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UNIVERSIDADE Ɖ COIMBRA

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FROM FACE PERCEPTION NEUROSCIENCE TO IDENTIFICATION OF FUNCTIONAL IMAGING MARKERS IN NEUROPSYCHIATRIC DISORDERS

VOLUME 1

Dissertação de Mestrado na área de Engenharia Biomédica, orientada pelo Professor Doutor Miguel Castelo Branco e apresentada ao Departamento de Física da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

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Abstract

The perception of faces is one of the basic aspects underlying social interactions. From a very young age we learn to interact with others by expressing various emotions and feelings that can be seen by expressions in other people's faces. Studying the neural correlates of emotion recognition is halfway to help people with difficulties in this important cognitive and affective domain.

Previous neurophysiological studies concerning brain responses to emotions often lead to controversial and even nonreplicable results.

We had two main goals for this work:

The first task is to try to replicate some studies already made in the area of emotion recognition and try to find some statistical differences between three emotions (Sad, Neutral and Happy) for the N170.

The second objective of this thesis is to understand and explain the reasons why some studies lead to controversial results and nonreplicable results, based on preprocessing differences. Assuming that the cognitive task is done properly, one of the remaining reasons for that is the possible use of different methods in the pre-processing steps.

Our task used 10 subjects (4 females, 6 males), where each subject performed 4 EEG runs and each run had around 6 minutes of recording. The task consisted in showing a neutral face to the subject and after a GAP showing an eye movement instruction. The instruction was a face with an emotion displayed concomitantly with an averted gaze. After that the participants should complete a saccade or an anti-saccade.

We used different methods of pre-processing: 1) interpolation; 2) Re-reference; 3) filters, baseline correction and epochs rejection and 4) Independent Component Analysis (ICA).

For the first objective we could replicate some previous studies and we found statistical differences between the different emotions for the N170.

For the second objective we concluded that every method of pre-processing has significant influence in the results, giving different amplitudes and latencies for ERP's. We also recommend that for a more reliable result one should use every method of pre-processing that we refer on this paper, and provide transparent reporting, given the impact of preprocessing.

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List of Abbreviations

(2-D) – Two-dimensional

ANOVA – Analysis of Variance

EEG – Electroencephalography

EPSP – Excitatory Postsynaptic Potentials

ERN – Error-related Negativity

ERP – Event-related Potentials

FDR – False Discovery Rate

FIR – Finite Impulse Response

fMRI – Functional Magnetic Resonance Imaging

ICA – Independent Component Analysis

ISPS - Inhibitory Postsynaptic Potentials

IIR – Infinite Impulse Response

MMN – Mismatch Negativity

Ms – Milliseconds

Hz – Hertz

SNR – Signal-Noise Ratio

µV – Microvolts

VPP – Vertex Positive Potentials

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: _________________________

Date: _________________________

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1. Introduction

This thesis will follow this structure: in chapter 1 we will present the introduction of this work where we will explain the context and the objective of the work and background concepts to better understand our aims; chapter 2 will explain the standard methods used to pre-processing and data analysis of electroencephalography (EEG); in chapter 3 we will compare our results with previous works; our results and discussion about the use of different methods in pre-processing data will be reported in the last chapter, chapter 4.

1.1 MOTIVATION AND OBJECTIVES

Since our birth that the perception of faces is important in social interactions. During our life we are constantly interacting with different people which express the most diverse emotions. Primary emotions include fear, happiness, sadness, anger, surprise or disgust. These types of emotions usually can be expressed by natural reactions seen on the faces of people. Studying the neural correlates of emotion recognition is halfway to help people with difficulties in this important cognitive and affective domain (e.g. ASD).

Previous neurophysiological studies concerning brain responses to emotions lead to controversial and often non replicable results. In order to explain those differences, there is ongoing discussion and experimental work trying to identify the reasons behind that. Assuming that the task is done properly, and the subjects fulfil adequate inclusion criteria, one should also consider as one of the reasons for getting different results the use of different methods in the pre-processing data. Maybe the digital filters used, or the reference used or even analysing different channels may lead to slight differences and this should be carefully considered. This is one of our goals in this thesis, to get a grasp on into which extent pre-processing methods may influence or not the amplitude and the latency of the results.

In this study we aim to understand factors influencing brain responses in facial emotions recognition and demonstrate how we should proceed to analyse them in order to get valid results. To do that we will systematically study pre-processing (analysis) methodological steps that may or may not influence the results. In this line, we will test different pre-processing approaches to suggest an analysis pipeline for better EEG results in the field of face emotion recognition research.

Our study aims to answer the following questions: are there significant differences in brain response to different emotions? What neural correlates can be discerned at the EEG level? How do the pre-processing steps influence the results obtained?

The first part of the work contains the explanation of the methods used in pre-processing and data analysis of EEG data. To pre-process the data, we used the following methods: 1) interpolation of channels, 2) reference tests, 3) filters, 4) baseline correction and epochs rejection and 5) Independent Component Analysis (ICA). To output the results, we used topography maps and plots of the Event-related Potentials (ERP). This second chapter can be used as a pipeline for replication of the results that will be described in chapter 3 and 4, which are the second part of this work.

In the next chapter, after pre-processing the data, we will compare responses to emotions (e.g. happy, sad and neutral faces) and compare our results to previously studies reported.

In the last part we focus on understanding the different effects of different pre-processing steps. We will test also different approaches to evaluate their impact in the latency and the peak amplitude of the ERP's. The ultimate question was: Can the use of different approaches in the EEG data pre-processing methods have significant impact on the peak amplitude and the latency of the ERP?

Summarizing, the main goals of this work are 1) To review different pre-processing methods, 2) Replication of previous studies on face processing and respective comparison of the results and 3) Discussion of the influence of distinct pre-processing approaches in ERP analysis of face processing signals.

1.2 ELECTROENCEPHALOGRAPHY (EEG)

Electroencephalography records the electrical activity of the brain and is a frequently used technique to assess its functioning. It measures the voltage fluctuations resulting from the activity from large populations of neurons and can be analysed in terms of time or frequency domain. The current of the electrical activity in the brain reach the

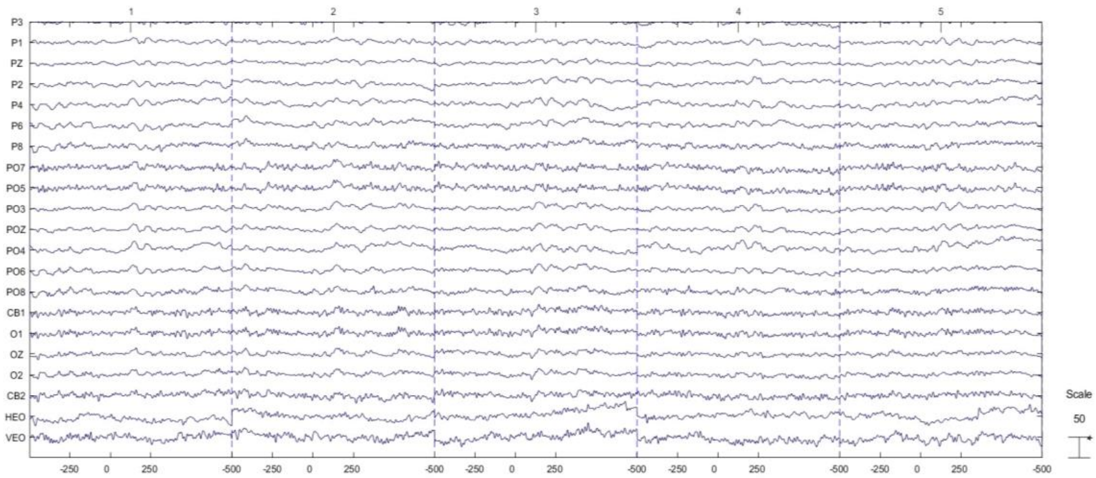


Figure 1. Example of an EEG recording trace before pre-processing.

scalp, and these fluctuations are recorded by EEG. [1][19]

The most important source that produces electrical flow that can be recorded at EEG is the synaptic activity. In order to be recorded far from its source, the electrical signal needs to be strong (originated from thousands of neurons), synchronized and long enough. This happens at synaptic activity in which the electrical signal is transmitted through axon or dendrites in electrical charges. Those electrical charges act like batteries and can be excitatory postsynaptic potentials (EPSP) or inhibitory postsynaptic potentials (IPSP), and they are an important source. ESPS are potentially caused by a depolarization where positive ions go from a negative extracellular field to the cellule and they depolarize the membrane. The opposite is called IPSP. [1][4][19]

This electrical activity can be recorded by EEG due to volume conduction. This volume conduction is the process associated with the flow of the electrical activity from the generator across the tissues to the electrodes of EEG, the main source being cortical. [1][19]

The EEG signal can be classified based on its frequency, amplitude and shape. The shapes are normally sinusoidal, and they are measured peak to peak. The most identified waves are 1) delta, 2) theta, 3) alpha, 4) beta and 5) gamma. [2]

Frequency, measured in Hertz (Hz), is one of the characteristics used to define the EEG rhythms, and varies along the different oscillations referred.[27]

Delta waves are characterized by being slow waves, with frequencies between up to 3Hz and normally associated to deep sleep without dreams and to relaxation periods. Usually they have the highest amplitude among the different types of brain oscillations. [3] [27]

Theta waves are slow waves and normally found in sleeping and meditation. They have frequencies between 3Hz-8Hz. [3][27]

Alpha oscillations are the most common and found waves on EEG. They have frequencies between 8Hz and 12Hz and they are obtained in stages of relaxation or in stages in which the subject is prepared to take an action at any moment. Visual activation, thinking, calculating, or doing other action makes this type of waves to be reduced. [2][3][27]

Beta oscillations have frequencies between 14Hz and 32Hz and are associated to visuomotor activity and situations of anxiety or actions that require attentional modulation. The higher the frequency the more state of anxiety. [27]

Gama oscillations are characterized to have frequencies higher than 32Hz and are found under particular cognitive tasks, in particular when the subject needs is under high cognitive load. [27]

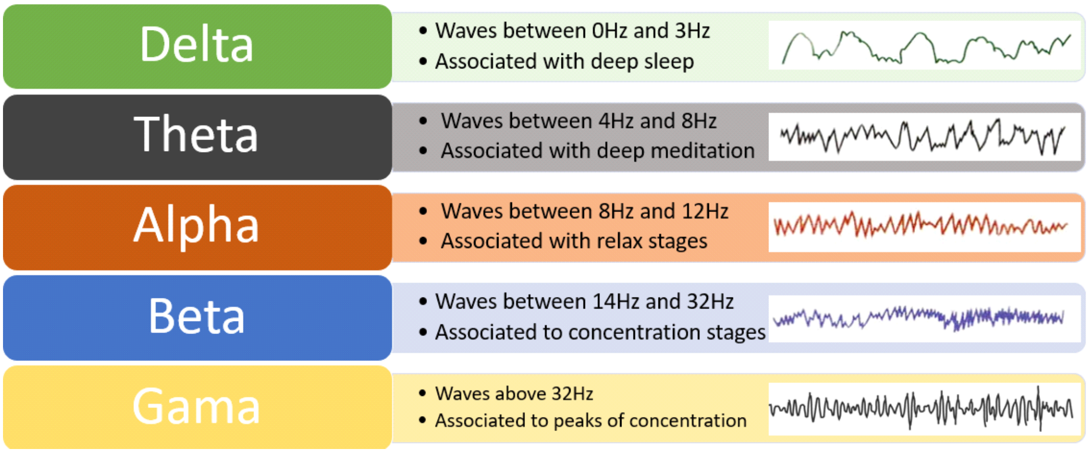


Figure 2. Different types of oscillations in EEG classified depending of their frequency.

EEG topographic plots can provide a 2-D image of the brain but it is impossible to infer the precise location of the EEG source only with the recording of the scalp. So, although EEG has a good temporal resolution in the order of milliseconds (ms), the main disadvantage of EEG is the limited spatial resolution. EEG can mainly record the brain activity in the superficial layers of the cortex and is incapable of identify specific brain locations.[1][19]

There are other complementary tools to provide brain imaging spatial information, such as functional magnetic resonance imaging (fMRI).

Compared with EEG fMRI provides a better spatial resolution but worse temporal resolution.

1.3 EEG APPLICATIONS IN BASIC RESEARCH AND CLINICAL APPLICATIONS

Since EEG is a non-invasive method that allows to record the electrical activity of the brain in a swift manner.

There are 5 principal areas where EEG technology can be applied: 1) Neuromarketing, , 2) Social Interaction studies, 3) Psychology and Neuroscience, 4) Clinical studies in neuropsychiatry and 5) Brain Computer Interfaces (BCI). [6]

Neuromarketing is a field of decision neuroscience that may use EEG to study the decisions and the reactions of the consumers and the brain's activity when they see some products or services and they decided to buy it. It is still a very controversial technique. [7]

Social neuroscience is another area of application of EEG research. A recent method called "hyperscanning" features recording of multiple data from different subjects during the activities that require social interactions to study inter-subject synchronization with the partners. [6][9]

In neuroscience and psychology, the EEG is often used to measure repeated events of the same conditions. The average ERP characterizes the brain response to each experimental condition. Our study case follows this approach, which is also often used in neuropsychiatric studies [6]

Brain computer interface is a device that receives the signals from the brain and translate them to an output machine that will execute the brain commands. In medicine area this is useful in disorders like amyotrophic lateral sclerosis. This can be also applied with military applications featuring the use of exoskeleton and an EEG cap to control the exoskeleton. [6][8]

1.4 EVENT RELATED POTENCIAL (ERP)

Event related potentials (ERP) are brain responses to visual, sensory, motor and cognitive stimulus or events and they can be recorded by EEG [13]

Visual ERPs can be divided in 2 different groups. Early waves that goes up to 100ms after the stimulus has been shown and represents the sensory events that depends from the physical parameters of the stimulus. Waves after 100ms are part of the other group and they generally are related to cognitive ERP´s. These waves are characterized by a specific peak latency and amplitude. [13]

1.5 DIFFERENT TYPES OF ERP'S WAVES

There are distinct types of ERP´s waveforms that are known by their interval of latency and their specific amplitude that are associated to a specific event. The ERP waveforms are described by a letter (N or P) that corresponds to their polarity (Negativity or Positivity) and after that by a number that corresponds to the latency where this peak can be found. P50, N100 or N1, N170, P200, N200, P300, N400, P600 or error-related negativity(ERN) are some waves that have been already studied . We will explain these ERPs and give a more in-depth explanation of the N170 wave and the ERN that are more important to understand our study-case. [10]

P50 is a positive ERP wave that usually can be found at 50ms after presenting the stimuli (figure 3) and it is used to study sensory gating, which means that this wave appears when a second redundant stimuli is produced right after the main stimuli. [10]

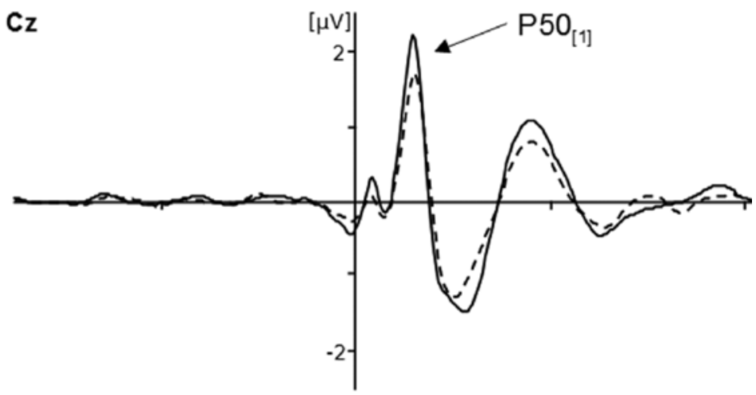


Figure 3.Exemple of a P50 wave. [25]

N100 or N1 wave is a wave that can be found between the 90ms and 200ms and has a negative peak (figure 4) and is associated with an unexpected stimulus that can be visual or auditory. The highest amplitude of this ERP can be found at Cz and its called "vertex potential". It also refers to matching processes and if the stimuli have been shown before. This wave can be found in our study case. [10]

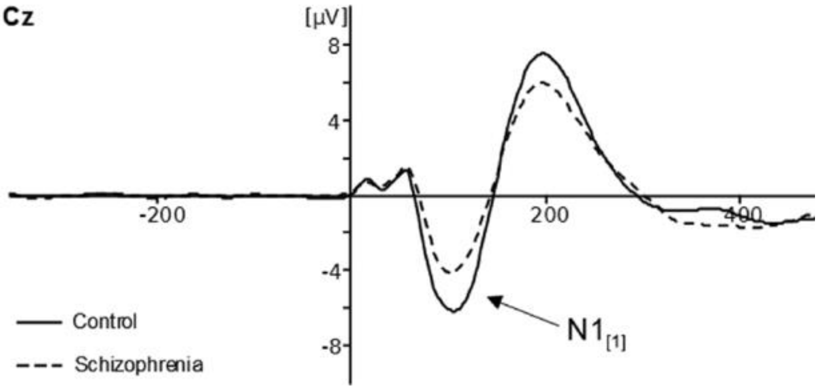


Figure 4. Example of N1 waves in clinical studies. [10]

P200 is a positive peak that is characterized by having 200ms of latency after the stimulus had been shown (figure 5). [10]

N200 is a negative peak around 200/350ms after presenting the stimulus (figure 5). This wave can be presented in three different types: N2a or Mismatch negativity (MMN), N2b and N2c. N2a or MMN represents the auditory stimuli in sensorial memory. N2b characterize the inhibition response and error monitoring. It requires stimulus attention and is often found in Go-NoGo tasks. N2c are related to visual attention tasks, the latency varies with the reaction time and can be found too in Go-NoGo tasks. [10][20][21]

P300 has his positive peak around 300 ms after the stimuli has been shown (figure 5) and represents the attentional response an unexpected target using the oddball paradigm. Different stimuli are shown randomly but one of them is shown less frequently. The objective is to see the response to the unfrequently stimulus. Shorter latencies are related with better mental performance. [10]

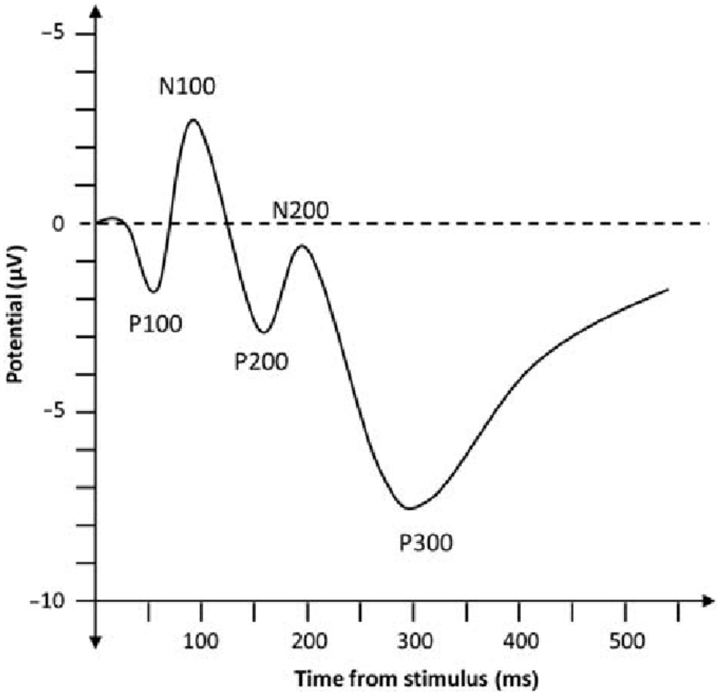


Figure 5. Examples of N100, P100, P200, N200 and P300. Note that the plot is with the negative voltages upward. [24]

N400 is a negative peak around 300-600ms and is the brain response to semantic incongruencies, be them auditory, visual, signals, smells, pictures or even faces (figure 6). [10]

A positive peak around 600ms is referred as P600. It is a brain response related to language domain, more specific with listening to or reading grammatical errors (figure 6). [10]

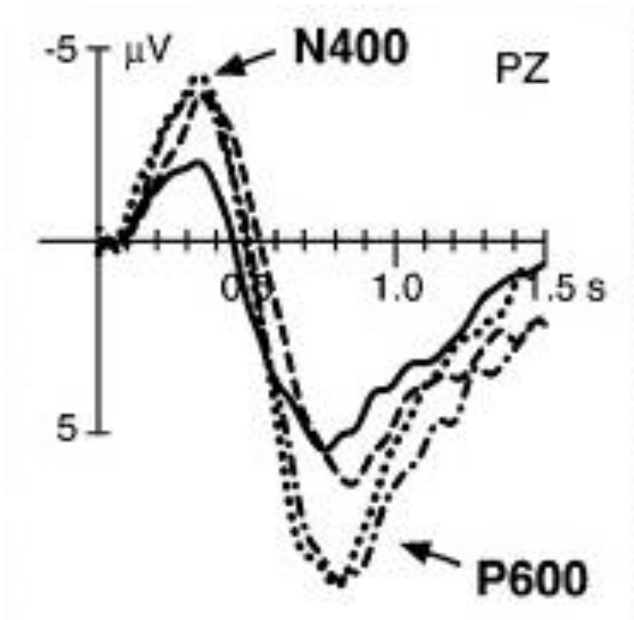


Figure 6. Example of N400 and P600. [23]

1.6 N170 AND ERROR-RELATED NEGATIVITY

N170 is a negativity deflection near 170ms after the stimuli were shown. Was described in detail by Shlomo Bentin in 1996 and his colleagues. He showed to his participants of his study a couple of images showing faces or parts of it and compared the ERP wave with the ERP wave of other objects. He concluded that in the comparing the two situations, the wave response of the images with faces had a sharp negativity with a peak at 170ms. The topographic map showed a maximal response to the occipitotemporal electrodes.

The N170/vertex positive potential (VPP) is very specific in recognition of faces. The amplitude of N170 of a face is significant different when compared with the N170 of an object. Besides that, the literature also defends that this component peaks between 140ms and 180ms and is better observed at bilateral occipitotemporal electrodes (more specific P7, P8, PO8 and P7). This was sustained by the study of Jeffreys (1996) and by the study of Joyce & Rossion (2005). Joyce & Rossion refer that the reference used to get the final results influence the amplitude of N170. We think that is relevant because one of our objectives is to understand the influence of pre-processing methods on the amplitude and latency of the N170 [11]

Jeffreys studied the general characteristics of VPP, the difference of VPP between faces and objects and the origin of the VPP. For that he studied 6 subjects, 4 male and 2 females with ages between 18 and 57 years. Two VEP's were recorded to every stimulus, each one being the average of two blocks, each one with 8 stimuli. The stimuli were presented for 250ms. The reference used was the earlobe electrode and was only recorded Fz,Cz, Pz and Oz. The epochs had 512ms of period and started 100ms before the stimuli had been presented. [12]

The results that they got were clear. The potential evoked to a face or non-face appears at a latency near 140ms, but sometimes can have some delay of milliseconds. However, they found that the peak is significantly different when comparing faces with non-face stimuli. In the non-face stimuli, this peak appears later and with a smaller amplitude (figure 7). Although these differences, they seem to have the same waveform and scalp distribution. This may indicate that both these responses have similar neural correlates even if the sources can be different. The last conclusion that they made was that the VPP was better observed at occipitotemporal electrodes. Some years later they found that N170 and VPP can be explained by the same neural activity and reflect the same process.[12]

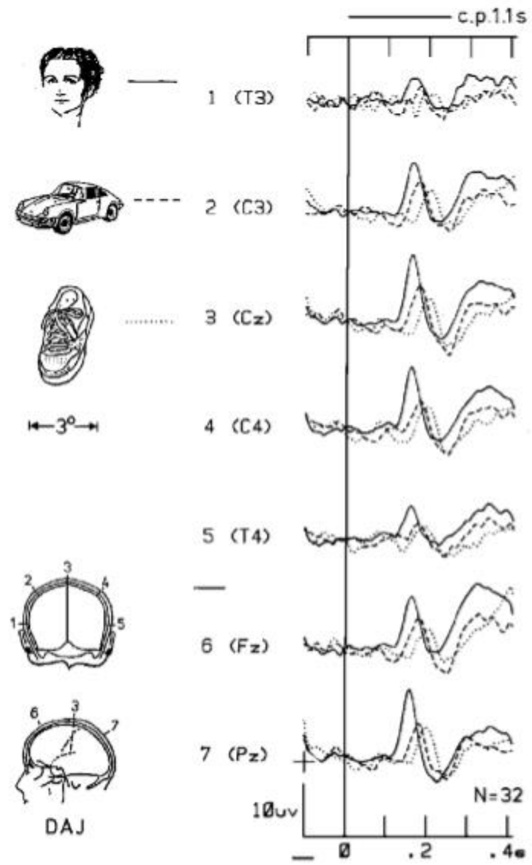


Figure 7. Figure taken from Jeffreys's study that shows the N170 comparing faces and non-faces responses. Full line corresponds to faces, dashed traces to car and dotted traces to a shoe.

If in the part that touches the origin of N170, what it is representing and where it can be better observed, the scientific community is practically in agreement but when it comes to significant differences in N170 of facial expressions, this is no longer the case. Some researchers had found larger amplitudes in N170 or latencies comparing facial expressions (neutral, happy, sad) that sustains the thesis that the brain can recognize different facial expressions (Batty & Taylor, 2003; Blau et al., 2007; Gur et al., 2002; Righart & de Gelder 2008a; Willis et al., 2009). However, the scientific community is not in full agreement with this thesis and some researchers have studies saying that there are not significant differences on N170 when comparing different facial expressions (Eimer & Holmes, 2007; Eimer et al., 2003; Holmes et al., 2005). The table 1 summarizes and shows some studies in this area and what results were observed. In the next paragraphs we will expose two studies by explaining their methods and their tasks and what they conclude for each hypothesis, two studies concluding that there is significant statistical difference at N170 and two more studies defending the opposite theory. The systematic literature review of Monteiro et al. summarizes well this controversy.

A Systematic literature review is a replicable method for searching relevant articles to review the state of the art using multiple search engines. To do that first we need to choose our bank of data, which in our case was Scholar Google and PubMed. After that we choose some relevant Key words related to our study. In our case we choose: "N170" (all fields); "re-reference" (all fields); "eeg filters" (all fields); "high-pass filters" (all fields); "emotion recognition" (all fields); "vep" (all fields); "emotions"; "happy" (all fields); "sad" (all fields); "happy vs sad" (all fields); "eeg" (all fields); "interpolation" (all fields); "ICA" (all fields). Before reading any article, we read the abstract to see if that article were on some way related to our work and the content were relevant for our research. After that, if the article was considered relevant, that article was completed read a noted their conclusions. We tried to search for different opinions in what matters of the difference on N170 of happy and Sad to show both sides. Then we search for articles that referred differences on ERP changing some methods. The resume of our search is in the next table and after that we will proceed to explain 6 relevant articles from this list. [11][49][50]

Table 1.Table of some articles searched

Authors	Year	Stimuli	Peak of ERP	Eletrodes where ERP was analyzed	Reference	Difference searched	Results
Acunzo et al.	2012	Faces: Neutral, Fear, Happy	Near 200ms (N170)	C1, CP1, CP2, P1, P2	Mastoids	Influence of high pass filter (no high pass; 0.1 Hz; 0.5Hz; 1Hz)	High pass filter influences the amplitude of N170
Almeida et al.	2014	Faces: Neutral, Happy; Angry, Fear Disgust	130ms-200ms (N170)	P7, P8	Average reference	Effect of emotions in N70	Amplitude varied significantly between emotions
Batty et al.	2003	Faces: Fear, Disgust, Sadness, Happy, Surprise, Neutral, Anger	140ms (N170)	P7, P8	Average Reference	Effect of emotions in N70	Amplitude varied significantly between emotions
Campanella et al.	2006	Faces: Neutral, Happy, Fear, Sad	160ms-230ms	T5, T6	Average reference	Effect of emotions in N170	No differences between emotions
Chen et al.	2012	Faces: Neutral, Happy, Sad	130ms-200ms (N170)	P7, P8	Earlobes	Effect of emotions in N170	Amplitude and latency varied significantly between emotions
Dubal et al.	2011	Faces: Neutral, Happy	130ms-190ms (N170)	P7, P8, P5, P4, P3, P1, P2	Average reference	Effect of emotions in N170	No differences between emotions

Eimer et al.	2003	Faces: Anger, Disgust, Sadness, Surprise, Fear, Happiness, Neutral	150ms-200ms (N170)	T5, T6	Earlobes	Effect of emotions in N170	No differences between emotions
He et al.	2012	Faces: Neutral, Happy, Sad, Angry	140ms-200ms (N170)	Fz, Cz, Pz	Mastoids	Effect of emotions in N170	No differences between emotions
Holmes et al.	2006	Faces: Neutral, Fear,	160ms-220ms (N170)	T5, T6	Earlobes	Effect of emotions in N170	No differences between emotions
Jiang et al.	2014	Faces: Neutral, Happy, Angry	130ms-200ms (N170	P7, P8	Average reference	Effect of emotions in N170	The reference influences the amplitude and latency of N170
Joyce et al.	2005	Faces, Cars	140ms-200ms (N170)	P7, P8	Mastoids, Earlobe, Nose tip, Average reference, vertebrae C7	Effect of reference at N170	The reference influences the amplitude and latency of N170
Lee et al.	2007	Faces: Neutral, Happy, Fear	140ms-210ms (N170)	PO8	Vertex	Effect of reference at N170	The reference influences the amplitude and latency of N170

Liljander et al.	2016	Ear stimuli	P50	Cz	Mastoids	Influence of high pass filter(0.1Hz; 0.5Hz; 1Hz; 2Hz; 5Hz; 10Hz)	High pass filter influences the amplitude and the wave
Morel et al.	2014	Faces: Neutral, Happy, Fear	100ms-200ms (N170)	P7, P8	FCz	Effect of reference at N170	The reference influences the amplitude and latency of N170
Rigoulot et al.	2011	Faces: Neutral, Fear	140ms-200ms (N170)	P7, P8	Average reference	Effect of reference at N170	The reference influences the amplitude and latency of N170
Tanner et al.	2015	Sentences	150ms-300ms; 300ms-500ms (N400); 500-800ms (P600)	C3, Cz, C4, CP1, CP2, P3, Pz, P4	Mastoids	Influence of high pass filter (no high pass; 0.1Hz; 0.01 Hz; 0.3Hz; 0.5Hz; 0.7Hz; 1Hz)	High pass filter influences the amplitude and the wave

The study of Batty & Taylor implemented a task where they showed to 26 adults, 13 males (10 right-handed) and 13 females (12 right-handed), images of faces expressing emotions or a neutral expression. The task used by them consisted in showing black and white images of adult neutral or emotional faces. To validate the classification of the emotion expressed in the image, this was presented to a group of 20 people that did not participate in the ERP study to classify them. For the image to be approved and used as a facial expression, it should be classified with the respective expression by 18 of the 20 participants. [13]

Figure 8. Figure taken from Batty & Taylor study and represents the faces used in their task.

The ERP tasks consisted in 3 blocks shown twice in random order. Each block had 70 images of facial expressions, 10 for each expression (fear, disgust, sadness, anger, neutral, surprise and happiness) and 15 images representing objects (e.g. cars) which were shown randomly. The luminance was equal for all expressions, images were 11x8 cm and they were shown on a computer screen with 50 cm from the participant for 500ms. Upon the presentation of hte series of images the participant had to respond with a mouse-click to the target stimuli (e.g. cars). [13]

ERP's were recorded from 32 scalp electrodes according to the international 10-10 system. The average reference was used as offline reference and the impedances were maintained below 5 kΩ. EEG was sampled by SynAmps for 1100ms with a band-pass of 0.1-30Hz. Trials with ocular activity were rejected. N170 was measured at P7 and P8.

In the results they found that the latency of N170 for this experiment was around 140ms and they varied with emotions where the faces expressing fear, disgust and sadness were later comparing with neutral, happiness and surprise. They also found some significant differences at the amplitude with a larger amplitude for fear and the lowest amplitude at neutral, although the effect sizes were rather small. The results are expressed in figure 10. [13]

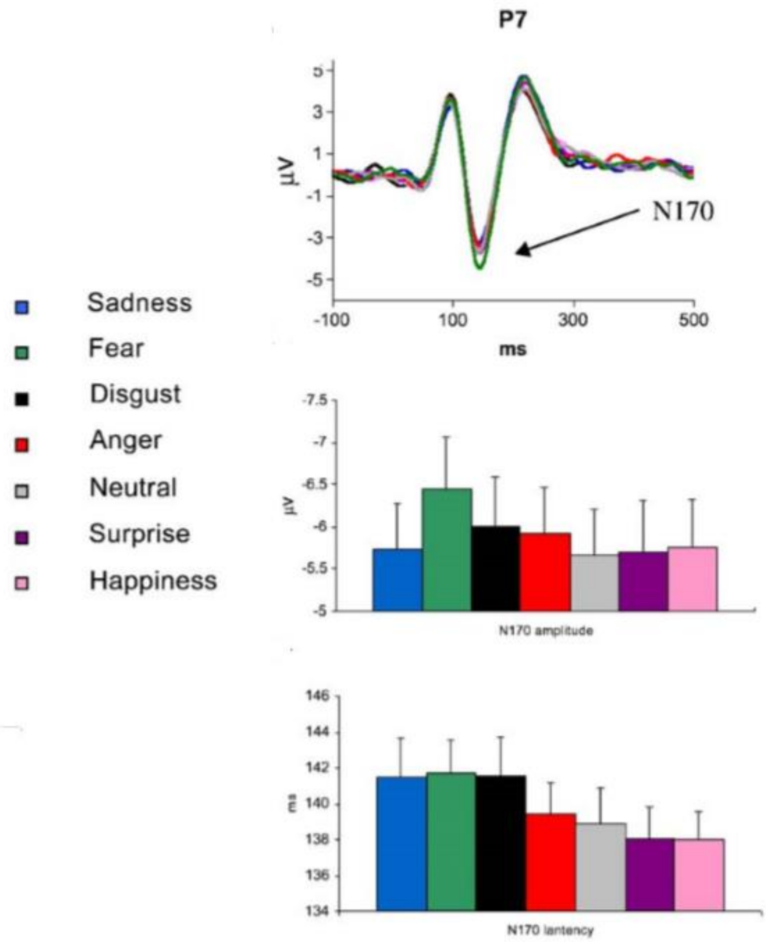


Figure 9.Figure taken from Batty & Taylor study. This represents the results from N170 in terms of amplitude and latency for the differents emotion that they used at their task.

They conclude that the different seven emotions produced different effects in amplitude and latency of N170. They also found that the emotion content of a facial detail is processed in a fast manner and is reflected in the N170. The last conclusion that they made about this problem was that the latency of N170 in negative emotions is larger than the neutral and positive ones. [13]

Chen et al. made a clinical study that is consistent with the previous one. They used three groups of patients, first one composed by people that they just had their first episode major depression (F-MD), second one composed by people that had recurrent major depression (R-MD) and the last one that was composed by 46 healthy patients (22 males, 24 females) which they used as control (HC). In the paper they used the HC group to compare the amplitude and the latency of N170 with the other two groups. They also statistically compared the different emotions that they used in the task for each group. We will just focus on the results that they obtain for the within group comparison that they made in the HC group. [14]

The task was an “emotional oddball paradigm” which consisted in showing images of faces to a patient that should react to the presence of emotional faces. The stimuli consisted in faces being neutral, sad, happy. More than one face from a different person has been shown to the participants. [14]

The stimuli were presented for 100ms and after that the subjects had around 1400ms to process the face by seeing a black screen, finishing a 1500ms time to answer to the stimuli. [14]

The EEG data were acquired using a BrainAmp MR portable ERP system, using 32 electrodes in the scalp which were placed according to the international 10-20 system. To the online reference they choose the ear electrodes. The data were filtered at 0.1 high pass and 30Hz low pass. Signals with more than 70µV were considered artifacts and rejected. Only trials being corrected were used in the analysis. They set a range time for the epochs of 200ms before the stimuli and 800ms after with a 200ms baseline time. To process all this, they used the Brain Vision Analyzer software. [14]

They restricted the analysis channels to P8 and P7 and being the statistical significance threshold set to P<0.05. [14]

Using all this method they conclude that in what matters to the N170 amplitude, the amplitude of happy faces was significantly higher than neutral and sad faces and amplitudes for neutral faces were significantly higher than sad faces. They also concluded that for the N170 latency the happy ones were significantly shorter than the sad and neutral ones. Figure summarizes shows the ERP results that they got. [14]

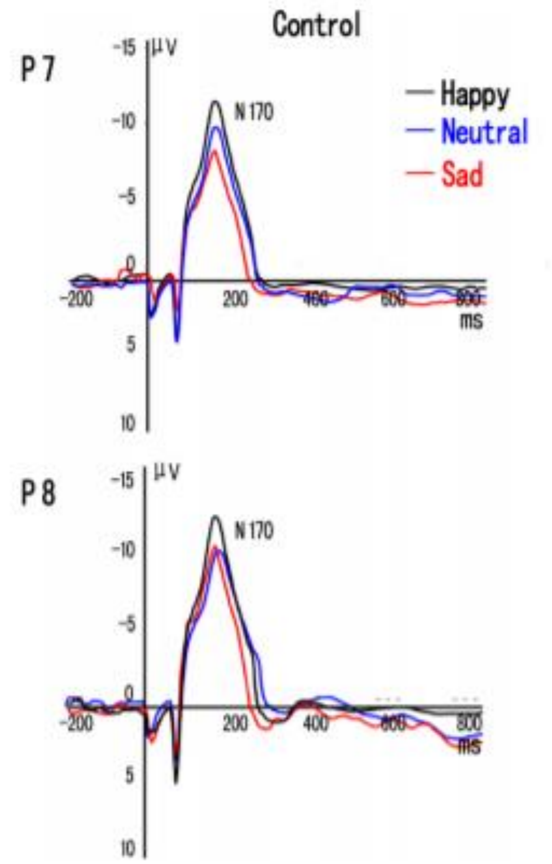


Figure 10. Figure taken from Chen study showing the results of N170 comparing the Happy, Neutral and Sad emotions at channel P7 and P8.

On the opposite side, there are some studies that did not replicate these results. They could not find any significant difference between the emotions and between emotions and neutral faces, so they defend that the is no effect in what matters of emotions present in the N170.

One of these studies is the project of Eimer et al. in 2003. For their study they used 14 participants (7 male and 7 female) with a range for the age between 18 and 54 years and an average age of 28.6 years. [15]

For the task they apply a stimulus that consisted in showing faces of 10 different people taken from a set of pictures. The pictures were with faces expressing angry, disgust, fear, happiness, sadness, surprise or being just neutral. The task consisted in showing 24 blocks, each block containing 80 trials and on each trial two faces showing the same emotion were presented to the subject with a fixation white cross separating them. In the first 12 bocks, the pictures were presented randomly in a computer screen, being 40 of that trials emotional faces and the other 40 trials a mix random of emotions and neutral faces. The last 12 blocks were a different task where the subjects should focus on the lines near the fixation cross and press a button when the length of them were different. The faces in this part of the task were irrelevant. The screen was with 70cm from the participant. All the stimuli were presented for 300ms and with an interval of 2000ms between two stimuli. [15]

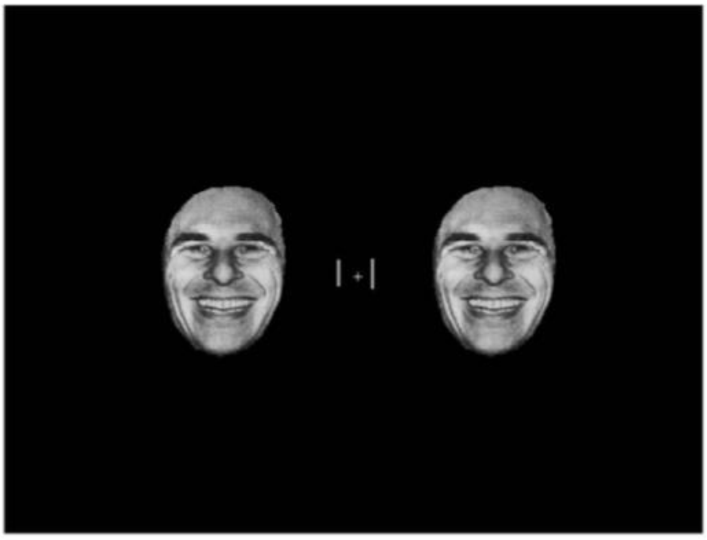


Figure 11. Figure taken from Eimer et al. study. It represents the stimuli presented in the task.

They used 21 electrodes displayed according to the 10-20 system and with an online reference at the earlobe electrodes. The impedance was kept below 5kΩ and the amplifier bandpass was 0.1 to 40Hz. No digital filters were used to the average data. The epochs were set with 800ms period time, 100ms before the stimuli and 700ms after the presentation of the stimuli with a baseline of 100ms before the stimuli. They rejected trials with too much eye movements, eyeblinks (Fpz above 60µV) and other artefacts where the voltage was above 80µV. To the facial task they used the channels T5 and T6 to analyse the N170 (they say that is in this channel where N170 is maximal) and they focused on the latency and the amplitude. [15]

In the results, first they made an average of the six emotions and compared to the neutral faces and then they compared each emotion with neutral faces. They found that N170 was maximal at T6 and T5 with a range of 150ms-200ms of latency. They got no statistically significant differences in the amplitude or latency, concluding that emotional content does not have any type of effect in N170 [15]

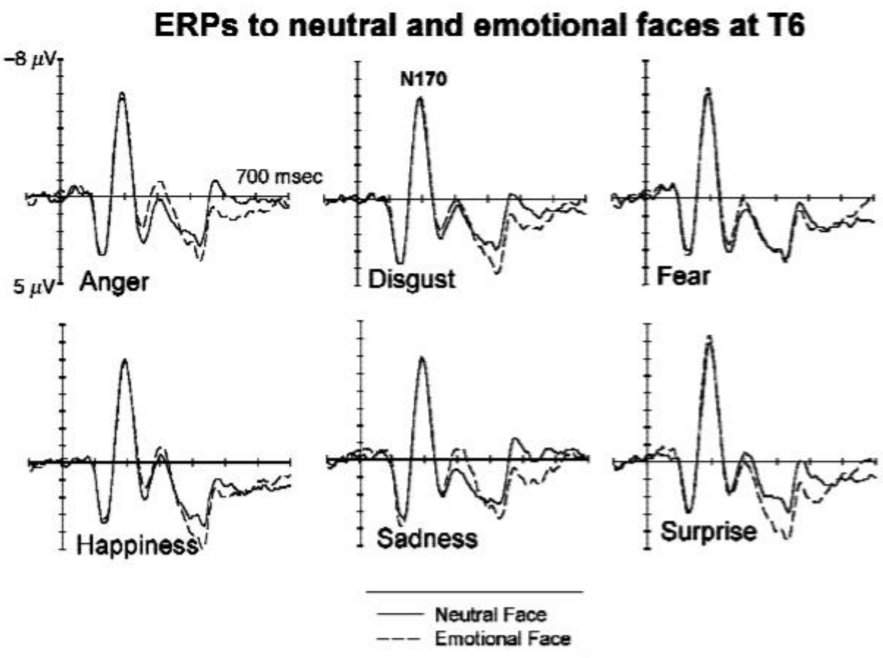


Figure 12. Figure taken from Eimer study. It represents the N170 comparing na emotion with a neutral face.

Holmes in 2006 performed a similar study. The objective was the same, to know if emotions have some effect or not in N170.

In his study there was 12 participants (4 male and 8 female) with a range of ages between 18 and 41 years and with an average age of 31 years. All subjects were normal and were right-handed. [16]

The stimuli were images of faces expressing fear or neutral of 10 different people. The faces were shown bordered by two lines, one on the right side of the image and one on the left side. These lines could have different lengths and all the possible arrangements were presented randomly. [16]

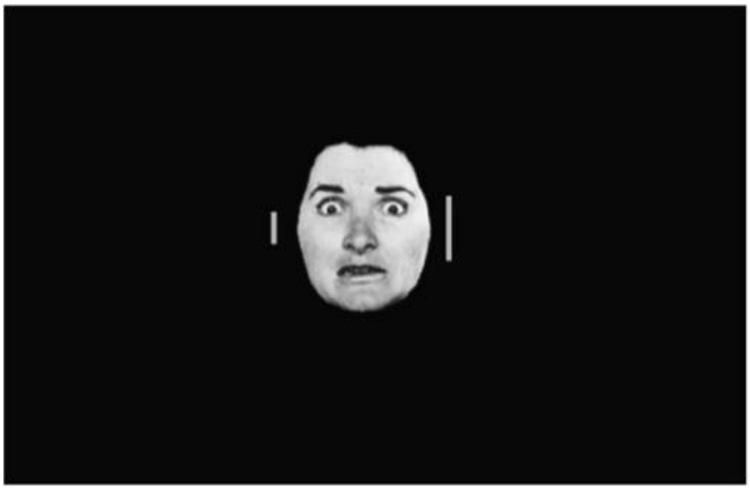


Figure 13. Figure taken from Holmes study. It represents the stimuli presented in the task.

The experiment had two tasks, one relative to the lines and one to the emotions. In the emotion task they had to click in a button with a finger when a face was immediately repeated in that block of images. The subjects had to perform two blocks and each one had 92 trials, each one showing a face. The stimuli were presented for

300ms with an interval of 1200ms. Each block had 12 immediately face repeated and the other 80 trials were 40 with a fear face and the other 40 with a neutral face. [16]

The EEG were recorded with 23 electrodes according 10-20 international system. The impedances were kept below 5kΩ. They used a baseline of 100ms pre-stimulus and only the trials that were non-repeated were counted as epochs. Trials with eye blinked and eye movement were rejected. [16]

To make the statistical analysis they used ANOVA's on the amplitudes in the range of latencies of 120-160ms, 160-220ms, 220-300ms and 300-700ms. The results were compared in the lateral electrodes (F3, F4, C3, C4, P3 and P4), in the midline electrodes (Fz, Cz and Pz) and the lateral posteriors electrodes (T5 and T6). [16]

They concluded that no significant amplitude effects were found at latencies between 120-160ms post stimulus. In the 160-220ms significant differences were found between neutral and fear faces at lateral electrodes and midline electrodes, concluding that fearful faces had a more positive ERP then neutral face. At lateral posterior electrodes (T5 and T6) they found no significant differences between emotions although the fear face was more positive then neutral one. They concluded that the N170 were not affected by emotions [16].

It is quite clear that relatively similar studies left to different results, which has led to a strong debate why this is the case. Assuming that that task differences are minimal, one of the reasons of getting different results may be the different methods in the pre-processing data. Maybe the digital filters used, or the reference used or even analysing different channels may lead to different results. This is one of our goals in this thesis, to evaluate if the pre-processing methods may influence or not the amplitude and the latency of an ERP.

The study of Joyce & Rossion (2005) refers that and focus on the influence of the reference. The mains goals of them was to investigate if the amplitude of N170 is dependent or not from the reference chosen and to what area N170 is maximal. [17]

In their study they used 16 participants which 10 were male and 6 were female with ages between 21 years and 39, being the average age at 27.5 years. [17]

The stimuli consisted in presenting 16 grayscale images of an object (a car) and 16 grayscale images of a face and 16 words (no more than 7 letters). The subject was at 100cm from the computer screen. They used that 46 images and another 46 inverted images, making a total of 96 trials. They were presented with 4 blocks, each block had 96 stimuli, having a rest period between blocks of 1 minute. The stimuli were presented for a period of 250ms, having an interval randomly that went from 1250ms to 1750 ms. The subjects had to press a button when the image appeared was inverted. [17]

The EEG was recorded using 53 electrodes using the 10-20 international system. Four additional electrodes were used to control the eye movements and the blinks. The online reference was a left mastoid. Additional reference recordings were used as the right mastoid, ear lobes electrodes, nose tip, right sternoclavicular junction and vertebrae C7. Bandpass filter online was used from 0.01 Hz to 100Hz. [17]

They used EEprobe 2.0 (ANT, Inc.) to analyse the data recorded. They filtered the data with a digital 30Hz low pass. They also rejected some epochs that had artifacts, eye movement and blinks. They also did different studies using different offline reference: average mastoids, average earlobes, non-cephalic, nose and common reference. [17]

They confirmed that the amplitude of N170 were higher at right hemisphere then the left hemisphere of the occipitotemporal electrodes. They also concluded that using the nose as reference, the amplitude of N170 would get higher. For all references, a positive peak appeared after the N170. This is corroborated by figure 14. [17]

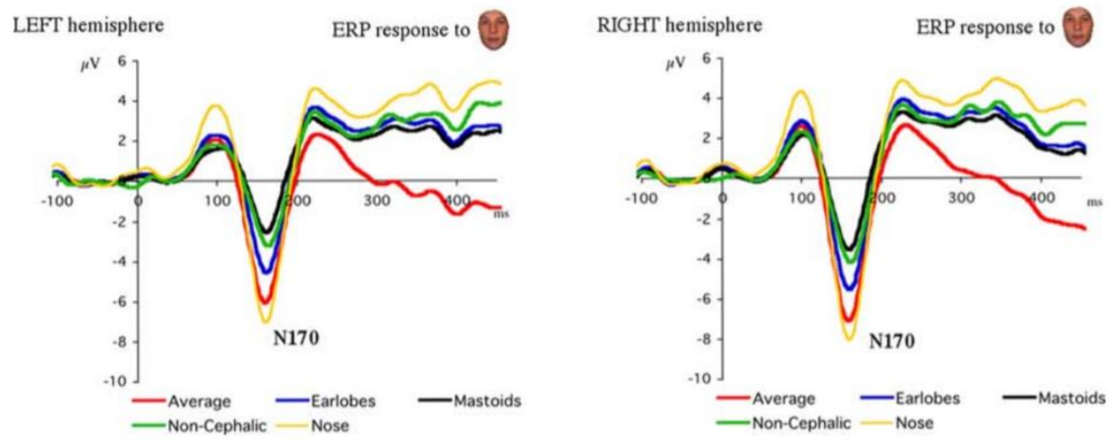


Figure 14.Figure taken from Joyce & Rossion study. It represents the N170 of faces comparing the different references at left hemisphere and right hemisphere of occipitotemporal eletrodes.

At the comparison of faces with cars and words the amplitude was relatively larger for faces when comparing with the other two categories. Again, the N170 was larger on the right hemisphere comparing with the left one. The latency of N170 was significantly different when compared words with cars, but not significant when compared with faces. Comparing faces with cars showed no significant differences on latency. [17]

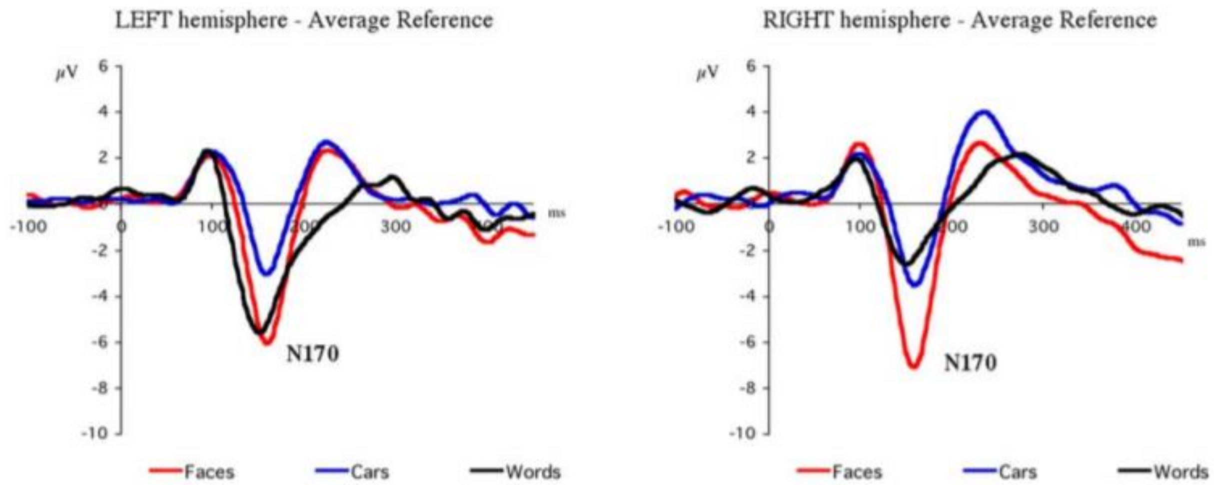


Figure 15. Figure taken from Joyce & Rossion study. It represents the N170 using average reference at left hemisphere and right hemisphere of occipitotemporal electrodes.

The N170 amplitude analysis showed pertinent significant effects of reference, hemisphere, Reference X category and Category X hemisphere. They found significant differences in the amplitude using nose vs the others and significant differences using the earlobes and the average vs the rest. Concerning category, faces had a larger amplitude than cars and words and words had larger amplitude than cars.

The interaction between category and reference showed that there was no difference between cars, faces or words using mastoid and non-cephalic references. Using average reference, all categories were significant different from each other. With the earlobe, only faces were larger than cars and with the nose reference faces were larger than cars and words. [17]

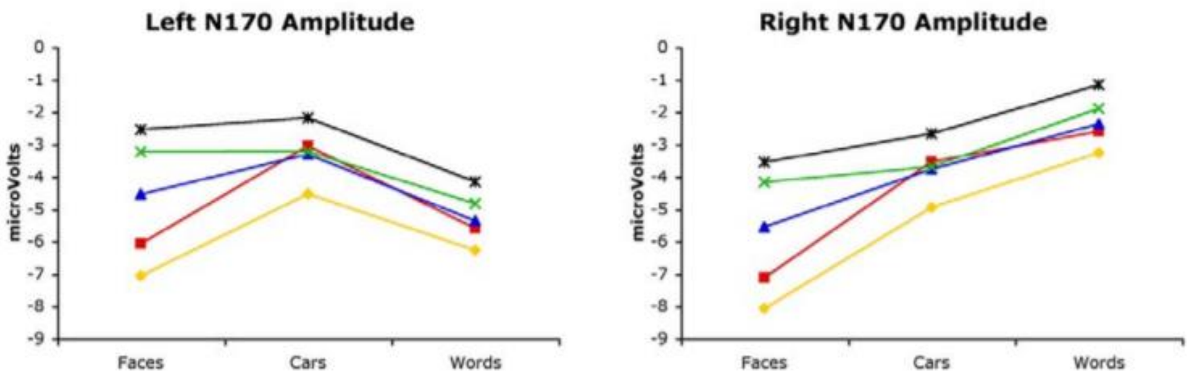


Figure 16. Figure taken from Joyce & Rossion study. This represents the different amplitudes of the three categories using different references. In yellow they used the nose as reference, red the average, blue the earlobes, green non-encephalic and black the m

They concluded that the N170 is dependent from the reference one chooses and choosing a wrong reference can have a significant impact. In general, they suggest using an average reference to improve power in the analysis. [17]

Another study that investigates whether pre-processing methods may lead to different results in the ERP analysis is the study of Acunzo (2012). In his study the authors address the influence of high-pass filters. [18]

They included 24 subjects, all right-handed. To complete the task, the subjects were submitted to an image showing a fixation point and near to that, an arrow pointing to the left or the right for 200ms. After this facial stimulus were presented to subjects for 300ms. Between the fixation and the facial stimulus there was a 750ms interval. The face could appear in the right side where the arrow was pointing or in the opposite side. To perform this task, the subject had to press a button when a happy face was in the direction of arrow. Happy, fearful, and neutral faces were shown as stimuli, divided in 352 trials with happy faces and 768 trials of neutral plus fearful faces equally divided. [18]

The EEG was recorded using 64 electrodes according the extended 10-20 international system, plus the 2 mastoids and using 4 electrooculographic (EOG) electrodes. [30]

To pre-process the acquired data, they used the EEGLAB toolbox. First, they re-reference the data using the mastoids. After that they passed to the filters where they low-passed the data with a cut-off frequency of 40Hz and they varied the high-pass frequency (0.05, 0.1, 0.5 and 1Hz) in order to study the principal effects of using high-pass filters. They used the FIR filter default of EEGLAB. They made the epochs, setting a baseline period of 100ms pre-stimuli. The last thing that they did was to remove epochs showing artifacts, eye movements and blinks. [18]

To analyse the ERP they used an average of 4 channels (P1, P2, CP1 and CP2), using ANOVA to compare the condition and the cut-off frequencies used. The epochs were set to -100ms pre-stimulus and 500ms post-stimulus.[18]

They concluded that high-pass filtering has effects on the N170, affecting the waveform as we can see in the figure 18. Increasing the cut-off frequency makes the positive peak after 200ms disappear. However, this is not the only effect of increasing the cut-off frequency, it also makes the amplitude of N170 smaller. [18]

The next thing that they examined was the scalp topography, concluding that the high-pass filter influences the scalp topography, which can give misleading information to researchers in source reconstruction. This can be seen in the figure 17, where "diss" means dissimilarity which goes from 0 to 2, where 0 means that the map of scalp topography are identical, and 2 means inverted maps. [18]

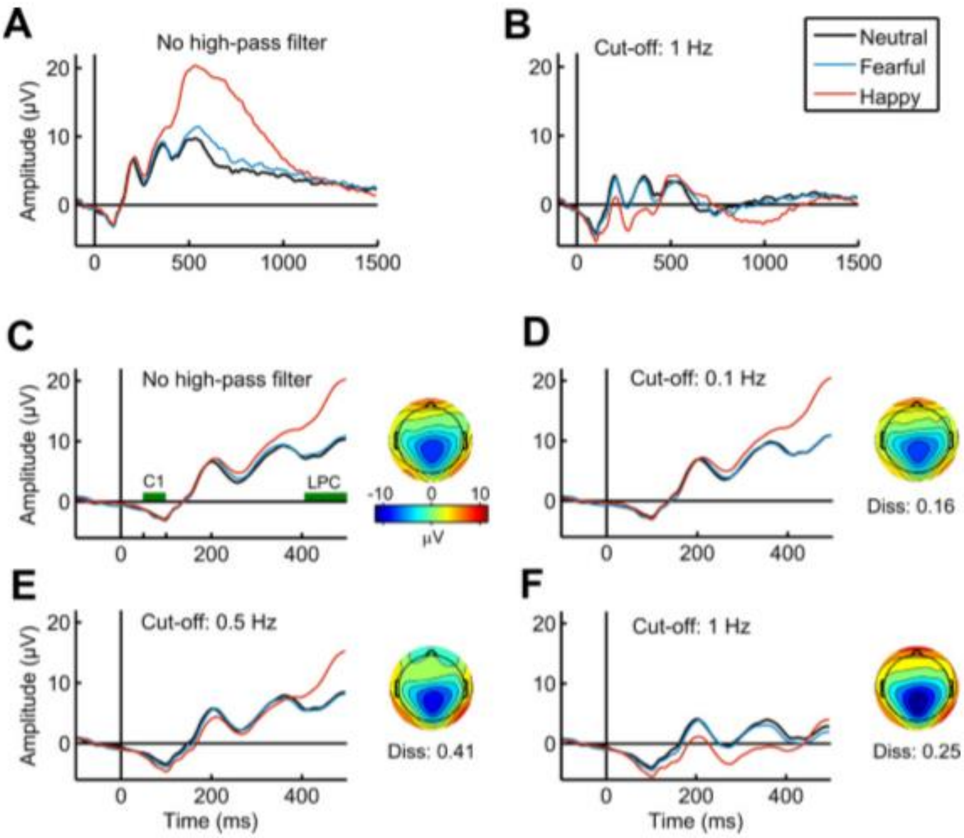


Figure 17. Figure taken from Acunzo's study representing the influence of high-pass filters in emotion recognition. This ERP is the average of CP1, CP2, P1 and P2.

Using the channel C1, they show the impact of different cut-off frequencies on amplitude and significance levels which can be seen in figure 18 [18]

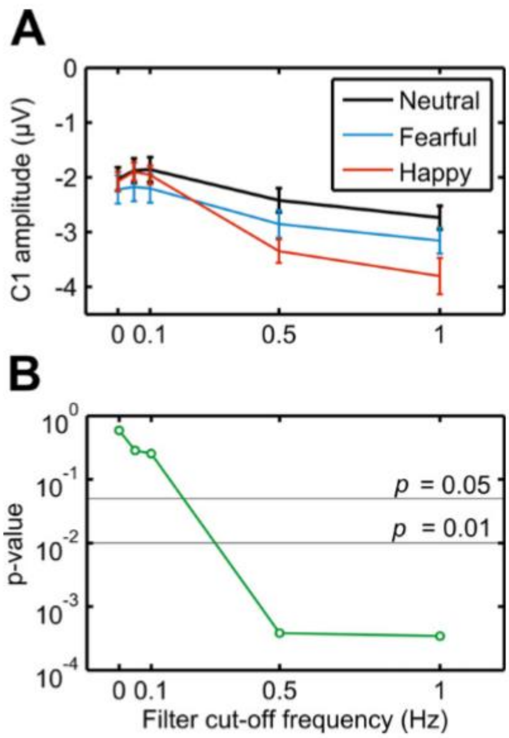


Figure 18. Figure taken from Acunzo study. Represents the statistical study for each condition.

The last thing they made was to test the correlation between the late positive peak around 500ms and the N170. The theory that they made was that late peak would contribute significant to the amplitude of N170. They found that correlation and said that the effects on N170 were because of that late positive subcomponent. [18]

Error negativity (NE) or error-related Negativity (ERN) was reported for the first time in 1991 by Michael Falkenstein and his co-workers and two years later by Gehring as an ERP wave associated with errors in tasks choices. The literature says that this ERN wave starts in the moment or sometimes a bit before the wrong choice had been choosen and peaks about 100ms after starting. The latency varies, even in the literature, because it depends on the response devices and the type of the task. The scalp distribution has is maximum at FCz which has his location in the middle of the scalp. This wave appears in trials that the subject has performed an error, irrespective of the type of task. [11]

ERN can be interesting to understand if the brain can predict the error even before it is consciously perceived. We give some relevance to this wave, because as you will see in the next chapters, our work can be applied to this type of studies.

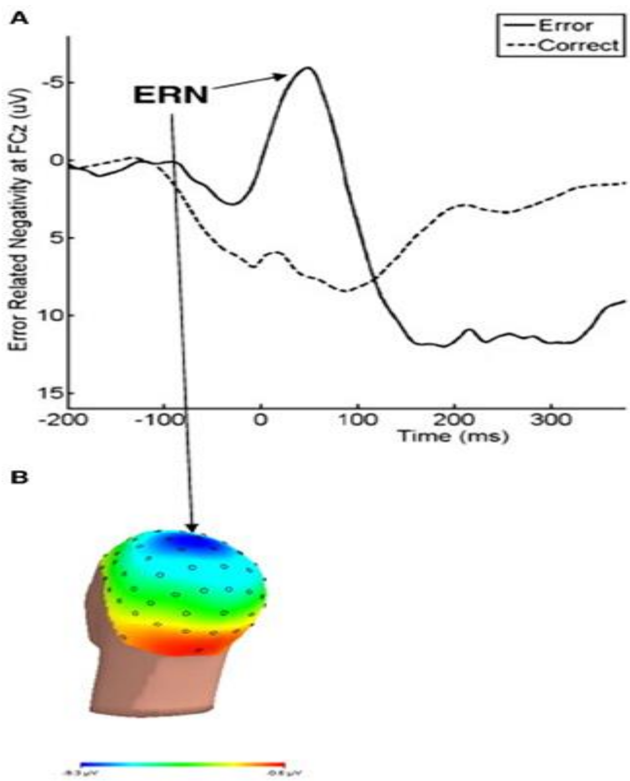


Figure 19. Example of a plot showing an ERN (A) and his respective topographic map (B) that shows the max gradient in the midline frontal central scalp locations, more precisely FCz. [22]

Chapter 2: Methods

In this chapter we will focus on describing and explaining which pre-processing methods and which analysis methods are used to analyse data from EEG signal. To realize the pre-processing methods, we use the scientific software MatLab and the EEGLAB toolbox. Our objective was to pre-process the acquired data and use it after that to compare the ERP between sad and happy and between different visual stimulation condition (sad/happy and neutral conditions. The pre-processing methods that we used and that we will explain are: 1) interpolation of channels, 2) re-reference, 3) filters, baseline correction and epochs rejection and 4) Independent Component Analysis (ICA). We will compare the conditions for the latency and amplitude of the peaks, using different corrections to evaluate if there are any significant statistical differences between them.

2.1 EEG PROCEDURE

2.1.1 Subjects

Our study case has 10 participants with 4 females and 6 males, in which 7 are right-handed and 3 is left-handed. The average age is 25,125 years old. We have done 4 EEG runs, each one with around 6 minutes of recording separate with breaks in order to the participants to rest and start again the task concentrated in it. The participants were from University or staff members.

2.1.2 Procedure

During subject scalp preparation for EEG acquisition, the scalp of the participant has been cleaned with cotton and alcohol in order to get it cleaner and disinfected to get better impedances, which is critical to achieve a good signal to noise ratio. After that the cap was placed on the participant scalp, we fill each electrode with conductive gel using a syringe and after connecting the electrodes we test the impedances. We make sure that the impedances were as low as possible with a quality threshold around 10 ohm.

The participant next performs the task. Participant was seated 60cm in front of monitor where the stimuli is presented. The stimuli were shown in a monitor with resolution of 1024x768 pixels. The stimuli were divided in 4 runs with 84 neutral faces, 42 happy faces and 42 sad faces presented randomly, getting a total of 168 happy and sad epochs for each participant to analyse offline.

Participants received an explanation of the task previously to the experiment. We explain that the task consists in a 5 seconds preparation and after it a neutral face is shown for 1 second. Is given a gap of 500ms with a black screen after it and then it is shown the instruction. The instruction given is random and this procedure is repeated until participants get 42 happy and sad instructions each. The instruction varies into 3 different tasks. If the instruction is a happy or sad face looking forward, the subject must perform a nogo task. If the instruction is a happy face looking to one side, the subject must perform a pro-saccade to the same side. A saccade is a ballistic eye movement of the participant in the same (saccade) or opposite (anti-saccade) direction of the face shown. If the instruction is a sad face looking to one side, the participant need to realize an anti-saccade which is the opposite of a saccade. After the instruction has been shown there will be a black screen only with a cross in the centre where the participant needs to fixate for an average 750ms. For last a target is shown for 200ms and after that participant must perform the anti-saccade or the saccade. The task is depicted in figure 20.

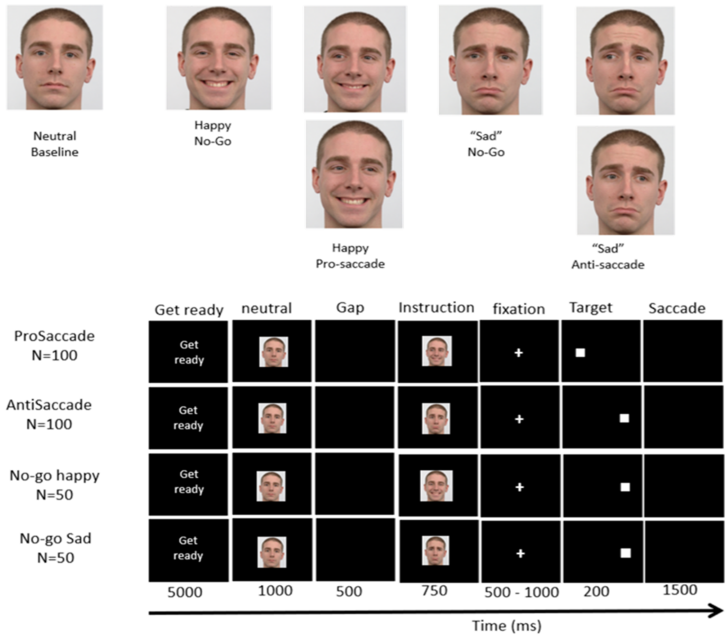


Figure 20. Experimental Paradigm.

2.1.3 EEG Recording

We used a 64 channel Quick-cap from Compumedics Neuroscan. The electrodes were positioned according to the extended 10/20 system. The SynAmps 2/RT amplifier had a sampling rate of 1024 which acquire the data through Scan 4.3 software (Compumedics Neuroscan). The impedance of the electrodes was maintained below 10 kiloOhms. Electrodes which had bad impedance or were acquiring to much noise were marked to be considered for exclusion or interpolation when the EEG was analysed. All electrodes were recoded related to Cz as online reference.

An eye tracker iView Hi-speed 120 with a sampling rate of 1250Hz was used to track the eye movements and make sure that the saccades and the anti-saccades were made.

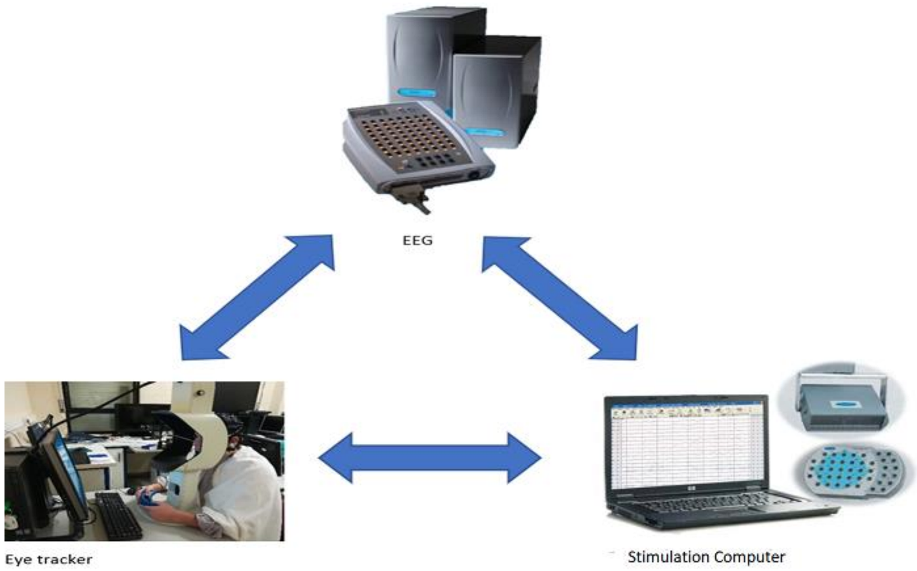


Figure 21. Tools used.

2.2 PRE-PROCESSING METHODS

2.2.1 Channel Interpolation

Interpolation of channels is a pre-processing method used on the EEG signal, and its function is to replace missing channels or replace channels that have a high noise level. Is important to interpolate these bad channels because they will affect the average electrode reference which will leave our data much noisier and will cause unnecessary rejections of epochs, leading to a inappropriate data analysis. This interpolation is done with the data of nearest channels using the spherical standard method and its recommended to be done early as possible in the analysis pipeline. First of all, we need to identify our bad channel or the missing channel (figure 22). In cases where more than one channel needs to be interpolated, we must interpolate all the channels at the same time to minimize the errors. If we want to interpolate two channels that are near to each other and this is not done at the same time, those channels will receive noise of the other during the single interpolation. [28]

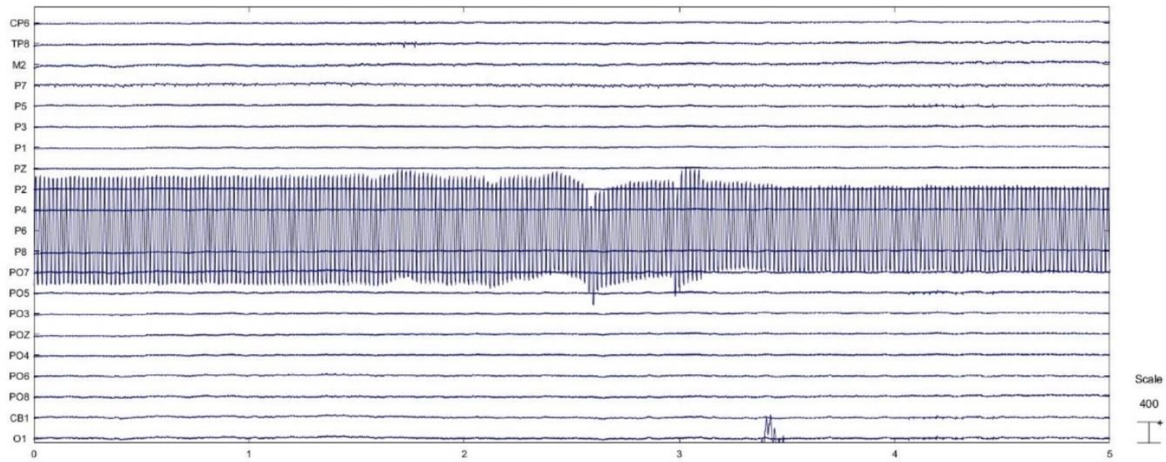


Figure 22. Example of a channel that needs to be interpolated (channel P6). It´s called a bad channel and the reason of that is because the electrode was not working properly during the data acquisition.

To interpolate we used the spherical standard method, which is the spherical spline surfaces. This method consists in a mathematical expression (1), where V ($\vec{r}$) is the potential at that point (relative to other point), c_0 and c_j are constants fit to the data, $g_m(x)$ a function (2), $\vec{r_j}$ the location of a measurement electrode on the spherical scalp and $\vec{r}$ the location of an arbitrary surface point. V ($\vec{r}$) is given by [29]:

$$V(\vec{r}) = c_0 + \sum_{j=1}^{N} c_j \, g_m\left(\hat{r} \cdot \hat{r_j}\right) \qquad (1)$$

Where the product $\left(\hat{r} \cdot \hat{r_j}\right)$ is the cosine of the angle between $\vec{r_j}$ and $\vec{r}$. The function $g_m(x)$ given by [29]:

$$g_m(x) = \frac{1}{4\pi} \sum_{n=1}^{\infty} \frac{2n+1}{(n(n+1))^m} P_n(x) \qquad (2)$$

Where $P_n(x)$ is the n^{th} degree Legendre polynomial. With the data that we had (1), we try to compare two different situations. [29]

The first plot (top left plot) shows the ERP data on channel P6 without doing the interpolation of that channel (figure 23). The second (top right plot) is the same data processing but after the interpolation (figure 23). Comparing the plots, the first is very noisy and the ERP cannot be identified. However, after interpolating that channel, the ERP becomes more visible and easier to identify since the bad data is no longer there and got replaced by the method explained before. This bad data not only influence the analysis on the channel that has artefacts but also has influence on the results of the other channels (figure 23, bottom plots).

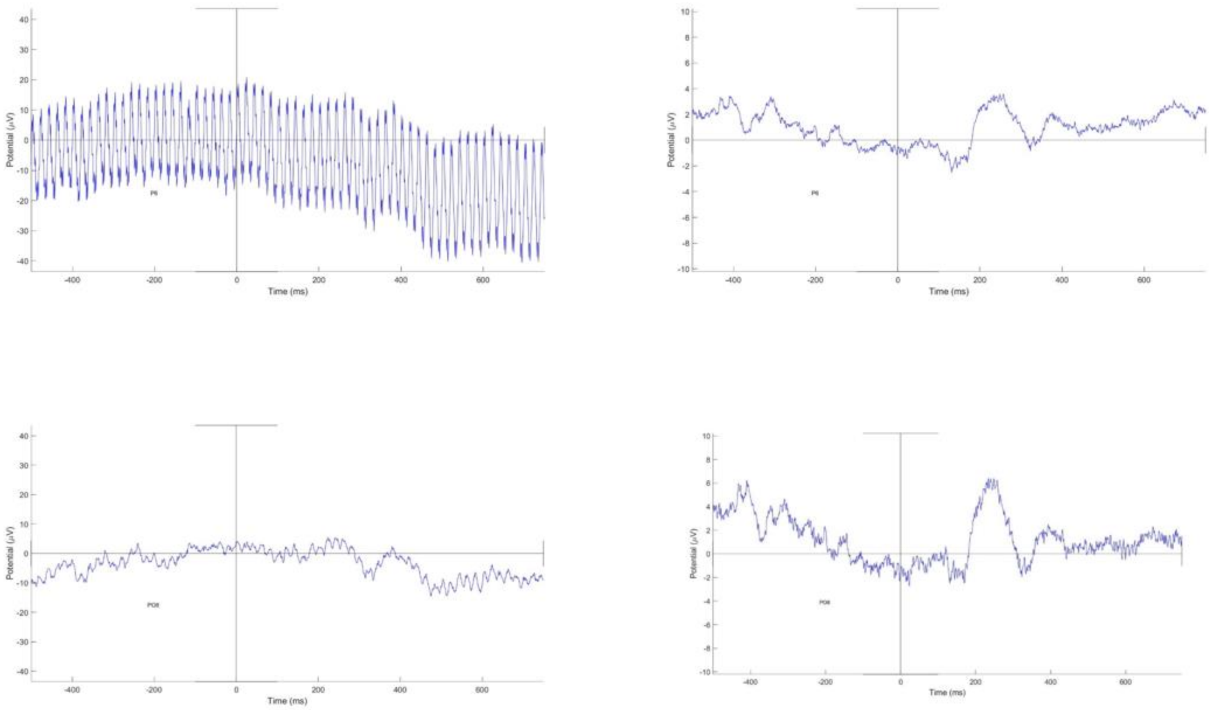


Figure 23. Top left plot: Example ERP of the bad channel P6 during data acquisition prior to interpolation; Top right plot: ERP of the channel P6 after the average reference and interpolation. ERP is now clear, and one can identify the different peaks (e.g. N200 and P300); Bottom left plot: Data analysis of the channel PO8 after doing the average reference and without interpolating P6; Bottom right plot: Data analysis of the channel PO8 after interpolating P6 and doing the average reference.

In this case we cannot identify the ERP on the top left plot of figure 23 because this channel is being affected by the re-reference method. Doing the re-reference including a channel that is very noisy does contaminate the data of the other channels.

It is very important to interpolate the missing channels and the channels that have much noise in order to get clean data for further processing, not only in that channel but in all channels that we are considering. [28]

2.2.2 Reference

EEG records the electrical potential resulting from the brain activity between two scalp points and is expressed in units of microvolts. The signal that we get from the channels is the difference in electrical potential between that specific channel and the reference electrode. The online reference channel is usually combined with a ground electrode and can be changed during the offline analysis to another reference system. The original reference is always the ground reference, where the ground electrode is connected to the ground circuit of the amplifier leading to some electrical noise that normally is not present in the scalp electrodes. Since the displayed signal that we get is the difference between the reference and the channel, subtracting the ground electrode to the electrodes of scalp we are adding electrical noise to the result.[31][32][33]

To decrease this potential source of noise, a second channel is used as an online reference. To change that we choose between all the electrodes of the scalp one to be the reference during the EEG recording. Doing this will reduce the ground noise and the signal will be in function of the online reference. [31]

$$[(channel - ground) - (online\ reference - ground)]$$
$$= channel - online\ reference$$

Typically, the online reference chosen is close to FCz or Cz but there is the possibility to choose a different reference after the recording, that is called offline reference in a process called re-referencing. It can be done by choosing another recorded channel or the average of a few channels. It is important to choose it wisely because this might influence the pre-processing and results. The voltage at that channels works in the same way as the online reference after changing from ground reference, but now the signal will be in function of the offline reference.[31][32]

$$[(channel - online\ reference) - (new\ reference - online\ reference] = channel - new\ reference \quad (10)$$

Choosing the reference is important because it might influence the amplitude and consequently the displayed signal of the channels that now are expressed in function of that reference channel. The location of the reference channel influences the topography of the EEG and the amplitudes of the other channels. Since the channel reference is the level zero voltage, changing it will directly affect the amplitudes of the channels because we use a new value at (10) and consequently the scalp topography shape will not be the same whereas the scalp topography is given by the amplitudes along the scalp by the different channels. It is also important to consider the region of interest that we are studying. The reference channel needs to be close enough to the area of interest and at the same time not too close. It needs to be close enough because is important that the reference channel has the same electrical noise as the channels in the region that want to study. This will allow to subtract error when re-reference and get cleaner data. This happens too with the reverse. If the reference channel that one is using has some noise and the region of interest not, that electrodes on the region will be contaminated by the noise of the reference channel. On the other hand, it should not too close because the difference in electrical potential between the electrodes around the reference and the reference channel will be around 0 since they both are affected by the same brain activity and consequently will reduce drastically the measured brain activity that we are recording since the signal will be subtracted during the re-reference.[31][32]

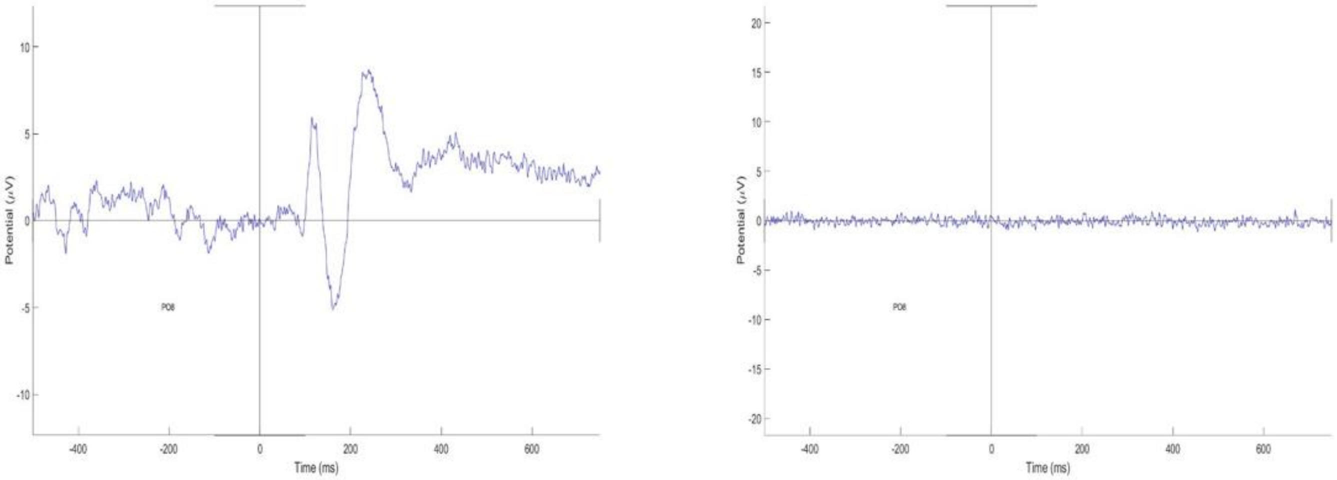


Figure 24. 24a (left plot): ERP results of channel PO8 using the channel P6 which is near to the region of the interest. 24b (right plot): Data analysis from channel PO8 using the average reference.

The figure 24b is an example of the previously explained problem. It corresponds to the data analysis of the channel PO8 after interpolating the missing channels and re-reference the data to the PO6 channel. These channels are near to each other and they are on the region of interest of this study since the task of this study was face recognition and it's expected to find an ERP at the right posterior channels as we will see in the next chapter. It is possible to realise that there is no ERP on the image and that is because the reference was chosen wrong, reducing the brain activity ERP result.

When we know that the region of the interest is in central areas of the scalp, we can use the average mastoids as reference. They are good because they do not record the brain activity of that region and at the same time, they are near to the other scalp electrodes picking up similar noise. If the region of the interest is lateral temporal areas, they are close enough to record the brain activity there and reduce the amplitude of the signal that one wants to study. To prevent this to happen in studies that we do not know the region of interest, we can use the average of all electrodes as reference. [31][32]

The average reference becomes mor optimal if between 64 and 128 electrodes are used to prevent the signal is not overrepresented in some regions of the brain and get the effects and the brain activity that we seek. It's important to cover all the scalp with electrodes to get a good performance of average reference and not reduce the amplitudes of the regions of interest in the brain. Here the new reference will be the average electrical activity of all electrodes along the scalp. The main advantage of this is that all channels will contribute equally to the new reference. This means that the signal amplitude will still be reduced when subtracting to the channels of the region of interest but on a smaller scale because all electrodes contributes to the subtracting reference which means that the expected signal will still be there to analyse. Using the average reference will also take in consideration the average error of all channels. This means that reference will consider the noise of each channel, average it and subtracting to each channel providing a smaller model of error for each channel. In summary, the average reference is a way to get a 'virtual' reference that is close to zero.[30][31][32]

The figure 24b is referent to the same study as the figure 24a and with the same pre-processing methods used. The only difference between them is that in the figure 24b we used average reference to re-reference. In this new image the ERP expected is now easy to identify because the signal was not significantly reduced by the reference and the error was subtracted effectively.

Summarizing, the choice of the reference is an important step that might influence the results. There is no perfect reference and the reference that is chosen need to consider several factors as the region of the brain activity that we want to study, the number of the electrodes used at the recording and the type of the analysis will be performed. Typically, the most used reference is the average reference because it approximates better to a ‘perfect reference’.[33]

2.2.3 Filtering

The next step of pre-processing after re-reference and interpolations is filtering the data. The purpose of filtering is to remove high-frequency and low-frequency artefacts and some frequency drifts that may appear. In the opposite side, previous literature state that the use of filters may lead to some signal distortions and can have significant influence in the latency and the amplitude of the peaks of the ERP. [34] That’s the reason why in the literature there are opinions that say filtering is a necessary step for data analysis and others say that is a mistake because will cause latency and amplitude alterations that will led to bias results. We will discuss these different opinions in the last chapter and use our results in order to arrive to a conclusion if filtering is good or not at pre-processing methods.

With filtering is important to preserve the signal but attenuate as much as possible the noise. The filters that we used were temporal which means that they attenuate the signal components in a specific frequency. There are different types of filters 1) High-pass, 2) Low-pass, 3) Band-pass and 4) Notch filter.

High-pass filters are characterized by letting pass the frequencies above the specific frequency chosen and reject the others. [36]

Lower-pass filters are the opposite of high-pass filters, they let pass the frequencies below and reject the others. [36]

Band-pass filter is a combination of high-pass and lower-pass filter, where we choose a lower and a higher edge of frequency pass band and the filter will let pass the frequencies between that range and reject the others. [35]

Notch filter also known as Band-stop filter is usually used when the record of EEG is contaminated by AC line voltage artefact. Is known that the line frequency is 50Hz, so it used the notch filter between the frequencies of 47.5Hz and 52.5Hz to reject the frequencies in that range. Using this is possible to clear the noise contamination by the electrical activity. [36]

We used Finite Impulse response (FIR) that is a type of digital filter characterized by an impulse response that becomes null after some finite time, in contrast with Infinite impulse response (IIR). To filter the data, we used a band-pass and the range was between 1Hz and 60Hz. [36] The lower edge was 1Hz because of the use of ICA and we will explain later why we must filter at 1Hz if we want to use ICA.

The filters should be applied to the continuous data and not only to the epochs. This occurs because the edge artefact of 0.5Hz last up to 6s, which is longer than the range of the epochs. Sometimes you can filter the ERP at 30Hz which can make it smoother and look cleaner but it in the other hand can corrupt the analysis.[5]

After filtering one should proceed to remove the noisy trials in a semi-automatic procedure. It's recommended to do it manually even if it is slower, but we don't take the risk of rejecting some trials that may have important data. If one aims to use the ICA, it is important to pay attention to do not remove the epochs with blinks.[5]

In the last chapter we will discuss this in more detail, the different filters we can use and what is the impact at the latency and the amplitude of the ERP.

2.2.4 Influence of ICA

Independent Component Analysis (ICA) is a tool used in EEG signal processing to reduce the artifacts and noise of the signal. Imagine that you have a room full of people with a series of micros recording their voices. What ICA can do is to separate that recording in each person's voices. Summarizing ICA is a tool that uses an algorithm to decomposes the signal in a number of components related to the number of channels recorded. This allows to decompose the EEG signal into components that might explain e.g. muscular, eye, cardiac or even other acquisition related artefacts. Having many electrodes on EEG allows to use blind source separation algorithms like ICA to isolate these different artifact sources. After this separation we can remove those components and get a cleaner result. It is important to note the e.g. If the channels recording eye movement/artifacts are too noisy, they should not be included on ICA.[45][46]

The literature says that if we want to use ICA, we should high pass filtering set between 1Hz and 2Hz before use it instead of pre-filtering with lower frequencies then 0.5Hz. It will give better results in terms of Signal-Noise Relation, accuracy and better gradients at ICA components, making it easier to identify what type of component is present. [43]

The reasons why ICA works better with a high pass filter at 1HZ is because ICA is toward high amplitude if the length of the data is finite. Plus, the information that we are seeking (from the neurophysiological point of view) is often between 3Hz and 80Hz and cutting of the left values will help ICA. [42][43]

There are different algorithms implemented in EEGLAB. In this study we used 'runica'.

Runica uses the infomax ICA algorithm of Bell and Sejnowski (1995) with a natural gradient feature of Amari, Cichocki & Yang, the extended-ICA algorithm of Lee, Girolami & Sejenowski and PCA dimension reduction [44]

After running ICA, we can sort the components by relative power or by percent power accounted for. The function that give us the relative power is given by:

$$rp\ (comp) = 100 \times \frac{Mean(back_{proj}^2)}{Mean(data^2)} \qquad (3)$$

Where $back_{proj}$ is given by:

$back_{proj} = \ comp_{map} \times comp_{activation(t)}$, for t in 'limcontrib' (4)

The function of percent power:

$$ppaf(comp) = 100 - 100 \times \frac{Mean(\left(data - \frac{back}{proj}\right)^2)}{Mean(data^2)} \qquad (5)$$

After choosing the function to sort the components, we need to choose the time range to rank the component contributions and the number of the components that we

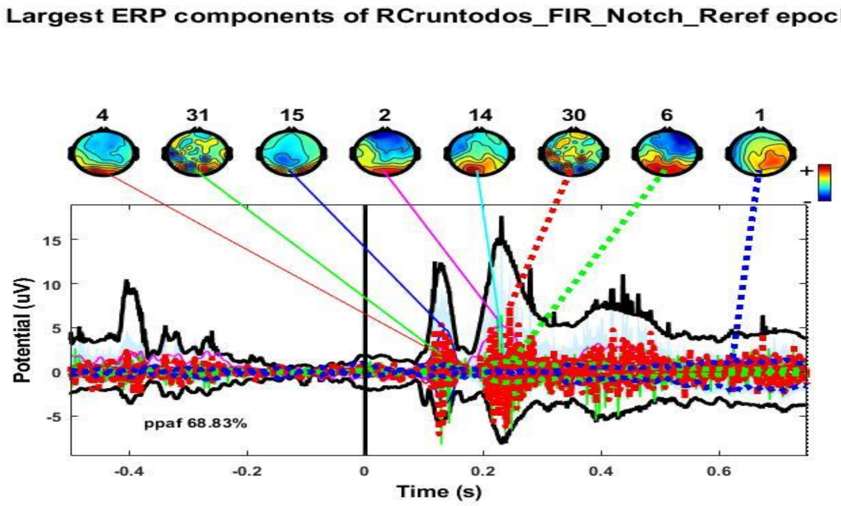


Figure 25. The eight most important components and the percentage of signal explained.

want.

In our study we used the function of the percent powered. The time range was between 0ms and 400ms with the 8 components because we already expected an ERP during this time window due to the nature of our task. From figure 25 we show, the eight most important components and the percentage of signal explained (68.83%) in that range. Note that, these components might be noisy components and in that case they should be removed.

To remove these components is important to know how to identify which ones are artifacts. One of the most common components to reject is the eye artifact component. This component is characterized by describing the eye movement and eye blinks and is a very common. It is easy for ICA to identify these artifact components because they are large in amplitude.[47]

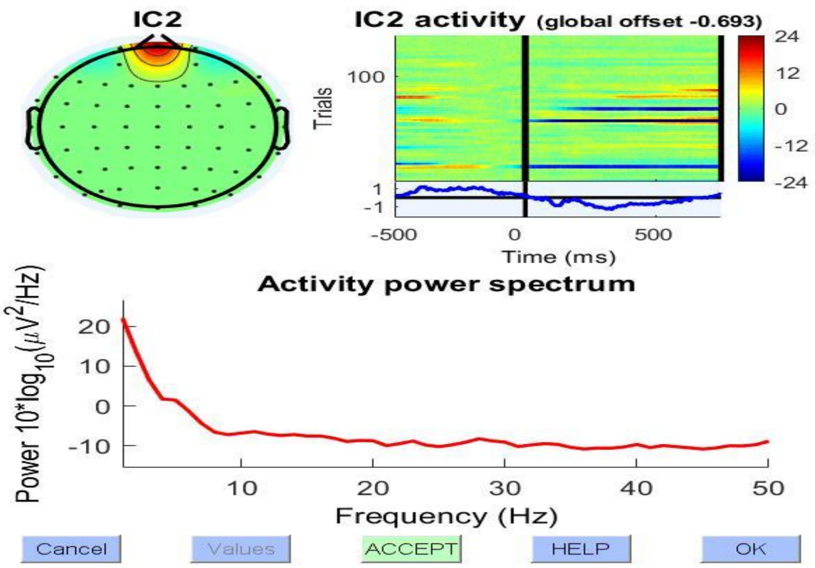


Figure 26. ICA component which represents an eye artifact.

The figure 26 shows an eye artifact component. Generally, in these components the activity power has the peak at frequencies below 10Hz and the scalp topography shows frontal activity near the eyes.[47]

Another typical artifact component found is the muscle component. This artifact appears because of the muscle activity or muscular movements from the patient and its important to remove or reduce it in pre-processing analysis.[47]

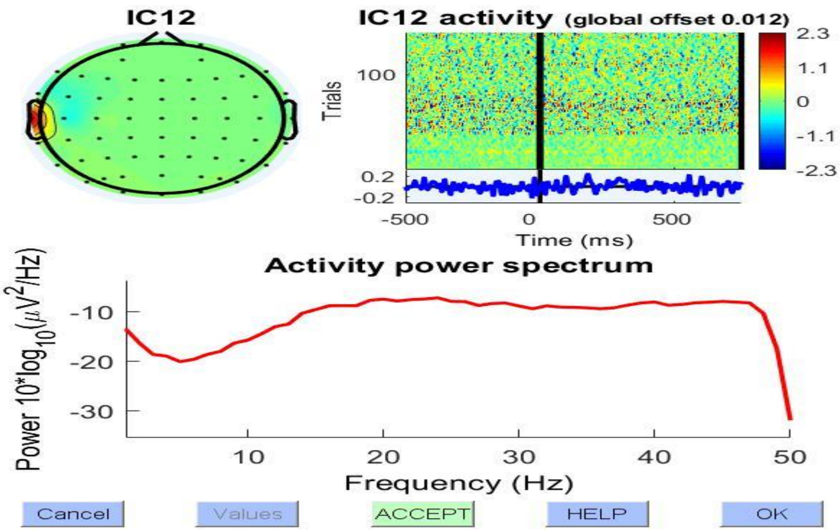


Figure 27. ICA component which represents a muscular artifact.

This muscular artifact component (figure 27) is characterized by scalp topography outside the skull, the peak of activity power spectrum at high frequencies and very noisy trials. [47]

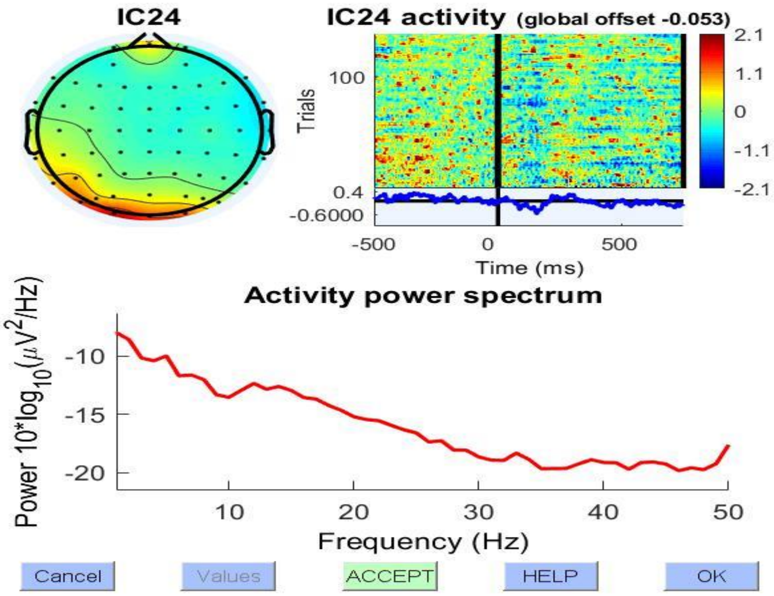


Figure 28. ICA component which represents a cardiac artifact.

The image 28 shows a cardiac artifact component. This artifact is generated by the electrical potentials of the heart and it is characterized by no peaks at activity power spectrum and with a scalp topography concentrated at back and outside the skull because of the distance of the heart. [47]

Even after removing the artifact components, is possible that the data analysis still has some noise and is not possible to identify the expected ERP. When this happens, we can search the components and find one that can represent the signal of interest. That component is called a brain component and is for example characterized by a peak near the frequency of 10Hz in activity power spectrum, with a dipolar scalp topography and with a visible ERP at the epoched data. [47]

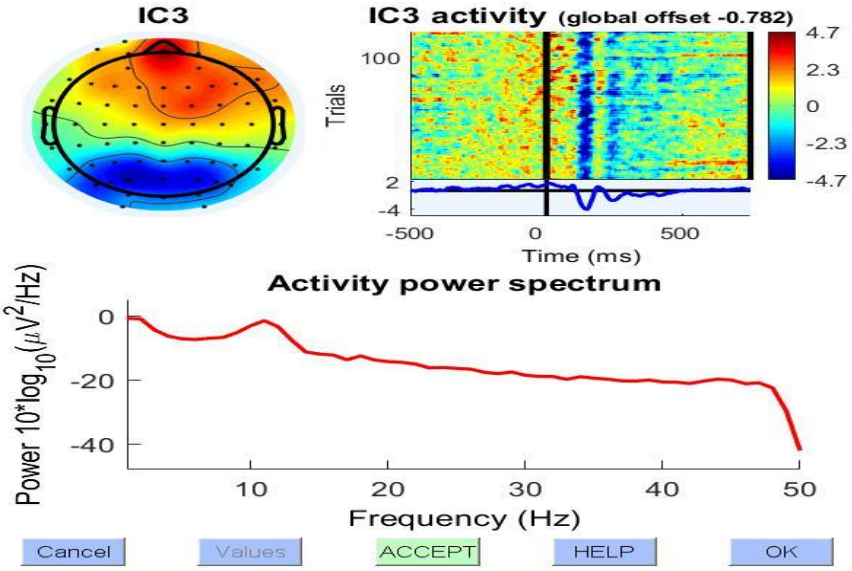


Figure 29. Good ICA component which can represent a signal of interest.

This last component represents the brain activity and it is caused by a brain source. Brain sources usually have frequencies between 1Hz and 30Hz and the most common peak frequencies are the 10Hz which represents the alpha waves. In the image 30 we can identify the ERP on the epoched data.[47]

Usually the number of components removed per data is limited. This happens because at the components there are a mix of noise and signal that we are looking for. Thus, if it is not clearly an artifact component or we have doubts, its better leave it instead of removing important signal. As Michael X Cohen says, an expert in this area, “it´s better to leave a little noise than remove real signal”. In general, only the first components are the ones important for inference. [5]

2.2.5 Topography and ERP's analysis

We will focus on two principal methods to analyse the results: topography and ERP´s.

Topography maps have the advantage of analysing the brain activity at some specific latency by mapping the brain activity. These maps provide some spatial representation of the electrical difference potential along the scalp. To understand how they work its important first to know how to interpret these maps and the spatial distribution of the electrodes.

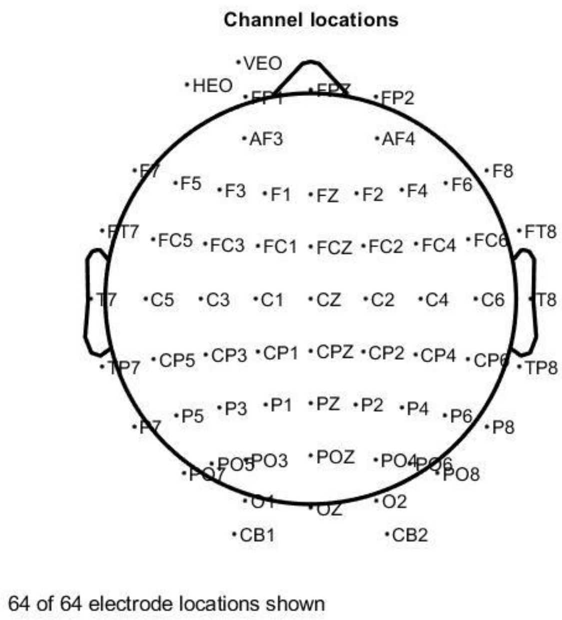


Figure 30. Channel location of the 64 channels used in a 2D projection.

This figure 30 shows the two-dimensional (2-D) spatial distributions of the 64 electrodes on a 10-20 international system. On the top front there is the nose and left ear and right ear are represented on the left and the right respectively at the image. Using the 2-D map representation, will give us with a spatial distribution of the activity changes over the scalp. Another way of representing this spatial information that we get from EEG data is using a three-dimensional map. There are advantages and disadvantages in both. The 3-D plots seem in general more precise and are easier to understand and see where on the head the different features are coming from. On the other hand its not possible to cover all the scalp with one plot, sometimes its needed 3 plots to cover the all the topographic area. The 2-D plots are harder to understand, but they can cover all the activity at the electrodes on the scalp with a single plot. At this work we will focus on the 2-D maps. [5][37]

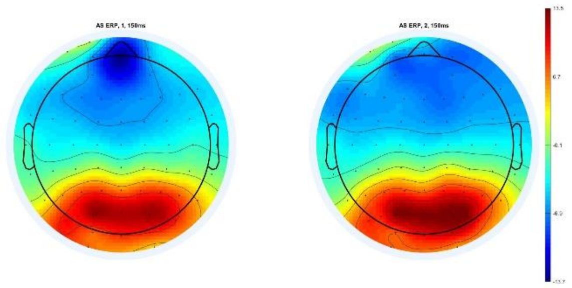


Figure 31. Topographic map showing the voltage gradient of the brain activity in a 2D projection

This scalp map was obtained by using the channels values, but it can be obtained by using the corresponding laplacian values. This map shows us the voltage at each channel at a specific time, giving us a map with a colour gradient from blue to red, where blue is the lowest voltage and red the highest voltage. This is useful to see the localization the brain activity when the target stimuli are shown. [5][37]

A dominant point-of-view of analysis of EEG data is to study the ERP. In ERP's it is represented the brain activity over a period from a single channel or cluster of channels locked to the beginning of the stimuli of interest.

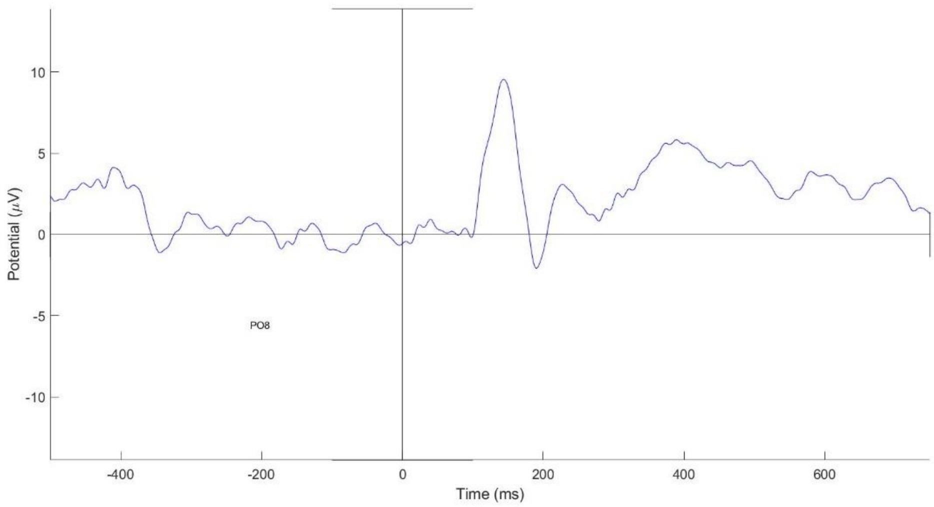


Figure 32. Graphical representation of an ERP type (N170)

The ERPs are the average of all epochs for the stimuli of interest in each channel. This graphic can be analysed by the latency of the ERP peaks or the peak amplitude of them. In the same data it differs from channel to channel as it depends the location of each channel. Sometimes the ERP cannot be identified in the ERP plot and one of the reasons can be because there is no significant brain activity at that electrode or because the pre processing was not done properly. These types of plots will help to know the exact time when the neural response occurs after the stimuli been shown, the amplitude of that response and if there is brain activity there. [5]

2.2.6 Computing statistics for studies with multiple groups and conditions

In EEGLAB it is possible create a study set where we can compare statistically different groups and conditions. This is done after applying the pre-processing methods in our EEG data. In our case we used this tool to compare the same group of 10 people with

3 conditions. The first one was the brain activity after showing a happy face, the second condition was with a sad face and the last one was with neutral face. With this tool we can plot the ERP's for each channel using the average of all subjects for each condition and compare them statistically using some corrections to see if there are any significant differences between the conditions. We can also plot the topographic map at some specific time to see the brain activity along the scalp.

We used permutation statistics for multiple comparisons without corrections for multiple comparison and after that with three different corrections for multiple comparisons. The corrections used was Bonferroni correction, Holmes correction and FDR correction.

Permutation statistics are nonparametric statistics, do not require distributional assumptions, so this does not depend of any kind parameters and does no define a fixed model. It requires more population to draw some significant results comparing with parametric statics. One more advantage of using nonparametric statistics is that we can use some correction for multiple comparisons. [40]

First, we must define a null hypothesis, that usually affirms that there is no relation between the conditions compared and we try to prove it that is wrong with a statistic test. After that we choose a p-value and it will give us the significant result of the test. In permutation tests for multiple comparisons we must have in mind the multiplicity of the different tests and that's the reason why we should apply correction to adjust and get a new p-value depending on how many tests we are assuming. [40]

The first and the most conservative correction that we use for multiple comparisons between the conditions was the Bonferroni correction. The objective of using this correction is to reduce the type 1 errors in statistics and correct the p-value for multiple comparisons. "Type 1" error is the first type of errors that are committed in statistics and is defined by false positives or in a simpler way of explaining is when we reject the null hypothesis even if is true. This correction tries to reduce this error by multiplying each probability by the total of number of independent tests performed [38]:

$$P(at\ least\ one\ significant\ result) = 1 - (1 - pvalue)^n$$

Where we define the p-value that we want for each test, also known as significance level, and the n is the number of the tests performed and the result is the probability of incorrectly reject the null hypothesis on the family of tests. [39]

To know the new correct P-value for the family we use the following equation with the p-value of the test that we already know:

$$Pvalue_{Bonferrini} = Pvalue_{test} * n$$

Where n is the number of independent tests performed.[38]

The second correction that is used for multiple comparisons is the Holm correction. This correction is weaker than the Bonferroni and despite being similar, is sequential. We organize the different values of the p-value of each test from the smallest to the highest and we do the same procedure but each time we test one p-value from a test we take it out on the next procedure. This continues until we find a non-significant test, or we performed all the tests. [38]

The last correction is called as false discovery rate (FDR) and is used for the same purpose of the previous corrections but is less conservative. [41]

When comparing multiple conditions, EEGLAB uses ANOVA instead t-test.[48]

ANOVA (Analysis of Variance) is a parametric test used to compare multiple groups for the same variable. It uses the F-test. To it works the samples need to be independents, get a normal distribution and the variance of the populations needs to be equal. We will use ANOVA in some studies. [26]

To conclude we want to know if there are any significant statistical differences in recognition of emotions. To know that we used the previous corrections and in the next chapter we will compare the results obtained with other studies in the same area.

2.2.7 Testing distinct pre-processing approaches

In this project we will analyse the amplitude and the latency of N170 using six different approaches with the two objectives: 1) Compare the 3 conditions used in the task; 2) Compare each task condition but varying the pre-processing methods.

For the first objective we will use the first study to compare Happy vs Sad, Happy vs Neutral and Sad vs Neutral. The pre-processing methods used were the same for each condition, 10 subjects used per condition with interpolation of the channels considered bad. We used average reference for offline reference, rejecting epochs considered as artifacts and setting a baseline of 250ms pre-stimulus. Digital filters were used, a band pass with a range of 1Hz and 60Hz. Notch filter were used too. Finally, before we analyse the results, we run ICA on the data, and we removed the components considered artifacts.

For the second objective, we compared for each task condition the different pre-processing methods that we used for the N170. To get that we did six different studies, the first one only junction all the data and without any pre-processing method; the second one adding the average reference and the interpolation; the third adding to the previous one filters; the fourth adding to the previous epochs rejection; the fifth adding ICA to the previous; and the last adding the rejection of the components of ICA. With this we want to study the impact of each method and how will this get significant effect in the final results. The next table will summarize the studies used in this project. Plus the studies on the table we also did one per condition do all methods but filtering at 30Hz instead of 60Hz.

Table 2.Studies made to analyse N170 through different conditions.

Study	Condition	Interpolation and Re-reference	Filter at 1Hz, 60Hz and Notch filter	Epochs Rejection	ICA	ICA Component rejection
1	Happy					
2	Happy	×				
3	Happy	×	×			
4	Happy	×	×	×		
5	Happy	×	×	×	×	
6	Happy	×	×	×	×	×
7	Sad					

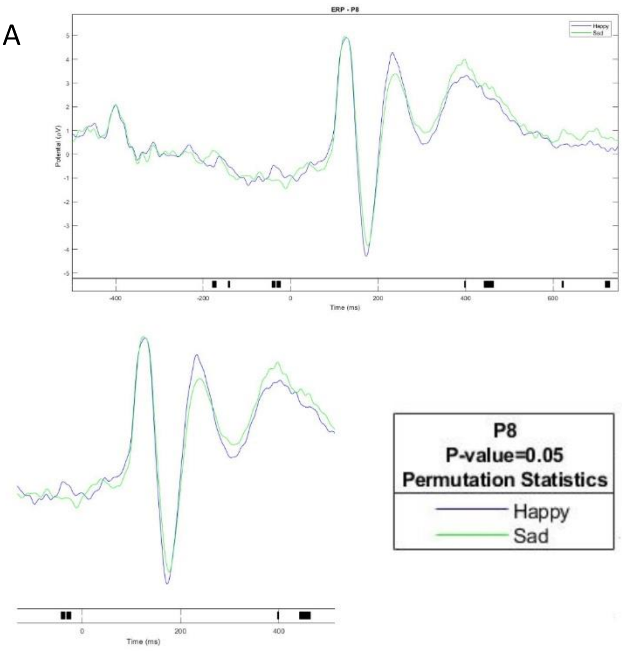
8	Sad	×				
9	Sad	×	×			
10	Sad	×	×	×		
11	Sad	×	×	×	×	
12	Sad	×	×	×	×	×
13	Neutral					
14	Neutral	×				
15	Neutral	×	×			
16	Neutral	×	×	×		
17	Neutral	×	×	×	×	
18	Neutral	×	×	×	×	×

Chapter 3: Results

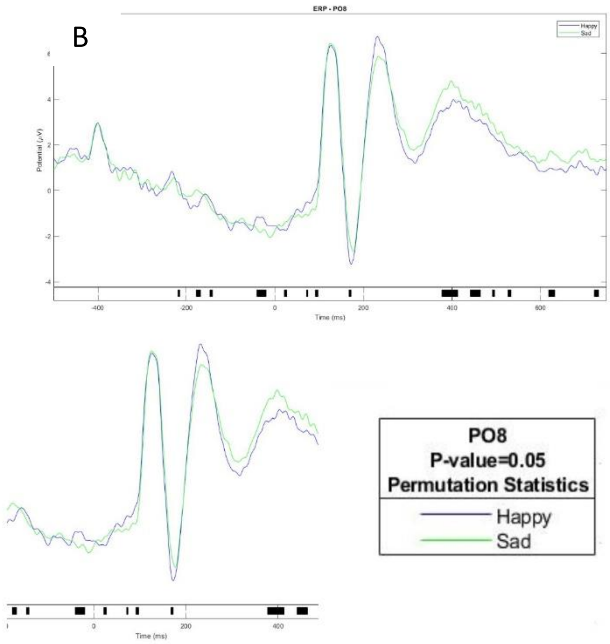
In the first part of our work, we tried to replicate previous studies where the ERP associated to faces were compared. We compared Happy vs Sad, Happy vs Neutral and Sad vs Neutral conditions. We focused our research on 3 channels: P8, PO8 and PO6. We used a toolbox from MATLAB, which is EEGLAB. For each condition from the data first we interpolate the channels with artifacts and re-reference the all data to average reference. After that we high pass filtered at 1Hz and low pass at 60Hz. We used a Notch filter between 47.5Hz and 52.5Hz. Finally, we took the epochs for each condition with a baseline of -250ms to 0ms and ran ICA after rejecting the epochs with artifacts. We finish our pre-processing removing some components of ICA. All this was the first part of the work. We used the study 6, 12 and 18 of the table.

First, we compared Happy with Neutral. Using a tool from MATLAB which is EEGLAB, we create a study with the data. We used permutation statistics to compare between conditions, using a randomization of 1000. To the p-value, first we choose 0.01 which mean statistically highly significant and if there were no differences, we change the p-value to 0.05 which means statistically significant. We focused our search only in 3 channels (P8, PO8 and PO6). The results for the first comparison are in the next figure.

A


ERP - P8
Happy
Sad
Potential (µV)
Time (ms)
P8
P-value=0.05
Permutation Statistics
Happy
Sad

B


ERP - PO8
Happy
Sad
Potential (µV)
Time (ms)
PO8
P-value=0.05
Permutation Statistics
Happy
Sad

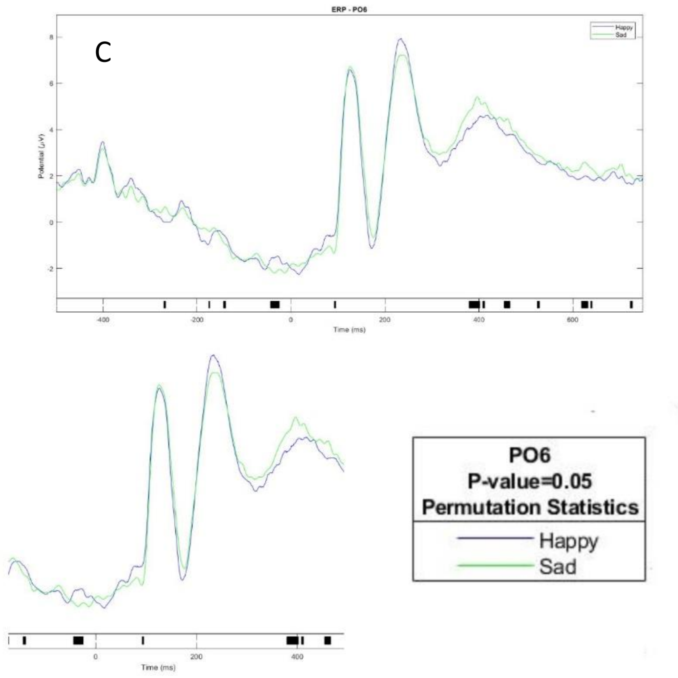


Figure 33. A: Comparison of the ERP between the two conditions (Happy in blue and Sad in green) for the channel P8, using permutation statistics with a p-value of 0.05. B: Comparison of the ERP between the two conditions (Happy in blue and Sad in green) for the channel PO8, using permutation statistics with a p-value of 0.05. C: Comparison of the ERP between the two conditions (Happy in blue and Sad in green) for the channel PO6, using permutation statistics with a p-value of 0.05. Note that the significant periods are represented in black above the time axis.

There was not found any significance differences using 0.01 as p-value and using p-values=0.05 there were differences in the amplitude of N170 on the channel PO8 between the conditions happy and sad but no difference was found in the P100 and P300 between these two conditions. After we proceed to see the topography map at 170ms, and the result is shown in figure 34.

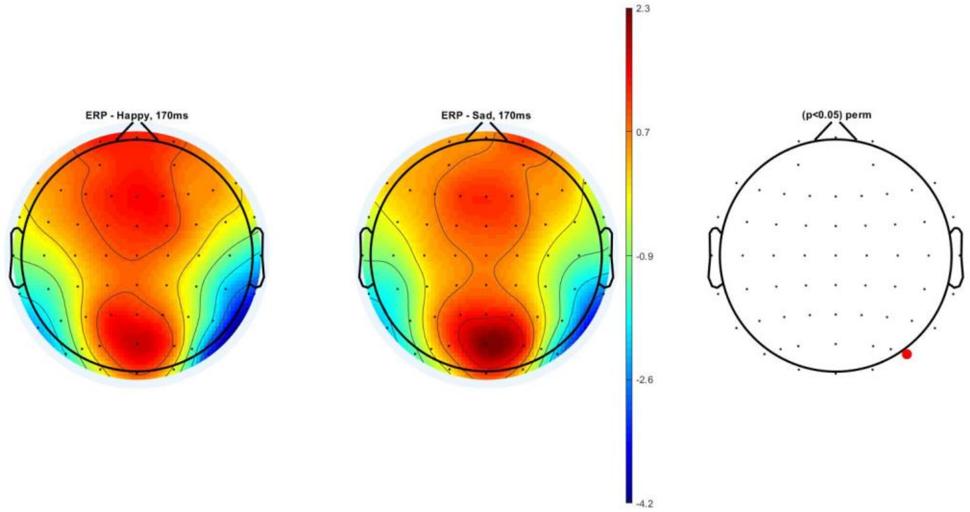


Figure 34. Topography map of the conditions happy and sad at 170ms with statistical plot using p-value=0.05.

This shows the gradient of the amplitude among the scalp between the conditions happy and sad at 170ms. It is possible to see that the only channel that shows significant differences is PO8. Is also possible to see that the amplitude of this negativity is higher in the occipitotemporal electrodes and lower at midline and front electrodes.

The next study was between the conditions of happy and neutral. We used permutation statistics with a p-value of 0.01 and 0.05 with a randomization of 1000 and we focused on the same previous channels and a new one (P8, PO8 and CP6). The ERP results are shown in the figure 35.

A

B

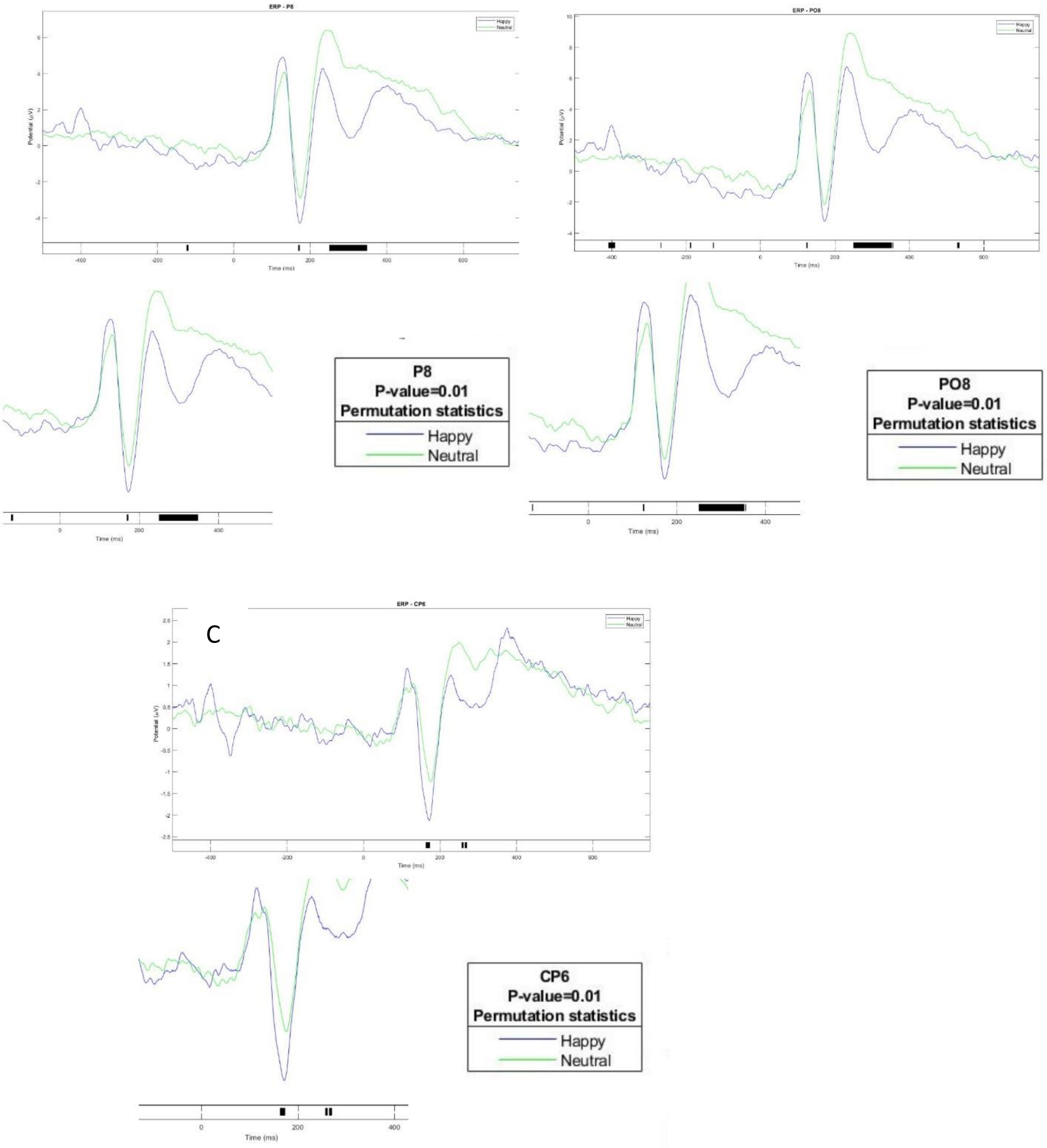


Figure 35. A: Comparison of the ERP between the two conditions (Happy in blue and neutral in green) for the channel P8, using permutation statistics with a p-value of 0.01. B: Comparison of the ERP between the two conditions (Happy in blue and Neutral in green) for the channel PO8, using permutation statistics with a p-value of 0.01. C: Comparison of the ERP between the two conditions (Happy in blue and Neutral in green) for the channel CP6, using permutation statistics with a p-value of 0.01.

Note that the significant periods are represented in black above the time axis.

For these comparisons we found statistical differences between the conditions using a more significant p-value (0.01). At P8 and CP6 channels we found that the amplitude of N170 were significant different between the condition Happy and Neutral. In the channel P8 we also found differences on the amplitude of the P300 as shown in the top left of the figure 36. For the last channel (PO8) we couldn't find any statistical difference for the N170 but for the P100 and P300 we could as we can see in the top right of the figure 36. To finish our analysis through MATLAB we proceed to see the topography map at 170ms. (figure 36)

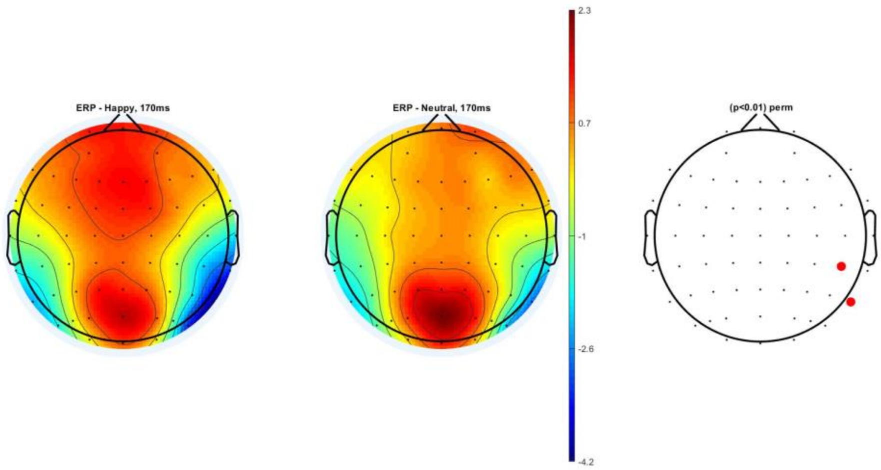


Figure 36. Topography map of the conditions happy and neutral at 170ms with statistical plot using p-value=0.01.

The last study between facial conditions was with neutral and sad faces. We used permutation statistics with a p-value of 0.01 and 0.05 with a randomization of 1000 and we focused on the same previous channels and a new one (P8, PO8 and PO8). The ERP results are shown in the figure 37 with a p-value of 0.05.

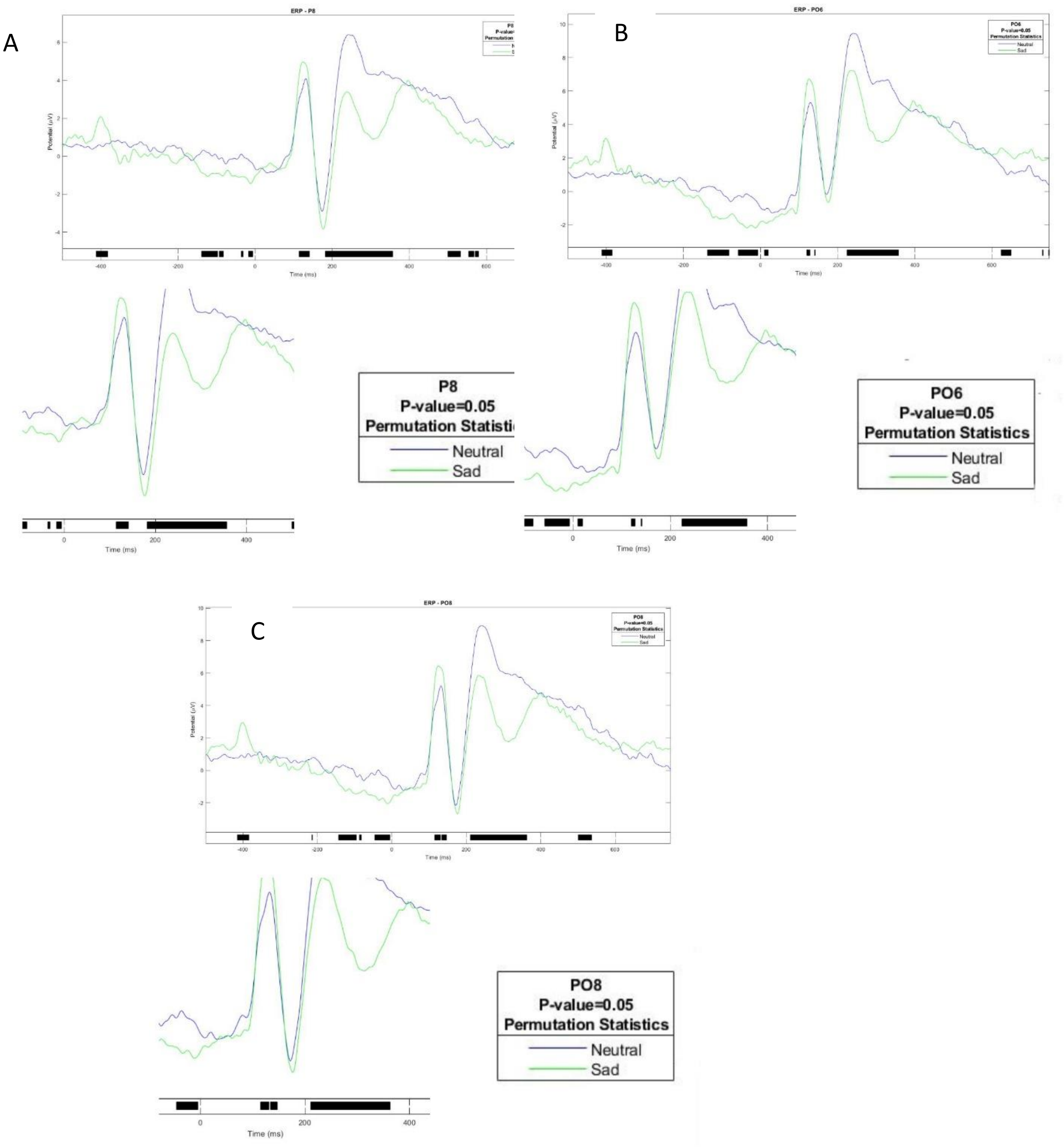


Figure 37. A: Comparison of the ERP between the two conditions (Neutral in blue and Sad in green) for the channel P8, using permutation statistics with a p-value of 0.05. B: Comparison of the ERP between the two conditions (Neutral in blue and Sad in green) for the channel PO6, using permutation statistics with a p-value of 0.05. C: Comparison of the ERP between the two conditions (Neutral in blue and Sad in green) for the channel PO8, using permutation statistics with a p-value of 0.05.

Note that the significant periods are represented in black above the time axis.

Between these two conditions we could not find any significant difference with a p-value of 0.01 for N170. With a p-value of 0.05 for the three channels chosen we could find significant differences among the amplitude of P100 and P300. For N170 we could only find this for the channel P8. To finish our analysis through MATLAB we proceed to see the topography map at 170ms. (figure 38)

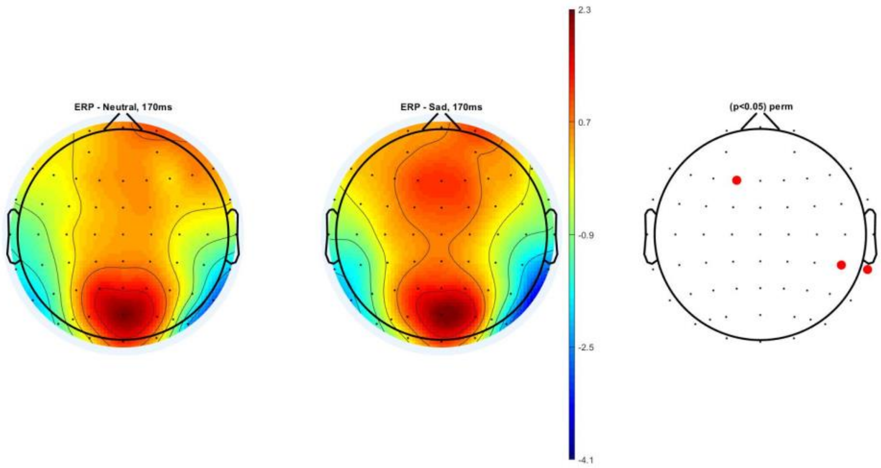


Figure 38.Topography map of the conditions neutral and sad at 170ms with statistical plot using p-value=0.05.

The second part of this work focused on the influence of each method of pre-processing used. We tried to understand if the alteration or the annulation of a single method could influence the amplitude or the latency of the ERP's. In order to see that, for each condition (happy, sad and neutral) we got the final result (ERP) and compare them using different pre-processing methods. For each facial condition we compared the ERP's of 7 different studies. First one using only the data acquired, second one applying to the data interpolations of missing channels and re-reference the data to average reference. Third one applying high-pass filter (1Hz), low-pass filter (30hz) and notch filter (47.5Hz-52.5Hz). The fourth study was applying epoch rejection to the data already filtered and re-referenced. The fifth was running ICA to the previous data. The sixth was rejecting components of ICA. And the last one was to do all the methods already referred but instead of filtering low pass at 60Hz, we filtered at 30Hz. We used parametric statistics (ANOVA test) with p-values of 0.05 and 0.01. We will focus on the channels where we search for N170 in the previous studies that we already showed the results (PO8, P8 and PO6). The first facial condition that we will show is the Happy condition.

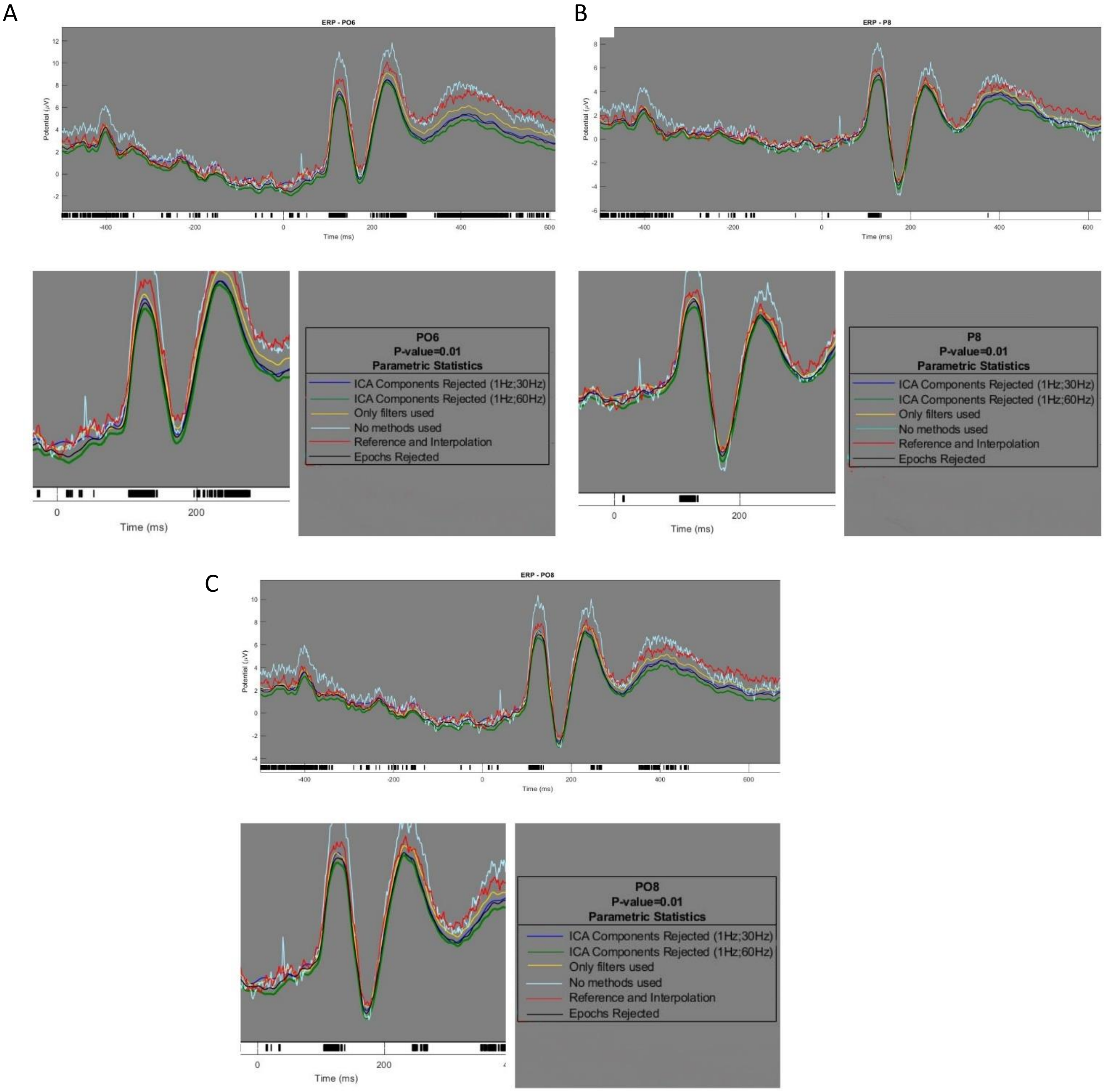
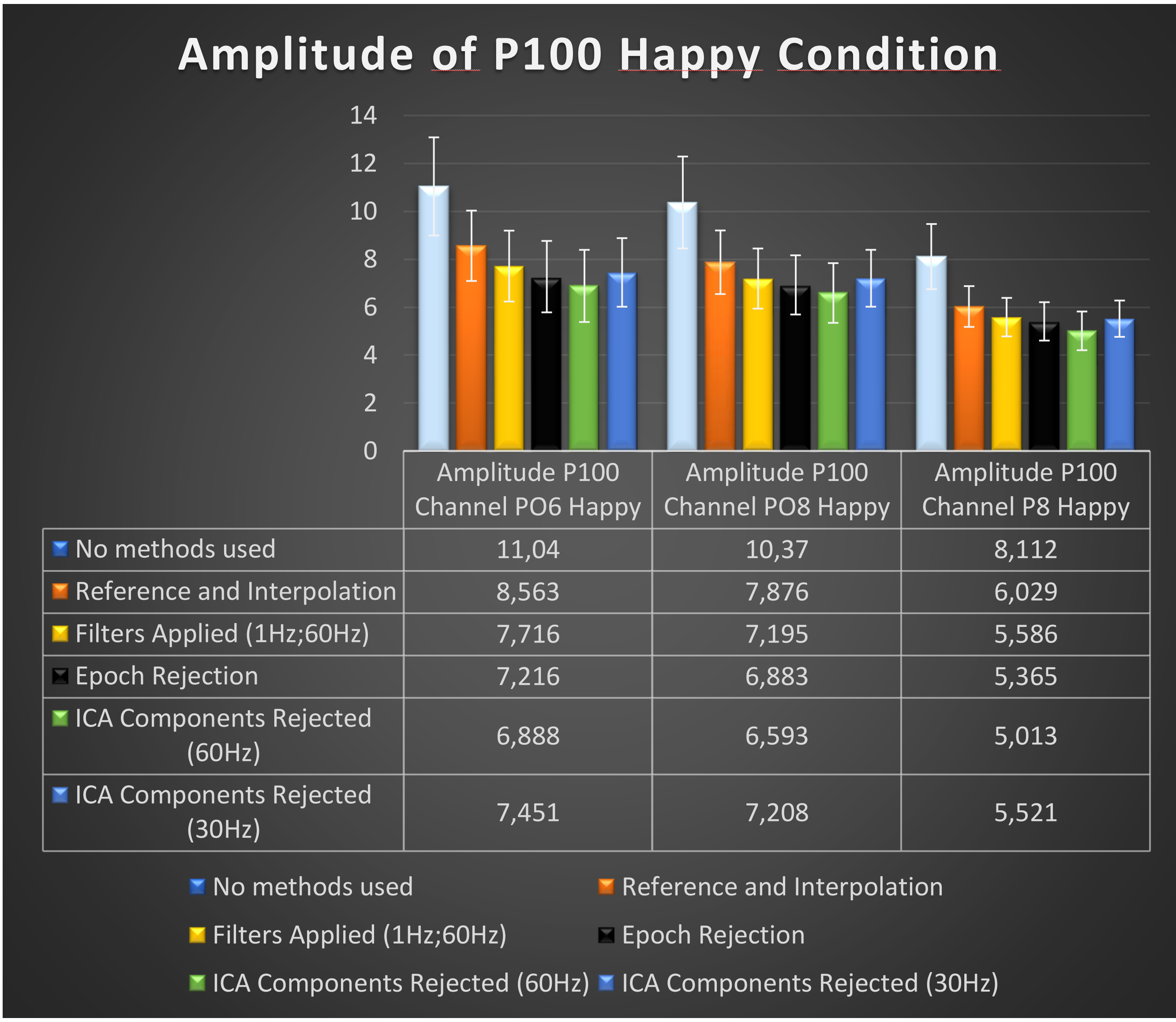


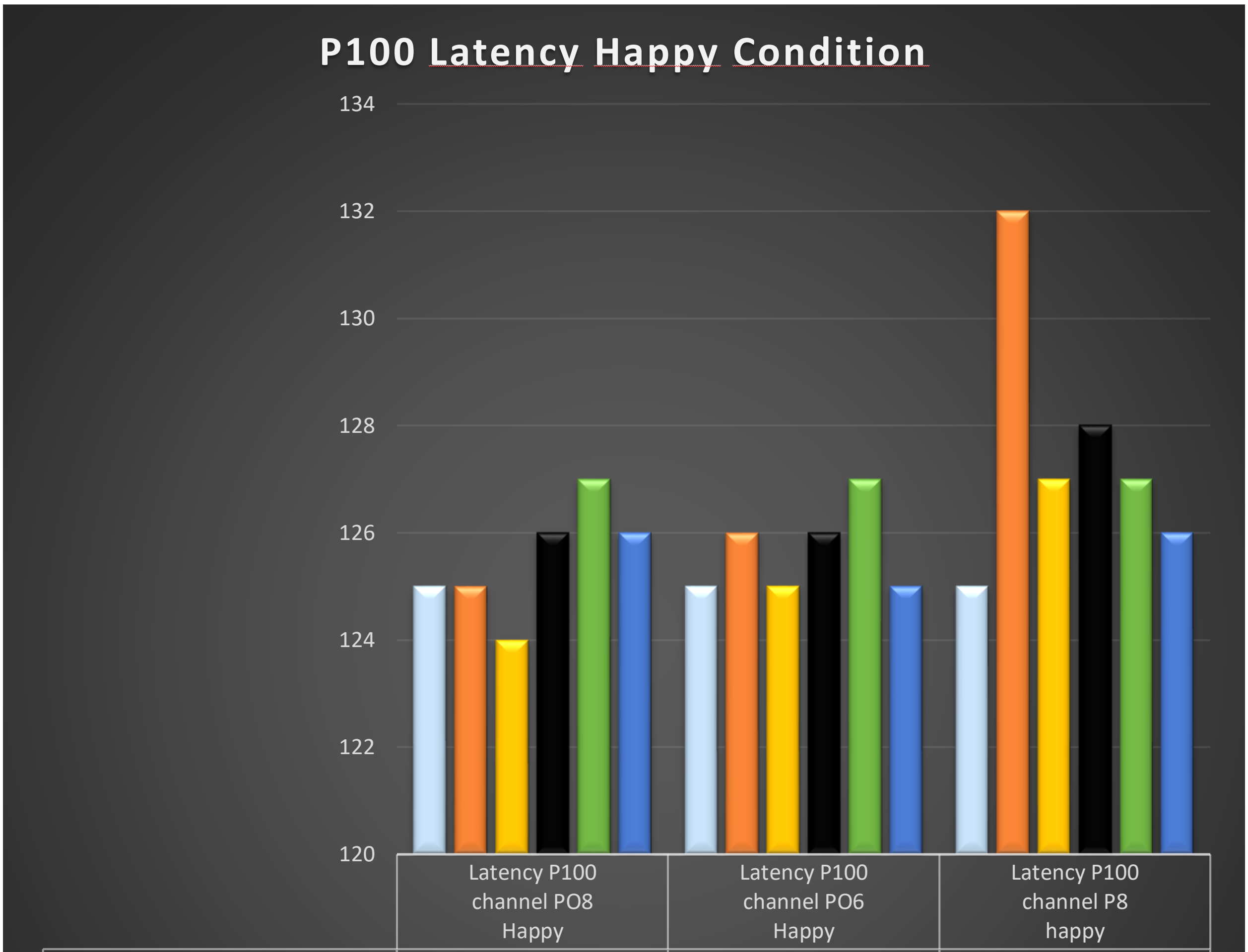
Figure 39. A: Comparison of the ERP between seven conditions for the channel PO6, using parametric statistics with a p-value of 0.01. B: Comparison of the ERP between the seven conditions for the channel P8, using parametric statistics with a p-value of 0.01. C: Comparison of the ERP between the seven conditions for the channel PO8, using parametric statistics with a p-value of 0.01.
Note that the significant periods are represented in black above the time axis.

The next figures show the values of the amplitudes and latencies of N170 and P100 for each channel showed previously.



	Amplitude P100 Channel PO6 Happy	Amplitude P100 Channel PO8 Happy	Amplitude P100 Channel P8 Happy
No methods used	11,04	10,37	8,112
Reference and Interpolation	8,563	7,876	6,029
Filters Applied (1Hz;60Hz)	7,716	7,195	5,586
Epoch Rejection	7,216	6,883	5,365
ICA Components Rejected (60Hz)	6,888	6,593	5,013
ICA Components Rejected (30Hz)	7,451	7,208	5,521

Figure 40. Values of amplitude in µV of P100 for Happy faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.



	Latency P100 channel PO8 Happy	Latency P100 channel PO6 Happy	Latency P100 channel P8 happy
No methods used	125	125	125
Reference and Interpolation	125	126	132
Filters Applied (1Hz;60Hz)	124	125	127
Epoch Rejection	126	126	128
ICA Components Rejected (60Hz)	127	127	127
ICA Components Rejected (30Hz)	126	125	126

Figure 41. Values of latency in ms of P100 for Happy faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

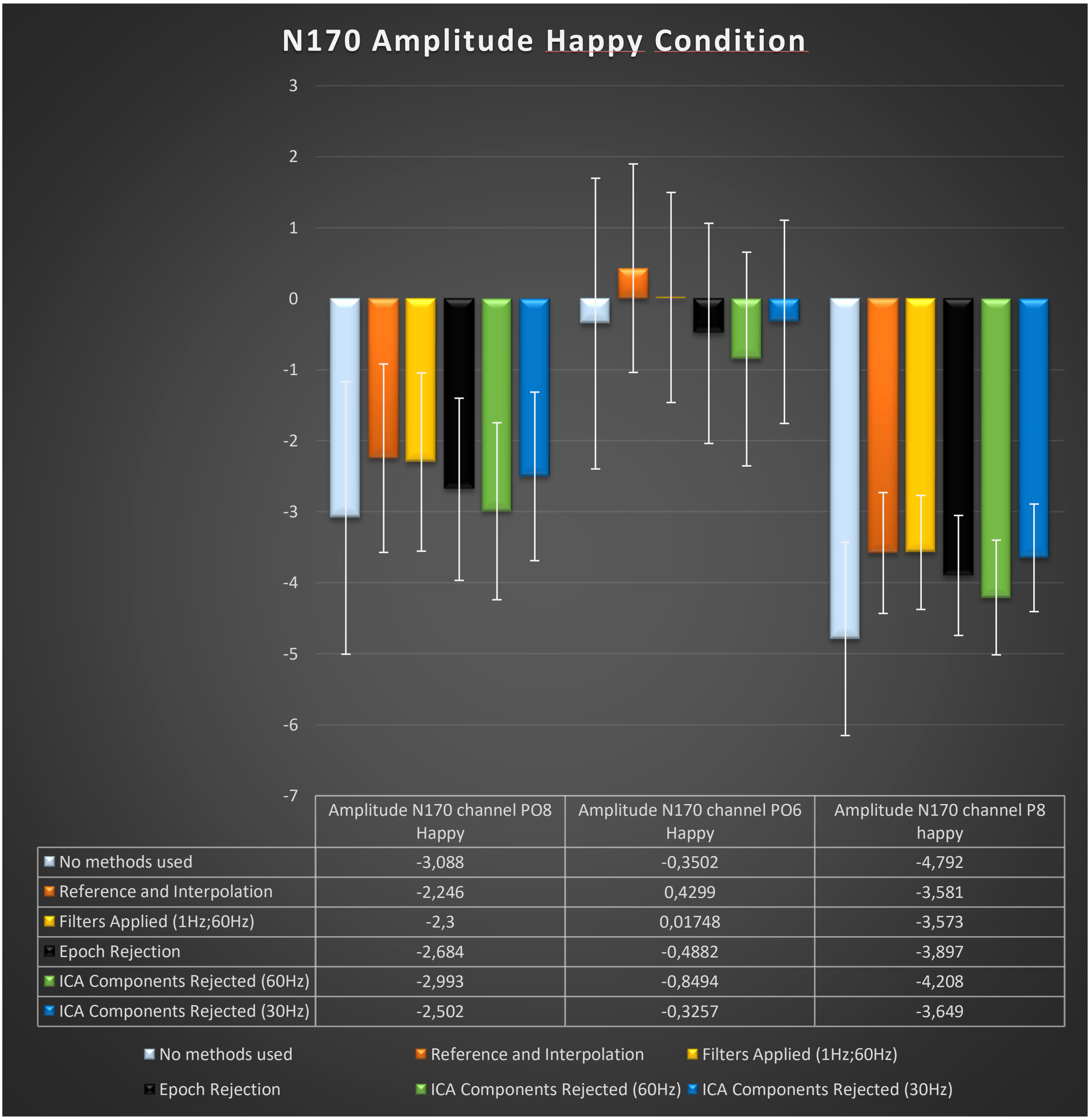


Figure 42. Values of Amplitude in μV of N1700 for Happy faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

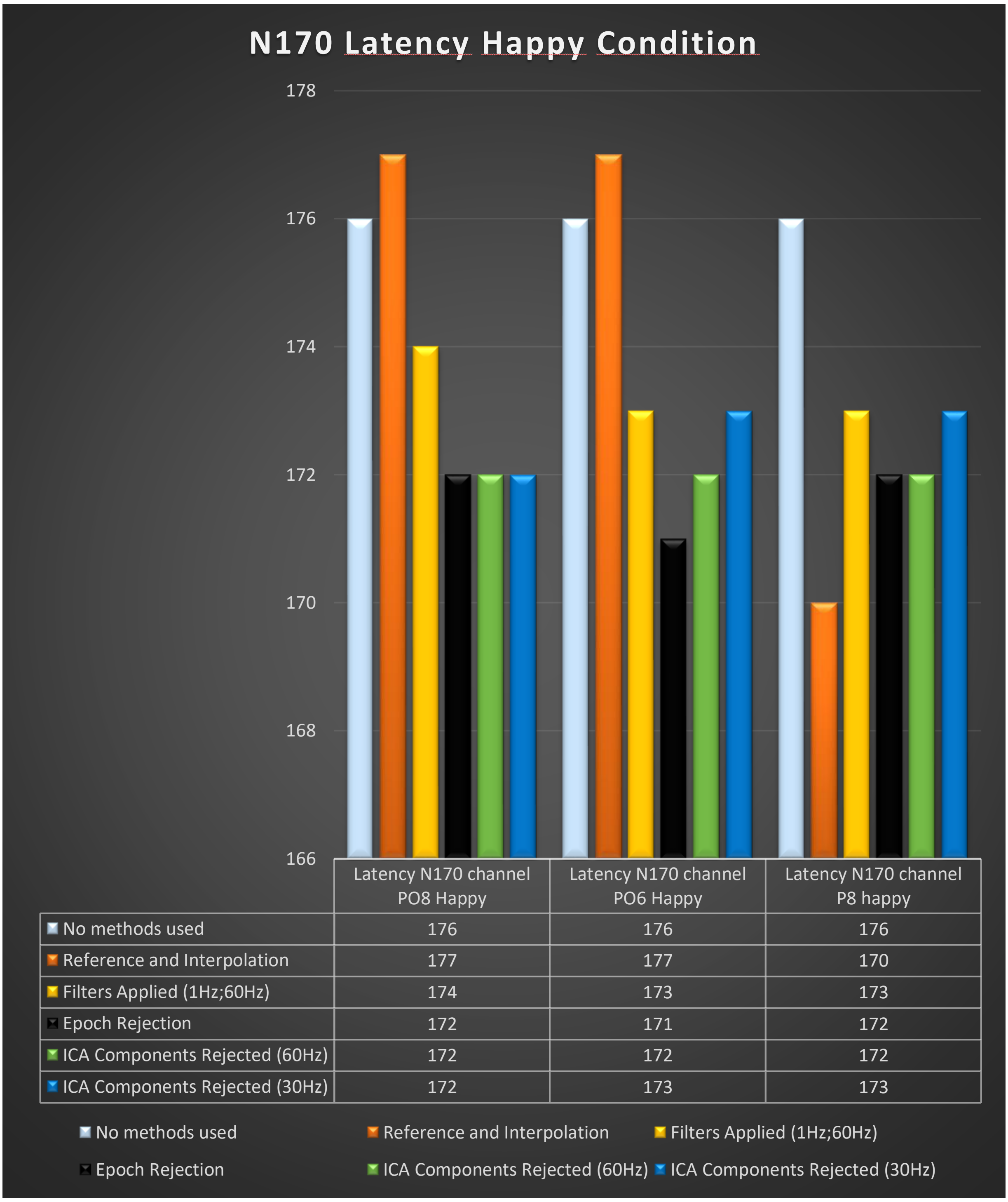


Figure 43. . Values of latency in ms of N1700 for Happy faces through seven variantons of pre-processing methods at channel P8, PO8 and PO6.

Through the figure 38 there are no significant differences for the N170 for all the three channels, but it is possible to find significant differences for P100, P300 and for the baseline (time before 0ms). Looking for the values of P100 amplitude (figure 40) it is possible to conclude that the addition of methods makes the amplitude of P100 decrease significantly. Also, good to refer that, filtering at 30Hz instead of 60Hz, makes the amplitude be a bit higher. When we look to the N170 the things are a bit different. The amplitude tends to increase when we use some methods. Comparing the filters (30Hz and 60Hz), the amplitude of filtering at 60Hz gives a higher amplitude. In terms of latency, it is not clear and not a uniform variance, but it suggests that for the P100, when we use more methods, tend to increase the latency and for the N170 tend to decrease.

Now the question that comes up after analysing these results is: Which of the six conditions gives the best results and which one should we use?

To answer the following question, we looked for the signal-noise ratio. The SNR represents the relation between the signal and the noise, and a higher SNR means a lower noise that in turn means a lower effect of the noise in the signal. What are we going to look for is the higher SNR for the P100 and the N170. [61]

The standard definition of SNR is ratio between the power of the signal to the power of the noise. It also can be measure by the following equation:

$$SNR = \frac{s^2}{E[N^2]}$$

Where s is the signal constant and E is the expected value for the N random noise. If the expected value for noise is 0, that value can be replaced with by the variance. [61]

An alternative definition can be used for calculating the SNR which is the coefficient of variation. It is also suggested by Von Frau Filipa Alexandra Campos Viola. (2011), which says that the SNR is given by the ratio of signal peak to the mean of the standard deviation of the baseline period.[60]

This signal noise is given by:

$$SNR = \frac{\mu}{\sigma}$$

Where μ means the peak of the amplitude and σ the mean of the standard deviation from the baseline period. [60]

Table 3. Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of P100 for Happy condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Components Rejected (30Hz)
Standard Deviation PO6	2.047	1.468	1.479	1.55	1.505	1.431
Standard Deviation PO8	1.918	1.327	1.255	1.283	1.246	1.187
Standard Deviation P8	1.36	0.8512	0.8028	0.8446	0.8076	0.7577
SNR PO6	5.393258	5.833106	5.217039	4.655484	4.576744	5.206848
SNR PO8	5.406674	5.935192	5.733068	5.36477	5.291332	6.072452
SNR P8	5.964706	7.082942	6.958146	6.352119	6.207281	7.286525

Table 4.Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of N170 for Happy condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Components Rejected (30Hz)
Standard Deviation PO6	2.047	1.468	1.479	1.55	1.505	1.431
Standard Deviation PO8	1.918	1.327	1.255	1.283	1.246	1.187
Standard Deviation P8	1.36	0.8512	0.8028	0.8446	0.8076	0.7577
SNR PO6	-0.17108	0.292847	0.011819	-0.31497	-0.56439	-0.2276
SNR PO8	-1.61001	-1.69254	-1.83267	-2.09197	-2.40209	-2.10783
SNR P8	-3.52353	-4.207	-4.45067	-4.61402	-5.2105	-4.81589

Analysing the values of the standard deviation that decrease with the using of pre-process methods and the lowest standard deviation belongs to the last condition (all pre-process methods used and filtered at 1Hz and 30Hz).

In what matters to the SNR of P100, for all the channels, the SNR increase after doing the reference and the interpolation but then decreases as new methods are added. Is still important to refer that the highest SNR for P100 is from the last condition (all pre-process methods used and filtered at 1Hz and 30Hz), which means that filtering at 60Hz and 30Hz have a significant difference in the final result for P100.

Referring to the SNR of N170, all pre-process methods added makes the SNR increase. The SNR of 60Hz is higher than the 30Hz. Also refer that the channel that has the highest SRN is the channel P8.

The next study shows the same conditions and variances of pre-processing methods used to be compared but using now sad faces as stimuli. We used parametric statistics again to compare them, p-value equal to 0.01 and we focused on the same channels (PO8, PO6 an P8). The next figure represents the ERP from the three channels.

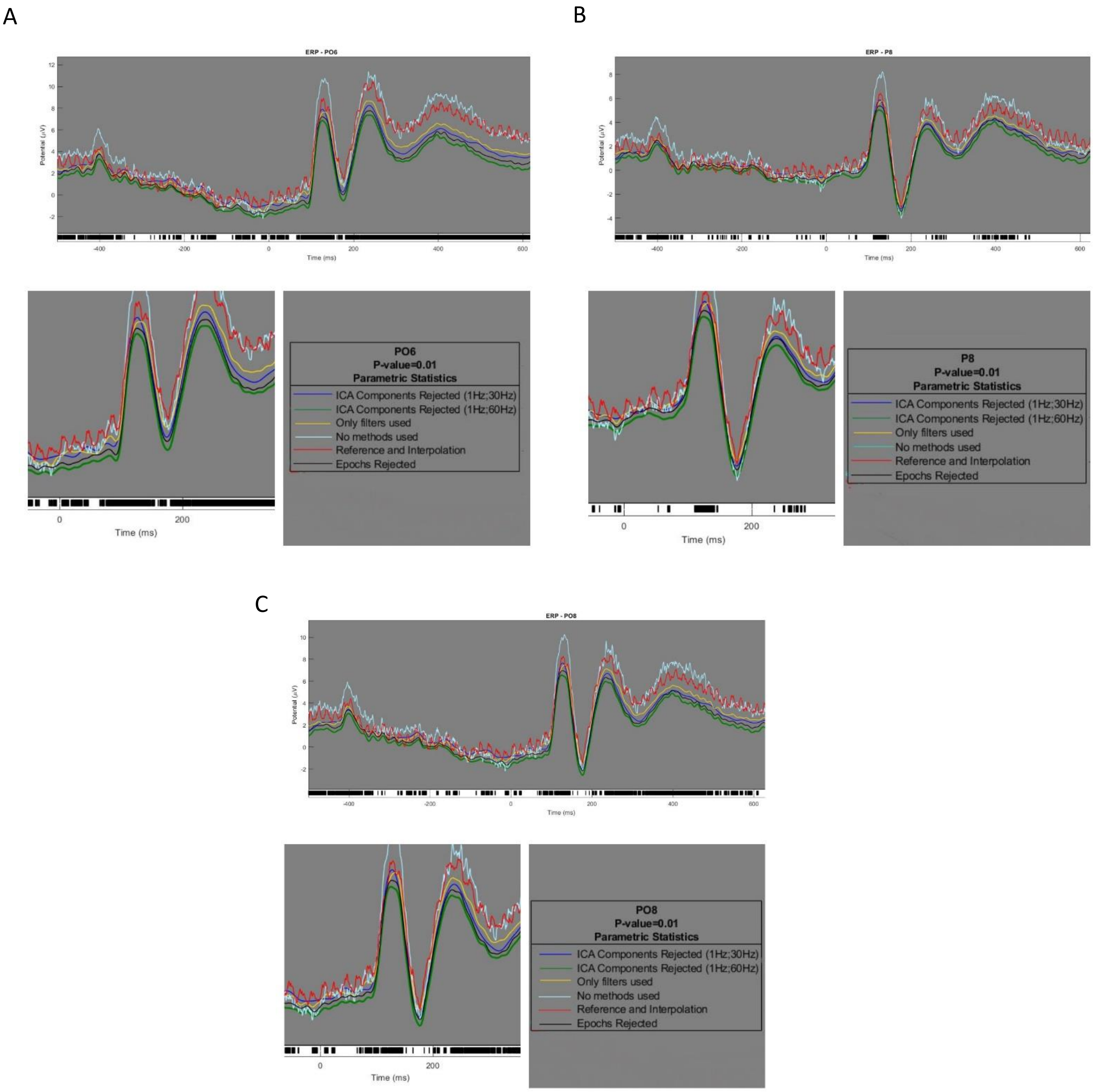
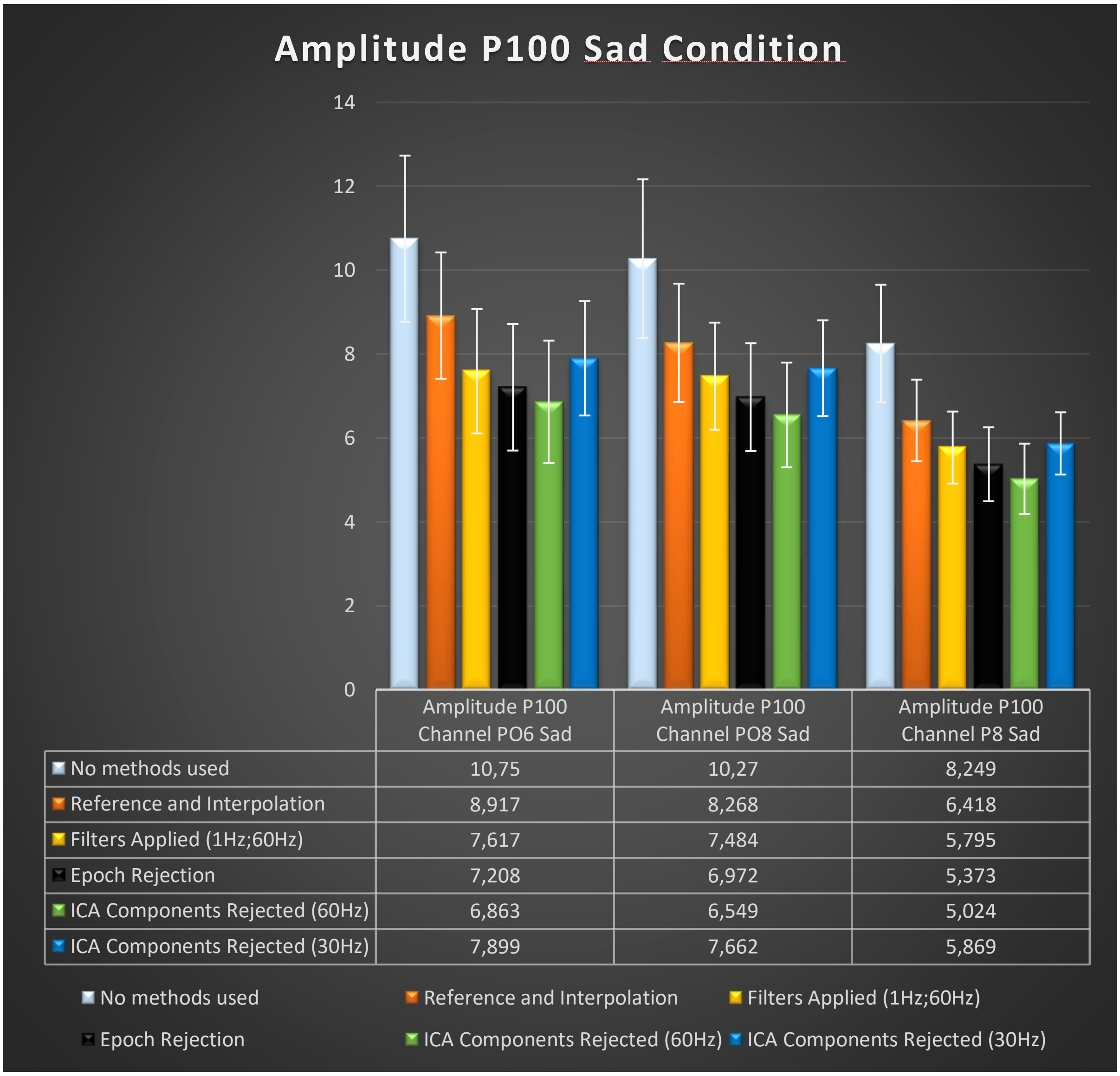


Figure 44. A: Comparison of the ERP between seven conditions for sad faces and for the channel PO6, using parametric statistics with a p-value of 0.01. B: Comparison of the ERP between the seven conditions for sad faces and for the channel P8, using parametric statistics with a p-value of 0.01. C: Comparison of the ERP between the seven conditions for sad faces and for the channel PO8, using parametric statistics with a p-value of 0.01.

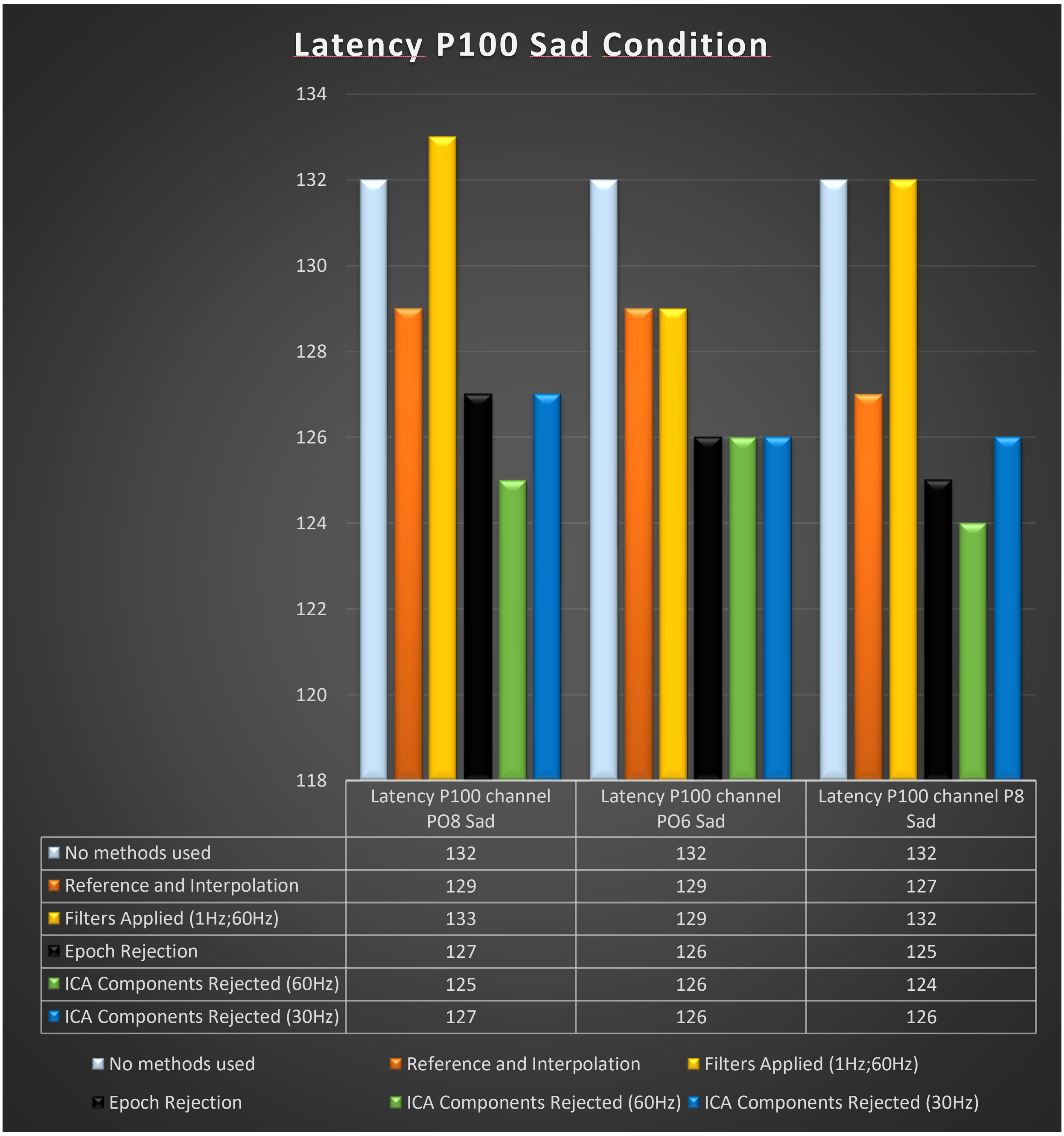
Note that the significant periods are represented in black above the time axis.

The next tables show the values of the amplitudes and latencies of N170 and P100 for each channel showed previously.



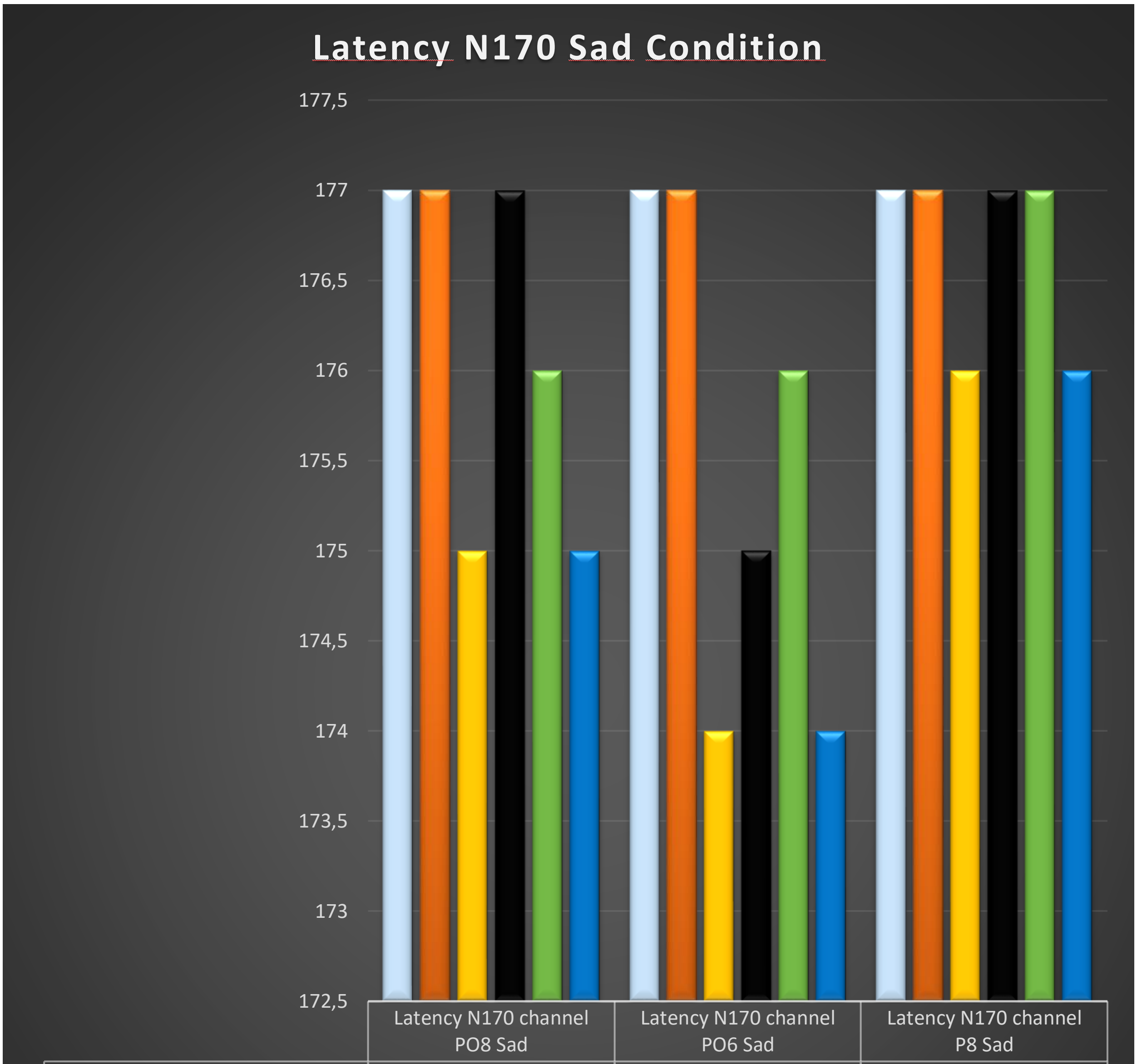
	Amplitude P100 Channel PO6 Sad	Amplitude P100 Channel PO8 Sad	Amplitude P100 Channel P8 Sad
No methods used	10,75	10,27	8,249
Reference and Interpolation	8,917	8,268	6,418
Filters Applied (1Hz;60Hz)	7,617	7,484	5,795
Epoch Rejection	7,208	6,972	5,373
ICA Components Rejected (60Hz)	6,863	6,549	5,024
ICA Components Rejected (30Hz)	7,899	7,662	5,869

Figure 45. Values of Amplitude in μV of P100 for Sad faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.



	Latency P100 channel PO8 Sad	Latency P100 channel PO6 Sad	Latency P100 channel P8 Sad
No methods used	132	132	132
Reference and Interpolation	129	129	127
Filters Applied (1Hz;60Hz)	133	129	132
Epoch Rejection	127	126	125
ICA Components Rejected (60Hz)	125	126	124
ICA Components Rejected (30Hz)	127	126	126

Figure 46. Values of latency in ms of P100 for Sad faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.



	Latency N170 channel PO8 Sad	Latency N170 channel PO6 Sad	Latency N170 channel P8 Sad
No methods used	177	177	177
Reference and Interpolation	177	177	177
Filters Applied (1Hz;60Hz)	175	174	176
Epoch Rejection	177	175	177
ICA Components Rejected (60Hz)	176	176	177
ICA Components Rejected (30Hz)	175	174	176

Figure 47. Values of latency in ms of N170 for Sad faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

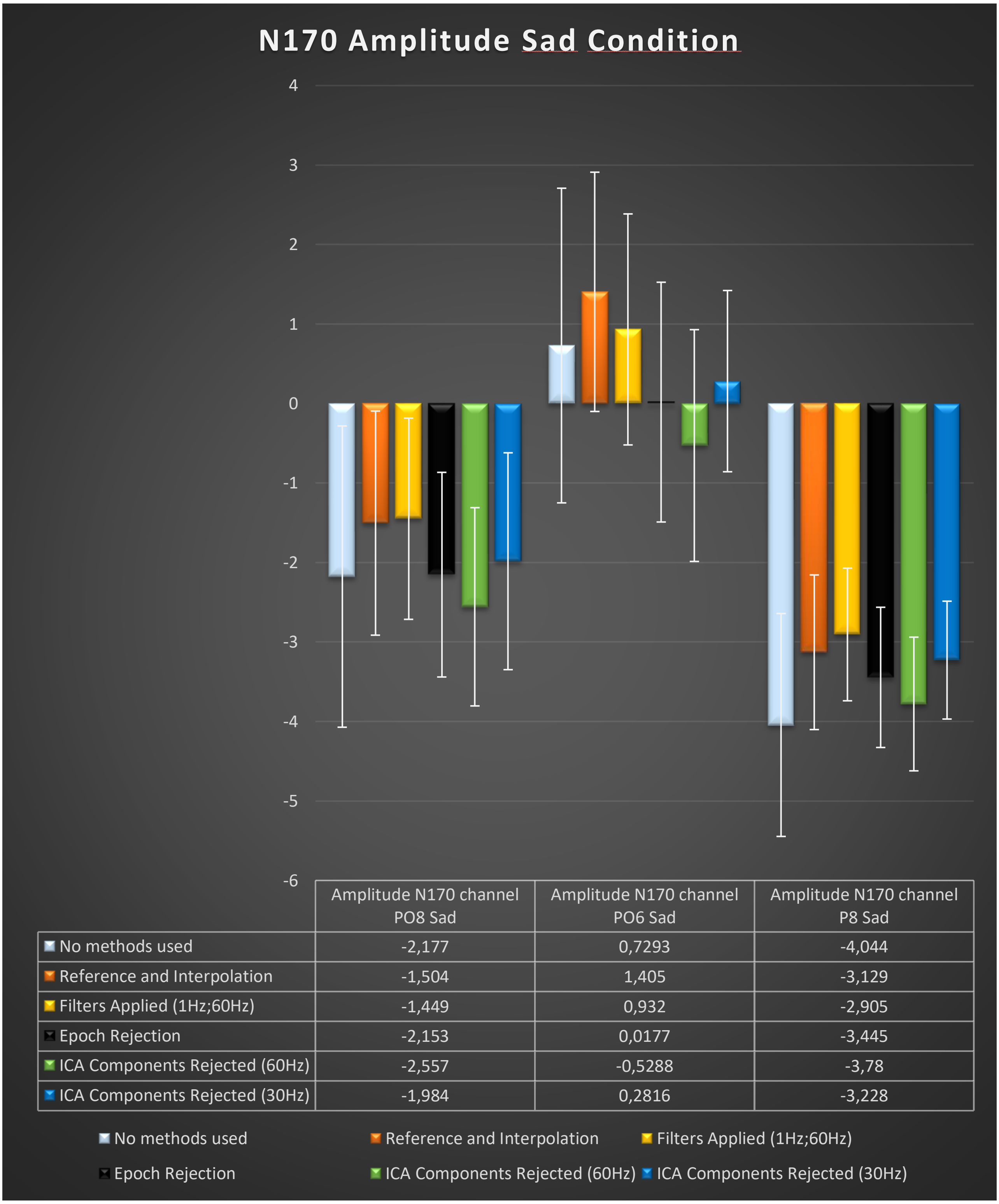


Figure 48. Values of Amplitude in µV of N170 for Sad faces through seven variations of pre-processing methods at channel P8, PO8 and PO6

Analysing the condition “Sad”, what we can see through the figure 44 that was found significant differences for the baseline, P100 and P300 for all the three channels (PO8, PO6 and P8) and significant differences for the channel PO6 to the N170 (using permutation statistics and p-value=0.01).

For the amplitude values of P100, tend to decrease significant for all three channels thought the sequence of the methods and the amplitude of filtering at 60Hz is lower than the amplitude of 30Hz.

To the N170, the opposite of the P100 occurs. The methods tend to increase the amplitude of N170 and the amplitude of filtering at 60Hz is higher than the amplitude of filtering at 30Hz. The PO6 seems to be the exception.

When we talk about the latencies, for the P100 it suggests that the use of methods make the latency decrease, but when talking about the latency of N1700 we could conclude anything since was very ambiguous.

Again, we use the SNR to clarify and analyse these results.

Table 5.Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of P100 for Sad condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Components Rejected (30Hz)
Standard Deviation PO6	1.979	1.505	1.453	1.508	1.458	1.364
Standard Deviation PO8	1.895	1.41	1.266	1.287	1.247	1.14
Standard Deviation P8	1.402	0.9725	0.8346	0.882	0.8408	0.7413
SNR PO6	5.432036	5.924917	5.242257	4.779841	4.707133	5.791056
SNR PO8	5.419525	5.86383	5.911532	5.417249	5.251804	6.721053
SNR P8	5.883738	6.599486	6.943446	6.091837	5.975262	7.917173

Table 6.Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of N170 for Sad condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Components Rejected (30Hz)
Standard Deviation PO6	1.979	1.505	1.453	1.508	1.458	1.14
Standard Deviation PO8	1.895	1.41	1.266	1.287	1.247	1.364
Standard Deviation P8	1.402	0.9725	0.8346	0.882	0.8408	0.7413
SNR PO6	0.368519	0.933555	0.641432	0.011737	-0.36269	0.247018
SNR PO8	-1.14881	-1.06667	-1.14455	-1.67288	-2.05052	-1.45455
SNR P8	-2.88445	-3.21748	-3.48071	-3.9059	-4.49572	-4.35451

Analysing the values of the standard deviation for the sad condition, it decrease with the using of pre-process methods and the lowest standard deviation belongs to the last condition (all pre-process methods used and filtered at 1Hz and 30Hz), with the exception of the STD of PO8.

In what matters to the SNR of P100, for all the channels, the SNR increase until we reject epochs but then decreases with epoch rejection and the rejection of ICA components. Is still important to refer that the highest SNR for P100 is from the last condition (all pre-process methods used and filtered at 1Hz and 30Hz), which means that filtering at 60Hz and 30Hz have a significant difference in the final result for P100.

Referring to the SNR of N170, all pre-process methods added makes the SNR increase for PO8 and P8. The SNR of 60Hz is higher than the 30Hz. Also refer that the channel that has the highest SRN is the channel P8. PO6 is the exception again.

The last study represents the same conditions and variances of pre-processing methods used to be compared but using neutral faces as stimuli. We used parametric statistics again to compare them, p-value equal to 0.01 and we focused on the same channels (PO8, PO6 an P8). The next figure represents the ERP from the three channels.

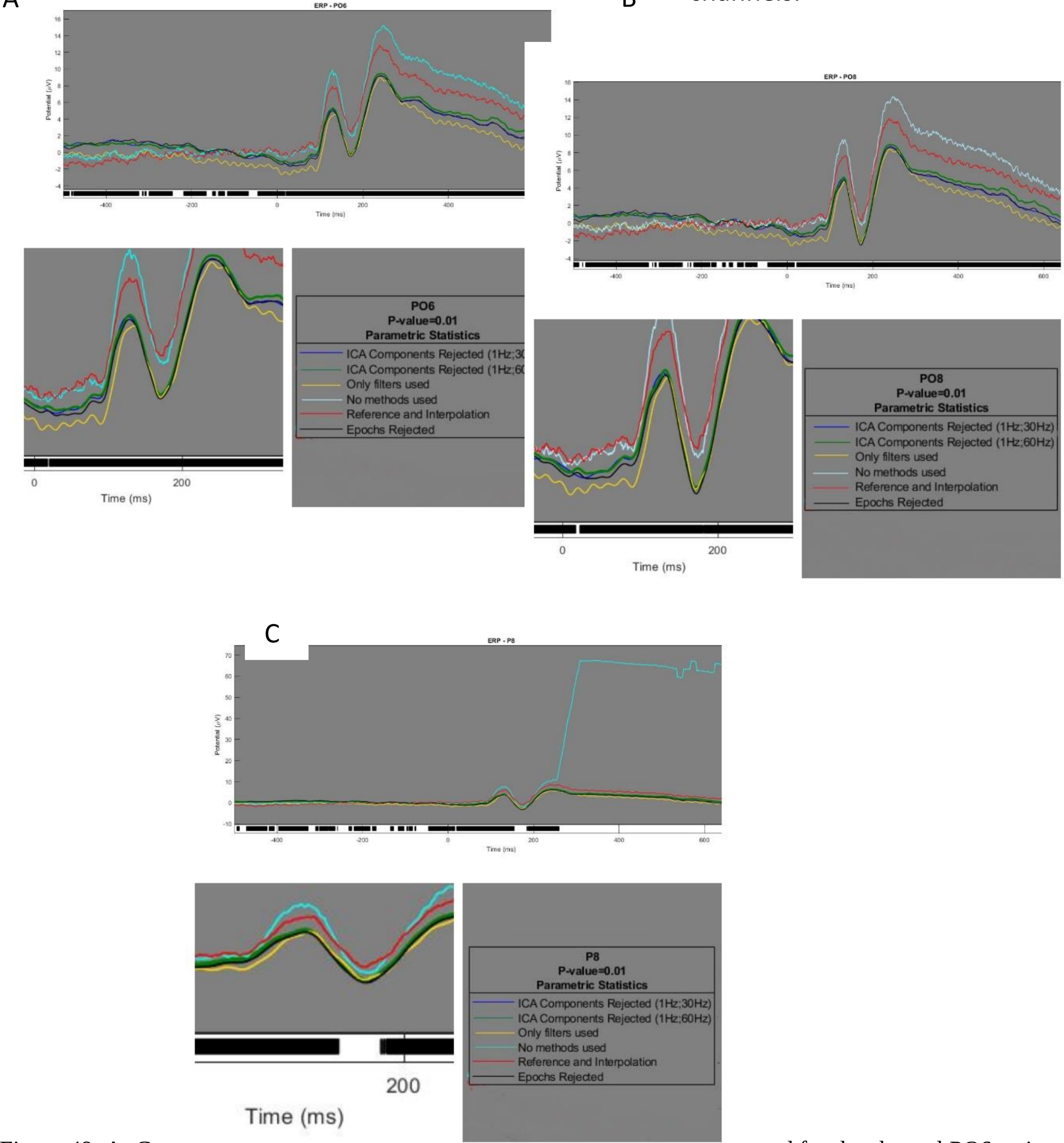


Figure 49. A: Comparison of the ERP between six conditions for neutral faces and for the channel PO6, using parametric statistics with a p-value of 0.01. B: Comparison of the ERP between the six conditions for neutral faces and for the channel PO8, using parametric statistics with a p-value of 0.01. C: Comparison of the ERP between the six conditions for neutral faces and for the channel P8, using parametric statistics with a p-value of 0.01.

Note that the significant periods are represented in black above the time axis.

The next tables show the values of the amplitudes and latencies of N170 and P100 for each channel showed previously.

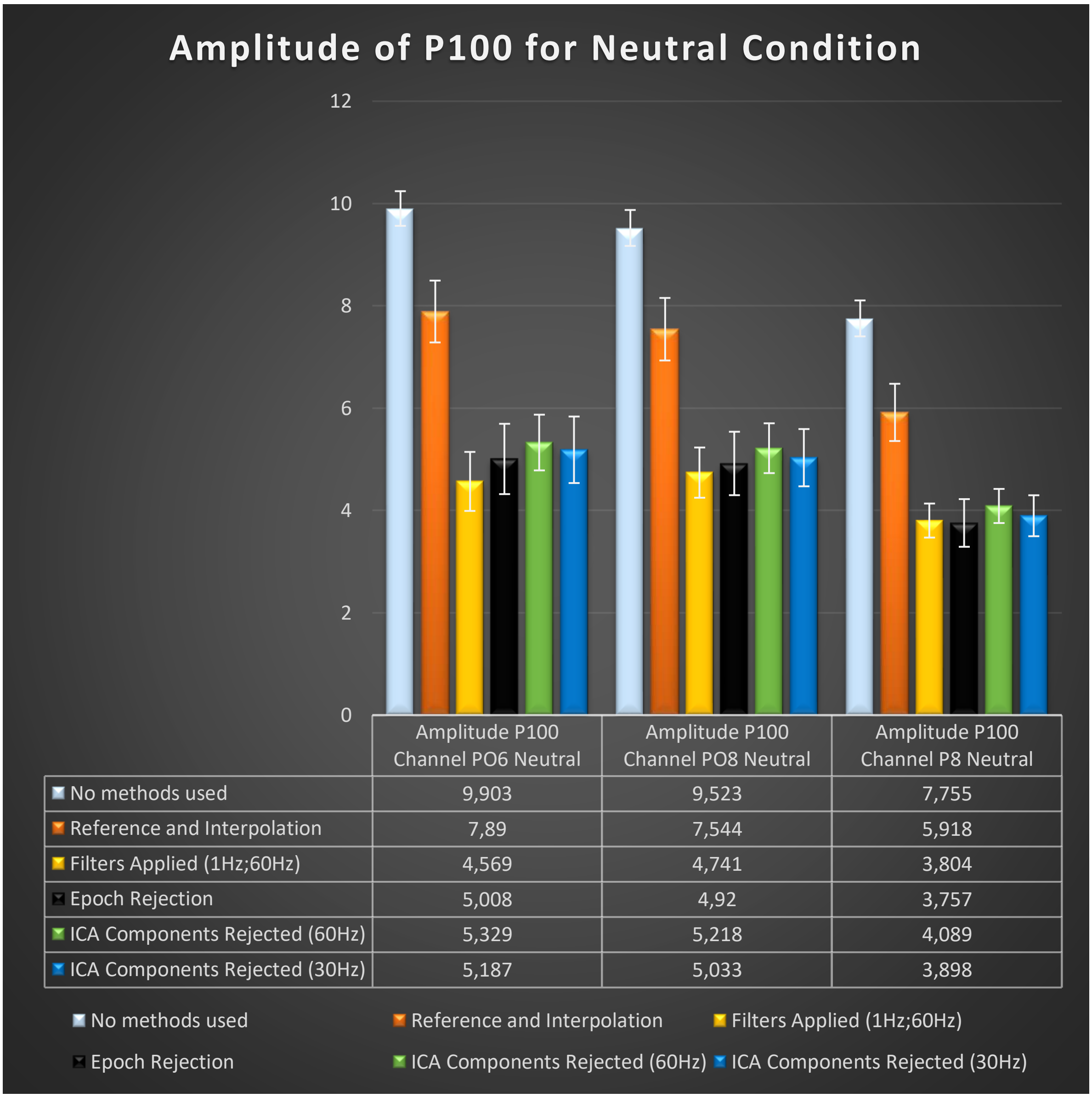


Figure 50. Values of Amplitude in µV of P100 for Neutral faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

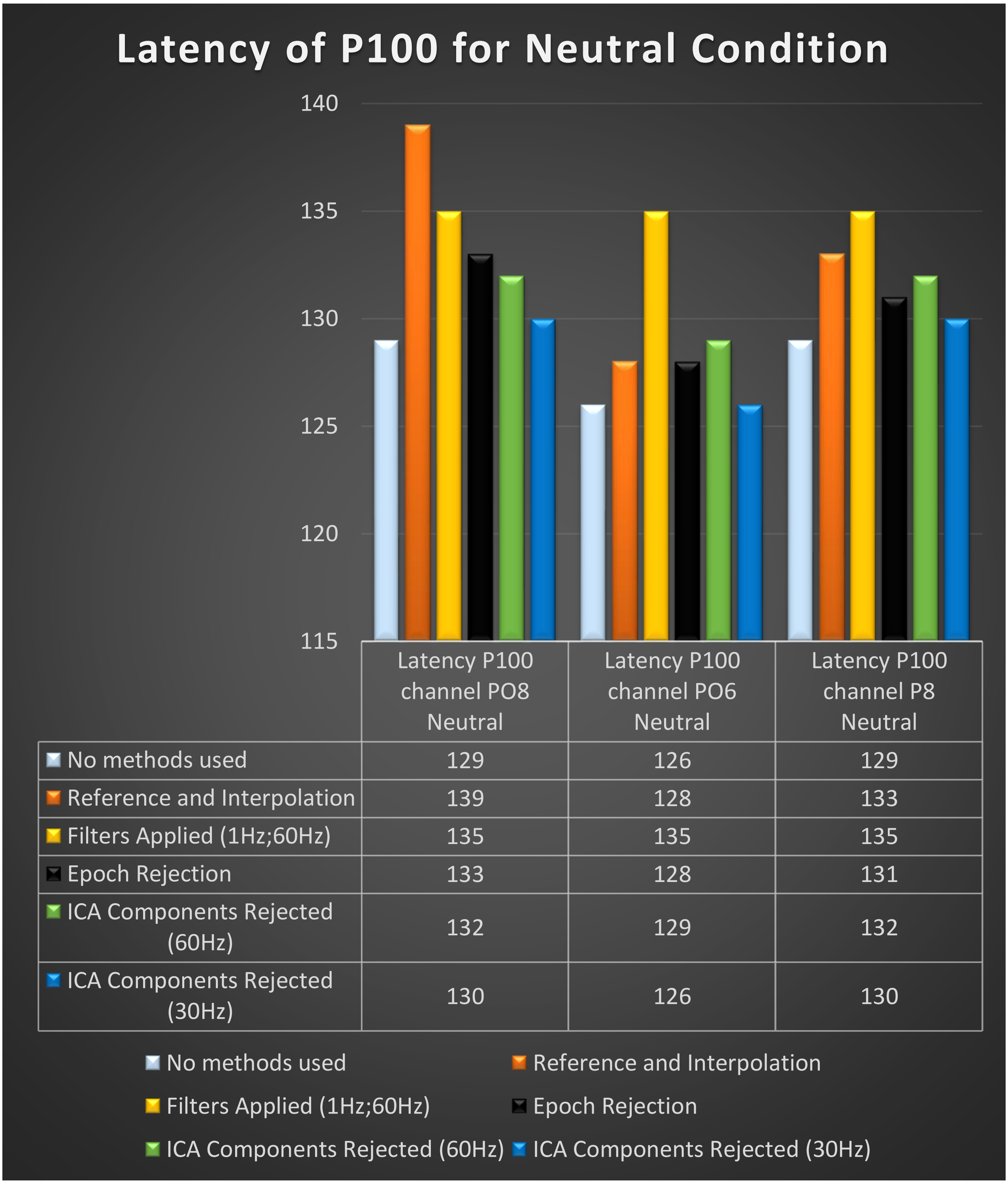


Figure 51. Values of Latency in ms of P100 for Neutral faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

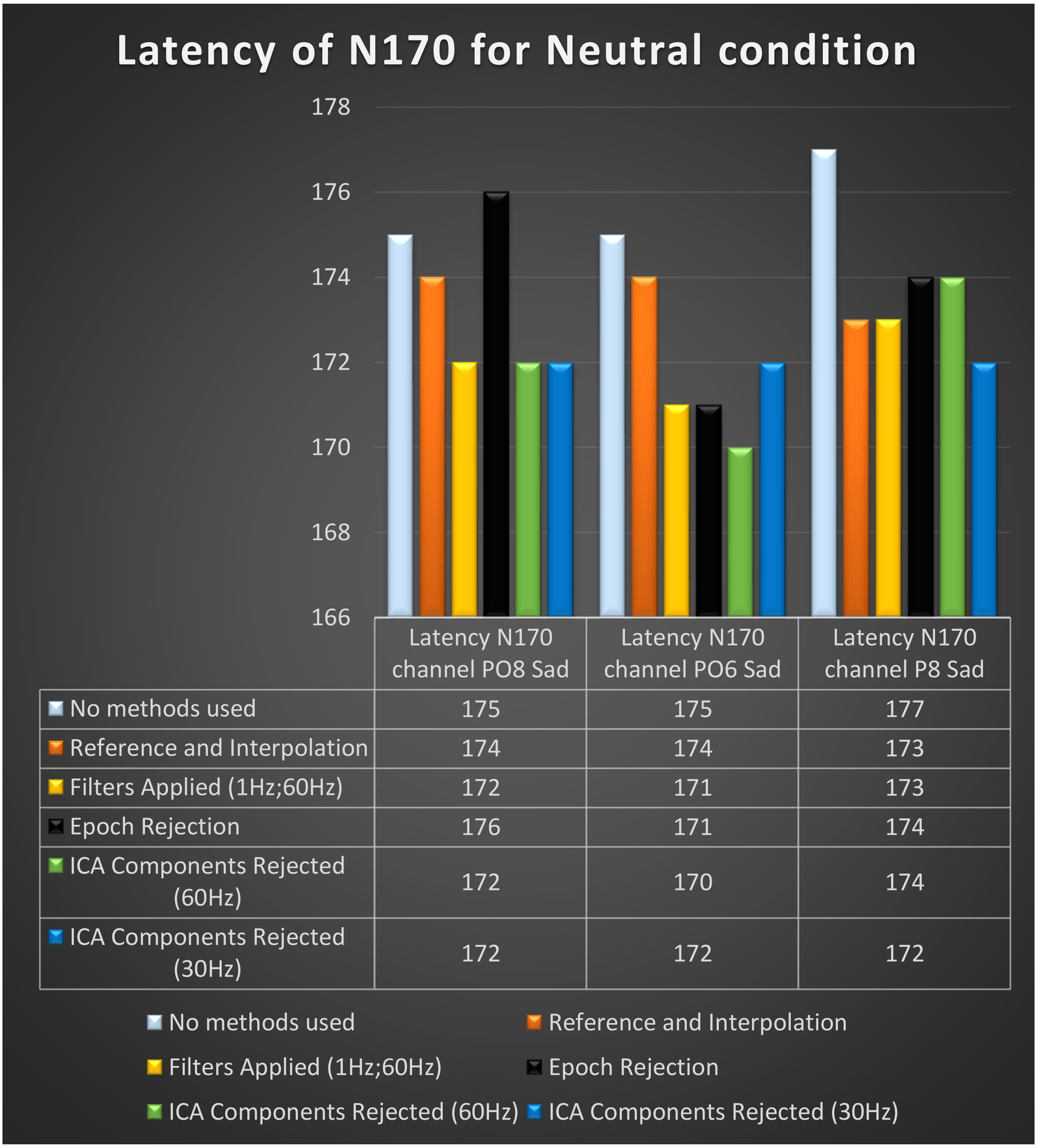


Figure 52. Values of Latency in ms of N170 for Neutral faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

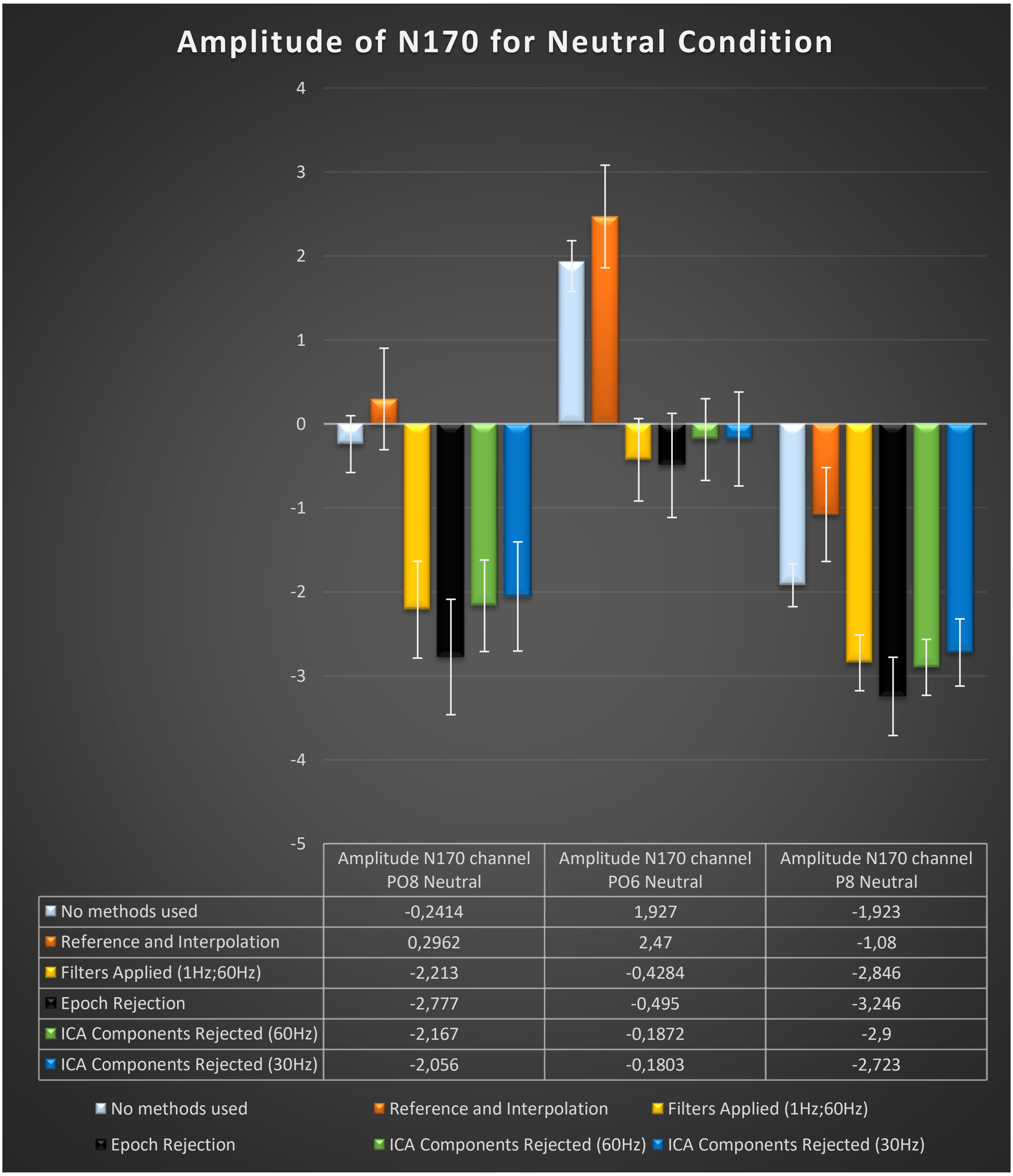


Figure 53. Values of Amplitude in µV of N170 for Neutral faces through seven variations of pre-processing methods at channel P8, PO8 and PO6.

The last analyse we made was to the neutral condition. Through the figure 49 was possible to see and found significant difference for almost all timeline, and more specific to N170, P100 and P300 for the channels PO8 and PO6. The channel P8 found only significant differences for P100 and P300 (using permutation statistics and p-value=0.01). Also refer that the number of trials is higher than the first two condition.

For the amplitude of P100, it decreases until the filters applied but after that starts to increase again. Filtering at 30Hz makes the amplitude be smaller than at 60Hz.

In terms of N170, in general, apply methods makes the amplitude increase. and the amplitude of filtering at 60Hz is higher than the amplitude of filtering at 30Hz. The PO6 seems to be the exception.

When we analyse the latency, we couldn't conclude anything because there was no pattern for both ERP's.

Table 7.Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of P100 for Neutral condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Components Rejected (30Hz)
Standard Deviation PO6	0.3386	0.6045	0.5766	0.6873	0.5447	0.65
Standard Deviation PO8	0.3513	0.6112	0.4907	0.6198	0.4867	0.5596
Standard Deviation P8	0.2546	0.5593	0.3326	0.466	0.3334	0.4
SNR PO6	29.2469	13.05211	7.924037	7.286483	9.783367	7.98
SNR PO8	27.10788	12.34293	9.661708	7.938045	10.72118	8.993924
SNR P8	30.45954	10.58108	11.43716	8.062232	12.26455	9.745

Table 8.Values of Standard deviation and SNR of PO8, PO6 and P8 for the amplitude of N170 for Neutral condition

	No methods used	Reference and Interpolation	Filters Applied (1Hz;60Hz)	Epoch Rejection	ICA Components Rejected (60Hz)	ICA Compon Rejected (30Hz)
Standard Deviation PO6	0.3386	0.6045	0.5766	0.6873	0.5447	0
Standard Deviation PO8	0.3513	0.6112	0.4907	0.6198	0.4867	0.5
Standard Deviation P8	0.2546	0.5593	0.3326	0.466	0.3334	
SNR PO6	5.691081	4.086022	-0.74298	-0.72021	-0.34368	-0.27
SNR PO8	-0.68716	0.48462	-4.50988	-4.48048	-4.45243	-3.67
SNR P8	-7.55302	-1.93099	-8.55683	-6.96567	-8.69826	-6.8

For the values of the standard deviation, applying the methods make the standard deviation decrease. It decreases less than the Sad and Happy condition and the values are closer because the number of trials for the neutral condition are higher than the other conditions.

In what matters to the SNR of P100, for all the channels, the SNR decreases with all the methods used. Is still important to refer that the highest SNR for P100 is from the first condition (no methods used). This is unexpected and can be justify by the higher number of trials which makes a lower STD and results on this SNR.

Referring to the SNR of N170, all pre-process methods added makes the SNR increase for PO8 and P8. The SNR of 60Hz is higher than the 30Hz. Also refer that the channel that has the highest SRN is the channel P8. PO6 is the exception again.

Chapter 4: Discussion

The first part of the work had the purpose of reproduce previous facial recognition studies and compare our results with them. We had the objective of acquire data from task related EEG and compare the impact of pre-processing on the ERPs. We compared three conditions: happy, sad and neutral faces. For this we focused our analysis on the N170 and the P100.

First, we compared happy vs sad using permutation statistics and a p-value of 0.05 as the statistically significance criterium. We found differences in the channel PO8 for N170. The rest of the channels had no differences between these conditions for P100 and N170. (figure 33)

Using the permutation statistics and a p-value of 0.01, we proceed to compare happy vs neutral faces. This time we found significant differences for N170 in channels P8 and CP6 and significant differences for P100 in the channel PO8. (figure 35)

The last comparison that we made was neutral faces vs sad faces with a more liberal p-value criterium of 0.05. We found statistically differences between for N170 for the channel P8 and for P100 for the channels PO8 and PO6, for this criterium.

In the terms of amplitude of N170 the amplitude of happy was found to be the highest and the neutral to be the lowest. For P100 the lowest amplitude was to neutral faces and the highest is hard to define, depending on the channel, although the amplitude of happy faces and sad faces was very close.

The topography for the 170ms is near the same for all conditions. The highest amplitude at 170 ms is found in the occipitotemporal electrodes (P8 and PO8 channels) We can find some amplitude changes in Frontal (F channels) Occipital area (O channels) and Parietal regions (P channels). That is expected since Occipital cortex is related with visual processing, the Frontal cortex with the problem-solving and decisions and Parietal area with visual attention. [62] Our task requires all these processing pathways. We associated the highest amplitude found at that latency (occipitotemporal electrodes) to face processing aspects. (figures 34, 36 and 38)

Basically, we could conclude that the peak around 170ms after the event (N170) is a highly replicable characteristic of face recognition. We also could conclude that the peak varies significantly in amplitude when the emotion change, which means that different emotions had a different amplitude suggesting that the brain can recognize different emotions. Happy emotions had a higher amplitude than sad emotions for N170. To summarize the first part, we also conclude that the best location to investigate the N170 corresponds to occipitotemporal electrodes because the amplitude is higher there at that time point.

Our study also corroborates some studies presented here at chapter one. This is the case for example for the study of Jeffreys (1996). That study concludes that there are a specific ERP for face recognition around 170ms after the event and that ERP can be seen better at occipitotemporal electrodes.

The next studies that our research support are the ones that suggest that different emotions on faces have different amplitudes for N170. Studies from Batty & Taylor, (2003), Blau et al., (2007), Gur et al., (2002), Righart & de Gelder (2008a) and Willis et al., (2009) report results in this line and our analysis support their findings. We also support Chen et al., (2014) findingsthat happy faces had a higher amplitude then sad face.

On the other side, our study does not support studies like Eimer et al., (2003) and Holmes and Eimer (2006) who had negative results.

These leads to the conclusion that replicability is still a problem in this domain. Considering that the problem is not the experimental process, there are two ways to justify these results. The first is the channels that they are looking for the differences. In the Eimer et al., (2003), for example, they focus on differences on channels different of ours. They report mainly results at T5/T6 channels and we only found differences at P8/PO8 and PO6 channels. However, it is likely that they also analysed the same channels we reported here, leaving unexplained this spatial mismatch

A potential problem can be the different methods used in pre-processing the data.

Our second objective of our work is to find out if the pre-processing methods have influence on the amplitude and which methods are the best and which ones can be recommended for use.

To accomplish that objectives, we compared for each condition the addition of pre-process methods to understand the influence of them. We started with no processing methods, and we finished with the removal of ICA components. In between we tested the reference and interpolation, the filters, and the epochs rejection.

The conclusion that we make from all this analysis is that the pre-processing methods have strong influence on the amplitude and statistical inference. Through the figures 39, 44 and 49 we can see that as we use more processing steps the amplitude of the P100 peak decreases, leading to a signal loss. On the other way the amplitude of N170 is preserved or even increases with the using of the methods. To understand if we were correctly using the methods and if they were giving positive feedbacks for the results, we examined the SNR value. We searched for an increase of SNR for every method, which would mean that even if we were losing signal along the methods, the ratio between signal and noise should increase.

Looking for the SNR results we can see that for the P100 the SNR increases until we reject epochs for the channels P8 and PO8. Doing the epoch rejection and the rejection of ICA components the SNR decreases. Also, applying a filter of 30hz instead of 60Hz gives a better SNR. For our results, we can conclude that rejecting epochs and applying ICA (removing components) may potentially remove more signal then noise. Is important to refer that this is internally valid to our data, but it may not have external validity.

In terms of N170, we can see that with every method increases the SNR. Filtering at 60Hz this time gives a better SNR then filtering at 30Hz and we can conclude that every method for the N170 removes more noise than signal.

Concluding, the best signal-noise ratio is given by the channel P8 for both ERPs. Another conclusion that we can make is that for each ERP, the influence of the high pass and low pass filter is visible in the results. Depending on what we are studying or what ERP we are looking for, we need to adjust the filters to have the more reliable results. If we are looking for the P100, the best choice that when can made that preserves more the signal and reduce more the noise is using all the methods already here described and filtering at 30Hz instead of 60Hz (Highest SNR); in what matters of N170 , what we recommend is to use all methods again but filter at 30Hz, since the signal has the highest amplitude (more signal than the others) and the noise is reduced to the minimum (SNR is the highest). This is valid to our data (internal validity), but it may not have external validity

Lastly, we want to recommend the use of the different pre-processing methods that we used here (reference, interpolation, filters, epoch rejection, ICA components rejection), since we prove that each one makes the results slightly better. Each method improves and increase the SNR (for the N170) which lend us to a better and a more reliable result in the end. As a guideline we recommend to use the interpolation of the bad channels first; reference using the average reference as second; using filters for all data (1Hz for ICA and 30Hz or 60Hz depending of the ERP that one wants to analyse); Epoch rejection; and for the last, run ICA and reject up to 4 or 5 bad components.

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