Natalia Novaes Pelizari Pinto

Functional connectivity measured by the global efficiency of the motor network is decreased in Parkinson's disease in comparison to healthy controls and essential tremor

Thesis presented to the Faculdade de Medicina, Universidade de São Paulo to obtain the degree of Doctor in Science

Program of Radiology

Supervisor: Prof. Dr. Ellison Fernando Cardoso

São Paulo

2020

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Diminuição da conectividade funcional medida pela eficiência global da rede motora: estudo comparativo entre doença de Parkinson, tremor essencial e voluntários sadios

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

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To all my patients

Acknowledgments

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Epigraph

“We live everything as it comes, without warning,
like an actor going on cold.
And what can life be worth if the first rehearsal for life is life itself?
That is why life is always like a sketch.
No, sketch is not quite the word,
because a sketch is an outline of something,
the groundwork for a picture,
whereas the sketch that is our life is a sketch for nothing,
an outline with no picture.
(...) What happens but once,
might as well not have happened at all.
If we have only one life to live,
we might as well not have lived at all.”

Milan Kundera, *The unbearable lightness of being*, 1984

Normatization Adopted

This dissertation is in accordance with the following norms:

References: adapted from the *International Committee of Medical Journals Editors* (Vancouver).

University of São Paulo, Medical School. Division of Library and Documentation. "*Guia de apresentação de dissertações, teses e monografias*." Elaborated by Anneliese Carneiro da Cunha, Maria Julia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 3ª ed. São Paulo: Divisão de Biblioteca e Documentação; 2011.

Abbreviations of titles and journals in accordance to the *List of Journals Indexed in Index Medicus*.

Summary

Glossary

AAL= Automated Anatomical Labeling Atlas

ACC= Anterior Cingulate Cortex

ALFF= Amplitude of Low Frequency Fluctuations

BOLD= Blood Oxygen Level Dependent

DBS= Deep Brain Stimulation

DMN= Default Mode Network

DTI= Diffusion Tensor Imaging

DWI= Diffusion Weighted Imaging

EEG= Electroencephalography

EPI= Echo Planar Imaging

ET= Essential Tremor

FAPESP= Fundação de Amparo à Pesquisa do Estado de São Paulo

FDR= False Discovery Rate

FEF= Frontal Eye Fields

fMRI= Functional Magnetic Resonance Imaging

FOG= Freezing of Gait

FOV= Field of View

GABA= Gamma-Aminobutyric Acid

GE= Global Efficiency

GPi= Internal Globus Pallidus

GT= Graph Theory

HCFMUSP= Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

HIAE= Hospital Israelita Albert Einstein

LED= Levodopa Equivalent Dose

MEG= Magnetoencephalography

MIBG= Metaiodobenzylguanidine

MNI= Montreal Neurological Institute Brain Atlas

MPRAGE= Magnetization Prepared Rapid Acquisition Gradient Echo

MRI= Magnetic Resonance Imaging

MSA= Multiple-System Atrophy

NEX= Number of Excitations

PD= Parkinson’s Disease

PDAR= Parkinson’s Disease form Akinetic-Rigid

PDT= Parkinson’s Disease Predominantly Tremulant

PET= Positron-Emission-Tomography

PPC= Posterior Parietal Cortex

ROI= Region of Interest

SMA= Supplementary Motor Area

SNR= Signal-to-Noise Ratio

SPECT= Single-Photon-Emission-Tomography

SPM= Statistical Parametric Mapping

STN= Subthalamic Nucleus

tDCS= transcranial direct current stimulation

TE= Echo Time

TMS= Transcranial Magnetic Stimulation

TR= Repetition Time

UPDRS= Unified Parkinson’s Disease Rating Scale

VIM= Ventrointermediate Nucleus of Thalamus

VLp= Ventrolateral posterior Nucleus of Thalamus

VMHC = Voxel-Mirrored Homotopic Connectivity

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Resumo

Pinto NMP. *Diminuição da conectividade funcional medida pela eficiência global da rede motora*: estudo comparativo entre doença de Parkinson, tremor essencial e voluntários sadios [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2020.

Doença de Parkinson e Tremor Essencial estão entre os transtornos do movimento mais prevalentes. No entanto, sua fisiopatologia não é completamente conhecida. A Ressonância Magnética Funcional tem se tornado uma ferramenta útil para estudo das funções cerebrais em indivíduos saudáveis e em patologias diversas. Dentre as diversas metodologias disponíveis para análise dos dados de ressonância funcional, optamos por aplicar a Teoria de Grafos, que é o campo da matemática que analisa redes complexas e tem sido aplicada à neuroimagem para quantificar os circuitos funcionais cerebrais. Através da Teoria de Grafos, podemos calcular a Eficiência Global da rede motora, que reflete a transferência efetiva de informação em uma rede de nós. Matematicamente, é expressa como o inverso da medida dos caminhos mais curtos entre os nós de uma rede. De acordo com dados da literatura recente a esse respeito, levantamos a hipótese de que a eficiência global da rede motora deveria estar diminuída na Doença de Parkinson em relação a voluntários sadios e a pacientes com Tremor Essencial, e que essa diminuição se correlacionaria com parâmetros clínicos, como níveis de tremor. Também levantamos a hipótese de que, em pacientes com Tremor Essencial, a conectividade do cerebelo deveria estar alterada em relação a controles e a pacientes com Doença de Parkinson. O objetivo principal desde estudo foi de avaliar a hipótese acima, bem como acessar as diferenças entre os subgrupos de Doença de Parkinson – Rígido-Acinética e Tremulante. Objetivos específicos foram de avaliar a conectividade par-a-par (*seed-to-voxel* e *ROI-to-ROI*), correlacionar dados de Eficiência Global a parâmetros clínicos, como escala de UPDRS[1], escala de tremor e Dose Equivalente de Levodopa. Cento e três sujeitos (54 PD, 18 ET e 31HC) foram incluídos no estudo. Todos os grupos foram submetidos a Ressonância Magnética estrutural e funcional. Imagens foram pré-processadas

[1] UPDRS – do inglês, Escala Unificada de Avaliação da Doença de Parkinson.

usando o programa CONN, sendo 9 indivíduos excluídos devido a artefatos de movimentação. As Regiões de Interesse (ROI) foram determinadas com base nos atlas "*Automated Anatomical Labeling*" e "*Harvard-Oxford*", além de ROIs criadas manualmente para Substância Negra e Núcleo Denteado. Tais ROIs foram selecionadas por sua relevância na Rede Motora. A análise estatística foi realizada com parâmetros conservadores. A análise através da Teoria de Grafos mostrou diminuição da Eficiência Global (GE) da rede motora na PD em relação a HC. As áreas que mais contribuíram para esta redução foram a área motora suplementar (SMA) esquerda e o giro pós-central bilateralmente. Para ET, não houve diferença na GE da rede motora em comparação a DP. No entanto, houve aumento da conectividade da rede cerebelar anterior em relação às outras ROIs da rede motora em comparação a PD. As escalas de tremor se correlacionaram positivamente com a GE da rede motora em ambos os subgrupos da PD, assim como na GE da substância negra esquerda em ambos subgrupos, sendo maior a correlação com a forma rígido-acinética. Concluímos que a conectividade funcional medida através da GE da rede motora está diminuída na PD em comparação a controles, especialmente devido à diminuição da conectividade da SMA esquerda e do giro pós-central bilateral. Esses achados corroboram a teoria de que há uma disfunção global da rede motora na PD, que não afeta somente os gânglios da base, mas também áreas associadas à modulação do movimento. Essas áreas poderiam ser novos alvos para terapias como Estimulação Magnética Transcraniana, e para posteriores estudos de neuroimagem funcional sobre a conectividade pré e pós intervenção em tais áreas.

Descritores: Doença de Parkinson;Tremor essencial; Imagem por ressonância magnética; Neuroimagem funcional; Transtornos dos movimentos; Córtex motor; Conectoma.

Abstract

Pinto NMP. *Functional connectivity measured by the global efficiency of the motor network is decreased in Parkinson's Disease in comparison to healthy controls and essential tremor* [thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2020.

Even though Parkinson's Disease (PD) and Essential Tremor (ET) are two of the most prevalent movement disorders and the most frequent causes of tremor, their pathophysiologies are not completely understood. In order to enlighten brain functioning in healthy brains and in diseases, functional magnetic resonance imaging has become a powerful tool. Graph theory is the mathematical field that analyzes complex networks, and has been applied to neuroimaging data to quantify brain's functional systems. One of its measures is Global Efficiency (GE), which reflects effective information transfer within a network of nodes and edges. It is mathematically expressed as the inverse of the shortest path length between nodes of a graph. According to previous literature data, we hypothesized that there would be a decreased GE of the motor network in PD patients when compared to controls and ET patients, and that this decrease would be related to clinical parameters such as tremor scores. We also hypothesized that ET patients would show different connectivity patterns from controls, specially involving the cerebellum. The major aim of this project was to evaluate the hypothesis above, and assess possible differences between PD subgroups. The specific goals were to evaluate pairwise metrics (*seed-to-voxel* and *ROI-to-ROI*), and to correlate the GE of the motor network to clinical parameters, such as the Unified Parkinson's Disease Rating Scale (UPDRS), tremor scores and Levodopa Equivalent Dose (LED). 103 subjects (54PD, 18ET and 31HC) were enrolled in this study. All the groups were submitted to structural and functional MRI. Images were pre-processed using the CONN software and 9 subjects were excluded from the study due to motion artifacts. Regions of interest (ROIs) were determined based on the Automated Anatomical Labeling Atlas (AAL) and Harvard-Oxford Atlas, and we manually created ROIs for the Denteate Nucleus and Substantia Nigra. ROIs were selected because they are important hubs on the motor network related to tremor. Statistical analysis was set to very conservative parameters.

Network analysis showed reduced GE of the motor circuit of PD in comparison to HC (p 0.042). Areas that most contributed for this reduction were left supplementary motor area and bilateral postcentral gyri. For ET, there was no difference in GE of the motor network when compared to PD. However, there was an increase in the connectivity of the anterior cerebellar network to the other ROIs of the motor network in the ET group when compared to the PD group. Tremor scores correlated positively with GE of the network in both PD subgroups, and also to the GE of the left substantia nigra in both subgroups, being greater in the PDAR group. We concluded that functional connectivity measured by the GE of the motor network is diminished in PD in comparison to controls, especially due to decreased connectivity of left SMA and bilateral postcentral gyrus. These findings corroborate to the theory that there is a global impairment of the motor network in PD, and it does not affect just the basal ganglia, but also areas associated with movement modulation. These areas could possibly be new targets for therapies such as transcranial magnetic stimulation and for posterior functional connectivity neuroimaging studies focusing on pre and post intervention in such areas.

Descriptors: Parkinson disease; Essential tremor; Magnetic resonance imaging; Functional neuroimaging; Movement disorders; Motor cortex; Connectome.

1. Introduction

James Parkinson described the disease that carries his name in 1817, on the manuscript “An Essay on the Shaking Palsy”(1). Parkinson’s Disease (PD) is the second most prevalent neurodegenerative disease worldwide, affecting over 5 million people, and around 1% of the population above 65 years old(2). Data is scarce about epidemiology in Brazil. One study was performed in 2005 at Bambuí[1], and found prevalence of 7.2% of parkinsonian syndrome amongst the elderly, of which 3.3/7.2 were due to Idiopathic PD, 1.1/7.2 due to vascular parkinsonism and 2.7/7.2 caused by medication(3). Since our population in Brazil is growing older, we expect an increase in the prevalence of PD in our society.

Cardinal signs of PD are resting tremor, rigidity, bradykinesia and postural instability. Tremor occurs in rest but can re-emerge in posture after a pause, and is not as responsive to dopaminergic replacement such as other parkinsonian features such as bradykinesia or rigidity. Other signs and symptoms can include hypomimia, decreased blinking, micrography, freezing of the gait and camptocormia(4). Even though the motor signs are the principal landmarks of the disease, the presence of non-motor symptoms usually account for the decrease in life quality. Pain, depression, autonomic dysfunction, sleep disorders, obstipation and cognitive decline are frequently present(5).

There is great clinical variability amongst patients with PD, and according to the preponderance of symptoms, we can divide them on two main groups: predominantly tremulant (PDT) or akinetic-rigid (PDAR)(6).

Pathological features are characterized by degeneration of dopaminergic neurons of substantia nigra’s *pars compacta*, associated with proteic inclusions called Lewy Bodies(7). We know that neurodegeneration is not restricted to the dopaminergic system, and it also involves cholinergic neurons of Basal Nucleus of Meynert, serotoninergic neurons of the Raphe Nuclei, brainstem, medulla, cerebral cortex and autonomic nervous system(4).

[1] Bambuí is a city in the State of Minas Gerais, in the Southeast of Brazil.

Braak et al. proposed a model of disease evolution that suggests that pathological changes begin on olfactory neurons and lower brainstem, subsequently ascend to upper brainstem and basal ganglia, and finally affect the cerebral cortex, as seen on the figure below(8).

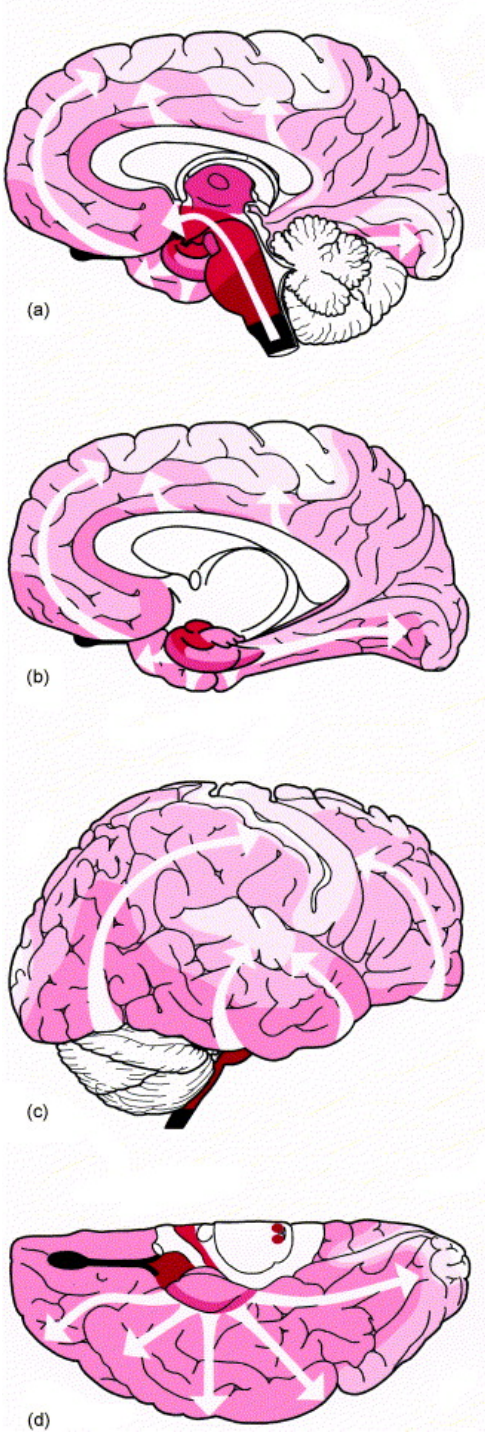


Figure 1: Stages of evolution of PD according to Braak et al. (8)

Although many studies have contemplated PD pathophysiology, the mechanisms that lead to neuronal degeneration are not completely understood. It is believed that PD is a product of the interaction between genes that make individuals more susceptible to the disease and environmental factors. Amongst them, rural life, exposition to pesticides and herbicides, and drinking water from a well were associated to an increased risk (9, 10), whereas tabaco smoking and caffeine consumption were protecting factors (11).

Several gene mutations have already been linked to PD, involving proteins such as alfa-synuclein, ubiquitin-carboxy-terminal hydrolase L1, parkin, LRRK2, PTEN-induced kinase, amongst others. Some of them are related to elimination of misfolded proteins, apoptosis, mitochondrial dysfunction, oxidative stress and inflammation, suggesting that PD pathology comprises a great series of processes that lead to neurodegeneration(12,13).

Neuroimaging techniques have been a growing field of study concerning neurodegenerative diseases, not only focusing on diagnosis, but also in the attempt to elucidate neuropathology and its mechanisms. PD diagnosis remains essentially clinic. However, Magnetic Resonance Imaging (MRI) helps to exclude differential diagnosis such as subdural hematoma, vascular parkinsonism and structural changes found in atypical parkinsonism, like Progressive Supra-nuclear Palsy and Multiple-System Atrophy (MSA), and helps to corroborate the clinical diagnosis. With the discovery of the “swallow tail sign” on mesencephalon using the Susceptibility-Weighted Imaging (SWI) sequence on 3T MRI, a great step was taken towards PD diagnosis with neuroimaging. The normal aspect of the nigrossome-1 on MRI looks like the tail of a swallow bird, being this feature lost in PD. This diagnostic test has a sensibility of 94.6% and specificity of 94.4% for detecting PD(14, 15), but it also does not differentiate PD from atypical parkinsonism(16).

SPECT using fluorodopa as a ligand helps to differentiate PD from medication-induced parkinsonism and ET, but it does not differentiate PD from atypical parkinsonism(17). MIBG-cardiac scintigraphy helps to differentiate PD and Dementia with Lewy Bodies from MSA, as it reflects post-ganglionic autonomic denervation present in PD but not in MSA(18). Transcranial ultrasound shows hyper echogenicity of substantia nigra in over 90% of Parkinson’s patients,

and it helps to differentiate PD from essential tremor and from atypical parkinsonism(19).

The term *tremore semplice essenziale* was firstly used by Burresi in 1874, when describing the case of an 18-year old patient with severe action tremor (20). Essential Tremor is one of the most prevalent Movement Disorder, affecting approximately 1% of the general population and 5% of the population older than 65 years of age(21). It is considered a more “benign” disease, since the tremor is the illness itself, and it was thought not to have cognitive, postural, autonomic and psychiatric comorbidities associated. This disease is characterized by a low amplitude and high frequency tremor (4 to 12Hz), bilateral, symmetrical or slightly asymmetrical, postural or kinetic, affecting mainly arms and legs(22). It can eventually also affect head, jaw, and vocal chords. There are studies that also point to other discrete impairments, such as mild gait ataxia, saccadic eye movements and even slightly dystonic features(23).

Family history is frequent, with a pattern of mendelian autosomal dominant inheritance with incomplete penetrance(24). First-degree relatives of ET patients have a 5-fold increased chance of having the condition than the general population. The disease has a broad clinical heterogeneity and there are no neuroimage alterations, biomarkers or anatomopathological findings that support its diagnosis. No individual gene mutation was found to explain ET, but a few *loci* were associated to the disease in chromosomes 3q13 (ETM1; OMIM:190300), 2p22-p25 (ETM2; IMIM:602134) and 6p23 (ETM3; OMIM:611456)(25-27). It is believed that this condition is a product of the interaction amongst multiple genes and environmental factors, such as alcohol consumption and heavy metal exposition(28).

Even though the disease is considered “benign”, tremor can sometimes be severe with great impact on life quality, functionality and social life, and new studies have shown that there could also be some degree of neurodegeneration implicated, with mild cognitive and psychiatric alterations, such as attention deficit, operational memory impairment, visuospatial dysfunction and diminishing of executive function. Physiopathology of these alterations could be due to changes in fronto-cerebellar circuits and decrease in cortical volume(29-31). Other non-motor features were also described, such as sleep disorders,

depression, anxiety, hearing and olfactory losses(23). Although the idea of neurodegeneration seems controversial, it may be supported by the fact that symptoms start insidiously, progresses over time and worsens with age(32).

The issue whether PD and ET should be compared is often addressed, considering that both diseases cause tremor, and, in most cases, are easy to differentiate based on clinical parameters. However, sometimes, it is difficult to distinguish them and, so far, there are no biomarkers that are exclusive to any of those diseases. According to Jankovic and Fekete(33), there is growing evidence that both diseases are pathogenically related. Some patients with PD might be misdiagnosed as ET initially, but there is evidence that prevalence of PD is higher in ET patients when compared to age-matched controls. Moreover, the DATATOP cohort study showed that ET and the tremulous form of PD could have a common pathogenic process (6).

Several fMRI studies have already been performed in order to investigate the motor network in PD and ET, mostly focusing on pairwise comparisons such as seed-to-voxel and voxel-to-voxel connectivity. Graph theory (GT) is the mathematical field that analyzes complex networks, and has been applied to neuroimaging data to quantify brain's functional systems(34). One of the measures of GT is Global Efficiency (GE), which is a frequently used metric to study integration within brain networks. GE is mathematically expressed as the inverse of the shortest path length, and it reflects effective information transfer within a network.

A smaller number of studies regarded GE in PD and ET, with conflicting results. While most studies showed decreased GE of the motor network, some found no abnormalities or even increased GE.

According to what is known about PD pathophysiology, we hypothesized that there would be a decreased global efficiency of the motor network in PD patients when compared to controls and ET patients. This decrease would be related to clinical parameters such as tremor scores. We also hypothesized that ET patients would show different connectivity patterns from controls, specially involving the cerebellum, possibly with increased connectivity to some areas in the motor network.

Since our study has a large number of participants, great quality equipment, short Repetition Time (TR), and used very strict pre-processing parameters and conservative statistical analysis, it is hoped to contribute to clarify some of these controversies.

2. Goals

2.1 Main Goals

The main goal of this study was to evaluate the functional connectivity measured by the global efficiency of the motor network in PD and compare it to ET and healthy controls (HC). Besides, it aims to assess possible differences between PD subgroups.

2.2 Specific Goals

2.2.1 First Specific Goal

To evaluate Seed-To-Voxel Connectivity of ROIs of the Motor Network.

2.2.2 Second Specific Goal

To evaluate ROI-to-ROI Connectivity amongst ROIs of the Motor Network.

2.2.3 Third Specific Goal

To correlate the global efficiency of the motor network to clinical parameters such as the Unified Parkinson's Disease Rating Scale (UPDRS), tremor scores and Levodopa Equivalent Dose (LED).

3. Literature Review

We have carried an extensive literature review over tremor physiology in both diseases in order to better comprehend the circuits implicated and to better define the targets of this research. Therefore, in this section we will summarize relevant models that seek to explain underlying physiology of both diseases, and data found from important previous reports on tremor physiopathology and on preceding neuroimaging studies.

3.1 Normal function of the Basal Ganglia Circuitry

The following figure is a schema of the normal function of the basal ganglia and both direct and indirect pathways.

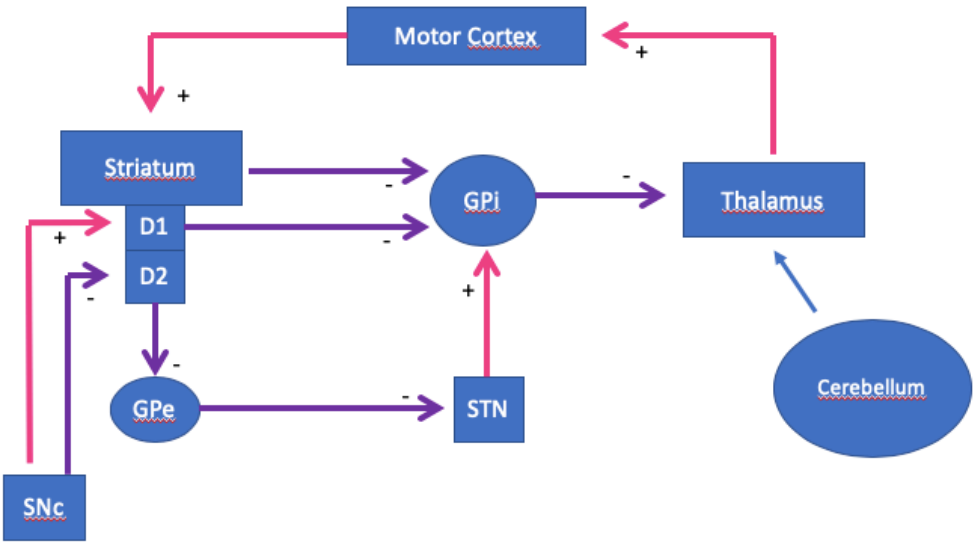


Figure 2: Model of normal function of the basal ganglia. Adapted from Campbell, William W. DeJong's Neurological Examination (35). Pink arrows indicate excitatory connections. Purple arrows indicate inhibitory projections.

The striatum receives glutamatergic (excitatory) projections from the cortex, and has inhibitory efferences to the GPi and GPe. There are two pathways that convert to the thalamus. The direct pathway is mediated by D1 receptors in the striatum, which receive dopaminergic projections from the Substantia Nigra pars compacta. Dopamine stimulates D1 receptors in the striatum, which increases inhibition over the GPi. This inhibition of the GPi results in less inhibition

of the thalamus, which in turn results in stimulation of cortex and facilitation of movements. Dopaminergic stimulation of D2 receptors in the striatum inhibits the GPe, in the indirect pathway. GPe inhibits the STN, which stimulates the GPi, increasing the inhibition over the thalamus. Therefore, dopamine facilitates movement through stimulation of the direct pathway, and through inhibition of indirect pathway. Consequently, in PD, since there is a lack of dopaminergic neurons, movements are impaired due to diminished function of the direct pathway and increased function of indirect pathway, as we can see on the figure below.

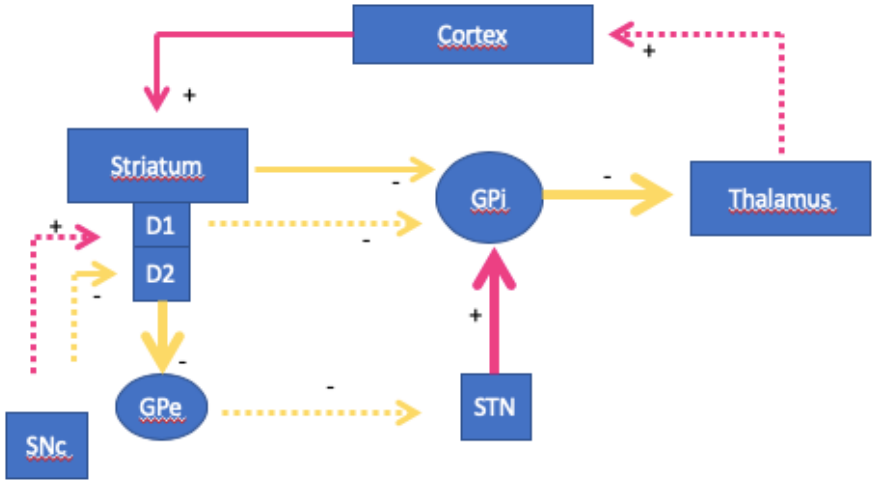


Figure 3: Basal Ganglia Dysfunction in PD. Dotted arrows indicate decreased function, while thicker arrows indicate increased function

There are also glutamatergic projections from the cortex directly to the STN, which increase the GPi activity, in the hyperdirect pathway(36). This pathway is important to prevent premature responses in case of conflict between different pathways, inhibiting undesired motor activity.

3.2 Models of Tremor Pathophysiology in PD

Tremor physiology in PD is complex and not completely understood. There is a hypothesis that the dopamine deficit in the striatum-thalamus-cortical system

is somehow compensated by hyperactivity of the cerebellum-thalamus-cortical circuit, and that would lead to tremor. Previous Positron-Emission-Tomography (PET) and Single-Photon-Emission-Tomography (SPECT) studies that measure dopamine concentration on putamen showed that it does not correlate to the presence of tremor(37).

Functional Magnetic Resonance Imaging (fMRI) studies correlated tremor with activity in putamen, pallidum, cerebellum, ventrointermediate nucleus of the thalamus (VIM), motor and premotor cortices(38). The cerebellum-thalamus-cortical circuit seems to be responsible for tremor amplitude, while basal ganglia such as Subthalamic Nucleus (STN) and Internal Globus Pallidus (GPi) would be responsible for tremor initiation, since they oscillate at the same tremor frequency. The decrease in dopaminergic projections would increase GPi activity, which would result in hyperpolarization of neurons that receive projections from GPi in the thalamus(39). This increase of inhibition from GPi over thalamic nucleus would, by its turn, increase the chance of generating activity in rhythmic neuronal groups in the thalamus(40). This is known as the “dimmer-switch” hypothesis, in which the basal ganglia would generate the trigger for tremor and the cerebellum-thalamus-cortical circuit would modulate its amplitude(41). This theory was later revisited by Helmich in 2018(42). In the thalamus, tremor-related activity was localized in the Ventrolateral nucleus in its posterior part (VLp), which receives afferents from the cerebellum. In the cerebellum, tremor was related to lobules V and VI, which is the sensorimotor portion. In the cerebral cortex, tremor was associated to activity in primary motor cortex and premotor cortex, and also in the somatosensory cortex, with an important role in tremor amplitude. Basal ganglia did not relate to tremor amplitude, but to onset of tremor episodes.

The Role of STN is less clear, since it is anatomically too small to be analyzed in fMRI, but electrophysiological studies have shown synchronization at double tremor frequency(43), and increased coherence with motor cortex during tremor(44). Furthermore, STN-DBS at near-tremor frequency is capable of entrain the tremor, suggesting its role in maintaining tremor rhythm(45).

3.3 Models of Tremor Pathophysiology in ET

There is large evidence of cerebellar dysfunction in ET: spectroscopy studies showed reduction in N-acetilaspartate in cerebellum of ET patients(46); voxel-based-morphometry showed cerebellar atrophy(47); anatomopathological studies showed a loss of Punkinje cells, dendritic edema of these cells and an increase in their axonal ramifications(48).

The neurotransmitter probably implicated is gamma-aminobutyric acid (GABA), which is a molecule secreted by Purkinje cells that inhibits the denteate and other efferent neurons of cerebellum, aiding in motor control. In ET, there can be GABA deficiency or alterations in its cerebellar receptors(49, 50). One of the theories is that degeneration of Purkinje cells would lead to GABA deficiency and, consequently, to decrease of inhibition over circuits related to fine motor control. Data that support this hypothesis are: smaller density of Punkinje cells in cerebellar cortex from ET patients(51); results from experiments of mice with knockout GABA receptor that developed postural and kinetic tremor(52); and cerebrospinal fluid of ET patients who showed decreased GABA concentration(53). Additional evidence of gabaergic impairment is that tremor improves with drugs that increase GABA activity, such as benzodiazepines, gabapentine, primidone, and alcohol.

Another possible mechanism for tremor initiation is related to the instability of the feedback circuits of movement, which allows for modulation of peripheral movement according to central command and also to the movement itself. Short feedback loops are formed by the alfa-motoneurons in the spinal cord, and longer loops go further until cerebellum, brainstem and motor cortex. Therefore, the simultaneous presence of multiple feedback loops increases the opportunity for instability in the system, which can be oscillatory and facilitate tremor generation(37).

Transcranial Magnetic Stimulation (TMS) studies were able to modulate tremor, which reinforces the role of the cerebral cortex in this pathology(54). Deep Brain Stimulation studies revealed that low frequency stimulation of the

ventrolateral nucleus of the thalamus was able to modulate tremor amplitude(45), supporting its important role in the disease.

3.4 Basic Functional Neuroimaging Concepts

Functional MRI (fMRI) has been used in the last decades to study healthy brains as well as neuropathology. It is a non-invasive method that does not require contrast or radiation, which allows measures of the Blood Oxygen Level Dependent (BOLD signal). When hemoglobin is bind to oxygen, it is diamagnetic, whereas it is paramagnetic when not oxygenated. When a region is active, it consumes oxygen, and causes an increase in the blood flow. The ratio oxy/deoxyhemoglobin rises, which causes less distortion of the magnetic field and increases the signal, measured as BOLD signal. (55). We assume that regions that have the same pattern of oxygen consumption are functionally correlated, or connected.

3.4.1 Acquisition parameters

The first parameter to consider when performing a functional MRI study is the *magnetic field* strength of the scanner, which is usually 1.5T, 3.0T or 7.0T. The higher the magnetic field strength, the higher the spatial resolution and the BOLD effect. However, the presence of more artifacts has to be considered.

In order to detect the BOLD signal, T2* weighted MRI acquisitions should be performed. Other techniques such as changes in cerebral blood flow or cerebral metabolic rate of oxygen can be alternatively used. Data are usually collected on the entire brain, either in a sequential or in an interleaved slice acquisition(55).

The *Repetition time* (TR) is the time taken to acquire one brain volume in the EPI sequence. Longer TRs are associated to greater sensitivity to motion, while short TRs improve the temporal resolution of the technique. *Echo time* (TE) is the interval between the application of the radiofrequency excitation pulse and signal acquisition. For the 3T field, it is usually 30ms. *Field of View* (FOV) reflects

the area being covered in one slice, and *Matrix* means the number of data points in each plane(56).

Flip angle is the amount of rotation of the net magnetization during a pulse. It is usually chosen to be the *Ernst angle*, of around 90º [2].

3.4.2 Artifacts

Artifacts in fMRI can be due to equipment related issues – such as pulse sequence (spatial distortions), field inhomogeneities, radiofrequency interferences – or to physiological noise (cardiac pulsation and respiratory movements, for example). Head movement can also generate motion artifacts.

Many strategies are used to minimize artifacts, such as recording respiratory and cardiac rates and use such measures as regressors, shorten the TR, and using band-pass filtering. Many softwares have also been developed to minimize their impact, such as the ART, which was used in this project.[3]

3.4.3 Pre-processing parameters

Pre-processing are a series of steps performed before statistical analysis in order to guarantee image quality, exclusion of artifacts, and to ensure that images are comparable to each other.

All images have to be converted into the same file format. Images should be visually screened do detect tumors, strokes, haematomas or other lesions that would impair their analysis, as well as visible artifacts.

Normally the first few seconds and volumes of scanning are discarded in order to allow for stabilization of gradients. Then *slice-timing correction* adjusts the time-course of each voxel data in its respectively slice to match its timing.

Motion correction is fundamental in order to couple each voxel to its anatomic area. Images are *realigned* to a reference volume using a rigid body

[2] From mriquestions.com, on July 18th, 2020.

[3] View at: http://www.nitric.org/projects/artifact_detect/, last accessed in July 18th, 2020.

transformation, with x, y and z rotations and translations. It is important to stablish a threshold of tolerance of movement, and images with motion greater than the dimensions of one voxel should be discarded(57). *Nuisance variables* can be used in order to help motion correction, such as signal from the cerebrospinal fluid or white matter. *Scrubbing* is a method to exclude noise from head movement, based on measures of head displacements and whole-brain BOLD signal displacements.

Normalization is a process in which images are fitted into a template image, such as the Tailarach or MNI atlas. This procedure allows for comparisons amongst different subjects(56).

Spatial smoothing and *filtering* are processes in which data points from one voxel are averaged with their neighbors, blurring the sharp edges. It allows increases in the signal-to-noise ratio (SNR), but reduces spatial resolution. The *Gaussian kernel* determines the extent to which the data is smoothed(55).

3.4.4 Functional Magnetic Resonance Imaging Modalities

Connectivity comprises structural connectivity, functional connectivity, effective connectivity and dynamic connectivity. Structural brain connectivity implicates anatomic links amongst structures, while functional connectivity denotates association of nodes that are not necessarily anatomically connected. Effective connectivity implies a direct influence of one area over another(34). Dynamic connectivity, which is primarily used for task-based fMRI, allows exploration of the brain as a dynamic system, with estimation of effective connectivity according to the hemodynamic response influenced by external experimental variables(55).

Functional connectivity studies can be performed while the patient is laying still with, with eyes closed or open, and not performing any specific task, which is called resting state fMRI(58), or while the individual is answering to cognitive or motor tasks. Regions of Interest (ROIs) are the areas that will be focused on for functional evaluation.

Connectivity can be accessed through many modalities of analysis. The Seed-to-Voxel analyses will evaluate the connectivity of one ROI to the rest of the brain; ROI-to-ROI analyses will calculate connectivity values between two ROIs. Network analyses are more complex and require sophisticated analyses tools, such as Dynamic-Independent Component Analysis and Graph Theory.

Graph theory (GT), as previously mentioned, is the mathematical field that studies the complex interaction of nodes linked by connections(34). It was first described by Leonard Euler in 1736, when trying to solve a mathematics problem about the seven bridges of Königsberg (now Kaliningrad, Russia), and a way of walking around the city crossing each bridge once and only once. Euler proposed a mathematical solution organized as a graph of nodes and edges[4].

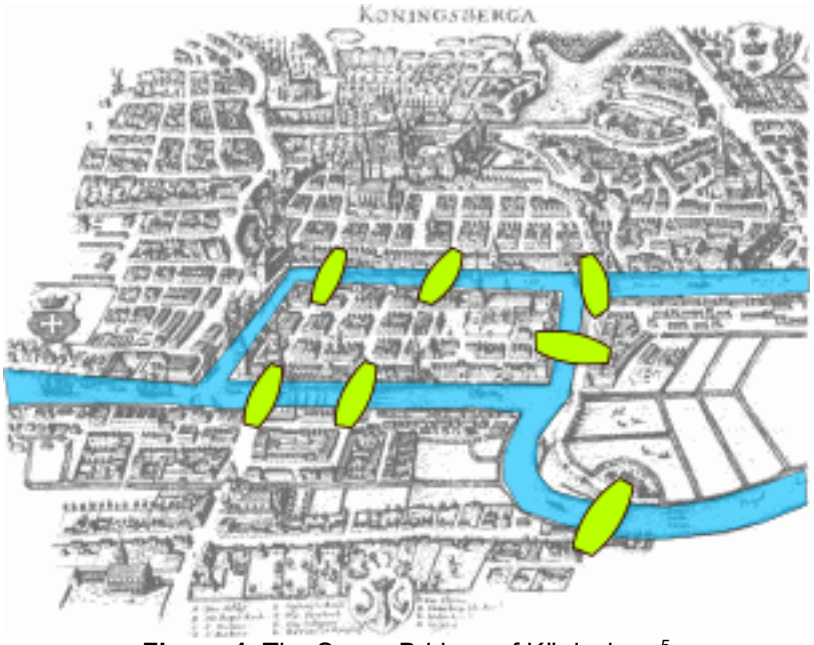


Figure 4: The Seven Bridges of Königsberg[5]

One of the measures of GT is Global Efficiency. It reflects effective information transfer within a network of nodes (i.e., ROIs) and edges (i.e., correlations or "paths" between nodes)(59). It is mathematically expressed as the

[4] Euler, Leonhard (1736). "Solutio problematis ad geometriam situs pertinentis". Comment. Acad. Sci. U. Petrop 8, 128-40.

[5] Downloaded from: https://en.wikipedia.org/wiki/Seven_Bridges_of_Königsberg on July 28th, 2020.

inverse of the average shortest path length in a graph *G* to all other nodes in the graph, which means the minimum number of edges that the information takes to travel from one point to another. Short path lengths mean high global efficiency. Consequently, a network with high Global Efficiency (GE) means that connection amongst the hubs of the network is made through short path lengths. Other measures from GT are local efficiency, betweenness centrality, cost, average path length, clustering coefficient and degree.

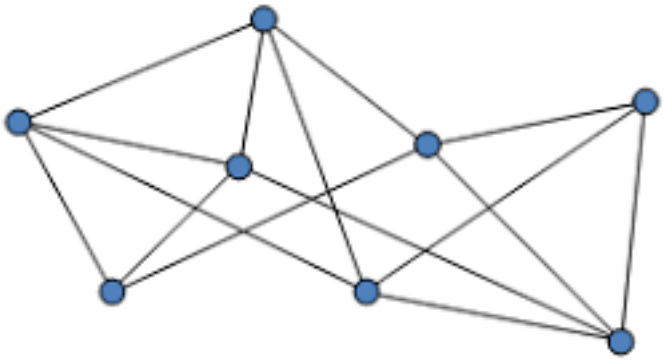


Figure 5: Example of a *graph*[6]

3.5 Previous fMRI Studies in PD and in ET

The literature on fMRI has grown exponentially in the last few years. In this section, important data concerning fMRI in PD and TE that were relevant to tremor circuitry will be summarized.

3.5.1 Pairwise metrics studies in PD

A study performed by Chen et al. compared the amplitude of low frequency fluctuations (ALFF)[7] in PDAR patients to PDT patients and HC. Compared to

[6] Downloaded from https://uh.edu/engines/epi2467.htm on July 28th, 2020.

[7] ALFF is a method that measures signal on a voxel by voxel basis. It reflects local spontaneous activity, and exhibits different properties of the BOLD signal. 55.Soares JM, Magalhães R,

controls, results showed that patients with tremulant form of PD had greater ALFF in the posterior cerebellum contralateral to the more affected side by tremor. Patients with PDAR form had lower ALFF in putamen bilaterally and posterior cerebellum, and higher ALFF in cortical areas. Comparing PDT to PDAR, those who presented tremulant predominant form had higher ALFF on bilateral putamen and posterior cerebellum, and lower ALFF on bilateral temporal gyrus and left parietal lobe. In all patients, posterior cerebellum ALFF correlated positively to tremor scores, and putamen ALFF scores correlated negatively to rigidity and bradykinesia scores, as well as to Hoehn & Yahr scale(60). It could be questioned, from these studies, if there would be a cerebellar hyperfunction to compensate the basal ganglia hypofunction. Or whether in the PDAR form, cerebellar hypofunction would be responsible for postural alterations. In this direction, a study with Deep Brain Stimulation (DBS) in pedunculopontine nucleus showed, by PET, an increase in blood flow to cerebellum and an improvement of gait and postural symptoms in PD(61).

Still comparing PDT and PDAR, a paper published by Hu, X. et. al. using Voxel-Mirrored Homotopic Connectivity (VMHC[8]) indicated that patients with the PDT form presented decreased VMHC in posterior cerebellum, and PDAR patients exhibited lower VMHC in the precentral gyrus, suggesting that functional coordination between homotopic brain areas is compromised in both subgroups of the disease (63).

Another study performed by Helmich, R.C. et. al. used fMRI, SPECT and peripheral recording with electromyography, and indicated that PDT patients had increased functional connectivity between GPi and putamen to the cerebellum-thalamus-cortical circuit(38). They have also shown that pallidal dopamine depletion correlated with clinical tremor severity, and that GPi, GPe and putamen were momentarily activated at the beginning of tremor episodes, while cerebellum-thalamic circuit co-oscillated with tremor amplitude.

Moreira PS, Sousa A, Ganz E, Sampaio A, et al. A Hitchhiker's Guide to Functional Magnetic Resonance Imaging. Front Neurosci. 2016;10:515.

[8] VMHC is a connectivity metric that compares each voxel in one hemisphere to its mirrored counterpart in the other (62. Zuo XN, Kelly C, Di Martino A, Mennes M, Margulies DS, Bangaru S, et al. Growing together and growing apart: regional and sex differences in the lifespan developmental trajectories of functional homotopy. J Neurosci. 2010;30(45):15034-43.

When comparing denteate connectivity, using voxel-wise resting-state fMRI, also in subgroups of PD, Ma, H. et. al. observed that PDT patients had increased connectivity from bilateral denteate nucleus to anterior lobe of cerebellum, and diminished connectivity to pre-frontal cortex bilaterally. Denteate connectivity to posterior cerebellum was correlated to tremor severity. Therefore, their study showed higher dentato-cerebellar connectivity and lower dentato-prefrontal connectivity in PD (64).

Gao, L. and Wu, T., in 2016, published a review on functional network disruption in PD. Regarding bradykinesia, it has been shown that there is weaker connectivity between striatum and cortical areas such as the SMA and premotor cortex. Concerning Freezing of Gait (FOG), it has been related to impairment in frontal cortical regions, basal ganglia, and the midbrain (65).

3.5.2 Pairwise metrics studies in ET

Morphometric and functional analyses performed in a group of ET patients compared to healthy controls showed increased BOLD signal in cerebellum in healthy subjects. A subgroup analyses of ET patients with and without resting tremor indicated that those who presented it had GPi dysfunction, which is consistent to data reported from PD patients' resting tremor, what suggests a role of this structure in this type of disorder(66).

An elegant study performed by Buijink, A. et. al. coupling electromyography and fMRI has detected that tremor during motor tasks has an excitatory effect on the connection from cerebellar lobule V to the thalamus, and decreased functional connectivity between cortical and cerebellar motor regions. The decreased in functional connectivity correlated to intensity of tremor. Also, the increase in connectivity from cerebellum to thalamus correlated to severity of tremor(67).

Finally, other fMRI study by Passamonti, L. et al. performed in ET patients indicated alterations in posterior cerebellum, dorsolateral pre-frontal cortex and parietal lobules, which could explain not only motor symptoms but also some degree of cognitive impairment associated to ET, such as verbal working memory. In this experiment, ET patients exhibited greater cerebellar response in

crus I and lobule VI when compared to controls during attentional-demanding working memory tests (29).

3.5.3 Network metrics using Graph Theory

In order to better visualize the main findings of recent studies that used Global Efficiency metrics, Table 1 is presented:

Paper Title	Year of Publication	Number of participants (Parkinson /Controls)	Main results	Network Analyzed	Result of GE
Graph Theory and Network topological metrics may be the potential biomarker in Parkinson's Disease (68)	2019	9/7	Increases of global efficiency, cost, and degree in frontoparietal PPC[9] (R) network, but decreases of local efficiency, clustering coefficient, and average path length in salience ACC[10], dorsal attention FEF[11] (L), and salience Insula (R) networks, respectively. Suggests that the graph theory and the network topological metrics measurement may be the potential biomarkers in PD to evaluate the disease progression and to monitor therapeutic results.	Frontoparietal PPC, salience ACC, dorsal attention FEF, salience insula	Increased in PPC, decreased in the others.
Iron-related nigral degeneration influences functional topology mediated by striatal dysfunction in Parkinson's disease(69)	2019	90/38	PD patients showed significantly increased global efficiency ($p = 0.013$) but fairly preserved local efficiency ($p = 0.214$) (network-striatum, pallidum and thalamus). Iron accumulation in the inferior SN had a significantly positive correlation with functional global efficiency ($r = 0.250$, $p = 0.021$) and negative correlations with functional path length ($r = -0.238$, $p = 0.028$) and clustering coefficient ($r = -0.295$, $p = 0.006$) in PD patients.	Striatum-pallidum-thalamus	Increased
Dynamic Graph Theoretical Analysis of Functional Connectivity in Parkinson's Disease: The	2019	69/29	PD subjects had a lower variability in the Fiedler Value[12], modularity, and global efficiency, indicating both abnormal dynamic global integration and local segregation of brain networks in PD. They were shown to be related with disease	Cerebellum cortex, Thalamus, Caudate, Putamen, Pallidum, Hippocampus, Amygdala, Accumbens,	Decreased variability in GE

[9] PPC= Posterior Parietal Cortex

[10] ACC= Anterior Cingulate Cortex

[11] FEF= Frontal Eye Fields

[12] Fiedler Value corresponds to the second smallest eigenvalue (the algebraic connectivity) of the Laplacian matrix of a graph G. 71. Weisstein EW. "Fiedler Vector." From M*athWorld* --A Wolfram Web Resource. [Available from: https://mathworld.wolfram.com/FiedlerVector.html.

Importance of Fiedler Value (70)			severity and other clinical variables including age.	cingulate, frontal, cuneus, entorhinal, fusiform, inferiorparietal, inferiortemporal, lateraloccipital, lateralorbitofrontal, lingual, medialorbitofrontal, middletemporal, parahippocampal, paracentral, parsopercularis, parsorbitalis, parstriangularis, pericalcarine, poscentral, posteriorcingulate, precentral, precuneus, rostralanteriorcingulate, rostralmiddlefrontal, superiorfrontal, superiorparietal,, suepiortemporal, supramarginal, transversetemporal, insula.	
Apomorphine-induced reorganization of striato-frontal connectivity in patients with tremor-dominant Parkinson's Disease (72)	2019	16	Reduction of tremor symptoms was mirrored by a significant increase in overall connectivity strength and reorganization of the modular structure of the basal ganglia and of the fronto-striatal module. They also found an increase in the centrality of motor and premotor regions.	Crossley's Template[13]	Increased centrality induced by drug.
Global and Subnetwork Changes of the Structural Connectome in de novo Parkinson's Disease (75)	2018	23/38	PD patients showed lower global efficiency and global clustering coefficient compared with healthy controls. Structural brain network of early-stage medication-naïve PD patients is altered relative to healthy controls in such a way that it allows for less integration (global efficiency) and segregation (clustering coefficient) of information processing.	DMN, frontoparietal, sensorimotor and attention networks.	Decreased GE
Relationship between Cerebrospinal fluid biomarkers and structural	2018	132/61	Global measures (but not local efficiency) and CSF α-synuclein were significantly lower in PD patients. Global efficiency and clustering coefficient correlated positively with α-	Whole Brain, based on the AAL atlas.	Decreased GE

[13] Crossley's Template is a whole brain template with similarly-sized regions (638 nodes). 73. Crossley NA, Mechelli A, Vértes PE, Winton-Brown TT, Patel AX, Ginestet CE, et al. Cognitive relevance of the community structure of the human brain functional coactivation network. Proc Natl Acad Sci U S A. 2013;110(28):11583-8, 74. Ramirez-Mahaluf JP, Medel V, Tepper Á, Alliende LM, Sato JR, Ossandon T, et al. Transitions between human functional brain networks reveal complex, cost-efficient and behaviorally-relevant temporal paths. Neuroimage. 2020;219:117027.

brain network properties in Parkinson's Disease **(76)**			synuclein, $A\beta_{42}$, and total tau CSF levels. Furthermore, these CSF biomarkers showed no significant association with the UPDRS-III score.		
Impaired topographic organization in cognitively unimpaired drug-naïve patients with rigidity-dominant Parkinson's Disease (77)	2018	20/20	PDAR patients presented the small-world property [14], and abnormalities at the nodal level (E_{nod}[15], N_{Deg}[16], and N_{Bet}[17]) but not at the global level (C_p [18], L_p [19], E_{loc} [20], and E_{glob} [21]). Results revealed lower nodal centralities mainly in the occipital lobe and areas of the limbic system, and higher nodal centralities in distributed frontal and temporal regions. The decreased nodal efficiency of occipital regions was negatively correlated with UPDRS-III scores.	Whole Brain, based on the AAL atlas, divided into 90 nodes.	No abnormalities in GE
Functional Brain Connectome and its relation to Hoehn and Yahr Stage in Parkinson's Disease (79)	2017	153/81	The functional connectome in PD showed abnormalities at the global level (ie, decrease in clustering coefficient, global efficiency, and local efficiency, and increase in characteristic path length) and at the nodal level (decreased nodal centralities in the sensorimotor cortex, default mode, and temporal-occipital regions; $p < .001$, FDR corrected). Further, the nodal centralities in left postcentral gyrus and left superior temporal gyrus correlated negatively with UPDRS-III score ($P = .038$, FDR corrected, $r = -0.198$; and $P = .009$, FDR corrected, $r = -0.270$, respectively) and decreased with increasing Hoehn and Yahr stage in patients with PD.	Whole Brain, based on the AAL atlas, divided into 90 nodes.	Decreased GE
Impaired brain network architecture in newly diagnosed Parkinson's Disease based on Graph	2017	26/19	Nodal degree, global efficiency, local efficiency and characteristic path length consistently revealed disruptive sensorimotor network, and visual network to a less degree in PD. By contrast, default mode network (DMN) and cerebellum in PD showed higher	278 nodes from a whole-brain parcellation defined by Shen et al (81).	Increased GE

[14] Small World Property is a mathematical term that indicates a Graph G has a high clustering coefficient and a small characteristic path length. 78. Mehlhorn H. SF. Small-World Property. In: Dubitzky W., Wolkenhauer O., Cho KH., Yokota H. (eds) Encyclopedia of Systems Biology. : Springer. New York, NY.; 2013.

[15] Enod= Nodal efficiency

[16] Ndeg= Nodal degree

[17] NBet= Nodal betweeness

[18] Cp= Clustering coefficient

[19] Lp= Path Lenght

[20] Eloc= Local Efficiency

[21] Eglob= Global Efficiency

Theoretical Analysis**(80)**			nodal degree, global efficiency and local efficiency, and lower characteristic path length. Global and local efficiency in the midbrain was higher in PD excluding substantia nigra. PD group also exhibited lower cluster coefficient in the subcortical motor network (thalamus and caudate nucleus). No significant correlation was found between topographic properties and motor severity.		
Levodopa modulates small-world architecture of functional brain networks in Parkinson disease (82)	2016	21/20	Patients off medication showed no significant changes in global efficiency and overall local efficiency, but in a subnetwork analysis did show increased local efficiency in Executive (p=0.006), and Salience (p=0.018) networks. Levodopa significantly decreased local (p=0.039) efficiency in patients except within the Subcortical network where it significantly increased local efficiency (p=0.007). Levodopa modulates global and local efficiency measures of small-world topology in PD suggesting that degeneration of nigrostriatal neurons in PD may be associated with a large-scale network reorganization, and that levodopa tends to normalize the disrupted network topology in PD.	226 nodes comprising 10 intrinsic brain networks	<u>No changes</u> in GE *off* medication.
Alteration of Brain Functional Networks in Early-Stage Parkinson's Disease: A Resting- State fMRI Study (83)	2015	26/30	PD patients exhibited abnormal global properties, characterized by lower global efficiency. PD patients exhibited increased nodal centrality, primarily in the bilateral pallidum, the inferior parietal lobule, and the medial superior frontal gyrus, and decreased nodal centrality in the caudate nucleus, the supplementary motor areas, the precentral gyrus, and the middle frontal gyrus. There were significant negative correlations between the Unified Parkinson Disease Rating Scale motor scores and nodal centralities of superior parietal gyrus. These results suggest that the topological organization of the brain functional network was altered in early-stage PD patients who received antiparkinson treatment, and it was speculated that the antiparkinson treatment may affect the efficiency of the brain network to effectively relieve clinical symptoms of PD.	Whole Brain, based on the AAL atlas, divided into 90 nodes.	<u>Decreased</u> GE

Distinguishing patients with Parkinson's Disease Subtypes from Normal controls based on Functional Network Regional Efficiencies (84)	2014	25/20	Local and global efficiencies were measured and used to distinguish subgroups of PD patients from HC. Network regional efficiency could discriminate among individual PD subgroups and HC. Regions involved were basal ganglia, limbic regions, cerebellum, and others. Global efficiency performance was dependent on inclusion of the cerebellum in the analysis. This study suggests that cerebellum may play different roles in pathologies of different PD subtypes.	AAL atlas with and without the cerebellum, parcellated into 1024 random anatomical regions of interest.	GE was able to differentiated PD from HC when cerebellum was included in the network. But it does not specify if GE was greater or lower.

Table 1: Summary of Main Results of recent literature review concerning Global Efficiency Metrics in Parkinson's Disease

As it may be observed in Table 1, several studies have already addressed Global Efficiency metrics in Parkinson's Disease and Essential Tremor, with conflicting results. While most works showed decreased GE in comparison to controls, others found no abnormalities or even increased GE. Also, areas involved in network abnormalities varied greatly amongst them, with some evidence of disruption in connectivity of cerebellum, thalamus, basal ganglia and cortical areas. Moreover, as summarized in the table above, the ROIs and networks analyzed were heterogeneous amongst the studies.

Since PD is associated with a reduction of the motor activity, and based on the previous studies which, in its majority, pointed out to a decreased global efficiency, our hypothesis was more driven towards a diminished GE of the motor network in PD in comparison to controls.

As we have argued previously, our study provides a large number of participants, it can rely upon results obtained with a high-quality equipment, it was conducted according to rigorous pre-processing and very conservative statistical parameters. This set of conditions allowed us to explore such literature gap and look further into Global Efficiency metrics in ET, PD and its subgroups, seeking to contribute to better understanding the underlying mechanisms of each group.

4. Methods

4.1 Patient Selection

A convenience sample of patients treated at the Movement Disorders Clinics at *Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* (HCFMUSP)[22] and at the *Associação Brasil-Parkinson*[23] was invited to participate in this study, which was approved by the local ethics committee (Online registration n° 8197, CAPPesq n° 0700/11). All individuals signed the Term of Consent and their identities in the study were protected by the use of numeric codes. The study was financed by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo)[24], Protocol Number 2011 / 18747 – 0.

Inclusion criteria were:

- for PD patients: idiopathic PD, according to the Parkinson's UK Brain Bank criteria[25] (85), Hoehn & Yahr[26] I or II (86); all PD patients were evaluated in the ON state.
- for ET patients: the Movement Disorders Society diagnostic criteria[27] for ET(22), absence of parkinsonism and exclusion of medication-induced tremor;
- healthy controls: absence of neurological diseases, age pairing with patients' group.

Exclusion criteria were:

[22] Clinics Hospital of Medical School of São Paulo University

[23] Brazilian Parkinson Association

[24] Foundation for Research Support of São Paulo State

[25] Criteria available in the Annex A.

[26] Scale available in the Annex B.

[27] ET diagnostic criteria available in the Annex C.

- impossibility of performing an MRI due to claustrophobia or cardiac pacemaker;
- use of ancient metallic prothesis;
- presence of severe movement disorder that would impair imaging analysis;
- intracranial structural abnormalities, such as tumors or previous vascular lesions.
- excessive head motion detected during image pre-processing (more than 3 standard deviations from the mean intensity in the session or composite head movement that exceeded 2 mm from the previous image or volumes with a global-signal z value>9).

All patients and controls were examined by the Movement Disorders specialists from the Movement Disorders Clinics of HCFMUSP, and were classified by the Unified Parkinson's Disease Rating Scale (UPDRS)[28] (87) and by the Hoehn & Yahr scales(86).

All patients and controls were submitted to MRI scans in two hospitals - HCFMUSP and *Hospital Israelita Albert Einstein* (HIAE)[29]. Functional images were performed in the latter. PD patients were scanned while under effect of Levodopa (ON state). This decision could pose questions about whether the results of the fMRI would be due to the pathophysiological changes in PD brain or due to medication effects(88). However, many patients do not tolerate the MRI scan on OFF state (without effect of medication), due to tremor, axial rigidity, pain and discomfort. Moreover, analysis of fMRI during the ON state would be closer to patient daily activities scenario. The Levodopa Equivalent Dose (LED) was calculated for each subject using the Birmingham University conversion formula, as shown in table 1(89):

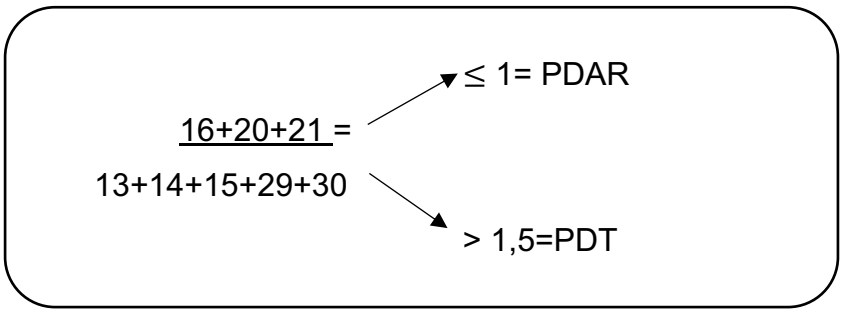
[28] UPDRS available in the Annex D.

[29] Israeli Hospital Albert Einstein

Drug	Convertion Factor
Immediate release Levodopa	X 1
Controlled release Levodopa	X 0,75
Entacapone (or Stalevo)	LD x 0,33
Pramipexole	X 100
Selegiline	X 10
Amantadine	X 1
Bromocriptine	X 10

Table 2: Levodopa Equivalent Dose according to the Birmingham University conversion formula

PD patients were further subdivided into two subgroups: (i) the tremulant-predominant group (PDT) and (ii) the akinetic-rigid group (PDAR), according to criteria proposed by Jankovic, shown below: calculating the media between the sum of the UPDRS items associated with tremor (16, 20 and 21) and the sum of items associated to rigidity, gait and postural instability (13, 14, 15, 29 and 30). Patients with average equal or below 1 were classified as PDAR and patients with average above or equal to 1.5 were classified as PDT(6).



4.2 Image Acquisition

The fMRI scans were performed at Siemens Trio Scanner 3.0T MR with a 32 channels head coil and a gradient of 45mT/m at HIAE in São Paulo, Brazil. Whole brain volumes were acquired by the echo planar imaging (EPI) multi-band accelerated sequence with TR (“repetition time”) of 600ms(90). BOLD sensitive images were acquired on T2*-weighted sequences. Acquisition parameters were:

40 axial cuts, slice thickness= 2.5mm, TE= 31ms, NEX= 1, Flip angle= 90º, Bandwidth= 2290Hz/px, FOV= 210mm, matrix size= 84x84, voxel dimension= 2.5mmx2.5mmx2.5mm. All images were acquired while patients were lying still with eyes closed (resting state). For co-registration and normalization, structural images with high resolution were also acquired (MP2RAGE).

4.3 Image Pre-Processing

Data were preprocessed and analyzed using the CONN toolbox version 17.b (91), with a standard MNI152 pipeline and parameters. CONN is a Matlab based software that analyses functional connectivity in resting state and during tasks. Preprocessing steps included the realignment and unwarping, slice-timing correction, segmentation, normalization, outlier detection, and smoothing with a Gaussian kernel of 4mm. Nuisance variables were based on scan motion censuring (discarding volumes with displacement >2mm and global-signal z-value >9; no subjects were excluded), 12 realignment parameters, white matter and cerebrospinal fluid signals. Band-pass filtering (0.008-0.09Hz) and nuisance variables were regressed out using a simultaneous bandpass approach(92).

4.4 ROI selection

Regions of interest (ROIs) and seeds were determined based on the Automated Anatomical Labeling Atlas (AAL)(93) and on the Harvard-Oxford Atlas(94).

The AAL Atlas has 120 structures derived from a single subject brain MRI provided by the Montreal Neurological Institute (MNI).

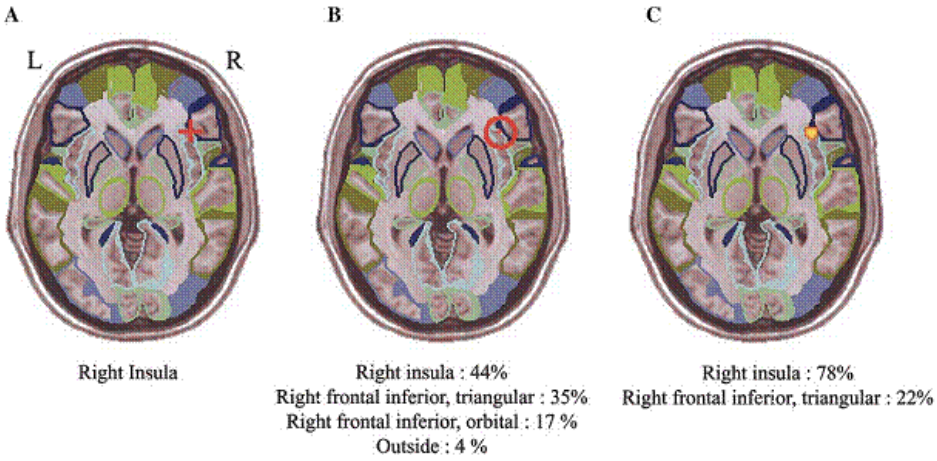


Figure 6: Example of the Automated Anatomical Labelling Atlas (AAL)(93)[30]

The Harvard-Oxford Atlas has a probability distribution for each brain region, with cortical parcellations derived from the *Maximum A Posteriori* (MAP) estimate, covering 48 cortical and 21 subcortical structural areas.

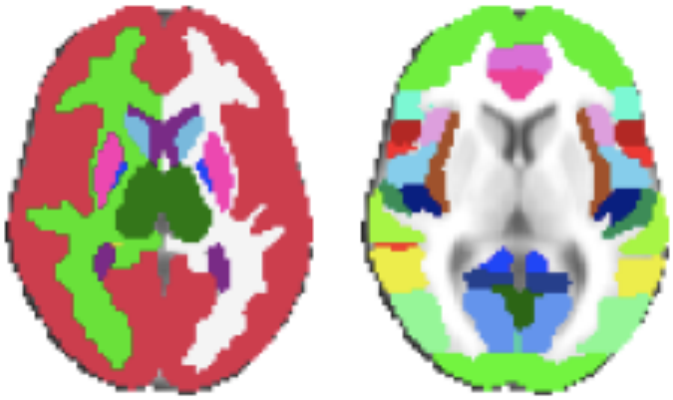


Figure 7: Example of the Harvard-Oxford Atlas[31]

[30] Last Downloaded on July 7th, 2020.from: https://web.archive.org/web/20150211005316/http:/www.cyceron.fr/index.php/en/plateforme-en/freeware

[31] Last Downloaded on July 7th, 2020 from: http://ftp.nmr.mgh.harvard.edu/pub/dist/freesurfer/tutorial_packages/centos6/fsl_507/doc/wiki/Atlases.html

We manually created ROIs from coordinates previously used in other studies for the Denteate Nucleus and Substantia Nigra (95, 96) using MatlabR2018b. We studied ROIs relevant to the motor network that would be somehow implicated in the tremor pathophysiology, bilaterally: denteate nucleus of cerebellum, substantia nigra, thalamus, caudate, putamen, pallidum, pre-central gyrus, post-central gyrus, supplementary motor area, and cerebellar networks anterior and posterior. ROIs are displayed in figures 8 and 9.

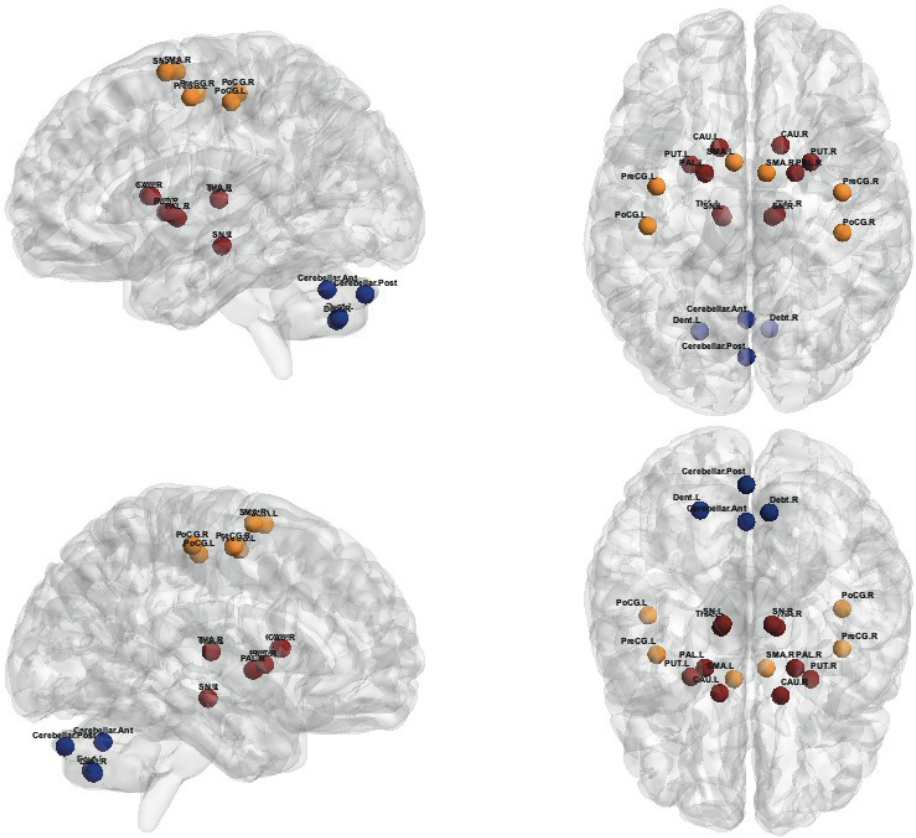


Figure 8: Regions of Interest (ROIs): In orange, cortical ROIs (Pre-central gyrus right and left, Post-central gyrus right and left, Supplementary Motor Area right and left); in red, subcortical ROIs (caudate right and left, putamen right and left, pallidum right and left, thalamus right and left); in blue, cerebellar ROIs (cerebellar anterior network, cerebellar posterior network and denteate nucleus right and left). Colors are just for didactic purpose, they do not infer degree of connectivity or size of the ROIs.

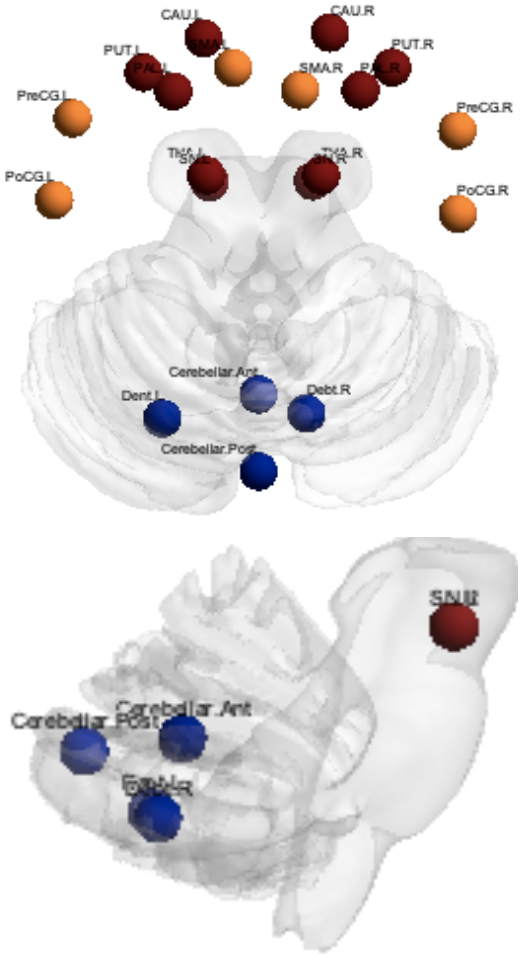


Figure 9: Cerebellar ROIs in blue

4.5 Connectivity estimation and analysis

For each individual, connectivity correlation maps were produced by extracting the mean BOLD time course from voxels within each seed and computing Pearson's correlation coefficients between that time course and 1) the time course of all other voxels (for seed-to-voxel analysis) and 2) the mean time course of each ROI. Correlation coefficients were converted to normally distributed *Z*-scores using the Fisher transformation to allow second-level General Linear Model analysis. Two sample *t*-tests were performed on the Fisher transformed r-maps to examine differences in resting-state functional connectivity between each one of the patients' groups and the Control group. Group-level effects were considered significant if they exceeded a peak amplitude of $p >$

0.001 uncorrected, and a family-wise error-corrected cluster extent threshold of $p < 0.05$. For ROI-to-ROI connectivity, we established a threshold to ROI-to-ROI connections by intensity, with p-FDR<0.05 at seed level correction, two-sided, enabling permutation tests. We compared all groups (PD x HC, ET x HC, PD x ET, and the subgroups of PD – PDAR x PDT, PDAR x TE, PDAR x HC, PDT x TE, PDT x HC). ROIs chosen for the seed-to-voxel and the ROI-to-ROI analyses were, bilaterally: substantia nigra, denteate nucleus, pallidum, putamen, caudate, thalamus, precentral gyrus, postcentral gyrus, supplementary motor area, and cerebellar networks anterior and posterior. We opted to evaluate all possible metrics concerning the connectivity, from the simplest Seed-to-Voxel analysis and then moved on to the whole brain analyses as a network, which was more in the direction of our previous hypothesis.

Graph theory analyses were also computed using the CONN toolbox. We focused on the global efficiency metric since it has been shown to be one of the most robust measures to assess integration properties of brain networks(97). The unweighted ROI-to-ROI correlation matrices of the motor network (one for each participant) were first submitted to a threshold at a cost value of k=0.15. Global efficiency indices were submitted to a threshold at p-FDR<0.05 in a two-sided analysis based on correlation scores. Independent sample t-tests were performed to examine differences between groups on global efficiency scores (PD, ET, and HC), two at a time. Statistical analyses were conducted by using JASP with p<0.05, one-tailed.

Clinical parameters (UPDRS, tremor scores alone from UPDRS and levodopa equivalent dose) were analyzed as covariates for the PD group to estimate the impact of disease severity and medication on the network connectivity.

5. Results

5.1 Clinical and Epidemiologic results

A total of 135 patients were included and performed all the clinical evaluations and MRI. The initial group was composed of 72 PD patients, 21 ET patients and 42 HC. After pre-processing, 32 patients were excluded due to artifacts of acquisition or movement. The final sample was composed of 54 PD patients, 18 TE patients and 31 HC. An ANOVA test was performed and there was no relevant statistical difference of age amongst groups (F1.010, p0.368). In PD patients, the predominance of symptoms was in the right side in 31 subjects, and left side in 22 subjects.

Groups	Number of subjects	Median Age	Standard deviation	Std. Error	Minimal Age	Maximal Age
HC	31	63,32	11,726	2,106	47	89
PD	54	64,91	9,098	1,238	48	86
ET	18	68,78	7,488	1,765	55	78
Total	103	65,11	9,806	0,966	47	89

Table 3: Demographic data divided per group and age

Groups were not paired by gender, as shown in table below (p<0.001):

		Group			
Gender		**PD**	**HC**	**ET**	**Total**
f	Count	12.00	22.00	10.00	44.00
	%	24.0 %	75.9 %	66.7 %	46.8 %
m	Count	38.00	7.00	5.00	50.00
	%	76.0 %	24.1 %	33.3 %	53.2 %
Total	Count	50.00	29.00	15.00	94.00

Table 4: Gender distribution per group

We calculated the average of UPDRS scores and LED in the PD group, being the results shown in Tables 5 and 6:

Descriptive Statistics PD group

	UPDRS	LED
Valid	47	50
Missing	3	0
Mean	52.00	563.1
Std. Deviation	16.53	377.8
Minimum	22.00	0.000
Maximum	90.00	2421

Table 5: Mean UPDRS scores and LED for PD group

Descriptive Statistics

	LED		UPDRS	
	PDT	PDAR	PDT	PDAR
Valid	19	24	19	23
Missing	7	7	7	8
Mean	450.4	671.9	49.58	52.57
Std. Deviation	241.9	475.6	18.94	13.93
Minimum	0.000	0.000	22.00	25.00
Maximum	1050	2421	90.00	78.00

Table 6: Mean LED and UPDRS scores in subgroups of PD

5.2 Image Quality Control

After scrubbing, we excluded 9 subjects from the study, once they had more than 25% of invalid scans (more than 125 volumes). The patients were

excluded from the following groups: 4 from the PD group, 2 from the HC group and 3 from the ET group (7.4% of PD group, 6.45% of HC group and 16.66% of ET group).

The mean motion from each group is shown on the following table:

	Mean_Motion		
	HC	**PD**	**ET**
Mean	0.1519	0.1650	0.1466
Std. Deviation	0.05063	0.05340	0.04352
Minimum	0.07070	0.08427	0.09237
Maximum	0.2671	0.3024	0.2288

Table 7: Mean motion in each group. HC: Healthy Control; PD: Parkinson Disease; ET: Essential Tremor

The difference of the mean motion amongst the three groups was calculated by ANOVA and was not significant (F 1.036, p 0.359).

The number of Invalid Scans after scrubbing was similar amongst the three groups, as shown in the table 8 below (p 0.20):

Descriptive Statistics

	Invalid Scans		
	PD	**ET**	**HC**
Valid	50	15	29
Mean	38.44	24.67	29.59
Maximum	116.0	76.00	121.0

Table 8: Invalid Scans in each group after scrubbing. HC: Healthy Control; PD: Parkinson Disease; ET: Essential Tremor

5.3 Connectivity Results

5.3.1 Seed-to-Voxel Analyses

Seed-to-Voxel analyses was performed using the following seeds, bilaterally: denteate nucleus, substantia nigra, thalamus, pallidum, caudate nucleus, putamen, supplementary motor area, pre-central gyrus, postcentral gyrus, and cerebellar networks anterior and posterior. No statistically significant difference was found amongst groups.

5.3.2 ROI-to-ROI Analyses

ROI-to-ROI analyses were performed separately in pairs of two ROIs each time, amongst the following ROIs, bilaterally: denteate nucleus, substantia nigra, thalamus, pallidum, caudate nucleus, putamen, supplementary motor area, pre-central gyrus, postcentral gyrus, and cerebellar networks anterior and posterior. There was no difference amongst groups.

5.3.3 Graph Theory Analyses

Graph Theory network analysis amongst ROIs listed above showed reduced Global Efficiency (GE) of the network on PD Group compared to HC ($t(77)=-1.749$, $p=0.042$). The average GE of the network in PD patients was 0.0231, versus 0.0297 for controls. Left SMA had the lowest GE (0.022 FDR-corrected) in the PD group, followed by Postcentral gyrus left (0.0496 FDR-corrected) and Postcentral gyrus right (0.0496 FDR-corrected).

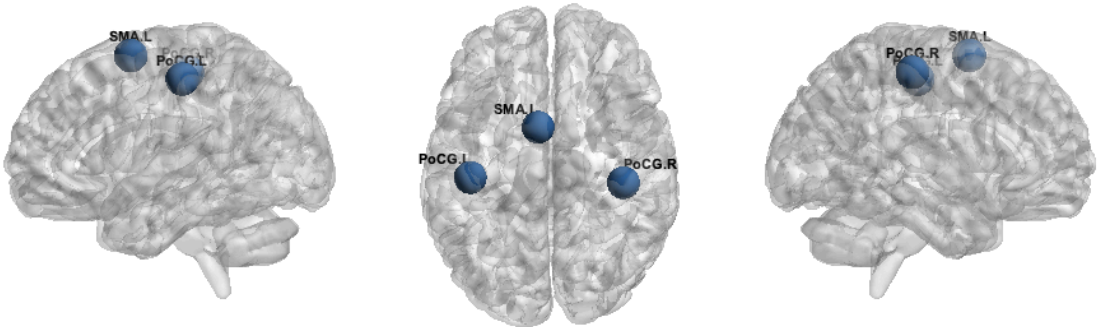


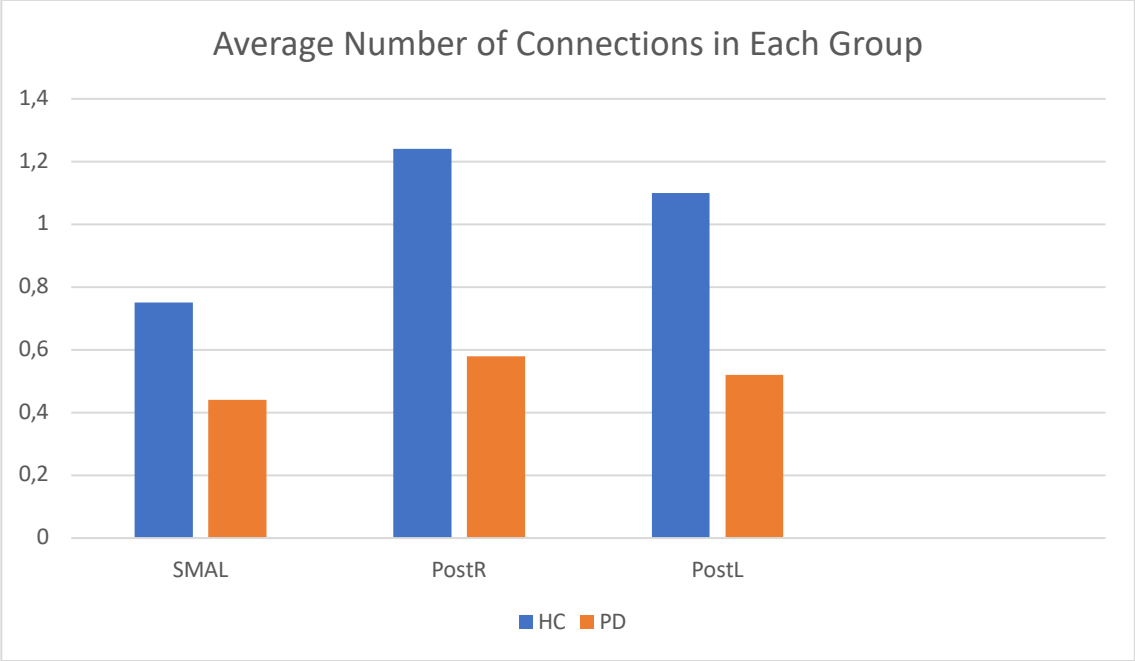
Figure 10: Results of Global Efficiency of the motor network in the PD group *versus* HC group. Blue areas are Postcentral gyrus right and left and left SMA

In order to better interpret these results of global efficiency, particularly concerning the meaning of the ROIs displayed on the CONN output results, we calculated the average number of connections for each ROI of the network obtained by the adjacency matrix, as seen in the table 9, below:

	Post R	Post L	SMA L
PD	0,58	0,52	0,44
HC	1,24	1,103	0,75

Table 9: Average number of connections for each ROI

These values seem to confirm that the average number of connections is larger in the HC group. Therefore, a reduced global efficiency in PD is possibly driven by a lower number of connections of these three nodes (left SMA, Left and Right Postcentral gyrus) within the motor network in this group, as shown on Graphic 1.



Graphic 1: Representation of the average number of connections in each group. On the *x axis*, the ROIs that were responsible for the difference in GE between the two groups. On the *y axis*, the average number of connections in each group

For Essential Tremor, there was no difference in GE when compared to HC, neither to PD. There was also no difference between the two subgroups of PD to each other, as well to ET and to HC.

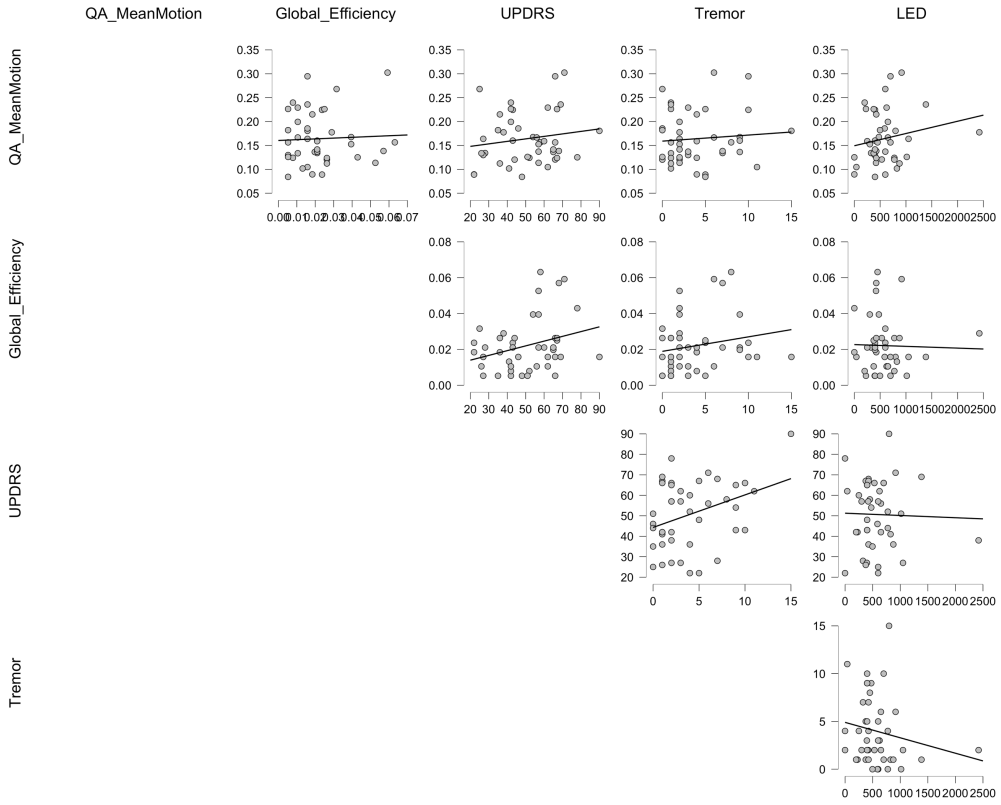
5.4 Covariates Analysis

UPDRS Scores did not correlate to GE in PD groups. Tremor scores alone correlated positively with GE of the network in both PD subgroups, but were not statistically different between themselves (p 0.75). Tremor scores correlated positively to the GE of the left substantia nigra in both groups, and were statistically different between themselves (p=0.001), being greater in the PDAR group.

LED was also used as a covariate to study the possible influence of the medication on GE, but no statistically significant result was found.

In order to exclude motor artifacts as possible confounders for connectivity analysis, we evaluated the correlations of mean motion and GE, and found no relation between them.

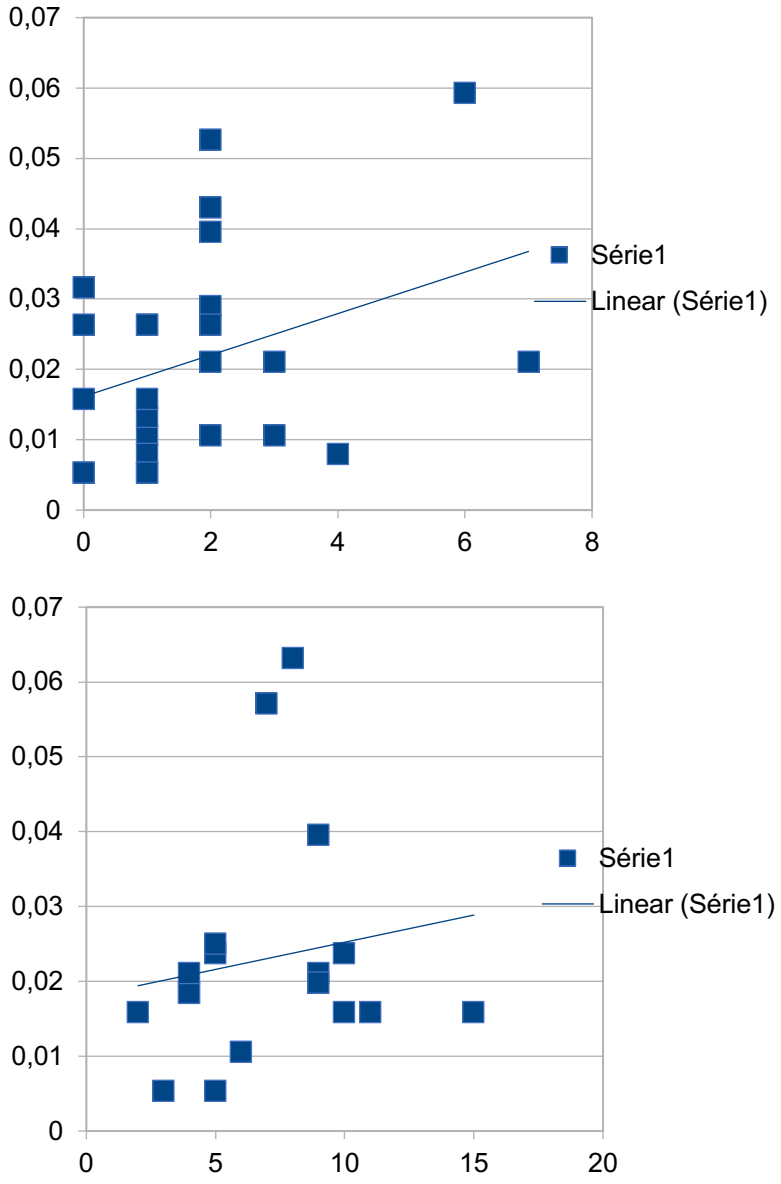
The Graphic 2 shows the correlations amongst Global Efficiency and Image Acquisition Parameters (mean motion) and Clinical Characteristics such as UPDRS, tremor scores and Levodopa equivalent dose (Spearman correlation).



Graphic 2: Representation of correlation matrices amongst Mean Motion, Global Efficiency, UPDRS, Tremor scores and LED

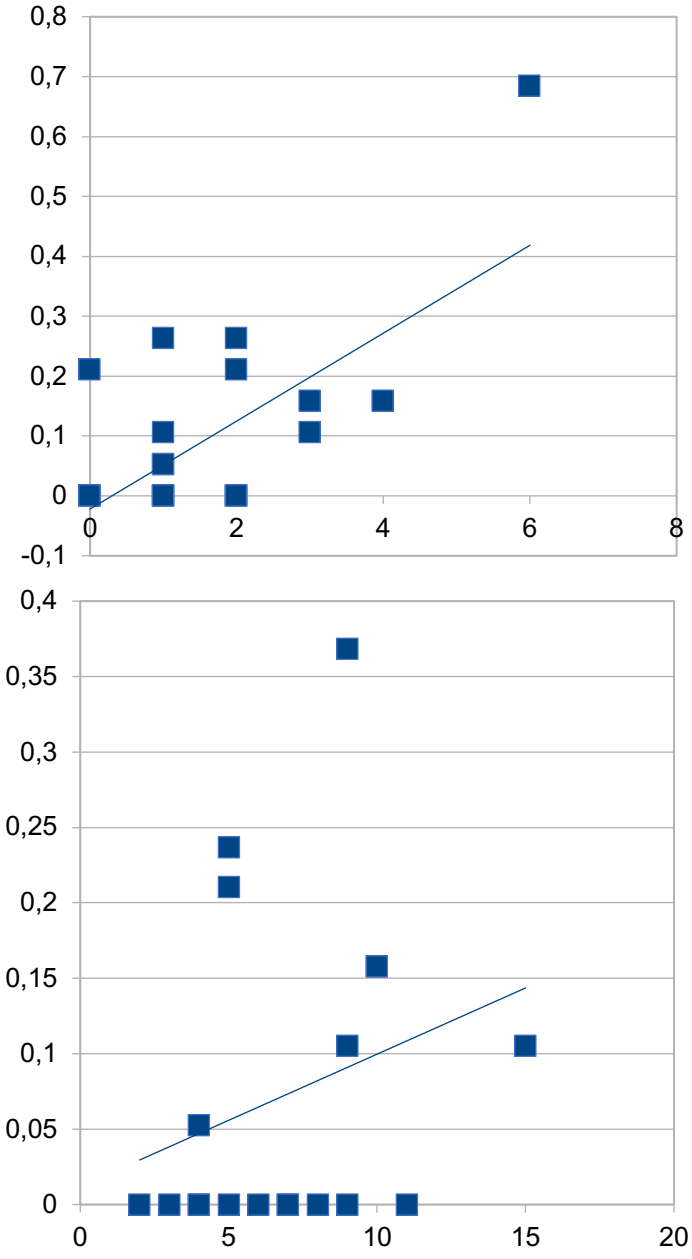
We can observe on the graphic above that there seems to be a positive correlation between UPDRS and Tremor Scores and Global Efficiency values.

The graphics below represent the correlation of the tremor scores (x axis) with the global efficiency (y axis) on the two subgroups of PD. Groups were not different from each other (p 0.097).



Graphic 3: Representation of tremor score (x axis) and global efficiency (y axis) in PD subgroups (PDAR on top, PDT on bottom)

The graphics below represent the correlation of the global efficiency of the left substantia nigra and tremor scores on the two subgroups of PD. Groups were significantly different from each other (p 0.001).



Graphic 4: Representation of left Substantia Nigra GE in both PD subgroups (PDAR on top, PDT on bottom). GE of the left substantia nigra on the y axis and tremor scores on the x axis

It is worth to note, however, that if we exclude the subject who showed an abnormally high GE in the PDAR group, this difference would disappear (p 0.433).

6. Discussion

According to our previous hypothesis, motor network connectivity measured by the GE is decreased in Parkinson's disease in comparison to healthy controls. Areas that most contributed to this diminished connectivity were the left SMA and bilateral postcentral gyrus. Tremor scores correlated positively to the GE of the network in PD, and influenced the connectivity of the left substantia nigra differently in both groups, especially in the PDAR. However, since it was an outlier driven result, we therefore considered that groups were not different from each other, and these values need to be further explored.

However, from what we know so far about the disease phenomenology, we expected that pairwise comparisons evolving these ROIs would also show connectivity alterations. We speculate that our study did not show such changes because our patients were scanned under the effect of levodopa. Another reason would be that very strict pre-processing parameters were used in order to control for motion and artifacts. Moreover, our statistical analysis was very conservative, according to current tendencies of the international neuroimaging community, admitting $p<0.001$ with FDR corrections to estimate connectivity in seed-to-voxel analyses and $p<0.05$ p-FDR corrected to ROI-to-ROI analyses.

Other studies have also evaluated the GE of the motor network, with conflicting outcomes. One study analyzed longitudinally 16 PD patients and showed decreased connectivity between the SMA, pre and postcentral gyri when compared to controls (98), which are in accordance with our results. In addition, another group with a larger number of participants demonstrated that GE is decreased in PD patients when compared to HC, and such decrease correlates with cerebrospinal fluid biomarkers (CSF), such as alfa-synuclein, $A\beta_{42}$ and total TAU. Such data suggest that functional imaging and measurement of CSF biomarkers together can bring a better understanding of PD pathogenesis(76). However, Hou et. al. (77) and Berman et. al. (82) found no global level abnormalities, while Guan et. al.(69) have found increased GE, which correlated positively to iron accumulation in the inferior SN.

It is known that SMA is one of the main areas that receive projections from the basal ganglia(99), playing a direct role in movement control thru direct projections to the spinal cord, and also throughout interactions with other structures to help control postural stability, bimanual coordination, sequences of movements and initiation of internally generated movement. Previous studies have reported SMA connectivity disruption in PD, with controversial results. A few studies showed enhanced connectivity of the SMA to the putamen and amygdala(100) and increased pre-SMA activation in early PD patients while performing self-initiated movements(101). Another study also using graph theory to measure network interactions showed decreased connectivity in SMA, left dorsolateral prefrontal cortex and left putamen(102). The latter study compared PD ON and OFF medication, and the decreased connectivity of SMA persisted on both states. Other methods such as PET and SPECT have also shown decreased SMA activation in PD in comparison to controls during internally triggered movements(103), and increased activation in SMA in PD patients under DBS(104) or apomorphine(105). Therefore, SMA may play an important role in PD physiopathology and should be considered as a target in neuromodulation studies.

The postcentral gyrus is the primary sensorial area, although it is questioned if it could also be involved in tremor modulation because of its peripheral proprioceptive information for the movement feedback loops. Another issue concerns wether its strong connections with the thalamus would play a role in tremor control. A previous research has shown diminished activation in somatosensory cortex in PD after tactile stimulation(106). A systematic review and meta-analysis assembled thirty studies, with a total of 854 PD patients, and emphasized the role of postcentral gyrus as a critical region in PD. They have found an increased functional connectivity in post-central gyrus in PD compared to healthy controls(107).

Contrary to our expectations, global efficiency was positively correlated to tremor scores. One study found no relation of GE and motor severity(80), while another, with the use of apomorphine, described a reduction of tremor mirrored by an increase in overall connectivity strength. However, both studies used

network measures different from what we have done (72). Therefore, the biological interpretation of these values is not straightforward.

Regarding ET, the fact that the connectivity of the anterior cerebellar network was increased to the other regions of the motor network when compared to PD reinforces its role in the pathogenesis of tremor is this disease. The cerebellum integrates multimodal sensorimotor inputs from the cortex, vestibular nuclei and spinal cord and modulates the information thru outputs to the cerebral cortex, across the cerebello-thalamo-cortical tract, so that movement is harmonic and smooth (108). One fMRI study using graph theory analysis found disruption in the efficiency of the overall brain functional network in ET, involving multiple areas of the brain. In the global level, ET patients exhibited lower small-worldness values than HC, and at the regional level, showed higher values of GE in several cortical and cerebellar areas(109).

Several studies have addressed the cerebellum as a treatment target for ET through neuromodulation. A recent review by França, C. et al. summarized different types of intervention such as DBS, TMS and tDCS in the treatment of movement disorders. Their findings suggest that cerebellar neuromodulation could be a therapeutic choice to ameliorate tremor in ET. In addition, cerebellar neuromodulation was effective in other diseases such as cerebellar ataxia and dystonia. It is believed that cerebellar stimulation improves movement disorders because of its influence on the motor cortex – M1 receives excitatory input via the dentato-thalamo-cortical pathway – and because of changes in blood flow and metabolism(110).

A limitation of our study, as already mentioned and justified, is that PD patients were scanned under the effect of Levodopa to minimize motion and discomfort during scanning. There have been previous reports in the literature suggesting that levodopa could be a confounding factor for connectivity analyses(88), with a tendency to “normalize” network measures such as local efficiency(82). A network study comparing patients in ON and OFF states showed that levodopa normalized degree centrality abnormalities specially in occipital regions and postcentral gyrus(111). However, we used LED as a covariate to study its effect on the GE of the network, and found no correlation.

Our groups were not gender balanced. There is a study that has previously shown sex-related differences in PD(112). Women with PD present a milder disease phenotype and start disease at an older age compared to man, possibly due to neuroprotective effects of estrogen. Also, women tend to have a longer life expectancy than man. Therefore, these variables could explain why there are more homebound women with advanced PD. They are also more likely to be widowed or single compared to men, and to lack caregivers. Men had a higher mean UPDRS score and higher rate of dementia. We do not believe sex-related differences played an important role in our study, since the inclusion criteria were based on clinical parameters, such as having a low score of I or II in the Hoehn & Yahr scale, which already excluded patients with severe symptoms. However, we cannot completely discard that the differences we found were not related to gender.

An additional limitation of the study is that we did not have any co-registration of fMRI and electrophysiological parameters concerning tremor, such as accelerometers or surface motion-detection electrodes. Therefore, the possibility that the changes found on connectivity analyses were due to movement artifacts cannot be completely excluded. However, motion parameters from MRI were used as a covariate, and patients who moved a lot during the scan were excluded (patients that had more than 25% of invalid scans).

Another limitation was the small sample of ET patients, possibly due to the fact that patients were recruited at a tertiary movement disorder center, which tends to refer non-disabling cases to secondary services.

Finally, another pitfall is that we have not sub-segregated thalamus nuclei, because the atlas we chose for segmentation did not offer this option. However, it is known from previous works that the VIM nucleus of the thalamus is usually implicated in tremor physiology(38). In our opinion, these sub nuclei are too small and could not survive the kernel gaussian filter and subsequent analysis.

We believe that one of the strengths of our study is that we used very strict pre-processing parameters in order to control for motion and artifacts. Moreover, our statistical analysis was very conservative, according to current tendencies of the international neuroimaging community. Another strong point, besides strict

pre-processing and statistics, was our large sample, high-quality equipment and very short TR (0.6s).

The results lead us to speculate whether the areas implicated in the decreased connectivity – SMA and postcentral gyrus – could be possible targets for TMS or tDCS. Future studies should focus on pre and post intervention on those areas to evaluate a possible increase in GE, which in turn could be translated into clinical improvement.

Regarding the collected data, we could also correlate global efficiency of other networks – such as the Default Mode Network (DMN) or the salience network – to neuropsychological data such as the Mini-Mental State Examination, depression clinical scales (Hamilton, Beck and Zung), and the presence of hallucinations.

Yet another point of discussion concerning the fMRI field is its potential as a diagnostic tool or biomarker. A review on that matter has shown that a pattern characterized by increased activity in temporal cortex, parietal lobule, precuneus, cerebellum and thalamus, and a decreased activity in SMA, occipital cortex, middle frontal gyrus and striatum had an accuracy of 90% in discriminating PD from HC. Another study by Zhang, D. et. al. suggested that local, but not global efficiency, was able to differentiate PD subtypes. The performance of GE in distinguishing the PD subtypes depended on the presence or not of the cerebellum in the network analyzed(84).

Therefore, our current work demonstrates decreased connectivity of the motor system in PD in comparison to controls, which we hope brings a relevant contribution to a growing body of studies on the network disruption in Parkinson's disease and Essential Tremor. Hopefully, it might also contribute to the development of new strategies of intervention. It is expected that the work will also help the international research community on this quest for a better diagnosis on PD based on fMRI(113).

7. Conclusions

We concluded that functional connectivity is decreased in PD group when compared to HC.

Pairwise comparisons (Seed-to-voxel and ROI-to-ROI) did not show statistically significant differences in PD, ET and HC groups according to our parameters of functional analysis.

Graph theory network analyses showed that functional connectivity measured by the Global Efficiency of the motor network in PD is decreased in comparison to HC. This decrease is due specially to the role of the left supplementary motor area and post-central gyrus bilaterally.

Global Efficiency of the motor network was not different between HC and ET.

Tremor scores alone correlated positively with GE of the network in both PD subgroups, but were not statistically different between them (p 0.75). Tremor scores correlated positively to the GE of the left substantia nigra in both groups, and were statistically different between them (p=0.001), being greater in the PDAR group.

8. Annexes

8.1 Annex A

UK Parkinson's Disease Society Brain Bank Diagnostic Criteria

Step 1. Diagnosis of Parkinsonian Syndrome

- Bradykinesia
- At least one of the following
    - Muscular rigidity
    - 4-6 Hz rest tremor
    - postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction

Step 2. Exclusion criteria for Parkinson's disease

- history of repeated strokes with stepwise progression of parkinsonian features
- history of repeated head injury
- history of definite encephalitis
- oculogyric crises
- neuroleptic treatment at onset of symptoms
- more than one affected relative
- sustained remission
- strictly unilateral features after 3 years
- supranuclear gaze palsy
- cerebellar signs
- early severe autonomic involvement
- early severe dementia with disturbances of memory, language, and praxis
- Babinski sign
- presence of cerebral tumor or communication hydrocephalus on imaging study
- negative response to large doses of levodopa in absence of malabsorption

- MPTP exposure

Step 3. Supportive prospective positive criteria for Parkinson's disease

- Three or more required for diagnosis of definite Parkinson's disease in combination with step one
    - Unilateral onset
    - Rest tremor present
    - Progressive disorder
    - Persistent asymmetry affecting side of onset most
    - Excellent response (70-100%) to levodopa
    - Severe levodopa-induced chorea
    - Levodopa response for 5 years or more
    - Clinical course of ten years or more

8.2 Annex B

Hoehn & Yahn Scale

Stage	Hoehn and Yahr Scale
1	Unilateral Involvement only, usually with minimum or no functional disability
2	Bilateral or midline involvement without impairment of balance
3	Bilateral disease: mild to moderate disability with impaired postural reflexes; physically independent
4	Severely disabling disease; still able to walk or stand unassisted
5	Confinement to bed or wheelchair unless aided

8.3 Annex C

Essential Tremor MDS Diagnostic Criteria

Essential tremor

1) isolated tremor syndrome of bilateral upper limb action tremor

2) at least 3 years' duration

3) with or without tremor in other locations (e.g., head, voice, or lower limbs)

4) absence of other neurological signs, such as dystonia, ataxia, or parkinsonism.

Essential tremor plus: Tremor with the characteristics of ET and additional neurological signs of uncertain significance such as impaired tandem gait, questionable dystonic posturing, memory impairment, or other mild neurologic signs of unknown significance that do not suffice to make an additional syndrome classification or diagnosis. ET with tremor at rest should be classified here.

Exclusion criteria for ET and ET plus:

- Isolated focal tremors (voice, head)
- Orthostatic tremor with a frequency >12 Hz • Task- and position-specific tremors
- Sudden onset and step-wise deterioration

8.4 Annex D

Unified Parkinson's Disease Rating Scale (UPDRS)

I. MENTATION, BEHAVIOR AND MOOD

1. Intellectual Impairment
0 = None.
1 = Mild. Consistent forgetfulness with partial recollection of events and no other difficulties.
2 = Moderate memory loss, with disorientation and moderate difficulty handling complex problems. Mild but definite
impairment of function at home with need of occasional prompting.
3 = Severe memory loss with disorientation for time and often to place. Severe impairment in handling problems.
4 = Severe memory loss with orientation preserved to person only. Unable to make judgements or solve problems.
Requires much help with personal care. Cannot be left alone at all.

2. Thought Disorder (Due to dementia or drug intoxication)
0 = None.
1 = Vivid dreaming.
2 = "Benign" hallucinations with insight retained.
3 = Occasional to frequent hallucinations or delusions; without insight; could interfere with daily activities.
4 = Persistent hallucinations, delusions, or florrid psychosis. Not able to care for self.

3. Depression
1 = Periods of sadness or guilt greater than normal, never sustained for days or weeks.
2 = Sustained depression (1 week or more).
3 = Sustained depression with vegetative symptoms (insomnia, anorexia, weight loss, loss of interest).
4 = Sustained depression with vegetative symptoms and suicidal thoughts or intent.

4. Motivation/Initiative
0 = Normal.
1 = Less assertive than usual; more passive.
2 = Loss of initiative or disinterest in elective (nonroutine) activities.
3 = Loss of initiative or disinterest in day to day (routine) activities.
4 = Withdrawn, complete loss of motivation.

II. ACTIVITIES OF DAILY LIVING (for both "on" and "off")

5. Speech
0 = Normal.
1 = Mildly affected. No difficulty being understood.
2 = Moderately affected. Sometimes asked to repeat statements.
3 = Severely affected. Frequently asked to repeat statements.
4 = Unintelligible most of the time.

6. Salivation
0 = Normal.
1 = Slight but definite excess of saliva in mouth; may have nighttime drooling.
2 = Moderately excessive saliva; may have minimal drooling.
3 = Marked excess of saliva with some drooling.
4 = Marked drooling, requires constant tissue or handkerchief.

7. Swallowing
0 = Normal.
1 = Rare choking.
2 = Occasional choking.
3 = Requires soft food.
4 = Requires NG tube or gastrotomy feeding.

8. Handwriting
0 = Normal.
1 = Slightly slow or small.
2 = Moderately slow or small; all words are legible.
3 = Severely affected; not all words are legible.
4 = The majority of words are not legible.

9. Cutting food and handling utensils
0 = Normal.
1 = Somewhat slow and clumsy, but no help needed.
2 = Can cut most foods, although clumsy and slow; some help needed.
3 = Food must be cut by someone, but can still feed slowly.
4 = Needs to be fed.

10. Dressing
0 = Normal.
1 = Somewhat slow, but no help needed.
2 = Occasional assistance with buttoning, getting arms in sleeves.
3 = Considerable help required, but can do some things alone.
4 = Helpless.

11. Hygiene
0 = Normal.
1 = Somewhat slow, but no help needed.
2 = Needs help to shower or bathe; or very slow in hygienic care.
3 = Requires assistance for washing, brushing teeth, combing hair, going to bathroom.
4 = Foley catheter or other mechanical aids.

12. Turning in bed and adjusting bed clothes
0 = Normal.
1 = Somewhat slow and clumsy, but no help needed.
2 = Can turn alone or adjust sheets, but with great difficulty.
3 = Can initiate, but not turn or adjust sheets alone.
4 = Helpless.

13. Falling (unrelated to freezing)
0 = None.
1 = Rare falling.
2 = Occasionally falls, less than once per day.
3 = Falls an average of once daily.
4 = Falls more than once daily.

14. Freezing when walking

0 = None.
1 = Rare freezing when walking; may have starthesitation.
2 = Occasional freezing when walking.
3 = Frequent freezing. Occasionally falls from freezing.
4 = Frequent falls from freezing.

15. Walking
0 = Normal.
1 = Mild difficulty. May not swing arms or may tend to drag leg.
2 = Moderate difficulty, but requires little or no assistance.
3 = Severe disturbance of walking, requiring assistance.
4 = Cannot walk at all, even with assistance.

16. Tremor (Symptomatic complaint of tremor in any part of body.)
0 = Absent.
1 = Slight and infrequently present.
2 = Moderate; bothersome to patient.
3 = Severe; interferes with many activities.
4 = Marked; interferes with most activities.

17. Sensory complaints related to parkinsonism
0 = None.
1 = Occasionally has numbness, tingling, or mild aching.
2 = Frequently has numbness, tingling, or aching; not distressing.
3 = Frequent painful sensations.
4 = Excruciating pain.

III. MOTOR EXAMINATION

18. Speech
0 = Normal.
1 = Slight loss of expression, diction and/or volume.
2 = Monotone, slurred but understandable; moderately impaired.
3 = Marked impairment, difficult to understand.
4 = Unintelligible.

19. Facial Expression

0 = Normal.
1 = Minimal hypomimia, could be normal "Poker Face".
2 = Slight but definitely abnormal diminution of facial expression.
3 = Moderate hypomimia; lips parted some of the time.
4 = Masked or fixed facies with severe or complete loss of facial expression; lips parted 1/4 inch or more.

20. Tremor at rest (head, upper and lower extremities)
0 = Absent.
1 = Slight and infrequently present.
2 = Mild in amplitude and persistent. Or moderate in amplitude, but only intermittently present.
3 = Moderate in amplitude and present most of the time.
4 = Marked in amplitude and present most of the time.

21. Action or Postural Tremor of hands
0 = Absent.
1 = Slight; present with action.
2 = Moderate in amplitude, present with action.
3 = Moderate in amplitude with posture holding as well as action.
4 = Marked in amplitude; interferes with feeding.

22. Rigidity (Judged on passive movement of major joints with patient relaxed in sitting position. Cogwheeling to be ignored.)
0 = Absent.
1 = Slight or detectable only when activated by mirror or other movements.
2 = Mild to moderate.
3 = Marked, but full range of motion easily achieved.
4 = Severe, range of motion achieved with difficulty.

23. Finger Taps (Patient taps thumb with index finger in rapid succession.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or

arrests in ongoing movement.
4 = Can barely perform the task.

24. Hand Movements (Patient opens and closes hands in rapid succesion.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

25. Rapid Alternating Movements of Hands (Pronation-supination movements of hands, vertically and horizontally, with as large an amplitude as possible, both hands simultaneously.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

26. Leg Agility (Patient taps heel on the ground in rapid succession picking up entire leg. Amplitude should be at least 3 inches.)
0 = Normal.
1 = Mild slowing and/or reduction in amplitude.
2 = Moderately impaired. Definite and early fatiguing. May have occasional arrests in movement.
3 = Severely impaired. Frequent hesitation in initiating movements or arrests in ongoing movement.
4 = Can barely perform the task.

27. Arising from Chair (Patient attempts to rise from a straightbacked chair, with arms folded across chest.)
0 = Normal.
1 = Slow; or may need more than one attempt.

2 = Pushes self up from arms of seat.
3 = Tends to fall back and may have to try more than one time, but can get up without help.
4 = Unable to arise without help.

28. Posture
0 = Normal erect.
1 = Not quite erect, slightly stooped posture; could be normal for older person.
2 = Moderately stooped posture, definitely abnormal; can be slightly leaning to one side.
3 = Severely stooped posture with kyphosis; can be moderately leaning to one side.
4 = Marked flexion with extreme abnormality of posture.

29. Gait
0 = Normal.
1 = Walks slowly, may shuffle with short steps, but no festination (hastening steps) or propulsion.
2 = Walks with difficulty, but requires little or no assistance; may have some festination, short steps, or propulsion.
3 = Severe disturbance of gait, requiring assistance.
4 = Cannot walk at all, even with assistance.

30. Postural Stability (Response to sudden, strong posterior displacement produced by pull on shoulders while patient erect with eyes open and feet slightly apart. Patient is prepared.)
0 = Normal.
1 = Retropulsion, but recovers unaided.
2 = Absence of postural response; would fall if not caught by examiner.
3 = Very unstable, tends to lose balance spontaneously.
4 = Unable to stand without assistance.

31. Body Bradykinesia and Hypokinesia (Combining slowness, hesitancy, decreased armswing, small amplitude, and poverty of movement in general.)
0 = None.
1 = Minimal slowness, giving movement a deliberate character; could be

normal for some persons. Possibly reduced amplitude.
2 = Mild degree of slowness and poverty of movement which is definitely abnormal. Alternatively, some reduced amplitude.
3 = Moderate slowness, poverty or small amplitude of movement.
4 = Marked slowness, poverty or small amplitude of movement.

IV. COMPLICATIONS OF THERAPY (In the past week)

A. DYSKINESIAS

32. Duration: What proportion of the waking day are dyskinesias present? (Historical information.)
0 = None
1 = 1-25% of day.
2 = 26-50% of day.
3 = 51-75% of day.
4 = 76-100% of day.

33. Disability: How disabling are the dyskinesias? (Historical information; may be modified by office examination.)
0 = Not disabling.
1 = Mildly disabling.
2 = Moderately disabling.
3 = Severely disabling.
4 = Completely disabled.

34. Painful Dyskinesias: How painful are the dyskinesias?
0 = No painful dyskinesias.
1 = Slight.
2 = Moderate.
3 = Severe.
4 = Marked.

35. Presence of Early Morning Dystonia (Historical information.)
0 = No
1 = Yes

B. CLINICAL FLUCTUATIONS

36. Are "off" periods predictable?
0 = No
1 = Yes

37. Are "off" periods unpredictable?
0 = No
1 = Yes

38. Do "off" periods come on suddenly, within a few seconds?
0 = No
1 = Yes

39. What proportion of the waking day is the patient "off" on average?
0 = None
1 = 1-25% of day.
2 = 26-50% of day.
3 = 51-75% of day.
4 = 76-100% of day.

C. OTHER COMPLICATIONS

40. Does the patient have anorexia, nausea, or vomiting?
0 = No
1 = Yes

41. Any sleep disturbances, such as insomnia or hypersomnolence?
0 = No
1 = Yes

42. Does the patient have symptomatic orthostasis? (Record the patient's blood pressure, height and weight on the scoring form)
0 = No
1 = Yes

8.5 Annex E

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Global Efficiency of the Motor Network is decreased in Parkinson's Disease in Comparison to Essential Tremor and Healthy Controls

Abstract

Background: Graph Theory (GT) is a mathematical field that analyzes complex networks that can be applied to neuroimaging to quantify brain's functional systems in Parkinson's Disease (PD) and Essential Tremor (ET).

Objectives: To evaluate the functional connectivity (FC) measured by the Global Efficiency (GE) of the motor network in PD and compare it to ET and Healthy Controls (HC), and correlate it to clinical parameters.

Methods: 103 subjects (54PD, 18ET, 31HC) were submitted to structural and functional MRI. A network was designed with Regions of Interest (ROIs) involved in motor function and GT was applied to determine its GE. Clinical parameters were analyzed as covariates to estimate the impact of disease severity and medication on GE.

Results: GE of the motor circuit was reduced in PD in comparison to HC (p 0.042). Areas that most contributed to it were left supplementary motor area (SMA) and bilateral postcentral gyrus. Tremor scores correlated positively with GE of the motor network in PD subgroups. For ET, there was an increase in the connectivity of the anterior cerebellar network to the other ROIs of the motor circuit in comparison to PD.

Conclusions: FC measured by the GE of the motor network is diminished in PD in comparison to HC, especially due to decreased connectivity of left SMA and bilateral postcentral gyrus. This finding supports the theory that there is a global impairment of the motor network in PD, and it does not affect just the basal ganglia, but also areas associated with movement modulation.

Introduction

Even though Parkinson's Disease (PD) and Essential Tremor (ET) are the most prevalent movement disorders and the most frequent causes of tremor, their pathophysiologies are not completely understood. Tremor physiology involves disruption in several areas of motor network, specially the cerebello-thalamo-cortical circuit and the basal ganglia. Functional Magnetic Resonance Imaging (fMRI) has been used to enlighten the knowledge of how brain networks are disrupted in these diseases.

In PD, it has been demonstrated that the interaction among different brain areas are implicated in the genesis of oscillatory activity that underlies clinical symptoms. According to Helmich et al., the cerebello-thalamo-cortical network would be the responsible for generating tremor in PD, triggered and modulated by the basal ganglia network(114). Deep Brain Stimulation (DBS) paradigms with closed-loops that stimulates the internal globus pallidus (GPi) after detecting activity in the motor cortex considers the modulation of these network properties rather than stimulating a single structure alone(115), advocating that DBS would exert its function reducing the abnormal oscillatory activity in the cortico-basal ganglia network. The latter study also suggested that such oscillations are pathological, giving that targeting directly this activity with double-tremor frequency, led to a greater alleviation of parkinsonian symptoms. Therefore, the study of motor networks in pathological state can bring new therapeutic prospective such as DBS or Transcranial Magnetic Stimulation (TMS) focusing not in determined targets, but in network modulation.

Previous fMRI studies have shown that motor network is affected in ET, with diminished functional connectivity between cerebellum and cortex that correlates with symptoms intensity, and also an increased connectivity between right cerebellar lobules I-IV and left thalamus(67). These findings suggest that cerebello-dentato-thalamic activity and cerebello-cortical connectivity are disrupted in ET. There is large evidence of cerebellar disfunction in ET: cerebellar stroke can ameliorate ipsilateral tremor (116); spectroscopy studies showed reduction in N-acetilaspartate in cerebellum of ET patients(46); voxel-based-morphometry showed cerebellar atrophy(47); anatomopathological studies showed a loss of Punkinje cells, dendritic edema and an increase in their axonal ramifications(48). In line with that, one open-label study performed repetitive TMS of the cerebellum and showed that not only the clinical tremor scores improved but also the functional connectivity of the cerebello-thalamo-cortical network was reestablished(117). Moreover, it has been long known that Ventral Intermediate (VIM) DBS partially restores the cerebello-thalamo-cortical pathway and reduces tremor(118). A recent systematic review of cerebellar neuromodulation through different techniques in movement disorders suggests that cerebellar modulation improved tremor in ET(110).

Several fMRI studies have already been performed in order to investigate the motor network in PD and ET, mostly focusing on pairwise comparisons such as seed-to-

voxel and voxel-to-voxel connectivity. Graph theory (GT) is the mathematical field that analyzes complex networks, and has been applied to neuroimaging data to quantify brain's functional systems(34). One of the measures of GT is Global Efficiency (GE), which is a frequently used metric to study integration within brain networks. Mathematically, efficiency is defined as the inverse of the path length, which means the minimum number of edges that the information takes to travel from one point to another. Therefore, short path lengths mean high GE.

So, the first aim of this project was to evaluate the functional connectivity measured by the global efficiency of the motor network in PD and compare it to ET and healthy controls (HC), and to assess possible differences between PD subgroups; the second aim was to correlate the global efficiency of the motor network to clinical parameters such as the Unified Parkinson's Disease Rating Scale (UPDRS), tremor scores and Levodopa Equivalent Dose (LED). We hypothesized that there would be a decreased global efficiency of the motor network in PD patients when compared to controls and ET patients, and that this decrease would be related to clinical parameters such as tremor scores. We also hypothesized that ET patients would show different connectivity patterns from controls, specially involving the cerebellum.

Methods

Patient Selection

Patients treated at the Movement Disorders Clinic of *Hospital das Clínicas da Universidade de São Paulo* and *Associação Brasil Parkinson* were invited to participate in this study, after its approval by the local ethics committee (Online registration n° 8197, CAPPesq n° 0700/11). All individuals signed the Term of Consent. Inclusion criteria for PD patients were: to have idiopathic PD, according to the UK Brain Bank Criteria(85), and Hoehn & Yahr I or II(86); for ET patients, the diagnostic criteria established by the Movement Disorders Society (22), absence of parkinsonism and exclusion of medication-induced tremor. For healthy controls, criteria included absence of neurological diseases, and age pairing with the patients' group. (Table 1) Exclusion criteria were impossibility of performing an MRI, a severe movement disorder that would impair imaging analysis, and intracranial structural abnormalities.

PD patients were classified by the UPDRS (Unified Parkinson's Disease Rating Scale)(87), and were submitted to MRI scans while under effect of Levodopa (ON state). This decision could pose questions whether the results of the fMRI would be due to the physiopathological changes in PD brain or due to medication effects(88). However, many patients do not tolerate the MRI scan in OFF state (without effect of medication), due to tremor, axial rigidity, pain and discomfort. Moreover, analysis of fMRI during the ON state would be closer to patient daily activities scenario. The Levodopa Equivalent Dose (LED) was calculated for each subject using the Birmingham University conversion formula and it was used as a covariate in statistical analysis. Patients were subdivided into two

subgroups – tremulant-predominant group (PDT) and akinetic-rigid group (PDAR), according to criteria proposed by Jankovic (6). We calculated the average between the sum of the UPDRS items associated with tremor (16, 20 and 21) and the sum of items associated to rigidity, gait and postural instability (13, 14, 15, 29 and 30). Patients with average equal or below 1 were classified as PDAR and patients with average above or equal to 1.5 were classified as PDT.

Image Acquisition

fMRI scans were performed at Siemens Trio Scanner 3.0T MR, with 32 channels head coil and gradient of 45mT/m. Whole brain volumes were acquired by the echo planar imaging (EPI) multi-band accelerated sequence with repetition time (TR) of 600ms(90). BOLD sensitive images were acquired on T2*-weighted sequences. Acquisition parameters were: 40 axial cuts, slice thickness= 2.5mm, TE= 31ms, NEX= 1, Flip angle= 90º, Bandwidth= 2290Hz/px, FOV= 210mm, matrix size= 84x84, voxel dimension= 2.5mmx2.5mmx2.5mm. All images were acquired while patients were lying still with eyes closed (resting state). For co-registration and normalization, structural images with high resolution were also acquired (MP2RAGE).

Image Pre-Processing

Data were preprocessed and analyzed using the CONN toolbox version 17.b(91), with a standard MNI152 pipeline and parameters. Preprocessing steps included realignment and unwarping, slice-timing correction, segmentation, normalization, outlier detection, and smoothing. Nuisance variables were based on scan motion censuring (discarding volumes with displacement >2mm and global-signal z-value >9; no subjects were excluded), 12 realignment parameters, white matter and cerebrospinal fluid signals. Band-pass filtering (0.008-0.09Hz) and nuisance variables were regressed out using a simultaneous bandpass approach(92).

ROI Selection

Regions of interest (ROIs) were determined based on the Automated Anatomical Labeling Atlas (AAL)(93) and Harvard-Oxford Atlas(94). We manually created ROIs for the Denteate Nucleus and Substantia Nigra(95, 96) using MatlabR2018b. They were chosen due to their relevance to the motor network and that would be somehow implicated in the tremor physiopathology: denteate nucleus, cerebellar networks anterior and posterior, substantia nigra, thalamus, caudate, putamen, pallidum, precentral gyrus, postcentral gyrus and supplementary motor area. ROIs are displayed in figure 1.

Connectivity estimation and analysis

Graph theory analyses were also computed using the CONN toolbox. We focused on the global efficiency metric since it has been shown to be one of the most robust measures to assess integration properties of brain networks(97). It reflects effective information transfer within a network of nodes (i.e., ROIs) and edges (i.e., correlations or “paths” between nodes)(59). It is mathematically expressed as the inverse of the average shortest path length in a graph *G* to all other nodes in the graph. The

unweighted ROI-to-ROI correlation matrices of the motor network (one for each participant) were first submitted to a threshold at a cost value of k=0.15. Global efficiency indices were thresholded at p-FDR<0.05 in a two-sided analysis based on correlation scores. Independent sample t-tests were performed to examine differences between groups on global efficiency scores (PD, ET and HC), two at a time. Statistical analyses were conducted by using JASP with p<0.05, one-tailed.

Clinical parameters (UPDRS, tremor scores alone from UPDRS and levodopa equivalent dose) were analyzed as covariates for the PD group to estimate the impact of disease severity and medication on the network connectivity.

Results

Differences between clinical diagnosis on the Global Efficiency of the motor network

Global Efficiency of the motor network was reduced in PD Group when compared to HC ($t(77)=-1.749$, $p=0.042$). The average GE of the network in PD patients was 0.0231, versus 0.0297 for controls. Left SMA had the lowest GE (0.022 FDR-corrected) in the PD group, followed by Postcentral gyrus left (0.0496 FDR-corrected) and Postcentral gyrus right (0.0496 FDR-corrected) (Figure 2). In order to better interpret these results of GE - particularly what is the meaning of the ROIs displayed on the CONN output results - we calculated for each ROI of the network the average number of connections obtained by the adjacency matrix, as shown in figure 3. These values confirm that the average number of connections is larger in the HC group. Therefore, a reduced global efficiency in PD is possibly driven by a lower number of connections of these three nodes (left SMA, Left and Right Postcentral gyrus) within the motor network in this group.

For Essential Tremor, there was no difference in GE of the motor network when compared to HC nor to PD. However, there was an increase in the connectivity of the anterior cerebellar network to the other ROIs of the motor network in the ET group when compared to the PD group. There was also no difference between the two subgroups of PD themselves, neither when compared to ET and HC.

Covariates Analysis

UPDRS Scores did not correlate to GE in PD groups. Tremor scores alone correlated positively with GE of the network in both PD subgroups, but were not statistically different between them (p 0.75). Tremor scores correlated positively to the GE of the left substantia nigra in both groups, and were statistically different between them ($p=0.001$), being greater in the PDAR group.

LED was also used as a covariate to study the possible influence of the medication on GE, but no statistically significant result was found.

In order to exclude motor artifacts as possible confounders for connectivity analysis, we evaluated the correlations of mean motion and GE, and found no relation between them.

The data that support the findings of this study are available on request from the corresponding author.

Discussion

According to our previous hypothesis, motor network connectivity measured by the GE is decreased in Parkinson's disease in comparison to healthy controls. Areas that most contributed to this diminished connectivity were left SMA and bilateral postcentral gyrus. Tremor scores correlated positively to the GE of the network in PD, and influenced the connectivity of the left substantia nigra differently in both groups, especially in the PDAR group.

Other studies have also evaluated the GE of the motor network, with conflicting outcomes. In accordance to our results, one study analyzed longitudinally 16 PD patients and showed decreased connectivity between the SMA, pre and postcentral gyri when compared to controls(98). In addition, another group with a larger number of participants demonstrated that GE is decreased in PD patients when compared to HC, and such decrease correlates with cerebrospinal fluid biomarkers (CSF), such as alfa-synuclein, $A\beta_{42}$ and total TAU. The data suggest that functional imaging and measurement of CSF biomarkers together can bring a better understanding of PD pathogenesis(76). However, Hou et. al. (77) and Berman et. al. (82) found no global level abnormalities, while Guan et. al.(69) have found increased GE, which correlated positively to iron accumulation in the inferior SN.

We know SMA plays a direct role in movement control thru direct projections to the spinal cord, and also throughout interactions with other structures to help control postural stability, bimanual coordination, sequences of movements and initiation of internally generated movement. Previous studies have reported SMA connectivity disruption in PD, with controversial results. A few studies showed enhanced connectivity of the SMA to the putamen and amygdala(100) and increased pre-SMA activation in early PD patients while performing self-initiated movements(101). Another study also using graph theory to measure network interactions showed decreased connectivity in SMA, left dorsolateral prefrontal cortex and left putamen(102). The latter study compared PD ON and OFF medication, and this decreased connectivity of SMA persisted on both states. Other methods such as PET and SPECT have also shown decreased SMA activation in comparison to controls during internally triggered movements(103), and increased activation in SMA in PD patients under DBS(104) or apomorphine(105). Therefore, SMA may play an important role in PD physiopathology and should be considered as a target in neuromodulation studies.

The postcentral gyrus is the primary sensorial area, but we wondered if it could also be involved in tremor modulation because of its peripheral proprioceptive information for the movement feedback loops. Another question is if its strong connections with the thalamus would play a role in tremor control. One previous research has shown diminished activation in somatosensory cortex in PD after tactile stimulation(106). A systematic review and meta-analysis englobed thirty studies, with a total of 854 PD patients, and emphasized the role of postcentral gyrus as a critical region in PD. They have found an increased functional connectivity in post-central gyrus in PD compared to healthy controls(107).

The fact that SN connectivity was influenced differently by tremor scores in both subgroups of PD could raise the question to whether this area would be implicated in the differences of clinical manifestation between them. Furthermore, contrary to our expectations, global efficiency was positively correlated to tremor scores. One study found no relation of GE and motor severity(80), and another research using apomorphine described a reduction of tremor mirrored by an increase in overall connectivity strength, but they did not use the same network measures as we did(72). Therefore, the biological interpretation of these values is not straightforward.

Regarding ET, the fact that the connectivity of the anterior cerebellar network was increased to the other regions of the motor network when compared to PD reinforces its role in the pathogenesis of tremor is this disease. The cerebellum integrates multimodal sensorimotor inputs from the cortex, vestibular nuclei and spinal cord and modulates the information thru outputs to the cerebral cortex, across the cerebello-thalamo-cortical tract, so that movement is harmonic and smooth. (108) One fMRI study using graph theory analysis found disruption in the efficiency of the overall brain functional network in ET, involving multiple areas of the brain. In the global level, ET patients exhibited lower small-worldness values than HC, and at the regional level, showed higher values of GE in several cortical and cerebellar areas(109).

A limitation of our study, as already mentioned and justified, is that PD patients were scanned under the effect of Levodopa to minimize motion and discomfort during scanning. There have been previous reports in the literature suggesting that levodopa could be a confounding factor for connectivity analyses(88), with a tendency to “normalize” network measures such as local efficiency(82). A network study comparing patients in ON and OFF states showed that levodopa normalized degree centrality abnormalities specially in occipital regions and postcentral gyrus(111). However, we used LED as a covariate to study its effect on the GE of the network, and found no correlation. Another limitation was the small sample of ET patients, possibly due to the fact that patients were recruited at a tertiary movement disorder center, which tends to refer non-disabling cases to secondary services.

We believe that one of the strengths of our study is that we used very strict pre-processing parameters in order to control for motion and artifacts. Moreover, our

statistical analysis was very conservative, according to current tendencies of the international neuroimaging community. Another strong point, besides strict pre-processing and statistics, was our large sample, high-quality equipment and very short TR (0.6s).

Therefore, our current work demonstrates decreased connectivity of the motor system in PD in comparison to controls, giving a relevant contribution to a growing body of studies on the network disruption in Parkinson's disease and Essential Tremor, and hopefully might contribute to the development of new strategies of intervention such as DBS or TMS to better treat PD and ET.

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1. Research Project: A. Conception: Prof. Dr. Ellison F Cardoso, Prof. Dr. Edson Amaro and Prof. Dr. Egberto R Barbosa. B: Organization: Prof. Dr. Ellison F Cardoso. C: Execution: Dr. Fabiana C Hirata supervised all the MRI acquisitions. Dr. Luciano Melo and Dr. Natalia P Novaes were responsible for patient's recruitment and clinical evaluation.
2. Connectivity and Statistical Analysis: A. Design: Dr. Ellison F Cardoso, Dr. Joao R Sato. Execution: Dr. Joana B Balardin, Dr. Natalia P Novaes. C: Review and Critique: Dr. Joao R Sato.
3. Manuscript Preparation: A. Writing of first draft: Dr. Natalia P Novaes. B: Review and Critique: Dr. Joana B Balardin, Dr. Ellison F Cardoso

Financial Disclosures of all authors

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10. Milestones of this Project

I have started my *Movement Disorders Fellowship* at Hospital das Clínicas da Universidade de São Paulo in 2013. Our Movement Disorders group has an enormous number of patients and some of the best specialists in the field in Brazil. It is a group largely involved in research, teaching, as well as in excellent patient care. Due to our strong collaboration with the Radiology department, I had the opportunity to engage in this endeavor in 2014.

This research was part of a larger project from Institute of Radiology and Institute of Neurology of HCFMUSP and *Instituto Israelita de Ensino e Pesquisa*[33] from HIAE, involving a large group of patients and researchers. It was supported by *FAPESP* - Protocol number 2011/18747-0.

Our group has previously published two papers from this same study. The first used the Diffusion Tensor Imaging (DTI) technique to study substantia nigra as a possible marker for PD. A meta-analysis was also performed to compare it to the rest of data available in literature, and the conclusion was that fractional anisotropy of substantia nigra DTI is not a biomarker of PD(119).

The second paper was about the Default Mode Network, studied from resting state fMRI in PD in comparison to HC, and it showed decreased connectivity from precuneus to motor system regions in PD(120).

An important step of this process was the *CONN toolbox course* at Harvard (Boston, U.S.A.), in 2018, at the Athinoula A. Martinos Center for Biomedical Imaging, which enabled me to better use the software and all its functionalities for connectivity analyses. Part of my trip was sponsored by HIAE. The course was taught by Dr. Susan Whitfield-Gabrieli and Dr. Alfonso Nieto-Castanon, the creators of CONN.

Another landmark was my internship at *Hôpital de La Pitié-Salpêtriére* in Paris, in 2018, under the supervision of Prof. Emmanuel Flamand-Roze and Prof. Marie Vidailhet, where I had the opportunity to observe other fMRI projects,

[33] Israeli Institute of Teaching and Research

discuss the subject with several researchers, and enlarge my clinical experience in Movement Disorders.