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Deep recordings of brain activities in dystonic patients: preliminary results

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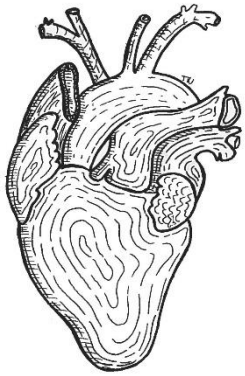
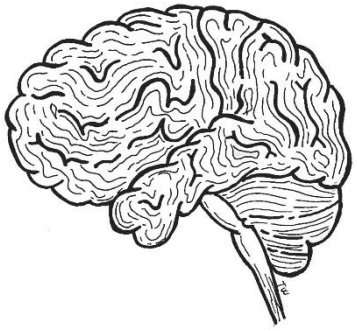
Ringraziamenti

Alla fine di ogni percorso i ringraziamenti sono un obbligo da cui nessuno può esimersi. Questo elaborato non sarebbe stato possibile senza l'aiuto della mia relatrice e i ragazzi del Sanger Lab.



Non banalizzando e non limitando ringraziamenti alla sola tesi, ringrazio sinceramente i miei genitori e mia sorella che mi hanno supportato in questi anni non solo economicamente. Paola sostenitrice delle avventure universitarie e non, senza lei preparare le materie sarebbe stato tanto noioso e poco proficuo! Le sere a sostenerci a vicende e le giornate presame passate insieme sono uno dei ricordi più belli che mi porterò dietro!



Capitolo importante è senza dubbio casa Gozzano, la casa più bella di Milano. Alessandro e 'Michele' mi hanno sostenuto dal primo giorno di convivenza, sopportandomi e alleggerendo le fatiche universitarie, le notti a suonare in balcone e le, ahimè, passate feste di casa Gozzano sono indelebili nella mia mente. Non posso non escludere: Sara, Tea, Jacopo, Emanuele, EB, BSBE e tanti altri.

Abstract

Dystonia in children patients is different than adults: etiopathogenesis and history of disorders change; disorder can compromise acquisition of movement skills and children development; coexist different motor disorder; dystonia in children tends to become generalized and symptoms have variability also in same form of the syndrome; it is difficult to choose the best therapy for patients and there are anatomical differences between brain in adults and in children.

Sanger's Lab from University of Southern California, in collaboration with Los Angeles Children Hospital, are focalizing its studies on data acquired from deep brain. Specifically, patients involved are children and they are affected by secondary dystonia.

In patients with secondary dystonia application of deep brain stimulation do not provide same benefits than patients with primary dystonia. Data acquired from patients, after surgical implantation of electrodes, used for deep brain treatments, are combined with videos. Each video reports a patients' frame of recovery in the hospital.

This thesis starts from video analysis for aiming basically to distinguish three aspects of dystonic patients: *spasm* (and *involuntary movements)*, *rest* and *voluntary movements*. Each patient's activity has been divided in periods and for each period has been created an Excel table containing all information about *condition* of patients. Results of video analysis have been used to creates video table in which is reported a summary, in percentage, of information included in video analysis. Sanger's lab has used these to make statistical analysis with detected spikes, joining videos and neural activity.

Sommario

La distonia infantile presenta caratteristiche diverse rispetto a quelle presenti nei pazienti adulti. Oltre alle differenze nell'eziopatogenesi e nei disturbi, nei pazienti non adulti sono talvolta compromesse le capacità di acquisizione dei movimenti, coesistono diversi disturbi motori e si riscontra una forte variabilità nei sintomi sebbene i pazienti siano affetti dalla stessa forma di distonia. Altre differenze rimandano alle difficoltà legate alla scelta della terapia più adatta ed alle differenze anatomiche.

Il Sanger Lab dell'University of Southern California, in collaborazione con il Los Angeles Children Hospital, sta focalizzando i suoi studi sui dati acquisiti dai nuclei più interni del sistema nervoso coinvolti nello studio della distonia. Nello specifico i pazienti studiati sono affetti da distonia secondaria e non sono adulti. La terapia utilizzata su questi soggetti è la stimolazione elettrica dei nuclei più profondi del cervello, anche se, come mostrato in letteratura, i benefici della terapia non sono gli stessi rispetto a quelli ottenuti, in seguito alla deep brain stimulation (DBS), su soggetti con distonia primaria.

Nel presente elaborato i dati relativi all'attività neuronale sono stati uniti con i dati provenienti dall'analisi dei video. Durante il ricovero in ospedale ogni paziente è stato filmato sia durante le sue attività quotidiane sia durate i momenti in cui era sottoposto ad analisi e test. L'attività di ciascun paziente è stata divisa in periodi e per ciascuno di essi è stata creata una tabella contenente tutte le informazioni sulle condizioni del paziente. L'obiettivo è di distinguere le fasi in cui il paziente è soggetto a spasmi o movimenti involontari, dalle fasi in cui è a riposo o quelle in cui volontariamente si muove. I dati provenienti da queste analisi sono stati usati dal team del Sanger Lab per le analisi statistiche che uniscono l'attività neuronale dei pazienti alle informazioni ottenute dall'analisi video.

CHAPTER 1: Introduction

In this chapter it will be introduced general concepts about dystonia, possible categories described in literature about the syndrome, therapies used for dystonic patients and diagnosis used in medicine. It will focus on secondary dystonia, since patients involved in this study are affected by this specific syndrome. It will examine the Deep brain stimulation. The technique will be described at the begin paying specific attention on history of electric stimulation, describing main concepts of the procedure used for treating dystonia and focusing on developments and updates. The chapter ends describing the work done on dystonic patients in Los Angeles Children Hospital. The hospital's team, in collaboration with the Sanger Lab's team, is studying syndrome by deep brain recording. All their studies about dystonia have constituted a starting point for this thesis.

1.1 Dystonia syndrome

Dystonia is neurological syndrome, that causes movement disorder characterized by intermitted or sustained muscle contractions, abnormal movement, tremors, involuntary spasm and anomalous posture. It may involve a single muscle, a group of muscles or a group of muscles throughout the body.
Sometimes it can be also painful and can affect both adults and children of all ages but generally in childhood dystonia tends to progress to generalization while in adulthood usually tends to concentrate in a specific district or body portion [11] [12] [13].

Dystonia symptoms are different and depend upon the form of dystonia. May include foot cramp or tendency for one foot to turn, after writing several lines the hand may drags or a worsens. In other cases, the neck may turn or pull involuntarily, especially when the person is tired or under stress, both eyes might blink rapidly and uncontrolled, they could close by spasms, patients could also have tremor or difficulties speaking.

In some cases, dystonia can affect only one specific action, while allowing others to occur unimpeded, it's the case of some musicians have dystonia when using his/her hand, for example, to play an instrument, but not when using the same hand to type. Activities and movements can change better or worse the phenomenology of the syndrome. It occurs, for example, when a tactile stimulus or voluntary movement on patient can reduce the effects: in this case feature is observed in many dystonia syndromes [11].

Dystonia is related to a malfunction of basal ganglia or other brain region that controls movement as thalamus, cerebellum and cerebellar cortex. Patients have abnormalities in the brain ability to process neurotransmitters that help cells in the brain to communicate with each other and have also abnormalities in the way of the brain to process information and generates command to move [12].

The choice and assessment of various therapeutic interventions is difficult for many reasons (*figure 1*). First of all, dystonia's symptoms are difficult to evaluate and quantify and it is a syndrome with different causes, with different distribution in patient's anatomy and with heterogenous clinical manifestations that creates variable disability [8]. Moreover, some patients have spontaneous remissions.
There are many forms of dystonia and many manifestations of the syndrome [13]:

- *Blepharospams* (affects the muscles of the eyelids and brow)
- *Cervical Dystonia* (affects neck and sometimes the shoulders)
- *Oromandibular dystonia* (forceful contractions of the face, jaw, and/or tongue)
- *Spasmodic dysphonia* (vocal cords)
- *Hand dystonia* (affects fingers, hand and/or forearm)
- *Lower limb dystonia* (affects leg, foot and/or toes)
- *Musician's dystonia* (task-specific)
- *Generalized dystonia* (generalized dystonia is characterized by twisting of the limbs and torso)
- *Dopa-responsive dystonia*
- *Myoclonus dystonia* (hereditary form of dystonia that includes prominent myoclonus symptoms)

- *Paroxysmal dystonia and dyskinesias* (episodic movement disorder, abnormal movements occur only during attacks)
- *X-linked dystonia-parkinsonism* (hereditary form of dystonia that involves symptoms of parkinsonism)
- *Rapid-onset dystonia-parknsonism* (hereditary form of dystonia that includes symptoms of parkinsonism)
- *Secondary dystonia* (triggered by factors such as trauma, medication exposure, toxins)

We can classify it according: anatomical distribution, age and cause.

Anatomical distribution classifies syndrome considering the body distribution. If one single part of the body is involved, the syndrome is called Focal dystonia (*cervical dystonia*, *blepharospasm*, *spasmodic dysphonia*, *oromandibular dystonia*, *brachial dystonia*). Instead, Segmental dystonia (*meige syndrome*, *craniocervical dystonia*, *bibrachial dystonia*) affects two or more contiguous part of the body. Otherwise, dystonia is called Multifocal if the two parts of body involved are not close each other. Finally, when it is involved the trunk plus two parts of the body it is labelled Generalized dystonia.

Age classification distinguish Early-onset (≤ 26) and Late-onset (> 26). The first one usually affects leg or arm, while Late-onset affects neck or cranial muscles and tends to remain focal or segmental. In Early-onset patients, dystonia gradually affects more than one limb and in general becomes generalized.

Even if causes of dystonia are not identified in every patient, we can distinguish the syndrome according the etiological classification and identify two principal categories: primary or idiopathic dystonia and secondary or symptomatic dystonia.

- In primary dystonia causes are unknown. More recent studies highlight the lack of apparent neuropathology in definition of primary dystonia and difficulties in identification of the etiology. Nonetheless, this represent a challenge for new technology. For example, the diffusion of tensor magnetic resonance imaging is expanding the ability to detect structural changes.

Research tried to define the class of "Primary" dystonia focusing in clinical features rather than the pathological changes. It has been defined '*Primary pure dystonia*' if there are no identifiable exogenous causes and if it there is unique evidence of syndrome except for tremors and occurs torsion dystonia[1] [6].

It had been defined '*Primary plus-syndrome'*, disorder in which torsion dystonia is combine with other disorder like parkinsonism or myoclonus. Instead, if there is alternance between normality and torsion dystonia, it is defined '*Primary paroxysmal dystonia*'.

Most common forms of primary dystonia are focal, affect a single part of body and come on during the adult life. Generally, it is related with mutation of gene DYT1 (mutation of this gene doesn't contribute with secondary dystonia).

- Secondary dystonia may be related exogenous events. It could be accompanied by other neurological deficits or with Parkinson's disease and other parkinsonian disorders or dystonic phenomenology in another movement disorder. The pathology will be examined in depth in the following paragraph.

Several clinical manifestations and causes do not make simple the diagnosis. A possible approach is genetic test batteries, but they are expensive and not reliable. Another strategy is the *"red flag"* approach, in this case it used the identification of telltale clinical features (as corneal Kayser-Fleischer ring or liver disease in Wilson's disease) for the diagnostic test. This strategy is not always useful because dystonic disorders are lack of telltale features. Reliable methods are based on study of clinical phenomenology, it excludes that the disorder may be mimic dystonia and it is delineated the clinical syndrome according to four dimensions (age at onset, body distribution, temporal pattern, associated features).

Currently there are not medications to prevent Dystonia, but there are treatments that can be selected according the patient and the symptoms. In some case treatments may improve posture and relieve the pain when the dystonia symptoms are

[1] It is characterized by painful muscle contractions and involuntary distortion

more serious, for example when it compromises respiration or provokes muscle breakdown, the treatments can save the life.

- *Physical therapy* is used to control and improve the posture and prevent contractures. Usually it does not consider the use of braces but especially in children cases it may utilize "sensory trick". In other case, it might use hand devices or immobilize the healthy part of the body to induce patient to use sick ones. Sometimes dystonia is characterized by impaired sensory perception thus sensory training treatment may be good therapy. In this category belongs also neurophysiological technique as repetitive transcranial magnetic stimulation at low frequencies or neck-muscle vibration of the contracting muscle. Many patients seem to appreciate physical therapy, benefits are temporary but there are no reliable studies justify regular application.
- *Medical therapy* foresees the use of different drugs according the kind of dystonia and patient condition. Some consider the use of dopamine-related drugs (antidopaminergic drugs) as levodopa, even if most patients have limited improvement with it, only few patients have substantial improvement and complete resolution of dystonia. They augment or suppress dopaminergic transmission in the basal ganglia. In past most clinical trials used dopamine-receptor-blocking drugs but the poor response and side-effect (sedation, parkinsonism, tardive dyskinesia) have limited the use of it. Anticholinergic drugs as trihexyphenidyl, benztropine, biperiden, ethopropazine, orphenadrine and procyclidine are useful for treatment of generalized and segmental dystonia. These drugs block muscarinic acetylcholine receptors in the basal ganglia but can cause drowsiness, confusion, memory difficulty and hallucinations. GABA-related drugs as alprazolam, chlordiazepoxide, clonazepam, diazepam amplify transmission through GABA receptors. Typical side effect includes sedation, impaired mentation, impaired coordination and depression.

  Other pharmacological treatments use several drugs as muscle relaxants or morphine sulfate, phenol, chemomyectomy with muscle necrotizing drugs even if the benefits are not well-designed.

Finally use of Botulinum toxin is considered one of the most powerful therapeutic tools in the treatment of ophthalmic disorder and neurological disorder, such as dystonia syndrome. The benefit of this toxin is mainly due to its mechanism of action of blocking the release of acetylcholine into neuromuscular junction stopping overactive muscles. The choice of good dose is not simple because it provokes weakness and involvement of nearby muscles that may be difficult to balance, it may cause also resistance to antibodies.

- *Surgical treatment* has been used for long time but recently is reused. The most used technique is the deep brain stimulation (DBS) and usually the main target for stimulation is the internal globus pallidus (GPi). This treatment is favorite compared with other ones, because it has low risk of complications and because it provides customization of the parameters. This treatment will be examined in detail in the following paragraph. Another kind of surgical treatment is peripheral surgeries, this procedure aims to stimulate the extra-spinal sectioning of nerves to specific muscles. It is used before botulinum treatments, to destroy overactive muscles or nerves controlling them. Side effects are permanent somatosensory loss or dysesthesia, muscle atrophy and weakness, dysphagia.

In recent years research have found information about dystonia and about genetics. Large number of genes related to syndrome have been identified (many have been identified using the "DYT" nomenclature). Including proteins that have different functions as chaperones (DYT1), transcription factors (DYT6), structural proteins (DYT11) and enzymes involved in dopamine biosynthesis (DYT5). A limit in knowledge is how system-level changes in brain function responsible for abnormal patterns of movements are changed by these molecular and cellular changes, in fact one of the "missing step" in dystonia is the lack of information regarding neuropathology [6], this basically constitutes a barrier for the progress of the treatments and the develop of therapies for the syndrome.

Another limit is also that the number of cases of human dystonia which have been studied remains extremely few.

A collateral improvement of dystonia is related to education and counseling. For many years patients are frequently misdiagnosed and in some cases syndrome has

been confused with psychiatric problem. Patients need education and counseling, these are important for regaining trust so that they are inclined to accept recommendations [8]. Achieving best results and diffusing good outcomes about the syndrome may help patients to go beyond the frustration and mistrust, that sometimes accompanied the treatments. As often the approach used for treating syndrome is a 'trial and error' approach and in addition patients may be affected by depression, anxiety and social withdrawal.

1.1.1 Secondary dystonia

Secondary dystonia can be related with environmental causes by focal brain lesions of various origin especially cerebral palsy. Lesions can be provoked by neurodegenerative disorders, metabolic disorders of the central nervous system or several drugs and chemicals that affect the basal ganglia, thalamus and brain stem after an injury (in *Table 1* a list of disorders causing secondary dystonia)[10].

The clues suggesting that dystonia is secondary can be:

early onset of speech abnormality, dystonia symptoms appears with rest at onset, Hemidystonia, presence of abnormalities other than dystonia on neurological examination or general medical examination (for example: ataxia, parkinsonism, dementia, seizures, myoclonus, visual loss etc.), non-physiological findings suggesting a psychogenic basis (false weakness, false sensory loss, inconsistent or incongruous movements), abnormality on brain imaging, abnormality in results of laboratory assessment.

It can be distinguished several kinds of dystonia:

- Associated with inherited neurological disorders (dystonia-plus syndromes, degenerative diseases)
- Symptomatic of an exogenous or environmental cause
- Associated with Parkinson's disease and other parkinsonian disorders
- Dystonic phenomenology in another movement disorder.

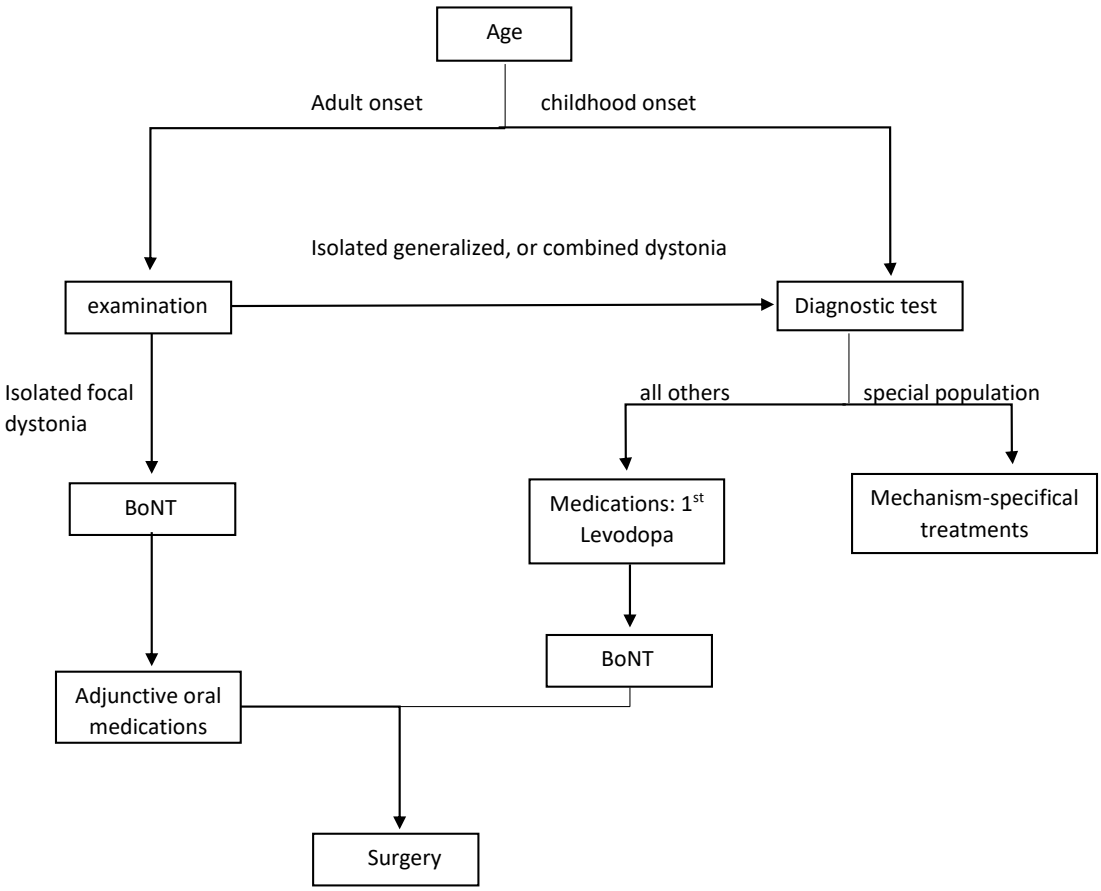


Figure 1. Method for evaluation of dystonia diagnosis [8].

Dystonia-plus syndrome includes: *Dopa-responsive dystonia*, *myoclonus-dystonia* and *rapid-onset dystonia-parkinsonism*.

Dopa-responsive dystonia caused by heterozygous mutations in GCH1 classified as DYT5, usually is highly tractable, symptoms generally worsen in the day and improve with sleep and it may develop parkinsonism. *Myoclonus-dystonia* is rare, it is characterized by prominent myoclonic jerks, affecting arms, neck and trunk. It typically exhibits in childhood or adolescence and it is caused by mutation in the SGCE gene classified as DYT11. *Rapid-onset dystonia-parkinsonism* is another rare type of dystonia, it is frequent in adolescence or early adulthood. In this case the responsible gene DYT12 it is recently identified. Other subcategories of secondary dystonia are related to cases in which there is a histopathological evidence of brain

degeneration. Inborn errors of metabolism cause many of these disorders which are autosomal recessive [2] [10].

An example is the Wilson's disease results from mutation in the ATP7B gene or the juvenile parkinsonism which is caused by mutations of parkin gene. Usually diagnosis of secondary dystonia may proceed if the primary dystonia is excluded or there are clear evidences that syndrome can be associated to secondary dystonia. Basically, test is the MRI or specific test as measure of BH4, neopterin or dopamine metabolities in cerebrospinal fluid (*dopa-responsive dystonia*).

At rest dystonic movements are more frequent in secondary dystonia, while patients affected by primary dystonia are affected by the presence of tremor (it was observed in 60% of patients with primary and in only 24% of those with secondary dystonia), chronic inflammatory process, or peripheral trauma (positioned in the area later affected by dystonia), in addition to sensory trick and development of spontaneous remissions [18]. Another difference is that secondary dystonia has higher incidence of dystonic posturing even if it is not evident however jerky clonic contractions are more frequent in primary dystonia.

[2] Autosomal recessive is one of several ways in which a trait, disorder, or disease can be passed down through families

LIST OF CAUSES OF SECONDARY DYSTONIA taken from [10]

(*Table 1*)

Hereditary disorders associated with neurodegeneration	*Autosomal dominant* Huntington's disease Machado-Joseph disease (SCA3) Other SCA subtypes (SCA2, SCA6, SCA17) Familial basal ganglia calcification (Fahr's disease) Dentatorubral-pallidoluysian atrophy Neuroferritinopathy Frontotemporal dementia Neuronal intranuclear inclusion disease *Autosomal recessive* Juvenile Parkinson's disease Wilson's disease Aceruloplasminaemia Pantothotenate kinase-associated Neurodegeneration (Hallervorden-Spatz) Neuroacanthocytosis Ataxia with vitamin E deficiency Ataxia-telangiectasia Ataxia with oculomotor apraxia Sulfite oxidase (molybdenum cofactor) deficiency Triosephosphate isomerase deficiency Guanidinoacetate methyltransferase defi ciency Infantile bilateral striatal necrosis Cockayne's disease Lysosomal storage disorders GM1 gangliosidosis GM2 gangliosidosis (hexosaminidase A deficiency) Niemann-Pick type C Metachromatic leukodystrophy Krabbe's disease Neuronal ceroid lipofuscinosis Amino and organic acidurias Glutaric acidaemia type I Homocystinuria Propionic acidaemia Methylmalonic aciduria Fumarase deficiency Hartnup disease *X-linked recessive* Lubag (X-linked dystonia parkinsonism) Lesch-Nyhan syndrome Deafness-dystonia-optic atrophy syndrome (Mohr-Tranebjaerg syndrome)

	Pelizaeus-Merzbacher disease Rett's syndrome *Mitochondrial* Leber's hereditary optic neurop- -haty Mitochondrial encephalomyopathy with lactic stroke-like acidosis and episodes (MELAS) Myoclonic epilepsy with ragged- -red fibres (MERRF) Leigh's syndrome
Dystonia-plus syndrome	Dopa-responsive dystonia Myoclonus-dystonia Rapid-onset dystonia-parkinso- -nism
Acquired/exogenous causes	*Medication* Dopamine receptor-blocking agents Antiepileptic agents Levodopa Dopamine agonists Calcium-channel blockers (cinnarizine, flunarizine) *Toxins* Manganese Carbon monoxide Carbon disulphide Methanol Wasp sting *Perinatal cerebral injuries* Cerebral palsy Kernicterus *Vascular lesions* Stroke Arteriovenous malformation *Infection* Encephalitis Subacute sclerosing panencephalitis HIV/AIDS Abscess *Brain tumours* *Paraneoplastic syndromes* *Demyelination* Multiple sclerosis

	Pontine myelinolysis *Trauma* Head trauma Cervical cord injury Peripheral injury (including complex regional pain syndrome) *Structural* Atlanto-axial subluxation Klippel-Feil syndrome Syringomyelia Arnold-Chiari malformation
Parkinson's disease and other parkinsonian disorders associated with dystonia	Parkinson's disease Progressive supranuclear palsy Corticobasal degeneration Multiple system atrophy
Other movement disorders exhibiting dystonic phenomenology	Tic disorders Familial paroxysmal kinesigenic Dyskinesias Familial paroxysmal non-kinesigenic dyskinesias Episodic ataxia syndromes

1.2 Deep brain stimulation

1.2.1 History

Electrical stimulation has been always used to manage the nervous symptoms and neurological disorders. Under ancient Roman emperor Claudius, physicians believed that the use of electric ray on the cranial surface may be a remedy for headache (*Torpedo torpedo* and *Torpedo nobiliana*, a characteristic of these fishes is the capability to produce electric discharge). In eighteenth century electrical stimulation was used for treatment of seizures, depression and cure of pain. While in early ninetieth electrical stimulation was used by Giovanni Aldini on the exposed human cerebral cortex of decapitated criminals. He also discovered that it evoked horrible facial grimaces. These studies were fundamental to understand brain function and to use stimulation techniques. In 1809 Luigi Rolando started to do electrical experiments on animals, he was followed by Eduard Hitizing and Gustav Fritsch that

studied electrical stimulation on dogs. In 1872 David Ferrier found, in monkeys, points of the brain related to movements after stimulation.

First experiments on human, in 1874, was made by Robert Bartholow. In Italy it was made in 1882 by Ezio Sciamanna, who performed experimentations on a trepanned patient who had a traumatic brain injury. The year after Alberto Alberti made an experiment on a woman with an eroding tumor of the skull, the gravity and the step of the tumor allowed him to access to dura mater surface. Other accurate studies were made by the British surgeon Victor Horsely until 1950 when Wilder Penfield gave a complete and clear idea about brain stimulation of the human cortex and a real accurate representation of the human brain functions, including motor and somatosensory areas. In 1938 Ugo Cerletti started to use the technique as treatment for severe psychosis, it was used as treatments for schizophrenia and other mental illness and only years later (1950) it was used for pain control. The Studies on stereotactic lesional functional neurosurgery were important for the determination of the structures around ventricles of basal ganglia, they were useful for the precise localization of the targets (Ernest Spiegel and Henry Wycis) for treating the dyskinetic disorders and tremor in Parkinson's disease. In 1963 Natalia Petrovna Bekthereva published a study about use of multiple electrodes implanted in sub-cortical structures for hyperkinetic disorder; while Carl Wilhem Sem-Jacobsen used the electrodes in thalamus, for recording and stimulate patients with epilepsy and psychiatric disorders. The introduction of L-dopa in 1960 involved a drastic decline of the surgical treatment of Parkinson's disease and only ventral intermediate and globus pallidus were used as target points. Although DBS remained used for treatment of psychiatric and pain control surgery. In 1991, Benabid, Blond and Sigfried studied the technique for tremor in thalamus and the year before Laitinen used DBS for Parkinson in globus pallidus, they showed that the technique is safer than thalamotomy which is not always tolerated because may provoke speech problems and swallowing deficits. DBS in 1994 was established for Parkinson disease, the stimulation had as target site the GPi, and in following years it started the diffusion of treatment for other syndrome as dystonia. First case reported for dystonia is in 1999 but with dramatic results, only the following year GPi DBS was effective for patient with DYT1 primary dystonia. Between 2003 and 2010 other targets were considered as caudate

nucleus, the sub-thalamic nucleus, the cerebellum, the centro-median nucleus of the thalamus and the hippocampus, DBS for dystonia is extend also for non-DYT1 dystonia, studies showed greater improvements in children than adults. Lately other diseases and disorders are treated with DBS as Tourette syndrome, psychiatric disorders (depression and obsessive-compulsive disorder), but also obesity, eating disorders and drug resistant hypertension. Even if it is not still used for pain a group of researches in Milan used DBS to study cluster headache while a recent study aims to use DBS after severe traumatic brain injury.

1.2.2 General concepts

The deep brain stimulation consists in the use of chronic electrical stimulation of specific brain region by electrodes. Usually a pacemaker-like unit called implantable pulse generator (IPG) is connected via a subcutaneous wire with four contacts, implanted in a target point [25], in *Table 2* a list of main syndromes and target regions for the treatment. Deep brain stimulation is not used only for treating dystonia but for others syndrome as Parkinson's disease (PD), essential tremor, a wide variety of neurological and psychiatric conditions including epilepsy, obsessive-compulsive disorder (OCD) and major depression. IPGs are voltage-dependent usually, the current that flows on electrodes will depend on impedance of them which have a variable impedance because may we have electrochemical changes in electrode-brain interface [24]. Pulse train duration, amplitude, location, frequency and regularity are parameters to modulate in order to obtain benefit from the treatment. These parameters can be designed with computer models which are based on electrodes location, desired stimulation target and current spread in brain tissue [24]. Research is working on next generation of DBS which aims to have more flexible parameters and patterns, good results and control of stimulation current for responding to neural activity [25].

Targets of stimulation are deep brain structure rather than cortical areas (*Table 2*), they can change according the syndrome. In dystonia the target area are globus pallidus internus (GPi) but also the subthalamic nucleus (STN) or the ventolateral thalamus (Voa). The time response to DBS can change, for ventral intermediate (Vim) thalamus benefit of tremor occurs over seconds, as in subthalamic nucleus (STN)

for tremor. While for rigidity and bradykinesia times changes from minutes to hours [25]. The time course of symptom reliefs, when stimulation is initiated, is mirrored by the time course with which symptoms return, for example in dystonia the deep brain stimulation of globus pallidus internus (GPi) may provoke an improvement in phasic dystonia movements in short time, instead several months of treatments are required for tonic symptoms [25].

Researches show that thalamic neurons that receive predominantly excitatory afferents may be excited by stimulation, other ones show that recorded neuronal activity in the downstream nuclei brought an increase in firing, proving that output from the stimulated nucleus increased. The most plausible explanation for the progressive time course improvement of dystonia after GPi DBS is that it reflects gradual brain reorganization (plasticity). This hypothesis is compatible with the notion that dystonia is a disorder with anomalous plasticity, in which abnormal neural organization is led by a complex set of physiological abnormalities [23].

It is plausible that DBS in GPi increases output from the stimulated nucleus and activates surrounding fiber pathway, activating a complex pattern that modulates the entire basal ganglia. Although the local electrical effects, DBS provides mutations in neurochemical changes as release of neurotransmitters locally and throughout the stimulated network, for example stimulation of the anterior thalamus may increase the release of adenosine or in caudate nucleus results show the production of dopamine.

One of the limit of the treatment is related to the fact that the precise neuroanatomical substrate for the clinical benefits and side effects of DBS remains difficult to understand and it is still object of study. Determination of position electrodes is based on magnetic resonance imaging or alternatively, preoperative magnetic resonance imaging fused with postoperative computed tomography, while stimulation targets derived from prior clinical experience. From a neural point of view, we consider that there are many factors influencing the neural element stimulation. Indeed, stimulation does not act predominantly on the soma but on axon and dendrites near the electrode [25]. Considering this aspect if a neuron, distant from the electrode, has a dendritic or axonal process in proximity to the electrodes the result

is that may be stimulated faster than another one that is adjacent to the electrode [25]. It has been demonstrated that good results are obtained by stimulations of also adjacent fiber tracts surrounding or running through the stimulated site. For example, nigrostriatal, pallidothalamic, cerebellothalamic and pallidonigral fiber tracts may be activates by stimulation of STN and could contribute to the therapeutic effects of DBS [24].

Another limit is to combine the expected outcomes for patients with dystonia with other neurological features. Even if there are few information about treatment, patients should be aware about the possible failure of treatments. As treatment foresees surgical implantation, it may cause complication or infection, electrode lead displacement, or extension fractures. Stroke, pain, allergic reaction, temporary tingling in the face and limbs, slightly paralysis, problem to speech or vision, jolting or shocking sensation, dizziness and/or loss of balance, reduced coordination and difficulty to concentrate are all side effects that syndrome may cause on patient. Another not completely clear aspect of DBS is that stimulation of GPi has not significant effect on mood or cognition in dystonia patients, but several suicides cases have been reported after the treatment.

1.3 Children's Hospital Los Angeles experience and main goal

Children's Hospital Los Angeles's team has decided to apply and analyze effects of DBS in children and young adults affected by secondary dystonia.

Patients ages ranges from 6 to 20 years, they are both female and male. Usually there are two phases: one in which there are implanted temporary electrodes in multiple candidate: Ventralis Intermediate (Vim), GPi, STN, Ventralis oralis anterior and posterior (VoaVop). After that he/she is monitored for several days and a team of specialists will test how he/she reacts to stimulation and analyzes the neural activity under specific situations with and without electrical stimulation. In the second phase of the treatment, electrodes will be implanted in one or two targets.

Principal goal of the team is not only to understand the effect of stimulation on secondary dystonia patients but also to increase knowledge about the syndrome. As said patient is monitored for the whole hospital recovery and biological signals as

EMG or neural activity. Patients are continuously recorded for all days to better understand symptoms and reactions to the test.

Main limits that Children's Hospital Los Angeles's team have to deal with are:

- the lack of patients and cases of syndrome in order to obtain general results.
- Etiology and age of patients are heterogenous.
- Lack information about main target points for stimulation.
- Results are evaluated with Burke-Fahn-Dystonia rating scale and Barry-Albright dystonia scale probably it could be used other scales for evaluating severity of dystonia.

The starting point of this work are videos analysis, methods used for obtaining this analysis will be explained in following chapter. In each analysis of each it is reported symptoms of the syndrome such as tremor or spasm for each patient are and how he/she interacts with external environment. All these aspects can represent the base for future works, in which video's results are combined with spike activity, understanding if there is correlation between a specific condition of the patient (as *rest* or *movement*) and neural activity.

DEEP BRAIN STIMULATION INDICATIONS AND TARGETS taken from [25]

(*Table 2*)

Indication	**Target(s)**
Epilepsy	ATN, seizure focus (cerebellum, CN, STN, hippocampus, CM, CC, Loc, MB)
Essential tremor	Vim, (STN)
Obsessive-compulsive disorder	VC/VS, (ALIC, NAc, STN, ITP)
Parkinson's disease	GPi, STN, (PPN)
Primary dystonia	GPi, (STN)
Addiction	NAc, STN
Alzheimer's dementia	NBM, fornix
Anorexia	Cg25
Chorea	GPi
Chronic pain	PAG, VPL/VPM
Cluster headache	PH
Depression	Cg25, ALIC, NAc
Holmes tremor	Vim, STN
Impairment of consciousness	CT
Obesity	VMH, LH
Schizophrenia	NAc/VS, VTA
Tinnitus	LC
Tourette's syndrome	CM thalamus, GPi, ALIC, NAc

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CHAPTER 2: Material and methods

In this chapter it will be showed a brief description of dystonia and neural recording studies, results obtained in literature about neural activity in dystonic patients and models created. It is added also a brief description of the methods used to classify the grade of severity of the syndrome. This will be followed by description of data used in this work, the creation of the video tables, the theoretical description of the neural analysis and the processing used for raw data. After a general overview of the spike sorting there is a brief description of standard algorithm used for detecting spikes, analyzing algorithm applied in data for the statistical analysis which aims to combine video tables and spike activity.

2.1 Protocol

This thesis is based on the analysis of two kind of data: video and neural recordings from deeper site in the brain. For each patient is associated a huge dataset, it basically is constituted by:

- videos of his/her recovery at Children's Hospital of Los Angeles
- the neural activity recordings (during the test and during the neural stimulation)
- EMG signals recorded during test.

All these data are used to study different aspects of dystonia, providing the possibility to analyze the syndrome from different points of view.

Each patient's dataset is constituted by one or more '*periods*', each period identifies a portion of his/her hospital recovery and the duration can change from few minutes to hours. Each patient has implanted four electrodes in left side and four in right side to catch neural activity. The interested regions are GPi1, GPi2, VA, VIM and VoaVop. The raw data coming from these electrodes are recorded, in particular it has been considered all the recordings in which subject was exposed to *'movement test'* to evaluate his/her capacity to move voluntary or his/her capacity to interact during the electrical stimulation. Team's members ask to patient to move a limb

portion or drawing something than catch something or simply test different stimulation parameters on patient to evaluate his/her capacity to react. All the neural activity coming from the deep site is acquired and processed.

For analysis it has been used only data in which patients do not under electrical stimulation and in which they are making a voluntary movement, for two reasons: 1) data with stimulation are noisy and difficult to analyze; 2) neural activity during movement may be correlate in future studies with EMG signal for having a complete overview of the situation. For each period it has been associated the corresponded video, each video has been analyzed and it has been obtained the Excel table that will be used to do statistical consideration explained in the following paragraphs.

For this work it has been decided to use videos. It has been analyzed videos of 6 patients (5 males and 1 female). The idea was to combine the results from video analysis with results coming from neural activity by a statistical method. The aim is not only adding new information about dystonia in children patients but also validating the instruments and methods used.

2.2 Researches and studies about deep brain recordings

2.2.1 Dystonia overview

It is very challenging understand functionality of interested brain regions in dystonic patients, both because information available are few both because electrophysiological characteristics have variability for each patient. Differences in phenotype and in etiology between several kind of dystonia (primary and secondary, focal and generalized etc.) are not clear and research aims to compare activity in the same brain region [47] [38] [39]. Indeed, some studies showed that GPi firing rate changes according etiology. In particular a primary group, containing cases in which neurological disease and structural neuroimaging is normal, it has been compared with a second group, containing cases in which disorder is symptomatic of a brain insult, in the last case the firing rate is lower than first one. In GPe firing rate in first group is higher than second one [52]. Researchers have often taken advantage from

the implantation from the deep brain stimulation to record neural activities and analyze sites as GPi, Vim or VL. Each region could be a source information, for example recent analysis (2018) have been shown that changes in Vim could contribute to develop of dystonia [32].

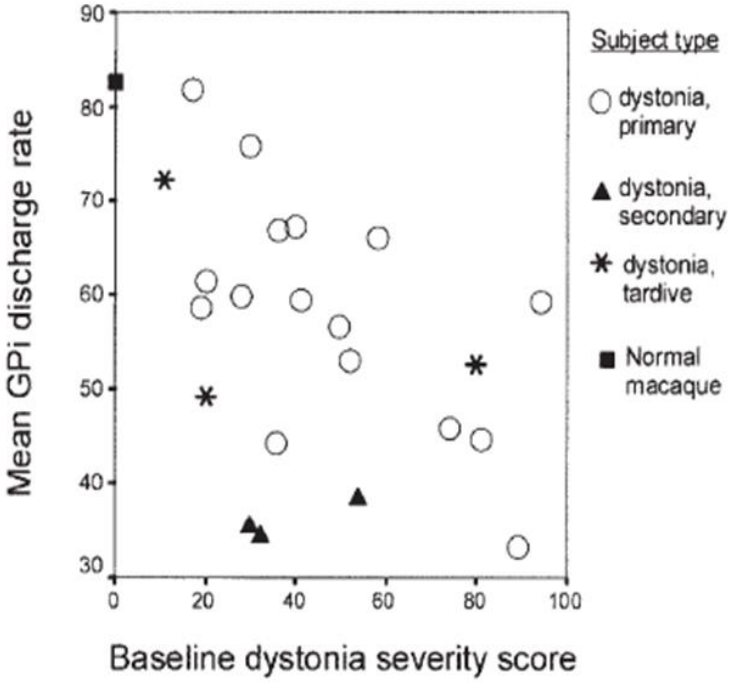

Figure 2. GPi mean neuronal firing rates scatterplot. Severity it is evaluated with Burke–Fahn–Marsden Dystonia Rating Scale-movement (BFM-M). This scale evaluates the dystonia impairment but do not provide evaluation of functional ability. Taken from [46].

Others have analyzed difference between discharge rate in GPi between primary and secondary dystonia (*figure 2*). It seems that patients with primary dystonia have a higher discharge rate than patients affected by secondary dystonia, in the first case values are 57.8 ± 1.5 Hz while in second case is 34.0 ± 3.5 Hz [46] (same conclusion could not deduct about discharge in GPe).

The study of deep brain recordings provides more specific information, about spikes and their shape, firing rates, single neural activity or provides information about an extended activity of neurons, potential in a group of neurons (local field potential, LFP) or oscillatory activity. Researchers aim sometimes to join these aspects [37] as the pathophysiology of disorder as dystonia (but also in Parkinson) is subjected to changes in firing patterns and oscillatory activity (beta and theta activity) [40]. Local field potential at low frequency (minus 12 Hz) in GPi shows high activity [35] [45].

Abnormal oscillatory in specific range frequency may be corelated with malfunction and disorder of basal ganglia, for example at low frequency, oscillation activity increases [41] than other frequency range as it showed also in *table 3*.

Dystonia symptoms are strongly correlated with movements and they can relate syndrome with EMG signal. One distinguishable aspect compared to normal subject is that in dystonia patients, regardless the etiology, there is an activity at low frequency in EMG signal [32], moreover many studies shows the significant correlation between EMG and neural frequency as shown in *figure 3* in which discharge activity increases with the muscle activity [47].

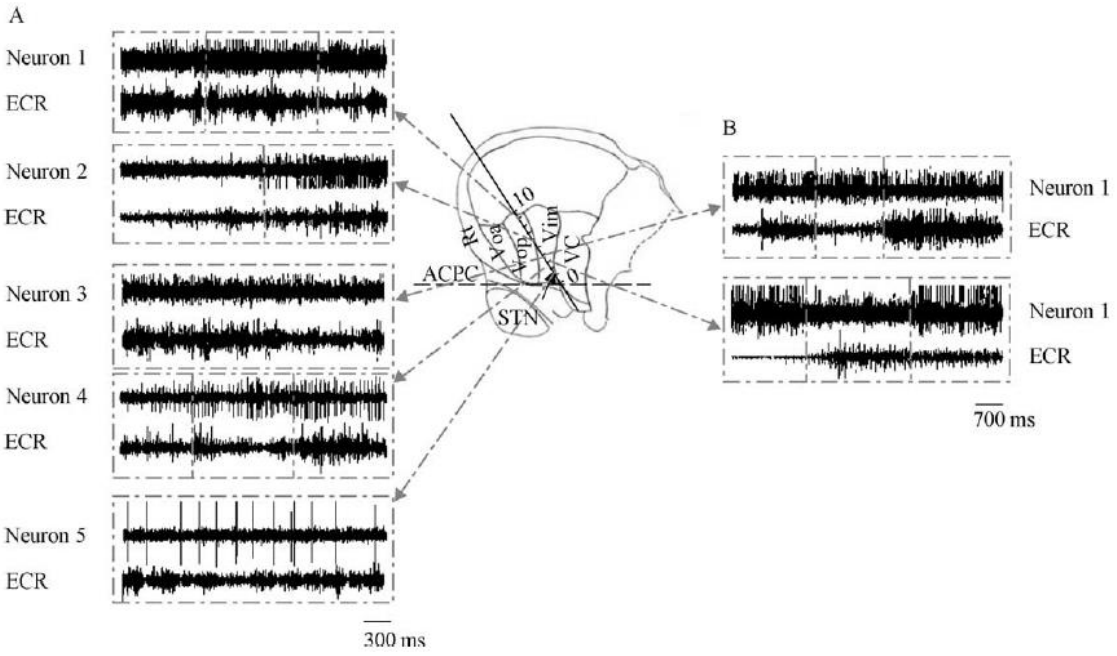

Figure 3. It shows neural activity related to EMG in thalamus along the depth of the trajectory target of Vop/Vim. Five neurons are related with ECR (extensor carpi radialis) muscles. The first four neurons are synchronized with ECR muscles, the last neuron activity is correlated to dystonia tremor and its neural activity discharged in burst at low frequency. Taken from [47].

Another differences it is that cells respond to movements of more one joints differently. Number of deep cells activated it is sensible higher than in others patient with tremor or pain, as shows in *figure 4*.

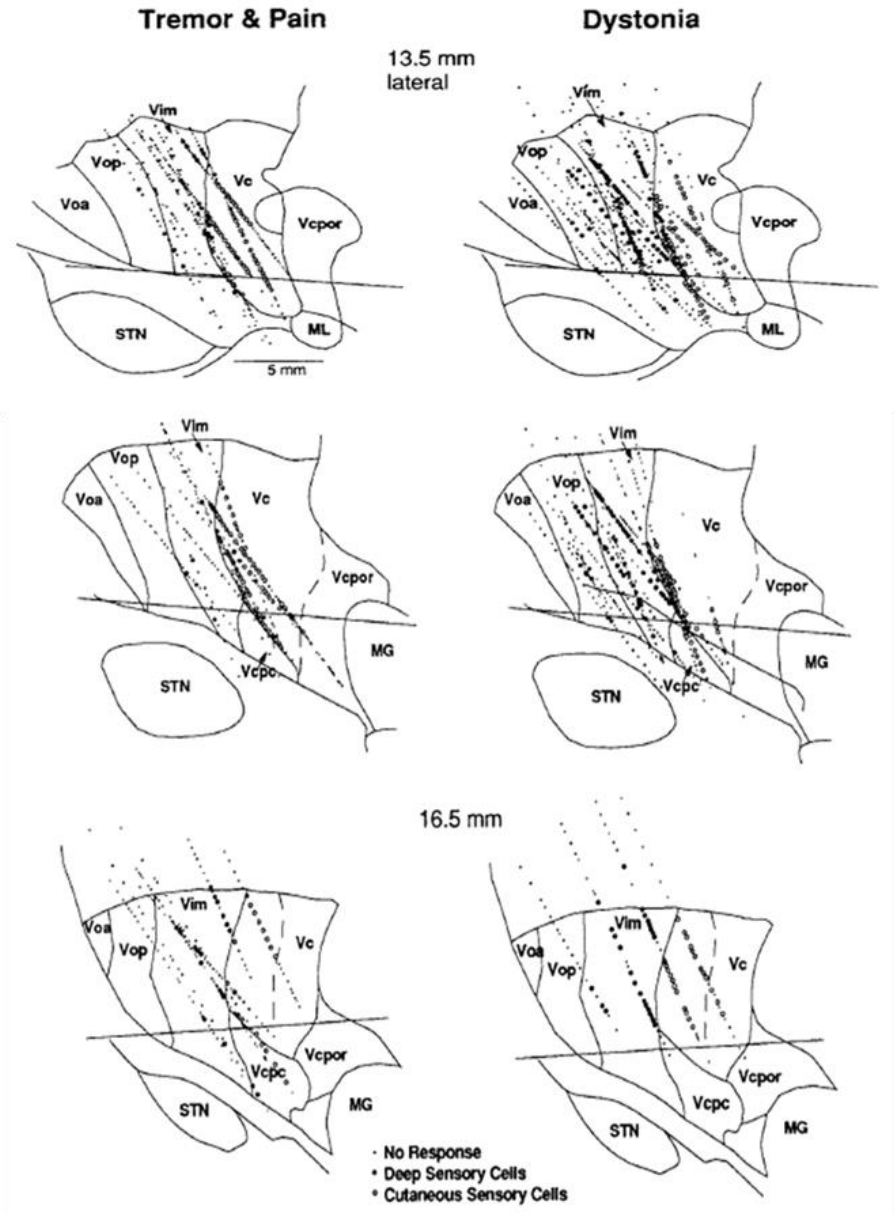

Figure 4. In the two category (pain and tremor, dystonia) it is represented the location of cells. Taken from [32].

Important role in dystonia is fulfilled by basal ganglia and its connections. Cortical area is the origin of them but also target points in which they return after that they divide between thalamus and basal ganglia. Connection involve not only GPi but also STN which receives excitatory input from cortical areas and inhibitory input from external globus pallidus [41]. In cortical area there are many circuits which have different function, as the motor circuit.

Its projections from cortical area go to the putamen, from there two outputs go to the basal ganglia sites the GPi and the substantia nigra (SNr), they also called *direct* and *indirect* pathways. This last one by means GPe and STN sends an inhibitory bundle from D_2 (there are neurons of the putamen which project gamma-aminobutyric acid (GABA)ergic inhibitory bundles) to Gpi and SNr at the same time, model is not so simply because projections from STN return to GPe and from GPe to GPi and SNr. The *direct* pathway instead uses D_1 (putaminal neurons) and the effect of SNc dopaminergic excitatory monosynaptic on it. GPi sends its inhibitory pathways on motor thalamus, ventralis anterior (VA), ventralis lateralis oralis (VLo), centromedian (CM) nuclei and parafascicular nuclei of the thalamus which projections turn to the striatum (*figure 5*) [38]. Excitatory bundles of GPi go to primary motor and arcuate premotor areas, but also supplementary motor area and premotor area. SNr sends excitatory projections to prefrontal cortex while inhibitory ones to ventralis anterior magnocellularis (VAmc).

This is only a simplification of the motor circuit because it has been identified other sub-circuits which have their origin in motor cortex, supplementary and arcuate premotor cortex, involving thalamus and basal ganglia. This sub-circuits are important because are related to develop of abnormal movements. The study and the creation of this model (*figure 6* [38]), proposes different hypothesis about how motor control is related with basal ganglia. One of these hypotheses is based on the idea that inhibition of GPi/SNr by means the *direct* pathway and excitation of GPi/SNr by indirect pathway provokes scaling of movements [39].

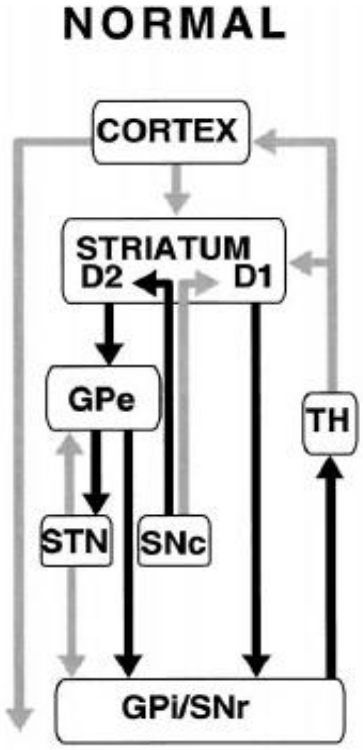

Figure 5. Representation of basal ganglia in thalamus. Model consider changes in mean discharge rate of neurons. Inhibitory fibers are represented by black lines, instead excitatory by grey one. GPe: globus pallidus external; GPi: globus pallidus internal; STN: subthalamic nucleus; SNr: substantia nigra pars reticulata; SNc: substantia nigra pars compacta; D1 and D2: dopamine receptors. Taken from [38].

If neurons in that sites are inhibited would facilitate movement because thalamocortical excitatory projections, which goes to the cortex, are disinhibited, instead if neurons are activated thalamocortical projections are inhibited as movements. Thus, balance of output of GPi/SNr and input of the same one could scale the movement.
Involuntary movements are produced by alteration of temporal coding of neural signals, it is important because it gives a precise information of transmission in neural system. Signal in cortex from the thalamus are disrupted by abnormal spatiotemporal patterns of synaptic afferent activity provoked by altered spontaneous activity, that changes receptive fields in GPi and thalamus. General concept is that alteration of cortical motor area in dystonia is maybe caused by pallidal neural activity, this one changes transmission of thalamocortical, winded receptive fields in pallidum and thalamus, may provoke the inability to select specific muscles.
Involuntary movements may be provoked also by increase of synchronization of neural activity, the increase may change the equilibrium impeding also the voluntary movements [39] [38].

In general, in *hypokinetic* (regards the reduced power of movements) or *hyperkinetic* (excessive involuntary movement, dystonia fits well with this model [38]) movement disorders may be provoked by presence of abnormalities in the described

circuits. *Figure 7* [39] shows a complex model of primary dystonia during *rest* and during *movement*. At *rest* neural activity in thalamus and in globulus pallidus is reduced (*figure 7.B*), during movement instead activity in globulus pallidus continues to reduce and while in thalamus activity increase (*figure 7.C*). Cortical and brainstem output are disrupted by this reduction and consequently it occurs the disordered movement of the syndrome.

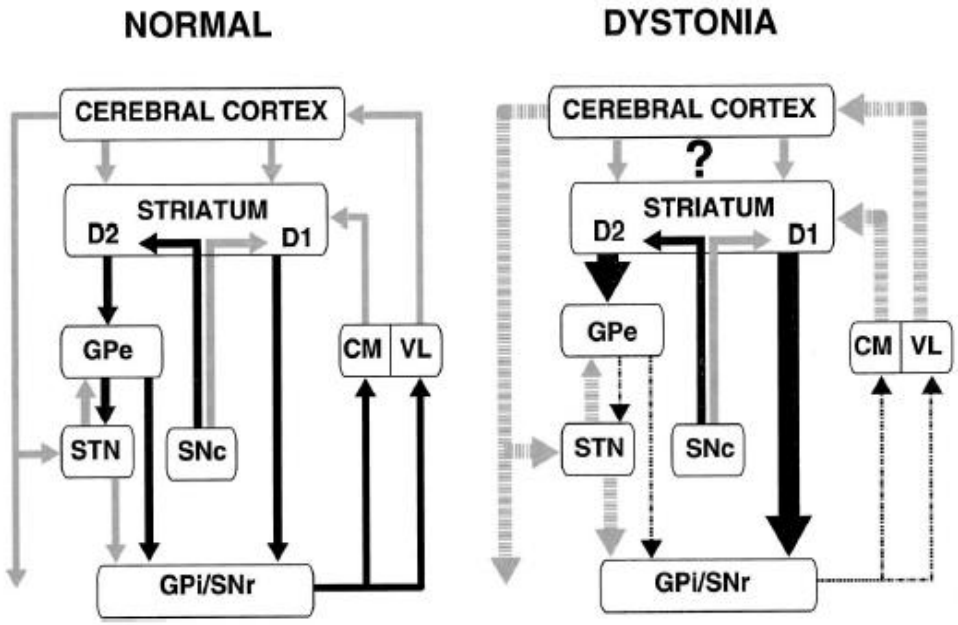

Figure 6. MEA: midbrain extrapyramidal area; STN: subthalamic nucleus; VL: ventral lateral nuclei; CM: center median nucleus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; D1: dopamine 1 eceptor subtype; D2: dopamine 2 receptor subtype. This model is based on decrease in mean discharge rate and altered patterns of neuronal activity in the external and internal segments of the globus pallidus (GPe and GPi). The width lines represent change in mean discharge rate between dystonia case and normal case. Wider or thinner line indicate increase or decrease of mean discharge rate respectively. Taken from [38].

A good dystonia model considers all changes in basal ganglia, thalamus and cortex than normal subjects. Increment, in inhibitory output from striatum to GPe and GPi, is the clear explanation of changing rates in GPe and GPi by *direct* pathways and *indirect* pathways. Striatum is inhibited also by center median nucleus (CM)

of the thalamus by means the *direct* pathways. Disinhibition of STN could increase the metabolic activity in GPi, this can be caused by lowered neuronal activity in GPe, or it can be caused by increased of GPi activity always via *direct* pathway [38]. The mean discharge rate it is most influenced by inhibitory output from *direct* pathway, at the same time STN increases its activity and in GPi neurons altered their receptive field. Irregular grouped discharges neurons are exactly due to changes in mean frequency and receptive field of GPi neurons [38]. Unfortunately changes in neural activity doesn't still contribute to understand knowledge about the development of dystonia.

Another difference with normal subject is that dystonia patients have discharge of GPi, Vop/Vim, irregular with intermittent pauses [47] [38] without distinction between primary or secondary dystonia as shows in *figure 8*. Sometimes there is also burst behavior in discharge [38] [39] [41] [46] and often in GP, STN and EP (entopeduncular nucleus) the neural activity is dominated by irregular and busty activity as shown in *figure 9* [43]. Even if these are clear aspects is not clear what is the relation between changes of neural activity and development of dystonia.

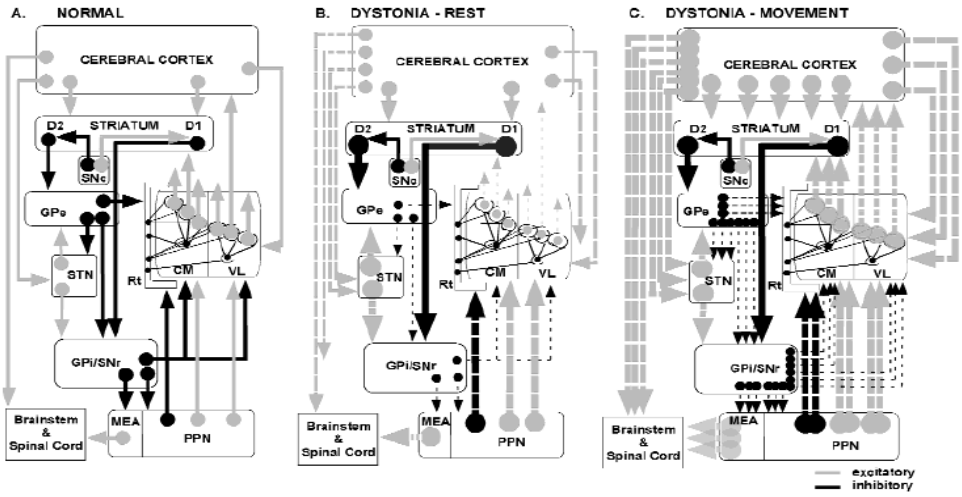

Figure 7. CM: centromedian; VL: motor thalamus; Rt: reticular nucleus of the thalamus; PPN: pedunculopontine; MEA: midbrain extrapyramidal area. Multiple lines with different lengths that exit from a nucleus indicates asynchronous neural activity; multiple broken lines with different lengths indicate altered patterns of asynchronous neural activity; multiple broken lines of the same length indicate altered pattern of synchronous activity. The amount of neural activity is represented by width of the lines. Taken from [39].

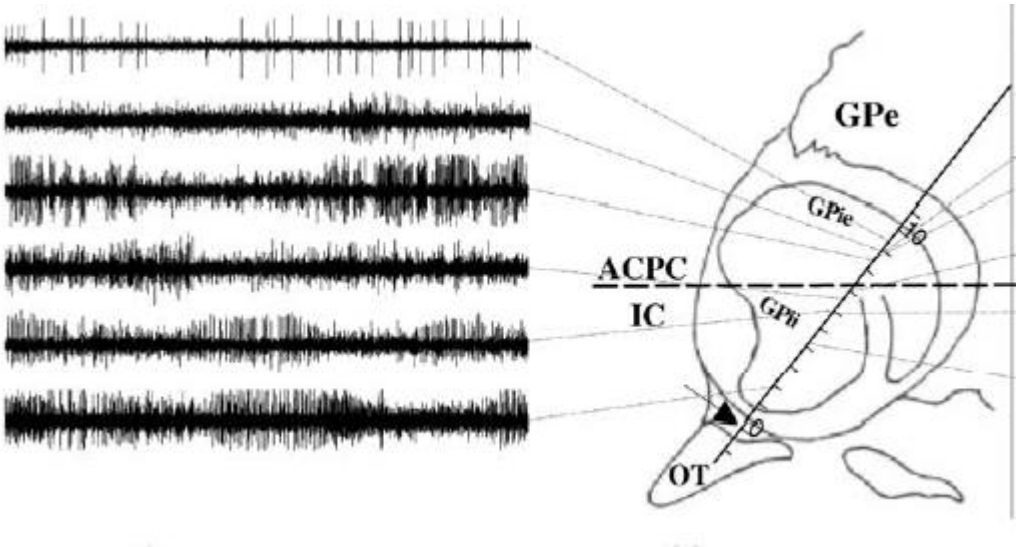

Figure 8. Discharge of neural activity in dystonic patient. Taken from [47].

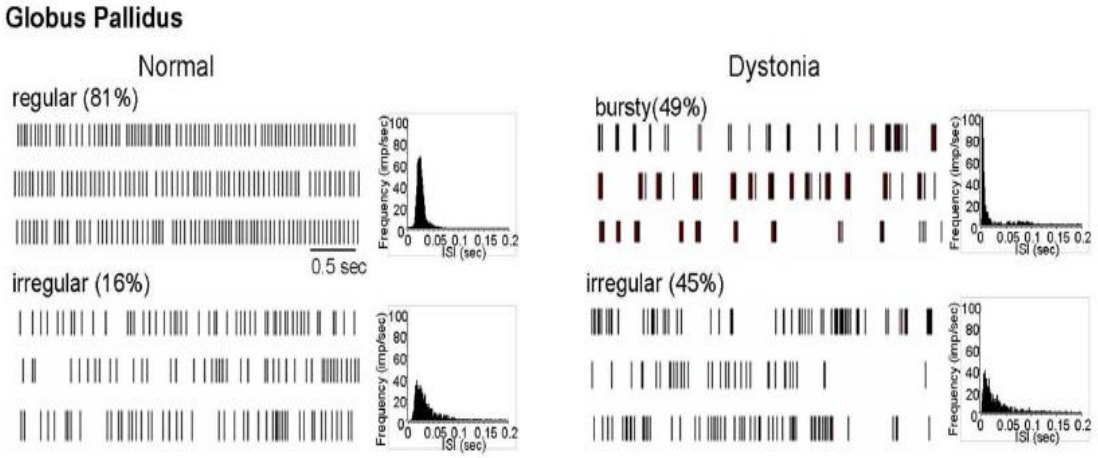

Figure 9. Raster and ISI histogram of discharge in globus pallidus. Taken from [43].

GENERAL RESULTS (*Table 3*)

Region	**Number of patients**	**Number of neurons**	**Dystonia Type**	**Measure-ments**	**Parkinson**
STN *(rest)* [41]	9* 20**	9* 19**	primary	Mean firing rate: 26.70 Hz (SD 6.3)	Mean firing rate: 31.70 Hz (SD 13.5)
STN *(voluntary movement)* [41]	9* 20**	9* 19**	primary	Mean firing rate: 23.60 Hz (SD 13.3)	Mean firing rate: 35.20 Hz (SD 15.6)
GPi [52]	14	123	contains cases in which neurological disease and structural neuroimaging is normal	Median firing frequency: 13.5 Hz	-
GPe [52]	14	31	contains cases in which neurological disease and structural neuroimaging is normal	Median firing frequency: 13.5 Hz	-
Gpi [52]	22	109	contains cases in	Median firing	-

			which dis-order is sympto-matic of a brain insult	frequency: 9.6 Hz	
GPe [52]	22	39	contains cases in which dis-order is sympto-matic of a brain insult	Median firing frequency: 7 Hz	-
GPi [40]	-	173* 168**	Cervical	Mean firing rate: 71.4±2.2	Mean firing rate: 91.7±3.0
GPe [40]	-	39* 58*	Cervical	Mean firing rate: 62.6 ± 4.8	Mean firing rate: 56.7 ± 4.4
GPi [38]	-	26	generalized	Mean discharge rate: 50 ± 20.5 Hz	-
GPe [38]	-	44	generalized	Mean discharge rate: 37.7 ± 28.0 Hz	-
Gpi [47]	20	48	primary	Mean discharge fre-quency: 42.1 ± 23.0 Hz	-
GPi [47]	20	47	secondary	Mean discharge fre-quency:	-

				47.8 ± 19.5 Hz	
GPi [46]	22* 15**	302* 151**	-	Mean discharge: 55.3±1.3 Mean frequency of significant oscillations: 6.0±0.5 Hz	Mean discharge: 95.2±2.3 Mean frequency of significant oscillations: 6.2±1.4 Hz
GPe [46]	15* 5**	151* 39**	-	Mean discharge: 54±1.9 Mean frequency of significant oscillations: 7.2±1.5 Hz	Mean discharge: 56.6±3.5 Mean frequency of significant oscillations: 4.1±0.4 Hz
STN [41]	9 * 20**	62 * 143**	primary	Significant oscillation: 32.2% (0-200 Hz) 6.5% (3-30Hz) 0.0% (13-30 Hz)	Significant oscillation: 39.9% (0-200 Hz) 20.3% (3-30Hz) 6.3% (13-30 Hz)
GPi [46]	16	225	primary	Mean frequency of significant oscillations: 5.8±0.5 Hz; Mean rate: 57.8±1.4 Hz	-
GPi [46]	3	27	secondary	Mean	-

				frequency of significant oscillations: 59.1±2.1 Hz Mean rate: 34.0±3.5 Hz	
GPe [46]	9	99	primary	Mean frequency of significant oscillatios: 10.2±3.2 Hz Mean rate: 53.4±2.4 Hz	-
GPe [46]	3	29	secondary	Mean frequency of significant oscillations: 3.7±0.3 Hz Mean rate: 52.1±3.6 Hz	-

**for dystonia; **for parkinson*

2.2.2 Dystonia severity classification

It can be useful evaluate dystonic syndromes or neurodegenerative disease. There are several scales for comparing as *Burke-Fahn-Marsden Dystonia Raiting Scale-movement (BFM-M)* used for evaluating children patients (Children's Hospital Team uses also this scale). It aims to estimates presence and severity of involuntary movements. Disadvantage of this scale is that does not consider the presence of other impairments and confuse symptoms with disabilities. Estimation is divided in two sub-test, one related to movement and another one related to disability subscale. In first one it is evaluated dystonia in nine body portions, at each it assigned score from 0 to 5. The second subscale considers 7 daily activities, for each it as-

signed score as previous case (*Table 4 taken from* [31], Appendix A). Other classification systems are based on evaluation of functional profiling and functional ability of patients as *Gross Motor Function Classification System (GMFCS), Manual Ability Classification System (MACS) and Communication Function Classification System (CFCS)*. These classifiers are used especially for celebral palsy but lately it has been used also for dystonic case, methods can be mixed for providing a different interpretation of the results [44]. Another scale used in dystonia case is *Barry-Albright Dystonia scale (BAD)* in which it is considered 8 body regions, it is assigned a score for each one considering only abnormal posture and movements. Results are not influenced by weakness, reflexes o other movements disorder [42] (*Table 5 taken from* [42], Appendix A). *Undefined Dystonia Rating scale (UDRS)* is another method for classification in which each body district has a detailed estimation and eliminating speech and swallowing rating estimation that could have subjective interpretation [31] (*Table 6 taken from* [31], Appendix A). Finally, *Global Dystonia Severity Rating Scale (GDS)* aims to assign a score from 0 to 10 at each body area [31] (*Table 7 taken from* [31], Appendix A).

2.3 Data

2.3.1 Video analysis

The quantity of video available is huge, patients were filmed for all day long during the permanence at Children's Hospital. Information that it can be extracted from videos are several because they report a lot of moments of patient's life in the hospital. As said I analyze only videos in which patient was exposed to a 'movement test' both because it was recorded neural activity both because patients during the test had electrodes to record EMG activity. I analyze that kinds of videos because in future analysis it could be interesting join the EMG signals and the neural signals with video results.

Each video it has been analyze considering:

- If it is day or night
- Time clock

- Patient condition (*rest*, *presence of spasm*, *sleep*)
- Which side of the body patient moves (*left*/*right*)
- If patient is under drugs administration
- If doctor, nurse, team, parents move or interact with him/her (*passive stimuli*)

All points aim to provide much information about patient and his/her interactions with external word.
The analysis in the specific has been organized with an Excel paper and each condition has been marked with 1 or 0 according it was 'verified' or not, in *figure 10* an example.

Column called *'Patient'* reports what patient was doing. If he/she was interacting with team's members, if he/she was speaking or was sleeping. All kinds of relevant information have been written in that section.

Following two columns *'mum/dad'* and *'others'* report what parents or medical staff were doing and if they were making something that can influence the condition of the patient.

Rest is a condition in which patient do not move voluntary or involuntary, no tremor or spasm, all cases in which patient was motionless.

Spasm column includes all the conditions in which patient had an involuntary movements, tremors or non-controlled movements.

Volitional movements column includes all cases in which it was clear that movements were intentional. Even if in many cases it was difficult understand the classification of the movement.

Right and *Left* columns were added to mark which body side is moved.

In *Passive stimuli* it marked if someone touched or moved patient, changes his/her position in the bed or interacts with him/her.

Sleep column indicates if patient is awake or not.

Medicine administered has been inserted to indicate if doctors or nurses give drugs to patient, they could influence the patient's behavior.

Given the huge database I decided to no classify uncertain movement, in which it is not clear if movement was voluntary or not, although in that case I have marked the body portion interested to movement. Each raw of vides table analysis has a temporal distance of ten seconds, thus every ten seconds I analyzed what patient did and what happened around him.

day/night	clock time	video time	Patient	mum/dad	others	REST	SPASM	VOLITIONAL	RIGHT	LEFT	PASSIVE STIMULI	SLEEP	Medicine administered
day	10:05:45	00:00:00	move slowly	touches him						1	1		
day	10:05:55	00:00:10	move slowly	touches him						1	1		
day	10:06:05	00:00:20	move slowly	touches him					1	1	1		
day	10:06:15	00:00:30	move slowly	touches him					1	1	1		
day	10:06:25	00:00:40	move slowly	touches him				1		1	1		
day	10:06:35	00:00:50	move slowly	touches him					1	1	1		
day	10:06:45	00:01:00	move slowly	touches him					1	1	1		
day	10:06:55	00:01:10	move slowly	touches him					1	1	1		
day	10:07:05	00:01:20	no information										
day	10:07:15	00:01:30	no information										
day	10:07:25	00:01:40	moves					1		1			
day	10:07:35	00:01:50	moves						1	1			
day	10:07:45	00:02:00	no information										
day	10:07:55	00:02:10	moves		say him to move			1		1			
day	10:08:05	00:02:20	moves		say him to move			1		1			
day	10:08:15	00:02:30	short sapsm				1			1			
day	10:08:25	00:02:40	rest			1							
day	10:08:35	00:02:50	rest		say him to mo	1					1		
day	10:08:45	00:03:00	short spasm, moves		say him to move		1	1		1			
day	10:08:55	00:03:10	short spasm, moves		say him to move		1	1		1			
day	10:09:05	00:03:20	short spasm, moves		say him to move		1	1		1			

Figure 10. An example of excel table created for each period of each patient. First column indicates if it is day or night, second and third columns indicate respectively time of recording and video time. Patient column describes what patient are doing, instead following two columns report how parents, doctors or others interact with patient. All other columns are marked with condition is verified otherwise cell is not marked.

2.3.2 Micro-electrodes acquisition

Usually electrodes for neural recordings can change in geometry, material and then electrical characteristics. For example, microelectrodes range from 10 and 20 μm, with a resistance of 0.1 to 0.5 MΩ at 1000 Hz to record activity in Gpi, Vim/Vop and STN [47], others use Platinum-Iridium, glass-coated with a tip of diameter of 2 to 4 μm and an impedance of 0.5 to 1.0 MΩ (at 1000Hz) [38]. For this project it has been

used the *MM16C-SP05X-000 of AD-TECH MEDICAL INSTRUMENT CORPORATION*. Each one is constituted by ten microelectrodes and eight macroelectrodes as represented in *figure 11*.

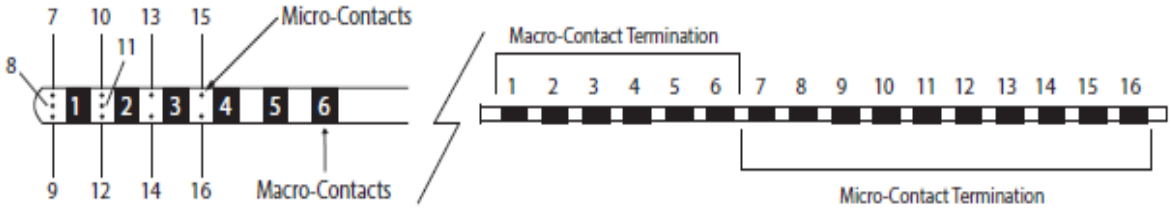

Figure 11. General representation of MM16C-SP05X-000 by AD-TECH MEDICAL INSTRUMENT CORPORATION.

Signal is from microelectrodes is sampled at 244414 Hz while from macroelectrodes is sampled at 30517 HZ. After acquisition of the signal from microelectrodes, signal is pre-amplified by custom circuit showed in *figure 12*. In passive channels (macroelectrodes for stimulation) R1 is placed and R3 and R4 are omitted. Instead if the channels are active (microelectrodes for recordings) R3 and R4 are placed and R1 is omitted. Acquired signal is send to the *RZ2 Bio-amp processor*, it is composed by the *PZ5 Neurodigitizer* for the amplification, analogue-digital conversation, filtering (high pass filter, anti-aliasing filter) and the *RS4 Data Streamer* for storing data.

2.4 Software analysis

The acquired raw data have been analyze with MATLAB software, in *figure 13* and *14* one signal coming from one electrode. Pre-processing has been constituted by first phase in which it has been reduced noise from data and selected a frequency band, followed by second phase in which filtered data have been used for extract spikes.

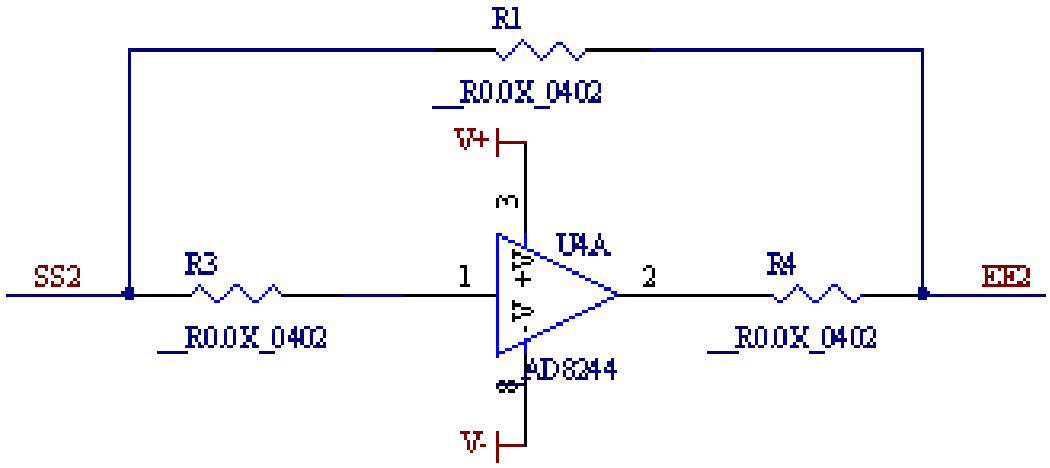

Figure 12. Custom circuit used after signal acquisition from electrodes.

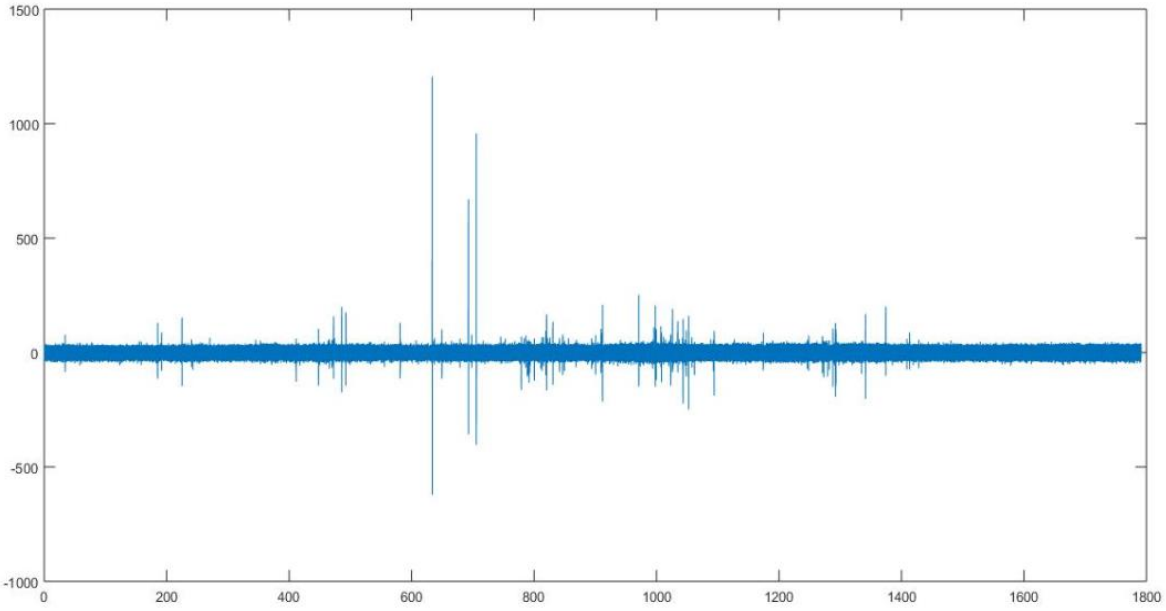

Figure 13. Data in time domain from micro electrodes located in the left side of STN, patient 55.

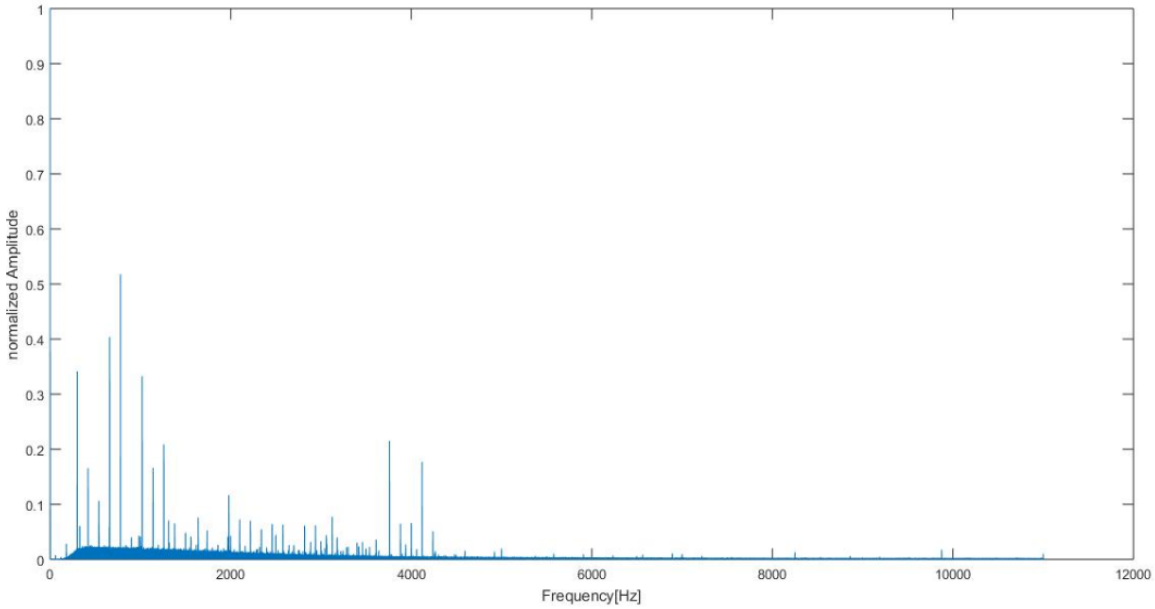

Figure 14. Data in frequency domain from micro electrodes located in left side of STN, patient 55.

2.4.1 Processing

The recorded raw data are affected by different noise sources: motion artifacts, wired noise, instrumentation noise, biological noise. First step, after acquisition, is processing of signal to easily worked with it. The standards studies prefer use of two filters before the spike detection. One for deleting the wire interference at 60 HZ and a pass-band to select only the interested signal portion other use also amplification if signals have low amplitude. The bandpass filter can change values: 300-10000 Hz, 200-1000 Hz, 500-5000Hz. Although the choose of the filter minimizes the effect of the noises, it may introduce artifacts in our signals and influence relationship between intra-action potential and extra-cellular action potentials or alters spikes discrimination of pyramidal and inhibitory neurons [49].

Usually it is used IIR filters (Elliptic, Butterworth or Chebyshev filters it is not important the kind of filters the results are similar), which has the main advantage of computational economy than FIR filters. Another difference between IIR and FIR is that FIR is not suitable for filtering a continuous signal. Major limits are that IIR are causal and cannot provide in general a linear phase response while non-casual filter

provides a signal that is similar to original one but it is smoother and has small ripple (*fig.15*).

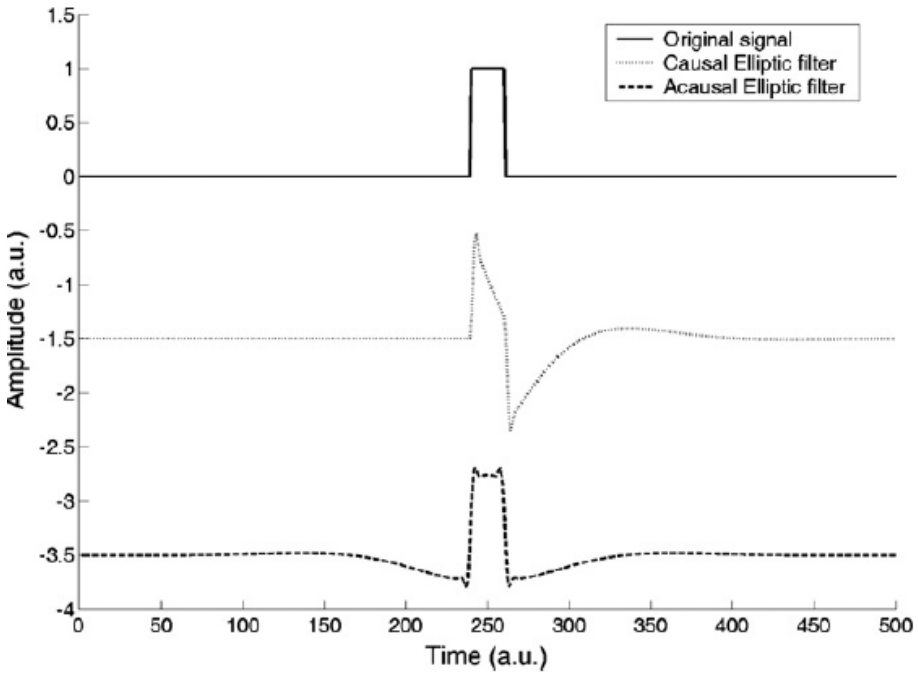

Figure 15. First signal is the original signal, second one is signal after casual elliptic filter. It introduces distortion than last signal which it has been applied a noncausal filter. Taken from [49].

We can note differences in spikes (*fig.17*), it represented in following three cases: a) non-casual filter in the range 300-3000 Hz, b) a casual filter in range 300-3000 Hz and c) a casual filter in range 600-3000 Hz. The distortions introduced by casual filter is more notable in short frequency range. Casual filters (FIR) not only distort spike shapes, but also change the appearance of artifacts and make them similar to real neural data. In *figure 16* is showed casual filtering, as it is showed it introduces negative peak in spike representation than other spikes obtained by other filters [49]. Usually times and shapes of spikes, overcome a fixed amplitude threshold, or continuous data coming from microwaves are recorded by hardware acquisition system. One problem is that it requires a large storage capacity and sophisticated data processing for recording continuous data which is applied non-casual filters without shape distortions offline.

During neural recording it is preferable getting feedback signal even if recent hardware capabilities and algorithmic implementations make the analysis of such volumes of data feasible [49]. Compromise is between off-line recordings which allows to get continuous data for spike detection and sorting without feedback response and on-line recordings which provides neural feedback but change spikes.

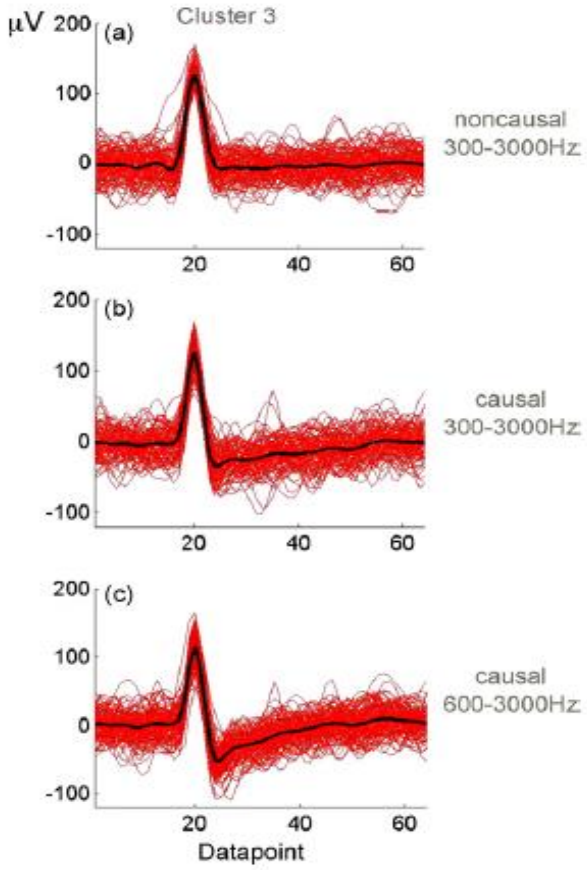

Figure 16. Spike and artifacts with three different filters. Taken from [49].

2.4.2 Data processing

The raw data in this thesis have been filtered with notch filtered to eliminate the wired interference and its harmonics, then it has been selected a frequency band between 300-3000 Hz using a Butterworth filter of 4 orders, an example in *figure 18*. The filtered data was used for spike detection and sorting described in following paragraphs.

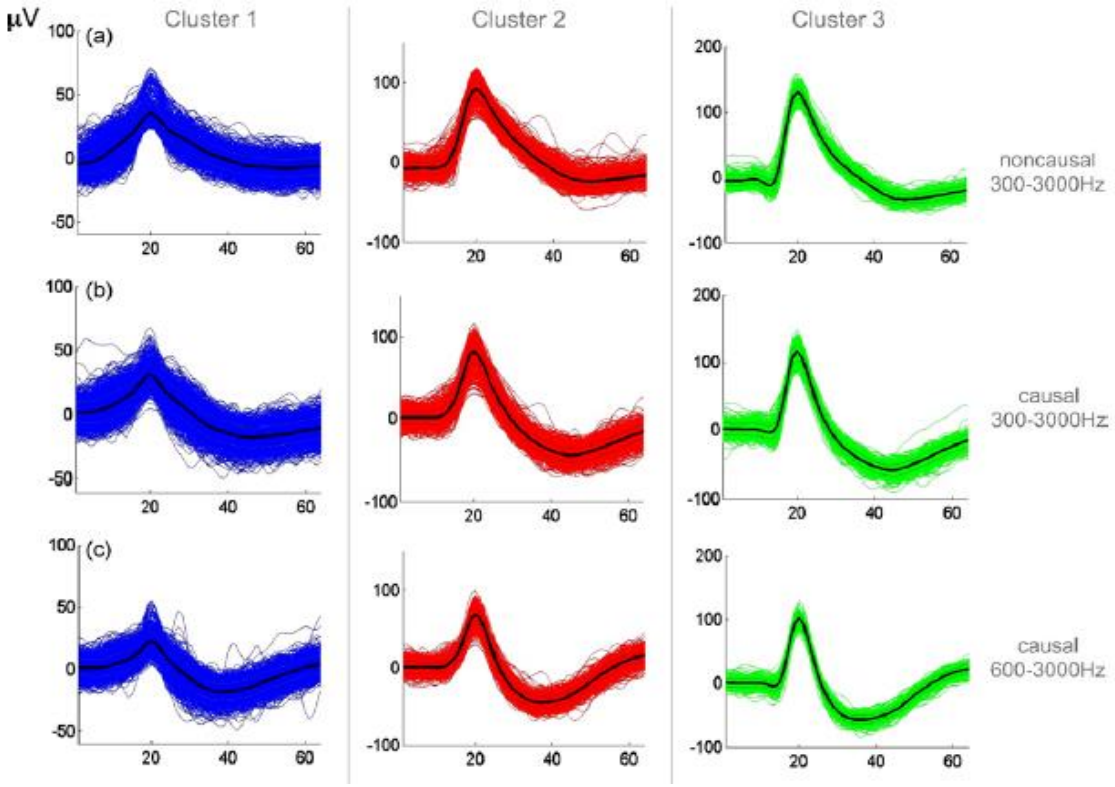

Figure 17. It shows three spikes clustering obtained from the use of different filters. Casual filter introduces, as expected, signal distortion. Taken from [49].

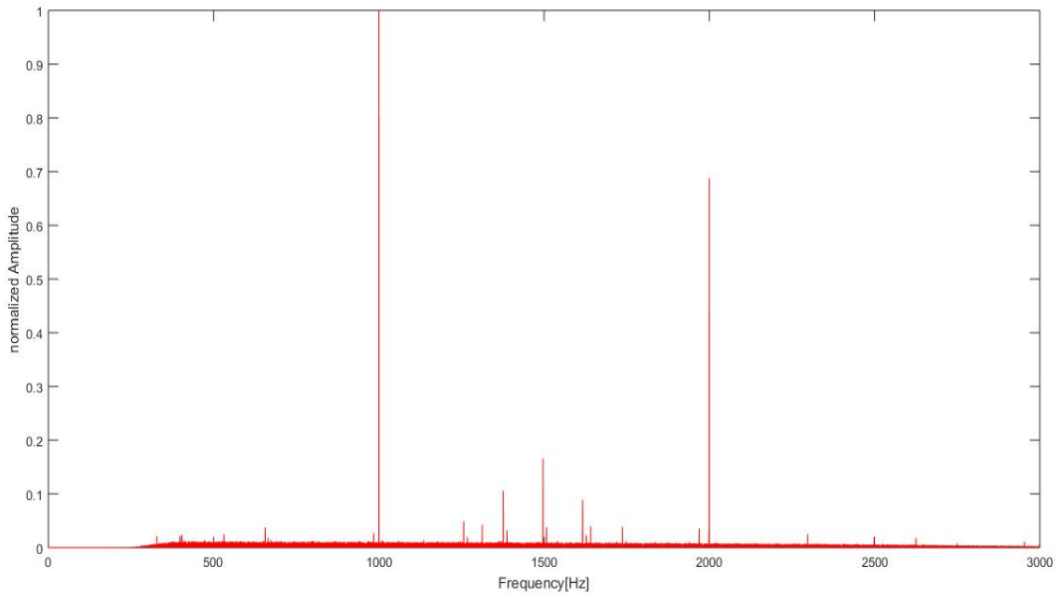

Figure 18. Data from left GPi after filters, patient 354.

2.4.3 Spikes

Each electrode may detect the spikes of a single neurons. Each neuron has the capability to produce spikes with specific shape and it depends basically by the distance between neuron and recording site but also by the morphology of dendritic tree. All similar spikes are gather together in a cluster, this is process is called spike sorting and it needs a spike sorting algorithm. First algorithm used the amplitude to discriminate neurons, then improvements provide to use of window for separating, defining group in which are contained spike shapes. Other detection systems choose a characteristic of the spike shape using this one to separate all spikes.

More complex methods take advantage from filtering, extraction of feature and then manual or automatic clustering. Sometimes it cannot be so simple record spike coming from single neuron, it can overlap more than one spike, creating multiunit clusters. It is recognizable because the shape is not standard, amplitude is low and it has a different refractory period (*figure 19*).

Another source which obstacles the spikes recognition, as it changes the spike form, is noise.

It has been demonstrated that number of neurons detected are minus than real number, there are several explanations: probably some neurons do not fire, tissue may be damage by implanted electrodes, probe inserted may produce an electrical insulation or simply technologies and algorithm used are not yet able to show a complete picture of the neuron organization. *Figure 20* gives an idea of detected spikes than the real number of neurons. Detected neurons are red, blue and cyan one. These are clustered for their shape, instead grey neurons in the ray of 50 μm are not detected.

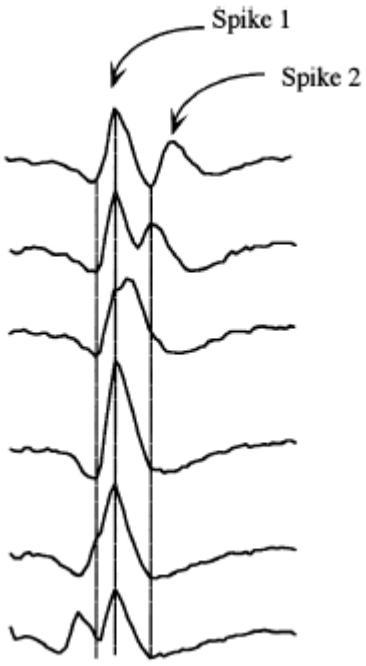

Figure 19. Summation of two spikes. Taken from [33].

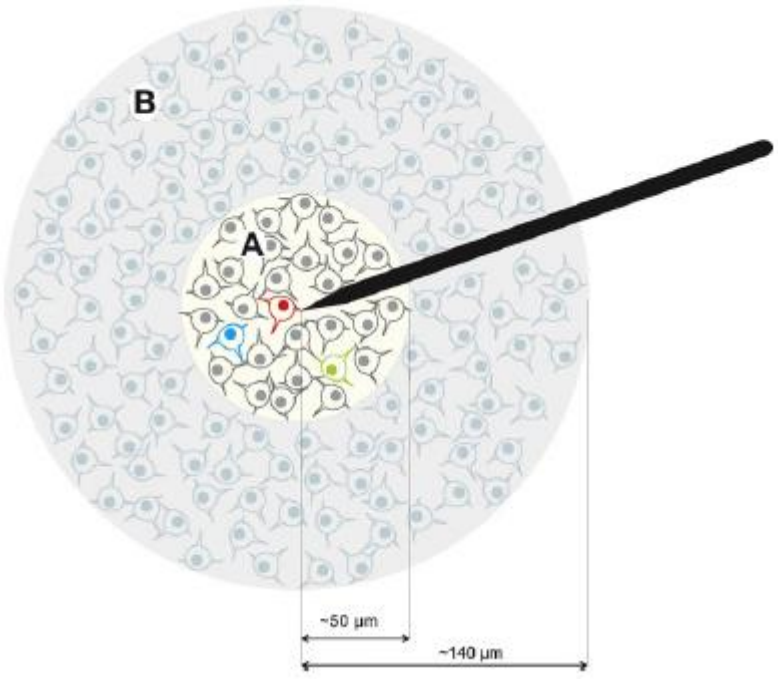

Figure 20. Extracellular neuronal recordings. Taken from [29].

2.4.4 Spike sorting: algorithm

Spike sorting algorithm detects single neuron activity, as said this is not so simple because often it relies on template that may move from real situation or it foresees feature extraction from signals and they use it to classify spikes neurons. For feature extraction may be used the wavelet coefficients or principal component analysis.

Also clustering methods may be variable, it can be used *K-mean* method or *super-paramagnetic* clustering. In *Table 9* a list of algorithms with the concerning wave-form and clustering techniques used.
There are two types of neural sorting: *off-line* and *on-line* algorithm. When it is used the first one it is recorded the raw signal and it gets spikes, even if it provides better accuracy it requires a large storage and much time for elaboration. Instead *on-line* algorithm provides spikes during the acquisition, this one is useful when it requires a real-time analysis but produce a lot of data (about 10 gigabytes per day).

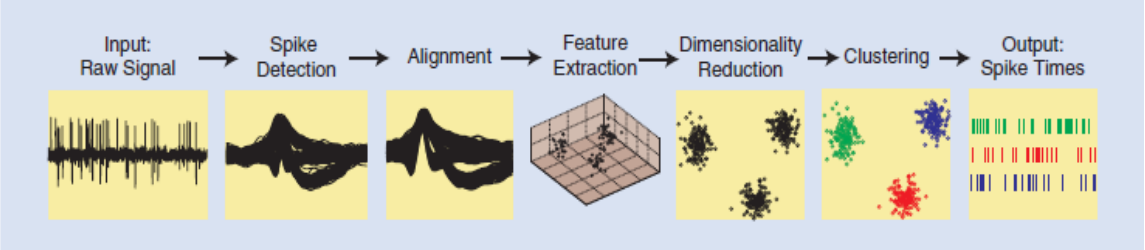

Figure 21. Steps to obtain single spike. Taken from [40].

Method for spike detection may be classified also as *manual,* not completely autonomous, and *automatic/unsupervised*, that does not need of user to work. There is a mixed category, *semiautomatic*, for example all the methods whose requests to set a threshold manually but after that they start to work automatically.
Another distinction in spike algorithm is based on *adaptivity*. Often neural signal is not stationary and it is required an algorithm that adapts itself to the environment. On opposite there are methods that are static and do no change during acquisition. There are also systems in the middle of the two categories which preserve a regular behavior but need of training period frequently.
Clustering method may be *parametric*, any algorithm whose data have a fixed structure, and *nonparametric,* instead data are identified according an arbitrary form and shape.
Since better performances are obtained by complex algorithm, often it is important having good compromise between accuracy and complexity.
In any case all algorithms are composed by two steps: *pre-emphasize* period of the signal and *application* of certain threshold used to detect the spikes.

The first spike sorting was made with hardware system. Processing was performed by hardware and the spike was detected with a Schmitt trigger. It still used this system today, it has been modified including *absolute-value* before comparison and automatic threshold computation. Another kind of spike-detection algorithm uses change of *energy signal* for detecting, it is favorable because it detects spikes only when they increase energy or amplitude and does not takes account frequency, moreover it is simple to implement. Other algorithms use *template*, it is compared

with the recorded signal, if correlation exceeds a threshold the spike is detected. Similarly, detection can be made also using wavelet transform. In this last method is very diffused because it is simple implement wavelet (it is sufficient a filter banks) and complexity is relatively low. In *figure 22* comparison of three methods, the signal-noise-ratio is better in case (c) and detection is less sensitive to threshold for wavelet method.

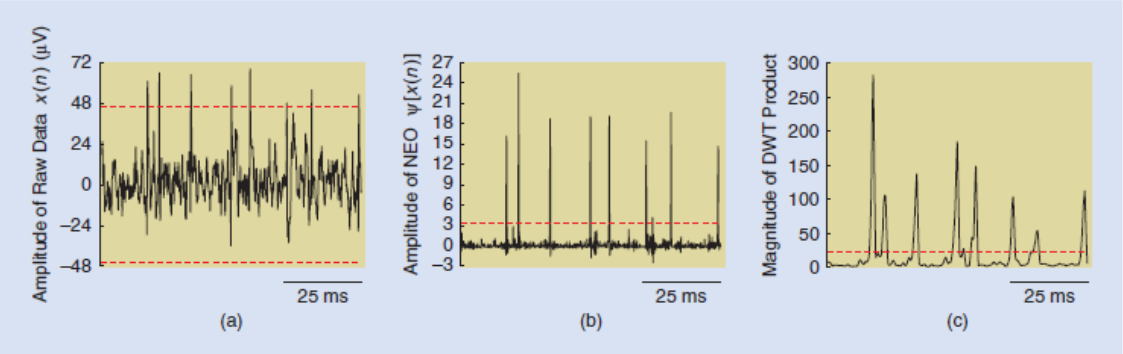

Figure 22. (a) Absolute-value methods, (b) energy signal methods (called also NEO), (c) wavelet method. Red dashed lines indicate the threshold. Taken from [51].

After each spike is detected following step is the alignment, this one is done before the classification, threshold crossing is the point used for alignment. This step is followed by feature extraction. Some algorithms use spike width, maximum amplitude, or peak-to-peak amplitude. All these are simple but can be influenced by noise, indeed robust method foresees use of principal component analysis (PCA). It can be combined with wavelet, obviously it increased accuracy and cost. Intermediate methods are DD method, that is simplified version of wavelet method it is computed the slope of sample point, and IT method, that foresees classification according spikes areas under positive and negative phase [51].

One problem in spike sorting system is the reduction of dimensionality. Firstly, because high dimensionality needs big storage and complex system for clustering, even if the performances are better, it can degrade the clustering introducing mistakes and noise. Dimensionality can be reduced with uniform sampling, or it can be cho-

sen the features to divide clusters and analyze all clusters distribution. If distribution is multimodal it indicates that the chosen feature is good to cluster spikes and in the dataset is present more than one population. There are also algorithms for reducing dimensionality: *Lilliefors*, that computes the distribution and applies a statistic test, *Hartigan's test*, that testes the multimodality by a statistical test or *maximum-difference test* which aims to find features that provides the high variability [51].

Final step is clustering, it is not simple and maybe it is the most complicated part of all the process. At the beginning clustering was made manually, today sometimes software proposes to user to define cluster boundaries, other mixed automatic and manual techniques, spike waveforms are assigned according the window/s intersected, chosen by user.

Complex but fast algorithm is *K-means*, it based on distance, this involves that all cluster will be spherical, but it is not unsupervised method, moreover it is not simple determine the number of neurons and it is not useful for real-time application.

Valley seeking is an unsupervised clustering algorithm, it computes the normalized density derivative and finds peaks of it, defining the regions between peaks as boundaries, this algorithm is nonparametric and can assign to a cluster also data having nontrivial shape, but it is not real-time and hardware is very complex. Same benefits and drawbacks are typical of *superparamagnetic clustering* (SPC), it always an unsupervised algorithm, in which data is similar to a granular magnet and a spin is assigned to each point. Low temperature and high temperature heated the model, at high temperature system appear confused with random spins ('paramagnetic region'), instead at low temperature opposite behavior will be obtain ('ferromagnetic region'). Clusters are showed in region ('superparamagnetic region') in which spins are aligned within the same high-density and spins are not aligned in different high-density regions, this algorithm will be explained more in details in following paragraph. When it required an online algorithm and a good compromise between accuracy and complexity can be used the *Osort Clustering*. It is efficient and does not need a lot of memory but a big data processing in real-time, moreover it uses distance metric for making decisions, so it has the same drawback of *k-means* method.

Spike sorting may be obtained with other techniques which exclude features extractions and clustering methods. Other methods foresee use of *Gaussian* mixture or *t-distribution* mixture models and combine statistical and probability theory for clustering or aim to create a model of noise using *maximum-likelihood* or *Bayesian estimation*.

After sorting it can be useful test if results are good or not, this is translated in evaluation of cluster separation. If two spikes, coming from two neurons, belong to a single cluster, it is possible evaluating the inter-spike and if it is below the minimum refractory of the period (2-3 ms). If difference is high it can be evaluated as measure of the insufficient separation of the single unit [38].
An alternative is checking the variance, if variance of the spike-shape is not uniform it indicates the insufficient unit isolation [36]. Another way to evaluate it is based on quantification of distance between clusters to understand if two or more clusters can be mix together. Anyway, in many cases the goodness is evaluate by user common sense and considering the available data-set.

SPIKE SORTING ALGORITHM (*Table 8* taken from [25])

Clustering	**Feature extraction**	**Authors**
Fuzzy-C-means	Wavelet	*Letelier andWeber*
Expectation maximization	PCA	*Harris et al.*
Fuzzy-C-means	Waveforms	*Zouridakis and Tam*
K-means	Wavelet	*Hulata et al.*
Manual cluster cutting	PCA	*Egert et al.*
Expectation maximization	PCA	*Shoham et al.*
Superparamagnetic clustering	Wavelet packet coefficients	*Quiroga et al.*
Template matching	-	*Rutishauser et al.*
Fuzzy-C-means	Linear Discrimination analysis	*Cho et al.*

Expectation maximization	PCA	*Adamos et al.*
K-means	Zero crossing	*Awais and Andrew*
Hierarchical clustering	PCA	*Biffi et al.*
Bayes	Wavelet	*Takekawa et al.*
Fuzzy-C-means	Discrete derivative	*Gibson et al.*
Density-based clustering	PCA	*Cheng et al.*
Valley-seeking	PCA	*Liu et al.*
Gray relation analysis	Wavelet	*Lai et al.*
Expectation maximization	PCA, wavelet, geometrical features	*Bestel et al.*
K-means, template matching	Wavelet	*Yuan et al.*
Fuzzy-C-means	PCA	*Oliynyk et al.*
Expectation maximization K-means, fuzzy-C-means, Manual cluster cutting	DWT, PCA, peak-to-peak	*Kwon et al.*
1D clustering	Geometrical features	*Englitz et al.*
K-means	FSDE	*Paraskevopoulou et al.*
Expectation maximization	PCA, DWT, geometrical features	*Nick et al.*
Manual amplitude window	-	*MCRack (Multi Channel Systems GmbH)*
Template matching, Manual cluster-cutting, K-means, Gaussian mixture models	PCA	Spike2
Expectation maximization K-means, valley-seeking	PCA	Off-Line Sorter

2.4.5 Quiroga algorithm

Spikes used in this work were obtained using Quiroga algorithm.
This algorithm follows the main steps of the standard algorithm. It has a first step in which will be identify spikes, then it will extract useful feature and finally it will use clustering method.
Two theoretical knowledge are at the base of Quiroga algorithm: *Wavelet* and *Super-paramagnetic clustering*.

Wavelet transform is determined by convolution between interested signal and a Wavelet function. This transformation has the advantage to provide a good resolution both in time and frequency instead other transforms which provide a representation only in one domain. Moreover, Wavelet transform can be applied also at non-stationary signals. Changing the parameters (a and b) of Wavelet function it is equivalent to change the scale or shift the function.

$$\psi_{a,b}(t) = |a|^{-\frac{1}{2}}\psi\left(\frac{t-b}{a}\right) \qquad (1)$$

Changing the scale of the function and computing the convolution with the signal allows to decompose signal at different scales and obtaining a multiresolution decomposition, this is implemented by Mallat algorithm. Quiroga uses a decomposition in four level and *'Haar'* function for its compactness and orthogonality.

Super-paramagnetic clustering is implemented using a Potts model, this model is like Ising model but each particle, in this case, will have q possible states. In this model each point represents a feature of each spike, and it is assigned randomly one of the q states. Interaction between points it is based on *k-nearest* neighbors and it is defined by:

$$J_{ij} = \begin{cases} \frac{1}{K} exp\left(-\frac{\|x_i - x_j\|^2}{2a^2}\right) \\ 0 \end{cases} \qquad (2)$$

(x_i and x_j: points near to each other; a: average nearest-neighbors distance; k: number of nearest neighbors)

From this formula is simple note that similar feature, and so spikes, have strong interaction. Each point will change state in another one in a random way, probability of change depends by temperature, using Wolf algorithm temperature (T) will change for *N* Monte Carlo iterations. This probability is described by following formula:

$$p_{ij} = 1 - exp\left(-\frac{J_{ij}}{T}\delta_{s_i,s_j}\right) \quad (\delta_{s_i,s_j}\text{: point-point correlation}) \quad (3)$$

During one single interaction points can change state only one time and all neighbor points that change state in one interaction create a 'frontier'. For each point of this frontier is applied the probability equation, if some point will change state frontier is update until it does not change anymore. Closer points will change state together, similarly at two points that belong to the same cluster.
Important role in cluster realization is assumed by temperature. In fact, probability function depends by temperature, for high values of temperature the probability to change state is low and the spin changes state randomly (paramagnetic phase), for low values instead the probability increased and spin change at the same time for all points (ferromagnetic phase). There is an intermediate zona (superparamagnetic phase) in which the spin will change at the same time but only for a group of spins. This behavior may be compare with cluster: for low temperature, it created one single cluster (points change state all together), for high temperature are created more clusters (points change state randomly), for intermediate temperature it created different clusters with different points (only some points change state together).

The raw data used for Quiroga algorithm have been previously filtered with a Butterworth filter between 300 and 6000 Hz *figure 23 (A)*. After this the second step is detection *figure 23 (B)*, it is fixed a threshold with the following formula:

$$Th = k * \sigma_n \qquad (4)$$

$$\sigma_n = median\left\{\frac{|x|}{0.6745}\right\} \quad (5)$$

Literature suggests a value between 3 and 5 for K. *X* is the signal after filter application while σ_n represents standard deviation of background noise.
Feature extraction is the third step (*figure 23 (c)*), for each spike are obtained 64 coefficients, shape of our signal is distributed in these coefficients. To separate spikes, it is needed having the coefficients that have a multimodal distribution, it was evaluated by deviation from normal distribution using the Kolmogorov-Smirnov test.
In the specific it compared the cumulative distribution of available data with a reference distribution with the same mean and variance, in this case the reference distribution is the gaussian distribution. It computed difference between the two terms and choose the 10 coefficients with largest difference. Last part of Quiroga spike sorting algorithm is clustering (*figure 23 (D)*). It runs the clustering algorithm for varying temperature and it localizes the superparamagnetic phase. In this phase algorithm creates new clusters with large number of members increasing temperature, in particular it changes from 0 to 0.2 for 200 times increasing the temperature of 0.01 and it is chosen the highest one where clusters contain more than 60 points. In this work it has been used this algorithm, combined the Principal component analysis during application of wavelets function and extraction coefficients. The spike activity obtained after clustering it has been represented in time by raster plot.

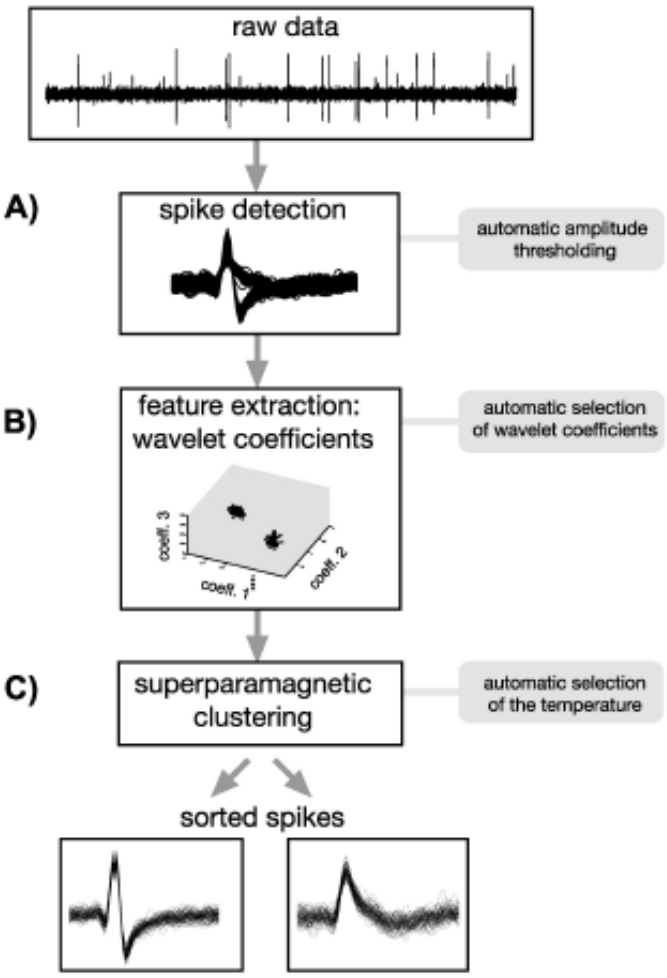

Figure 23. Main steps of Quiroga algorithm. Taken from [48].

2.5 Statistical analysis

Obtained video analysis and raster plot the key point is combine all results and compare them finding something relevant, to do this has been used a statistical method. This method will provide: firing rates as inverse of inter-spike time, distribution of firing rates, mean firing rates for each brain region and finally regression analysis. Statistical analysis considers *rest* condition, *spasm* condition, *voluntary movement* and which *side* of part is moved. The temporal scan of each event is related to temporal subdivision in the video. Information from video analysis is related with spike

detection in each electrode and each lead, it is created *dummy variables* that is marked with 1 when there is a spike otherwise is 0.
The statistical method used is the regression, it is applied for each region and it used the logarithmic scale for computation of firing rate:

$$lograte = c_0 + C_1 * D_{spasm} + C_2 * D_{voluntary} \qquad (6)$$

C_1 and C_2 are coefficients of dummy variable D_{spasm} and $D_{voluntary}$, *rest* condition is considered baseline.

After the creation of the regression model is evaluated the goodness of the model and the statistical significance. Goodness is verified by *R-squared*, which expresses the variance between data and accuracy of the model, or by the *analysis of residual,* which represents the analysis on error distribution or analysis on error variance or independence of errors from independent variables. In this project it has been used the *R-squared* and *Adjusted R-squared* this last one is a variation of *R-squared*. For significance is used the *F-test* which aims to demonstrate that two normal distribution have the same variance and after it is used *t-test* that aims to verified if mean values moves away from referment value.
It is measured *AIC* and *BIC*: *AIC* is *Akaike's information criterion* and provides an estimate of the quality of the model in terms of complexity and adaptability; *BIC* (*Bayesian information crit*erion) is a criterion for select a model between parametric models, it is based on *AIC* and *Likelihood*.
It has been used also *Durbin-Watson test* that shows if there is autocorrelation in residuals. Instead for evaluating the goodness-of-fit is performed the *Jarque-Bera test* based on Kurtosis and skewness. In appendix B some details about statistic test.

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CHAPTER 3: Results

In this chapter it will be presented results from video analysis of all 6 patients. In collaboration with Sanger's Lab from USC, it has been applied the statistical analysis on *period 1* of *patient 55* and *period 3* of *patient 354*. It are reported results of statistical analysis and average firing rate of two periods. Firing rates are computed considering right and left side of the brain, comparing it with, respectively left and right body side involved in the movement. Analysis of average firing rate has been focusing also considering brain areas and conditions of patient as annotated in video analysis.

3.1 Results

3.1.1 Video results

Video tables are obtained from video analysis, for each patient has been computed the percentages of *rest* condition, *spasm* condition, *volitional movement*, which *side* is moved, *passive stimuli* and *not classified* events. Each period lasts at maximum 1 hour, if duration of video was more than 59 minutes it has been divided. It has been added a column about recording time. All patients have been analyzed during '*movement test*' (as said, during this test has been requested patient to move one limb and test his/her capacity to execute a voluntary movement).

The aim of these tables is to constitute a practice and simple measure of video analysis independently from the statistical analysis. At the same time the subdivision of periods and the structure of the table could provide a tool for comparing video analysis directly with raster plot without statistical analysis.

Analyzing these tables, variability is noted not only between patients but also between periods of the same patients. Sometimes percentages of *rest* or *spasm* is clearly greater than other percentages or sometimes percentage of *not classified* events is not derisory, since in some cases it has been difficult determine the real condition of the patient. Syndrome affects patient in different way and it is not al-

ways clearly distinguishable if movement is voluntary or not, all these considerations have been taken into account during video analysis, anyway all this information are contained indirectly in following tables.

Patient 55 does not report a lot of spasms, percentage in both periods is very low (4,4 % and 2,5 %). *Patient 393* has very low percentages of *rest* condition and *voluntary* percentages in all periods that are characterized by many *spasm* and *involuntary movements*. Other patients as *139*, *332* and *354*, have not big difference between *rest* percentage and *spasm* percentage, with the only exception of periods 3 and 5 of *patient 332* and period 1 of *patient 354*. In *patients 278* there is a relatively high percentage in *not classified* column if compared with *rest* and *spasm* columns, it is caused by camera movements that provide few information to determine exact condition of the patient.

Patient 139 (*Table 9*)

Patient 139 (male)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classified
Period 1	09:48:29/ 10:03:59	10,6 %	9,57 %	0 %	4,25 %	20,2 %	7,44 %	79,7 %
Period 2	10:05:45/ 10:50:15	26,4 %	36,5 %	26,1 %	44,7 %	55,5 %	35 %	22,7 %
Period 3	10:54:07/ 11:06:17	18,9 %	51,3 %	21,6 %	66,2 %	68,9 %	40,5 %	22,9 %
Period 4	11:09:09/ 11:30:59	29,5 %	28 %	15,9 %	34,8 %	45,4 %	35,6 %	33,3 %
Period 5	11:32:21/ 12:04:51	28 %	28 %	9,18 %	37,2 %	54 %	86,7 %	38,2 %
Period 6	13:02:03/ 13:26:53	54,6 %	9.33 %	0 %	13,3 %	24,6 %	60,6 %	36 %
Period 7	14:28:32/ 14:31:42	60 %	25 %	0 %	25 %	35 %	30 %	15 %
Period 8	14:53:40/ 15:15:50	38 %	32 %	11,9 %	25,3 %	55,9 %	6 %	25,3 %

Period 9	08:32:57/ 08:45:27	12,1 %	16,2 %	1,35 %	36,4 %	51,3 %	9,45 %	71 %
Period 10	08:46:06/ 08:50:56	6,66 %	26,6 %	3,33 %	73,3 %	73,3 %	6,7 %	63,3 %

Patient 55 (*Table 10*)

Patient 55 (female)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classified
Period 1	10:32:47/ 11:32:37	66,6 %	4,4 %	15,5 %	17,2 %	16,9 %	19,1 %	15 %
Period 2	11:32:53/ 12:32:43	97,2 %	2,5 %	0,27 %	0,83 %	3, 05 %	13,3 %	0,5 %

Patient 278 (*Table 11*)

Patient 278 (male)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classified
Period 1	12:25:45/ 12:35:55	27,4 %	37 %	22,5 %	37 %	66,1 %	19,3 %	33,8 %
Period 2	12:38:19/ 12:48:29	32,2 %	9,67 %	19,3 %	37 %	53,2 %	40,3 %	46,7 %
Period 3	14:08:36/ 14:18:56	34,9 %	44,4 %	9,52 %	30,1 %	60,3 %	28,5 %	17,4 %
Period 4	14:30:31/ 14:38:41	36 %	24 %	2 %	40 %	62 %	22 %	40 %
Period 5	14:40:27/ 14:51:47	37,6 %	26 %	2,89 %	55 %	43,4 %	23,1 %	36,2 %
Period 6	15:17:03/ 15:34:13	46,1 %	20,1 %	0 %	30,7 %	47,1 %	49 %	33,6 %

Period 7	16:00:52/ 16:08:32	19,1 %	36,1 %	0 %	51 %	53,1%	0 %	44,6 %
Period 8	15:35:44/ 15:57:04	20,1 %	29,4%	20,1%	59,6%	37,2%	2,32 %	34,1 %
Period 9	13:53:33/ 13:58:43	21,8 %	46,8%	28,1%	50%	56,2%	53,1 %	18,7 %
Period 10	15:23:11/ 15:36:20	14,8 %	39,5%	4,93%	38,2%	71,6%	16 %	41,9 %
Period 11	15:37:07/ 15:43:07	16,2 %	18,9 %	0 %	56,7 %	54 %	2,70 %	0,64 %
Period 12	10:34:42/ 10:55:12	15,3 %	46,8 %	15,3 %	61,3 %	57,3 %	19,3 %	35,4 %
Period 13	15:43:54/ 15:49:44	30,5 %	22,2 %	16,6 %	41,6 %	50 %	30,5 %	44,4 %
Period 14	10:59:45/ 11:13:35	29,7 %	13 %	16,6 %	54,7%	35,7%	48,8 %	46,4 %
Period 15	11:29:11/ 11:41:01	18 %	38,8 %	29,1 %	72,2 %	55,5%	20,8 %	33,3 %

Patient 393 (*Table 12*)

Patient 393 (male)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classi-fied
Period 1	13:48:20/ 14:06:20	0 %	92,1 %	0 %	94,7 %	93,9 %	33,9 %	2,75 %
Period 2	14:57:33/ 15:57:23	0 %	20 %	0 %	29 %	25 %	12,2 %	80,2 %
Period 3	15:57:33/ 16:48:23	0.98 %	21,9 %	1,63 %	38,6 %	37,7 %	0 %	77,1 %
Period 4	10:35:42/ 11:27:32	0 %	96,7 %	11,2 %	98,7 %	98,7 %	21,5 %	3,20 %
Period 5	13:01:01/ 14:00:51	0.83 %	56,1 %	0 %	54,4 %	55,2 %	34,1 %	43 %
Period 6	14:01:01/ 15:00:21	0 %	75,9 %	0 %	75,9 %	76,4 %	14,8 %	24 %

Period 7	15:34:36/ 16:02:26	0 %	97,6 %	0 %	100 %	100 %	55,3 %	2,38 %
Period 8	16:04:31/ 16:34:41	7,69 %	62,6 %	3,29 %	82,4 %	90,6 %	41,2 %	28,5 %
Period 9	10:34:14/ 11:04:34	3,82 %	58,4 %	1,09 %	92,3 %	91,2 %	51,3 %	37,7 %
Period 10	14:48:56/ 15:14:36	2,58 %	54,8 %	0 %	96,7 %	96,7 %	18,1 %	42,5 %

Patient 332 (*Table 13*)

Patient 332 (male)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classified
Period 1	11:12:49/ 12:12:39	48,3 %	39,7 %	0 %	38,6 %	33,3 %	99,4 %	11,9 %
Period 2	12:12:49/ 13:12:39	38,9 %	40 %	5,83 %	35,8 %	36,1 %	16,6 %	20,5 %
Period 3	13:12:49/ 14:12:39	84,7 %	12,5 %	0 %	10,8 %	8,61 %	31,6 %	2,77 %
Period 4	14:12:49/ 15:04:49	40,2 %	36,1 %	2,87 %	29 %	32,5 %	81,1 %	22 %
Period 5	15:05:04/ 15:56:54	70,8 %	21,7 %	0 %	19,5 %	20,5 %	73,7 %	7,37 %
Period 6	15:57:14/ 16:40:44	38,5 %	39,3 %	0 %	22,9 %	36,6 %	93,1 %	22,1 %
Period 7	09:37:39/ 10:00:19	27 %	24 %	20,4 %	35 %	27 %	92,7 %	35 %
Period 8	10:00:37/ 10:43:47	34,6 %	29,3 %	3,07 %	31,9 %	29,6 %	100 %	35 %

Patient 354 (*Table 14*)

Patient 354 (male)	Hour	Rest	Spasm	Volitional movement	Right	Left	Passive stimuli	Not classi-fied
Period 1	10:27:09/ 11:25:49	32,5 %	67,1 %	19,8 %	38,5 %	62,3 %	29,1 %	0,28 %
Period 2	11:25:58/ 12:25:48	53,8 %	45,2 %	13,3 %	25,8 %	42,7 %	25,8 %	0 %
Period 3	12:26:02/ 13:25:52	43,8 %	52,2 %	0,27 %	29,7 %	48 %	40 %	3,61 %
Period 4	13:26:05/ 14:25:55	63,3 %	36,6 %	0 %	21,9 %	32,7 %	40,5 %	0 %
Period 5	14:26:08/ 15:25:58	48,3 %	51,1 %	0 %	26,6 %	48,3 %	3,05 %	0,55 %
Period 6	15:26:11/ 16:24:21	58 %	42 %	0 %	18,2 %	39,4 %	18,8 %	0,28 %

3.1.2 Statistical analysis results

Information for each patient are many, both in terms of videos and in terms of spikes. In following paragraphs, the average firing rates computed during statistical analysis.

From firing rates of patient 55 and 354 following histogram has been obtained:

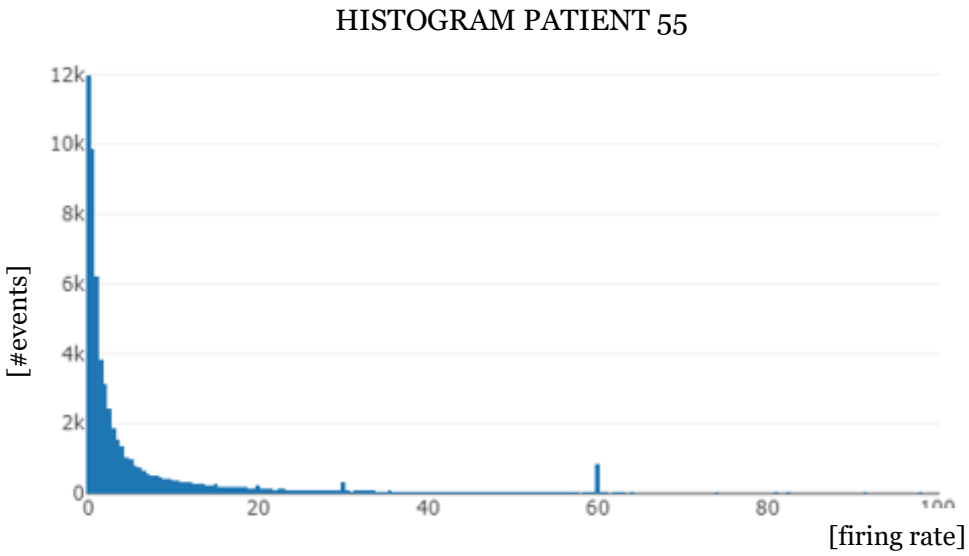

Figure 24. Histogram for patient 55.

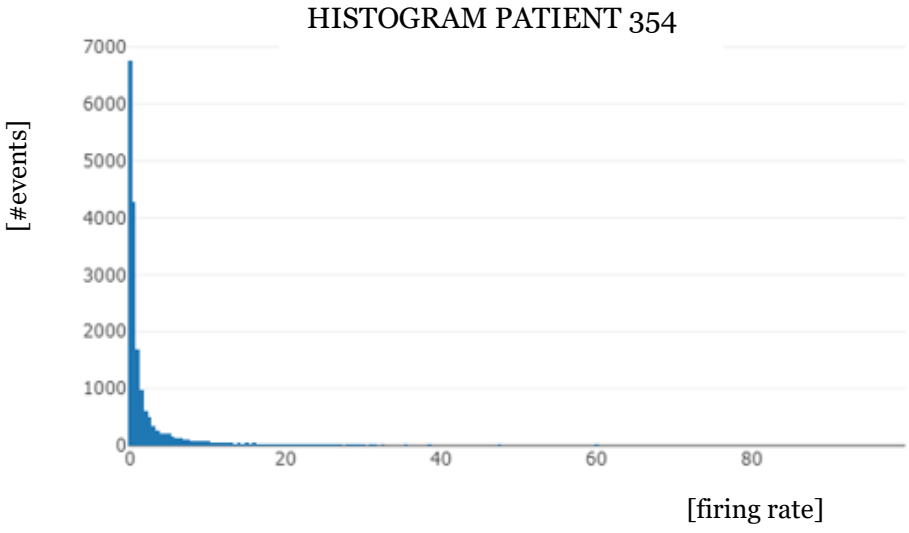

Figure 25. Histogram for patient 354.

3.1.3 Right brain

Average firing rates of all brain regions in right side have been compared with left side of the body. In *figure 26*, is represented the variability of averaging firing rates between conditions of the patient. Average firing rates have been computed between each regions and conditions of the patient always selecting spikes involving right side of the brain and left side of the body (*figure 27*). *Voluntary movement* assumes great values of firing rates in STN region, GPi and VIM have about the same values while Voa is more active during *spasm*. From comparison between condition of the patient and brain regions it has been computed table of *figure 28*. In this case there is a higher average firing rates associated to STN during *voluntary movement* in left side.

Analysis of patient 354 are reported in *figure 29* showed the activity of right side of the brain compared with left side of the body, in this case values and standard error are similar, except just for *voluntary movements* in left side of the body.

There is a big activity during *voluntary movement* in VIM region (*figure 30*), there is huge variability both between regions and between conditions of the same region and it is absent activity in GPi. In *figure 31* we can note high mean firing rates for VIM during *voluntary movement* involving both portion of the body

AVERAGING FIRING
RATE PATIENT 55

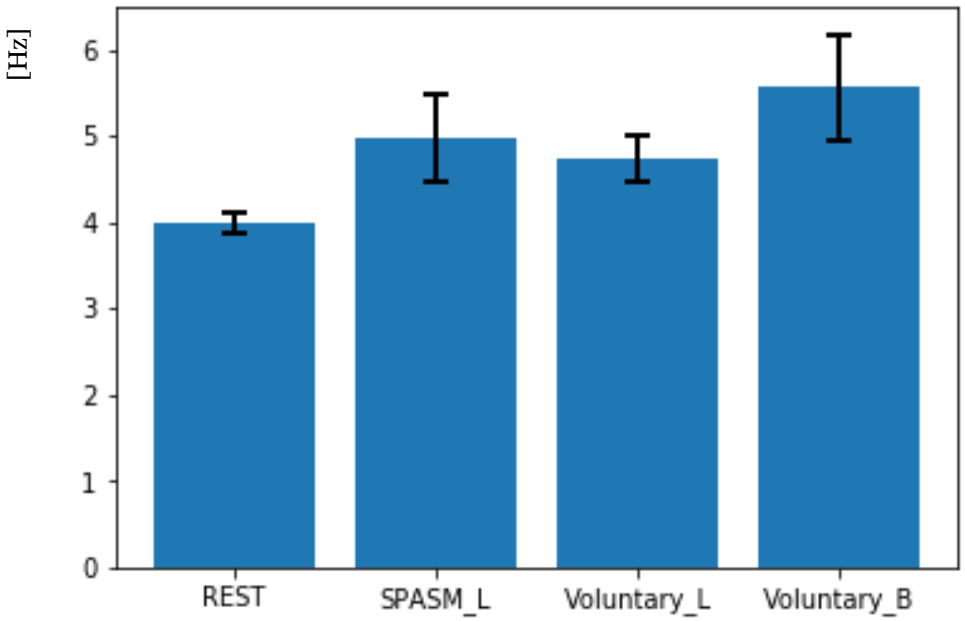

Figure 26. Average firing rates for patient 55, comparison between right side of the brain and left side of the body; SPASM_L indicates spasm in left side, VOLUNATARY_L indicates voluntary movements in left side; VOLUNTARY_B indicates voluntary movements in both sides. The bar is the standard error.

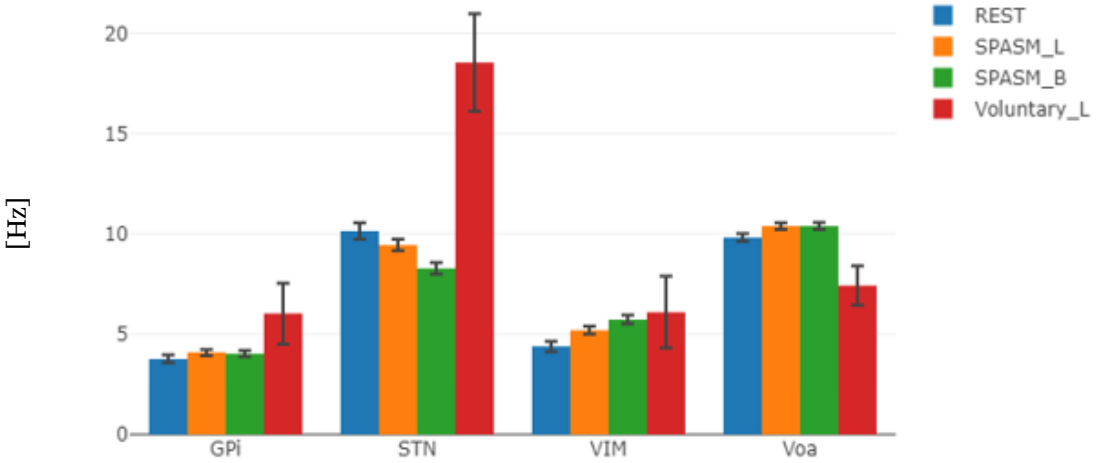

Figure 27. Average firing rate for patient 55 of four right brain regions compared with conditions of the patient.

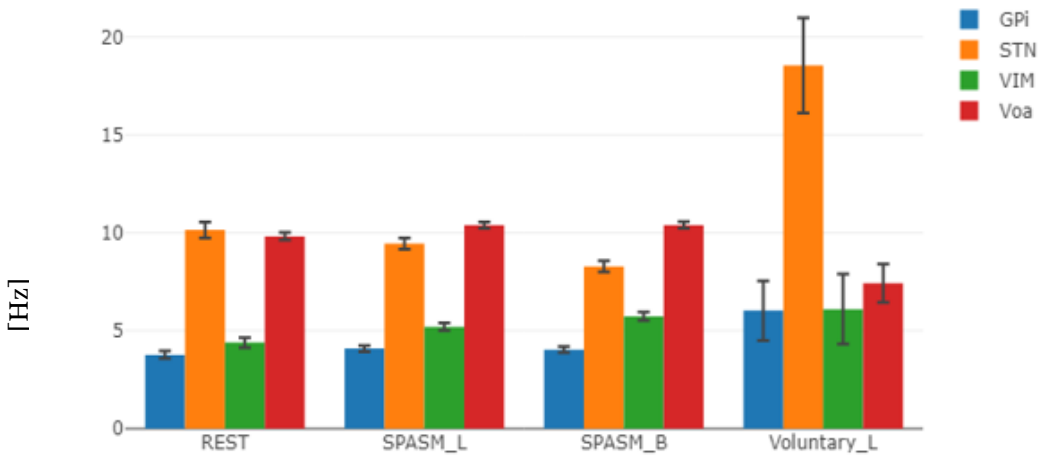

Figure 28. Average firing rate for patient 55 of four conditions of the patients compared with four right brain regions.

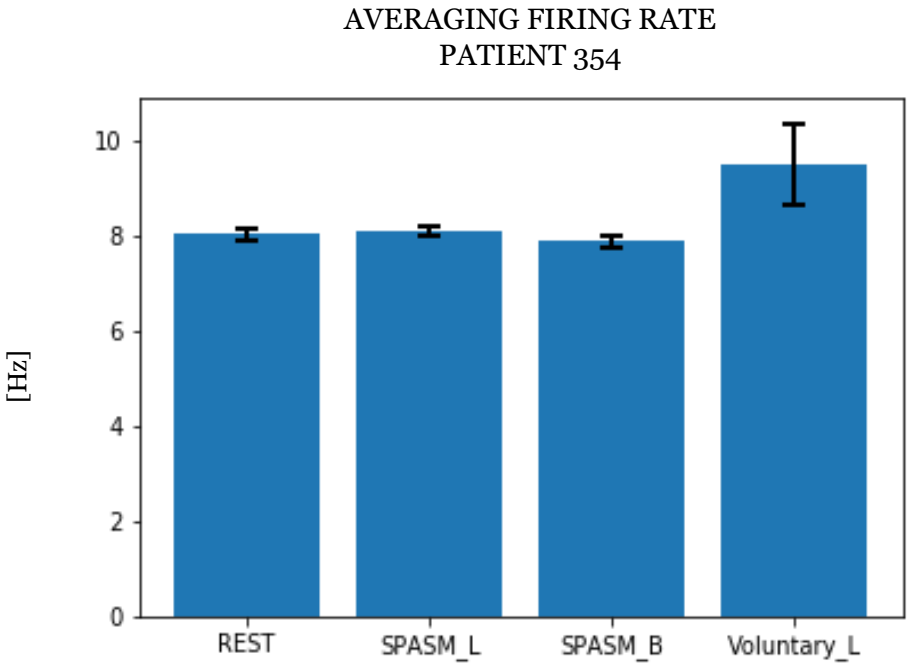

Figure 29. Average faring rates for patient 354, comparison between right side of the brain and left side of the body; SPASM_L indicates spasm in left side; SPASM_B indicates spasm in both sides; VOLUNTARY_L indicates voluntary movements in left side. The bar is the standard error.

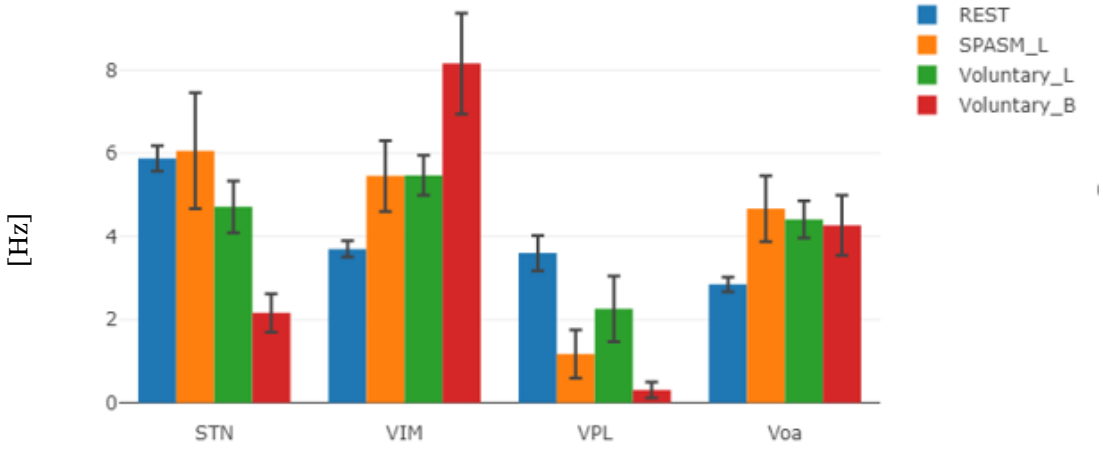

Figure 30. Average firing rates for patient 354 of four right brain regions compared with conditions of the patient.

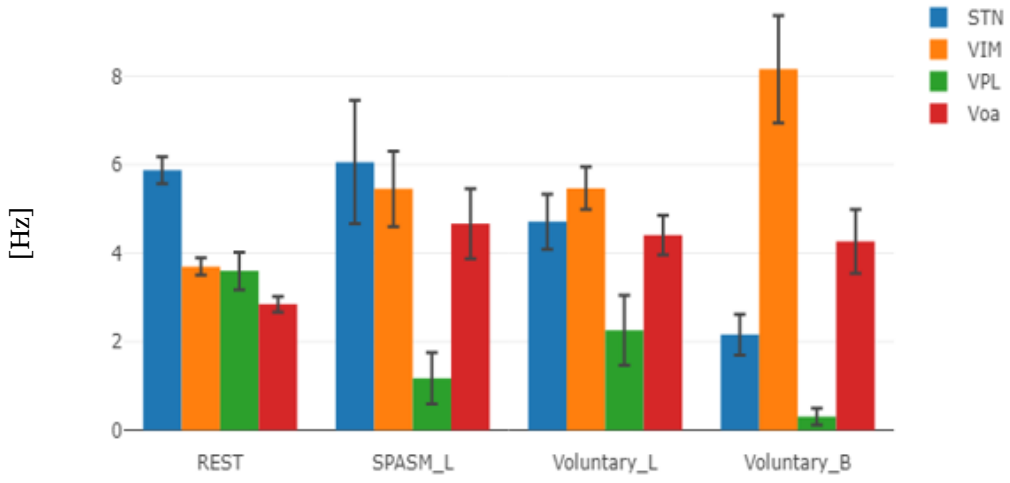

Figure 31. Average firing rates for patient 354 of four conditions of the patient compared with four right brain regions.

3.1.4 Left brain

Same analysis is obtained for left side of the brain compared with right side of the body. In this case *rest* average firing rates have greater value than other categories and standard error is lower than standard error in other ones (*figure 32*). Looking

figures 33 it is highlighted a high value for *rest* condition in all regions than other conditions, *spasm* is almost absent in ventral posterolateral nucleus. Moreover, for this patient, as well as the previous figures, there is no activity in globus pallidus and anything relevant has been recorded for that area. In *figures 34 Patient 55* has still high values in *rest* condition especially in VIM than other conditions.

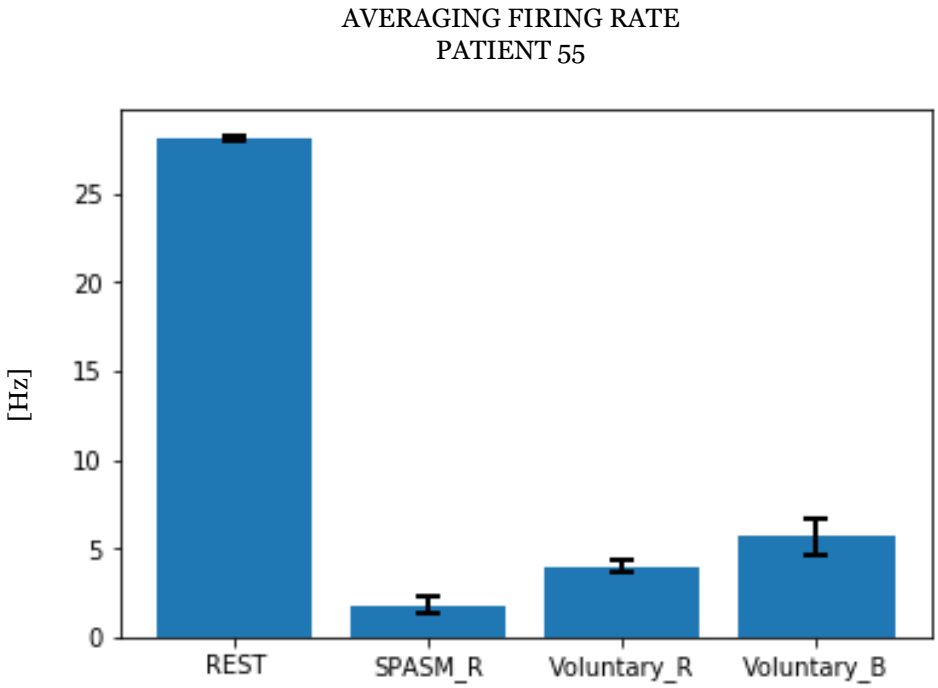

Figure 32. Average firing rates for patient 55, comparison between left side of the brain and right side of the body; SPASM_R indicates spasm in right side; VOLUNATARY_R indicates voluntary movements in right side; VOLUNTARY_B indicates voluntary movements in both sides. The bar is the standard error

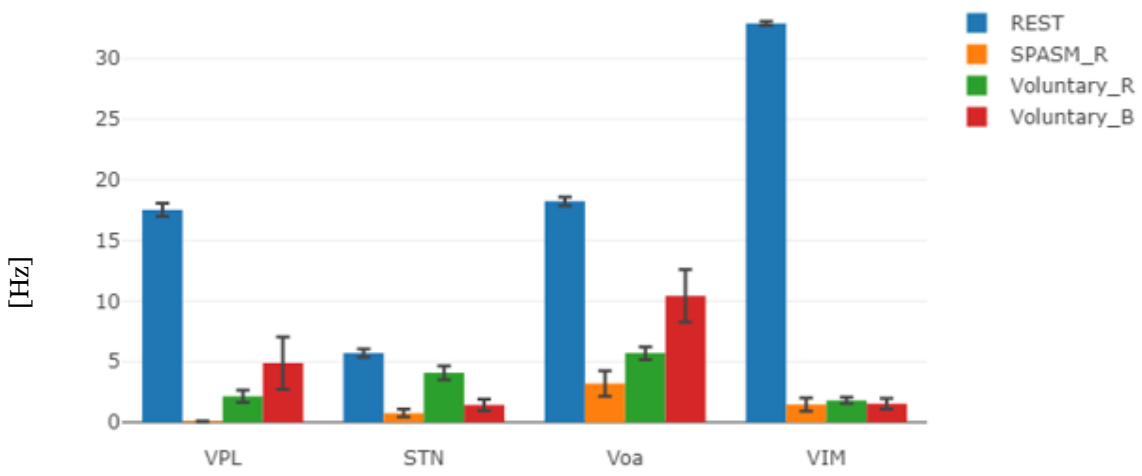

Figure 33. Average firing rates for patient 55 of four left brain regions compared with conditions of the patient.

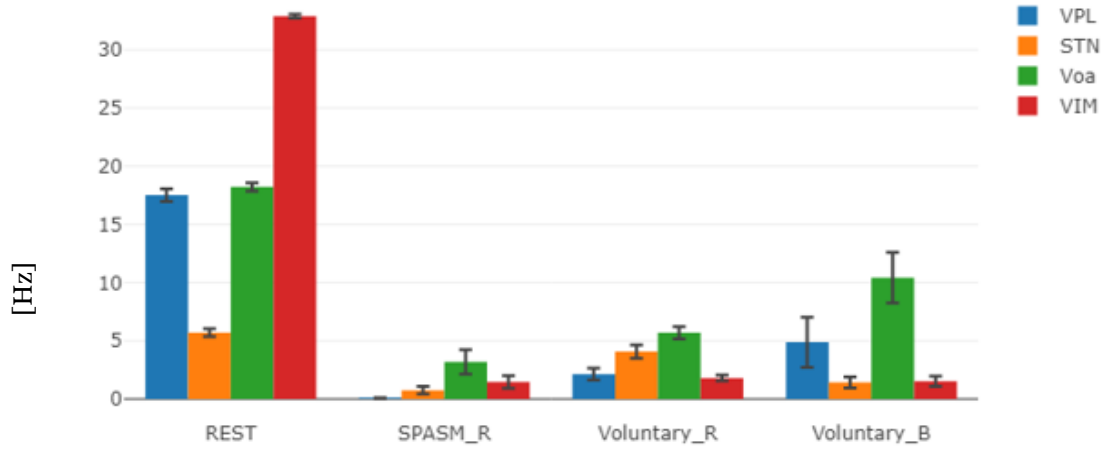

Figure 34. Average firing rates for patient 55 of four conditions of the patient region compared with four left brain regions.

Same analysis is made for patient 354. In *figure 35* it is analyzed the condition related to the left brain in comparison with right side of the body, values obtained are similar for all categories. *Voluntary movements* in *figure 36* are absent, it shows no

great variability between *rest* and *spasm* in each region and GPi remains region with lowest values. Instead, great activity has been recorded in Ventralis Oralis anterior (Voa) in all conditions of *figure 37*.

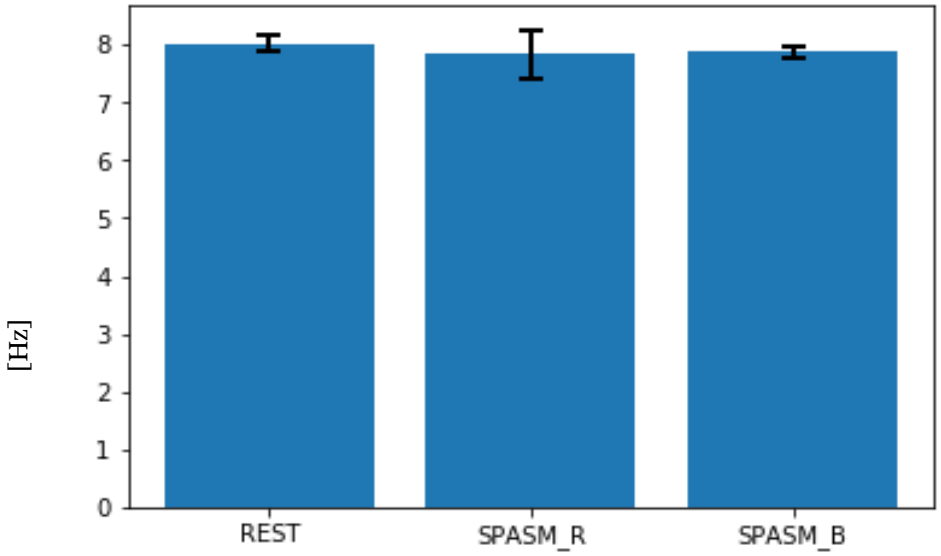

Figure 35. Average firing rates for patient 354; comparison between left side of the brain and right side of the body; SPASM_R indicates spasm in right side; SPASM_B indicates voluntary movements in both sides. The bar is the standard error.

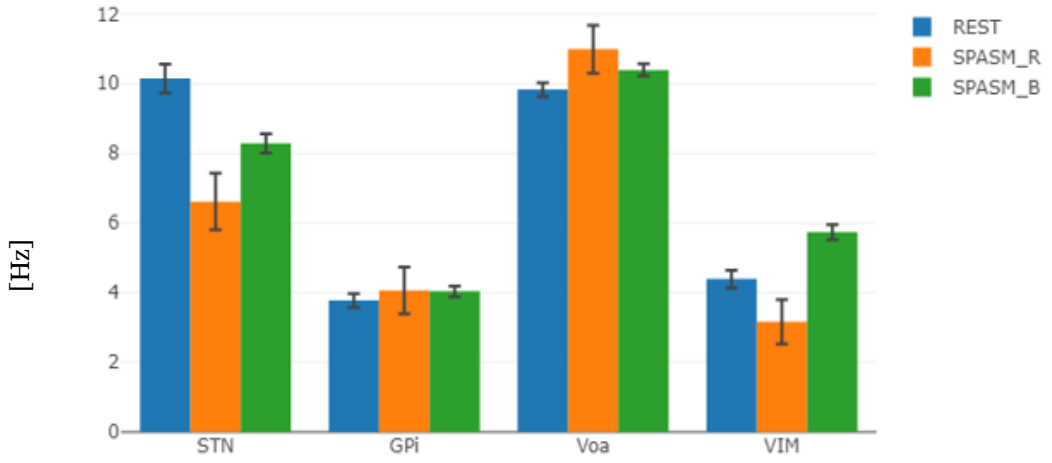

Figure 36. Average firing rates for patient 354 of four left brain regions compared with conditions of the patient.

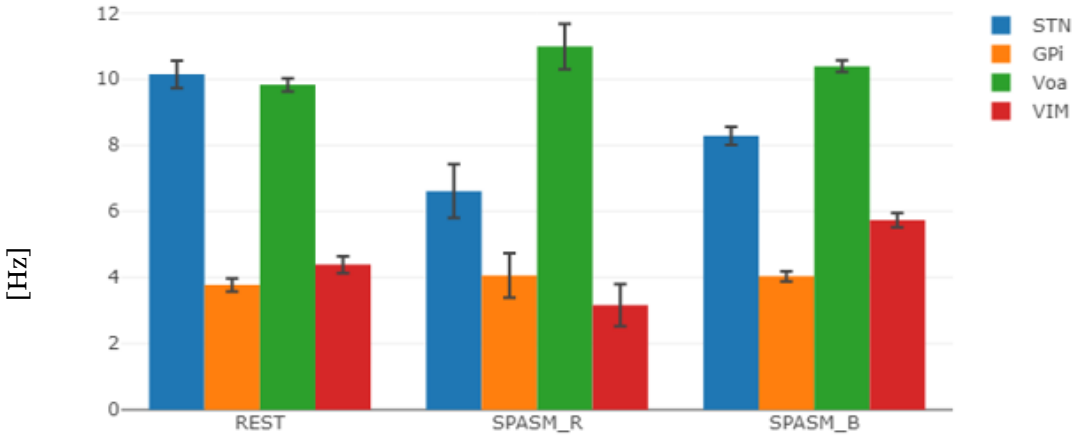

Figure 37. Average firing rates for patient 354 of conditions of the patient region compared with four left brain regions.

3.1.5 Aggregated data

It has been provided also an aggregation between two patients, obtaining same results than before *figure 38* and *39*, followed by statistical tables (*Table 15, Table 16*).

These last ones show all results obtained from regression analysis and results of statistical test as *R-squared* or *F-statistic*. *Table 15* is referred to results related to left side of the brain compared with right side of the body, whereas *Table 16* is referred to right side of the brain compared with left side of the body. Looking *t-values* it can be noted that spasm in left side is significative (*Table 16*) but same conclusion does not be apply to for in right side of the body (*Table 15*). Combined results of averaging firing rate show that *rest* condition remains with higher values in VIM and Voa, instead GPi activity remains low (*figure 40*). Activity in Voa is elevated in almost all conditions and VPL is almost absent in all conditions except for *rest* and *voluntary* conditions (*figures 41*). In the mixed analysis referred to the right side of brain compared to left side of the body, patient's condition with greater activation is *spasm*, that assumes great values in almost brain regions, except just for VPL (*figure 42*). In this nucleus *spasms* have low values or are absent (*spasm* in right side). Also, in diagrams concerned the distribution of firing rate's area according to conditions of the patient (*figure 43*), *spasm* assumes great values especially in Voa and STN, whereas activity in VPL results low.

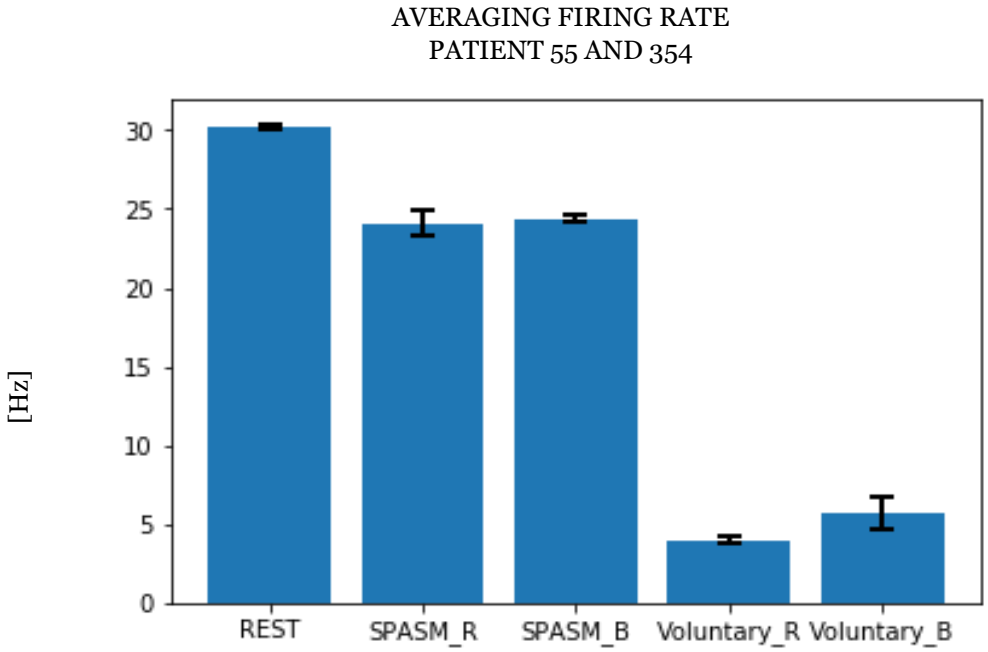

Figure 38. Average firing rates for patients 55 and 354, in the specific regions of left side of the brains with right side of the body. SPASM_R indicates spasm in right side; SPASM_B indicates spasm in both side; VOLUNATARY_R indicates voluntary movements in right side; VOLUNTARY_B indicates voluntary movements in both sides. The bar is the standard error.

AVERAGING FIRING RATE
PATIENT 55 AND 354

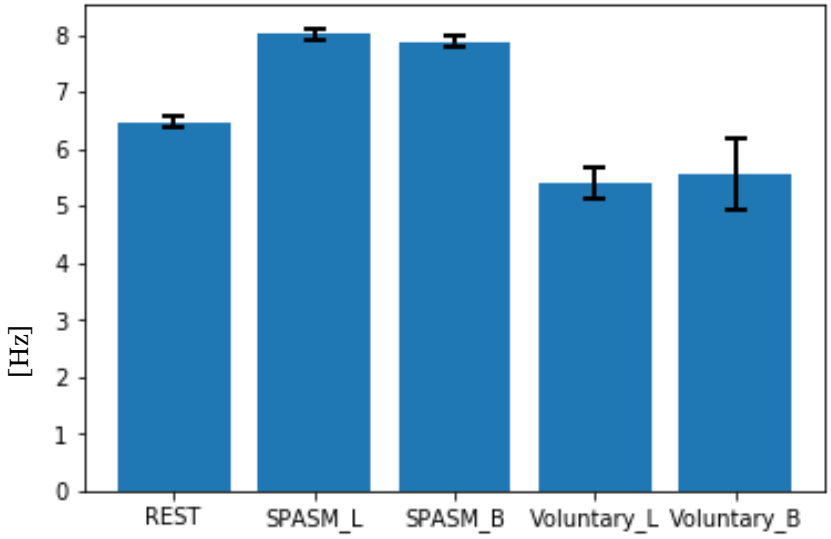

Figure 39. Average faring rates for patients 55 and 354, in the specific regions of right side of the brains with left parts of the body. SPASM_R indicates spasm in right side; SPASM_B indicates spasm in both side; VOLUNATARY_L indicates left voluntary movements in right side; VOLUNTARY_B indicates voluntary movements in both sides. The bar is the standard error.

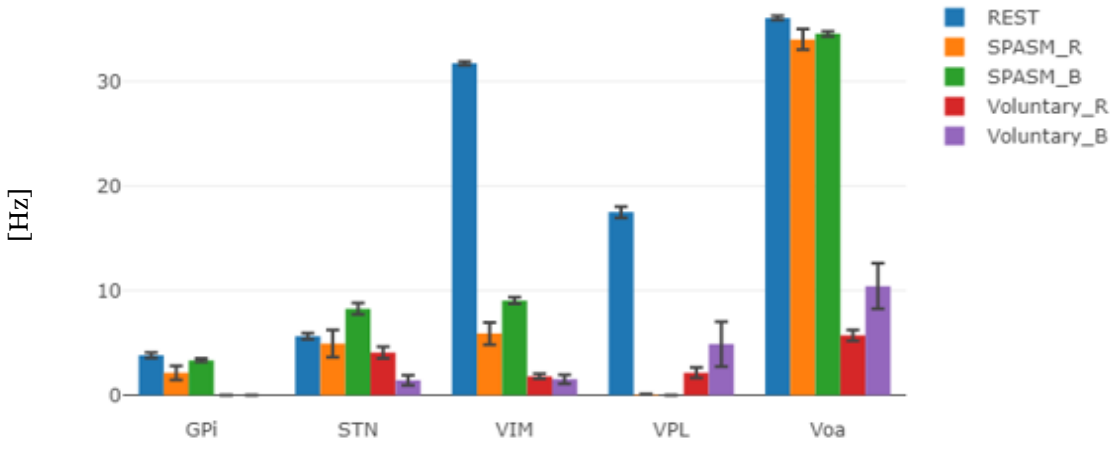

Figure 40. Aggregated average firing rates for patients 55 and 354 of left brain regions compared with conditions of the patient.

REGRESSION TABLE LEFT SIDE OF THE BRAIN Vs. RIGHT SIDE OF THE BODY *(Table 15)*

OLS Regression Results

Dep. Variable:	LogRate	R-squared:	0.033
Model:	OLS	Adj. R-squared:	0.033
Method:	Least Squares	F-statistic:	764.4
Date:	Sun, 24 Jun 2018	Prob (F-statistic):	0.00
Time:	07:15:58	Log-Likelihood:	-2.1048e+05
No. Observations:	90191	AIC:	4.210e+05
Df Residuals:	90186	BIC:	4.210e+05
Df Model:	4		
Covariance Type:	nonrobust		

	coef	std err	t	P>|t|	[0.025	0.975]
Intercept	1.9855	0.010	206.123	0.000	1.967	2.004
SPASM_R	-0.7145	0.071	-10.023	0.000	-0.854	-0.575
SPASM_B	-0.4771	0.020	-23.561	0.000	-0.517	-0.437
Voluntary_R	-2.7888	0.058	-48.036	0.000	-2.903	-2.675
Voluntary_B	-2.8875	0.158	-18.329	0.000	-3.196	-2.579

Omnibus:	9664.704	Durbin-Watson:	0.678
Prob(Omnibus):	0.000	Jarque-Bera (JB):	13114.503
Skew:	-0.931	Prob(JB):	0.00
Kurtosis:	2.857	Cond. No.	19.5

REGRESSION TABLE RIGHT SIDE OF THE BRAIN Vs. LEFT SIDE OF THE BODY *(Table 16)*

OLS Regression Results

Dep. Variable:	LogRate	R-squared:	0.014
Model:	OLS	Adj. R-squared:	0.014
Method:	Least Squares	F-statistic:	276.7
Date:	Sun, 24 Jun 2018	Prob (F-statistic):	1.16e-236
Time:	07:15:58	Log-Likelihood:	-1.6745e+05
No. Observations:	77435	AIC:	3.349e+05
Df Residuals:	77430	BIC:	3.350e+05
Df Model:	4		
Covariance Type:	nonrobust		

	coef	std err	t	P>|t|	[0.025	0.975]
Intercept	-0.0716	0.013	-5.651	0.000	-0.096	-0.047
SPASM_L	0.4807	0.019	25.888	0.000	0.444	0.517
SPASM_B	0.4471	0.019	23.693	0.000	0.410	0.484
Voluntary_L	-0.3492	0.042	-8.284	0.000	-0.432	-0.267
Voluntary_B	-0.0942	0.096	-0.980	0.327	-0.282	0.094

Omnibus:	1349.281	Durbin-Watson:	1.230
Prob(Omnibus):	0.000	Jarque-Bera (JB):	825.690
Skew:	-0.099	Prob(JB):	5.06e-180
Kurtosis:	2.534	Cond. No.	13.9

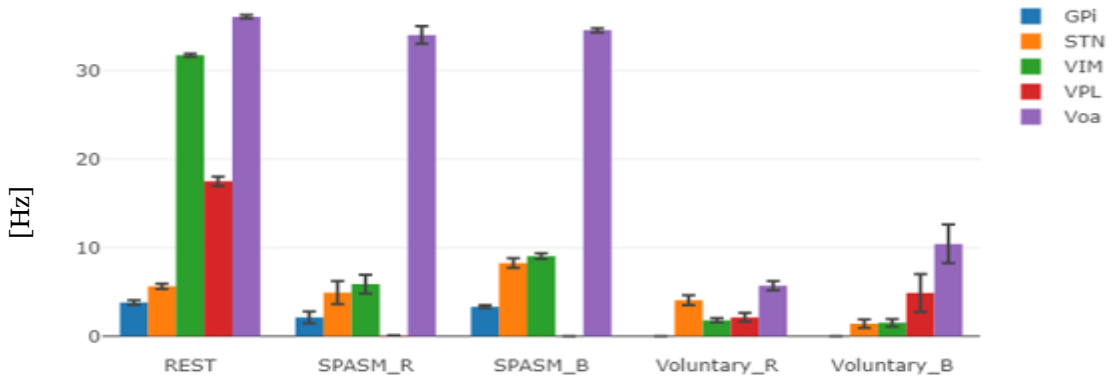

Figure 41. Aggregated average firing rates for patients 55 and 354 of conditions of the patient compared with left brain regions.

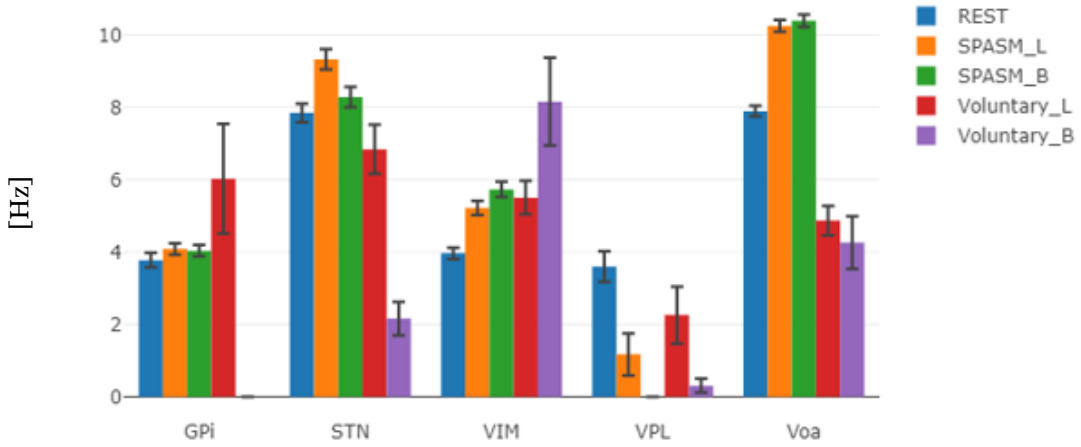

Figure 42. Aggregated average firing rates for patients 55 and 354 of right brain region compared with conditions of the patient.

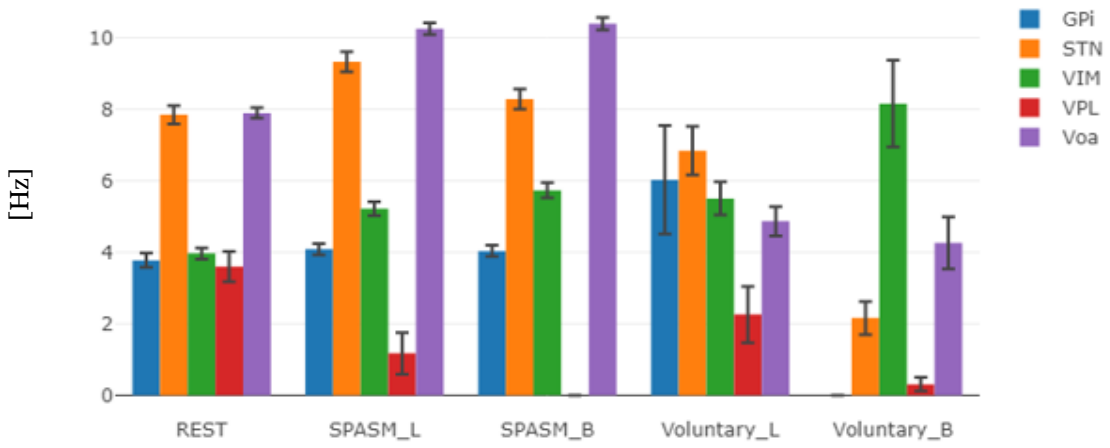

Figure 43. Aggregated average firing rates for patients 55 and 354 of four conditions of the patient compared with right brain regions.

CHAPTER 4: Conclusions

In this chapter results of *chapter 3* will be commented and compared them with results found in literature. It will suggest also updates and alternatives focusing on other possible approaches to work with neural recording.

4.1 Discussion

From analysis of the averaging firing rate, there is a great variability both in brain regions involved and in conditions of the patient analyzed. It is difficult to deduce general conclusions for lack of data analyzed with statistical method. The averaging firing rate, showed in literature, considers more patients, several kinds of dystonia, not only secondary. Furthermore, literature does not always consider all nuclei on which this work has focused.

Literature and researches concentrated their studies on analyzing of internal globus pallidus and external globus pallidus as they are mainly involved in dystonia process. In [52] median firing rate is between 13.5 Hz and 9.6 Hz, in [40] values are greater 71.4 ± 2.2 even if patients are all affected by Cervical dystonia or 55.3 ± 1.3 for [30]. For secondary dystonia values are about 47.8 ± 19.5 Hz [47], even if analyzed patients are twenty, in [46] the three analyzed patients report 34.0 ± 3.5 Hz. In the results of this work the GPi has not big activity and in some case averaging firing rate was zero or about zero. Mean firing rates of GPi reaches values between 0 and 6 Hz, greater values in right side of the brain during left *voluntary movements*. In STN mean firing rates was reported about 26.70 Hz (SD 6.3) [41] in *rest* and 23.60 (SD 13.3) [41] during *movement*, comparing these results with our data values are different. In right portion is lower than 10 Hz both for *rest* both for *movement* and, analyzing also single results referred to specific side of the brain, there is no averaging frequency that can amount to that value.

The highest averaging firing rate has been recorded in Voa region but only in one patient, in this case it is more than 30 Hz, both in *rest* condition and in *spasm* condition. These results are not reliable since they are obtained only from two patients and only for two periods of only one hour, but methods and analysis used might

constitute a starting point for applying techniques in many patients and, at the same time, for improving techniques and methods used.

4.1.1 Future directions

First step may consist in extending statistical analysis to all patients and to all periods, so that comparison with literature data will be more reliable. In this way it will be simple evaluate the methodologies used for neural activity recording and spike analysis, possibility to join other analysis or simply confirm the goodness of the methods used. Another goal is to verify efficient of spike detection and sorting. The acquisition system is without doubt a delicate point, especially in spike sorting analysis, which requires accurate study of the data. It is important to have data without artifacts in order not to use many filters avoiding deteriorating signal waveform and spike shape. Better acquisition system may provide better results and accurate clusters. It could be used other system over *RZ2 Bio-amp processor* or other kinds of electrodes.

As said actual systems do not record all activity of neurons of the area, not providing accurate information and realistic picture of neural activity. Important role is constituted by pre-processing and processing of the neural signal that can modify the real nature and change the real spike waveform. A possible improvement could limit filters application, that change signal waveform inevitably. Another change is related to algorithm used for spike detection and sorting. As it is showed in *chapter 2* there are several kinds of algorithms and, testing available data with other algorithm, might confirm the application of Quiroga algorithm or might suggest using of other ones. Validated the goodness of the acquisition and data analysis, analysis can be enriched with analysis of local field potential or EMG signal, studying the syndrome from another point of view. It could be applied also statistical analysis on periods in which patient was under electrical stimulation. These periods were very noisy, artifacts do not allow to obtain all spikes and waveform was often changes. Good processing of these could be useful for obtaining desiderated spikes, focusing on effects of deep brain stimulation.

4.2 LFP

Neural activity, by the study of the spikes activity, provides specific information on single neuron but it has also several problems as described in *chapter 2*. There are other ways to analyze brain electrical activity as local field potential.

Local field potential or micro-EEG gives a measure of electric potential of a small group of neurons in deep brain region, contrary to EEG which records potential from the scalp or ECoG which records from cortical surface. The study of LFPs is divided in frequency bands and computation of them is simpler than spike detection, because spike detection and sorting algorithm or clustering are not necessary but in addition to standard filters for pre-processing is sufficient the Fourier transform, or Wavelet transform to obtain measure of neural population field potentials. Standard methods to obtain LFPs foresees that signals are down-sampled and applicated a lowpass filter with a cutoff at 300 Hz. It can be used the Fourier transform computing the power spectrum or it can be used the windowed Fourier transform. Signals are decomposed in bands, the choice of the bands is arbitrary and in general are: 0-4 Hz (delta), 4-8 Hz (theta), 8-12 Hz (alpha), 12-24 Hz (beta), 24-100 Hz (gamma). If it is necessary, a more accurate analysis, could be used the Wavelets transform.

It is interesting to highlight how LFPs change in low and high frequencies of power spectra during tics in patients affected by Tourette syndrome, in three patient's conditions: *rest*, *voluntary movements* and *tics* [53]. This should be encouraging for applying the LFP analysis also in dystonic patients or in available data used in this work, as subdivision of data and analysis organization foresees a similar study based on condition of the patient.

LFP activity could be compared with EMG activity as for spike detection, it can be compared with *movements* and *rest*. It has been showed [54] that, in patient affected by Myoclonus-Dystonia, in *rest* condition activity of LFP between 3 to 15 Hz has a similar behavior than spectrum of sternocleidomastoid (SCM) muscle and is less correlated with tibialis anterior (TIM) activity as showed in *figure 44* [54].

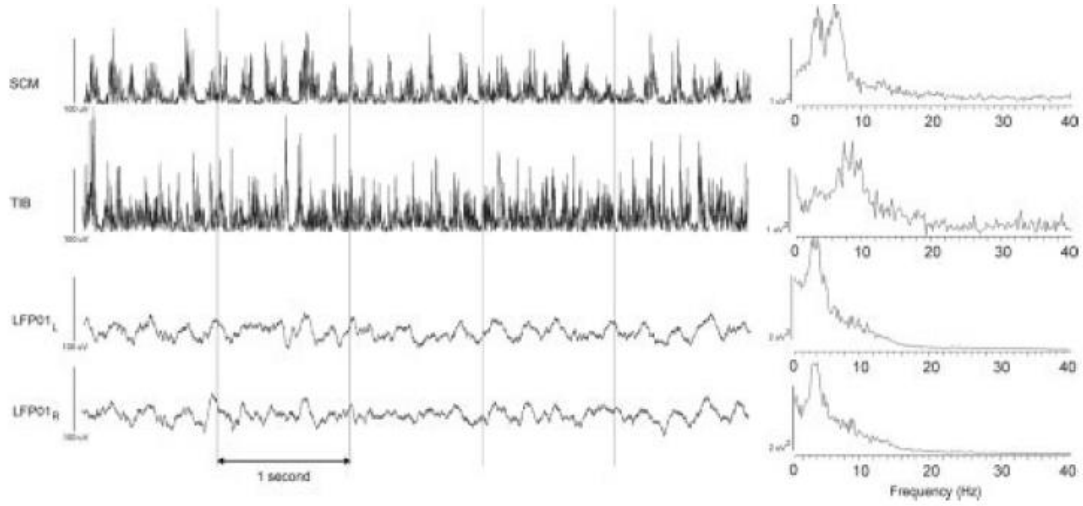

Figure 44. It is compared raw signal and power spectrum between SCM and TIM muscles and LFP activity in left and right globus pallidus. Taken from [45].

In dystonia LFP is often associated with high level in GPi in the band between 4 and 10 Hz [59] [58] and decreasing of power in the band between 11-30 Hz. Instead for Parkinson disease the behavior it is opposite in the same frequencies bands. Opposite patterns of LFP distinguish two diseases as shown in *figure 45* [58], different peak amplitude for same band frequency differentiates Dystonia from Parkinson disease.

LFPs could be related to spike activity by spike-triggered average (STA) [57] that it is obtained computing cross-covariance between LFP and spike train, dividing result for number of spikes. Another way is compared the neural activity and LFPs by using broadband power [56] rather than use LFPs power spectrum in narrow band as shows in *figure 46,* in which it is represented also the spike activity. Research has always analyzed the LFP power spectrum in narrow band, in that case neuron activity is synchronized with spiking activity at low frequency spike activity rates is negatively related to power spectrum and gamma oscillations are negatively correlated with a high number of neurons, quite opposite for high frequency. In broad band LFPs have oscillations in all frequencies and spikes activity is positively related with LFPs activity.

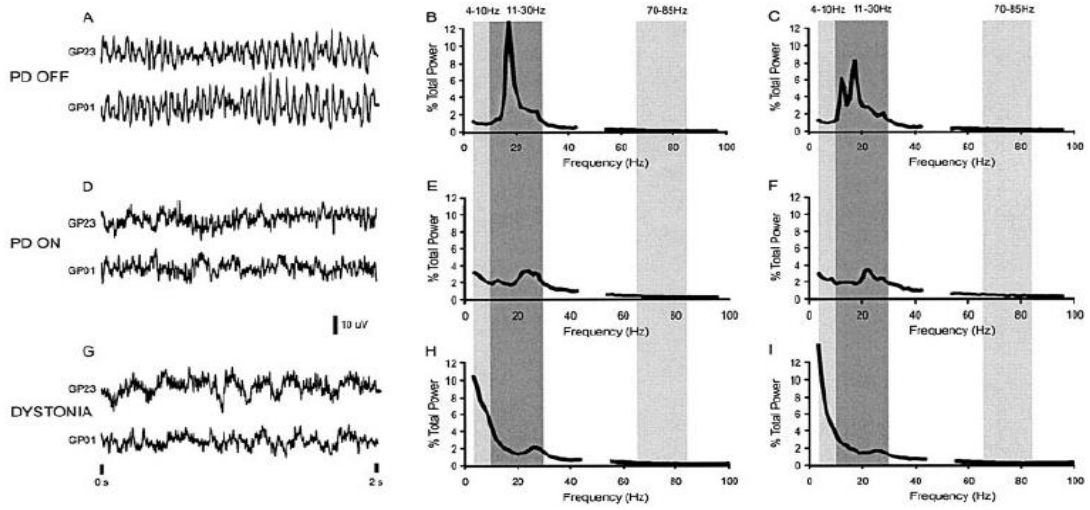

Figure 45. A-B-C with no treatment and D-E-F after treatment, they are referred to Parkinson disease, G-H-I they are referred to dystonia. The power graphics have been subdivided in three bands,4-10 Hz, 11-30 Hz and 65-85 Hz. Graphs B-C-E-F-H-I are referred to two different contacts. Taken from [58].

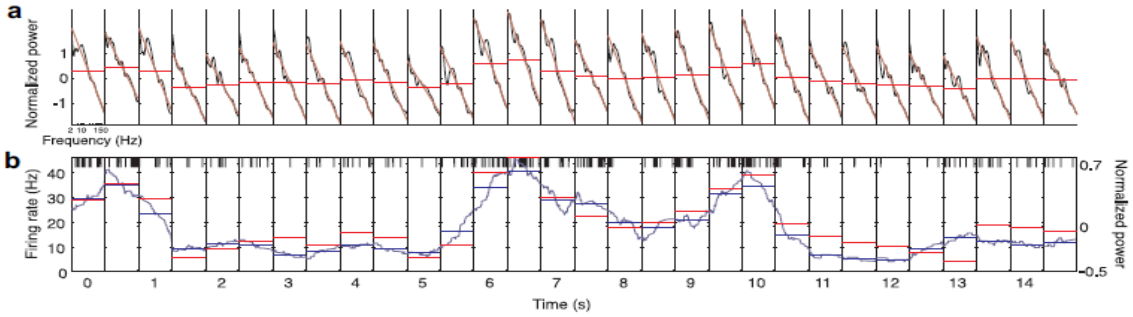

Figure 46. a) In black the LFPs power spectrum, red line represents the mean broadband LFP power, divided for epochs. b) It is represented neuron's spike activity in upper portion of the diagram instead blue lines are mean firing rates. Taken from [56].

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Appendix A

FAHN MARDSEN RATING FACTORS (*Table 4 taken from* [31])

Region	Provoking factor	Severity factor	Weight	Product
Eyes	0-4	x0-4	0.5	0-8
Mouth	0-4	x0-4	0.5	0-8
Speech and swallow	0-4	x0-4	1.0	0-16
Neck	0-4	x0-4	0.5	0-8
Arm(R)	0-4	x0-4	1.0	0-16
Arm(L)	0-4	x0-4	1.0	0-16
Trunk	0-4	x0-4	1.0	0-16
Leg(R)	0-4	x0-4	1.0	0-16
Leg(L)	0-4	x0-4	1.0	0-16
Sum	0-4	x0-4	1.0	Max 120

Factor/area/rating	Criteria
I. Provoking factor	
General	
0	No dystonia at rest or with action
1	Dystonia only with particular action
2	Dystonia with many actions
3	Dystonia on action of distant part of body or intermittently at rest
4	Dystonia present at rest
Speech and swallowing	
1	Occasional, either or both
2	Frequent either

3	Frequent one and occasional other
4	Frequent both
II. Severity factor	
Eyes	
0	No dystonia
1	Slight: Occasional blinking
2	Mild. Frequent blinking without prolonged spasms of eye closure
3	Moderate. Prolonged spasms of eyelid closure, but eyes open most of the time
4	Severe. Prolonged spasms of eyelid closure, with eyes closed at least 30% of the time
Mouth	
0	No dystonia present
1	Slight. Occasional grimacing or other mouth movements (e.g., jaw opened or clenched; tongue movement
2	Mild. Movement present less than 50% of the time
3	Moderate dystonic movements or contractions present most of the time
4	Severe dystonic movements or contractions present most of the time
Speech and swallowing	
0	Normal
1	Slightly involved; speech easily understood or occasional choking
2	Some difficulty in understanding speech or frequent choking
3	Marked difficulty in understanding speech or inability to swallow firm foods
4	Complete or almost complete anarthria, or marked difficulty swallowing soft foods and liquids
Neck	

0	No dystonia present
1	Slight. Occasional pulling
2	Obvious torticollis, but mild
3	Moderate pulling
4	Extreme pulling
Arm	
0	No dystonia present
1	Slight dystonia. Clinically insignificant
2	Mild: Obvious dystonia, but not disabling
3	Moderate. Able to grasp, with some manual function
4	Severe. No useful grasp
Trunk	
0	No dystonia present
1	Slight bending; clinically insignificant
2	Definite bending, but not interfering with standing or walking
3	Moderate bending; interfering with standing or walking
4	Extreme bending of trunk preventing standing or walking
Leg	
0	No dystonia present
1	Slight dystonia, but not causing impairment; clinically insignificant
2	Mild dystonia. Walks briskly and unaided
3	Moderate dystonia. Severely impairs walking or requires assistance
4	Severe. Unable to stand or walk on involved leg

BARRY-ALBRIGHT DYSTONIA SCALE (*Table 5 taken from* [42])

Body region	**Points**
Eyes: signs of dystonia of the eyes include: prolonged eyelid spasms, and/or forced eye deviations	0 – Absence of eye dystonia 1 – Slight. Dystonia less then 10% of the time and does not interfere with tracking 2 – Mild. Frequent blinking without prolonged spasms of eye closure, and/or eye movements less than 50% of the time. 3 – Moderate. Prolonged spasms of eyelid closure, but eyes open most of the time, and/or eye movements more than 50% of the time that interfere with tracking, but able to resume tracking 4 – Severe. Prolonged spasms of eyelid closure, with eyes closed at least 30% of the time, and/or eye movements more than 50% of the time that prevent tracking * – Unable to assess eye movements
Mouth: signs of dystonia of the mouth include: grimacing, clenched or deviated jaw, forced open mouth, and/or forceful tongue thrusting	0 – Absence of mouth dystonia 1 – Slight. Dystonia less than 10% of the time and does not interfere with speech and/or feeding 2 – Mild. Dystonia less than 50% of the time and does not interfere with speech and/or feeding

	3 – Moderate. Dystonia more than 50% of the time, and/or dystonia that interferes with speech and/or feeding 4 – Severe. Dystonia more than 50% of the time, and/or dystonia that prevents speech and/or feeding * – Unable to assess mouth movements
Neck: signs of dystonia of the neck include: pulling of the neck into any plane of motion: extension, flexion, lateral flexion or rotation	0 – Absence of neck dystonia 1 – Slight. Pulling less than 10% of time and does not interfere with lying, sitting, standing and/or walking 2 – Mild. Pulling less than 50% of the time and does not interfere with lying, sitting, standing and/or walking 3 – Moderate. Pulling more than 50% of the time and/or dystonia that interferes with lying, sitting, standing and/or walking 4 – Severe. Pulling more than 50% of the time and/or dystonia that prevents sitting in standard wheelchair, standing and/or walking (e.g. requires more than standard head rest for seating) * – Unable to assess neck movements
Trunk: signs of dystonia of the trunk include: pulling of the trunk into any plane of motion: extension, flexion, lateral flexion or rotation	0 – Absence of trunk dystonia 1 – Slight. Pulling less than 10% of the time and does not interfere with lying, sitting, standing and/or walking

	2 – Mild. Pulling less than 50% of the time and does not interfere with lying, sitting, standing and/or walking 3 – Moderate. Pulling more than 50% of the time, and/or dystonia that interferes with lying, sitting, standing and/or walking 4 – Severe. Pulling more than 50% of the time, and/or dystonia that prevents positioning in standard wheelchair, standing and/or walking (e.g. requires adapted seating system to control posturing, such as ASIS bar) * – Unable to assess trunk movements
Upper extremities: signs of dystonia of the upper extremities include: sustained muscle contractions causing abnormal posturing of the upper extremities (one for left upper extremity one for right upper extremity)	0 – Absence of upper extremity dystonia 1 – Slight. Dystonia less than 10% of the time and does not interfere with normal positioning and/or functional activities 2 – Mild. Dystonia less than 50% of the time and does not interfere with normal positioning and/or functional activities 3 – Moderate. Dystonia more than 50% of the time and/or dystonia that interferes with normal positioning and/or upper extremity function

	4 – Severe. Dystonia more than 50% of the time and/or dystonia that prevents normal positioning and/or upper extremity function (e.g. arms restrained in wheelchair to prevent injury) * – Unable to assess upper extremity movements
Lower extremities: signs of dystonia of the lower extremities include: sustained muscle contractions causing abnormal posturing of the lower extremities (one for left lower extremity one for right lower extremity)	0 – Absence of lower extremity dystonia
	1 – Slight. Dystonia less than 10% of the time and does not interfere with normal positioning and/or functional activities 2 – Mild. Dystonia less than 50% of the time and does not interfere with normal positioning and/or functional activities 3 – Moderate dystonia more than 50% of the time and/or dystonia that interferes with normal positioning and/or lower extremity weight bearing or function 4 – Severe dystonia more than 50% of the time and/or dystonia that prevents normal positioning and/or lower extremity weight bearing and/or function (e.g. cannot maintain standing due to severe dystonia at ankles)

* – Unable to assess lower extremity movements

UNIFIED DYSTONIA RATING SCALE (*Table 6 taken from* [31])

Factor/area	**Criteria**
Duration	
0	None
0.5	Occasional (_25% of the time); predominantly submaximal
1.0	Occasional (_25% of the time); predominantly maximal
1.5	Intermittent (25–50% of the time); predominantly submaximal
2.0	Intermittent (25–50% of the time); predominantly maximal
2.5	Frequent (50–75% of the time); predominantly submaximal
3.0	Frequent (50–75% of the time); predominantly maximal
3.5	Constant (_75% of the time); predominantly submaximal
4.0	Constant (_75% of the time); predominantly maximal
Motor severity	
Eyes and upper face	
0	None
1	Mild: increased blinking or slight forehead wrinkling (_25% maximal intensity)
2	Moderate: eye closure without squeezing or pronounced forehead wrinkling (_25% but _50% maximal intensity)

3		Severe: eye closure with squeezing, able to open eyes within 10 seconds or marked forehead wrinkling (_50% but _75% maximal intensity)
4		Extreme: eye closure with squeezing, unable to open eyes within 10 seconds or intense forehead wrinkling (_75% maximal intensity)
	Lower face	
0		None
1		Mild: grimacing of lower face with minimal distortion of mouth (_25% maximal)
2		Moderate: grimacing of lower face with moderate distortion of mouth (_25% but _50% maximal)
3		Severe: marked grimacing with severe distortion of mouth (_50% but _75% maximal)
4		Extreme: intense grimacing with extreme distortion of mouth (_75% maximal)
	Jaw and tongue	
0		None
1		Mild: jaw opening or tongue protrusion _25% of possible range or forced jaw clenching without bruxism
2		Moderate: jaw opening or tongue protrusion _25% but _50% of possible range or forced jaw clenching with mild bruxism secondary to dystonia
3		Severe: jaw opening and/or tongue protrusion _50% but _75% of possible range or forced jaw clenching with pronounced bruxism secondary to dystonia
4		Extreme: jaw opening or tongue protrusion _75% of possible range or forced jaw clenching with inability to open mouth
	Larynx	
0		None

1	Mild: barely detectable hoarseness or choked voice or occasional voice breaks
2	Moderate: obvious hoarseness or choked voice or frequent voice breaks
3	Severe: marked hoarseness or choked voice or continuous voice breaks
4	Extreme: unable to vocalize
neck	
0	None
1	Mild: movement of head from neutral position _25% of possible normal range
2	Moderate: movement of head from neutral position _25% but _50% of possible normal range
3	Severe: movement of head from neutral position _50% but _75% of possible normal range
4	Extreme: movement of head from neutral position _75% of possible normal range
Shoulder and proximal arm (right and left)	
0	None
1	Mild: movement of shoulder or upper arm _25% of possible normal range
2	Moderate: movement of shoulder or upper arm 25% but _50% of possible normal range
3	Severe: movement of shoulder or upper arm 50% but _75% of possible normal range
4	Extreme: movement of shoulder or upper arm 75% of possible normal range
Distal arm and hand including elbow (right and left)	
0	None
1	Mild: movement of distal arm or hand _25% of possible normal range

2	Moderate: movement of distal arm or hand 25% but _50% of possible normal range
3	Severe: movement of distal arm or hand 50% but _75% of possible normal range
4	Extreme: movement of distal arm or hand 75% of possible normal range
Pelvis and proximal leg (right and left)	
0	None
1	Mild: tilting of pelvis or movement of proximal leg or hip _25% of possible normal range
2	Moderate: tilting of pelvis or movement of proximal leg or hip 25% but _50% of possible normal range
3	Severe: tilting of pelvis or movement of proximal leg or hip 50% but _75% of possible normal range
4	Extreme: tilting of pelvis or movement of proximal leg or hip 75% of possible normal range
Distal leg and foot including knee (right and left)	
0	None
1	Mild: movements of distal leg or foot _25% of possible normal range
2	Moderate: movements of distal leg or foot 25% but _50% of possible normal range
3	Severe: movements of distal leg or foot 50% but _75% of possible normal range
4	Extreme: movements of distal leg or foot 75% of possible normal range
Trunk	
0	None
1	Mild: bending of trunk _25% of possible normal range

2	Moderate: bending of trunk 25% but _50% of possible normal range
3	Severe: bending of trunk _50% but _75% of possible normal range
4	Extreme: bending of trunk _75% of possible normal range

GLOBAL DYSTONIA SEVERITY RATING SCALE (*Table 7 taken from* [31])

Each body area is rated from 0 to 10:	**Ten body areas are tested:**
0 No dystonia present in that body area	Eyes and upper face
1 Minimal dystonia	lower face
5 Moderate dystonia	jaw and tongue
10 Most severe dystonia	larynx
	neck
	shoulder and proximal arm,
	distal arm and hand including elbow
	pelvis and upper leg
	distal leg and foot
	trunk

Appendix B

R-squared:

$$R^2 = \frac{ESS}{TSS} = 1 - \frac{RSS}{TSS} \qquad (7)$$

$$ESS = \sum_{i=0}^{n}(\hat{y}_i - \bar{y})^2 \qquad (8)$$

$$TSS = \sum_{i=0}^{n}(y_i - \bar{y})^2 \qquad (9)$$

y_i: observed data

$\bar{y}$: average

$\hat{y}_i$: estimated data from regression model

Adjusted R-squared

$$\bar{R}^2 = 1 - (1 - R^2)\frac{n-1}{n-k-1} \qquad (10)$$

n: sample size

k: total number of explanatory variables

AIC

$$AIC = 2k - 2\ln(\hat{L}) \qquad (11)$$

BIC

$$BIC = -2\ln(\hat{L}) + kln(n) \qquad (12)$$

k: number of parameters in the model

$\hat{L}$: maximum value of Likelihood

Durbin-Watson

$$d = \frac{\sum_{t=2}^{T}(e_t - e_{t-1})^2}{\sum_{t=1}^{T} e_t{}^2} \qquad (13)$$

e_t: residual

Jarque-Bera

$$JB = \frac{n}{6}\left(S^2 + \frac{(K-3)^2}{4}\right) \qquad (14)$$

n: sample size

$$S = \frac{\hat{\mu}_3}{\hat{\sigma}^3} = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^3}{\left(\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2\right)^{\frac{3}{2}}} \qquad (15)$$

$\hat{\mu}_3$: central moment of third order

$$C = \frac{\hat{\mu}_4}{\hat{\sigma}^4} = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^4}{\left(\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2\right)^2} \qquad (16)$$

$\hat{\mu}_4$: central moment of fourth order

Test t

It is a statistic test which aims to verify if mean value of a distribution moves away significantly from referment value.

P-value

In statistical hypothesis testing probability to obtain a result equal or greater magnitude, having null hypothesis true, than actual observed result is called *p-value*. When it is used the *p-value* it is fixed a null hypothesis and a threshold value, usually equally to 0.05. if p is major than threshold null hypothesis can be accepted. Instead if p is less than threshold null hypothesis is rejected.