 12 mm. The size of the filter is selected considering the dimensions of the image voxels such that small ROIs are not cleared away. The method consists on averaging the signal of the neighboring voxels falling within the filter in order to minimize the possibility of the signal of voxels corresponding to a specific region to fall outside it in the analysis.

Grand mean scaling: The scaling of the images was applied using "fslmaths" tool of FSL. This procedure is performed with the aim of removing intersession variability such that the signal of every timeseries is at the same scale. This method allows to easily compare the data across subjects.

Detrending: It is used to remove temporal drifts on each voxel derived from scanner overuse. The trend was removed considering a linear polynomial with AFNI package.

Band-pass filter: This step aims to eliminate the artifactual signals. The tool was implemented using «3Dbandpass» in AFNI such that frequencies between 0.009 and 0.08 HZ were conserved, which are the ones corresponding to our signal of interest, BOLD.

Residual calculation: Finally, the motion parameters estimated initially during motion correction are used as regressors in SPM to perform a general linear model (GLM) analysis for residual calculation.

Once the images are preprocessed, the functional connectivity among regions is computed using seed-based analysis. The results are provided as a symmetric connectivity matrix of 76x76 components (see figure 22) representing the connectivity of each single ROI analyzed with each of the other ROIs and the mean connectivity between each one and the rest of the regions of its network at each hemisphere. This concept is more clearly depicted in figure 21. In this example, dorsal attention network contains 3 regions; thus, the connectivity matrix will contain the scores of each of the 3 individual regions and also the mean connectivity of region 1 with 2 and 3 (in white), the mean connectivity of region 2 with 1 and 3 (in red), and the mean connectivity of region 3 with areas 1 and 2 (in black).

Before performing any analysis on this data, quality control must be done. For this reason, visual inspection of the final processed images is necessary to check that the registration of the functional image with the Yeo atlas has been correctly implemented (as shown in figure 20). Otherwise, the values of the correlation matrix would correspond to different ROIs.

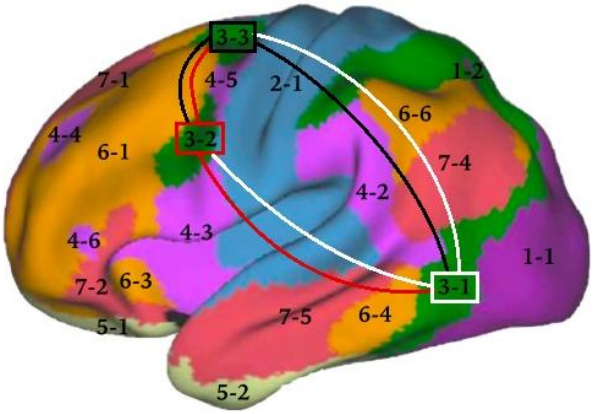


Figure 21. Example of the computation of connectivity scores of the correlation matrix containing the scores of each of the 3 individual regions of dorsal attention network (green) and also the mean connectivity of region 1 with 2 and 3 (in white), the mean connectivity of region 2 with 1 and 3 (in red), and the mean connectivity of region 3 with areas 1 and 2 (in black).

A MATLAB script was finally implemented in order to extract just the values of interest from the correlation matrix: the connections between ROIs of the same network (per hemisphere) but not the activity values of each single region.

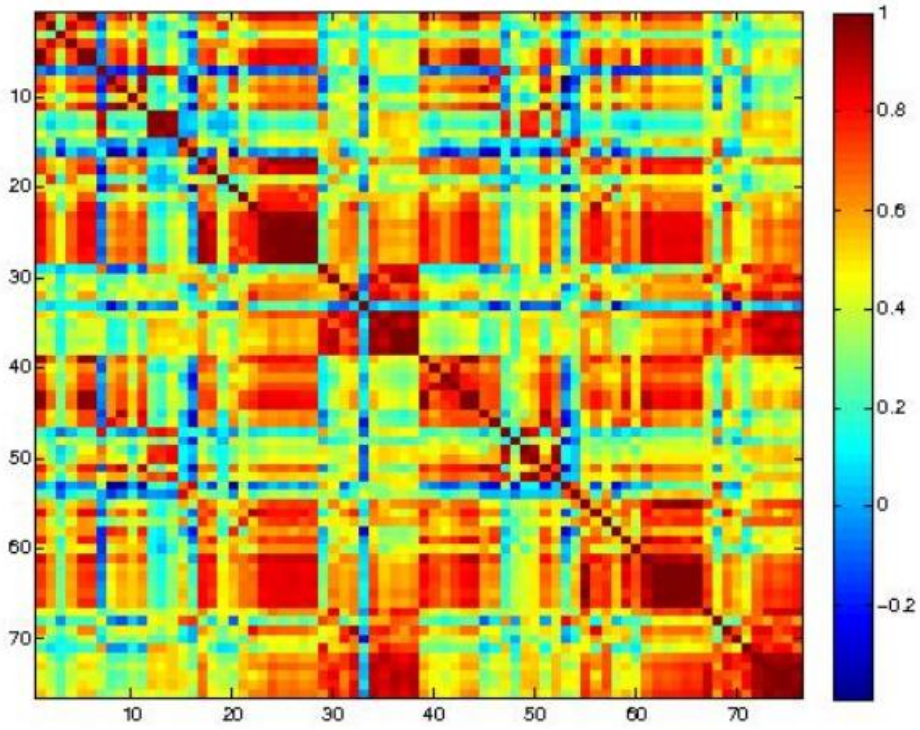


Figure 22. Symmetric correlation matrix displaying the connectivity of each ROI with each of the others and its mean connectivity with the rest of the ROIs belonging to its network. For example, as the first network analyzed is number 3, which contain 3 ROIs, the first row of the matrix represents the connectivity of ROI 3-1 with the rest of the regions and means of regions, the second one corresponds to 3-2, the third to 3-3, the forth to the mean of 3-2 and 3-3, the fifth to the mean of 3-1 and 3-3 and so on. Thus, the point [4,1] of the matrix has the value of the mean connectivity of region 1 with ROIs 2 and 3 of network 3.

3.5 *COMPUTATIONAL FACILITIES*

The image processing steps were implemented using configuration files for every subject, specifying all the parameters and file paths required to perform every process. However, completing each individual configuration file requires almost a day; thus, a cluster of up to 60 virtual machines (VM) available in the *Laboratorio de Imagen Médica* (LIM, Medical Image Laboratory) at the *Hospital General Universitario Gregorio Marañón* was used to accelerate the process.

The specifications of these VMs are the following:

- 30VMs of 1 core of 2 GHz, 6 GB RAM, 50 GB of storage capacity.
  Operating system: Debian 6.0.7, 64 bits.
- 30VMs of 1 core of 2.6 GHz, 6 GB RAM, 50 GB of storage capacity.
  Operating system: Debian 6.0.7, 64 bits.

The local computer and the remote machines of the cluster are connected using SSH (Secure Shell) protocol. Each user of the platform has access to the cluster via its own personal computer at the hospital using a Graphical User Interface (GUI). Using this interface, the user can remotely select the processes to run and the VMs needed (and available). In this project, several VMs were selected and the configuration files of each subject were automatically divided in these remote machines in order to run the processes in parallel.

The data required for the procedure is located in a common server that has direct connection with the cluster in order to enhance the efficiency of the data transfer. The users can access this server from their local computers. Once the procedure has finished, the results are stored back in the server and the unnecessary data is eliminated.

The overall scheme of the computational facilities described is depicted in the following figure.

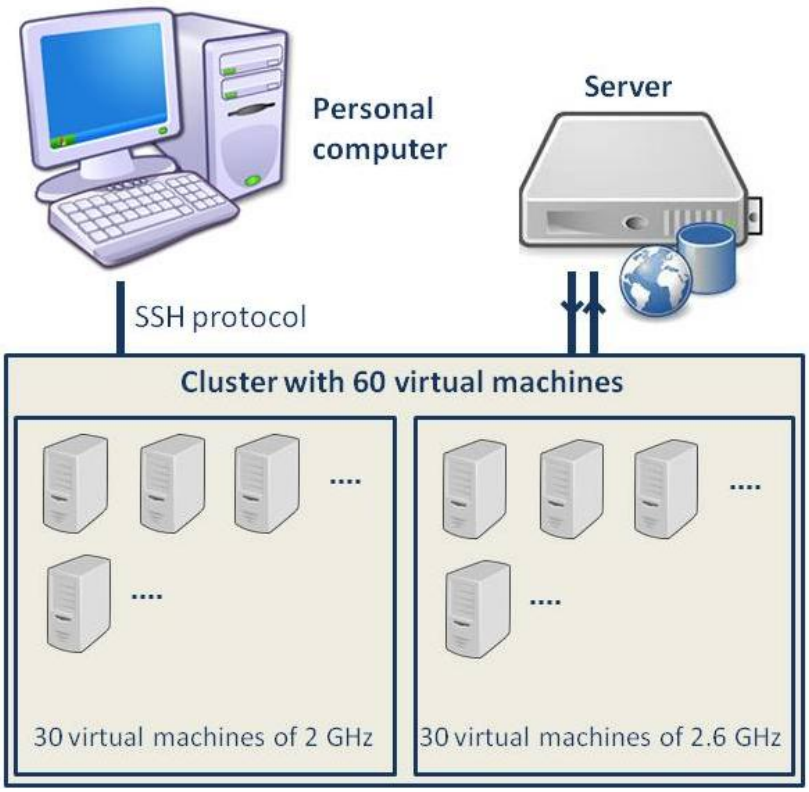


Figure 23. General scheme depicting the computational facilities used for the project at LIM in *Hospital General Universitario Gregorio Marañón*.

3.6 *STATISTICAL ANALYSIS*

An ANCOVA model was used in order to test the main hypothesis. Analysis of covariance (ANCOVA) allows testing the effects of both, categorical predictors (often called grouping variables or factors) and continuous predictors (typically called covariates) in the same model. Furthermore, ANCOVA is a useful model if the main interest relies on the effects of the categorical variable (i.e. between group differences) but there are also several variables that need to be controlled for.

This analysis was implemented using MANCOVA Toolbox for MATLAB (http://www.mathworks.com/matlabcentral/fileexchange/27014-mancovan/content/mancovan.m). The tool allows testing the effects of the covariates and the possible groups or interactions over the dependent variable (Y). The identification of sources of significance is done by analyzing the computed F-statictics and associated p-values of each independent variable in the model (covariates, groups and interaction, if present) for each column of the dependent variable. Regression coefficients, also computed in the function for each model, are standardized in order to ease comparisons and identify the direction of the correlations.

Four statistical models were implemented in order to test relation of functional connectivity with diagnosis, intellectual performance and symptoms. First of all, diagnostic differences in functional connectivity after controlling for age and sex were assessed. For this purpose, an Analysis of Covariance (ANCOVA) model with diagnosis as a between-group factor, age and sex as covariates and connectivity as the dependent variable was applied.

Next, the influence of functional connectivity alterations on the cognitive performance was studied. For this purpose, diagnosis, connectivity and the interaction diagnosis-by-connectivity were set as independent variables in an ANCOVA model for predicting IQ after controlling for age and sex.

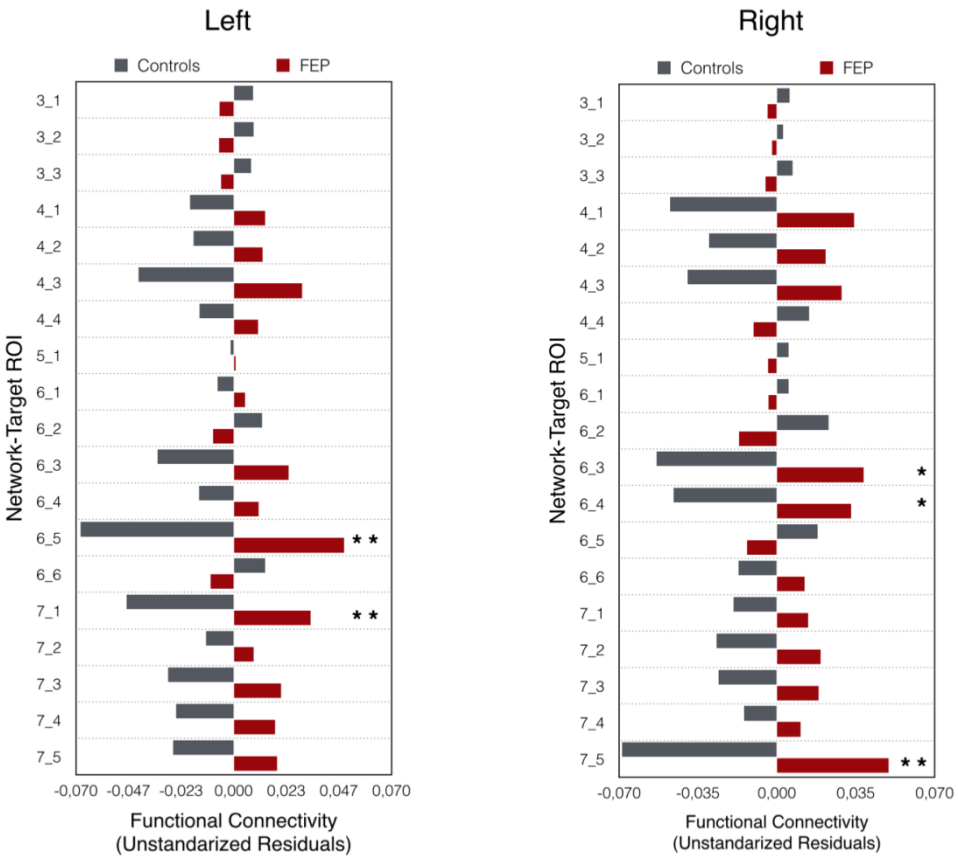
To examine within-group IQ-connectivity associations, an additional ANCOVA model was tested on each group separately, including as independent variable of interest connectivity and age and sex as covariates.

Finally, the relationship between functional connectivity and symptom severity (as measured by PANSS) was assessed in the FEP group. In this model, the independent variable of interest was connectivity, age and sex were included as covariates, and the dependent variable was the PANSS score.

4. RESULTS

Hypothesis 1: Patients with early-onset psychosis will have increased functional connectivity ('hyperconnectivity') with respect to healthy controls.

Figure 24 shows the mean connectivity values per group after controlling for the effect of age and sex. As observed, in the FEP group the functional connectivity between the target ROIs and the remaining network regions is in general increased when compared to healthy controls. These increases were statistically significant differences for five regions that were part of the FPN and DMN.



*Significant between group differences at alpha 0.10
** Significant between group differences at alpha 0.05

Figure 24. Mean connectivity values per group after controlling the effect of age and sex.

Specifically, the functional connectivity of the left cingulate-medial frontal (6-5), right insula (6-3) and right posterior middle/inferior temporal (6-4) within the FPN were higher in FEP. The same pattern was exhibited in the left medial prefrontal (7-1) and right temporal (7-5) ROIs within the DMN regions (see *Annex*, Table S1). Figure 25 displays F, p and standardized beta values for the effect of diagnosis on functional connectivity in these regions, as well as the dispersion of the connectivity values within each group. The increased functional connectivity of the right temporal region in patients is particularly evident.

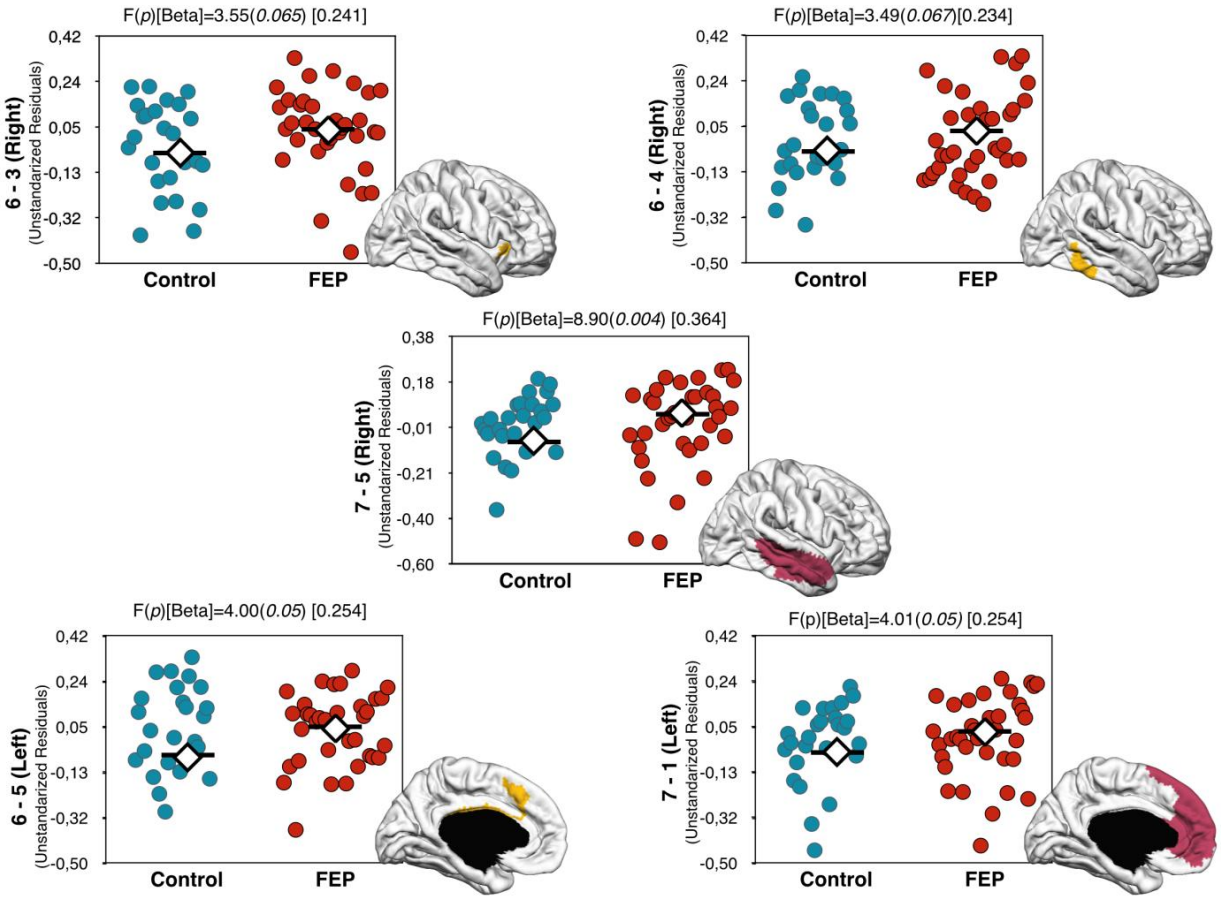


Figure 25. F, p and standardized beta values for the effect of diagnosis on connectivity in those regions were a significant effect was observed. In the ANCOVA model connectivity was the dependent variable, diagnosis was the independent variable, and age and sex the covariates. Plots display individual and mean connectivity values after controlling the effect of age and sex.

Hypothesis 2: Functional connectivity is differentially related to overall cognitive performance in FEP and controls.

After controlling for age and sex, there were significant diagnosis-by-connectivity interactions for the five regions studied with respect to intellectual performance (IQ), although there were no main effects of connectivity in none of them (see *Annex*, Table S2). As shown in Figure 26, functional connectivity of healthy controls tended to be insignificantly or positively related with IQ, that is, subjects with higher functional connectivity had higher IQ scores. In contrast, a higher functional connectivity in patients was associated with a poorer intellectual performance.

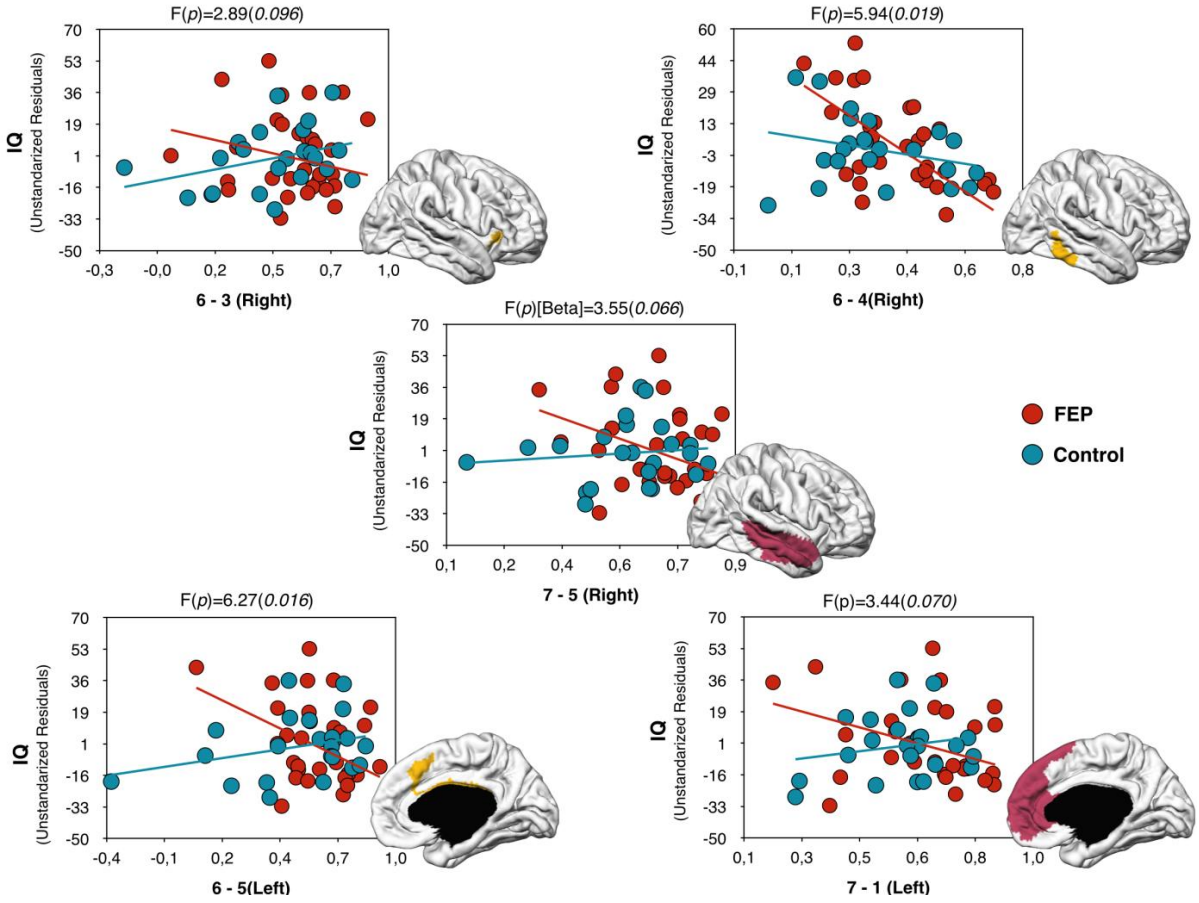


Figure 26. Dispersion plots for the connectivity-IQ relationships in FEP and controls after controlling for age and sex. F and p values for the interaction diagnosis-by-connectivity effect in the five regions considered are also displayed.

Table 3 includes the F, p and beta values denoting the main effect of connectivity on IQ in the five considered ROIs for patients and controls (see *Annex*, Table S3 for the whole set of regions). The five regions considered showed significant results in the control group, following a positive tendency except for the right posterior middle/inferior temporal (6-4) within the FPN, where negative relationships were found in both groups. In the case of patients, the highest effect was found for this latter region, while the lowest (and not significant) effect was observed in right insula (6-3). Taken together, the hyperconnectivity observed in the FEP group for these regions had a significant impact on intellectual performance: the higher the functional connectivity the lower the IQ.

Network: Target ROI			**6-3**	**6-4**	**6-5**	**7-1**	**7-5**
Right	Controls **Connectivity**	F	2.654	2.126			.253
		P	.120	.161			.620
		Beta	.355	-.365			.118
	FEP **Connectivity**	F	1.353	**16.234****			**4.653****
		P	.255	.000			.040
		Beta	-.238	-.625			-.433
Left	Controls **Connectivity**	F			1.971	.817	
		P			.176	.378	
		Beta			.311	.208	
	FEP **Connectivity**	F			**4.898****	**4.641****	
		P			.036	.040	
		Beta			-.419	-.458	

** Significant main effect of connectivity at alpha 0.05

Table 3. F, *p* and beta values for the effect of connectivity in the five overconnected regions on IQ. The ANCOVA model included as dependent variable IQ, and sex and age as covariates. Columns show these effects per Network and ROI. This ANOVA model was computed on Controls and FEP separately.

Hypothesis 3: Hyperconnectivity is positively related to symptom severity

Figure 27 shows that symptom severity (assessed with PANSS total scores) tended to be positively related to functional connectivity in the five regions. These associations were significant in the left cingulate-medial frontal (6-5) (within the FPN) and right temporal (7-5) (within the DMN). Annex (Table S4) includes the main effect of connectivity on PANSS total scores for the whole set of ROIs. As observed, higher functional connectivity was predictive of increased symptom severity, especially for the right hemisphere.

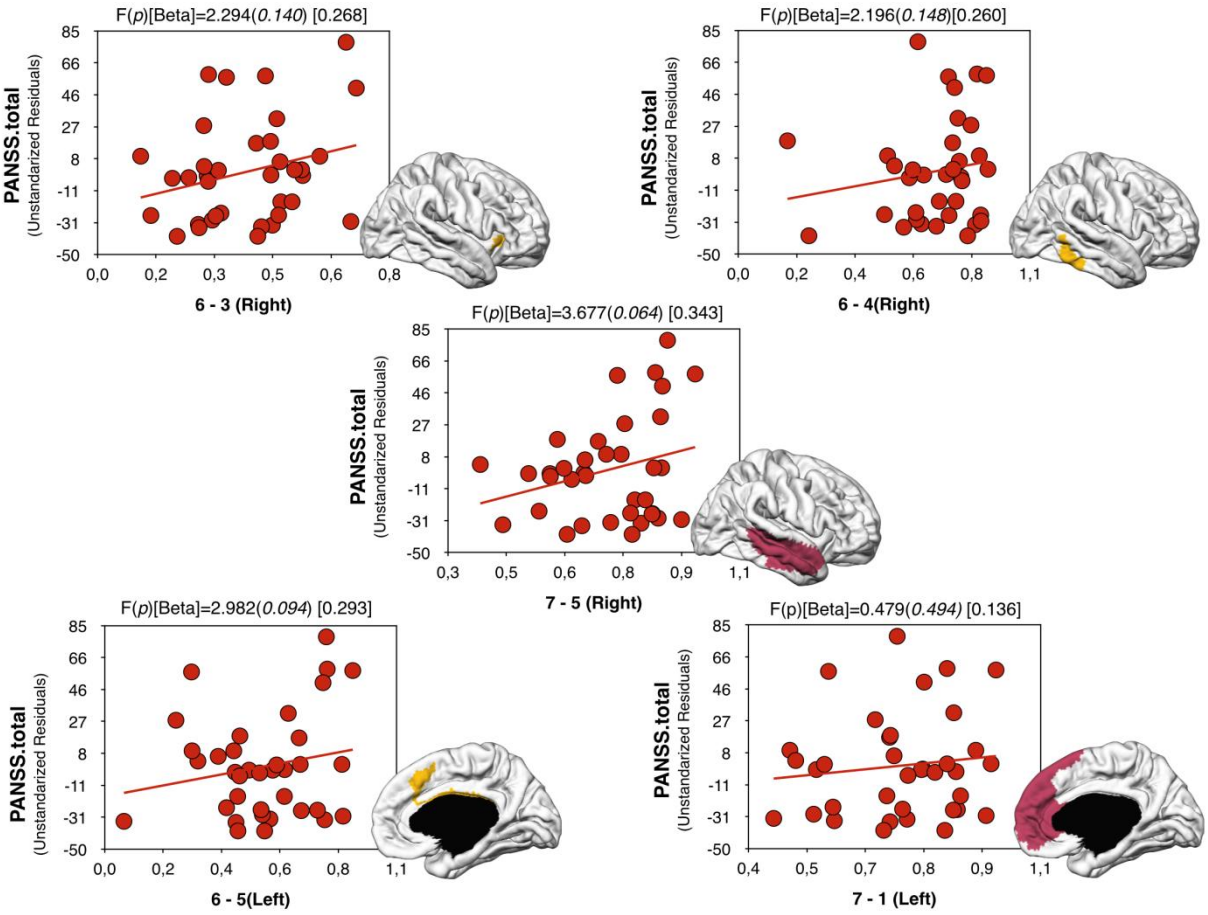


Figure 27. Dispersion plots for the connectivity-symptoms relationships in FEP after controlling for age and sex. F, p values and standarized beta values are also displayed.

5. DISCUSSION

5.1 *RESULTS DISCUSSION*

This study aims to use rs-fMRI to assess whether functional connectivity patterns are abnormal in youth suffering from FEP. After reviewing the literature three hypotheses were posed. First of all, patients were hypothesized to have increased functional connectivity with respect to healthy controls, a concept that was corroborated in most networks. After statistical inference, it was demonstrated that the FPN and DMN showed strong abnormal hyperconnectivity in particular. The second hypothesis stated that functional connectivity would be related to the cognitive impairments of FEP. A negative relationship was found between IQ and functional connectivity, i.e. patients with increased functional connectivity had more general cognitive impairment (lower IQ). Finally, the third hypothesis assumed altered brain connectivity to be linked to symptom severity. The results agreed with this idea, showing a positive relation between hyperconnectivity and symptoms.

5.1.1 Abnormal functional connectivity in FEP

The increased functional connectivity found in most networks among FEP with respect to healthy controls is consistent with previous findings in this field. Only in the Dorsal Attention network did the FEP group have decreased functional connectivity as was reported previously (Woodward et al., 2011). In contrast, left cingulate-medial frontal, right insula and right posterior middle/inferior temporal within the FPN as well as left the medial prefrontal and right temporal ROIs within the DMN showed hyperconnectivity in FEP patients when compared to healthy individuals. Other studies have also reported increased functional connectivity in the FPN in psychosis (Lui et al., 2011) although there are also findings showing decreased activity (Zhou et al., 2007).

Fornito et al. have analyzed the activity of this network during cognitive control tasks using graph analysis, corroborating the impaired connectivity patterns in the FPN reported in the current study (Fornito et al., 2011).

5.1.2 Functional connectivity of the DMN in FEP

On the other hand, much research has been devoted to DMN, due to its role on internal processing, its anti-correlation with task-positive networks and the evident stability it presents in rs-fMRI studies since it was first described in the 1990s. The hyperactivity of the DMN in FEP is robustly supported by a wide number of studies (Fryer et al., 2013, Satterthwaite et al., 2015), and dysfunction of the MPFC may be critical for the disorder (REF). Whitfield-Gabrielli and colleagues (Whitfield-Gabrieli et al., 2009) have reported decreased suppression of this region during a task, being more evident as severity of symptoms increases, together with increased general Default Mode connectivity. Furthermore, MPFC, ROI 7-1 in this study, often shows aberrant reduced connectivity with precuneous and posterior cingulated cortex, which also belong to DMN. On the contrary, our data reveals increased functional connectivity of this ROI with the rest of the regions in the network if compared to control subjects. Woodward et al. has instead shown increased connectivity of posterior cinculate cortex, key hub in DMN, with other regions like left inferior, middle frontal and middle temporal gyri.

5.1.3 Functional connectivity and cognitive impairment

The evaluation of cognitive performance with respect to functional connectivity revealed a negative correlation in FEP meaning that as connectivity increases in patients, IQ decreases. This is consistent with the hyperconnectivity found in patients and the typically reduced cognitive performance found in psychosis. Few studies have related IQ with functional connectivity in FEP. Empirical evidences suggests that the efficiency of networks predicts the intellectual performance, as shown by Song and colleagues (Song et al., 2009), that observed increases DMN efficiency in individuals

with higher IQ scores. The abnormal patterns of connectivity, the network segregation and the loss of modularization in and between FPN and DMN in FEP (Buckner, 2013, Baker et al., 2014) can be translated into a decreased network efficiency that would explain the lowered IQ scores of the patients.

5.1.4 Functional connectivity and symptom severity

Finally, the relation of clinical symptoms with connectivity was assessed for our dataset, revealing a coupling between increased PANSS scores and hyperconnectivity. This result is consistent with findings from Whitfield-Gabrielli (Whitfield-Gabrieli et al., 2009), showing a positive relation of hyperactivity and connectivity of the DMN with positive symptoms of early-onset schizophrenia. Contrasting findings are also reported by Alonso-Solís and colleagues (Alonso-Solis et al., 2012), which did not confirm any correlation with the connectivity of this network and symptom severity, or Bluhm (Bluhm et al., 2007), who related decreased DMN connectivity with positive symptoms. On the other hand, Lui and colleagues (Lui et al., 2010) reported increased PANSS scores associated with increasing temporo-putamen connectivity and decreasing temporo-precuneous connectivity; while positive correlation of negative PANSS scores with meso/paralimbic regions in schizophrenia was reported by Khadka (Khadka et al., 2013).

5.1.5 Functional hyperconnectivity in FEP

Despite the few number of studies investigating functional connectivity in youth with FEP, there is a general concept of globally increased connectivity of brain networks at the early stages of the disorder, particularly in the DMN. When the disease progresses, hyperconnectivity may change to hypoconnectivity. For example Anticevic et al. (Anticevic et al., 2015) found evidence for hypoconnectivity in chronic patients among prefrontal-thalamic regions, which show increased connectivity in FEP. How hyper- and hypoconnectivity relate to the disease is still far from understood. The working hypothesis is that hyperconnectivity results from an early life disease-related

insult that turns into hypoconnectivity as the brain worsens in later phases of the disorder (Satterthwaite et al., 2015).

5.2 *LIMITATIONS*

Apart from the cited general limitations involved in rs-fMRI, such as motion confounds, variability of analytical approaches and uncertainties of resting condition, there are also other restrictions that have constrained the efficiency and reliability of the result of this study.

The first issue to consider is the anisotropic voxel resolution of the acquired echo planar images with a particular large sized dimension in the z plane. This characteristic complicates, if not precludes, the assessment of small sized brain regions. In the z plane each voxel will contain information sampled over a large space, thus possibly including multiple smaller sized regions. Big voxels also disturb the accurate registration of smaller sized regions from atlases to the functional image as one single voxel can contain information of more than one smaller sized region from an atlas. As such, a smaller sized region cannot be meaningfully registered over the large sized voxel in the echo planar image.

The sample available for this study has also limitations. First of all, a large number of subjects is crucial to perform a powerful statistical analysis, which unfortunately was not the case. In order to obtain reliable results it is also important to reduce the potential heterogeneity of the sample. This way, age, sex, symptoms and even ethnicity or education level can be more precisely controlled for as to reduce confounding effects of these variables (that were later considered in the statistical models). Most of these features were considered by administering clinical, diagnostic and cognitive assessments, although the homogeneity of the sample can be still considered affected by factors like the 11-years age difference among individuals. It is important to note, however, that these patients are hard to recruit, e.g. undergoing an MRI scan can be a formidable test of endurance for a young patient with psychosis and many opt not to participate.

5.3 *FUTURE WORK AND ALTERNATIVE APPROACHES*

Given the cited limitations, the implementation of other approaches in order to analyze the images is a very recommendable future effort to consider. ICA seems to be a valid alternative to the top-down atlas-based approach applied in this project if more time is available in order to develop a good algorithm and enable student training.

Another useful consideration to implement in upcoming work is the assessment of longitudinal changes. For this purpose, the clinical and neurobiological progress of the subjects will be studied by acquiring new functional and structural images of the subjects at certain time intervals. These time differences must also be homogeneous across the sample, and clinical, diagnostic and behavioral assessment at these stages must be also performed. This way, it will be possible to evaluate the brain changes after FEP as well as the effect that changes in connectivity have in intellectual performance or symptoms.

6. CONCLUSIONS

The current project has analyzed the relation of functional connectivity in FEP patients with respect to healthy individuals, cognitive performance and symptom severity. For this objective, literature on technical aspects of rs-fMRI as well as the relationship between rs-fMRI and FEP was assessed. Combining knowledge from literature and practice, an analytic atlas-based approach to process rs-fMRI data of FEP and healthy controls was designed and implemented. This was the first analysis of rs-fMRI data performed at the *Hospital General Universitation Gregorio Marañón*, and the results show that it may be a useful technique to connectivity alterations in FEP.

Following the proposed workflow, the student started acquiring knowledge about rs-fMRI from the literature. Simultaneously, the student obtained handed on knowledge about processing of rs-fMRI data by practicing with an example data set in the processing environment at LIM.

After these training tasks, MRI scans were processed using the LIM processing environment, specifically designed for processing of functional data. For this stage of the project, configuration files were specifically designed for our subjects and launched on the cluster for fully-automated parallel preprocessing of all the images. When these steps were completed, the student performed quality control and validation of the preprocessing pipeline by checking the resulting images. Once validity of the steps was ensured, the derived correlation matrices could be used for subsequent statistical analyses. For this purpose, MATLAB scripts were designed by the student to successfully extract the values of interest for the statistical analyses, which were the mean connectivities among ROIs of resting state networks.

Next, four statistical models were developed to test the previously established hypotheses, selected according to previous literature. These models were

implemented in MATLAB using the MANCOVAN toolbox, for which the student created specific scripts.

Finally, the student interpreted the results in collaboration with psychologists, and specific figures were designed to clearly represent the findings. FEP patients showed hyperconnectivity with respect to healthy controls, and this was related to lower cognitive performance and increased symptom severity.

7. SOCIAL IMPACT

Mental health problems affect more than one third of the European population, being about 165 million people experiencing psychiatric disorders in a given year. Given the high number of affected individuals together with care costs and loss of productivity in workplace, this disorder places a huge economic burden on society and health care systems. In fact, Mental Health Economic European Network (MHEEN) has estimated that 2.000€ per year is the cost this disabling problem implies of every European household per year.

One of the main causes of this expensive management of mental disorders is due to the lack of proper stratification of patient conditions, such that treatment can be personalized in order to achieve optimal results. Consequently, much effort is now devoted to reduce the gap between clinical interventions and neuroscience pursuing translational research among these disciplines to ensure better patient classification.

For this reason, neuroimaging is shaping up as a potentially useful technique to understand the basis of these severe disorders, enabling the identification and categorization of different conditions and the development and testing of beneficial treatments and therapies. In the last decades, novel findings on psychotic disorder foundations using rs-fMRI have proven the value of this technique in the comprehension of brain organization and the roots of these health problems; discoveries that, incorporated to clinical settings, will be beneficial for patients, families, workers and government.

8. BUDGET

Human resources costs

Description	Number	Salary (€/h)	Hours	Cost (€)
Student	1	25	640	**16,000.00**
Internal supervisor	1	35	20	**700.00**
External supervisor	1	35	90	**3,150.00**
Engineers and psychologists	2	35	110	**3,850.00**
Total				**23,700.00**

Material costs

Description	Initial cost (€)	Cost per year (€)	Dedication	Total (€)
Personal computer	1000	200	8 months	**133.00**
MATLAB license (academic use)	500	100	6 months	**50.00**
Total				**183.00**

Total costs

Description	Cost (€)
Human resources	23,700.00
Material costs	183.00
Indirect costs	4,776.60
General costs	29.28
Industrial benefit	10.98
Total (without IVA)	28,699.86
IVA (21%)	6,026.97
Estimated total	**34,726.83**

Note: The cost of subject recruitment and assessment as well as image acquisition (technician an scanner costs) are not included in budget calculation as patient and control information were loaned from a previous study at the *Hospital General Universitario Gregorio Marañón*. The facilities (workplace, cluster), office supplies, Microsoft office pack or internet costs are neither considered for budget calculation as they are part of LIM services.

9. ANNEX

Network Target ROI		3_1	3_2	3_3	4_1	4_2	4_3	4_4	5_1	6_1	6_2	6_3	6_4	6_5	6_6	7_1	7_2	7_3	7_4	7_5
Right	F	.080	.022	.108	1.643	1.183	1.274	.287	.018	.058	1.047	**3.488***	**3.546***	.733	.396	.825	.889	1.280	.352	**8.901***
	p	.778	.883	.744	.205	.281	.264	.594	.894	.810	.310	.067	.065	.395	.532	.367	.350	.262	.555	.004
	Beta	-.036	-.019	-.042	.165	.139	.146	-.069	-.018	-.031	-.134	.234	.241	-.111	.082	.118	.123	.147	.078	.364
Left	F	.183	.181	.106	.234	.380	1.092	.264	.001	.152	.419	1.997	.415	**4.002***	.306	**4.014***	.454	2.017	1.167	.742
	p	.670	.672	.746	.631	.540	.300	.609	.972	.698	.520	.163	.522	.050	.582	.050	.503	.161	.284	.393
	Beta	-.054	-.055	-.042	.064	.081	.136	.066	.005	.051	-.085	.181	.084	.254	-.073	.254	.087	.181	.140	.112

* Significant at alpha 0.10 * Significant at alpha 0.05

Table S1. F, p and standardized beta values for the effect of diagnosis in the ANCOVA model including as dependent variable the connectivity, and sex and age as covariates.

Network Target-ROI			3_1	3_2	3_3	4_1	4_2	4_3	4_4	5_1	6_1	6_2	6_3	6_4	6_5	6_6	7_1	7_2	7_3	7_4	7_5
Right	Diagnosis	F	.318	.870	.004	1.150	.641	1.061	.575	.478	.098	.005	.071	.984	.668	1.384	.016	.089	.013	.006	1.153
		p	.575	.356	.948	.289	.427	.308	.452	.493	.756	.941	.790	.326	.418	.245	.901	.766	.908	.938	.288
		Beta	-.393	-.596	.044	-.273	-.306	-.233	-.319	-.266	-.215	-.046	.093	.288	-.616	-.541	-.094	-.114	.063	.041	.586
	Connectivity	F	.339	1.012	.009	.696	.014	.355	.247	.285	.040	.063	1.274	.610	.382	.890	.058	.028	.371	.410	.237
		p	.563	.319	.925	.408	.907	.554	.622	.596	.843	.803	.265	.438	.539	.350	.810	.867	.545	.525	.629
		Beta	-.131	-.230	.022	-.163	-.025	-.110	-.121	.127	.040	.059	.196	-.126	-.157	-.241	-.054	.033	-.107	-.135	.087
	Diagnosis -by- Connectivity	F	.015	.045	.610	.395	.156	1.008	.154	.272	.131	.508	**2.892***	**5.938***	.036	.046	.219	.862	.868	.837	**3.545***
		p	.904	.833	.439	.533	.695	.320	.696	.604	.719	.479	.096	.019	.851	.831	.642	.358	.356	.365	.066
		Beta	-.085	.140	-.530	-.197	-.172	-.277	-.178	-.216	-.253	-.429	-.664	-.806	.142	.113	-.373	-.390	-.531	-.519	-1.115
Left	Diagnosis	F	.046	.327	1.845	**7.275***	.760	**5.662***	**3.671***	2.283	.010	.050	.055	1.462	1.097	.672	.966	.471	.022	.170	**11.551***
		p	.832	.570	.181	.010	.388	.021	.061	.137	.922	.824	.815	.233	.300	.416	.331	.496	.884	.682	.001
		Beta	-.151	.414	-.636	-.551	-.291	-.526	-.722	-.653	-.075	.187	-.119	-.517	.361	-.462	.509	.582	.090	.210	-.593
	Connectivity	F	.338	.684	.082	1.844	.307	1.853	.702	.024	.222	.103	.028	1.893	.955	.026	.657	.261	.541	.068	.910
		p	.564	.412	.775	.181	.582	.180	.406	.877	.640	.750	.867	.175	.333	.872	.422	.612	.466	.795	.345
		Beta	.116	.179	-.052	-.263	-.113	-.270	-.204	-.032	.095	.080	-.037	-.291	.147	.042	.174	.122	-.131	-.047	-.168
	Diagnosis -by- Connectivity	F	.184	1.517	.157	.406	.240	.247	.567	.237	.263	.635	.434	.033	**6.270***	.001	**3.443***	1.512	.730	1.765	1.345
		p	.670	.224	.694	.527	.627	.621	.455	.628	.610	.430	.513	.857	.016	.981	.070	.225	.397	.190	.252
		Beta	-.304	-.887	.192	.165	-.188	.140	.332	.216	-.400	-.663	-.363	.085	-.917	.013	-1.071	-1.083	-.548	-.705	.253

* Significant at alpha 0.10 * Significant at alpha 0.05

Table S2. F, *p* and standardized beta values for the effect of diagnosis, connectivity, and diagnosis-by-connectivity, in the ANCOVA model including as dependent variable IQ, and sex and age as covariates. Columns include these effects per Network and ROI.

Network Target-ROI			3_1	3_2	3_3	4_1	4_2	4_3	4_4	5_1	6_1	6_2	6_3	6_4	6_5	6_6	7_1	7_2	7_3	7_4	7_5
Right	Controls **Connectivity**	F	.574	1.802	.033	1.623	.031	.763	.502	.539	.043	.048	2.654	2.126	.715	1.904	.214	.008	.877	.786	.253
		p	.458	.195	.857	.218	.863	.393	.487	.472	.838	.830	.120	.161	.408	.184	.649	.929	.361	.387	.620
		Beta	-.177	-.313	.042	-.292	-.041	-.201	-.165	.172	.049	.053	.355	-.365	-.191	-.307	-.113	.022	-.214	-.205	.118
	FEP **Connectivity**	F	.931	.998	1.576	**3.601***	.646	4.404	2.185	.013	.103	.693	1.353	**16.234***	.405	1.431	1.381	1.307	**3.908***	**5.844***	**4.653***
		p	.343	.327	.220	.068	.428	.045	.151	.909	.751	.412	.255	.000	.530	.242	.250	.263	.058	.023	.040
		Beta	-.208	-.201	-.278	-.372	-.180	-.414	-.300	-.022	-.068	-.162	-.238	-.625	-.131	-.254	-.248	-.225	-.414	-.457	-.433
Left	Controls **Connectivity**	F	.518	.935	.198	**4.078***	.678	**4.336***	1.977	.041	.375	.161	.052	**4.393***	1.971	.031	.817	.459	.802	.187	1.729
		p	.480	.346	.661	.058	.421	.050	.176	.841	.548	.692	.821	.050	.176	.863	.378	.506	.382	.670	.204
		Beta	.178	.246	-.103	-.425	-.189	-.442	-.345	-.047	.140	.094	-.054	-.448	.311	.041	.208	.156	-.202	-.101	-.288
	FEP **Connectivity**	F	.000	.884	.050	.389	2.113	.712	.001	.243	.058	.914	1.493	2.168	**4.898***	.070	**4.641***	2.266	**3.960***	**4.921***	.316
		p	.983	.355	.825	.538	.158	.406	.980	.626	.812	.347	.232	.152	.036	.793	.040	.144	.057	.035	.579
		Beta	-.005	-.206	.048	-.135	-.303	-.177	.006	.096	-.051	-.196	-.236	-.288	-.419	.052	-.458	-.315	-.417	-.437	.109

* Significant at alpha 0.10 * Significant at alpha 0.05

Table 3. F, *p* and standardized beta values for the effect of connectivity in the ANCOVA model including as dependent variable IQ, and sex and age as covariates. Columns include these effects per Network and ROI. This ANOVA model was computed on Controls and FEP separately.

Network Target-ROI			3_1	3_2	3_3	4_1	4_2	4_3	4_4	5_1	6_1	6_2	6_3	6_4	6_5	6_6	7_1	7_2	7_3	7_4	7_5
Right	PANSS Total	F	**4.327***	**4.338***	**3.342***	2.091	**3.385***	1.342	**3.897***	.016	**3.584***	**6.723***	2.294	2.196	1.817	**4.873***	**4.014***	**4.938***	**6.805***	1.274	**3.677***
		p	.046	.045	.077	.158	.075	.255	.057	.901	.067	.014	.140	.148	.187	.035	.054	.033	.014	.267	.064
		Beta	.367	.353	.327	.265	.344	.218	.345	-.022	.339	.418	.268	.260	.239	.400	.372	.380	.443	.208	.343
Left	PANSS Total	F	**5.849***	**5.831***	1.781	.116	.946	.000	1.157	.000	1.583	.525	4.260	.005	**2.982***	2.569	.479	.674	.241	2.551	1.714
		p	.021	.022	.191	.736	.338	.991	.290	.992	.217	.474	.047	.944	.094	.119	.494	.418	.627	.120	.200
		Beta	.426	.408	.248	-.063	.180	.002	.200	-.002	.230	.131	.346	-.013	.293	.273	.136	.157	-.092	.281	.230

* Significant at alpha 0.10 * Significant at alpha 0.05

Table S4. F, *p* and standardized beta values for the effect of connectivity in the ANCOVA model including as dependent variable symptoms (PANSS), and sex and age as covariates. Columns include these effects per Network and ROI. This ANOVA model was computed only in FEP.

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