Contents

Introduction

This thesis project is part of a partnership between the Department of Information Engineering in Padova (Padua) and the S. Camillo Hospital at Lido of Venice started in september 2009.

A group of patients of the hospital underwent a battery of psychological tests[1] aiming to define their neuro-psychical capabilities. At the same time, a neuro-physiological test consisting in stimulating the subjects by means of an auditory paradigm was administered to them. The response to the stimuli was extrapolated through an on-line acquisition system that acquired, pre-processed and stored the electroencephalogram (EEG)[2] traces of the subjects performing the test. In the literature, the EEG signals produced with such a methodology are called *evoked potentials*, as it will be deeper explained in chap. 1.

It could be said that both the tests aimed to distinguish classes among all the subjects: In this case, the classes were only two, one for the healthy and the other for the ALS-affected people. Following the above terminology, the expectation was to find the same subjects into the two classes, that is a confirmation from the neuro-physiological test to the former one. On the contrary, the outcomes from them did not completely agree. Probably, it was due to a knowledge lack in the meaning of this kind of EEG traces.

Indeed, the scope of the thesis work has consisted in extrapolating new parameters to substitute or be associated to those already used to discriminate a disease state from an healthy one.

A number of techniques were implemented to reach the initial goal: Firstly, the traditional time-domain analysis based on the average of several EEG single traces was exploited, as the literature almost always proposes, without finding any satisfactory outcomes. Then, the traces were filtered in the frequency-domain thanks to a frequency filter realized to remove oscillations that seemed not to have physiological meaning.

Finally, two new methods of EEG single sweeps processing were proposed: They were called *time warping* and *translation* and the basic common idea was to compensate every single trace for phase displacements and distortions, probably caused either by a tiredness or an inattention condition of the subjects carrying out the task.

In the next, a general overview about the evoked potentials will explain the background needed to understand the following chapters. Then, detailed information will be provided regarding the kind of subjects undergoing the tests, the type of paradigm employed and the system for the traces acquisition. But, the point of the thesis is made of chapters 3, 4, 5, and 6, where an explanation of the implemented methods of EEG signals processing will be presented. A chapter about the comparison ampng the different techniques, mentioned in the previous chapters, will conclude the document, although it will not end the research, as it will become clear in the following.

Chapter 1

Background: ERP_S and Generality about P300

Evoked Potentials can be described as electrical modifications that occur in the central nervous system (CNS) after the application of an external stimulus: They are measurable from the scalp and their fundamental characteristics (*latency* and *amplitude*) depend on the kind of stimulus[3].

There exist two types of evoked potentials: the *stimulus-related* or *exogenous* and the *event-related* (ERP_s) or *endogenous* or *cognitive* ones.

The first can be considered independent on the attention and vigilance status of the subject and they are registered during the sleep in a simpler way.

The cognitive potentials, instead, can be obtained only when the subjects focus their attention on a semantically relevant stimulus or different among the others: The ERP_s seem, thus, to be related to the steps of the identification that a person has to accomplish in response to the stimulus and to reach the required task. For their relationship with these cognitive processes, the ERP_s became one of the most investigated topics and was recognized as an interesting matter for the study of the superior cerebral functions diseases.

As their origin as concerned, they can be elicited by every kind of sensorial stimulus: although visual, somatosensorial and even olfactory stimuli are employed, the most common used is the auditory one.

The EEG traces registered in correspondence to auditory stimuli are made by several event-related components with short, medium and long latency, respectively. In fig. 1.1 is presented the typical response to a semantically relevant stimulus (usually called *target* stimulus).

In particular, five relevant deflections can be noted: three of them are positive and named with a P; the others are negative and named with an N. They occur in the range between 50 and 250 ms: Their expected occurrence is reminded by a number accompanying the previous letters. Thus, the *P1*

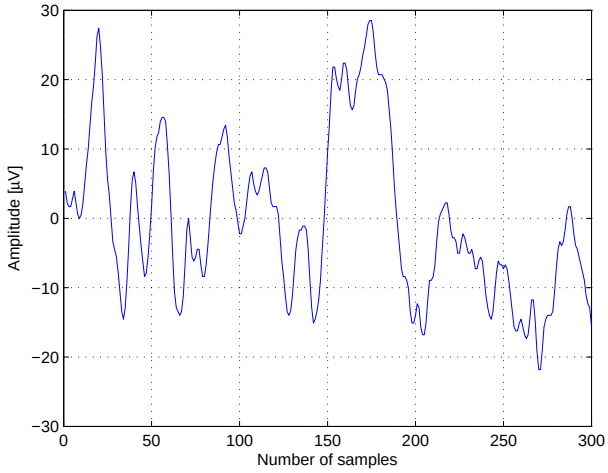

Figure 1.1: Typical EEG response to a TG stimulus.

deflection, for example, is the positive peak near 100 ms.

The above mentioned peaks are characterized by a larger amplitude and a larger latency in respect to the earlier stimulus-related components of the traces. For this reason, a longer time between two stimuli, the so-called *inter-stimuli interval*, has to be waited to make the generators of these long-term components able to show their response to the stimulus. A special role is played by the *N2* peak (probably resulted from the combination of more than one only component); analogously to the other peaks, its latency seems to be related to the reaction time and the attention status of the subject under test. Although these long-term components could have a connection with the cognitive processes performed by the subject under test, they are scarcely employed in the neurological field, maybe because of the uncertainty of their generators.

The most studied long-term component, instead, is the *P3* or *P300*, a large positive symmetrical deflection, mainly represented in the regions of the centre and the parietal lobe of the scalp. Similarly to the others, it could be registered only when the subject identifies a significant or different stimulus.

The simplest way to elicit it is the *classical active Odd-Ball 2 sounds*, exploited in this research work and discussed more deeply in § 2.2. Generally, with this kind of paradigm, the amplitude of the P3 takes values from 5 to 15 μV, while its latency is around 300 ms, even if it can vary in the whole range of [250, 500]ms. It has surely be known[3] that both the latency and the amplitude are strongly influenced by the occurrence of the stimulus, the *global sequence probability* (that is the number of target stimuli in the

whole sequence), the *local sequence probability* (that is the kind of stimulus preceding the target one), the semantical meaning of the stimulus and the type of the required task. The subject can be, indeed, informed or not to pay attention to a stimulus of semantical importance. In both the cases a P300 is elicited, but in a slightly different way[4]: in the absence of a pre-warning, a component known as *P3a* occurs with a shorter latency and localized in a more frontal region of the scalp. Otherwise, the *P3b* peak is found on the ERP and it appears mainly in the central and parietal lobes. Its meaning is not completely clear, but "it probably represents the voluntary end of a temporal period of processing of a stimulus by the associative cortical areas"[3]. Probably, the usually measured P3 is a sum of both the components, hardly distinguishable.

The P3 shape is not only influenced by the paradigm choice, but also by several physiological variables that determine the psycho-physical status of the subject. In particular, the P3 deeply depends on the conditions of vigilance, attention and precision in the task execution. Indeed, the decrease in one of the first two or the incorrect identification of the stimulus causes a decrease of the P3 amplitude, that could be so remarkable that can make the P3 peak unrecognizable. For this reason, it would be necessarily to administer the test in the morning for all the subjects, being sure that the subject has slept enough and checking the accuracy of the number of recognized target stimuli at the end of the test.

If the gender seems not to be an important factor, the age plays a fundamental role in modifying the P3 waveform. Indeed, since the amplitude is interpreted as an index for the set of resources of attention addressed to the fulfilment of the task and the latency as a measure of the velocity in classifying the stimulus, it can be understood that both a child and a elderly person produce a P3 with a longer latency and a lower amplitude than an adult. Therefore, it can be said that an ideal statistical approach would require a set of subjects uniformly distributed in every decade of age.

Actually, the thesis work included a typology of subjects distributed in a different way, as regard as both the controls, took as the reference group, and the patients. This could be explained because that was the available set of subjects at the S.Camillo Hospital and they are those that had previously undergone the battery of psychological tests whose results would be compared with the experimental ones from this work.

Chapter 2

Materials and Methods

2.1 Partecipants

The available set of subjects, as mentioned in the previous chapter, was made of seventeen healthy subjects and twenty-four patients of the S.Camillo Hospital with probable or define sporadic Amyotrophic Lateral Sclerosis (ALS)[1], a degenerative disease that damages the motor neural system.

Tab. 2.1 is filled in with the major characteristics of the two groups, chosen to match each other as mean age, education and other features as regards [5].

	CONTROLS	PATIENTS
number	17	24
age	56.88±15.92	55.13±13.74
range	23-81	29-73
gender(m/f)	12/5	19/5

Table 2.1: Subjects information.

They were firstly evaluated by different types of tests to define both their neuro-psychological status and physical disease. Indeed, as mentioned in the introduction, they firstly underwent a battery of psychometric tests that assess their *non verbal intelligence*, *attention*, *executive functions*, *memory* and *language* capabilities.

At the end, it was basically concluded that only cognitive difficulties in correctly reaching the goals of the tests characterize the patients' state, but the impossibility to success as the controls was discarded at all.

This result was not confirmed in a statistically significant way by the experimental measures from the electroencephalogram traces and that gave reason for the scope of this research work.

2.2 Paradigm

The subjects early described underwent a *classic Odd-Ball 2 sounds*, that fundamentally exploits the abilities of the subjects as the auditory discrimination and working memory as concerned.

Indeed, a sequence of alternated sharp(*beep*) and grave(*bop*) sounds were presented to the subject wearing stereophonic earphones. They follow each others pseudo-randomly: in fact, the beeps of frequency equal to 2kHz were presented as the *rare or target(TG)* stimuli twenty percent of the times, while the bops at 1kHz of frequency occurred eighty percent of the times and are called the *frequent or non target (NT)* stimuli.

The eliciting paradigm is *active* because the subject is educated to mentally count the number of TG stimuli occurring during each sequence of the test. In this way, the person waits for a semantically important stimulus and it is known that an increase in the P3 amplitude is thus achieved.

To make the recognizing process easier, an example of the sharp and of the grave sound is played at the beginning, before the actual test starts. Another shrewdness aiming to verify the attention and precision level of the subjects is to ask them about the number of TG stimuli heard during the test and check it with that showed by the electronic device that produces the stimuli. A too high discrepancy between the two values would be the index of a too low accuracy in the execution of the task and, in that case, the test should be repeated.

However, more than one sequence is administered to the subject in order to ensure the repeatability of the test. In each of them, about fifteen TG stimuli are presented, on average: Too much of them could bring the subject to develop an habit to the test, with subsequent decrease of the vigilance level, or increase their tiredness with probable decrease in the counting precision. On the other hand, the number of this kind of stimulus has to be large enough to ensure the statistical validity of the test.

Although the attention has been focused only on the TG stimuli properties till now, it is also important to know that after every NT event an EEG trace is produced and registered, as well. The two kinds of signals are both obtained with the same methodology and the same system for the *on-line* EEG acquisition. In the next chapter it is going to be explained the basic steps of the on-line processing, from the electrodes, used to catch the electrical modifications on the scalp surface, to the signals start point and object of the thesis work.

2.3 The On-Line Acquisition System

The on-line acquisition system is schematically represented by the blocks diagram of fig. 2.1(for details, read the manual user guide [6]).

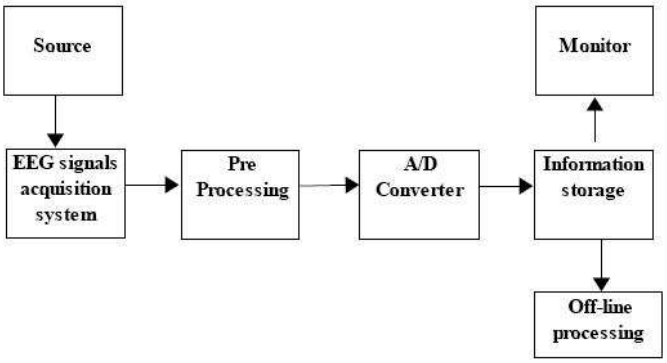

Figure 2.1: Blocks diagram of the on-line EEG acquisition system.

As mentioned above, a repeated auditory stimulation is administered to the subjects through stereophonic earphones: A sequence of 2 seconds-distanced 65 dB sounds, either sharp or grave, stimulate the coclear nerve of the subject undergoing the odd-ball test.

The auditory stimulation propagates along the neural system and allow the subject to identify whether the sound corresponds to a TG stimulus or not. In any case, the activation of neural cells (the *neurons*) produces electrical variations that could be registered outside the head: Precisely, a potential difference could be measured between each two points on the scalp surface.

The signal acquisition block operates this measurement by means of several electrodes. Each of them reveals the overall electrical activity of the area surrounding it, under the cerebral cortex. The spatial distribution of the P300 is such that is mainly evident in the central and parietal lobes: Therefore, three derivations, at least, have to be monitored in those regions (and only those channels were used in the following analysis). It was, thus, used a standardized system called *10-20* to localize the electrodes (see fig. 2.2). The three most important derivations were thus set as follows: one in the frontal lobe (FZ), the second in the central (CZ) and the last one in the parietal zone (PZ).

Actually, other derivations were used: In particular, the *electro-oculogram* (EOG) was registered in order to understand how strongly it had influenced every single trace. In fact, the blinking of the eyes and their movements can give rise to potentials much larger than the cognitive ones and they usually deeply distort the EEG traces on the other locations, especially the frontal one. For this reason, the traces for which the EOG is above a chosen threshold (e.g. 100μV) are discarded from the analysis. Other distortion factors are represented by the polarization of the electrodes and the scalp-electrode impedance. The shrewdness usually adopted to avoid these effects

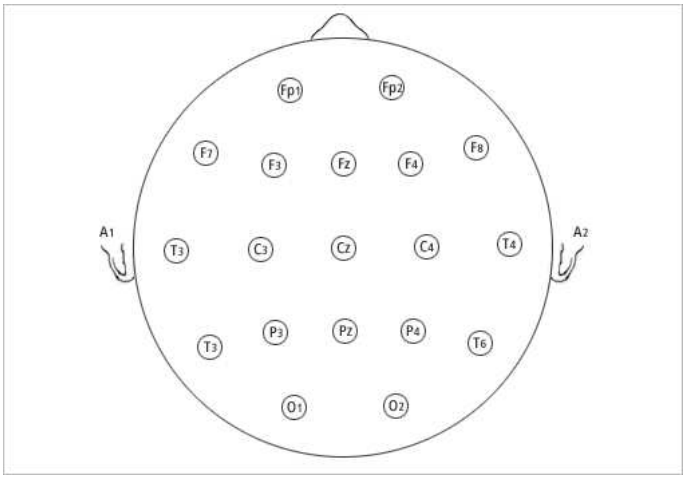

Figure 2.2: The standardized localization of the electrodes on the human scalp.

is to clean the scalp area in which the electrodes would be posted and to apply a gelatine on it, in order to limit the impedance inside the interval between 1 and 5 kΩ.

Further oscillations due not to cognitive processes can be found when an EEG trace is registered: a pre-processing step is, thus, needed. During this phase, an amplification action is employed to make the really low amplitudes of cerebral signals (in the order of tens of microvolts) more easily measurable. Moreover, a background noise affects the EEG acquisitions: It could be much larger than the cognitive potentials, so the signals are typically analogically pass-band filtered. The lower cut-off frequency is set to a value in the range of [0.1-0.3]Hz, while the higher is chosen between 30 and 100 Hz. Then, another filtering step could be included in the next *off-line* analysis: as explained in the following, a digital filter will be employed to remove other kinds of oscillations.

Moving forward in the blocks diagram, an analogical-to-digital (A/D) step is encountered: The current step is necessarily to make the following calculator able to recognize and store the acquired signals. Every analogical trace undergoes a double digitalization process: A quantization block is applied to the amplitudes scale, while a sampling with a sampling frequency (f_s) to the time dimension. The frequency f_s was chosen such that it respects the *Shannon's sampling theorem*[7]: As known, it states that to avoid *aliasing* phenomena, f_s has to be set to a value equal or greater than two times the maximum frequency component of the signal. Thus, for instance, an f_s equal to 200Hz or, alternatively, 1kHz was employed for the current analysis: Since the analog filter cuts all the frequencies above F_{co}=30Hz,

the Shannon's theorem ensures the absence of aliasing phenomena.

Therefore, using hose frequencies and knowing that each EEG acquisition takes 1.5 seconds, it can be noted that three hundreds or, alternatively, one thousand samples traces are obtained in output of the on-line system. It could be useful to notice that a *pre-stimulus* part of one hundred samples is always included in each trace. This piece of signal could be used as an indication of the baseline level of the brain activity of the subjects.

To conclude, the last block consists in the storage of the traces, with the help of a procedure that splits the acquire signals up into two different classes (TG and NT).

For the following developments of the work, it should be mentioned that each single sweep registered from one single channel (one electrode) after one stimulation will be called as *sweep* or *trace*, while the set of signals acquired after one stimulation in all the available channels will be named as *epoch*.

The set of signals from which this thesis has started were gathered for each subject (during the on-line acquisitions) into two matrices, one for the TG signals and the other for the NT ones. The latter represnt the start points for the *off-line* processing, that is precisely the topic of this thesis.

Thus, the next chapter will start to explain the first kind of analysis performed on those signals and developed in the time domain.

Chapter 3

Statistical Analysis In Time Domain

The overall aim of this research, that covered the thesis period and that will be probably carried on in the next future, is to find a characterization of the disease state of the ALS patients thanks to the comparison with the group of the controls. As said before, the thesis work started with a conventional study in the time domain: the EEG traces were, thus, simply processed by extrapolating few parameters that could identify the difference between the healthy subjects and the patients.

3.1 Grand Average

Tipically, the literature on ERPs, such as [5] and [8], focuses its attention on a time domain analysis of an average trace called *grand average*, that is obtained by summing (one channel per time) every single sweep considered good for the study registered on that channel all over the epochs and then dividing, sample by sample, the total by the number of traces taken into account.

In that, two different grand averages are computed: the first one is the average from the TG sweeps and the other is from the NT ones. Usually, these two mean traces are used for the analysis of ERPs: to be more specific, the clinical staff is used to evaluate the cognitive performance of a subject looking at the latency, defined as the interval of time from the beginning of the stimulus to the instant in which the peak appears, and the amplitude of the most neuro-physiologically meaningful peaks that appear in the TG grand average and already mentioned in cap.2. In particular, during the test sessions at the S.Camillo Hospital in which the subjects have undergone the classical ODD BALL 2 sounds test, a matrix *mrk* was computed being filled in with the latency and the amplitude of the *N1, P1, N2, P2, P3a* and *P3b* peaks.

The values in that matrix were computed thanks to a fuzzy algorithm [9] implemented by the research group of the Neuro-Physiology Laboratory of the hospital and, then, validated by the clinical staff. It works searching, in the grand average trace of each channel, for the peaks which are most likely one of the neuro-physiologically meaningful peaks of a typical P300 wave, by means of particular features that were previously computed. The reader can find all the details in [9], because the explanation of this algorithm is beyond the goal of this thesis. At the end, the fuzzy algorithm provides the values of those peaks as both the amplitude and the latency as concerned with a good approximation; but, then, the manual validation of a person of the clinical staff is however needed to confirm the automatic choice. Indeed, the variability of the subjects responses to each stimulus could be so high that it is often difficult to surely defined where the peaks are and even whether a trace refers to an healthy or to an ALS condition.

The aim of this first part of the analysis was to find, if possible, a statistically significant difference between the control group and the patients' one, processing data already acquired by the research group of the Laboratory. Thus, from the measured and validated values of the peaks, several plots and tables were produced for comparing the two groups of subjects, as regarding not only as the latency and the amplitude of the single peaks, but also the difference between each other.

First of all, two tables with the amplitude and latency mean values of the P3b peak, the most relevant peak among all were created from the TG grand average waveforms of controls and patients, respectively (see tabb. 3.1 and 3.2). Moreover, an important information, when working on a statistical set, is to know how significant the average is in respect to each instance of the population. To this scope, an information of standard deviation was included on the same tables in which the mean values were reported .

Controls	Amplitude [μV]	Latency [ms]
FZ	5.4190±5.1242	389.12±49.21
CZ	6.3817±7.0880	393.12±49.15
PZ	8.3482±7.0630	395.23±43.78

Table 3.1: P3b amplitude and latency for controls.

Patients	Amplitude [μV]	Latency [ms]
FZ	5.1540±4.9315	393.33±45.70
CZ	5.7585±4.7498	393.13±43.70
PZ	7.6604±4.2679	395.83±43.96

Table 3.2: P3b amplitude and latency for patients.

Although, globally, it can be seen from the previous tables that controls

show a larger mean amplitude and a shorter mean latency than patients (as expected), the values of the standard deviation are so high that it is really difficult to discriminate between an healthy subject and a ALS-affected one, when occurred individually.

Other methods were, then, employed with the aim of helping in distinguishing controls from patients, processing only their TG grand average curve: for example, the amplitude distance between different peaks was also computed. In particular, the difference between two components that characterize the ERP, the *P3b* and the *P3a* peaks mentioned in chap.2, was used to fill in tab. 3.3.

P3b-P3a Amplitude [μ**V**]	Controls	Patients
FZ	2.4789±2.2157	2.6702±2.3470
CZ	2.8422±3.3098	3.0915±2.6453
PZ	3.2453±2.9309	3.3513±2.1517

Table 3.3: Difference between P3a and P3b amplitude.

Analogously to the former table, it can be seen a general larger difference in the patients' case, but a really relevant standard deviation, as well, that does not allow to reach a satisfactory conclusion. A further attempt was to compare the amplitudes of the peaks all together. The figg. 3.1 and 3.2 can help in visualizing such a comparison: It shows, for each subfigure, the number of the subject on the rows and the name of the peak on the columns.

The idea behind this new representation was to have a look of the general distribution of the peaks in the amplitude scale: For each subject, the darker a box is, the larger the peak of the column is. This was the last attempt to find an evident difference between the healthy and the disease cases by means of the measured peaks only, and the visualization could help in that. On the contrary, this analysis did not lead to a relevant outcome, so it was decided to discard the use this kind of methodology.

The amplitude and the latency of the peaks are only partial information about the response of a subject to the stimuli. The complete knowledge is, instead, provided by the single traces registered during each stimulation. The literature, as said at the beginning of this section, is used to study the TG average curve resulting from the overall mean among all the TG epochs. Thus, in the following, it is going to be plotted the grand average of the three main channels (FZ, CZ and PZ) of one control and one patient[1],

[1] A choice had to be made, because of the large amount of figures otherwise available for each subjects: in this case, an example of healthy subject and one of the patients were chosen and all the three main channels will be presented. In the following chapters, instead, the attention will be pointed out more on the efficiency of some techniques of signal processing than on the difference between the channels, therefore one only channel will be taken into account. The most times it will be considered only CZ, because the P300 should be more evident on it.

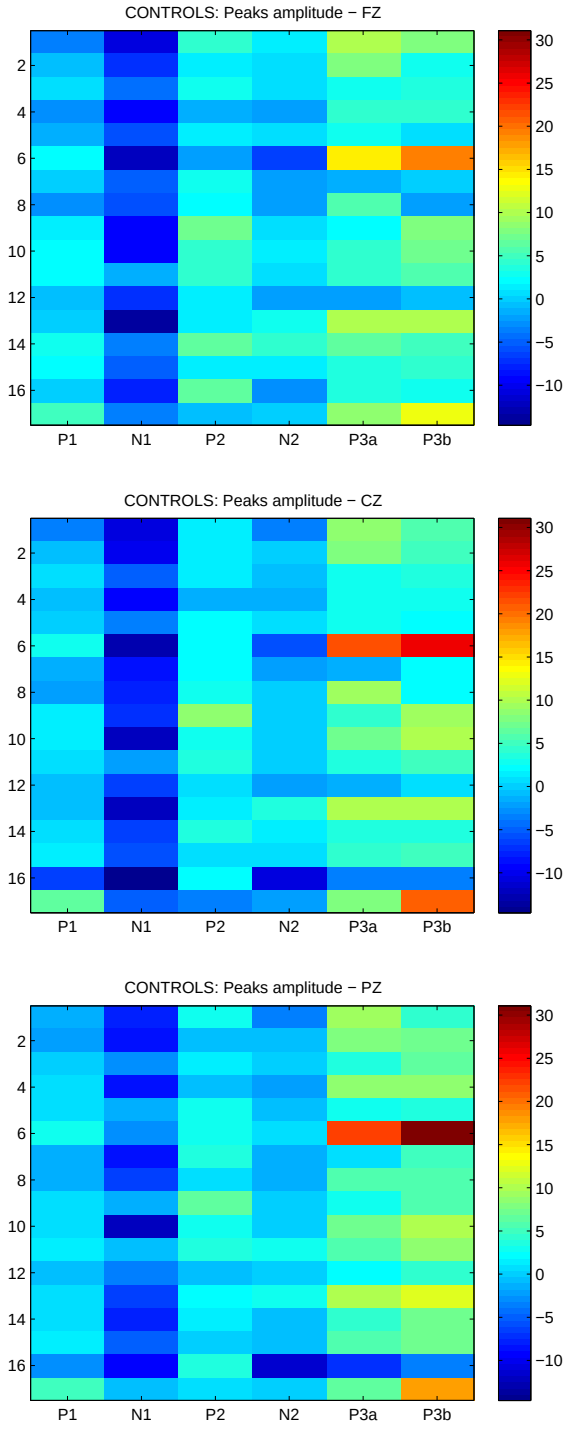

Figure 3.1: Peaks sequence amplitudes of control subjects.

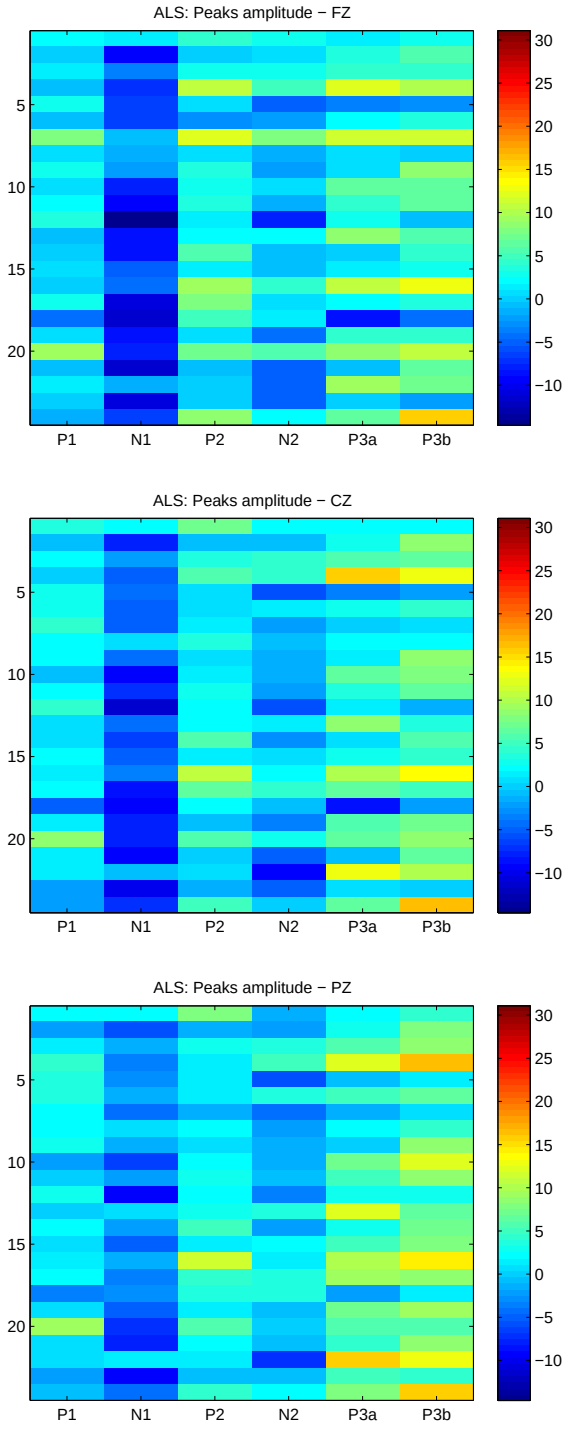

Figure 3.2: Peaks sequence amplitudes of the patients.

together with the standard deviation computed, sample by sample, as in the next formula:

$$SD(n) = \sqrt{\frac{\sum_{i=1}^{N} |x_i(n) - \overline{x}(n)|^2}{N-1}}$$

where N is the number of instances of the population, that is, the number of TG single sweeps used to compute the grand average, $x_i(n)$ is the n-th sample of the i-th single TG, $\overline{x}(n)$ is the correspondent sample on the average trace and, finally, n varies on the whole time interval of the EEG acquisition.

Actually, the three curves represented in figg. 3.3 and 3.4 are the TG grand averages (in the middle) and the point-to-point sum (or subtraction, the bottom one) of the grand average with standard deviation.

As it could be expected from the wide literature on the topic, the standard deviation assumes really high values and, plotting the so-called first difference (a kind of derivative) of each of the previous curves (see figg. 3.5 and 3.6), it can be seen that the standard deviation is not constant from time to time, but varies quite a lot during the EEG acquisition.

From a statistical point of view, a set of only such TG signals could be not characterized. Therefore, the next step was to try to take advantage from the knowledge of the NT traces, also: This is the idea behind the method showed in the next section.

3.2 Standardized Mean

The observation that no solution to the problem could be found only processing the TG curves and their peaks gave rise to the computation of a new statistical quantity that, somehow, considers both the TG and the NT characteristics of the ERPs. It was called *standardized mean* and was suggested by Hinterberger & al. in [10] on February 2005.

At the beginning of this thesis, an algorithm to compute the standardized mean was thus implemented using the article previously quoted as a starting point. Later, it was modified to fit the needs of the other members of the research team, the psychologists above all, that wanted to make statistical analysis over that new quantity.

The algorithm simply works as follows. Let us consider two stimulus conditions k, TG and NT (as in the example of fig. 3.7), and let us set k equal to 1 for the TG case and k equal to 2 for the NT. Moreover, let N_k be the number of trials in each conditions and $X^k_{t,n}$ the EEG amplitudes where t means the sample number at a time in the trial n.

In our study, a prestimulus period t_0 of one hundred samples is considered (see § 2.3) and its mean value is subtracted to the EEG signals in each trial, before starting the next analysis. Therefore, let $Y^k_{t,n}$ be the baseline corrected

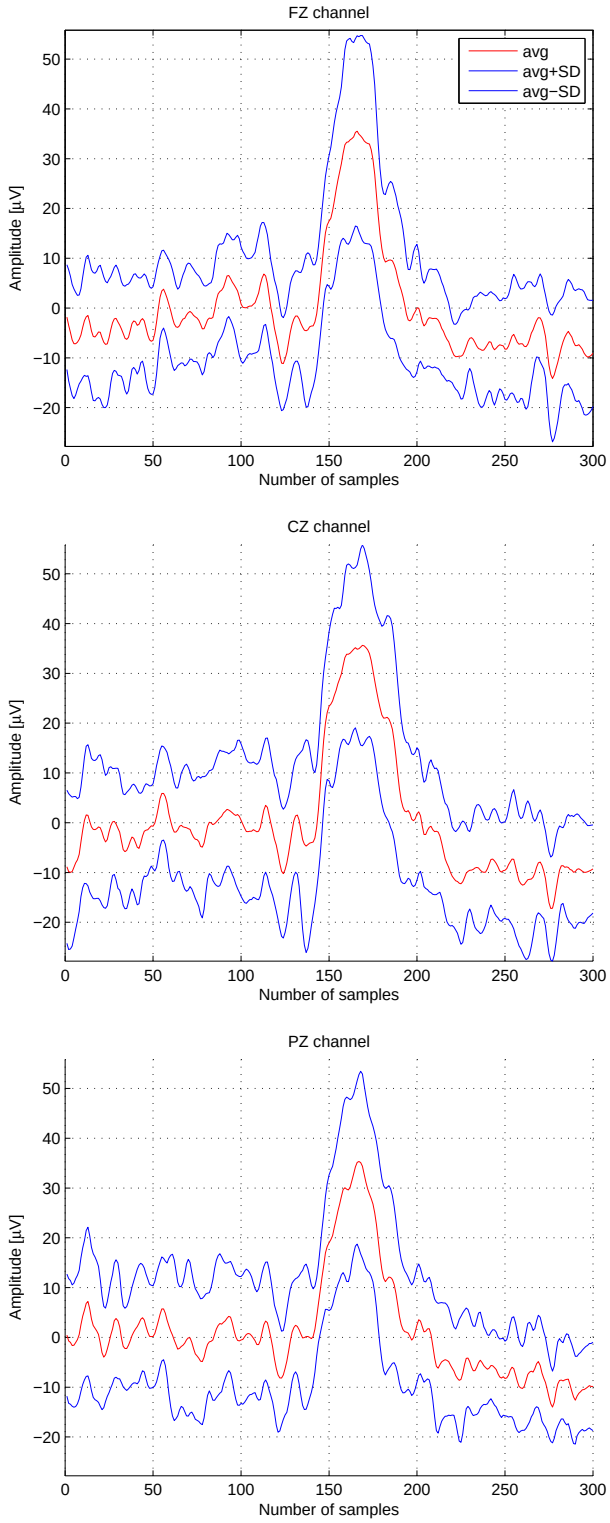

Figure 3.3: Example: Grand average and standard deviation traces for a control.

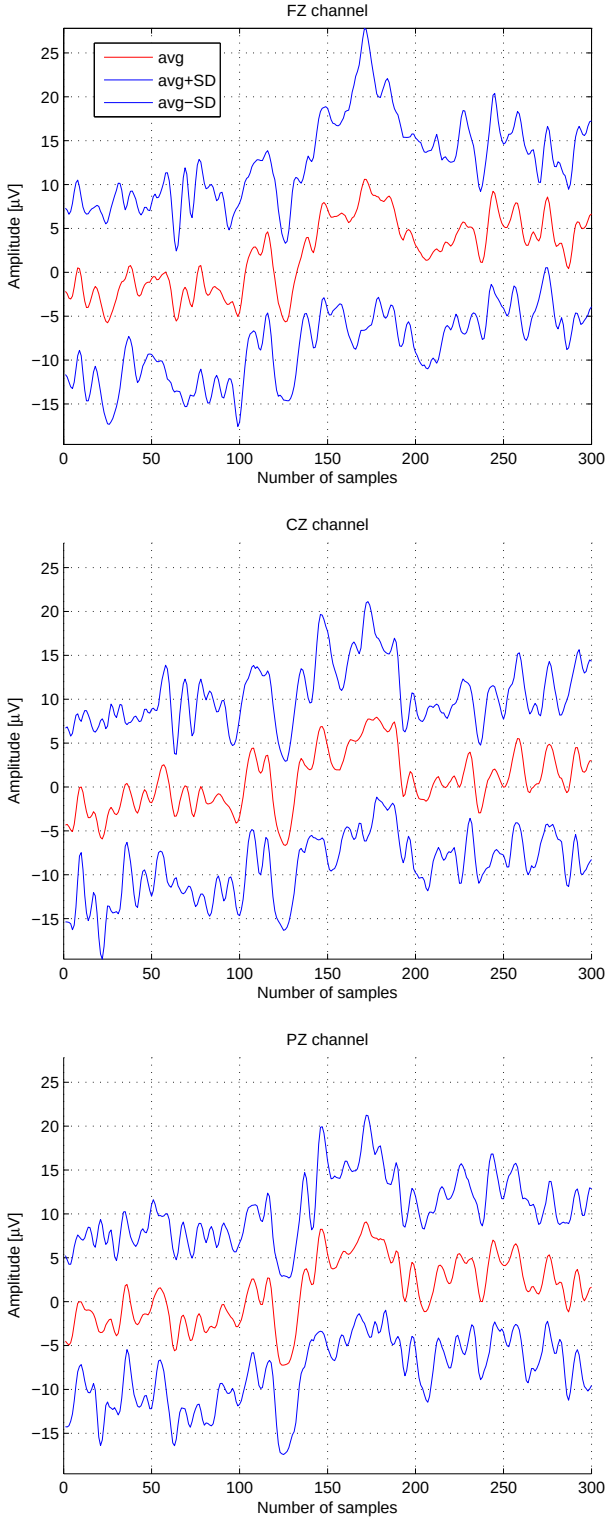

Figure 3.4: Example: Grand average and standard deviation traces for an ALS patient.

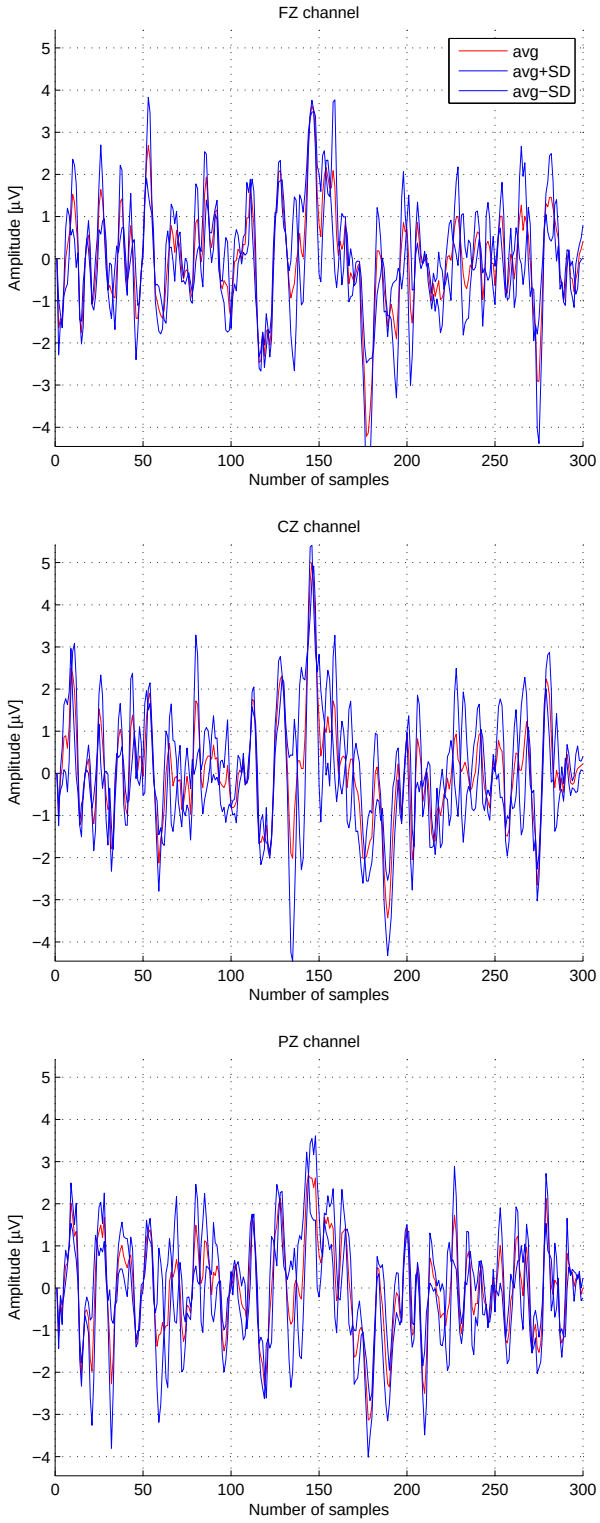

Figure 3.5: Example: Grand average and standard deviation derivative traces for a control.

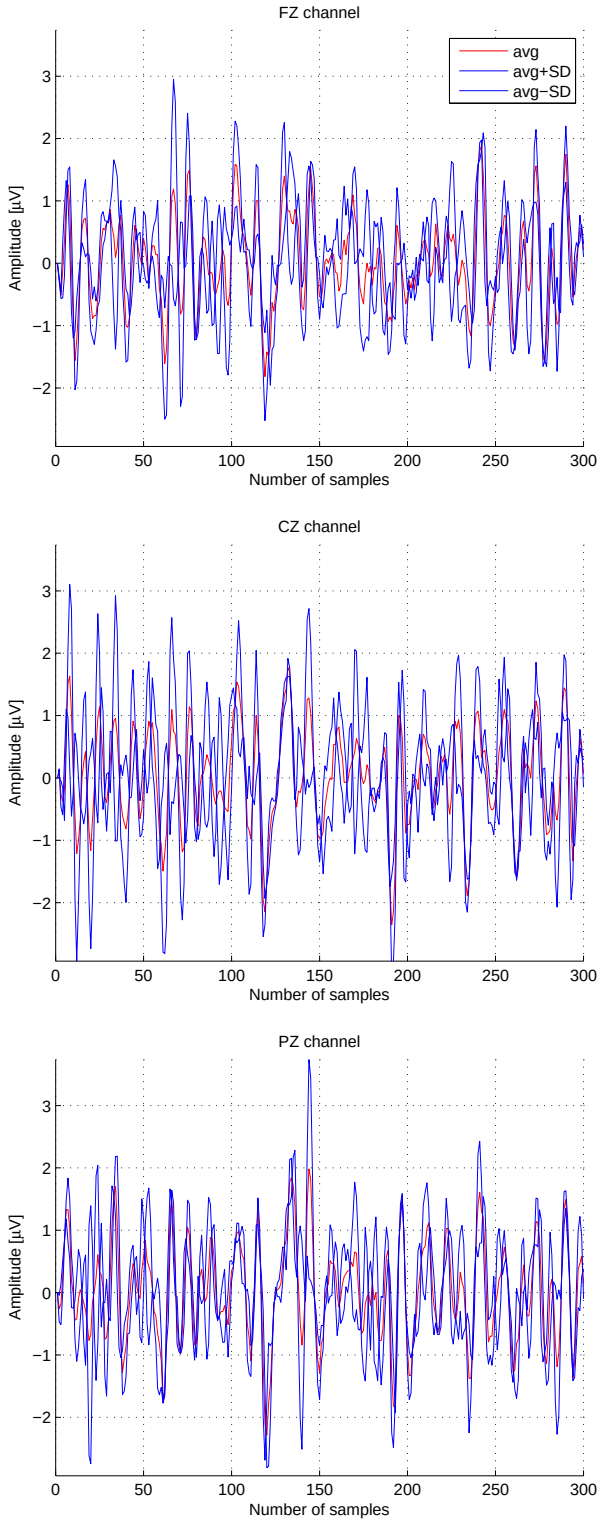

Figure 3.6: Example: Grand average and standard deviation derivative traces for an ALS patient.

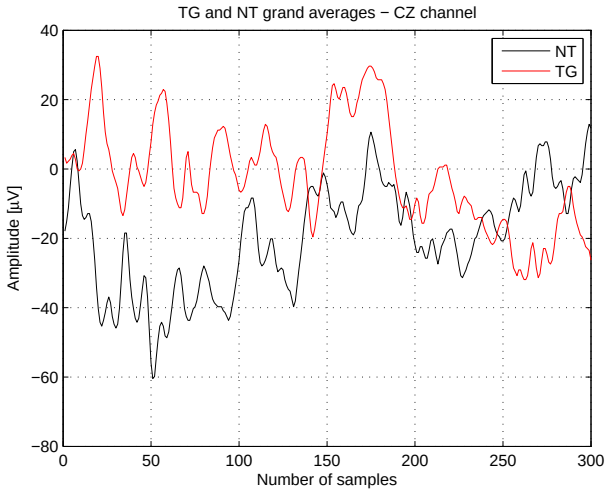

Figure 3.7: Example of TG and NT grand averages at the channel CZ for a control.

signal. It can be computed as:

$$Y^k_{t,n} = X^k_{t,n} - \frac{1}{L}\sum_{l=0}^{L-1} X^k_{t_0-l,n} \qquad k = 1, 2.$$

where the second term represents the mean baseline part.

All baseline corrected signals are then averaged for each condition as follows:

$$M^k_t = \frac{1}{N^k}\sum_{j=1}^{N^k} Y^k_{t,j} \qquad k = 1, 2.$$

The standard deviations S^k_t of each trial relative to the averaged signal are also calculated for the two conditions, separately, and serve as a data pool for the further steps of the algorithm.

Once the averaged and the standard deviation signals are estimated, the standardized mean can be evaluated on a proper interval of time. Thus, we obtain:

$$E^{(k_2-k_1)} = \frac{1}{N_p}\sum_{j=t_p-r}^{t_p+r} \frac{M^{k_2}_j - M^{k_1}_j}{\sqrt{{S^{(k_1)}_j}^2 + {S^{(k_2)}_j}^2}}$$

with $N_p = 2r + 1$, where t_p is the center of the chosen interval and r is its width.

Actually, in the implemented algorithm, the choice of r and t_p was initially fixed and, only after a later change, it was left to the user. Therefore,

in a first time, r was typically centered on 300 ms (the expected latency of the P300) and t_p was set to a value of 25 ms as Hinterberger & al. suggested in their article.

Afterwards, two simple functions were implemented in the more elaborated software used by the research group at the S.Camillo Hospital to process the ERPs. These two Matlab files make the users, that are mainly the psychologists of the research staff, able to carry on statistical analysis on the whole grand average traces, only by shifting the values of r and t_p given as input parameters of the two functions.

Finally, it has to be mentioned that a similar analysis was performed on each channel separately, paying attention that not always the same channels were acquired for all the subjects. Indeed, the first three channels, FZ, CZ and PZ were common for the whole set of subjects and only those were included in the remaining part of the study.

One more time a comparison between the values obtained for the controls and for the patients is presented thanks to a table: indeed, tab. 3.4 shows the mean value and the standard deviation of the *standardized mean* in each of the common channels for controls and ALS-affected subjects, respectively.

Standardized mean	Controls	Patients
FZ	0.2824±0.3017	0.2670±0.4181
CZ	0.3412±0.2592	0.3152±0.4210
PZ	0.4682±0.2868	0.4339±0.3776

Table 3.4: Standardized mean.

It could be expected that the values of the standardized mean were higher for the healthy subjects than for the patients, representing a kind of weighted difference between TG and NT waves. The reason for that is understandable thinking that controls should show better performance in distinguish a TG from a NT stimulus than a patient and, consequently, show a more evident distinction between the two kind of curves produced. Actually, it can be seen from tab. 3.4 that generally the above observation could be confirmed. But, one more time, the standard deviation is too high to lead to a valid conclusion, although it appears higher for patients than for controls as the literature had often stated.

3.3 Other Methods

Other methods using both TG and NT grand average traces are going to be outlined in the following, although without given any numerical results because of their poor performance. They hoped to find a new parameter that could characterize the set of the controls compared with that of the patients, exploited the diversity in the two conditions of stimulus.

Theoretically, as mentioned above, the difference between healthy and ALS-affected subjects should be evident in a better capability of the first ones to selectively focus their attention on the target stimulus and neglect the others as much as possible. To verify this, the area under the TG and the NT grand averages was thus computed for each subject and then a comparison between the target and the non target cases carried on, but without significant findings.

The full width at half maximum (FWHM) of the TG and the NT grand average traces was also calculated and the results for healthy people and ALS patients compared. But, the outcomes were not satisfactory, because of the large variability of the considered quantity within both the groups.

Finally, it can be observed that, in most cases, the P300 peak could be assimilated to a triangle; therefore, another possible method to characterize the required difference could be to compute its area in all the instances and to evaluate its variation from subject to subject. This computation was not actually performed during the thesis period, because it was already done by the research group at the S.Camillo Hospital with no relevant results.

Conclusion

As shown earlier, all these time domain and statistical analysis did not lead to find a significant differences between controls and patients, although a difference was found through psychological cognitive tests made by the psychologists of the research team at the I.R.C.C.S. S.Camillo Hospital. In particular, those tests demonstrated that patients can reach the right target of the experiment as the controls, but using more time and/or showing a *weaker* P300 response.

At this point of the research, it seemed to be useful to look for a method to elaborate each single sweep, in order to identify components due to cognitive processes and discard those due to some kind of artificial *noise*. With this goal, a spectral analysis was extensively employed. The following chapter will explain reasons, methodology and findings of that.

Chapter 4

Frequency Domain Analysis

Introduction

The poor performance of the statistical analysis in the time domain could be due to the limited number of subjects and of EEG traces available. But, since that set of subjects could be difficultly extended within a short period of time, like that of the thesis is, different methods of analysis were needed. The frequency domain analysis is, for example, one of the most important device through which every signal could be processed and deeply characterized: the idea that moved us in developing such an analysis was to exclude, first of all, the main noise component due to the neighbouring electrical devices and, then, remove (as much as possible) other artificial components that could distort the P300 pure nature.

4.1 Fourier Theory and Implementation

As mentioned before, the frequency domain analysis, also called *Fourier analysis*, was widely exploited to process the EEG traces (also in [11]). The theory behind this is based on the statement that every signal can be decomposed into its harmonic components, by means of a discrete or an integral sum, depending on whether its periodical nature or not.

Since the work was developed using a computer, a discrete version of this transformation should be exploited. A *Discrete Fourier Transform (DFT)*[12] was thus implemented, using the following formula:

$$X(k) = \sum_{n=1}^{N} x(n) exp(-j2\pi(k-1)(n-1)/N) \quad \text{with} 1 \leq k \leq N.$$

where X(k) with $1 \leq k \leq N$ is each of the N Fourier coefficients that characterize the signal x(n).

Its inverse is achieved by means of the next expression:

$$x(n) = \frac{1}{N}\sum_{k=1}^{N} X(k)exp(j2\pi(k-1)(n-1)/N) \quad \text{with} 1 \leq n \leq N.$$

where x(n) represents each of the N time domain samples that form the whole signal.

Computing the Fourier transform of a signal, an information about the amplitude, the frequency and the phase of each sinusoidal component constituting the overall trace becomes available.

The Fourier transform computation was accomplished in Matlab by the use of the *fft* command. This function implements the Fast Fourier Transform algorithm, that is an algorithm that efficiently computes the DFT. Then, since the transform is complex-valued, plotting its phase and magnitude with the help of *phase* and *abs* commands of Matlab, it is possible to extrapolate the information of magnitude, phase and frequency for each component, mentioned above.

An example of single sweep spectrum and grand average one for a control and a ALS-patient, respectively, are going to be plotted in the next (see figg. 4.2 and 4.1).

As it can be observed from the previously mentioned plots, the P300 is a slow wave. Thus, it is useful to limit all the spectra to an interval of frequencies in which they are significant, as shown in fig. 4.3.

Finally, it could be usefully observed that the frequency step in the spectra depends on the sample frequency f_s used to acquire the EEG signals: in the case in which f_s is 200 Hz, the frequency step is equal either to 0.39 Hz or 0.78 Hz (if the prestimulus is considered or not[1]), whether if f_s is 1 kHz, the step is 0.98 Hz.

Once the spectra had been computed, a kind a lowpass Fourier transform-based filter was applied on each single sweep.

4.2 Frequency Domain Filtering

Since the P300 is a slow wave, it is known from the literature ([13], [14] and [**?**]) that the peculiar frequencies that mainly characterize it are inside the range that covers all the frequencies till 3-5 Hz.

Actually, the filtering program asks the user for choosing a cut off frequency F_{co} above which discarding all the components: This process could be viewed as a kind of lowpass process with an adaptable cut off frequency. It is also clear that, if the users chose a cut off frequency lower than 5 Hz, they would distort the P300 nature.

[1] With f_s equal to 200Hz and a one hundred prestimulus samples a 512 samples-DFT is computed otherwise, without prestimulus, a 256-DFT is enough. In the case in which an f_s of 1kHz is employed, in any case a 1024-DFT is considered.

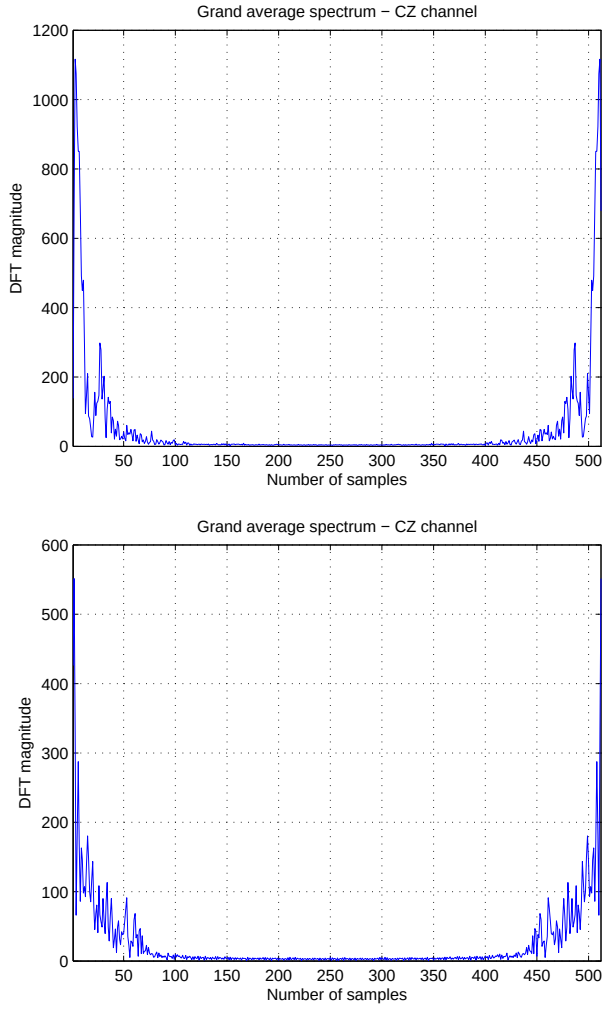

Figure 4.1: Grand average spectra of a control(left) and of a patient(right) at CZ channel.

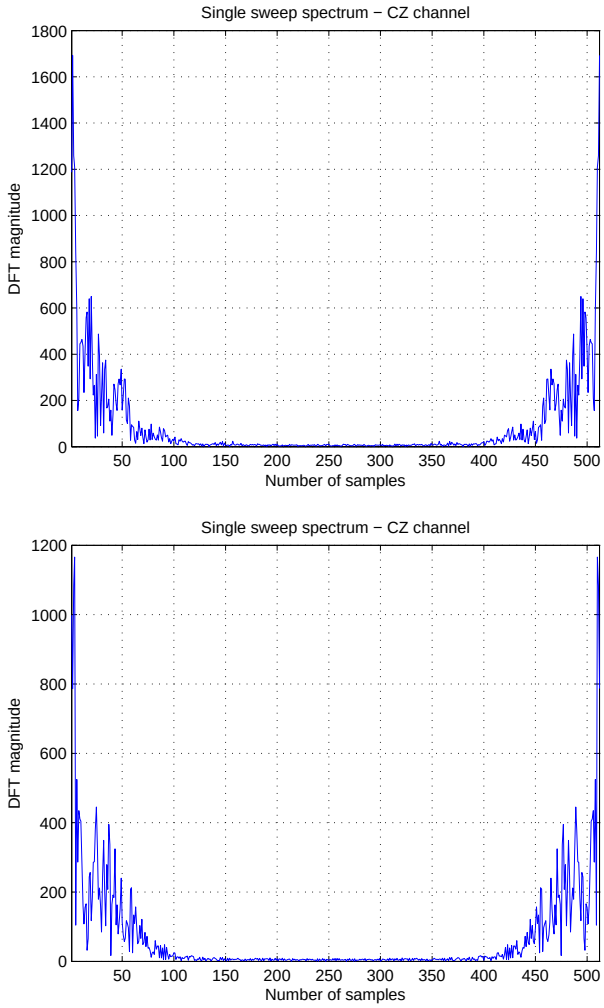

Figure 4.2: Example of one single sweep for a control(left) and of a patient(right) at CZ channel.

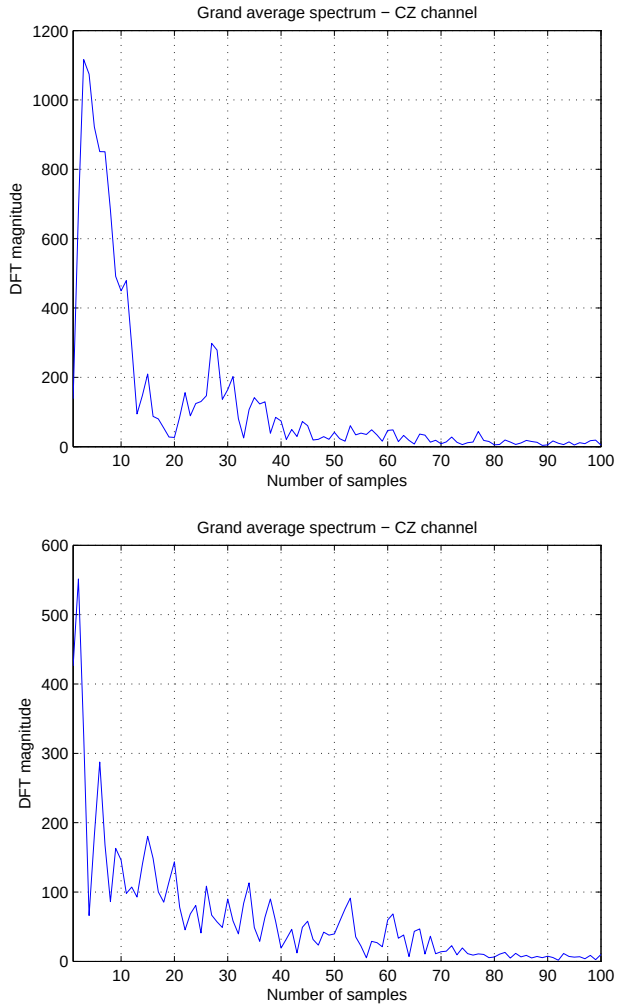

Figure 4.3: *Limited* grand average spectra of a control(left) and of a patient(right) at the CZ channel.

Moreover, another choice is left to the user: as mentioned earlier, in chap. 1, each signal has a prestimulus part made of one hundred samples. Therefore, the question is to choose whether considering or not the prestimulus portion of each trace. Soon, it will be clear that the outcomes strongly depend on this decision.

Let us, now, present the algorithm implemented to operate the filtering process on the single sweeps. It prepares the single traces to be filtered and operates the actual filtering action, passing through the following steps:

- The single sweeps are individually extrapolated from the appropriate matrix of the EEG data.
- If necessary, a zero-padding process is performed and each trace is extended by adding a number of zeros such that the resulting new trace has a number of samples equal to the closest higher power of two, in order to improve the efficiency of the DFT computation.
- The Fourier transform with the same number of samples of the corrispondent time domain trace is computed by using the fft algorithm.
- Then, the signal components with a frequency higher than the chosen F_{co} are excluded.
- The IDFT is applied by using the *ifft* command of Matlab that implements the inverse Fast Fourier Transform algorithm. Thus, the new filtered signal is obtained.

Then, all the filtered single sweeps were summed together to form the new grand average curve, one for each channel (FZ, CZ and PZ were only considered, as previously).

Thus, in figg. 4.4 and 4.5 the grand averages of two subjects, a control and a ALS patient, are shown with three possible levels of filtering, taking into account only the CZ channel.

As it can be seen, the most conservative result is achieved by setting F_{co} to 50 Hz, but in some cases a stronger action is needed to remove non physiological oscillations of the traces. In the next figg. 4.6 and 4.7, the same plots of above are presented, with the only difference that the prestimulus part of the signals was excluded.

Thus, it can be observed that the prestimulus part is strongly made by high frequency components: Indeed, filtering the highest frequencies in a signal with the prestimulus, a more relevant result is achieved than with the same level of filtering applied to the signal without it.

To confirm the above findings, it will be also plotted the output of the *complementary filter*, alternatively called *error filter*, that is the signal obtained considering only the frequencies excluded before (see figg. 4.8, 4.9, 4.10 and 4.11).

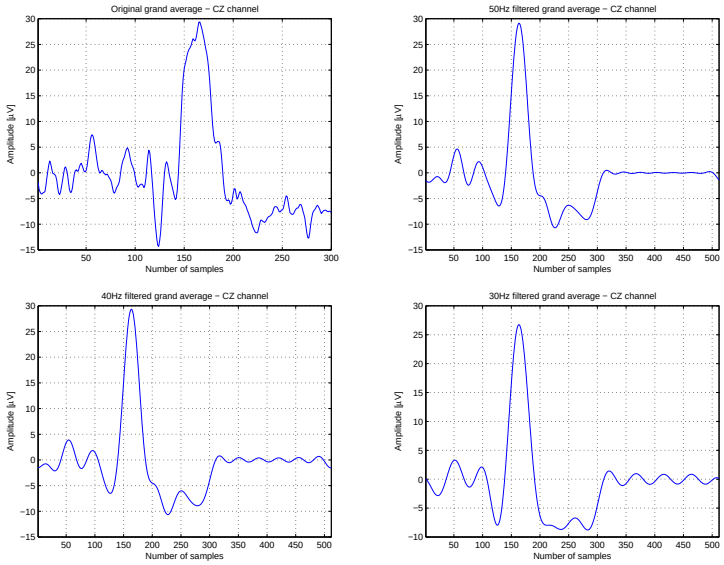

Figure 4.4: Original CZ channel grand average with prestimulus for a control and three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

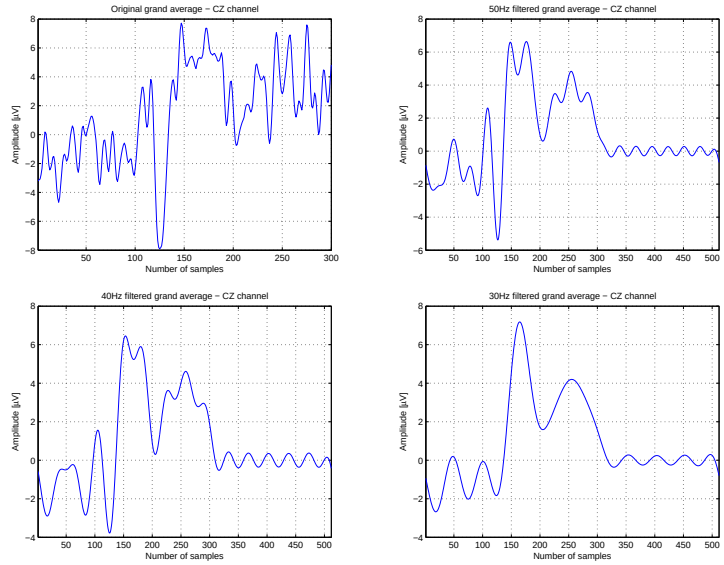

Figure 4.5: Original CZ channel grand average with prestimulus for a ALS-affected subject and three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

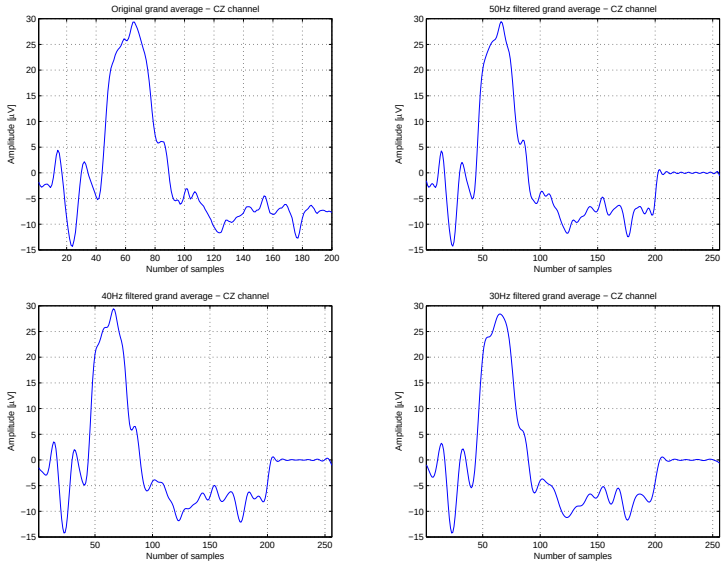

Figure 4.6: Original CZ channel grand average without prestimulus for a control and three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

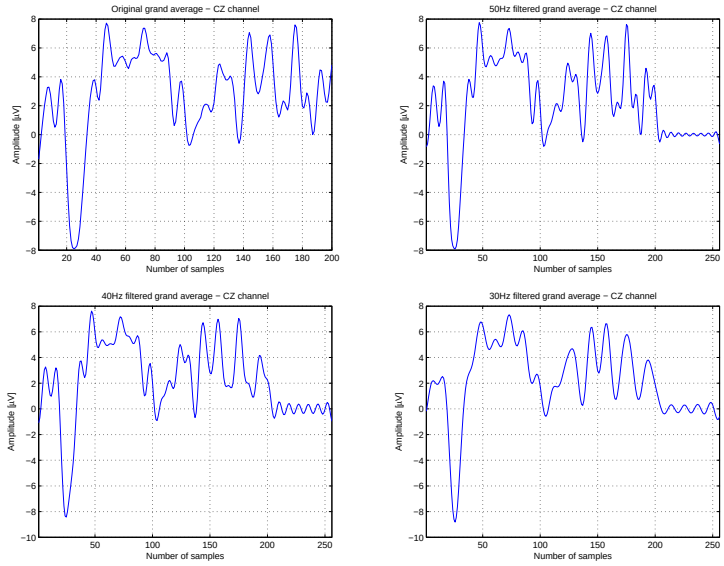

Figure 4.7: Original CZ channel grand average without prestimulus for a ALS-affected subject and three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

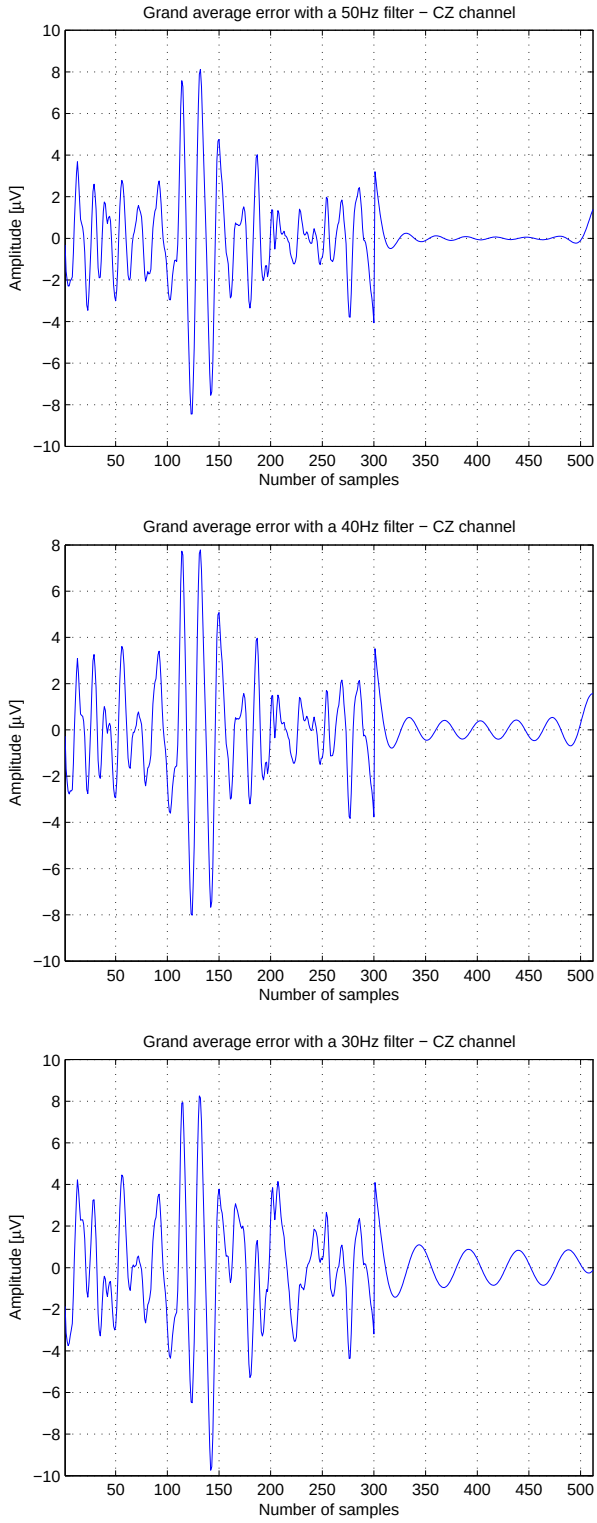

Figure 4.8: Error filter on the CZ channel for the grand average with prestimulus of a control and its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

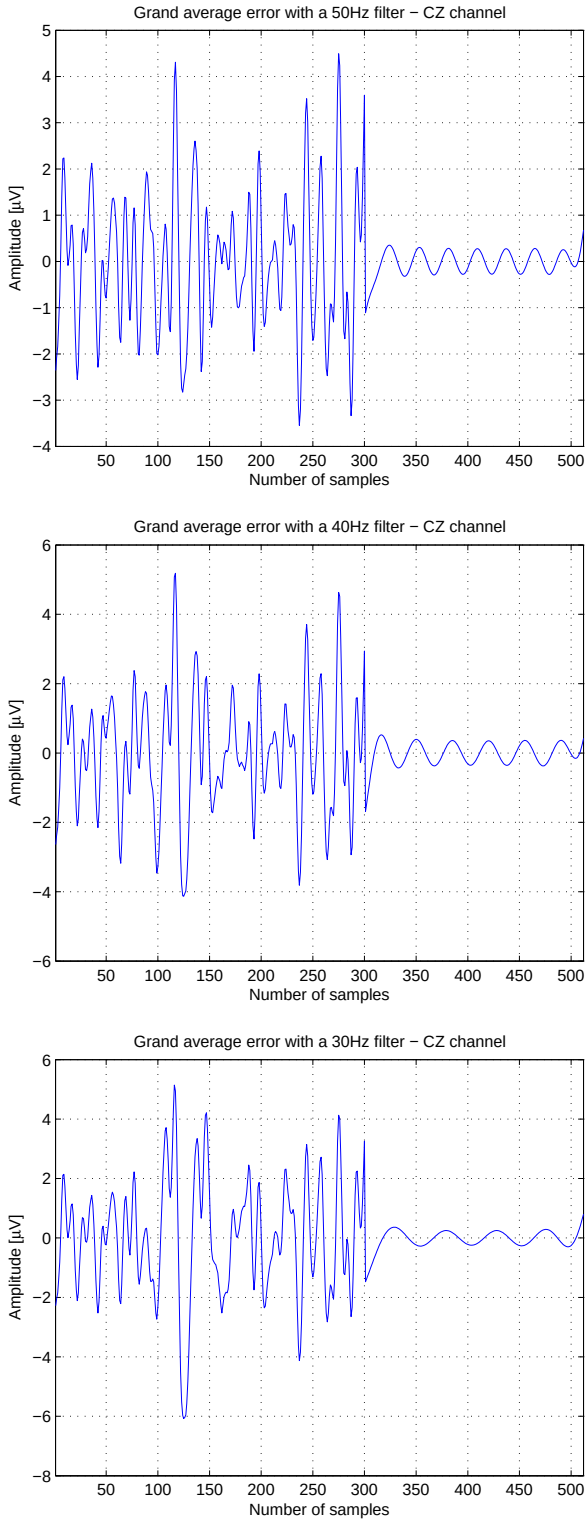

Figure 4.9: Error filter on the CZ channel for the grand average with prestimulus of a ALS patient and its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

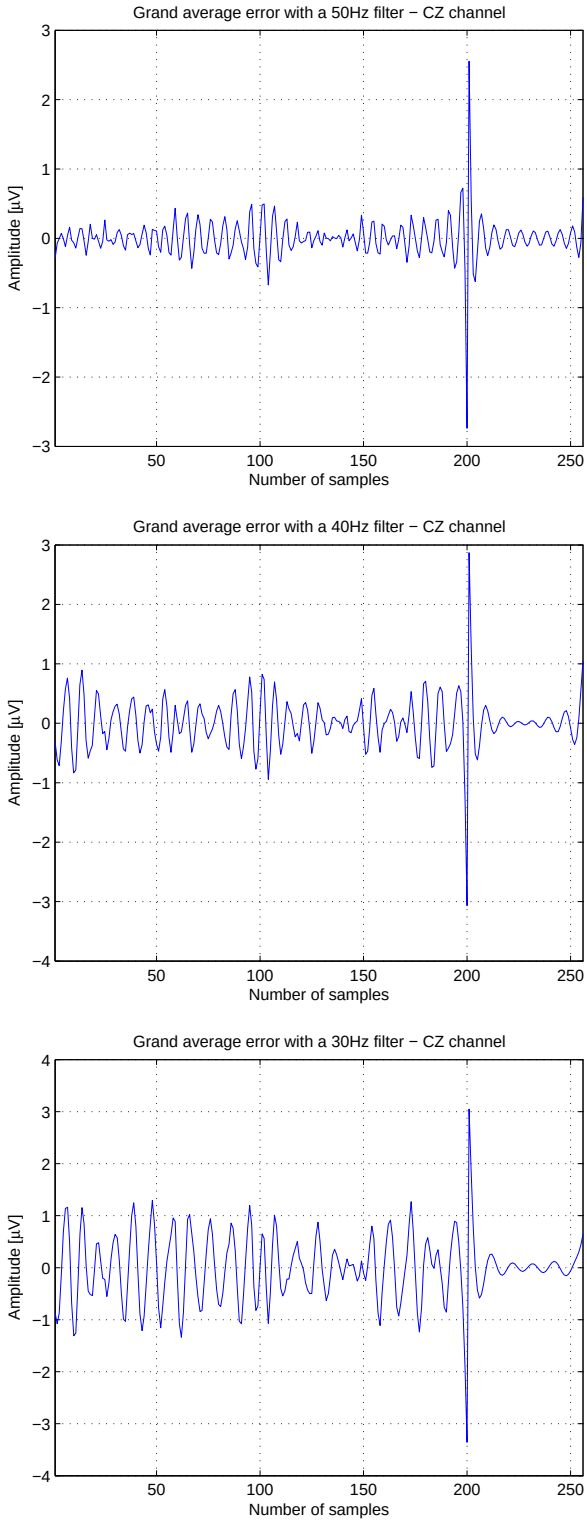

Figure 4.10: Error filter on the CZ channel for the grand average without prestimulus of a control and its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

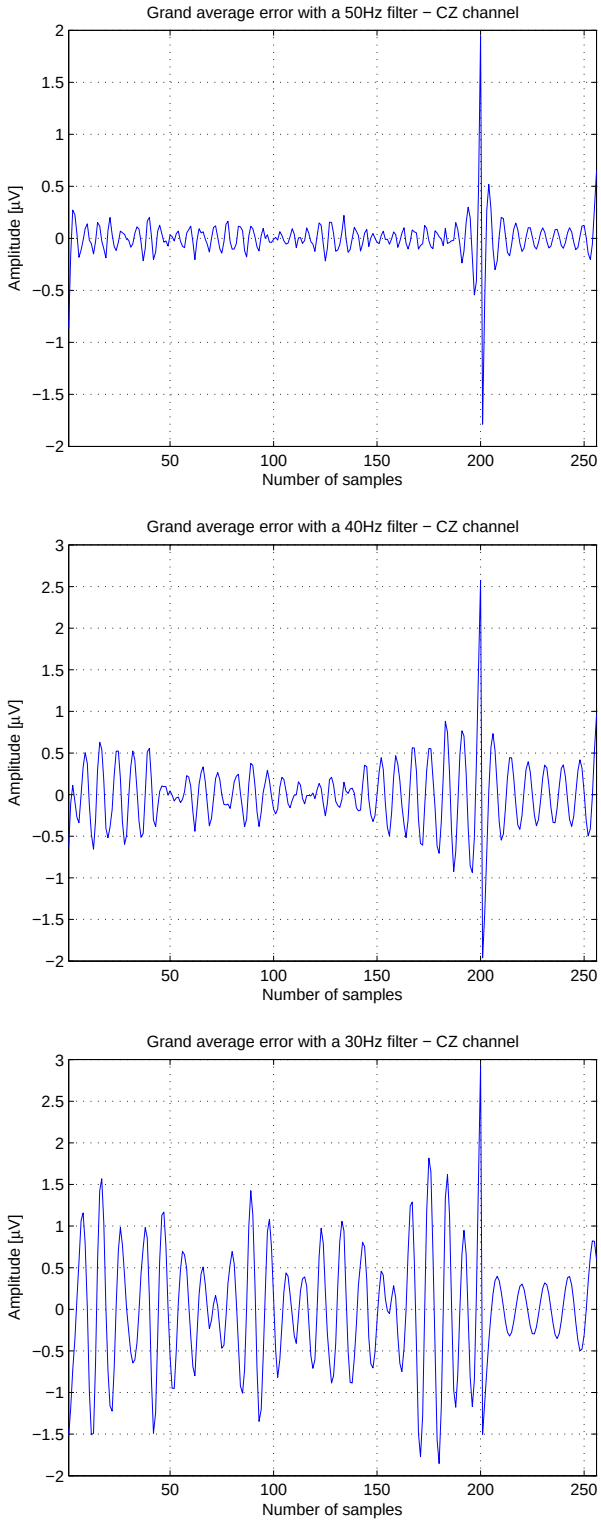

Figure 4.11: Error filter on the CZ channel for the grand average without prestimulus of a ALS patient and its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

It is again evident that, in the cases in which the prestimulus is taken into account, the impact of the error filter is more visible, than in the others. This could be explained saying that in the first part of the EEG acquisition, where no stimulus is applied yet, the most important contribute is due to the electrical field of the neighbouring electrical devices and to other oscillations probably not meaningful from a neuro-physiological point of view.

A measure of the quality and the efficiency of the method till now presented can be found in figg. 4.12 and 4.13, where the grand average curves of channel CZ of the same two subjects considered before are plotted with the information of a point-to-point standard deviation.

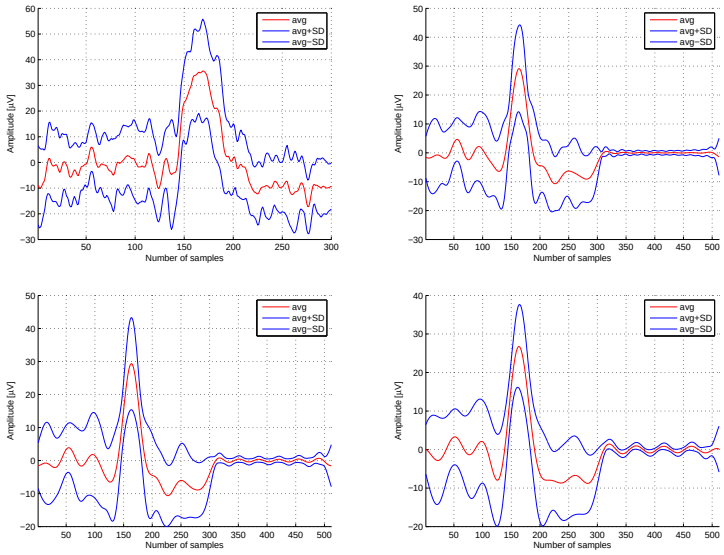

Figure 4.12: CZ channel grand average and standard deviation information for a control subject in the original case and with its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

The plots of the derivatives of the previous 40Hz filtered curves are shown in fig. 4.14. From that figure, it is confirmed that no fundamental improvements are achieved after the filtering as the standard deviation regards (indeed, compare with figg. 3.5 and 3.6).

Conclusion

It can be concluded that this frequency domain method could help in analyzing the ERP traces, because it can strongly remove *noisy* oscillation but, at the same time, it should be probably only the first step of a chain of passages towards a complete explanation of a P300 wave: Indeed, the standard deviation assumes, even after the filtering process, such high values that it

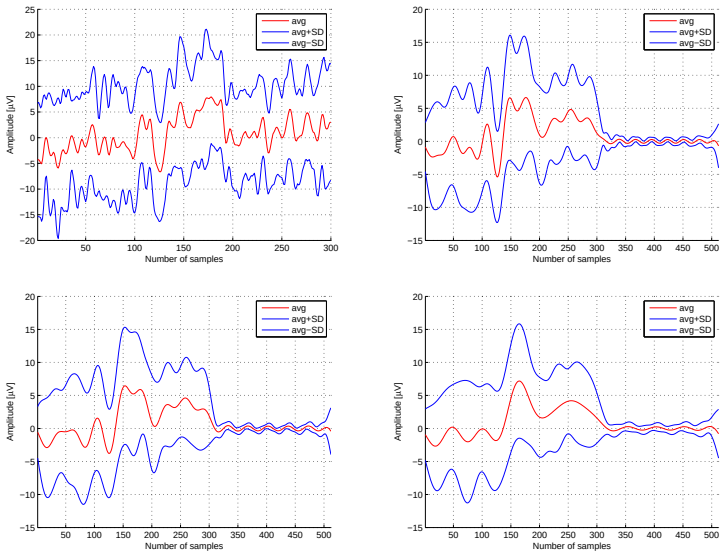

Figure 4.13: CZ channel grand average and standard deviation information for an ALS subject in the original case and with its three possible *levels* of filtering: above 50Hz, 40Hz and 30Hz, respectively.

is not possible to make a relevant statistical analysis on the filtered signal yet.

Thus, in the next, different strategies are going to be experimented in order to reduce the standard deviation within the set of traces of each subjects and, hopefully, to find features that could distinguish the group of the controls from that of the patients.

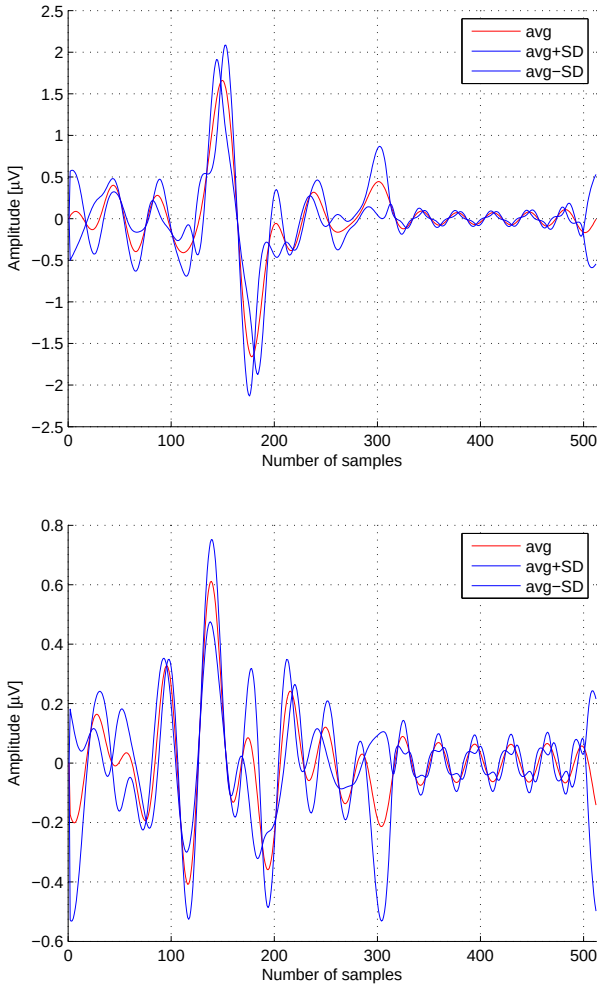

Figure 4.14: CZ channel grand average and standard deviation derivatives for a control subject (left) and a ALS patient (right) after filtering at 40 Hz.

Chapter 5

Time Warping

5.1 Neuro-Physiological Meaning

Besides noisy oscillations due to the electrical fields that could interfere with the on-line EEG acquisition system and other non neuro-physiological waves proper to the EEG background, other factors of distortion could arise from the psycho-physiological conditions of the subject that is undergoing the odd-ball test. This means that a modification of the P300 waveform could be also caused by the attentional level of the person and their tiredness state, besides several other variables can influence the test result, such as their expectation to either a TG or the NT stimulus.

In particular, in this chapter, a method that aims to reduce the variability of the P300 as the tiredness as concerned will be proposed. It was called *Time warping method*, because its goal is to remove as much as possible the dependence on a possible slowing down of the performance in the test due to a tiredness state that is peculiar for each trace of each subject. The idea behind is that every subject (controls and patients are supposed to behave in a similar manner) reacts to a stimulus with a certain delay, not necessarily constant, during the whole interval of time of the task. Moreover, this behaviour could be different from epoch to epoch, as well as from subject to subject. It can also be expected that the standard overall grand average can not take into account such a factor of distortion, probably omitting important contributions to the total signal. Therefore, in order to *normalize* the different reactions of each subject in every single sweep, a compression or, alternatively, an expansion was implemented in the time domain.

The algorithm works as explained in the next section.

5.2 Algorithm and Reference Sample Choice

Fundamentally, the point is to align each single sweep (in each channel) on a so-called *Mean* or *Reference Sample* chosen in an appropriate way. Then,

a distortion of the signal is operated depending on the reference point used as a center for all the traces.

In particular, the first step of the algorithm consists in finding the absolute maximum within the range between 250 and 500 ms, where the P300 is most expected to appear. This passage is performed thanks to a Matlab function implemented in [16] by searching for all the local maxima, choosing the largest one and, finally, setting the sample that corresponds to the absolute maximum in the interval of time mentioned above as the mean sample.

As an example, let us consider the grand average of the CZ channel of an healthy subject. If the algorithm that looks for the local maxima is applied, the plot of fig. 5.1 is found.

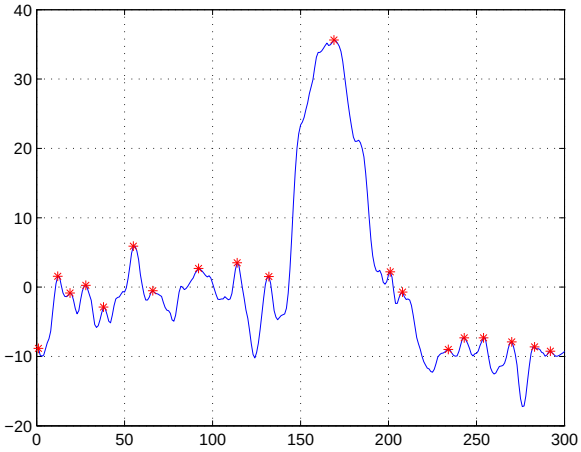

Figure 5.1: Local maxima on the CZ channel grand average of an healthy subject.

In that, the absolute maximum is localized in correspondence of the 170-th sample, that is consequently chosen as the reference sample for the time warping. It has to be thought that employing such a sample for the realignment basically means that it is assumed that a subject reacts, on average, with the latency of the original grand average curve. Equally likely, it would be possible to choose another sample as the reference one: For example, a different choice could consist in selecting the analogous peak of one of the first TG traces, that are supposed to be tiredness effects free.

Moving forward to the second step of the algorithm, the same maxima research is applied on each of the single traces of the subject in each channel, separately. Indeed, it has to be observed that the time warping or normalization process takes into account a conceivable different behaviour for different

channels: Thus, the method is distinctly applied for each of them. As an example, the fifth TG single sweep of the healthy subject under study shows several local maxima, identified by the algorithm in [16] and showed in fig. 5.2.

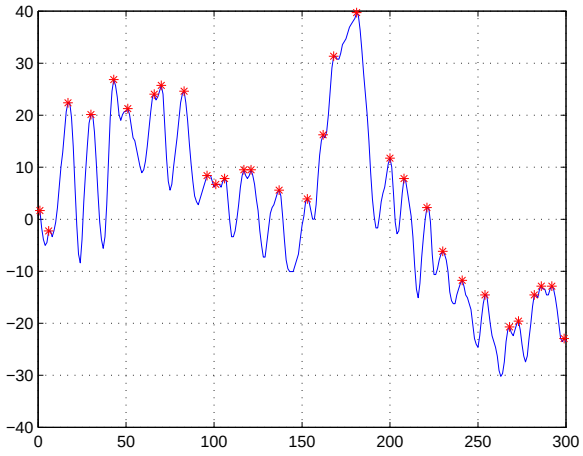

Figure 5.2: Local maxima of the fifth TG single sweep of the healthy subject considered for this example.

In a similar way in which the mean sample was earlier chosen on the grand average trace, the main peak (that is, the absolute maximum) of each single sweep within the window from 250 to 500 ms is considered.

Then, a so-called *normalization ratio* is computed for each TG trace as the ratio between the sample correspondent to the main peak of each one and the mean sample, common for all of them. It can be used as a measure of the severity of the time warping action.

The fourth, and final, phase creates new traces by linearly interpolating the original single sweeps on a new axis of samples equally spaced by a new step as large as the normalization ratio is.

To be more clear, let us consider the previous example. The curve achieved at the end of the process is reported in fig. 5.3.

It has to be noted that, after the application of this method, the length of the new single curves could be modified: They could become either shorter, as that in fig. 5.3, or longer, as in fig. 5.4, depending on the normalization ratio. To be precise, a compression of the signal is expected if that ratio is greater than one, otherwise an expansion takes place.

Once the algorithm is employed, a new set of single sweeps is available for each subject for the further analysis. The simplest way to continue the signal processing is to sum all the traces of a channel and dividing for their

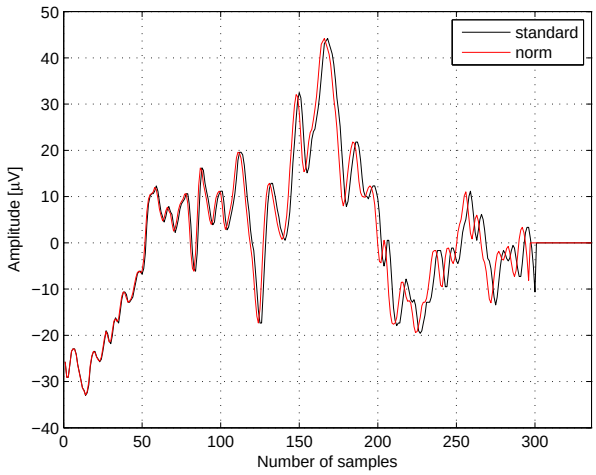

Figure 5.3: Time warped fifth TG single sweep of the CZ channel.

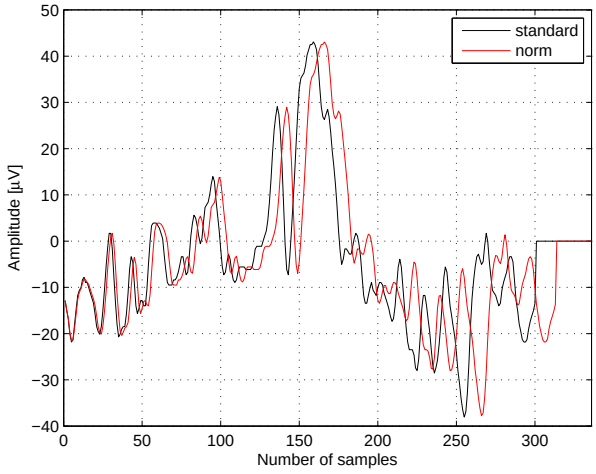

Figure 5.4: Time warped sixth TG single sweep of the CZ channel.

total number: It is thus achieved the new grand averages, three per subject (as three are the involved channels for each person).

5.3 Findings and Statistical Results

Theoretically, this time warping technique could, at least, correct for an uniform tiredness condition effecting the acquisition period of 1.5 seconds: Indeed, the normalization ratio is assumed as a constant during the whole interval of time.

A good measure of the efficiency of the process is provided by the plots of the new grand average curve with its relative standard deviation information, as in fig. 5.5.

From fig. 5.5, it can be noticed that a relevant improvement is reached: a reduction in the standard deviation in respect to the mean curve, at least in the neighbourhood of the main peak, could verify the effectiveness of this new strategy.

Several remarks could arise about this methodology: First of all, it can be guessed that the tiredness effects increase with the time progress of the test. But, gathering the values of the normalization ratios of all the subjects for all the tested sequences, it has to be stated that they seem not to follow a specific rule, that is no appreciable increase in those ratios is found along subsequent TG epochs.

Another hypothesis can be guessed as the relation between the level of ALS disease and that of the applied normalization: the disease should contribute to distort more the traces. Thus, higher mean values are expected for the patients into respect of the controls. The results of this investigation fill in tab. 5.1, where the statistics for the control and the patient of the previous example has considered[1].

Normalization ratio	Control	ALS-patient
FZ	3.1111±2.6943	11.7778±0.6939
CZ	4.8889±0.9623	7.5556±7.1983
PZ	11.7778±0.3849	10±9.6839

Table 5.1: Statistics on normalization ratio for all the subects.

A higher level of time warping seems to be necessary for the ALS patients, at least in the frontal and the central areas. This observation should be later verify for all the controls and all the patients, taking into account an analogous table for each subject. But, till now, the interest is more focused on highlighting the performance of this technique in the process of the EEG signals.

[1] A choice had to be made for space reasons: Indeed, a table analogous to tab. 5.1 can be filled in for every subject.

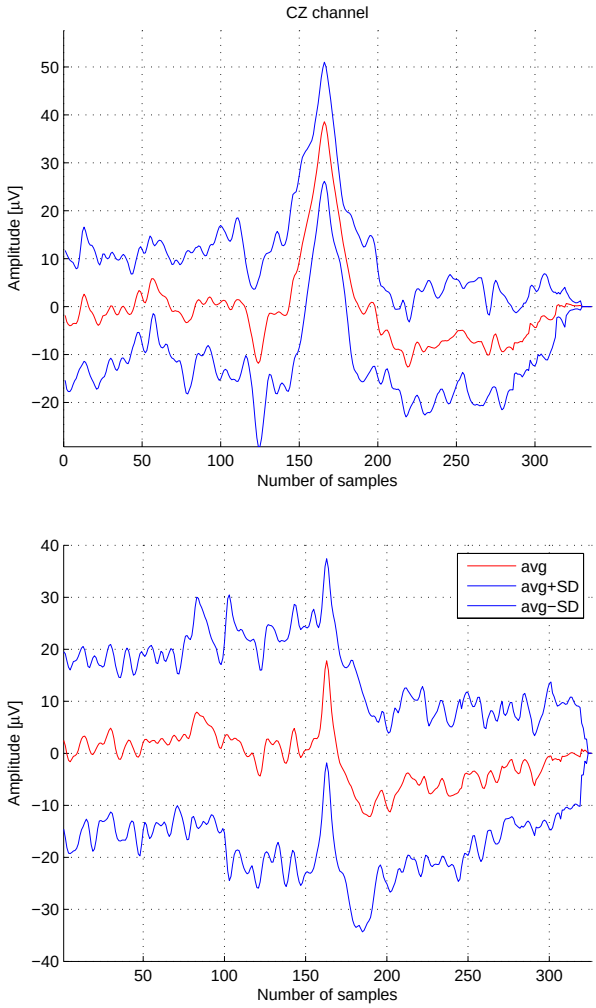

Figure 5.5: Time warped TG grand averages and relative standard deviations on the CZ channel of an healthy(left) and a ALS(right) subject.

Although some results have still no explanation, an improvement is nevertheless significant: in particular, the new grand averages (like that of fig. 5.5) show a very important peak within the range of [250, 500]ms that could be assimilated as the P300 peak without employing further algorithms to find it. Its amplitude is, indeed, really increased into respect of the original measured and validated P3 peaks, as it can be confirmed by comparing tabb. 3.1 and 3.2 with tab. 5.2. This could mean that, actually, a component of tiredness is strongly present in each EEG trace and has to be removed before starting any other statistical analysis.

P300 peak	Controls	Patients
FZ	16.0706±5.9377	17.1458±5.9315
CZ	18.4176±8.3347	18.0018±6.4720
PZ	19.2754±8.0853	20.6990±6.3981

Table 5.2: P300 peaks amplitude measured from the time warped grand averages.

It is still not clear if a good estimation of the latency of the P300 peak could be also derived from these new traces because, somehow, the reference sample was forced to be in a location previously set and fixed for all of them. But, a possible justification could be that this method has removed only psycho-physical conditions and not the cognitive ones: Therefore, in this case, the latency should be modified like this method modifies it. This is still an open question that will be probably one of the starting points for the future analysis.

Chapter 6

Translation

6.1 Neuro-Physiological Meaning

In the previous chapter, it was tried to exclude the tiredness influence on the EEG acquisitions; in the current one the focus is on the vigilance factor. Indeed, the level of attention plays a very important role in the origin and the determination of the shape of the P300 wave: Explicitly, it could create an initial delay in the subject response to the stimulus, whether they disregard the test for any reason. But once they come back to the highest vigilance state then, they should show an analogous *reaction speed* like any other aware subject.

The following analysis aims at excluding from each single sweep all the effects due to the inattention conditions and, at the same time, probably other unknown delaying causes.

6.2 Algorithm and Reference Sample Choice

The algorithm implemented to perform the latter methodology is quite similar to that used for the time warping technique discussed in the previous chapter.

Indeed, the first two steps are pretty the same: Let us consider a subject and one channel per time. The local maxima are searching on the TG original grand average curve and the largest one in the range between 250 and 500 ms is assumed to be the reference sample, alternatively called *Mean Sample*. Subsequently, the same maxima analysis is performed on each TG single trace and the highest maximum in the time window of above is taken into account for the following steps of the algorithm.

The third step consists in computing a quantity that characterizes the impact level of the method on the EEG curves: the *Translation Factor*. It was calculated by taking the difference between the sample that corresponds to the chosen maximum in the single trace and the reference sample of

the grand average. Obviously, this quantity could be positive or negative depending on the relative location of the two peaks considered on the single sweep and the mean trace. It can be noted that a positive value could be interpreted as a delayed response of the subject to the stimulus (compared with the mean response).

If the same example of the previous chapter is considered, the first two steps of this new methodology are again exemplified by figg. 5.1 and 5.2.

Then, the final step of the algorithm is to apply a rigid translation of each TG curve of an amount equal to the translation factor.

In the case of those control and patient used as examples, the outcome for two different TG single traces is reported in figg. 6.1 and 6.2.

Comparing the new translated traces with the corrispondent original ones, it can be noticed that, in the first case, a positive time shifting was operated while, in the second one, a kind of advance was corrected by a negative translation.

Once the new set of translated traces has been found, a new grand average could be computed for each subject (see fig. 6.3).

6.3 Findings and Statistical Results

As done in the time warping chapter, the new translated grand average and the correspondent standard deviation were computed and are reported in fig. 6.4.

It is clear that the standard deviation suffered a substantial reduction that could confirm the effectiveness of the process.

Besides the earlier general observation, a similar guess to that proposed at the end of the chapter about the normalization could be repeated here: In the disease case or after a certain number of stimuli, the subject should become less reactive, less careful in performing the test. From this point of view, it can be expected to find increasing values of the translation factor along subsequent epochs.

But, analogously to the normalization case, the distribution of the translation factor is not regular at all: It can vary with the subject, the channel and the number of the stimulus in a random way.

Moreover, the distinction between controls and patients seems to be highlighted thanks to this method. Indeed, from the results of tab. 6.1, where the mean translation factor for the healthy case and the ALS one is shown, a stronger shifting action seems to be necessarily in the latter case.

Generally, it can be concluded that for ALS patients a stronger translation action is needed than for controls and, moreover, that the variability in the disease cases is more important. The emblematic channel is the frontal one, in which a translation of more than twenty-five samples is applied to the patients' traces, with a standard deviation larger the half of the shift.

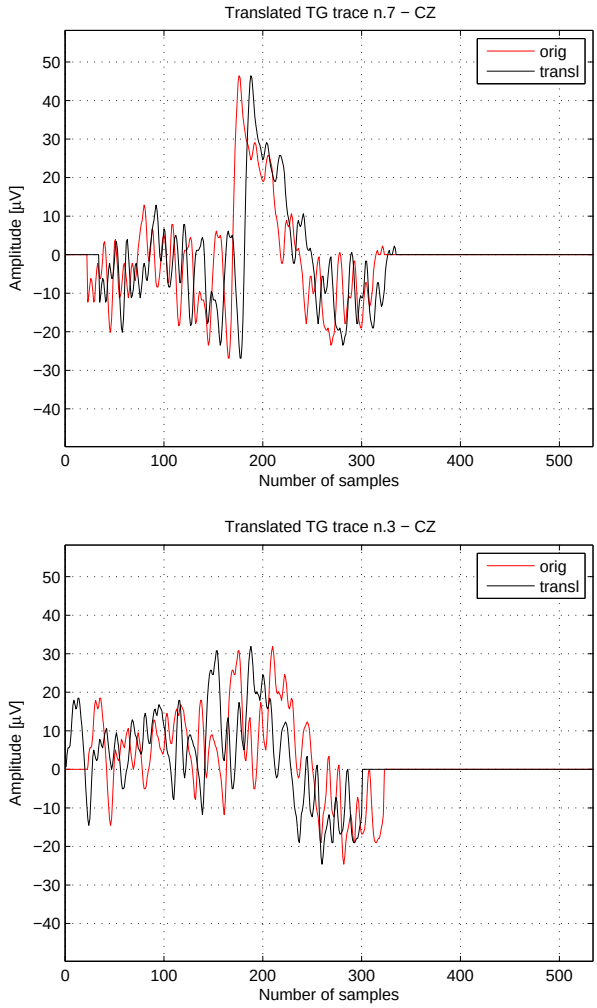

Figure 6.1: Two original and translated TG single sweeps on the CZ channel of an healthy subject.

Translation factor	Control	ALS-patient
FZ	7.4±6.7908	25.3750±14.5148
CZ	7±5.8919	11.1667±8.0954
PZ	6.6667±6.3770	9.6250±8.3239

Table 6.1: Statistics on the translation factor for all the subjects.

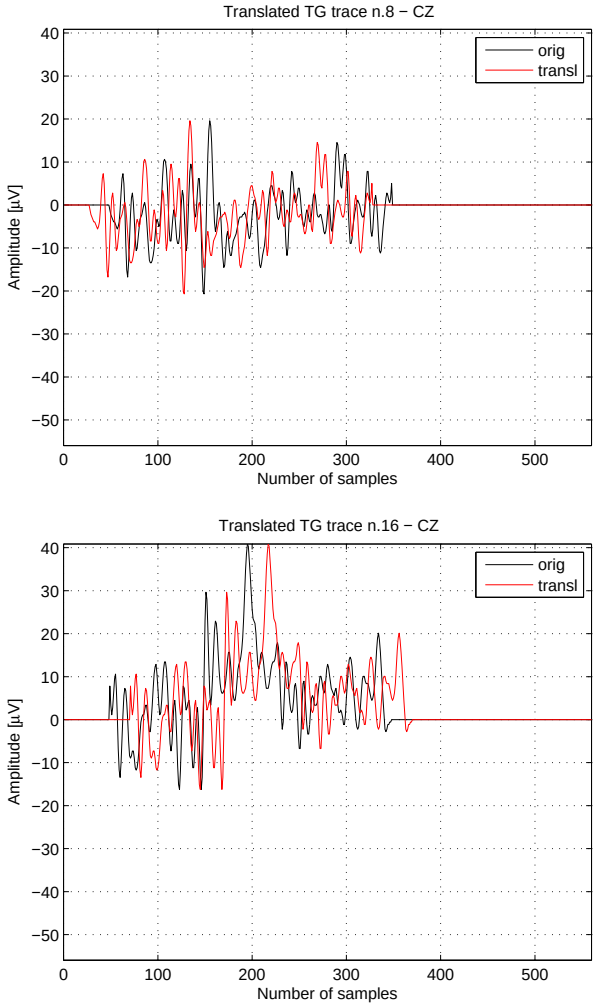

Figure 6.2: Two original and translated TG single sweeps on the CZ channel of a patient.

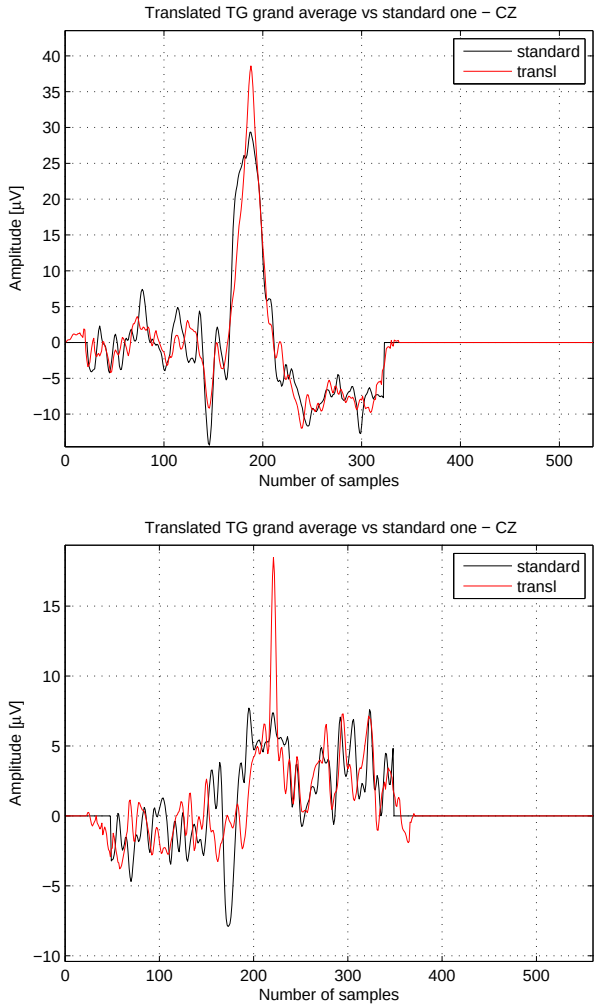

Figure 6.3: Translated TG grand averages compared with the standard ones on the CZ channel of an healthy(left) and an ALS(right) subject.

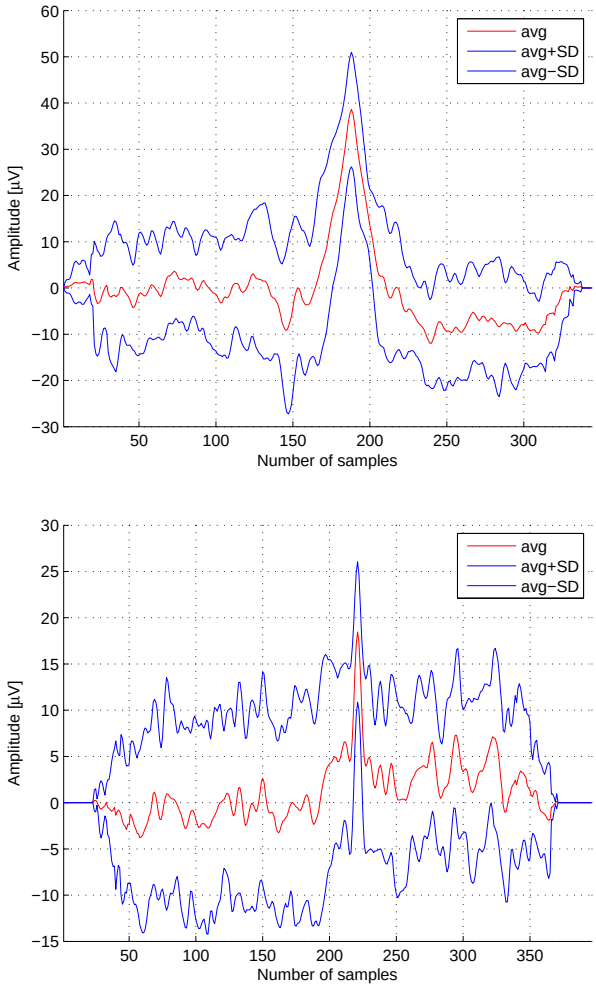

Figure 6.4: Translated TG grand averages and relative standard deviations on the CZ channel of an healthy(left) and an ALS(right) subject.

Although the high values of the standard deviation, the two classes of controls and patients remain distinct enough: The validity of this method could be thus increased by the previous observation about the translation factor. Finally, similarly to the time warping case, from the new translated grand averages, the P300 peak information can be extrapolated in a simpler way than what was done in the literature, because in the most instances a relevant peak is clearly identifiable as P300 deflection.

Thus, the tab. 6.2 can be filled in with the new mean amplitude values of the P300 peak.

P300 peak	Controls	Patients
FZ	11.5944±8.4266	10.3096±9.6858
CZ	13.6275±11.7095	9.9720±10.0695
PZ	8.2765±12.2566	9.8685±10.1944

Table 6.2: P3b peaks amplitude measured from the translated grand averages.

It can be further appreciated the improvement into respect of the original values of P300 amplitude validated at the S.Camillo Hospital and presented in chapter 3. As in chap. 5 case it happened, higher amplitude values can be found and the relevant reduction in the standard deviation from the mean measure could be considered as a proof of the utility of the method.

Chapter 7

Comparisons and Hints For Future Studies

7.1 Comparison among Different Methods

In this chapter the most important findings of this thesis work are going to be reviewed: The comparison among the three main methods used so far, the frequency-domain analysis, the translation and the time warping process, will be based on the grand average and the relative standard deviation information. Indeed, once the set of signals is optimized from a statistical viewpoint, further analysis can be more simply developed.

As known, a mean value together with a too large variance (as it happens for the original grand average sample by sample), can not be a valid estimate for the signals class that has given rise to it.

As an example, let us consider fig. 7.1, where the original TG grand averages are computed as the sum of the original EEG traces without any other process. The left panel shows the information about an healthy case, while the right one corresponds to an ALS-affected patient.

It can be seen from that figure, but it was also mathematically computed, that the standard deviation exceeds the 100% of the mean value in correspondence to some samples. This obviously means that the amplitude that the grand average reaches in such a sample is the mean of a set of samples (one for each single sweep) that lie in an extremely wide range of amplitude.

Therefore, took cognizance of this occurrence, the methods of chapp. 4, 5 and 6 were exploited in order to find a new set of traces that, once summed, produce a new grand average curve more representative of them.

Indeed, let us firstly take a look of fig. 7.2, whose plots were obtained by applying a filter with a cut off frequency set to 50 Hz. It is observed that a slight improvement is already visible into respect to the previous situation (fig. 7.1), although it is not the decisive solution, yet.

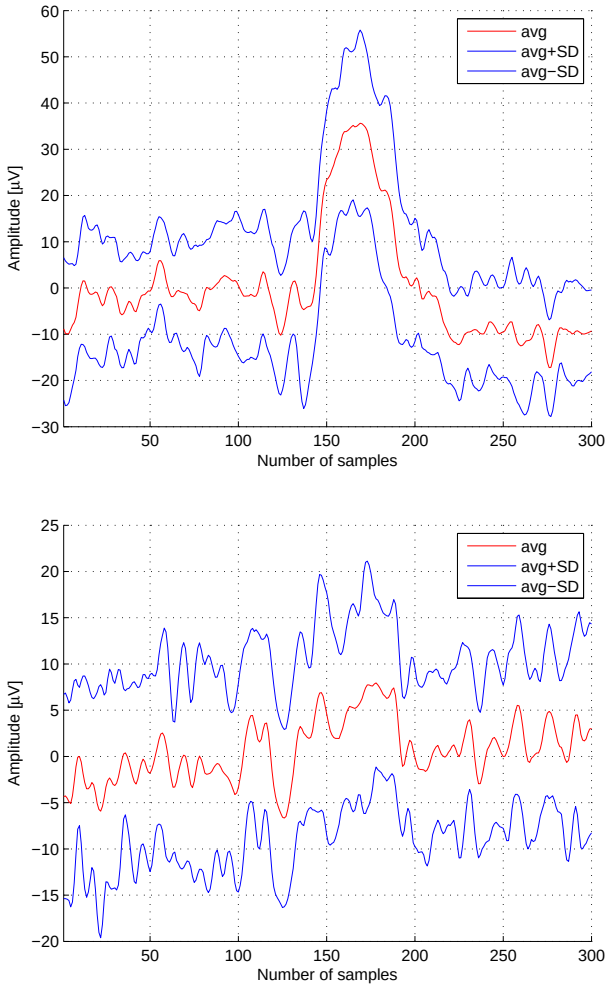

Figure 7.1: Original TG grand averages and relative standard deviations on the CZ channel for a control(left) and a patient(right).

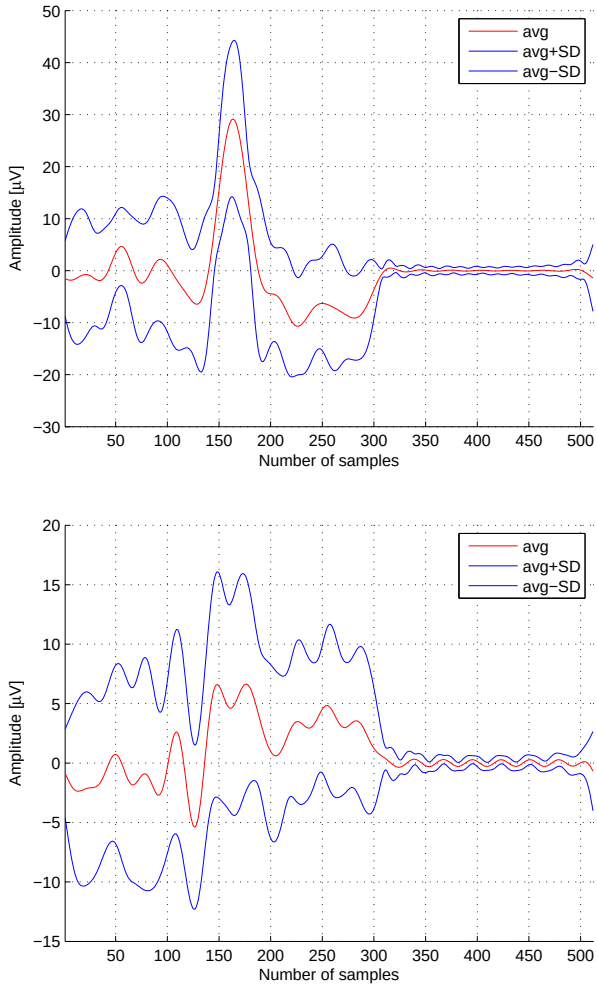

Figure 7.2: 50Hz Filtered TG grand averages and relative standard deviations on the CZ channel for a control(left) and a patient(right).

Looking for more relevant results, it is found that the best achievement using the available set of the EEG traces as a starting point is reached employing the time warping and the translation methods: They, indeed, allow to decrease considerably the standard deviation, as already mentioned in chapp. 5 and 6 and shown in figg. 7.3 and 7.4, even if mostly in the range surrounding the presumed P300 peak.

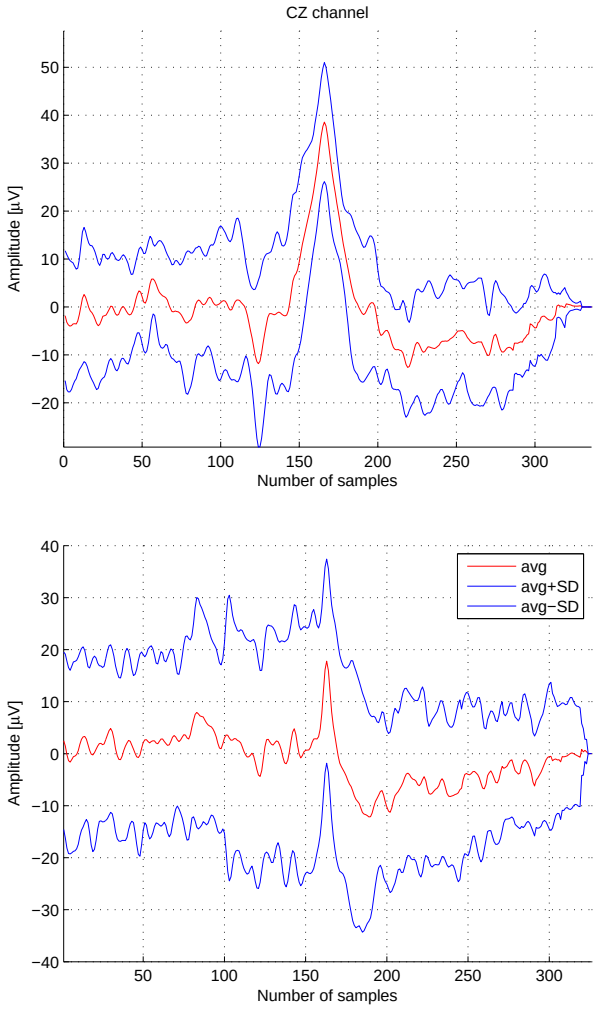

Figure 7.3: Time warped TG grand averages and relative standard deviations on the CZ channel for a control(left) and a patient(right).

The displayed improvement should enhance the validity of the above methods: The future developments on the topic could nevertheless bring us to modify those strategies, at least in some of their steps, with the goal of increasing their performance more and more.

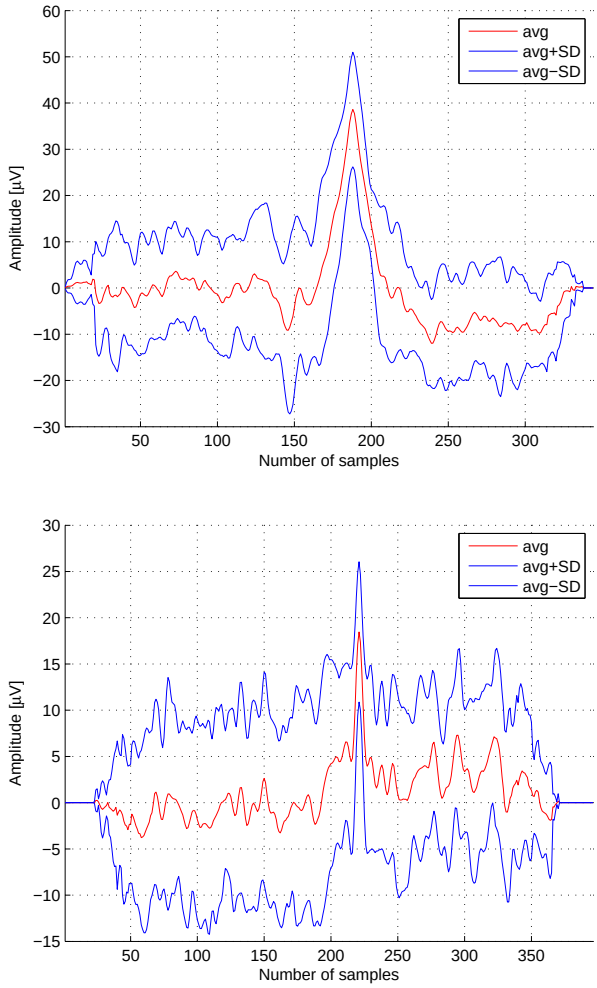

Figure 7.4: Translated TG grand averages and relative standard deviations on the CZ channel for a control(left) and a patient(right).

Another possible comparison could be made by plotting all the TG grand averages obtained from each of the three over-cited methods. In can be noted that all the strategies brought to emphasize the main peak in the [250, 500]ms range of each single sweep, counted as the P300 peak: As a result, each of the TG grand averages (especially the time warped and the translated ones) presents a very important peak amplitude. Figg. 7.5 and 7.6 can confirm this observation in two specific cases of an healthy subject and an ALS one.

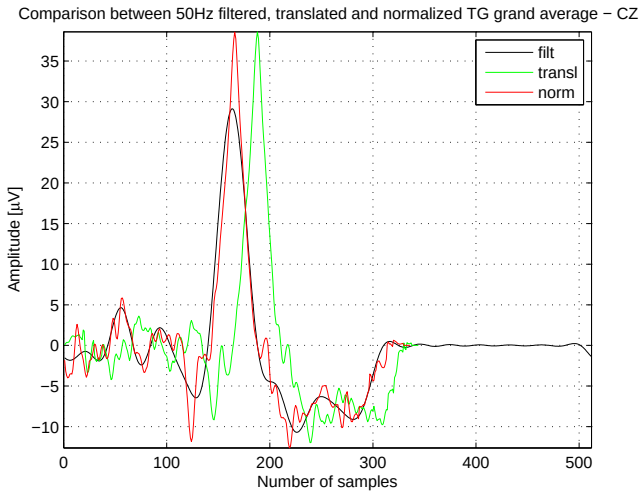

Figure 7.5: Comparison between the 50Hz filtered, the time warped and the translated TG grand averages of the CZ channel for a control.

From this perspective, it could be guessed that the best method to be exploited should be that which mostly stresses that peak, highlighting its location in the grand average curve. In this way, no further algorithm should be used to find the right P300 peak and a major effort could be involved to search for other useful parameters that characterize these ERPs.

7.2 Future Targets and Suggestions

The analysis presented so far has led to a uniform improvement of the set of the available signals, without be actually able to answer the initial question and target of the overall research project: Univocally identifying the P300 wave of an ALS-affected subject from that of an healthy one.

In fact, human EEG traces are singular, peculiar of each person and, depending on a number of variables, it is not classifiable[2].

Probably, what we are looking for is to study the effect of a set of causes acting in conjunction: thus, not the tiredness, the level of attention or other

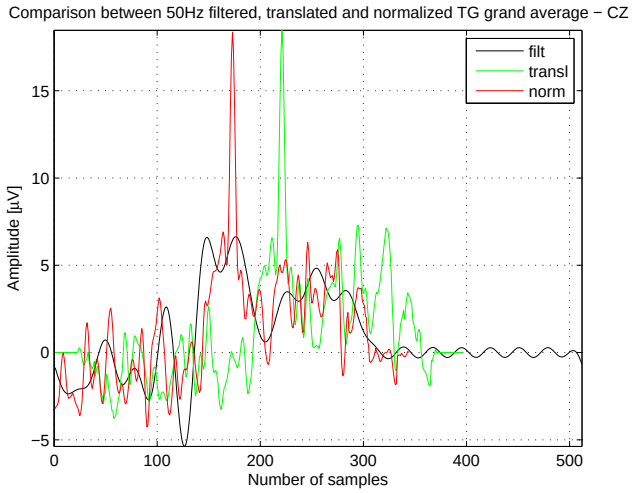

Figure 7.6: Comparison between the 50Hz filtered, the time warped and the translated TG grand averages of the CZ channel for a patient.

factors separately, but all together and inseparable among each other. In order to have a wider record of occurrences, it could be useful to extend the set of subjects, paying attention in considering a group of them uniformly spread as the gender and the whole age-domain as regard: As the literature[**?**] suggests, the same number of subjects has to be counted in every decade of age, half of them being male and the other half female.

Moreover, it would be useful if a baseline trace was registered for each subject in a rest condition for an interval of time long enough. With this information, it could be possible to know the background activity of the brain and, maybe, remove its contribution from the EEG traces, when a stimulus is then presented to the subject: Thus, hopefully, only the cognitive part of the EEG would remain to be analyzed.

Once gathered a new group of traces from this new hypothetical set of subjects, the methodologies proposed in this thesis could be employed again, with the scope to confirm the previously discussed outcomes and find new ones. Then, other methods already outlined or even only thought during the last months could be exploited, hoping to definitely discover such features that support the identification of the disease or healthy state of every new subject that undergoes the neuro-physiological test.

Conclusion

Moving from the initial and standard temporal analysis on the grand average curves to the time warping and the translation techniques applied on the

single EEG sweeps, relevant improvements seem to be reached.

If, on one hand, the frequency-domain filtering removed non physiological or, maybe, non cognitive-based oscillations, on the other hand, the latter two methods of processing allow us to find new grand averages more representative of the set of signals of which they are the overall sum. Indeed, applying those techniques, the standard deviation decreases by a relevant amount and only one really high peak appears in each grand average, simplifying its detection.

The average traces arisen after undergoing the time warping and the translation method could be probable considered as the overall mean P3 response to a TG stimulus, independently on the different tiredness and vigilance conditions of the subjects, variable during the test. Removing these factors should be the first step in the future procedure of discovering, more precisely, the deep meaning of the P3.

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