POLITECNICO DI TORINO

Master Degree Course in

Biomedical Engineering

Master Degree Thesis

FATIGUE IN MULTIPLE SCLEROSIS PATIENTS: INNOVATIONS IN THE ANALYSIS OF MUSCLE ACTIVATION DATA

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Abstract

Multiple sclerosis (MS) is a neurodegenerative disease and the most frequent cause of permanent disability in young adults, affecting about 2.5 million individuals worldwide. Causes of this disease are still unknown, but at the base there is a reaction of the immune system that triggers an attack against myelin.

The progression of MS disease is likely to depend on accumulated axon degeneration, and this generates neurological function impairment; in fact, tissue damage, due to the disease, causes an altered information transmission along cortico-cortical, cortico-spinal and cortico-subcortical connections.

Fatigue is one of the most distressing and common (affecting 75%-90% of the MS patients) symptoms of MS and a complex phenomenon of this disease. This phenomenon is important because it reduces quality of life, work performances and it has an impact on social interactions.

At present, pharmacological treatments of MS, although numerous and continuously evolving, are not reductive and the disease is disabling and irreversible. In any case, there are drugs and therapeutic strategies that can improve the quality of patient's life.

For the aim of the present study, it is important to mention Fampyra®, that acts on damaged nerve structures, preventing the charged potassium particles from leaving the nerve cells; in this way, it allows the electrical impulse to continue propagating along the axons.

The efficacy of fampridine is evaluated both with magnetic and electrical stimulation and assessing gait functions by administering some tests like 25 Feet Walk Test.

The aim of the present study is to support and help doctors in assessing patients' conditions before and after administrating Fampyra® and to make the whole assessment

procedure automatic thanks to an algorithm specifically studied to analyse muscles activation signals.

The study was performed on 10 MS patients (average age 48.4), with different levels of severity of the disease (average EDSS: 4.5), subject to a trial of the drug Fampyra® at the Azienda Ospedaliero-Universitaria San Luigi Gonzaga of Orbassano (TO).

The executed test consists in 2 electrical impulses for recording peripheral conduction times and 5 transcranial magnetic stimulations to record motor evoked potentials.

During stimulation, 5 muscles for each leg have been analysed: vastus medialis, vastus lateralis, tibialis anterior, peroneus longus and flexor hallucis brevis.

After having recorded both peripheral and central muscle activation signals, areas and latencies have been measured and a parameter, Inter Trial Variability (ITV), has been calculated. The evaluation of Fampyra® efficacy is based on this ITV parameter because it may indicate if conduction is improved or not.

Other methods to evaluate patient's conditions have been suggested in this work; this decision is due to the instability of data that has been noticed during the study.

Countermeasures taken have been the normalization of signals to reduce the variability related on peak values, the application of Dynamic Time Warping algorithm to stimulation signals and the measure of the energy of error signal. These two last methods have been used to assess the reliability of the results.

Finally, a score has been defined for each patient to help doctor to assess changes in patient conditions before and after drug administration.

The final assessment is based on the discussed score, on fatigue and clinical scales results and on walking tests perfomances.

Results exhibit a lower variability in normalized signals than in those non-normalized and it has been proved that high values of DTW distance and energy of the error signal are related on different signals morphology.

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List of acronyms

MS: Multiple Sclerosis;

TMS: Transcranial Magnetic Stimulation;

MEP: Motor Evoked Potential;

CMCT: Central Motor Conduction Time;

PCT: Peripheral Conduction Time;

CMAP: Compound Motor Action Potential;

HVES: High Voltage Electrical Stimulation;

ITV: Inter-Trial Variability.

Chapter 1

Introduction

1.1. Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune and neurodegenerative disease [1], during which damage and loss of myelin occur in multiple areas (hence the "multiple" name) of the central nervous system.

This disease is the most frequent cause of permanent disability in young adults (the range of its onset is 28 - 31 years), affecting 2.5 million individuals worldwide [2].

MS causes are still unknown, but at the basis there is a reaction of the immune system that triggers an attack against myelin; this attack consists of an inflammatory process that affects circumscribed areas of the central nervous system and causes the destruction of myelin and the oligodendrocytes, specialized cells which produce it.

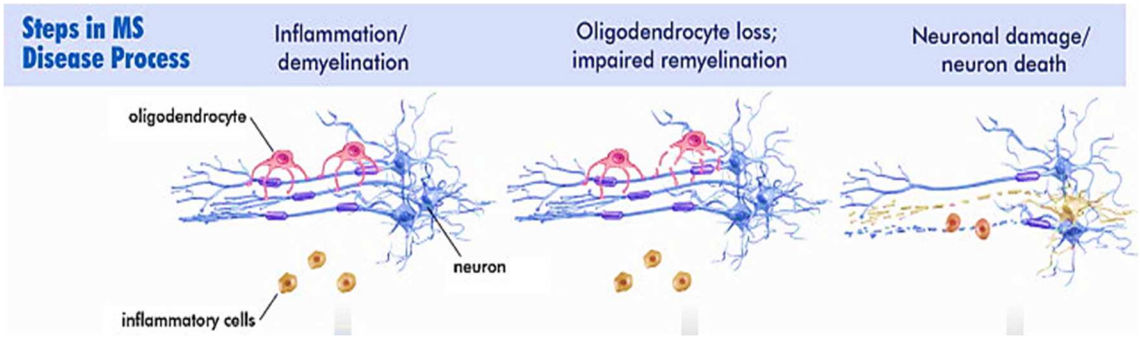


Figure 1.1 - Development of MS disease [3].

These areas of myelin loss (or "demyelination") (Fig. 1.2) also referred to as "plaques", can be disseminated anywhere in the cerebral hemispheres (Fig. 1.3) with a preference for the optic nerves, the cerebellum and the spinal cord. [5]

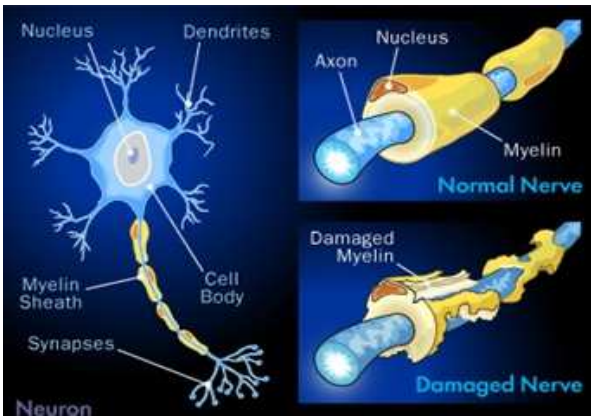


Figure 1.2 – Axon demyelination [7].

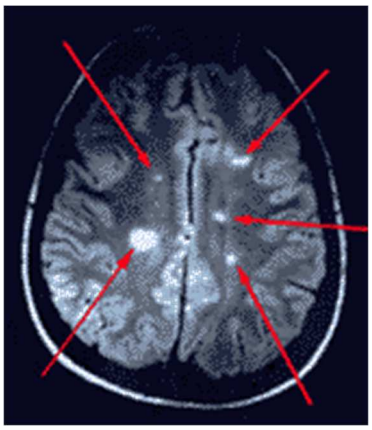


Figure 1.3 – Plaques in cerebral hemispheres [6].

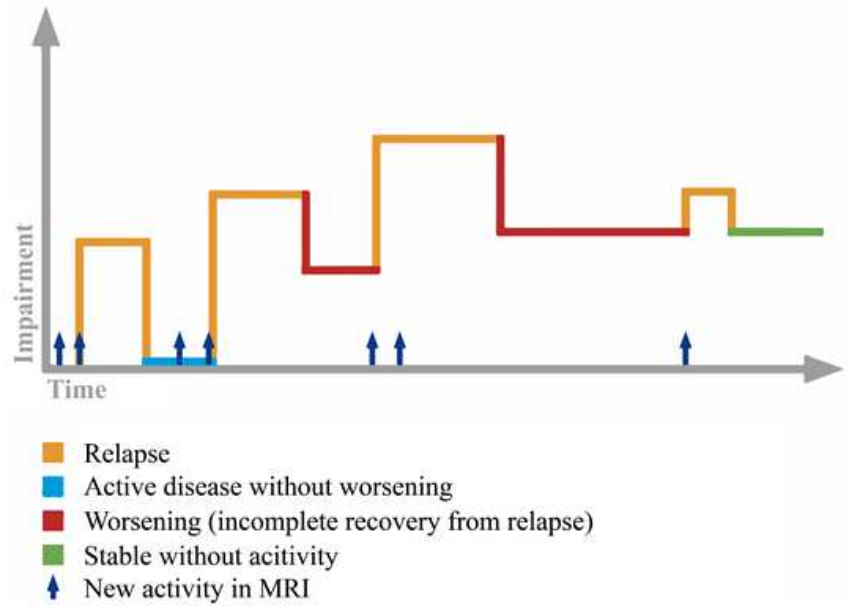
The progression of MS disease is likely to depend on accumulated axon degeneration, and this generates neurological function impairment; in fact, tissue damage, due to the disease, causes an altered information transmission along cortico-cortical, cortico-spinal and cortico-subcortical connections [8].

Diagnosing MS is a complicated process: it is mainly necessary to demonstrate the presence of central nervous system lesion dissemination [9]; this demonstration is based on both clinical and MRI findings.

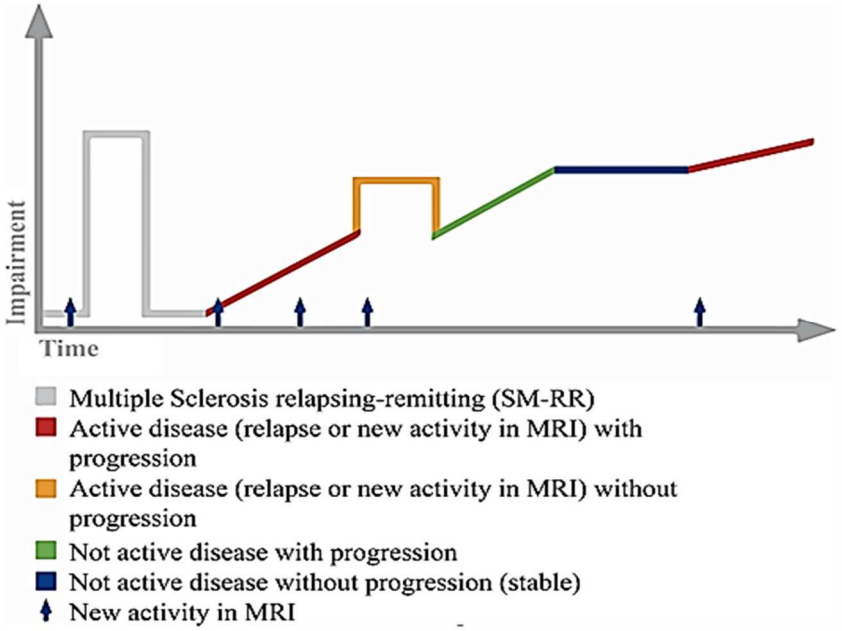
Moreover, determining the type of MS should make clearer the communication between doctors and MS patients and it should improve the design, the recruitment and the conduction of clinical trials.

The five main courses in which Multiple Sclerosis is usually classified are:

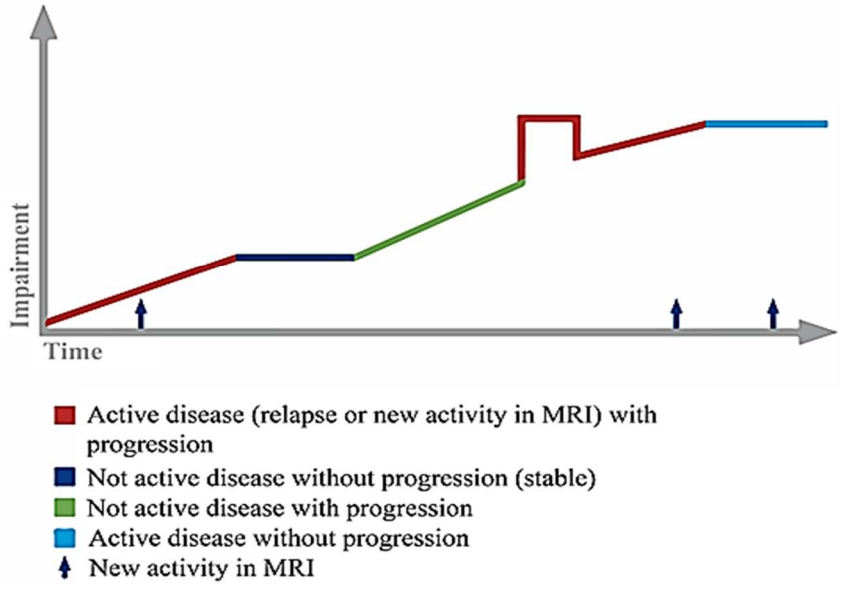
- Relapsing-Remitting (RR): it is the most common one (it affects 85% of MS population [5]) and it is characterized by acute episodes of illness (relapses), alternating with periods of complete or partial recovery (remissions);



- Secondary-Progressive (SP): it is the evolution of the relapsing-remitting form, and it is characterized by a persistent disability that progresses gradually over time without relapses.



- Primary-Progressive (PP): it is characterized by a deterioration of neurological functions since the appearance of the very first symptoms, in the absence of real relapses or remissions.



- Progressive- Relapsing (PR): it is characterized by progressive disease with occasional relapses.
- Benign MS: patient remains fully functional for at least 15 years after the onset.

1.2. MS fatigue

Fatigue is one of the most distressing and common symptoms of MS and a complex phenomenon of this disease.

This phenomenon is important because it reduces quality of life, work performances and it has an impact on social interactions [1].

The Multiple Sclerosis council for clinical practice defines fatigue as “a subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities” [12].

Despite its high frequency in MS population (75%-90% of the MS patients [7]), the pathophysiology of MS fatigue is still unclear, and several mechanisms [1] seem to influence it.

It has been seen that 50% of people with daytime fatigue also have nocturnal sleep disturbances [11] and therefore cannot recover the strength needed to face the day. Approximately 50% of people with MS have ambulatory impairments [11] and the only movement becomes a reason to waste energy.

Another element that influences fatigue is depression, which affects about 40% of people with MS.

It is also important to distinguish fatigue from fatigability, and fatigue in MS patients from fatigue in healthy people. Fatigue, in MS patients, interferes with daily activities and has a rapid onset, contrary to fatigue in a healthy person. Additionally, fatigue is a subjective sensation, while fatigability indicates objective changes in mental or physical performance [10].

Another distinction is that between "primary fatigue", that is directly related to the disease, because of damage to the central nervous system caused by inflammation, and "secondary fatigue" [4], that is most related to the emotional state, for example anxiety or depression, and to the presence of other conditions not necessarily directly related to multiple sclerosis, such as an infection, fever or a sleep disorder.

Measuring fatigue is difficult, but in the last years several scales have been produced which should measure both severity and subjective perception of fatigue [13]; among these, it is important to mention:

- Fatigue Severity Scale (FSS): it consists in nine items and it is focused on physical aspects of fatigue and how they affect daily life;

	Scores 1 = Strongly Disagree; 7 = Strongly Agree						
1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my three most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

Figure 1.4 – Fatigue Severity Scale (FIS) [14]

- Modified Fatigue Impact Scale (MFIS): it consists in 21 questions and it suggests the evaluation of cognitive, physical and psychosocial component of fatigue [1];
- Visual Analog Scale (VAS): it consists in a scale from 0 (no fatigue) to 10 (severe fatigue), in which patients should indicate the severity of fatigue;

Please mark an « X » on the number line that reflects the best your fatigue, with 0 refers to absence of fatigue and 10 refers to severe fatigue.

0 1 2 3 4 5 6 7 8 9 10

Figure 1.5 – Visual Analog Scale (VAS) [1]

- Expanded Disability Status Scale (EDSS): it has the aim to assess the level of disability in MS patients; it goes from 0, that corresponds to a normal neurological examination, to 10 [4]. This is not a fatigue scale, but a clinical assessment of the pathology.

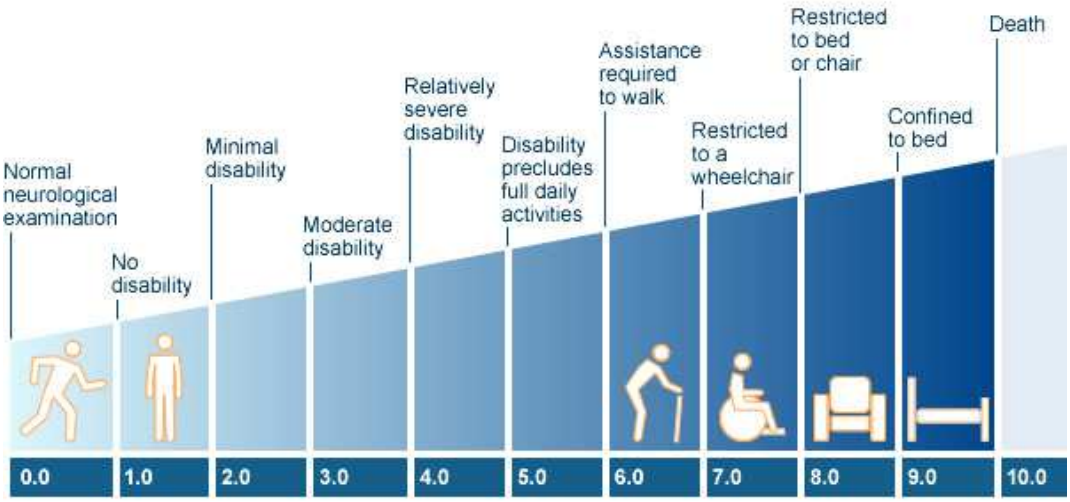


Figure 1.6 – Expanded Disability Status Scale (EDSS) [15]

There are energy saving strategies that are essential for the management of fatigue in multiple sclerosis [4]. Among these, learning how to balance activities and rest, with a schedule of activities to be carried out every day in order of priority, recognizing the signs of fatigue, learning to stop before reaching full exhaustion, making the work and the domestic environment comfortable in order to reduce energy expenditure and the use of relaxation techniques.

At present, pharmacological treatments of MS, although numerous and continuously evolving, are not resolutive and the disease is disabling and irreversible. In any case, there are drugs and therapeutic strategies that can improve the quality of patient's life.

1.3. Fampyra®

For the aim of the present study, it is important to mention Fampyra®, that contains a slow-release formula of 4-aminopyridine, which blocks potassium channels on the surface of nerve fibres.

It acts on damaged nerve structures, preventing the charged potassium particles from leaving the nerve cells; in this way, it allows the electrical impulse to continue propagating along the axons.

The recommended dose is one 10 mg tablet, taken orally, twice a day, 12 hours apart and the tablets should be taken on empty stomach.

Clinical benefits should be identified within 2 weeks of starting treatment with Fampyra®.

The efficacy of fampridine is evaluated both with magnetic and electrical stimulation, as it will be discussed successively, and assessing gait functions by administering some tests like 25 Feet Walk Test.

1.4. Aim of the study

The aim of the present study is to support and help doctors in assessing patients' conditions before and after administrating Fampyra® and to make the whole assessment procedure automatic thanks to an algorithm specifically studied to analyse muscles activation signals.

At the end, a new method more reliable and stable to assess patient's conditions, will be suggested.

1.5. Materials and Methods

The study was performed on 10 MS patients with different levels of severity of the disease subject to a trial of the drug Fampyra® at the Azienda Ospedaliero-Universitaria San Luigi Gonzaga of Orbassano (TO).

The executed test consists in 2 electrical impulses for recording peripheral conduction times and 5 transcranial magnetic stimulations to record motor evoked potentials; this procedure is necessary to evaluate the central motor conduction time that is an index of patient conditions.

Four patients have done the test before drug administration twice (a week apart), whereas the others only once; then, after two weeks and after having administered the drug, the test has been repeated.

During stimulation, 5 muscles for each leg were analysed: vastus medialis, vastus lateralis, tibialis anterior, peroneus longus and flexor hallucis brevis.

Chapter 2

State of art

2.1. Transcranial Magnetic Stimulation

To correlate fatigue in MS patients to neural activity, an emerging method is Transcranial Magnetic Stimulation (TMS).

TMS is based on the principle of electromagnetic induction: a powerful transient magnetic field, which induces electric currents in the brain, produces the non-invasive direct cortical brain stimulation.

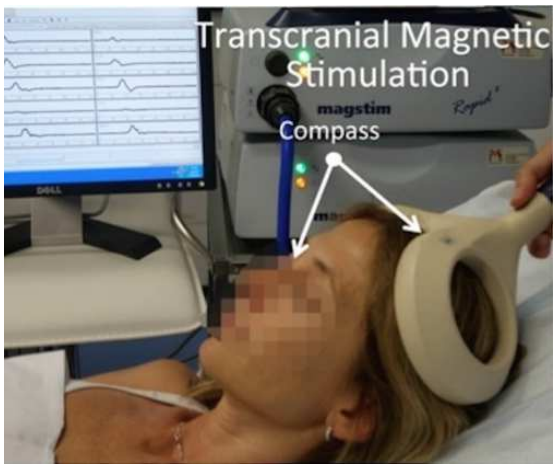


Figure 2.1 - Transcranial Magnetic Stimulation (TMS) [19]

During TMS, magnetic stimuli are delivered using a double cone coil that allows to stimulate the deep cortical regions; furthermore, the magnetic coil must be placed in a

point above the cortical leg motor area [16]; then this position is marked, and it must be the same throughout the whole procedure.

Nowadays transcranial magnetic stimulation is used for recording Motor Evoked Potentials (MEPs) to evaluate central motor conduction slowing in MS patients.

Corticospinal pathways excitability and integrity can be assessed using MEP amplitude and Central Motor Conduction Time (CMCT), but only a few TMS trials have been carried out in the context of MS fatigue and they have obtained mixed results.

2.2. Central Motor Conduction Time and MEPs variability

To calculate Central Motor Conduction Time (CMCT), as the subtraction of the total Peripheral Conduction Time (PCT) from the latency of corresponding MEP, Di Sapio et al. [17] used double cone coil TMS for recording MEPs in the same recording districts of Compound Motor Action Potentials (CMAPs) elicited by using High Voltage Electrical Stimulation (HVES).

In fact, in a recent study, W. Troni et al. [18] have demonstrated that CMAPs, used to calculate PCT, can be elicited using HVES of lumbo-sacral nerve roots at their origin from the spinal cord.

It is assumed that Multiple Sclerosis damage is central and not peripheral, for this reason the subtraction of PCT from MEP latency can be used.

A. Di Sapio et al. [17] have also demonstrated that MEP area decrease depends on conduction failure and the use of MEP area in clinical practice is difficult because of the area variability in serial recordings.

This variability can be reduced recording responses during controlled and defined voluntary muscle activation (Fig. 2.2) and using the average of few MEPs.

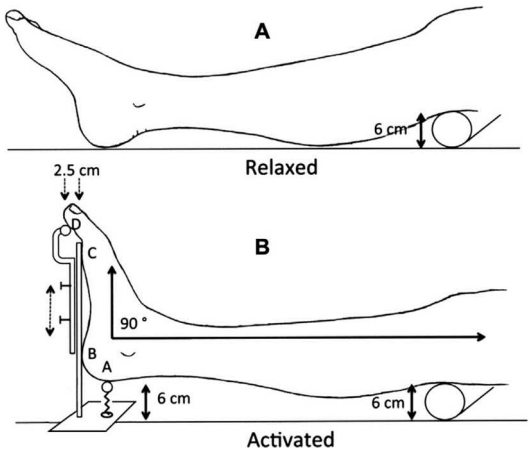


Figure 2.2 – Protocol for voluntary facilitation. A: relaxed position.
B: simultaneous activation of vastus medialis, tibialis anterior and flexor hallucis brevis. [17]

Even if it is possible to reduce variability of MEP latency and amplitude using MEP averaging and a standardized pattern of voluntary activation, Inter-Trial Variability (ITV) of motor responses to peripheral and transcranial stimulation makes their use in clinical practice impossible.

2.3. Inter-Trial Variability

W. Troni et al. [19] developed a strategy to reduce ITV in recording MEP and CMAP stimulations: it consists in detecting and controlling the factors that contribute to variability, like temperature, circadian changes of body temperature and the shift of the recording site.

Normalizing the measured ITV, they avoid also the instability due to a variable inversely related to the area size.

For controlling the shift of the recording site, W. Troni et al. [19] suggest a protocol in which the site for the stimulation is detected placing a multiple electrode array over the spinal cord (Fig. 2.3 and 2.4), more precisely over the dorso-lumbar tract.

Over the selected site a small round tattoo is drawn, as suggested by radiotherapy protocols, to allow an easier detection of the same stimulation site during the subsequent tests.

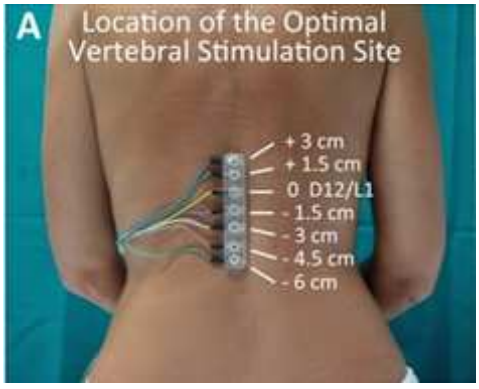


Figure 1.3 – Position of stimulation electrode.

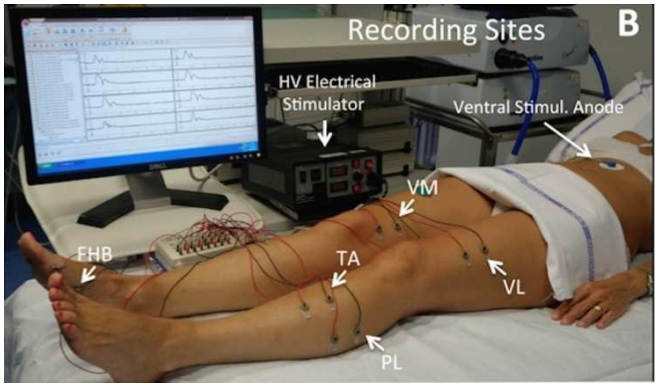


Figure 2.4 – Leg muscles activation signals recording.

To reduce variability due to the temperature, they suggest carrying out the test in a room set at 22°C and noting body temperature.

Finally, they indicate to record two CMAPs and, after having rectified them, to measure areas and latency.

ITV is calculated as:

$$\frac{\boldsymbol{V2 - V1}}{\boldsymbol{0.5 * (V1 + V2)}} * \boldsymbol{100}$$

where V1 and V2 are the areas or latencies of the responses to stimulation).

For recording CMAPs, once the electrode is placed, HVES is performed producing a rectangular pulse (t=50 µs and Vmax=1000V) and the current intensity is increased till the saturation of responses. Furthermore, as PCT, latencies were used.

For what concerns TMS and voluntary activation, MEPs are recorded using double cone coil. A stimulus with and amplitude between 50 and 200 µV is delivered in 10 recording

sites (5 on each leg); then stimuli, 150% above the threshold, are delivered. At the end of the procedure, 2 or 3 basal MEPs and 5 activated MEPs [17] are recorded.

2.3.1. Areas

Areas are determined, after having rectified signals, as the product ms*mV; subsequently, ITV is normalized to avoid potential bias; in this way, areas were evaluated using a new parameter, normITV:

$$\boldsymbol{ITV} * \frac{\mathbf{100} - (\boldsymbol{CV} * \boldsymbol{\Delta})}{\mathbf{100}}$$

where CV represents the ITV component inversely related to area for each unit of area (1 mVms), and Δ is obtained as the subtraction of mean area (between V2 and V1) from theoretical area value (as the mean area values from their population + 3SD) [20].

The procedure is the same for both central and peripheral stimulations. After having obtained all values of normITV, the ratio between MEP areas and RAD areas is evaluated and also in this case normITV is determined.

To assess if a muscle performance is improved or worsened after drug administration, every normITV value must be compared with a range determined by 5^{th} and 95^{th} percentile [19]: all that muscles belonging to that range are considered unchanged.

2.3.2. Latencies

For what concerns latencies (ms), they are measured on the graphs of muscle stimulations and for each value only ITV is then calculated; normalization in this case is not necessary.

Finally, similar to the area case, each value is compared with the range determined by 5th and 95th percentile [19].

Once ITV values are obtained for both central and peripheral signals, the last operation is to measure the difference between peripheral and central conduction times; these values of ITV are then compared with the respective range.

2.4. Gait Analysis

Gait Analysis is the systemic study of the human motion during walking.

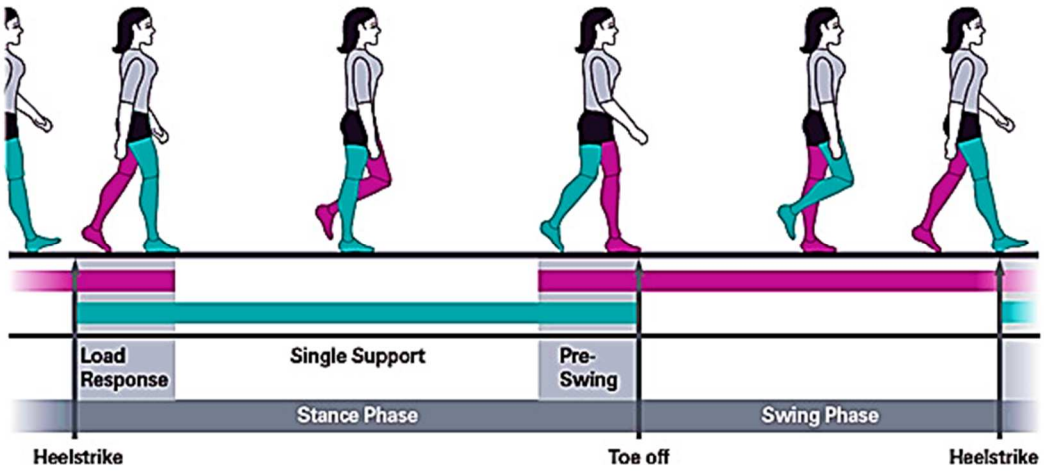


Figure 2.5 – Gait cycle [25]

Changes during walk yield some information about people health, useful for example, for diagnosis of Multiple Sclerosis or other neurodegenerative diseases [24], in fact, it helps assessing severity and progression of the disease and the efficacy of therapies.

The loss of functional ambulation is important also because of its frequency and its effects. In fact, following 45 years of MS, 76% of people with MS require an ambulatory aid and 52% bilateral assistance or worse [2].

For all these reasons, many tests to study walking capacity and physical activity in MS patients have been developed. In recent years, to evaluate walking performance in Multiple Sclerosis patients the 6-minute walk test (6MWT) [21] has been used.

In this work, both 25-foot walking test (25FWT) and 6-minute walking test (6'-WT) have been used.

The Multiple Sclerosis Functional Composite (MSFC) guidelines indicates that 25-foot walking test participants must "to walk at faster but safe speed" [22] over a 25-foot (7.62m) course. At the end of the test the time spent for making 25 feet is acquired.

For what concerns 6-minute walking test (6'-WT), the examination is performed by asking the patient to walk for 6 minutes along a hallway; during this test the patient can choose the effort intensity; he/she is invited to walk at preferred speed and he/she can stop or use the cane if he/she is used to do in daily life. At the end of the test, total distance is noted.

2.5. Automation of walking tests

The project to automate both walking tests is being carried out with a team of other engineers.

A mobile phone is tied to patient's ankle and, thanks to MATLAB® application, it allows to record data from accelerometer and gyroscope during the 6-minute walking test.

During this procedure, the mobile phone is connected via wi-fi to a computer that records the sensors data, and, thanks to a dedicated program, it analyses walking data extracting not only the covered distance as usual, but also, for example, walk velocity and steps frequency.

The aim of this procedure is to obtain not only the distance covered during patients walk and to automatize the procedure, but also to obtain other parameters that could help

doctors to assess severity of the disease and to have a wider idea about patient's conditions, in particular, about fatigue progression.

Chapter 3

Materials and methods

3.1. Introduction

The study has been carried out at Azienda Ospedaliero – Universitaria San Luigi Gonzaga in Orbassano (TO), in collaboration with a team made up of two neurologists and a neuropathophysiology technician.

It has been carried out on patients treated with Fampyra®, in a trial aiming to establish whether this drug improves patient conditions or not.

Ten patients have been analysed (3 women and 7 men) with an average age of 48.4 years (range 24 – 66) and an average EDSS score of 4.5 (range 1 – 6.5).

An algorithm has been implemented to automatize signals processing and provide an evaluation of patient conditions before and after Fampyra® administration.

The algorithm is implemented using MATLAB, version 2017b for Windows 10. It aims at emulating what doctors do after recording signals, measuring areas and latencies, for the evaluation of ITV.

Patients data have been extracted in ASCII format, from the computer used for recording stimulation signals.

The first six patients submitted to the trial have been subject to the stimulation test only once before the drug administration, whereas the other four patients have been submitted to the test twice, a week apart. For patients that have been submitted to two stimulations

before drug administration, the one closest to signal post-drug administration has been chosen for the sake of comparison and final evaluation.

This decision is due to a problem about data instability. In fact, as it will be explained in the next sections, a huge difference between signals pre- and post- drug administration may indicate an unreliable evaluation.

The algorithm allows one to choose data of patients to be analysed, and it measures, for both peripheral and central signals, areas and latencies, obtaining respectively normITV and ITV and implementing a preliminary assessment.

ITV measures the inter-trial variability, so it has been used to assess if patient's muscle performance is changed after drug Fampyra® administration; instead in this study, it has been used to highlight a problem related to the huge difference between signal before and after drug administration that will be explained in next sections.

3.2. Signals

Di Sapio et al. [17] define a protocol that consists in two electrical stimuli given to the patient to record peripheral muscle activation signals and at least five magnetic stimuli for obtaining central activation signals.

For peripheral signals, the protocol [17] indicates that the last epoch must be selected for the analysis, whereas for central signals, the average among the last five stimulations must be taken.

After having obtained the correct signal, the algorithm carries out the subtraction of mean value and first value; this last operation is done to allow signals to start from zero.

Finally, signals are rectified and, after this last operation they are ready to be used for ITV measure.

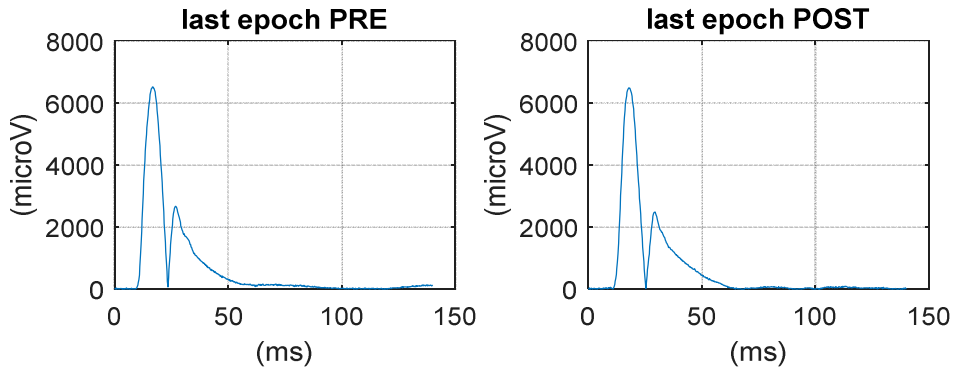


Figure 3.1 – Typical peripheral stimulation signal. Signal is referred to right Vastus medialis before (left) and after (right) drug administration.

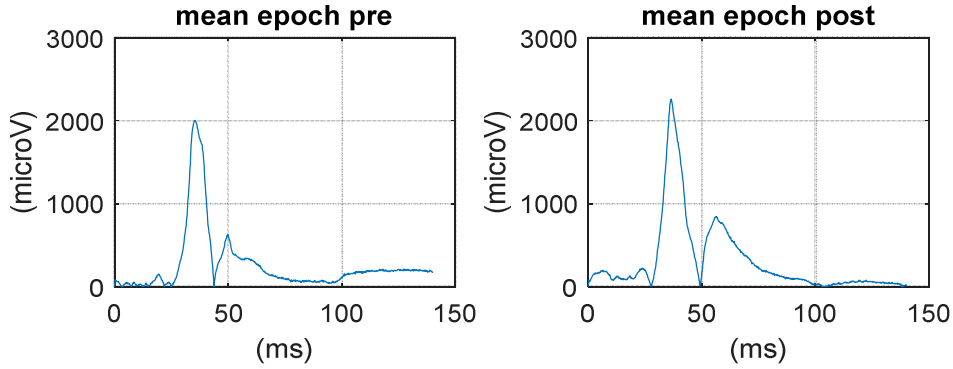


Figure 3.2 - Typical central stimulation signal. Signal is referred to right Vastus medialis before (left) and after (right) drug administration.

Central stimulation signals are in general more difficult to analyse because they are more heavily affected by noise than peripheral ones; this is because in Multiple Sclerosis central lesions are more likely than peripheral ones.

In accordance with doctors' opinion, signals that present a peak value below 50μV have been considered not meaningful and marked as "non-classified (NC)": actually, from these signals, it is impossible to obtain meaningful parameters or to recognize a useful waveform.

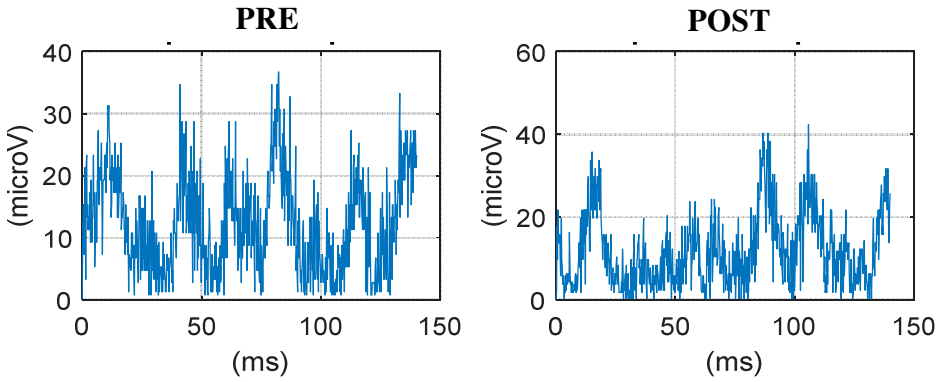


Figure 3.3 – Muscle activation signal lower than 50 µV. On the left it is shown the stimulation signal before drug administration, and on the right, for the same muscle, the signal after Fampyra administration.

For this kind of signals, areas have been measured, even though the signal has been classified as not meaningful, because this parameter can give anyway an idea about nervous transmission to muscles. On the contrary, latencies have not been determined, because the obtained measure, in this case, is unreliable.

For all other signals, i.e. for all signal with a peak above 50µV, the analysis has been carried out and all parameters, as ITV and normITV, have been obtained.

Finally, for evaluating whether patient conditions are improved or not after drug administration, it has been decided to give a score (the evaluation, as stated before, is carried out observing normITV for areas and ITV for latencies):

- -1 to worsened muscles;
- 0 to muscles that are not substantially changed;
- +1 to improved muscles.

For the assessment of each muscle, the same range (obtained considering 5^{TH} and 95^{TH} percentile) determined by W. Troni et al. [19] have been used, both for not normalized and normalized data.

Once every muscle has been evaluated, scores are summed up, keeping areas and latencies separated and obtaining a final score that can help doctor in final assessment.

For patients in which some muscle activation signals are classified as “not classified”, the final score is obtained as:

$$\frac{(10 - NC)}{10}$$

It is not possible to give a complete assessment of disease progression only considering this final score, but it must be related on all other patient’s parameters, as EDSS, fatigue scales scores and walking test results.

Fatigue in fact, is a complex phenomenon, and it must be evaluated considering as many different parameters as possible.

3.3. Instability data problem

Observing graphs derived from stimulation data, a problem has been noted: in some cases, the peak values in pre- and post-drug administration data, differ more than twice and this is not considered meaningful from the physiopathological point of view.

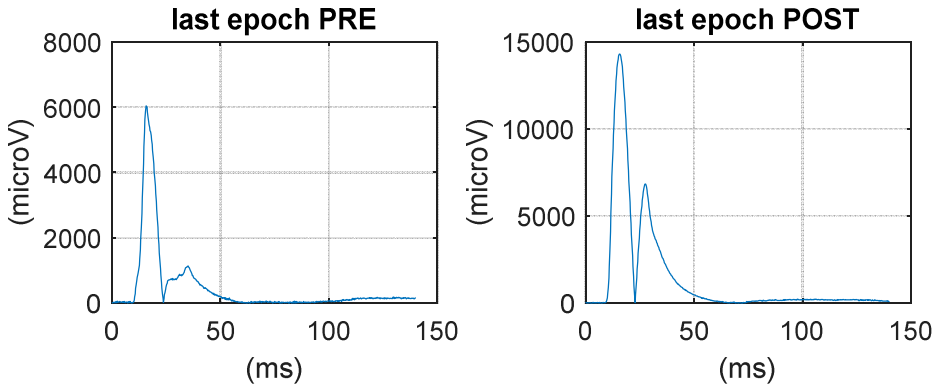


Figure 3.4 – An example that shows problem of the difference between peaks of graphs, before (left) and after (right) drug administration. Signals refer to peripheral stimulation and represent left Vastus medialis muscle.

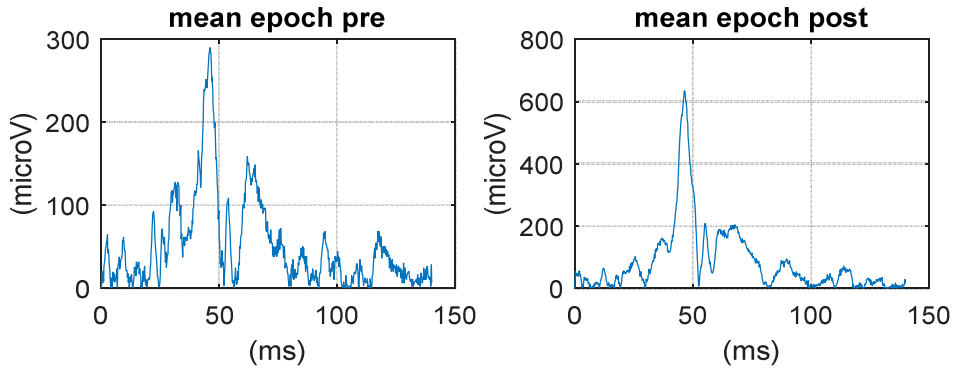


Figure 3.5 – An example that shows problem of the difference between peaks of graphs, before (left) and after (right) drug administration. Signals refer to central stimulation and represent left Tibialis anterior muscle.

Furthermore, this problem occurs with the same frequency (about 30% of data) for both peripheral and for central stimulation signals.

The reasons of this phenomenon are not clear; some hypotheses have been done together with doctors, but none of them appears completely satisfying.

The position of stimulation recording site is likely to have some impact on this problem, but this cannot be the only explication. What is clear is that the problem heavily affects the final assessment, because of its impact on ITV measures: it causes an unreliable measure of area (that depends on height other than base) and a consequent unreliable ITV and final evaluation.

The effects of the problem on final patient's assessment have been studied during this work and some alternative proposals have been tested and suggested.

3.4. Areas/Energy

In this study the signals energy (ms*mV^2) has been measured.

For each recorded signal, the total energy is measured and, in the same way as for areas, ITV and normITV are obtained.

Time axis measures always 140 ms because physiologically muscles activation occurs within this time; for this reason, differences in signal peak values affect energy measures as well as areas.

Affecting energy measure, the problem affects also ITV and consequently the final score.

The protocol suggested by Di Sapio et al. [17] includes also a ratio between MEP and RAD areas and, also for this parameter, ITV and normITV must be calculated.

In this work, ratios have not been considered because of the instability of data: this problem, as stated before, affects both central and peripheral areas measure, hence the ratio between these parameters results unreliable.

To avoid this problem or, better, to obtain a more reliable measure, some alternative techniques will be introduced in the following section.

3.5. Countermeasures to the peak variability problem

To avoid the problem about data instability, in this work some methods are suggested that, in addition to ITV, improve data reliability.

All these methods have been implemented with data of the same patients used for the measure of ITV, to allow the comparison of the results.

The proposed methods have been discussed with neurologists to better understand what can be more helpful for evaluating patient's conditions.

During discussion, signal morphology has emerged as a more important feature than values assumed by signals during stimulation tests. Hence a measure of signal morphological similarity is proposed.

Normalization has been suggested, in order to make ITV measures independent of peak signal values, hence results can be more reliable than those of the classical method.

Every parameter is affected by some instability, for this reason, having many different measures for patient has been considered the best strategy.

A final table with all measured parameters will be drawn for each patient, to allow neurologists to have an overview about patient situation before and after drug administration.

This final table has been used also to prove the instability of results caused by difference between signals.

In the table we summarize:

- Fatigue scales values;
- Walking tests results;
- Energy pre- and post - drug administration;
- Energy measured on normalized signals pre- and post - drug administration;
- NormITV;
- NormITV measured on normalized signals;
- Energy of error signal;
- DTW distance;
- ITV measured with latencies times.

3.5.1. Fatigue and clinical scales

First of all, it has been suggested to insert, together with the evaluation based on ITV parameters, also fatigue scale values (Tab.1).

Fatigue scales that have been considered are MFIS, FSS, VAFS and EDSS.

		MFIS	*FSS*	*VAFS*	*EDSS*
Patient		0-84	9-63	0-10	
1	Pre	30	NA	NA	5,5
	post	29	NA	NA	
2	Pre	48	52	1	6
	post	43	51	2	
3	Pre	NA	NA	NA	6
	post	NA	NA	NA	
4	Pre	37	57	3	3
	post	25	37	7	
5	Pre	28	44	4	4,5
	post	25	35	6	
6	Pre	33	41	6	1
	post	35	46	6	
7	Pre	16	31	5	3,5
	post	16	31	5	
8	Pre	49	54	10	6,5
	post	37	26	4	
9	Pre	59	60	8	3
	post	45	54	3	
10	Pre	9	50	7	6,5
	post	27	63	3	

Table 1 – Fatigue scale values for each patient of the clinical trial.

For each scale, a questionnaire has been provided to patient after every stimulation test, asking to spend some minutes to fill it out.

This has been made because, even though these scales are subjective they can provide an idea of patient's feeling related to fatigue improvement or worsening during the trial.

Final results derived from activation signals analysis, have been matched with results from severity fatigue scales made before and after drug administration.

The EDSS score is evaluated only once because it is a clinical evaluation and it is not expected to change in two weeks (that is the period between the pre- and post-drug administration measures).

Some patient did not fill out questionnaire for the evaluation of fatigue. In the respective cell, in the complete table (Tab. 2), this has been reported as “not available (NA)”.

3.5.2. Normalization

For solving the problem related to spikes level, graphs have been normalized (Fig.3.6 and 3.7) on respective peak values.

On each graph obtained by normalization, energy and consequently ITV and normITV have been determined in the same way as for analysing raw data.

Using this method, information about signal amplitude is lost, but on the other hand, this allows to obtain a more realistic evaluation about fatigue progression and to maintain signal morphology.

After having normalized and measured all parameters on the obtained signals, score has been defined for each analysed muscle, using same ranges (defined by 5^{TH} and 95^{TH} percentile) obtained W. Troni et al. [19].

Normalized signals have not been used for measuring latencies. Actually, no difference can be appreciated between latency values on normalized and not normalized graphs.

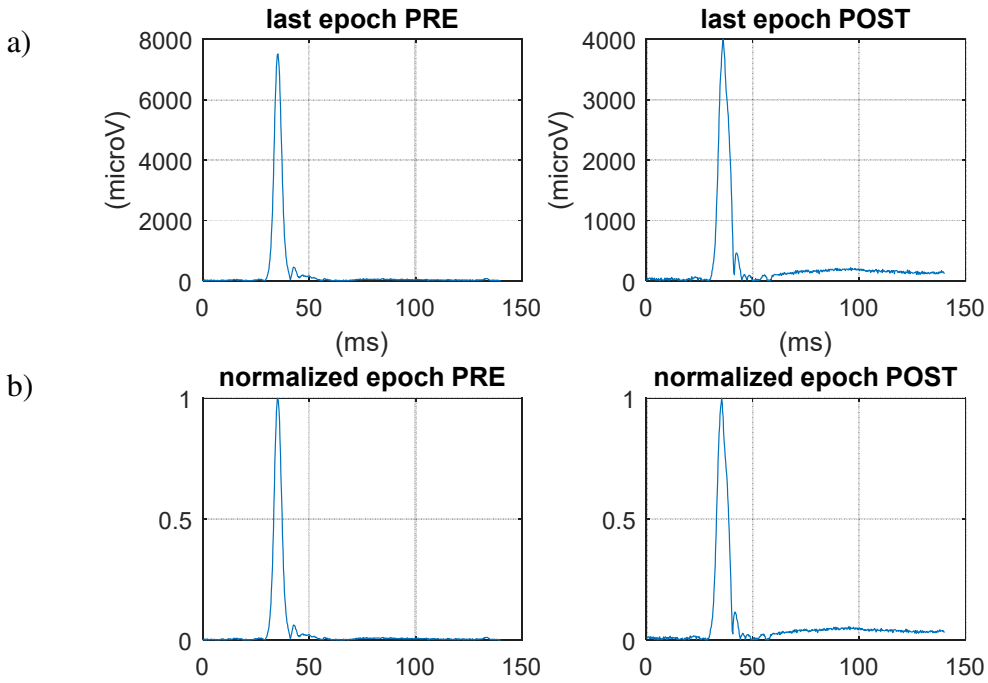


Figure 3.6 – a) Original signal before (left) and after (right) drug administration. b) The respective normalized peripheral stimulation signal.

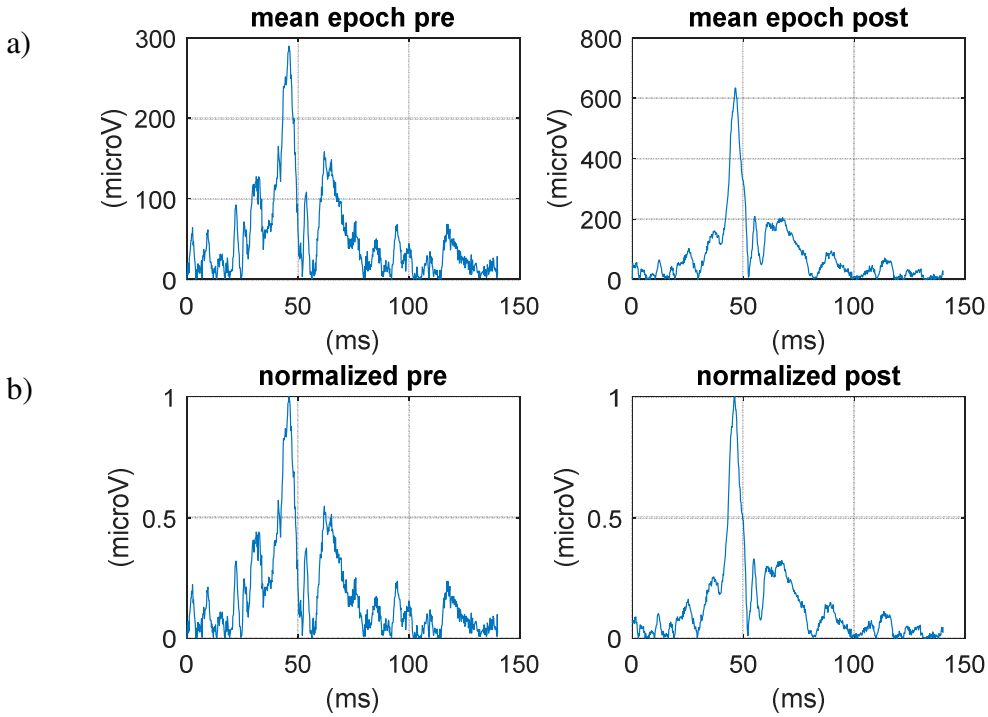


Figure 3.7 – a) Original signal before (left) and after (right) drug administration. b) The respective normalized central stimulation signal.

3.5.3. Dynamic Time Warping

Another method used to test reliability of ITV results, is the application of Dynamic Time Warping (DTW) algorithm to activation signals.

DTW is a non-linear normalization technique [26] used for determining similarity between two sequences that may differ in time and speed.

For evaluating their similarity, the algorithm takes two sequences and aligns them so as to minimize their Euclidean distance.

It returns two outputs: DTW distance, that is Euclidean distance between the two aligned sequences, and warping path, that specifies the optimal alignment between cycles [24].

This algorithm was thought to be used to analyse time sequences of video, audio and graphic data [23], then it has been successfully applied to gait analysis to compare two different gait patterns.

In this work, for the first time, it has been applied to compare stimulation signals, after having normalized them to avoid the already discussed problem that affects data spikes.

The basic idea is to consider the recorded muscle activation signal as two sequences which differ in speed and time.

Hence, DTW has been on normalized pre- and post- drug administration stimulation signals.

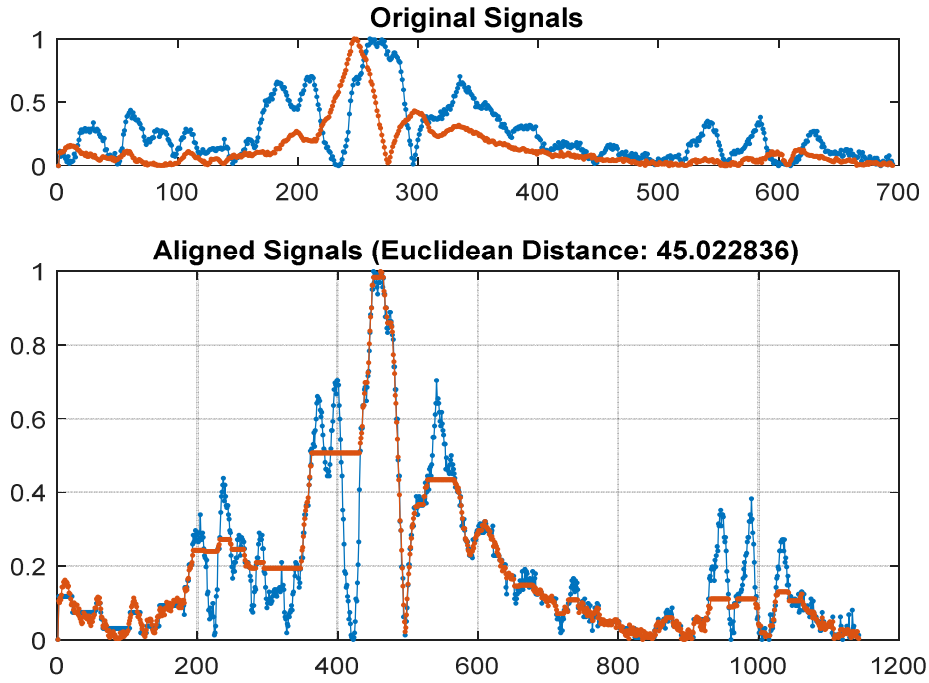


Figure 3.8 – Example of DTW algorithm applied to central activation signals.

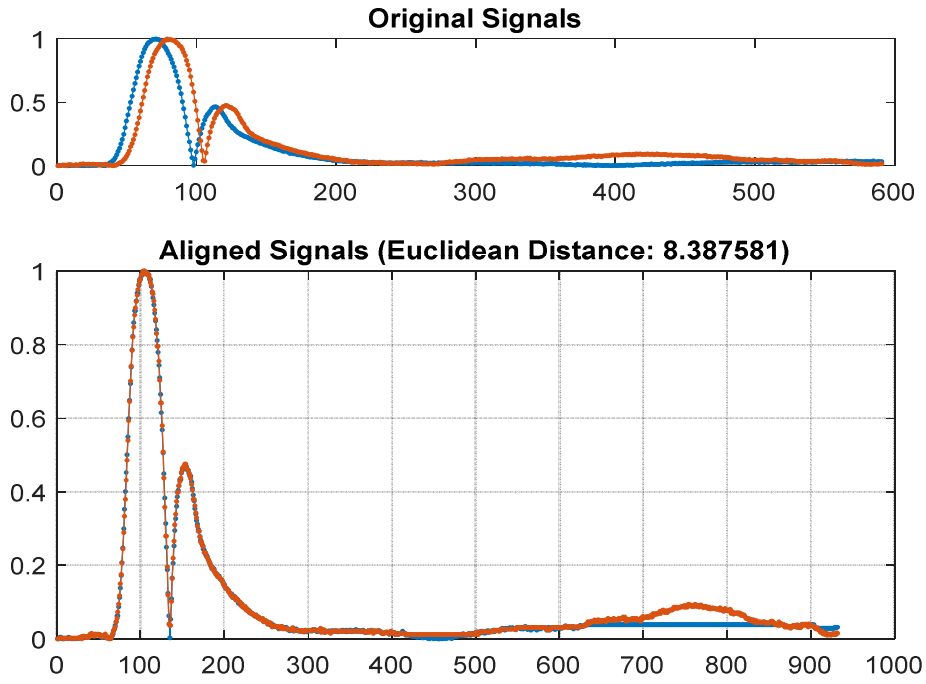


Figure 3.9 – Example of DTW algorithm applied to peripheral activation signals.

Moreover, the DTW algorithm allows one to speed up signal processing and to achieve a reliable parameter.

The algorithm, as stated before, returns the Euclidean distance between the two analysed sequences; this value has been used as a measure of reliability of results, based on ITV and normITV values.

In fact, from all peripheral data and for all central data of each patient, the average DTW distance has been taken as a reference: ITV of signals that have a DTW distance lower than average value has been considered reliable.

3.5.4. Energy of error signal

To confirm the reliability of results, together with DTW value, another suggested method is the energy of error signal.

Firstly, signals have been realigned considering peak values, to allow a more reliable measure. Error signal has been determined subsequently as the subtraction of post- drug administration signal from pre-drug administration stimulation signal, once both sequences have been normalized.

Energy (as ms*(mV^2)) has been evaluated and this last parameter, together with the others already presented, is used to support doctors in evaluating patient's conditions, evaluating the reliability of ITV and normITV parameters obtained from areas measure.

To decide if a result is reliable or not according to energy of error signal, as done for DTW distance, the average value among energy results, both peripheral and central signals, has been evaluated; all results with energy of error signal lower than mean value have been considered reliable.

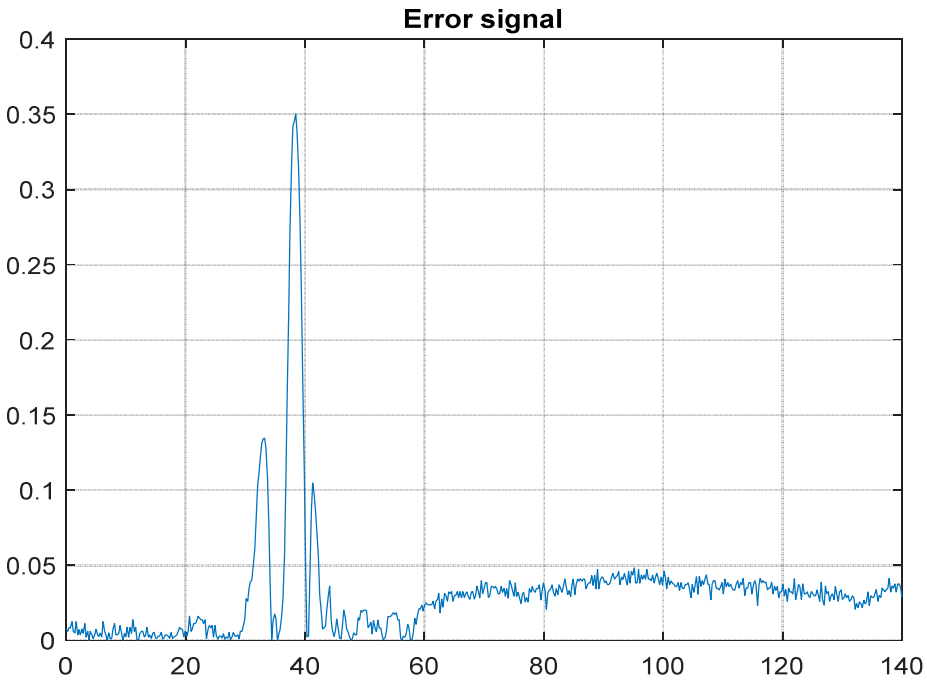


Figure 3.10 – Example of an error signal.

All the described parameter introduced for supporting medical evaluation of fatigue progresses are measured only for areas, because the discussed problem does not affect the research of muscle latency as much as for areas.

3.6. Latencies

Latency is defined as the time between the instant of stimulation and the beginning of the related response.

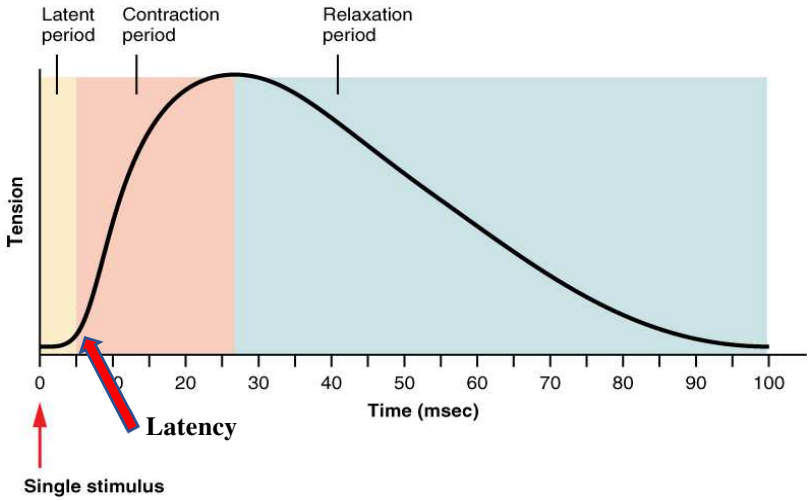


Figure 3.11 – Latency in a muscle activation signal [27].

Measure of latency is important to understand how long an impulse takes to be transmitted to respective muscle. In Multiple Sclerosis, in fact, impulse transmission is impaired and to quantify the severity of the injury, conduction time is a commonly used parameter.

Before searching latency time, signals have been filtered with a low-pass FIR filter to remove signal fluctuations that could result in a wrong measure.

Latencies have been determined for both peripheral and central muscle activation signals and, after having obtained all measures for all muscles, difference between RAD and MEP latency has been measured.

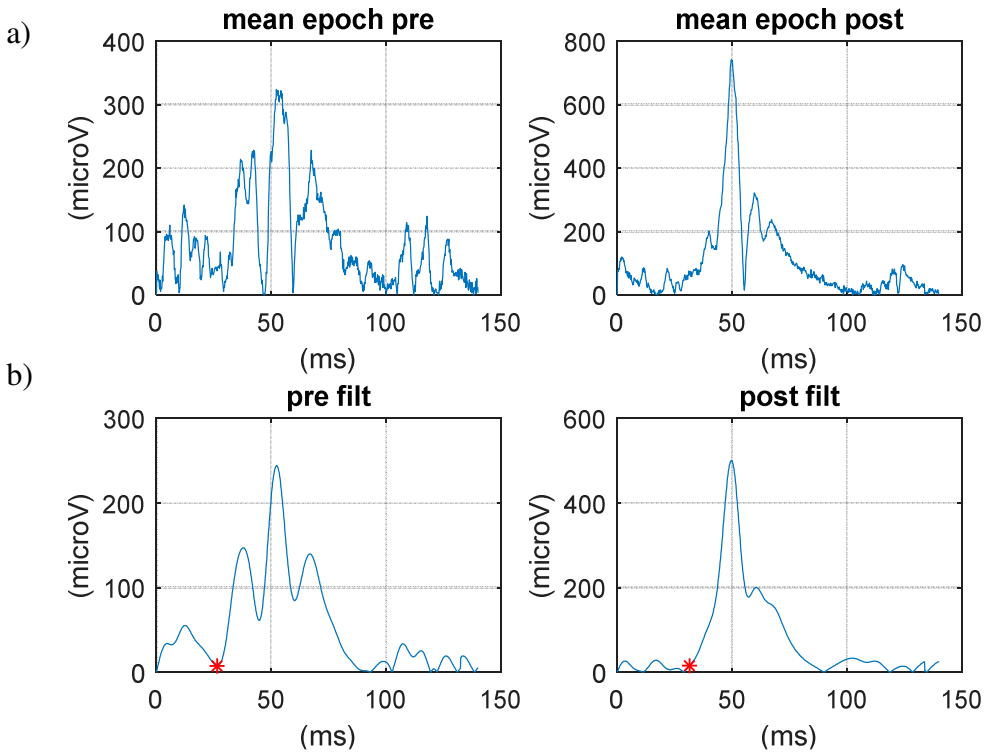


Figure 3.13 – An example of signal before (a) and after filtering (b). Graphs are referred to central stimulation. Red star represents the latency.

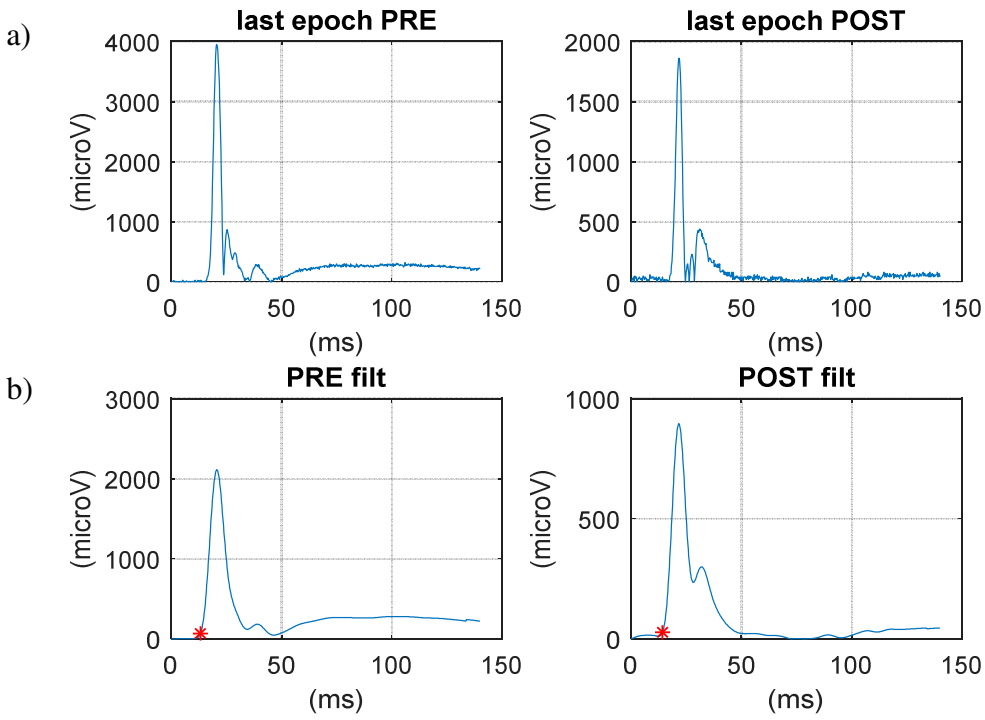


Figure 3.14 – An example of signal before (a) and after filtering (b). Graphs are referred to peripheral stimulation. Red star represents the latency.

The Central Motor Conduction Time indicates how long an impulse takes from central to peripheral conduction; this is representative of propagation time, hence is related to the progression of the disease in MS patients.

Latencies have been evaluated considering a percentage of peak value. Furthermore, to avoid fluctuations, a control on first milliseconds has been included. In fact, it is physiologically impossible that the activation point occurs before 10 ms, for this reason, if this happens due to various artifacts, the algorithm does not consider it, avoiding wrong results.

Threshold have been selected experimentally, considering physiologically realistic values.

This strategy suggests that also latencies are affected by problem of variability discussed before, but in this case the problem is not as severe as for areas.

Measures of latencies, in fact, have been compared with those obtained by doctors, and a good accordance has been noted. For this reason, latencies measures have been considered reliable despite the problem due to spikes differences and no countermeasures have been taken.

As stated before, latencies have been not evaluated on signals whose peak does not exceed 50 μV, because these signals have been considered non-meaningful: a signal so noisy means that electrical pulse does not reach muscle, so there is no significant contraction or response from the respective muscle.

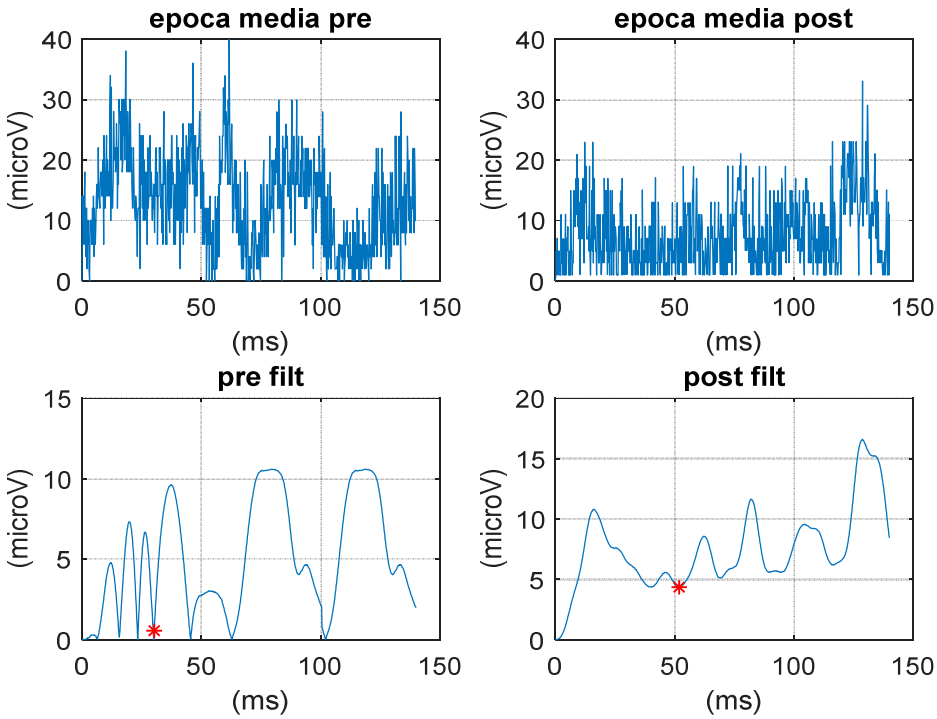


Figure 3.15 – An example of an unreliable measure of latency time due to a noisy signal, even filtered. In fact, signal dynamic results lower than 50µV both before (left) and after (right) drug administration.

Chapter 4

Results

In this section a complete overview of parameter assessing each patient of Fampyra® trial is presented. Each obtained parameter will be shown, and final assessment of patient conditions will be defined.

A patient with a score larger than 4 has been considered improved; on the contrary if results shown a score lower than -4, patient has been considered worsened and intermediate score denotes neither improved or worsened patient.

As for the 6-Minutes Walking Test, a patient has been considered improved if, after drug administration, he/she has walked more than 30% longer distance.

All ITV values for latencies and normITV for areas have been compared with the range used by Troni et al. [14], shown in following tables.

Recording Site	**RIV5th-95thPercentile**	**RIV5th-95thPercentile**	**RIV5th-95thPercentile**
VM	-9.6 / +9.1	-6.4 / +6.7	-12.2 / +15.1
VL	-9.7 / +13.6	-6.4 / +6.1	-14.9 / +11.5
TA	-6.4 / +5.2	-5.9 / +5.6	-14.8 / +13.8
PL	-6.7 / +6.9	-5.8 / +4.2	-12.4 / +12
FHB	-6.5 / +4.1	-7.6 / +3.5	-13.1 / +16

Table 2 – Ranges for latencies of peripheral signals (left), central signals (centre) and for CMCT (right)

Recording Site	RIV5th-95thPercentile	RIV5th-95thPercentile
VM	-29.1 / +26.6	-26.0 / +37.1
VL	-37.8 / +36.5	-21.3 / +35.0
TA	-21.7 / +28.0	-34.5 / +30.5
PL	-24.8 / +24.1	-24.1 / +34.8
FHB	-23.3 / +17.5	-31.7 / +24.5

Table 3 – Ranges for areas of peripheral signals (left) and central signals (right)

4.1. Patients

For patient's privacy each name has been replaced by a number, considering names in alphabetic order.

The most meaningful parameters have been summarized in a table for each patient. The meaning of every parameter is explained below:

- normITV: measured as defined by W. Troni et al. [19];
- normITVNorm: normITV measured on normalized signals;
- energyES: Energy of Error Signal;
- DTW_dist: Euclidean distance obtained from DTW algorithm;
- ITVlat: ITV measured with latencies values.

Abbreviations used for muscles name are:

- rt/lt VM: right/left Vastus Medialis;
- rt/lt VL: right/left Vastus Lateralis;
- rt/lt TA: right/left Tibialis Anterior;
- rt/lt PL: right/left Peroneus Longus;
- rt/lt FHB: right/left Flexor of Hallucis Brevis.

4.1.1. Patient #1

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-43,40	-9,50	13,20	1,79	-13,79
2	Lt VM	-2,66	-12,96	42,18	5,58	-25,80
3	Rt VL	-6,80	-40,04	69,35	8,17	176,47
4	Lt VL	17,04	14,38	69,54	9,43	7,41
5	Rt TA	55,78	14,45	**208,13**	**25,37**	-6,15
6	Lt TA	39,30	7,23	24,43	2,13	-8,45
7	Rt PL	34,14	-3,45	60,34	7,08	3,28
8	Lt PL	20,12	4,34	61,37	7,45	3,13
9	Rt FHB	**72,02**	**-5,56**	46,08	5,53	-3,15
10	Lt FHB	**-16,43**	**20,06**	135,11	7,37	97,22

Table 4 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
Pre	8180	6180	3190	3720	1660	3290	1810	2560	1310	1500
Post	6360	6840	4460	3780	2520	4340	2600	2960	3080	1060

Table 5 – Peak value of each peripheral signal

For this patient, mean DTW distance is 7.99 and mean energy of error signal is 72.97, so each muscle that presents a DTW distance and error energy larger than these values must be considered unreliable. In fact, for example, right Tibialis Anterior muscle (Fig. 4.1) exhibits high values of both DTW distance and energy of error signal (in boldface in Tab.4); this is due a difference between their peak values of about one thousand, and to a difference in the signal tails because of an artifact that prevents a waveform to return to zero. This problem heavily affects measures. DTW and energy values are able to highlight this artifact that makes a possible decision on these metrics unreliable.

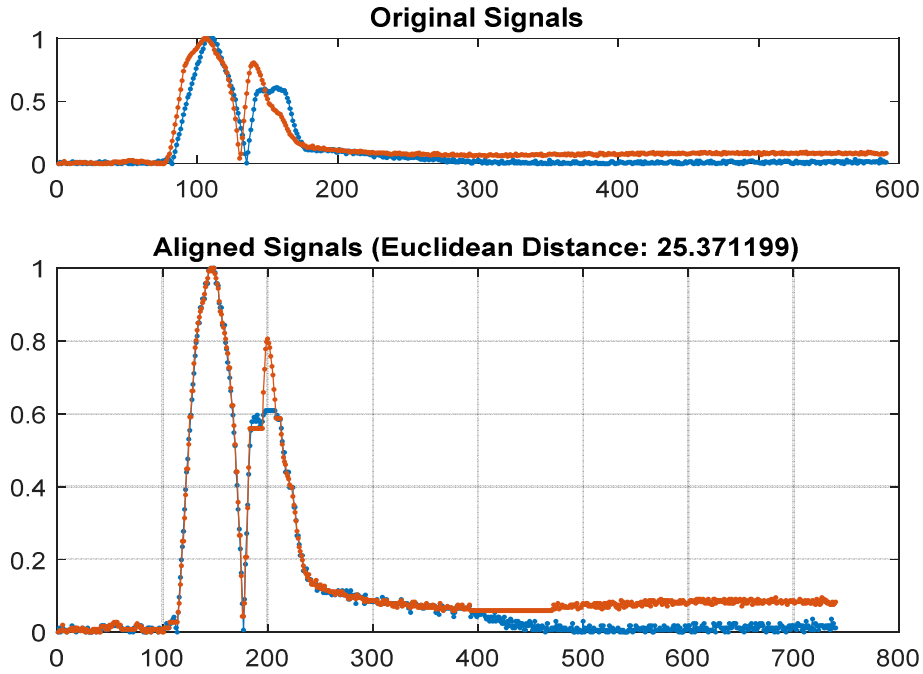


Figure 4.1 – Right Tibialis Anterior trend before (blue) and after (red) drug administration. Difference between signals is evident after 400ms.

It is also possible to notice that normITV values measured on non-normalized area and those measured on normalized signals are significantly different. This fact points out that waveforms before and after drug administration are different, mostly as for peak values and so final results must be carefully interpreted.

This phenomenon is evident for right muscle Flexor Hallucis Brevis, in which NormITV and normITVNorm differ also in sign. This is due to a difference of more than twice between peak values of respective signals (Tab. 5).

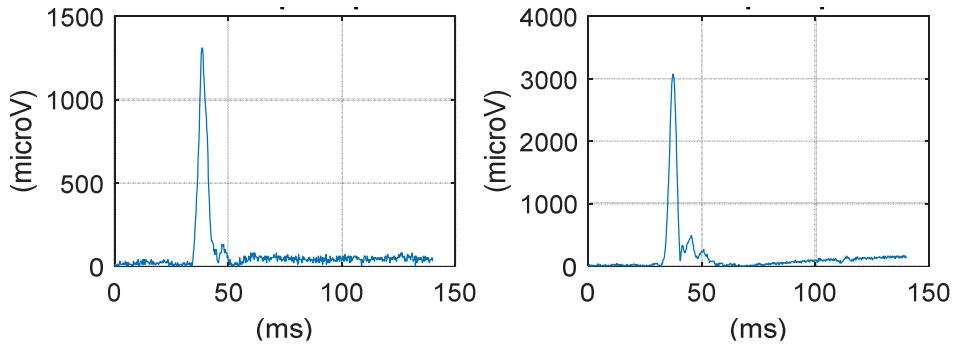


Figure 4.2 – Right Flexor of Hallucis Brevis trend before (left) and after (right) drug administration. A huge difference between respective maximum values can be appreciated.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlatc
1	Rt VM	-60,37	-17,01	136,62	14,22	4,88
2	Lt VM	-48,78	-2,48	74,31	11,83	11,17
3	Rt VL	-78,72	-47,13	917,97	33,83	59,92
4	Lt VL	**-128,82**	**44,09**	**596,22**	**29,62**	-26,48
5	Rt TA	56,12	33,37	633,74	17,70	-6,02
6	Lt TA	24,57	-6,82	460,55	20,04	4,13
7	Rt PL	55,72	18,52	687,67	24,72	-18,93
8	Lt PL	4,62	25,71	712,31	24,16	-10,39
9	Rt FHB	-10,12	13,23	1028,20	26,88	-2,02
10	Lt FHB	-46,91	9,45	102,63	9,37	-11,76

Table 6- Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	1772	1643	402	1405	864	459	348	293	465	1647
post	1354	1219	306	278	997	571	459	252	395	1121

Table 7 –Peak values of each central signal

For what concerns central stimulation signals, the largest difference between normITV and normITVNorm has been obtained for left Vastus Lateralis muscle; for this muscle DTW distance and energy of error exceed the average value (respectively 21.24 and 535);

this points out a different signals morphology before and after drug administration (shown in Fig.4.3).

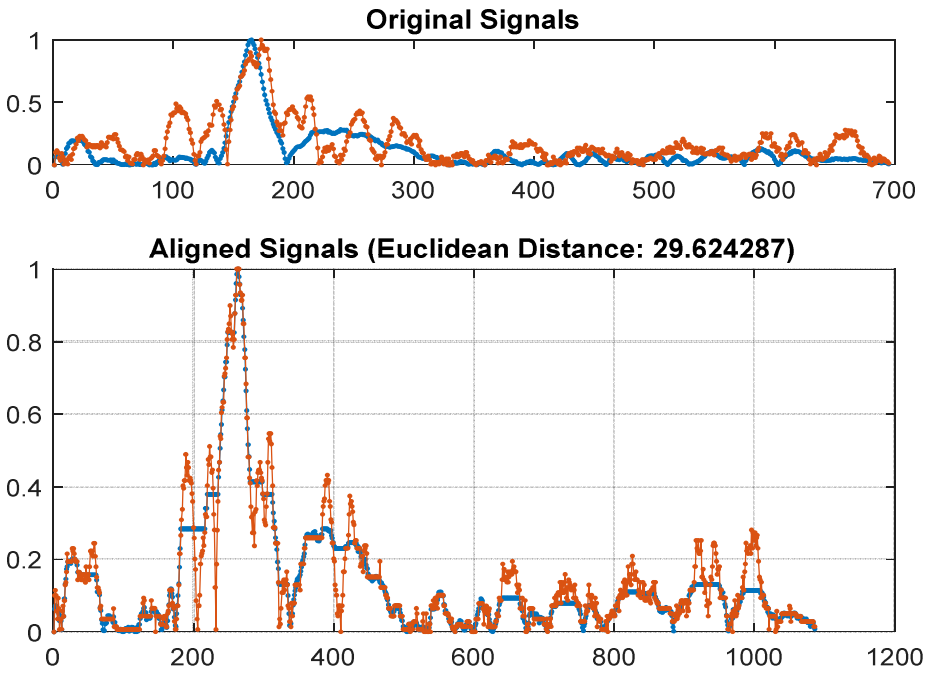


Figure 4.3 - Left Vastus Lateralis trend before (blue) and after (red) drug administration. The huge difference between signals morphology is evident in the presented figure.

When such a phenomenon occurs, a reliable assessment based on normITV is impossible. It is recommended to look at DTW and energy values: if they are high, normITV measured on normalized signals must be used to obtain a more reliable evaluation.

A possible explanation for this phenomenon is a wrong positioning of the stimulation electrode, but it is likely that this is not the only factor affecting this issue.

Latency results show that in four muscles conduction is improved, whereas in one case it is worsened, so the final score is 3.

Assigning scores as discussed in previous chapter, final patient evaluation has been summarized in the following tables:

	LATENCIES	AREAS	Norm AREAS	NC
RAD	1	3	0	0
MEP	3	-3	1	0
CMCT	0			0

Table 8 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
1	NaN	NaN	0	5,5

Table 9 - Fatigue scales scores

Table 8 shows also the difference between the results obtained analysing non-normalized signals and normalized ones: in RAD signals, the final score is 3; this means that at least 3 muscles are improved, but using normalized data no muscle is classified as improved.

There is also an incoherence between latencies and areas measured on non-normalized data mostly for what concerns MEP signals.

In summary this patient does not exhibit meaningful variations before and after Fampyra® administration, because no parameter changes in a substantial way.

Also, the 6-Minutes Walking Test does not show some meaningful improvement; in fact, the patient has walked more distance but not so much to be considered improved; for this reason, 0 has been assigned as the score for this test.

4.1.2. Patient #2

- **Peripheral stimulation signals**

For this patient, data about peripheral stimulation signals are not available.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	71,48	12,53	2054,84	61,55	**NC**
2	Lt VM	-23,29	-13,01	434,21	20,74	34,62
3	Rt VL	10,84	1,39	2164,39	41,15	40
4	Lt VL	-29,81	6,86	672,78	25,64	-34,06
5	Rt TA	143,01	16,36	2405,61	54,84	-47,56
6	Lt TA	63,80	31,60	1150,14	12,11	-23,49
7	Rt PL	48,64	-71,36	1292,15	39,44	0,49
8	Lt PL	1,72	-1,44	1116,44	32,81	-25,76
9	Rt FHB	-40,19	29,51	781,73	24,04	-5,06
10	FHB sx	-99,55	-42,91	2297,02	59,86	-35,88

Table 10 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
Pre	35,63	391,57	108,50	432,49	92,79	2742,83	301,89	362,20	952,52	580,09
post	56,68	364,72	115,94	334,14	532,37	2726,37	743,04	370,28	588,43	360,06

Table 11- Peak values of each central signal

There is one muscle, namely right Vastus Medialis that results not classified (NC), because signals have a peak amplitude less than 50µV (view figure 4.4).

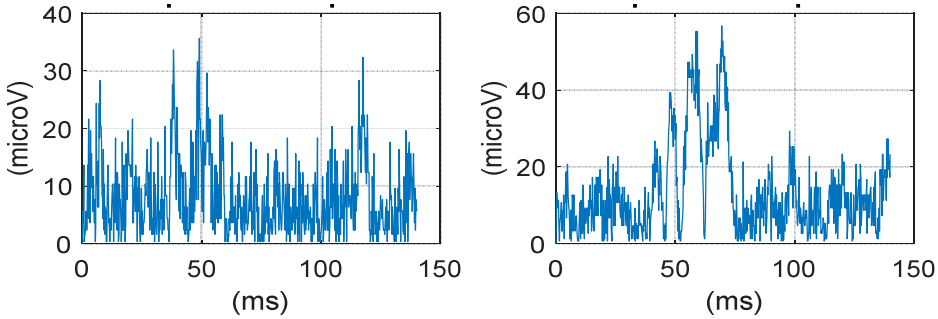


Figure 4.4 –Right Vastus Medialis trend before (left) and after (right) drug administration. Signal before drug administration has an amplitude lower than 50µV.

Mean DTW and energy of error signal are respectively 37.22 and 1436.93.

Five DTW values exceed this threshold, and four out of these five exhibit a difference between spikes more than twice. It is also important to notice that high DTW values

belong mostly on right muscles, which is likely to be more affected than left one in this patient, due to the specific localization of lesions.

Only for left Vastus Medialis and Tibialis Anterior, normITV values for normalized and non-normalized signals are similar.

Four ITV for latency values denote a possible improvement, whereas only one is worsened. So, the final score assigned to MEP latencies is 3.

	LATENCIES	AREAS	Norm AREAS	NC
MEP	3	1	0	1

Table 12 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
5	1	-1	0	6

Table 13 - Fatigue scales scores

Having obtained only central stimulation signals, it is impossible to perform a complete assessment of this patient's conditions. However, the available data, patient suggest that this patient does not show meaningful changes after Fampyra® administration.

Also, the 6 Minute Walking Test confirms this conclusion, but considering subjective fatigue scales, it seems that patient feels slightly improved. This has no confirmations in objective metrics.

4.1.3. Patient #3

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-129,29	-38,65	**2489,45**	**46,99**	-35,90
2	Lt VM	804,23	56,09	6622,55	102,63	-38,30
3	Rt VL	**9,45**	**-33,33**	1743,86	43,66	-6,45
4	Lt VL	-45,15	-29,43	955,27	22,99	18,18
5	Rt TA	-13,65	-2,49	698,90	7,28	26,57
6	Lt TA	-6,03	-13,59	238,61	11,49	-5,56
7	Rt PL	-0,59	-8,99	122,68	14,45	-3,77
8	Lt PL	-87,26	-74,98	7923,04	71,96	**184**
9	Rt FHB	-41,47	-5,81	83,84	7,85	-6,22
10	Lt FHB	-31,64	-11,33	281,37	12,30	0

Table 14 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	4010	6520	1760	3240	2700	4190	3130	1860	10310	5490
post	1550	9830	2640	2960	2460	4550	3390	1870	8190	4700

Table 15 - Peak values of each peripheral signal

Mean DTW distance is 34.16, whereas mean energy of error signal is 2115.96.

Comparing the obtained results with these two values, four signals exhibit DTW values higher than mean DTW and one of this, right Vastus Medialis, has also a difference more than twice between peak values.

This is another example of the instability data phenomenon already discussed.

Looking at Fig. 4.5, it is possible to appreciate that, besides the difference between spikes, also the signal morphology is significantly different in this case.

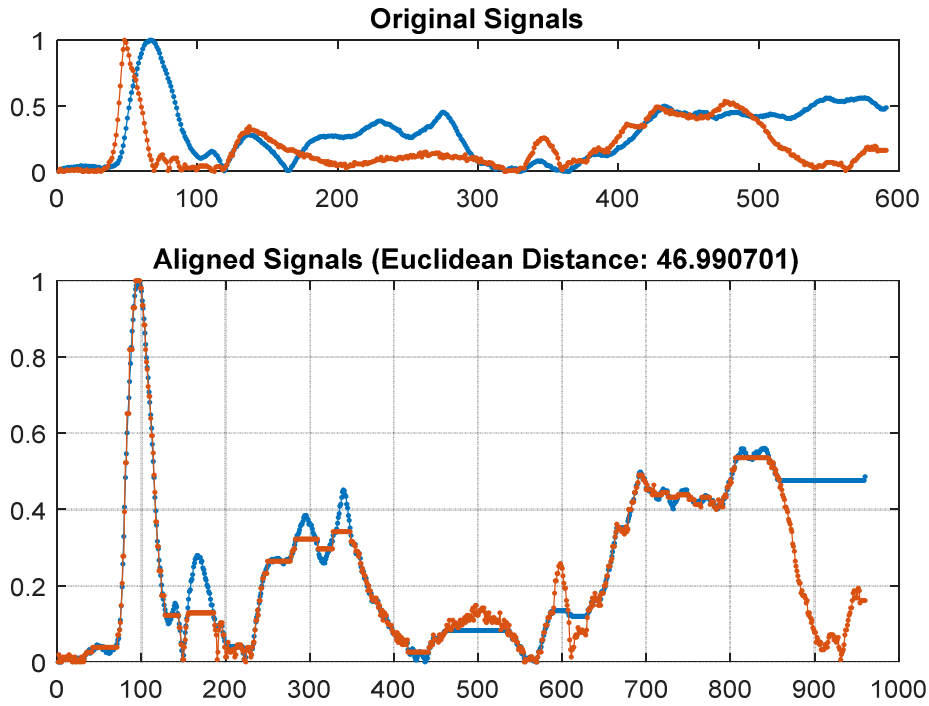


Figure 4.5 - Right Vastus Medialis trend before (blue) and after (red) drug administration. Notice the different morphology of signals that is highlighted also by DTW value.

Other two muscles however exceed the DTW threshold, with a difference that is less than twice, but quite significant; these are left Vastus Medialis and right Vastus Lateralis.

For what concerns right Vastus Lateralis, the difference in morphology is reflected also by the difference between the two normITV values that differs also in sign.

One muscle, left Peroneus Longus (Fig.4.6), have a very high ITV latency value, but also a DTW distance larger than mean value; this confirms that this result is unreliable.

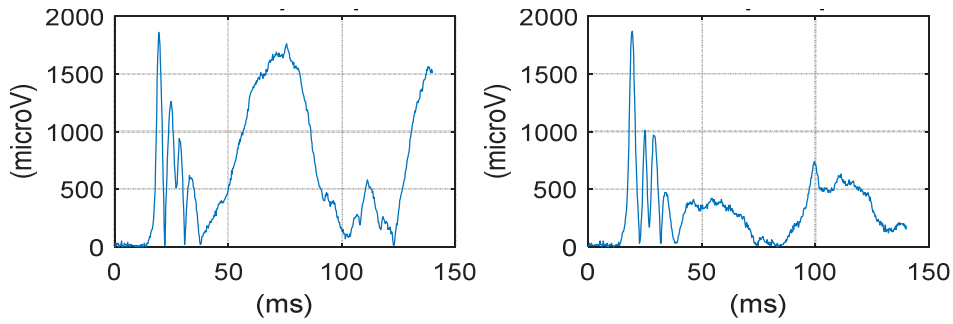


Figure 4.6 - Left Peroneus Longus trend before (left) and after (right) drug administration. Notice the difference between two signals morphology.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	63,25	-105,90	**8521,49**	**81,82**	-50,47
2	Lt VM	8,15	-53,62	1274,83	26,05	-3,47
3	Rt VL	108,66	-55,63	1419,07	49,19	-7,49
4	Lt VL	27,19	-36,64	610,45	18,74	0
5	Rt TA	89,98	-53,98	1029,99	25,71	-9,52
6	Lt TA	50,74	-13,90	200,80	14,42	-11,58
7	Rt PL	104,88	-23,25	949,04	35,21	-9,23
8	Lt PL	84,51	-30,86	1092,94	36,34	-0,87
9	Rt FHB	39,76	-24,15	450,54	9,41	36,99
10	Lt FHB	-5,41	-20,53	152,47	6,21	1,66

Table 16 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	146,45	642,29	170,95	804,08	295,27	2010,59	107,45	358,1021	718,56	1424,58
post	610,68	1008,81	685,09	1250,73	903,17	2878,29	323,7418	859,59	1118,83	1592,11

Table 17 - Peaks values of each central signal

For what concerns central stimulations signals, mean DTW distance is 30.31 and mean energy of error signal 1570.16.

Four muscles exhibit a DTW distance larger than mean value; for the same muscles it is also possible to appreciate a difference more than twice between peak value in pre- and post-drug administration signals.

Only one of mentioned signals, exhibits an energy of error signal exceeding the average values; this is confirmed by the graph (Fig.4.7); this muscle is right Vastus Medialis and, as for peripheral stimulation, a DTW distance larger than average value has been obtained.

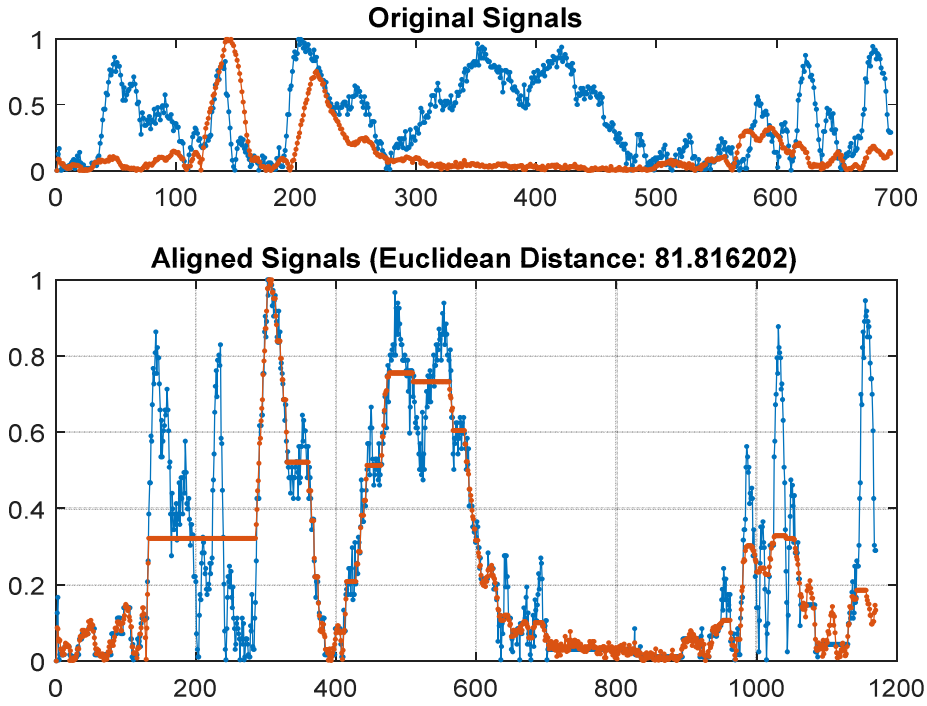


Figure 4.7 - Right Vastus Medialis trend before (blue) and after (red) drug administration

ITV in Vastus Medialis latency is quite large and this seems to denote a worsening, but, as stated before, the evaluation cannot be done without looking also at DTW distance and energy of error signal. These parameters, in fact, denote that this ITV value is unreliable because of the huge difference between signals morphology.

On the contrary, left Flexor of Hallucis Brevis signals are very similar (Fig.4.8), and the respective normITV values are similar as well, whereas the DTW distance and ITV values are lower than in the previous discussed cases.

This case is a clear example of what has been discussed before: the obtained results are strictly correlated with signals morphology. In fact, when the morphology is similar as in this case, normITV on not normalized or normalized signals leads to the same assessment, and DTW and energy values are lower than their respective average values.

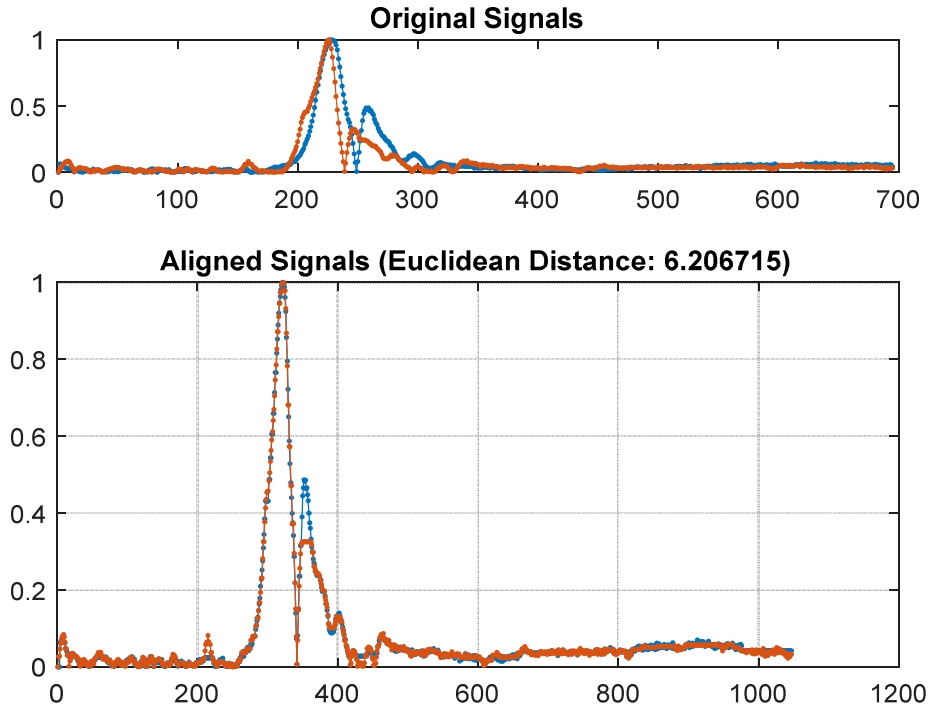


Figure 4.8 - Left Flexor of Hallucis Brevis trend before (blue) and after (red) drug administration. Figure highlights the similarity between the two waveforms.

Final scores are summarized in following tables (Tab.19 and 20):

	LATENCIES	AREAS	AREAS norm	NC
RAD	-1	-4	-1	0
MEP	4	7	-6	0
TOT	3			0

Table 18 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
NaN	NaN	NaN	-1	6

Table 19 - Fatigue scales scores

Scores derived from latencies and areas of non-normalized signals are consistent, whereas, comparing these results with those obtained using normalized signals, scores are almost opposite.

Moreover, it can be noticed that the scores based on normalized signals are consistent with 6 Minute Walking Test result; this last is rather reliable because a threshold of 30% is a very conservative parameter.

For this reason, even though looking at stimulation scores the situation seems to be confused, the 6 Minute Walking Test score clearly indicates that patient is worsened. This is in line with score based on normalized signals.

This patient is characterized by a high EDSS score, denoting a serious clinical condition

4.1.4. Patient #4

- **Peripheral stimulation signals**

		normITV	normITVNorm	err_energy	distNorm	ITVlat
1	Rt VM	26,05	-2,82	29,71	5,42	-4,88
2	Lt VM	257,16	67,71	2395,62	51,70	-4,44
3	Rt VL	149,11	92,08	3332,83	72,29	-73,68
4	Lt VL	-63,45	-38,57	364,94	20,68	**-111,11**
5	Rt TA	-33,72	7,40	186,57	15,21	-18,75
6	Lt TA	90,33	83,13	**7934,33**	**101,74**	-160
7	Rt PL	-23,50	36,23	417,94	17,14	66,67
8	Lt PL	96,02	90,34	**10574,04**	**46,16**	-9,90
9	Rt FHB	109,59	22,37	65,33	3,32	14,29
10	Lt FHB	113,13	27,08	161,60	10,57	9,52

Table 20 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre1	9840	10630	5950	2850	7070	3510	4560	3230	7830	7940
post	12010	11100	6270	2160	5140	2620	2590	2190	13800	12850

Table 21 - Peak values of each peripheral signal

The highest values of DTW distance (mean value: 34.42) and energy of error signal (mean value: 2546.29) have been obtained from left Tibialis anterior and left Peroneus Longus, but in neither case the difference between peak signal values exceeds twice. This could

mean that signals are different in morphology but not so much in amplitude; to better understand signal trends in this case is necessary to look at graphs.

Left Vastus Lateralis exhibits a very large ITV latency, but DTW distance and energy of error signals lower than average value (Fig.4.9).

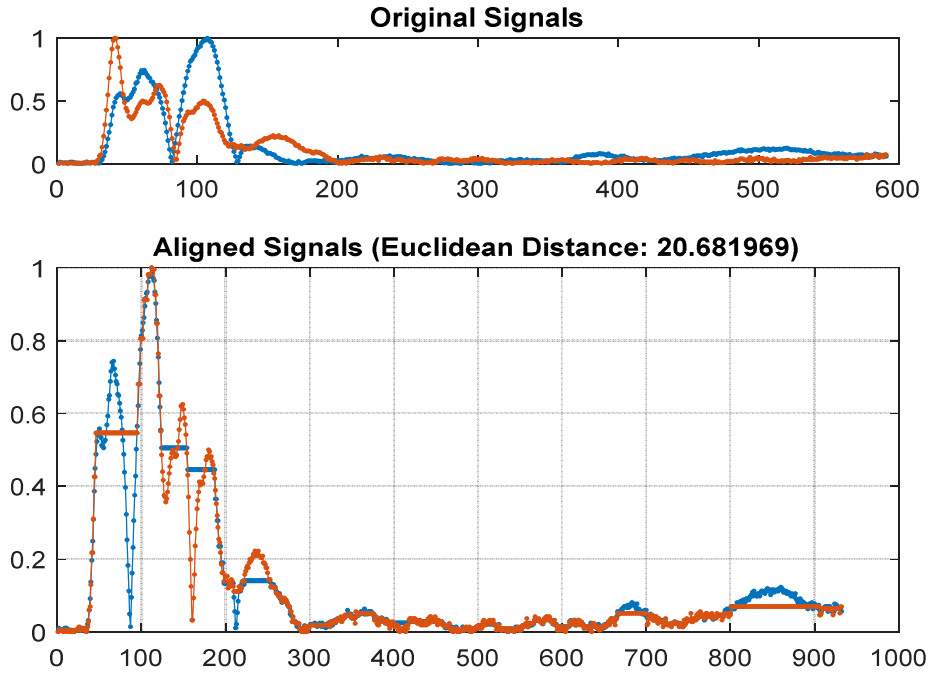


Figure 4.9 - Left Vastus Lateralis trend before (blue) and after (red) drug administration.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	8,20	16,62	5139,03	62,31	-155,76
2	Lt VM	64,91	70,80	5628,08	57,21	26,88
3	Rt VL	87,48	27,58	5991,89	68,64	NC
4	Lt VL	**3,92**	**3,53**	**2929,30**	**56,19**	-148,05
5	Rt TA	118,76	17,96	3300,94	55,44	NC
6	Lt TA	63,20	13,44	7186,56	78,72	-111,81
7	Rt PL	101,54	12,03	2364,21	43	64,39
8	Lt PL	126,91	50,49	4754,77	76,71	NC
9	Rt FHB	111,12	85,87	4189,39	107,19	-138,60
10	Lt FHB	118,02	23,41	4250,71	61,48	8,51

Table 22 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre1	64,27	92,24	44,18	117,27	36,81	96,38	51,60	46,92	128,87	53,03
post	60,54	87,30	74,27	117,59	107,70	140,93	115,75	133,15	181,01	145,73

Table 23 - Table shows maximum values of each central signal

Mean DTW value and mean energy of error signal are respectively 66.69 and 4573.49. Four DTW values are higher than the mean value, and two of these are defined as "Not classified"; this means that they are too noisy to be analysed.

The problem occurs in signal recorded before drug administration (Fig.4.10).

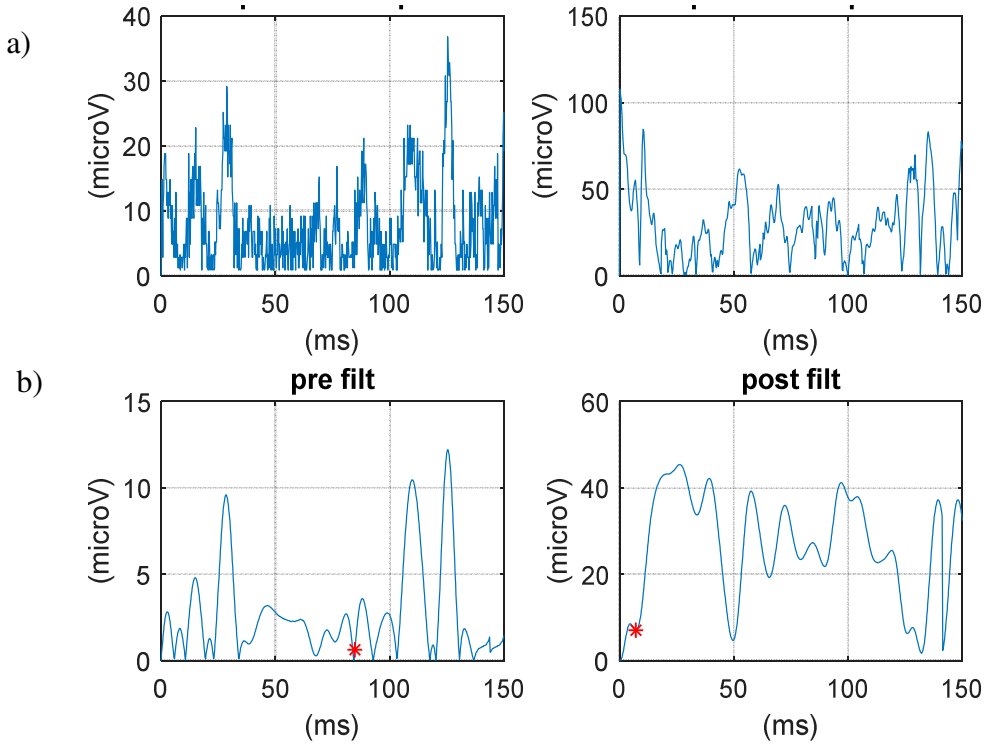


Figure 4.10 - Trend of a not classified signal. Red star defines the latency point. This is an example of a signal with an amplitude lower than 50µV

ITV values are also high, but the greatest values correspond to a high DTW distance, so they are considered unreliable. Latency measures are more reliable and easier to identify than areas. For this reason, when ITV are unreliable, all other parameters are unreliable too. This happens when the signal has a low amplitude: the threshold of 50 µV is very conservative, but there are also borderline cases that makes the signal analysis hard.

Left Vastus Lateralis exhibits consistent normITV values, but a high DTW distance even though it is below mean value. This fact could denote a different morphology of signals, similar but amplitude values.

Following table shows the final score for this patient:

	LATENCIES	AREAS	AREAS norm	NC
RAD	2	4	6	0
MEP	0,7	8	3	3
CMCT	1,4			3

Table 24 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
12	20	-4	0	3

Table 25 - Fatigue scales scores

Overall, the patient's conditions appear improved; in fact, results suggest a slight improvement of conduction after drug administration. This is felt by patient himself because the fatigue scales suggest an enhancement in physical and psychological conditions.

4.1.5. Patient #5

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	24,09	12,01	45,44	3,10	-20,9
2	Lt VM	**125,27**	**18,33**	97,94	9,67	3,51
3	Rt VL	-2,02	8,88	103,55	5,78	-49,1
4	Lt VL	72,21	2,20	250,35	8,26	3,77
5	Rt TA	94,42	28,50	312,75	11,77	16,54
6	Lt TA	33,18	11,46	86,38	6,71	1,40
7	Rt PL	-5,50	-10,64	39,16	8,45	-3,28
8	Lt PL	-4,56	21,47	134,43	16,77	-4,96
9	Rt FHB	84,57	28,82	342,16	10,91	7,69
10	Lt FHB	86,63	32,62	444,12	18,42	6,50

Table 26 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre2	11640	6040	4460	3430	3330	5010	6660	6010	4000	3060
post	12020	14310	4030	6570	6300	5820	7050	4710	6440	5300

Table 27 - Peak values of each peripheral signal

Mean DTW distance and energy of error signal are respectively 9.98 and 185.63.

Four signals exceed DTW mean value and mean energy of error signal value; these muscles also exhibit a huge difference between normITV measured on non-normalized area and normalized signals respectively.

Left Vastus Medialis shows a huge difference between peak values of signals and between normITV and normITVNorm, even though it is not characterized by high values of DTW and of error signal energy. This is likely to denote a difference only in signals morphology (Fig.4.11).

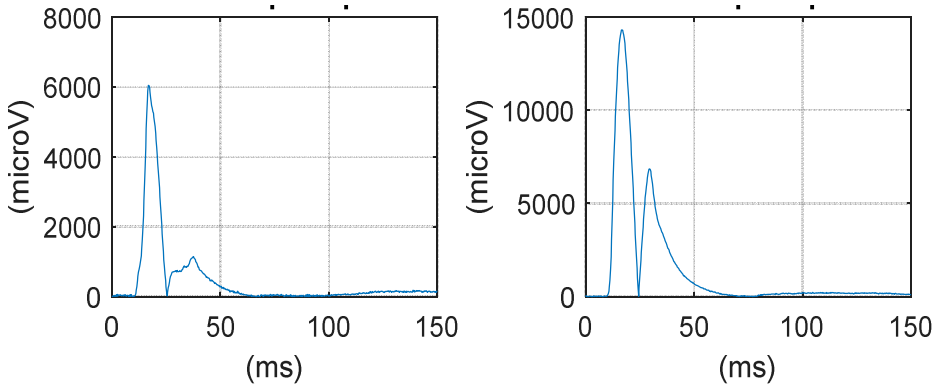


Figure 4.11 - Trend of left Vastus Medialis before (left) and after (right) drug administration. Graphs exhibit the difference between maximum values.

This fact confirms again that signal morphology is very important for the patient assessment and for normITV and ITV reliability.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	45,58	-34,49	1930,41	52,72	36,77
2	Lt VM	30,11	13,74	1449,60	31,14	76,44
3	Rt VL	13,12	15,13	2828,60	55,65	80
4	Lt VL	16,16	18,01	1314,69	32,28	**-111,6**
5	Rt TA	-11,90	14,40	4432,16	56,55	-33,33
6	Lt TA	106,48	-8,44	3196,93	76,07	**116,03**
7	Rt PL	80,03	-20,03	2755,43	55,50	27,59
8	Lt PL	29,63	-22,37	2095,46	40,18	-26,97
9	Rt FHB	87,51	14,58	1360,46	25,36	-15,91
10	Lt FHB	94,26	61,53	2874,47	52,88	0

Table 28 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre2	75,95	302,50	97,93	206,45	348,63	223,80	76,24	207,71	299,11	303,37
post	135,55	339,46	96,53	203,60	290,45	580,30	165,62	300,10	541,22	410,93

Table 19 - Peak values of each central signal

Mean DTW distance and energy of error signal are respectively 47.83 and 2423.82.

More than 50% of values exhibit DTW distance larger than average value and three of them have a huge difference between peak signals.

Two signals, namely left Vastus Lateralis and left Tibialis Anterior, exhibit a very high ITV latency. This suggests that signals are out of phase (Fig. 4.12).

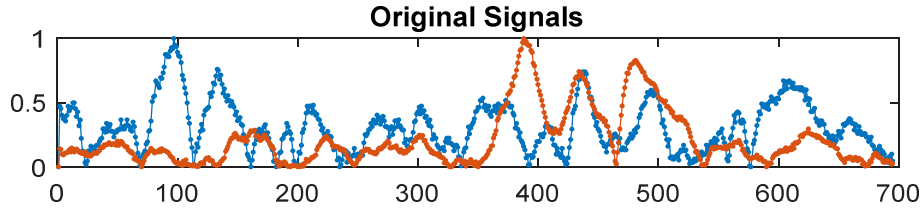


Figure 4.12 - Left Tibialis Anterior trend before (blue) and after (red) drug administration. As it is shown, signals are out of phase and this causes a ITV measure very high

On the other hand, left Flexor of Hallucis Brevis ITV reveal that signals are perfectly in phase, as suggested by the ITV value. But, for one of them, DTW distance and error signal

energy warn that this information may be unreliable because those values are higher than the average one.

Scores are summarized in following table.

	LATENCIES	AREAS	AREAS norm	NC
RAD	-1	6	3	0
MEP	-1	5	0	0
CMCT	2			0

Table 30 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
3	9	-2	0	4,5

Table 31 - Fatigue scales scores

Final scores suggest that this patient conditions are improved after Fampyra® administration, but looking at normalized data and DTW value, the assessment results unreliable.

In fact, there is a huge difference between final score measured on non-normalized and normalized signals.

Furthermore, the fact that this patient is not improved as much as it is suggested by analysing non-normalized data, is also witnessed by fatigue scales values; actually, this patient does not feel better so much.

4.1.6. Patient #6

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	39,48	13,72	52,32	4,36	**0**
2	Lt VM	44,46	23,49	71,74	5,67	-4,08
3	Rt VL	31,04	10,67	71,75	4,17	0
4	Lt VL	4,13	4,64	118,18	3,80	-15,4
5	Rt TA	17,33	7,71	217,72	6,26	0
6	Lt TA	52,77	9,67	277,48	3,62	21,85
7	Rt PL	-9,74	-1,31	52,48	4,25	3,70
8	Lt PL	21,21	2,10	54,81	4,77	1,87
9	Rt FHB	-5,81	-5,59	**69,31**	**11,41**	1,89
10	Lt FHB	-35,61	8,04	**124,19**	**13,19**	0

Table 32 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre2	4930	4840	3570	4100	4610	3890	2690	3780	6500	7060
post	6020	5690	4250	4070	4870	5380	2490	4480	6540	4900

Table 33 - Peak values of each peripheral signal

Mean DTW distance and mean energy of error signal are respectively 6.15 and 111.

In this case, only for both Flexors of Hallucis Brevis DTW and error signal energy exceed mean values, but no significant differences are found in signals peak values.

Some IVT latencies results 0; this means that signals start at the same time both before and after drug administration, as it can be seen by Fig. 4.11 (referred to right Vastus Medialis).

An ITV latency equals to 0 means that nothing is changed before and after drug administration.

This happens because there was nothing to improve in this patient conditions; in fact, looking at EDSS value, it is 1, i.e. this patient's clinical conditions are good, and he/she is hardly affected by MS motor symptoms.

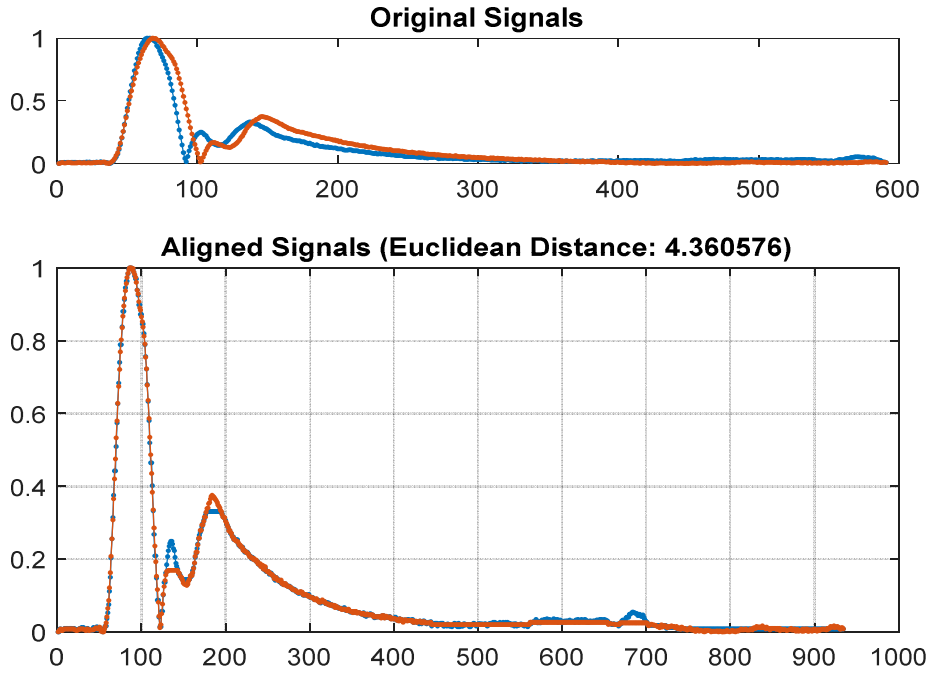


Figure 4.11 - Right Vastus Medialis trend before (blue) and after (red) drug administration. Signals are very similar and for this reason a low DTW is obtained.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-42,59	13,09	61,64	4,80	-10,70
2	Lt VM	42,37	6,23	35,10	4,38	2,27
3	Rt VL	17,33	3,73	57,37	4,11	0
4	Lt VL	2,69	5,43	156,67	7,26	0
5	Rt TA	85,31	3,19	53,95	3,72	0,78
6	Lt TA	49,67	9,24	164,93	3,66	0
7	Rt PL	-24,33	3,75	**1017,83**	**12,00**	-8,06
8	Lt PL	28,21	-20,13	157,57	**9,66**	4,26
9	Rt FHB	3,76	-38,69	**385,89**	**13,31**	14,33
10	Lt FHB	-39,18	-33,16	**499,90**	**17,48**	2,92

Table 34 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre2	1511,96	792,82	1329,60	882,09	4112,23	3305,99	842,51	**807,21**	1218,39	1238,75
post	1031,80	1020,61	1450,43	864,73	5010,46	3708,37	696,18	**1123,82**	1647,90	1212,62

Table 35 - Peak values of each central signal

Mean DTW distance and energy of error signal are respectively 8.04 and 259.08.

Left Peroneus Longus exhibits a huge difference between signals maximum values; this diversity is confirmed also by a high value of DTW.

Furthermore, high values of DTW and error signal energy are also present in right Peroneus Longus and for both Flexors of Hallucis Brevis, but for these muscles no meaningful differences between spikes are detected.

This may indicate that signals morphology is different, but they are similar in amplitude. An example is shown by Fig. 4.12.

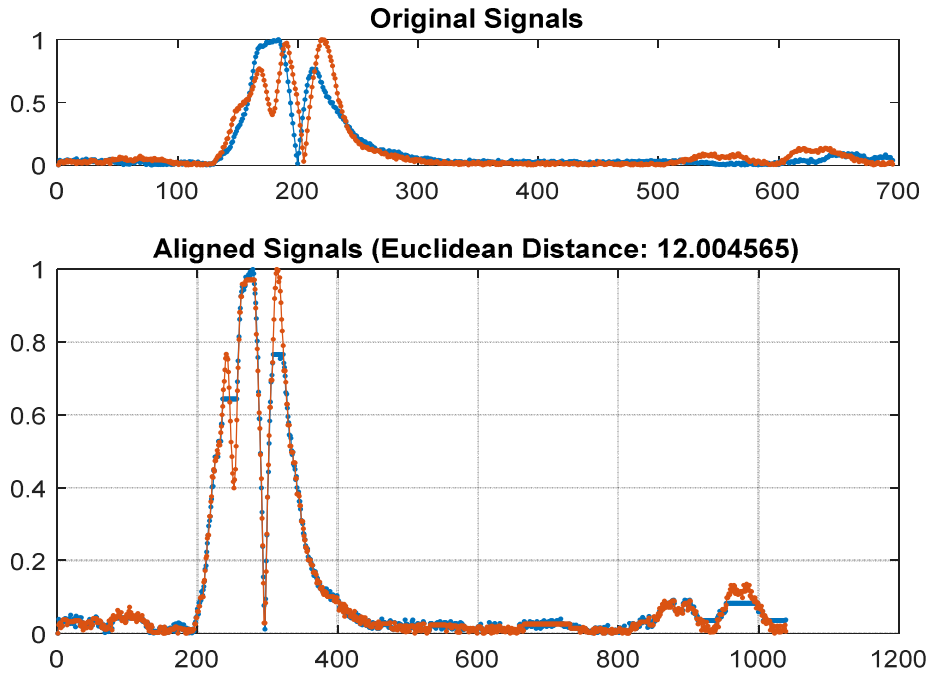


Figure 4.12 - Right Peroneus Longus trend before (blue) and after (red) drug administration. Signals differs in morphology but not in peak amplitude.

An example of a muscle that, before and after drug administration has not changed its behaviour is represented by left Vastus Lateralis. In fact, in this case, ITV latency is approximately 0 and DTW and error signal energy are very low. Muscle signal is shown by the following figure (Fig. 4.13).

All those factors point out that drug has not been effective, because before and after Fampyra®, administration no changes can be appreciated, and this also is confirmed by normITV values.

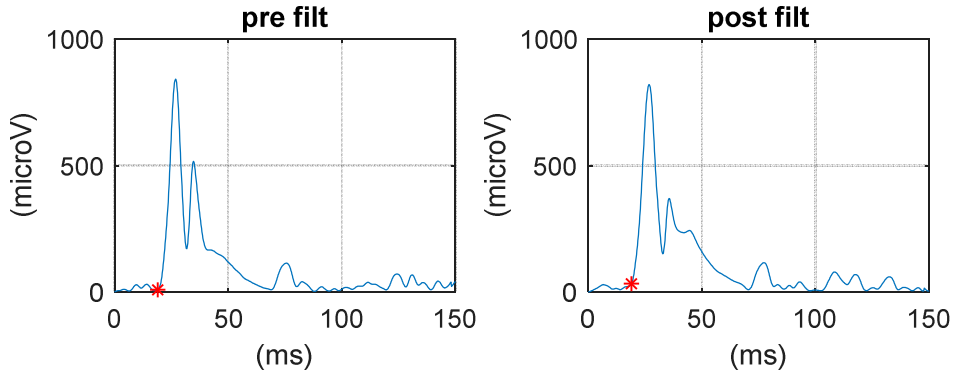


Figure 4.13 - Left Vastus Lateralis trend before (left) and after (right) drug administration. Red star represents latency.

Final scores are summarized in tables:

	LATENCIES	AREAS	Norm AREAS	NC
RAD	0	2	0	0
MEP	0	0	-2	0
CMCT	-2			0

Table 36 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
-2	-5	0	0	1

Table 37 - Fatigue scales scores

These results confirm that no meaningful changes can be appreciated between signals before and after drug administration.

Fatigue scales denote that also the patient does not feel better; on the contrary, he/she seems to feel quite worsened as it can be seen from fatigue scales scores.

The fact that this patient feels a worsening in fatigue progression, but from stimulation data no changes are found, highlights that fatigue is not only a motor phenomenon.

4.1.7. Patient #7

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	1,90	2,07	23,07	2,42	11,76
2	Lt VM	-13,82	8,60	8,67	2,28	19,72
3	Rt VL	5,48	1,23	26,28	5,47	20,9
4	Lt VL	-5,34	14,43	68,20	8,39	22,22
5	Rt TA	-89,76	26,41	395,82	17,99	-5,13
6	Lt TA	-85,00	11,18	367,83	18,81	-13,95
7	Rt PL	-59,69	21,00	178,69	16,02	-86,49
8	Lt PL	-79,88	-18,96	101,43	12,22	6,90
9	Rt FHB	-49,87	20,15	86,66	11,57	0,86
10	Lt FHB	-46,49	17,65	164,03	23,92	-6,11

Table 38 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	6520	9270	6720	6210	4690	3600	2920	3950	7510	5880
post	6490	7780	6920	5190	1270	1190	1280	1860	4000	3270

Table 39 - Peak values of each central signal

Mean DTW distance and energy of error signal are respectively 11.91 and 141.07.

The last five muscle signals exceed threshold of DTW distance and energy signal error; they also show a difference of almost twice between peak values. Instability of results can be appreciated also in the difference between normITV measured on normalized and non-normalized signals.

Such five muscles, at a first evaluation, could result worsened, but looking at DTW distances and normITV on normalized signals, some of them have been defined as improved.

This confirms the presence of instability that affects most of these data.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	80,00	-22,99	**447,78**	**25,04**	15,91
2	Lt VM	29,77	4,73	292,80	18,79	-21,60
3	Rt VL	57,32	-56,19	1273,26	45,02	17,42
4	Lt VL	-3,37	34,56	993,32	14,15	3,10
5	Rt TA	-43,38	49,21	1535,78	13,98	-0,95
6	Lt TA	98,05	17,55	155,49	6,86	**-34,18**
7	Rt PL	-53,67	-20,74	784,03	18,52	15,39
8	Lt PL	-66,71	55,91	601,02	13,71	-8,05
9	Rt FHB	26,15	47,91	533,31	24,22	-21,66
10	Lt FHB	58,24	-6,32	**505,26**	**21,52**	9,06

Table 40 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	289,50	2001,46	323,99	966,66	2146,49	1728,51	523,06	1371,16	597,37	459,68
post	633,32	2261,40	741,86	737,97	1216,70	2557,87	417,42	567,28	504,48	729,90

Table 41 - Peak values of each central signal

Mean DTW distance and energy of error signal are respectively 20.18 and 712.21.

The highest value of DTW distance results from right Vastus Lateralis signal; also, the energy of error signal is very high and in fact, difference between peak value of signals before and after drug administration exceeds twice.

The two values of normITV, for normalized and not normalized signals, are almost opposite too.

All presented data point out that the results are unreliable because the two signals have a very different morphology, as it can be seen by figure (Fig. 4.14).

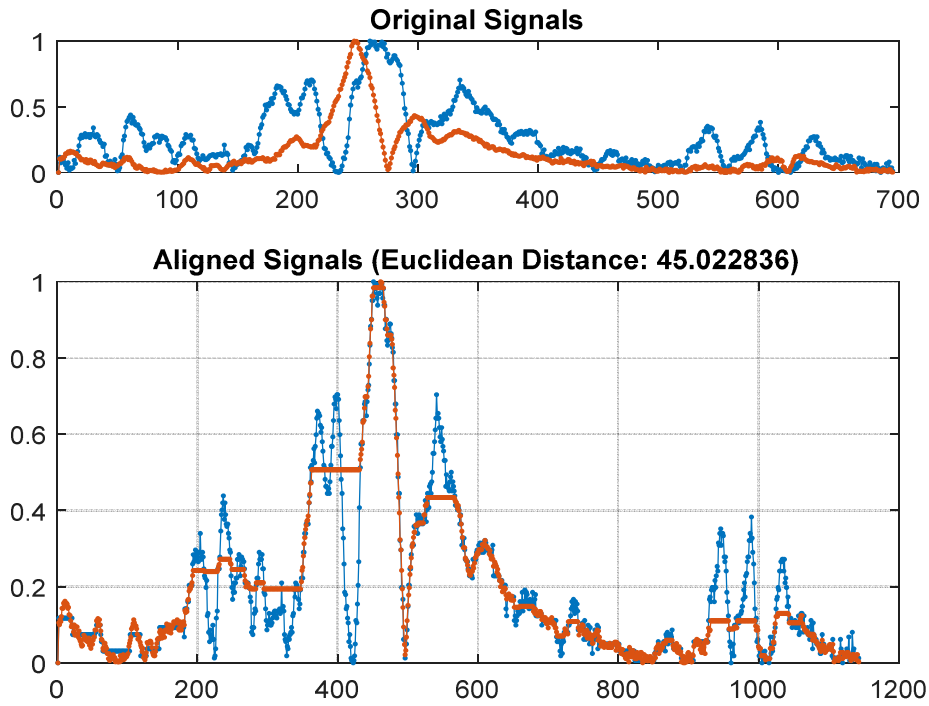


Figure 4.14 - Left Flexor of Hallucis Brevis trend before (blue) and after (red) drug administration. High DTW value indicates the unreliability of data.

This assessment is clear also for right Vastus Medialis and left Flexor of Hallucis Brevis, in which high DTW and error of energy signals witness the instability in normITV parameters.

As Fig. 4.15 shows, signals referred to left Tibialis Anterior are out of phase; this explains the negative ITV value that defined this muscle surely improved.

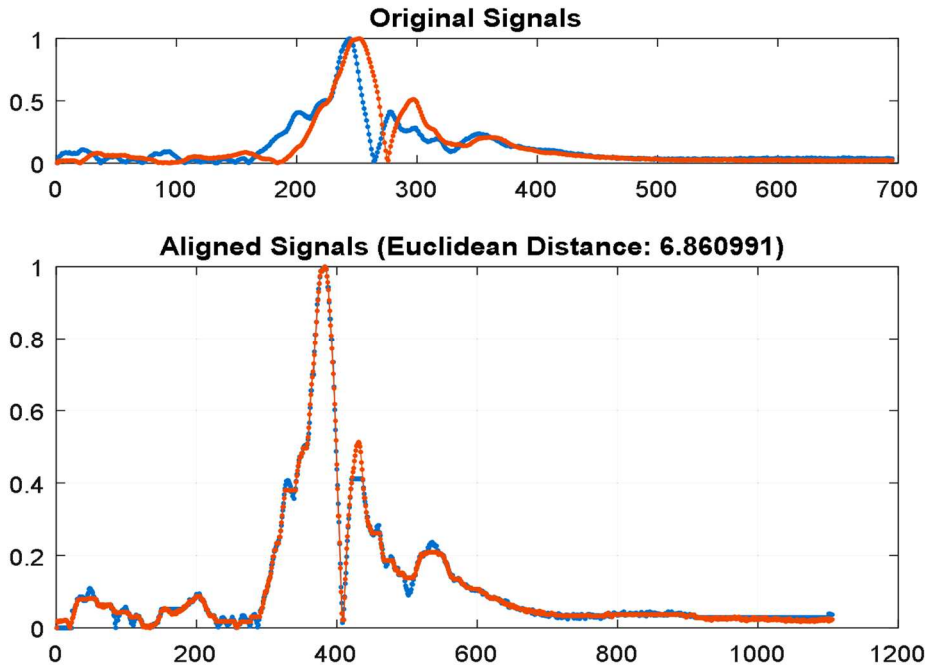


Figure 4.15 - Left Tibialis Anterior trend before (blue) and after (red) drug administration.

Final scores are summarized in following tables:

	LATENCIES	AREAS	AREAS norm	NC
RAD	-2	-6	2	0
MEP	0	2	2	0
CMCT	0			0

Table 42 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
0	0	0	0	3,5

Table 43 - Fatigue scales scores

Looking at areas on non-normalized signals, this patient seems to be worsened; on the other hand, looking at normalized areas, nothing seems changed.

This last assessment is confirmed by fatigue scales scores, that indicate that this patient does not feel better after Fampyra® administration.

Correlation between scores defined by normalized signals and those obtained on fatigue scales is another important indicator of non-normalized data instability.

As stated before, the final assessment cannot be done only looking at fatigue scales, but the presence of a correlation among all scores is a good indicator for better understanding the impact of the discussed problem on final evaluation.

4.1.8. Patient #8

- **Peripheral stimulation signals**

This patient does not have an evaluation of peripheral stimulation signals.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	9,80	-10,19	2475,15	66,17	NC
2	Lt VM	-41,36	-19,37	3859,82	64,88	**72**
3	Rt VL	-56,89	3,34	3144,20	68,78	NC
4	Lt VL	-40,69	-14,76	3555,98	81,03	NC
5	Rt TA	98,07	-69,23	2662,03	63,90	-41,94
6	Lt TA	-14,77	10,51	**273,45**	**9,82**	6,02
7	Rt PL	-115,24	-44,76	5098,99	83,68	NC
8	Lt PL	8,86	21,25	2754,07	70,94	NC
9	Rt FHB	7,68	54,16	**1570,58**	**55,65**	54,55
10	Lt FHB	23,89	53,78	2691,81	86,52	8,89

Table 44 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	36,73	73,45	54,03	39,96	85,88	691,81	65,47	54,45	476,01	209,14
post	42,28	62,50	34,56	33,05	338,93	580,73	29,42	49,85	336,11	166,21

Table 45 - Peak values of each central signal

Mean DTW distance is 61.14, whereas mean energy of error signal is 2808.61.

Five muscle activation signals results not classified; in fact, as it can be appreciated in Tab. 45, signal peak values do not exceed 50 μV. For all these signals, a high DTW value has been obtained.

For what concerns the other five, only two muscle signals exhibit a DTW lower than the mean value, namely left Tibialis Anterior and right Flexor of Hallucis Brevis.

The highest ITV is achieved for left Vastus Medialis; in fact, these signals are noticeably out of phase (view Fig. 4.16)

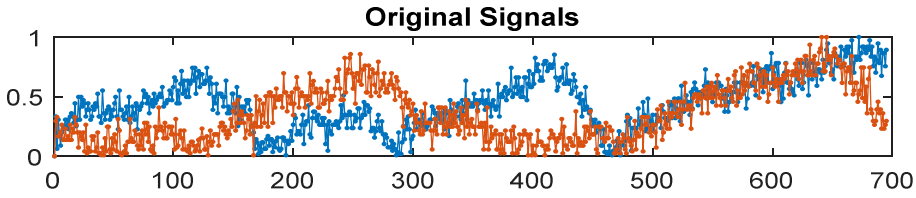


Figure 4.16 - Left Vastus Medialis trend before (blue) and after (red) drug administration

Final scores are summarized in the following table:

	LATENCIES	AREAS	Norm AREAS	NC
MEP	-1,5	-3	0	5

Table 46 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
12	28	6	1	6,5

Table 47 - Fatigue scales scores

The high number of “not classified” signals is probably due to the high EDSS score of this patient.

Looking at Tab. 46, this patient seems to be worsened; however, he/she feels improved, as reported in the subjective scales.

This divergence is probably due to the numerous of non-classified signals: a complete assessment based only on muscle stimulation signals cannot be carried out because many muscles do not yield meaningful data for the evaluation of patient conditions.

This is another situation in which, because of the high EDSS, the assessment cannot be based on stimulation scores as these cannot give a reliable result. Instead, the evaluation should be carried out considering fatigue scales and the 6 Minutes Walking Test.

These latter parameters point out that the patient conditions are improved after Fampyra® administration.

4.1.9. Patient #9

- **Peripheral stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-222,71	-64,85	**1846,12**	**54,06**	3,39
2	Lt VM	37,77	-4,69	24,83	3,89	2,90
3	Rt VL	18,37	13,86	129,70	9,71	-13,33
4	Lt VL	44,11	80,70	**3984,87**	**116,08**	-8,33
5	Rt TA	53,92	-6,80	311,09	14,43	8,26
6	Lt TA	105,20	45,69	1297,76	21,90	45,87
7	Rt PL	51,21	72,06	3495,04	70,51	1,94
8	Lt PL	99,39	62,52	1428,51	28,10	93,07
9	Rt FHB	3,44	-2,91	10,46	3,15	-3,28
10	Lt FHB	7,65	9,82	80,69	8,91	-3,33

Table 48 - Principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	11250	5790	3090	**4170**	690	790	1050	1170	6010	5240
post	7250	8060	3190	**2250**	1280	2580	770	2390	6360	5100

Table 49 - Peak values of each peripheral signal

Mean DTW distance and energy of error signal are respectively 33.07 and 1260.91.

For left Vastus Lateralis, high values of DTW distance and error signal energy have been obtained; in addition, also the difference between signals peak values is considerable. This situation denotes a huge difference in signal morphology (Fig. 4.17) mostly due to the fact that signal obtained after drug administration does not return to zero.

This is likely to be a recording error, but it is not clear how it happens; what is clear is that this problem affects the final assessment because muscle behaviour is erroneously classified as worsened. Again, for this reason, it is important considering DTW and the

energy of error signal to perform a more reliable and complete assessment of conduction measures.

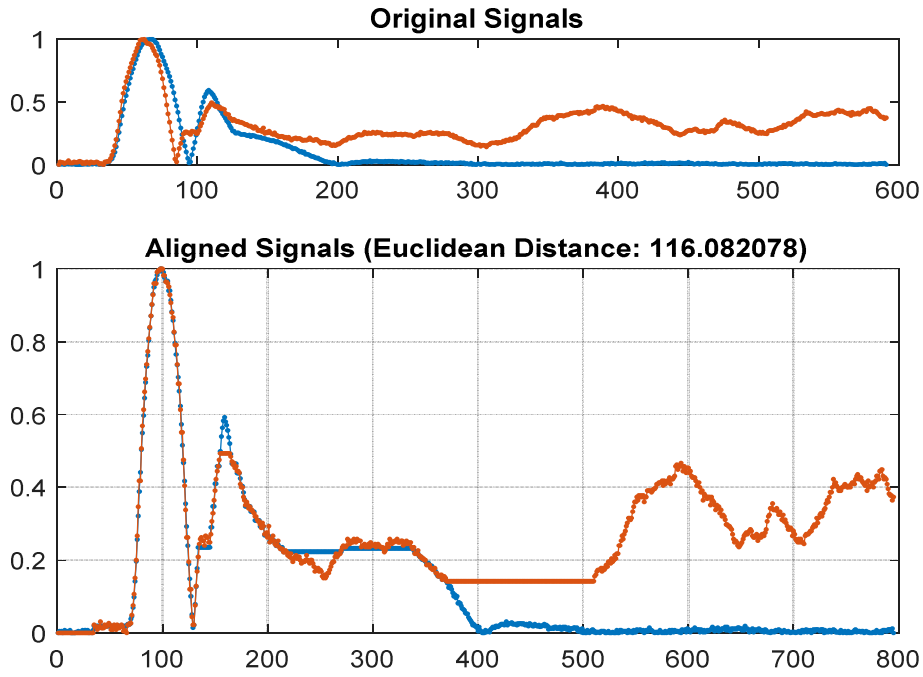


Figure 4.17 - Left Vastus Lateralis trend before (blue) and after (red) drug administration. It is clear the difference of morphology since a signal does not return to zero.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-218,62	2,08	20,14	5,71	1,09
2	Lt VM	130,97	1,61	143,60	9,51	-2,90
3	Rt VL	**10,07**	**25,92**	173,31	14,31	3,28
4	Lt VL	-164,56	68,03	**2172,06**	**58,43**	-4,08
5	Rt TA	41,24	-3,30	297,67	21,01	-2,14
6	Lt TA	45,01	-0,25	854,71	31,84	7,08
7	Rt PL	101,67	12,07	1127,09	30,38	-17,43
8	Lt PL	2,42	103,92	**5882,46**	**72,47**	-22,39
9	Rt FHB	-41,21	1,29	109,78	11,20	-18,82
10	Lt FHB	-10,85	11,31	224,49	12,31	-5,68

Table 50 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	9638,91	4351,65	3417,29	4935,55	4467,77	1733,68	713,85	1732,67	1730,79	1345,86
post	6717,80	6291,96	2958,48	1435,52	5144,37	2151,64	1335,71	693,42	1316,41	1157,42

Table 51 - Peak values of each central signal

Mean DTW is 26.72, whereas mean energy of error signal is 1100.53.

The highest values of DTW distance have been obtained for left Vastus Lateralis and left Peroneus Longus; for these two muscles, a large difference between peak signal values has been measured too.

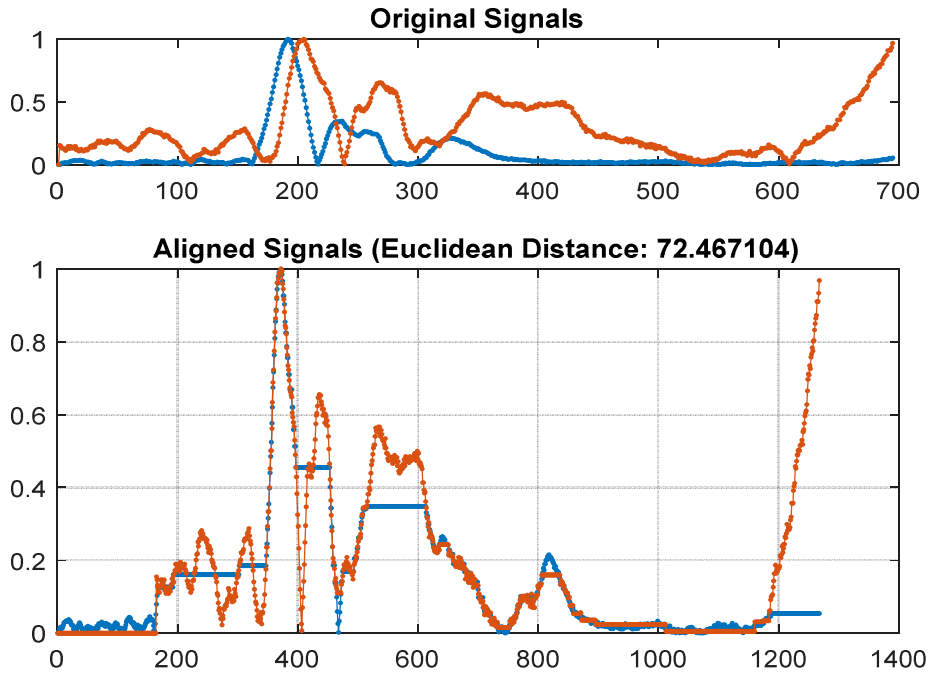


Figure 4.18 - Left Peroneus Longus trend before (blue) and after (red) drug administration

These differences also affect normITV values; in fact, in both signals notmITV measured on non-normalized data heavily differs from those measured on normalized signals.

For this patient, only in the case of right Vastus Lateralis two measured normITV are similar; this may mean such signals have a similar morphology (Fig. 4.19).

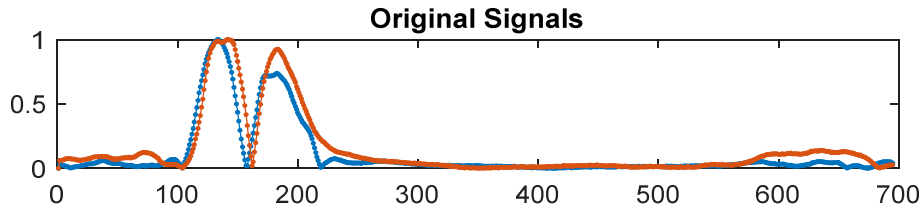


Figure 4.19 - Right Vastus Lateralis trend before (blue) and after (red) drug administration

The following table summarized final scores:

	LATENCIES	AREAS	Norm AREAS	NC
RAD	1	3	0	0
MEP	3	-3	1	0
CMCT	0			0

Table 52 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
1	NaN	NaN	0	5,5

Table 53 - Fatigue scales scores

Scores obtained working on normalized data are consistent with those derived from latencies, and they suggest that this patient conditions are not significantly changed.

4.1.10. Patient #10

- **Peripheral stimulation signals**

		normITV	normITVNorm	nergyES	DTW_dist	ITVlat
1	Rt VM	-52,30	10,32	80,53	10,91	-3,08
2	Lt VM	-29,36	4,80	34,42	4,42	2,90
3	Rt VL	-31,23	8,83	21,16	4,58	3,39
4	Lt VL	-61,88	-17,44	282,92	20,00	0
5	Rt TA	-13,04	-6,67	74,64	4,07	10,78
6	Lt TA	**29,94**	**25,77**	**991,07**	**26,88**	-6,13
7	Rt PL	-43,83	24,07	133,26	6,36	1,65
8	Lt PL	-34,05	-8,13	73,36	7,47	1,50
9	Rt FHB	-36,43	-5,37	138,86	10,67	-144,83
10	Lt FHB	-36,13	30,66	188,83	12,40	-3,37

Table 54 - Table summarizes principal indicators measured on peripheral stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	5900	6050	5950	3610	3200	1380	2830	3710	910	2690
post	3320	4500	4160	2300	3050	1430	1460	2920	670	1410

Table 55 - Table shows maximum values of each peripheral signal

Mean DTW distance and mean error of energy signal are respectively 10.78 and 201.90. Left Tibialis Anterior presents consistent normITV values, but high DTW distance and energy of error signal; this denotes an unreliable assessment caused by different morphology. This phenomenon can be observed also in Fig. 4.20.

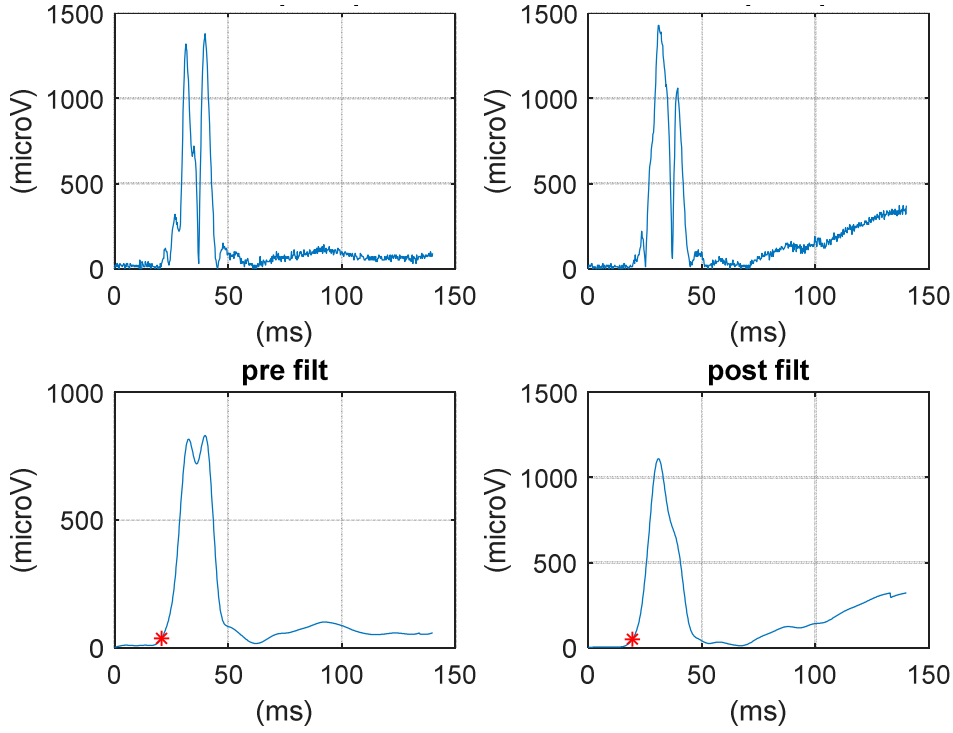


Figure 4.20 - Left Tibialis Anterior trend before (left) and after (right) drug administration. Red star represents latency.

Difference in signals morphology is present also in the post-drug administration signal, that after returning to 0 increases again. This is obviously an artifact, but its causes are not clear also for doctors.

For all other signals, normITV measured on normalized areas and normITV derived from non-normalized data are different, usually also in sign. This underlines the instability that affects non- normalized data.

- **Central stimulation signals**

		normITV	normITVNorm	energyES	DTW_dist	ITVlat
1	Rt VM	-14,02	-23,33	182,45	9,91	-22,70
2	Lt VM	-53,13	0,07	486,44	17,59	-33,90
3	Rt VL	1,14	44,08	315,99	12,21	-35,62
4	Lt VL	2,44	21,44	609,40	22,04	-4,11
5	Rt TA	28,43	44,21	982,72	18,65	6,71
6	Lt TA	7,75	-46,17	1087,53	35,84	-47,69
7	Rt PL	82,45	-6,79	152,02	7,40	1,61
8	Lt PL	34,22	-14,48	621,64	23,19	-1,56
9	Rt FHB	49,01	19,72	923,26	22,88	-61,54
10	Lt FHB	-62,30	28,52	**855,83**	**22,27**	-68,75

Table 56 - Principal indicators measured on central stimulation signals

	Rt VM	Lt VM	Rt VL	Lt VL	Rt TA	Lt TA	Rt PL	Lt PL	Rt FHB	Lt FHB
pre	787,08	605,84	822,47	347,45	476,94	133,56	377,19	170,06	357,93	**435**
post	845,29	412,79	601,58	303,60	421,49	197,53	753,20	240,51	443,67	**222,66**

Table 57 - Peak values of each central signal

Mean DTW distance is 19.20, whereas mean error signal energy is 621.73.

A huge difference in signals peak value has been obtained for left Flexor of Hallucis Brevis; this difference is confirmed by the high DTW distance and energy of error signal. This situation is due to a very different signal morphology (Fig. 4.21).

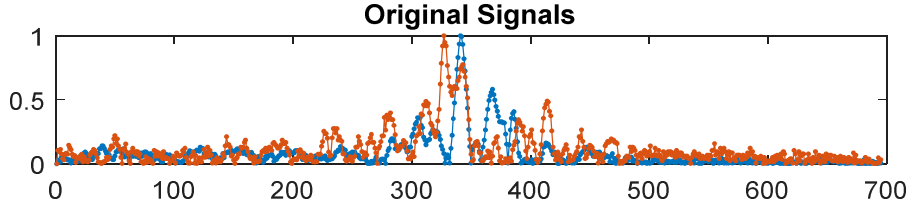


Figure 4.21 - Left Flexor of Hallucis Brevis trend before (blue) and after (red) drug administration

For both Flexors of Hallucis Brevis negative values of ITV latencies have been obtained too, but this is not due to improvement in patient's conditions, but instead to an initial artifact in signal before drug administration (Fig. 4.22). Causes of this phenomenon are unknow, probably this represents movement artifact.

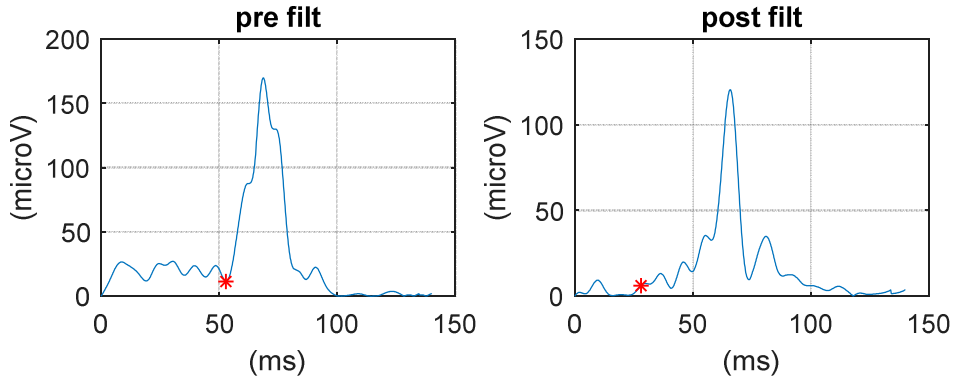


Figure 4.22 - Right Flexor of Hallucis Brevis trend before (left) and after (right) drug administration. Red star represents latency.

The same situation has been obtained also for left Tibialis Anterior; signals are very noisy and different of each other (Fig. 4.23), and this causes a wrong ITV latency measure.

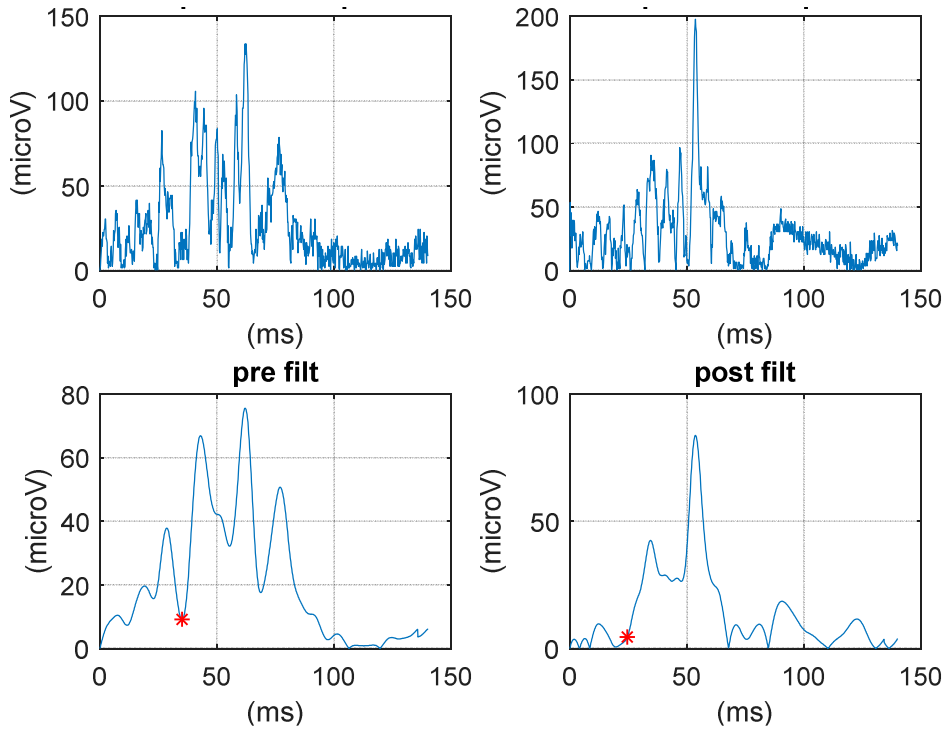


Figure 4.23 - Left Tibialis Anterior trend before (left) and after (right) drug administration. Red star represents latency.

Final scores about this patient have been summarized in following tables

	LATENCIES	AREAS	Norm AREAS	NC
RAD	0	-6	1	0
MEP	5	0	2	0
CMCT	-4			0

Table 58 - Scores derived from stimulation signals analysis

MFIS	FSS	VAFS	6MWT	EDSS
-18	-13	4	0	6,5

Table 59 - Fatigue scales scores

Looking at fatigue scales scores, this patient feels worsened. This assessment is confirmed by stimulation signals; in fact, considering central motor conduction times, scores suggest that most conduction muscle us worsened. On the contrary MEPs latencies and normITV denote a small improvement; this is probably due to the fact that the patient's feeling is not due to motor impairment.

Furthermore, problem about the artifact that affect central stimulation signals is probably due to the high EDSS score of the patient.

4.2. Normalized or not normalized signals

As discussed, results derived from non-normalized data differ from those obtained considering normalized signals.

The assessment obtained considering normalized signals is consistent with that defined by fatigue scales score; differently of the case in which non-normalized data are considered.

Every time that non-normalized normITV and normalized normITV are very different, a high DTW and energy of error signal values are obtained; this denotes an instability problem that affects results derived from non-normalized signals.

The described phenomenon is better explained by boxplots (Fig. 4.24 and 4.25) extracted using as population all muscles of all patients but keeping separated the central and peripheral stimulations signals.

Figures shows that normalizing the data decreases variability, almost keeping the mean value.

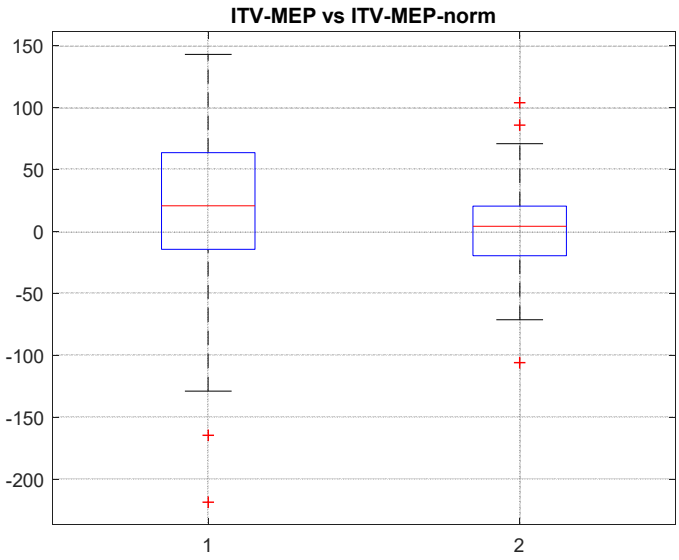


Figure 4.24 – Boxplots related to MEP signals considering non-normalized (left) and normalized (right) data.

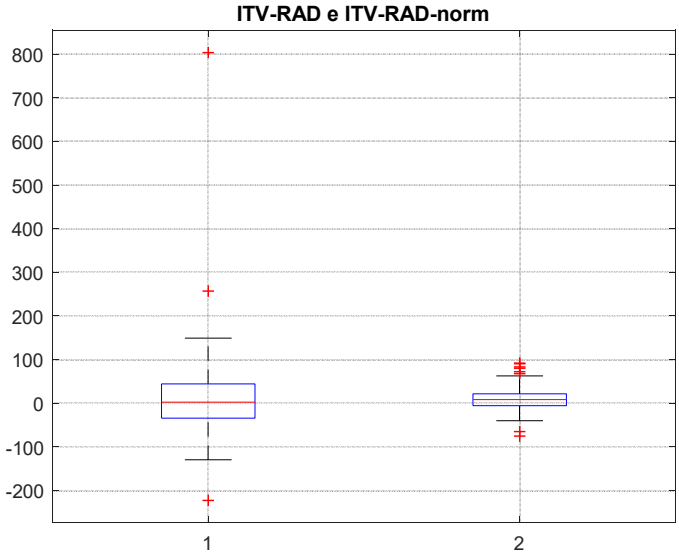


Figure 4.25 – Boxplots related to RAD signals considering non-normalized (left) and normalized (right) data.

Chapter 5

Conclusions

5.1. Comments and conclusions

The aim of this work was to study and analyse each MS patient for better understanding how data instability affects the results and consequently the final assessment.

The study has highlighted the importance of taking signal morphology into account; in fact, when signals before and after drug administration differ a lot, final results are unreliable and not consistent with those obtained from subjective fatigue scales and walking tests.

For example, when the signal to noise ratio is very low, due to a very impaired impulse transmission, any automatic algorithm is able to output a (latency or area) value anyway, but results are non-reliable.

This phenomenon happens especially when the patient under evaluation is characterized by a high EDSS score (typically more than 5). On the other hand, in patients with a very low EDSS score (1 or 2) no changes can be appreciated because such patients are hardly affected by motor impairments.

The absence of changes in patient conditions means the in these case, their possible perception of fatigue is not due to motor causes.

Another issue that affects data is represented by artifacts: in these cases, the algorithm returns unreliable results, but looking at graphs, such artifacts can be noted.

Final results, when artifacts occur, are not reliable and they must be interpreted considering what happens during signals recording. Causes of such phenomenon are not clear as it is not possible to perfectly control all possible confounding factors (e.g. temperature or recording site position).

This phenomenon should be more deeply studied, and some corrections should be found to allow measure to be more realistic.

Latency have been proven to be more reliable than area measures, and easier to determine, but this is possible only if patients EDSS is intermediate. In fact, for patients with high EDSS, also latency measures are unreliable due to very low SNR.

Due to this fact, when an ITV value is unusually high or low, all other features must be considered carefully.

A good result obtained during this work is the correlation between scores derived from fatigue scales and walking tests and those derived from normalized signals.

An exception occurs when patient has a very low EDSS, as it can be seen for patient 6; in these cases, fatigue is not a motor fatigue but only psychological and cannot be evaluated using muscle stimulation.

On the other hand, for cases in which patients shows a EDSS more than 5, the only reliable measure is that obtained by 6 Minutes Walking Test, because the threshold for considering a patient improved or worsened is very selective, whereas results determined with muscles stimulation are affected by instability.

The selectivity of the walking test threshold is the reason why in most cases, the score related to this test is 0, but when this does not happen, the result is very reliable.

5.2. Future developments

During this study it has been chosen to normalize signals to avoid instability, but at the same time, thanks to DTW and energy of error signal, the importance of signals morphology has been highlighted.

Future development could address methods for better taking into account signals morphology to allow a more reliable assessment of patient's conditions and to better help neurologists in this evaluation.

Another improvement could address the latency determination: in this study, the chosen method was to put a threshold as a percentage of the peak value of the signal. This method is affected, although in a limited way, by the discussed instability problem, so it could be refined to obtain a more precise and stable measure.

Looking at the obtained results, also the threshold to decide whether to consider a signal meaningful or not could be reconsidered, because it has been noted that a signal with an amplitude of about 100 μV contains very little useful information.

For what concerns the 6 Minute Walking Test, during this work only the distance walked has been considered. In future, developments other significant features could be extracted from walking paths and correlated with stimulations data and fatigue scale outputs.

The more parameters are considered, the more reliable and complete the final assessment can be. For this reason, a team is still working on walking tests, to allow doctors to better evaluate patient conditions using more significant features.

As for the technique employed in this work, it must be said that also neurologists have appreciated the discussed issues and are discussing about better protocols and/or metrics to refine their evaluations.

In summary the main results achieved are:

- To point out the sensitivity in selecting the stimulation site;
- To discriminate the reliability of scores depending on EDSS class;

- To reveal measure error of unknown origin and to give an indication of reliability of the achieved scores.

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