BIOMEDICAL SIGNALS

LAB SESSION 7 WITH MATLAB
SYNCHRONIZED AVERAGING (ERP)

Objectives:

- To study the effect of synchronized averaging on Event Related Potentials (ERP).
- To study the effect of the number of trials in repetitive stimulation for synchronized averaging.
- To study the effect of a poor alignment in synchronized averaging.

1. Event Related Potentials (ERP)

Electroencephalography (EEG) provides an excellent medium to understand neurobiological dysregulation, with the potential to evaluate neurotransmission. Time-locked EEG activity or event-related potential (ERP) helps capture neural activity related to both sensory and cognitive processes.

Event-related potentials (ERPs) are very small voltages generated in the brain structures in response to specific events or stimuli. They are EEG changes that are time locked to sensory, motor or cognitive events that provide safe and noninvasive approach to study psychophysiological correlates of mental processes. Event-related potentials can be elicited by a wide variety of sensory, cognitive or motor events. They are thought to reflect the summed activity of postsynaptic potentials produced when a large number of similarly oriented cortical pyramidal neurons (in the order of thousands or millions) fire in synchrony while processing information. ERPs in humans can be divided into 2 categories. The early waves, or components peaking roughly within the first 100 milliseconds after stimulus, are termed 'sensory' or 'exogenous' as they depend largely on the physical parameters of the stimulus. In contrast, ERPs generated in later parts reflect the manner in which the subject evaluates the stimulus and are termed 'cognitive' or 'endogenous' ERPs as they examine information processing. **The waveforms are described according to latency and amplitude**. The peak amplitude value relative to pre-stimulus baseline and latencies, defined as the time taken to reach the peak value after stimulus presentation.

ERPs waveforms

The **P2** (also called P200) is an early visual component which peaks at approximately 150–200 ms over occipital regions. P2 amplitude is smaller when perceptual load demands increase. For instance, increasing the number of irrelevant stimuli within a display caused a diminished P2 over the parietal–occipital region. Finding a decrease in P2 amplitude under conditions of cognitive load, such as working memory maintenance, would suggest that less attention was allocated for target detection in the secondary task. Therefore, significant changes in P2 amplitude can be expected, indicating that early

attentional processing in the visual cortex is diminished in the high working memory load condition. More information is available at the Wikipedia.

The **N2** potential (also called N200) is a negative deflection peaking at about 200 msec after presentation of stimulus. Alcohol induces an increase in the latency of N2 for example, and Schizophrenia patients have demonstrated reduced P2 and N2 amplitudes. More information is available at the Wikipedia.

The **P3** (also called P300) wave was discovered by Sutton et al. in 1965 and since then has been the major component of research in the field of ERP. For auditory stimuli, the latency range is 250-600 msec that is maximal over the central–parietal region for most adult subjects between 20 and 70 years of age. The latency is usually interpreted as the speed of stimulus classification resulting from discrimination of one event from another. Shorter latencies indicate superior mental performance relative to longer latencies. P3 amplitude seems to reflect stimulus information such that greater attention produces larger P3 waves. A wide variety of paradigms have been used to elicit the P3, of which the "oddball" paradigm is the most utilized where different stimuli are presented in a series such that one of them occurs relatively infrequently — that is the oddball. The subject is instructed to respond to the infrequent or target stimulus and not to the frequently presented or standard stimulus. Reduced P3 amplitude is an indicator of the broad neurobiological vulnerability that underlies disorders within the externalizing spectrum (alcohol dependence, drug dependence, nicotine dependence, conduct disorder and adult antisocial behavior).

The P3 is associated with shifts in attention that update representations in working memory. P3 amplitude decreases when attention is directed away from the current target. The P3 is also sensitive to demands placed on working memory. High working memory load would affect attentional ERP responses over the occipital and parietal regions. A decrease in P3 amplitude over the parietal region is expected when cognitive load is increased.

There are important relationships of this ERP with compensatory mechanisms provided by neurophysiological studies. In an investigation using the P3 and a sample of 89 **Multiple Sclerosis** patients and their controls, the patient group showed an enhanced P3 frontal amplitude in a choice reaction time task. Interestingly, the amplitude of the P3 correlated positively with cognitive performance in the patient group but not in the controls. In addition, a neurophysiological study measuring the P3 found increased ERP in patients relative to controls. Furthermore, cognitive performance was positively correlated with P3 amplitude, and patients displaying low amplitudes were more cognitively impaired. No such correlation between P3 amplitude and performance was observed in the control group.

Moreover, P3 reduction has been found in manic psychosis. Latency prolongation and amplitude reduction were seen in chronic bipolar patients. Reduced amplitude of P3 has been seen in depressed patients, mainly with suicidal ideations, psychotic features or severe depression.

From the other side, the analysis of the stimulus-locked averages also showed alterations in stimulus perception and categorization in the **Schizotypal personality disorder** (SPD) group. The amplitude of the P2, an exogenous component associated with stimulus identification, was reduced in the SPD sample. SPD individuals also show larger P3 latencies.

One of the most robust neurophysiological findings in schizophrenia is decrease in P3 amplitude. P3 is often smaller in amplitude and longer in latency in patients who have been ill longer. P3 latency was

found to be increased in schizophrenic patients but not in their first-degree relatives. In longitudinal analyses, P3 amplitude is sensitive to fluctuations in the severity of positive symptoms, independent of medication, and to the enduring level of negative symptom severity.

Regarding **neurotic disorders**, studies show that individuals with spider and snake phobias showed significantly larger P3 amplitudes than healthy controls when exposed to pictures of their feared objects, indicating enhanced processing of stimuli that reflect critical fear concerns.

In **panic disorder** an enlarged frontal P3 to distractor stimuli among patients has been reported using a three-tone discrimination task, supporting the hypothesis of dysfunctional prefrontal-limbic pathways. ERPs elicited by threat-relevant stimuli support the existence of an attentional bias, showing larger amplitude of P3 and slow waves in response to fear-related words or pictures in subjects with high-trait anxiety or **anxiety disorders** when compared with healthy controls.

Obsessive compulsive disorder (OCD) patients are seen to have significantly shorter P3 and N2 latencies for target stimuli and greater N2 negativity when compared with normal controls. However, there are no significant relationships between these ERP abnormalities in OCD patients and the type or severity of their OCD symptoms. An increased P3 latency in OCD patients has been found but no difference in amplitude.

Patients showed significant reduction in the amplitudes of P3 during **dissociative disorders** compared with the levels at remission. The latency of P3 remained unchanged. The amplitudes of P3 might be a state-dependent biological marker of dissociative disorders.

More information is available at Wikipedia.

In healthy subjects, several studies have reported some relationships between N2, P3 and personality. A consistent result of these studies is that introverts exhibit higher P3 amplitude than extroverts. P3 amplitude is weakly correlated (positively) to the self directedness dimension. Longer N2 latency may be associated with higher harm avoidance score. N2 amplitude is negatively correlated to persistence. This indicates that lower N2 amplitude may be related to a higher persistence score.

In conclusion, ERP constitutes a millisecond-by-millisecond record of neural information processing, which can be associated with particular operations such as sensory encoding, inhibitory responses and updating working memory. Thus, it provides a noninvasive means to evaluate brain functioning in patients with cognitive disorders and is of prognostic value in few cases. ERP is a method of neuropsychiatric research which holds great promise for the future.

2. Error correction tasks

Error monitoring and error correction are important executive functions which help humans to adapt to their environment and anticipate, learn, correct and mend the consequences of their actions. These functions are useful to avoid possible accidents (e.g., while driving) and to monitor error-prone situations.

The peak of the error-(related) negativity (ERN) is an ERP observed approximately 60–100 ms after the erroneous response in averages computed time-locked to the subject's response. The ERN is maximal when accuracy is stressed but, in some circumstances, is present even when errors are not consciously detected.

The ERN has been interpreted as a physiological correlate of the error detection process proper (that is, the more salient an error is, the more pronounced the ERN will be). Other authors have highlighted the role emotional and motivational aspects of the error in the generation of the ERN. Furthermore, a recent theory views the ERN as a particular case of activation of a neural system responsible for detecting conflict. This theory postulates that the system that generates the ERN is active in trials in which two competing responses are activated. Supporting this view, a second scalp potential, the N2, which is observed in the stimulus-locked averages as descried above, has been found to be enhanced in correct responses to high conflict trials, e.g., incongruent flanker stimuli (see below). There is a relationship between the ERN and corrective behavior. The increased ERN amplitude found for very fast corrected errors as compared with slow corrections suggested an involvement of the process underlying the ERN in the correction and compensation of erroneous responses.

In the last two decades, the performance monitoring system has been studied intensively using more specific neurophysiological measures than the P3, such as the error-related negativity or ERN. This component of the ERP is observed following behavioral errors. The ERN has been interpreted as a correlate of the error detection process. It has a frontocentral topography and its generators have been located in the anterior cingulate cortex and adjacent structures in the frontal lobe.

Regarding neurological disorders, clinical status can be defined by the Expanded Disability Status Scale (EDSS) [16]. Additionally, the **Multiple Sclerosis** Severity Score (MSSS) can be obtained. The more negative or larger the ERN, that is, the greater the neural recruitment, the longer the patients stayed relapse-free. On the contrary, those patients showing smaller (less negative) ERN values had greater EDSS and MSSS scores, indicating a worse clinical status.

Additionally, a significant positive correlation between the EDSS score and amplitude of the ERN at Cz and Pz have been found; and between the MSSS and amplitude of the ERN at Cz and Pz. That is, the smaller the ERN (the less negative its value), the higher the impairment

Studies in psychiatric populations have shown reduced ERN in patients with **schizophrenia** indicating deficits in the neural system involved in the generation of this component. These reductions have been proposed as an endophenotypic marker for schizophrenia spectrum disorders.

In healthy volunteers, typical and atypical antipsychotics reduce ERN amplitude, suggesting the blockade of the reinforcement-learning signals and impairs behavioral monitoring. Low ERN amplitude values are observed in schizophrenia, and these abnormal measures normalize following the administration of atypical antipsychotics such as risperidone. ERN can be used as a correlate of behavioral monitoring.

More information is available at the Wikipedia.

3. LAB EXERCISE 1

3.1 Experimental protocol: Eriksen Flanker task stimuli

A choice reaction time task, the Eriksen flanker task was used. Participants were required to respond to the center letter of a 5 letter array, designated as “target”, with either a left-hand or right-hand response.

Additional letters flanking the target letter either favored the target response (compatible trials, HHHHH or SSSSS called congruent) or primed the other response (incompatible trials, HHSHH or SSHSS called incongruent). To optimize the number of errors produced, 60% of the trials were incongruent.

Stímulus	Stimulus code	Response Code
HHHHH	1	1 (left)
SSHSS	2	1 (left)
SSSSS	3	8 (right)
HHSHH	4	8 (right)

As can be seen, there are four types of stimuli with their corresponding codes (1, 2, 3 and 4) but only two types of response (1 and 8). We call stimuli 1 and 3 congruent and 2 and 4 incongruent. It has been shown that the reaction time for incompatibles is statistically longer than for compatible ones (that is, the stimulus processing time is longer if the lateral letters "flankers" do not coincide with the central one).

The possible combinations are reduced to:

Correct answers:

Stímulus	Response
1	1 (congruent)
2	1 (incongruent)
3	8 (congruent)
4	8 (incongruent)

Incorrect answers:

Stimulus	Response	Correction
1	8	1
2	8	1
3	1	8
4	1	8

As can be seen, to obtain the averages in the incorrect responses we use only those incorrect responses that have been subsequently corrected by the volunteer, that is, those in which the incorrect response code is followed by another correct response code. This is done in this way because the ERN has been associated with the conscious detection of the commission of the error. That is, when the volunteer realizes that he was wrong. Before the test, the volunteers are instructed to correct all their wrong

answers. So in practice, the correct incorrect answers tend to predominate over the uncorrected ones. For this reason, the incorrect ones do not distinguish between congruent or incongruent.

Each stimulus array subtended about 2.5° of visual angle in width, and a fixation cross was presented in the middle of the computer monitor just below the target letter in the array. The duration of the stimuli was 100 ms with a random stimulus onset asynchrony between 900 ms and 1100 ms. Letter/hand assignments were counterbalanced between subjects. Prior to the first experimental session, participants were trained with 200 trials to reach a reaction time (RT) baseline level. Stimuli were presented in four groups of 50 trials and the experimenter monitored the percentage of choice errors. Participants were encouraged to respond faster until a certain percentage of errors was committed. The goal of this procedure was to aim for a reaction time that would yield approximately 10-15 % of errors. The experiment proper consisted of 6 blocks of 4 minutes and 200 stimuli each. A 30 second rest period was allowed between blocks.

In order to standardize behavior across subjects and treatments, participants were encouraged to respond to the stimuli as fast as possible and to correct their errors as fast as possible whenever they detected them. In a previous study by our group we found that encouraging or discouraging error correction has an influence on ERN amplitude. By encouraging all subjects to correct their errors we avoided the confounding factor of differences in ERN being due variations in the nature of the error (perceived vs. non-perceived). It also had the advantage of yielding a very informative behavioral parameter: the percentage of erroneous responses that were later corrected.

3.2 EEG recording

The electroencephalogram (EEG) was recorded continuously from the scalp using gold electrodes placed at 31 standard positions in the following order: Fp1, Fp2, F7, F3, Fz, F4, F8, FT7, FC3, FC4, FT8, T3, C3, Cz, C4, T4, TP7, CP3, CP4, TP8, T5, P3, Pz, P4, T6, PO3, PO4, O1, O2, LEFT mastoid channel, RIGHT mastoid.

There is data from a healthy volunteer performing the **Eriksen Flanker task stimuli** at the Virtual Campus ATENEA**.** It is composed of 31 EEG channels, all stimuli and corrections with their code and times associated with the visual stimuli and also the subject response. Electrode impedances were kept below 5 kOhm. The electrophysiological signals were filtered with a bandpass of 0.1-35 Hz and digitized at a rate of 250 Hz.

3.3 Routines available

Using the function

>> tableSIG=promedioStimulusLocked(name,pair,channel);

Stimulus-locked ERPs were generated. These epochs were created around the time point when each stimulus was presented (considering 400 ms before to 1 s later, this is, 1.4 seconds duration for each

epoch). They appear in the matrix *tableSIG* as row vectors. Baseline correction is performed subtracting the mean activity between 100 and 0 ms prior to stimulus presentation from the subsequent activity. Stimulus-locked averages are used to study stimulus identification and categorization using the P2, N2 and P3 components of the ERP. By averaging several epochs/repetitions from *tableSIG*, the P2, N2 and P3 can be identified and quantified. Subsequently, *tableSIG* output can be created separately for correctly-responded congruent and incongruent stimuli. For the former, the pair input associated with the stimulus-response code is [1 1;3 8]. For the latter, the pair to be considered is [2 1;4 8]. Later, the P2, N2 and P3 components will be studied in each of these two types of averages.

There is also a rejection of artifactual epochs. Volunteers generate other signals that overlap or completely obscure the EEG: they blink (high-amplitude, low-frequency artifact that is highest at the frontal electrodes), forehead wrinkles, yawn, or jaw clenching (higher-frequency muscle artifacts), etc. Before and during the test they are instructed to minimize these movements, but they cannot be totally avoided. To reject the artifacts, a series of electrodes are chosen and the amplitude of the signal is looked at, and if it is greater than a threshold (± 75μV in the VEOG channels, Fp1, Fp2, Fz, Cz and Pz) the epoch is totally rejected.

The records have been acquired taking as a reference an electrode located next to the right eye (this channel does not appear in the record since it is the reference channel). However, it is usual to refer the potentials to electrodes located behind the ears and called "mastoids". The registration with respect to a canal located next to the right eye is usually less artifactual than the registration with respect to the mastoids (the ocular artifact is corrected to some extent). However, the ultimate potential is with respect to the mastoids. For this, once the evoked potential has been obtained, it is re-referenced (for all channels) with respect to the average of the R_mast and L_mast channels that are in the register, that is, with respect to (R_mast + L_mast) / 2.

Name input is the file name with the complete path as a string (between 'xxx'). *Channel* input is the lead number according to the list mentioned above.

Using the function

>> tableSIG=promedioResponseLocked(name,triplet,channel);

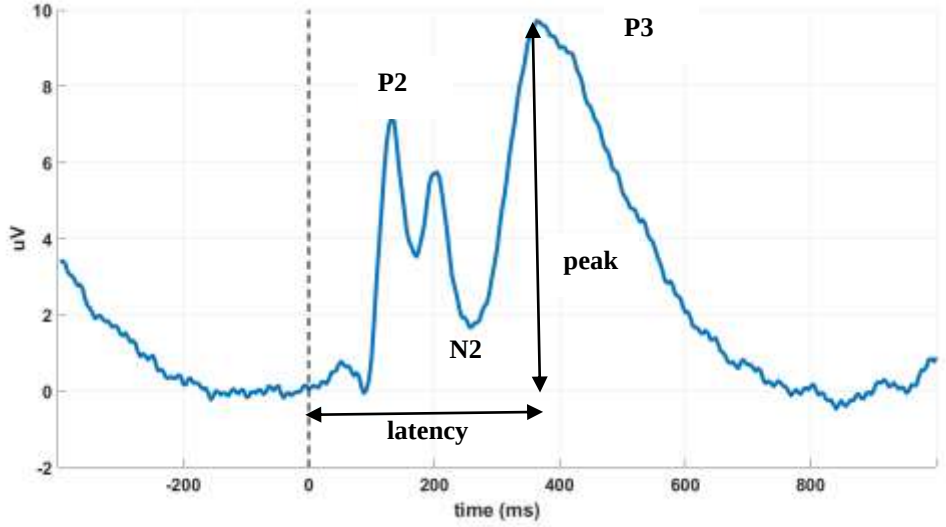
Response-locked EEG epochs were generated to evaluate the ERN component. These epochs were created around the time point when participants emitted a response (button press). Each epoch was also 1400 ms long, starting 400 ms before the subject's response and ending 1000 ms thereafter. They appear as row vectors in the *tableSIG* output as well. Epochs were also baseline corrected subtracting the mean activity between 100 and 0 ms prior to emission of the response. In addition to same artifact rejection procedure described above was applied.

By averaging several or many Response-locked EEG epochs, the ERN is expected to be observed, In this case, all corrections are considered (both congruent and incongruents) must be considered because of the limited number of cases. Thus, the triplet input must be [1 8 1;2 8 1;3 1 8;4 1 8].

3.4 ERPs identification

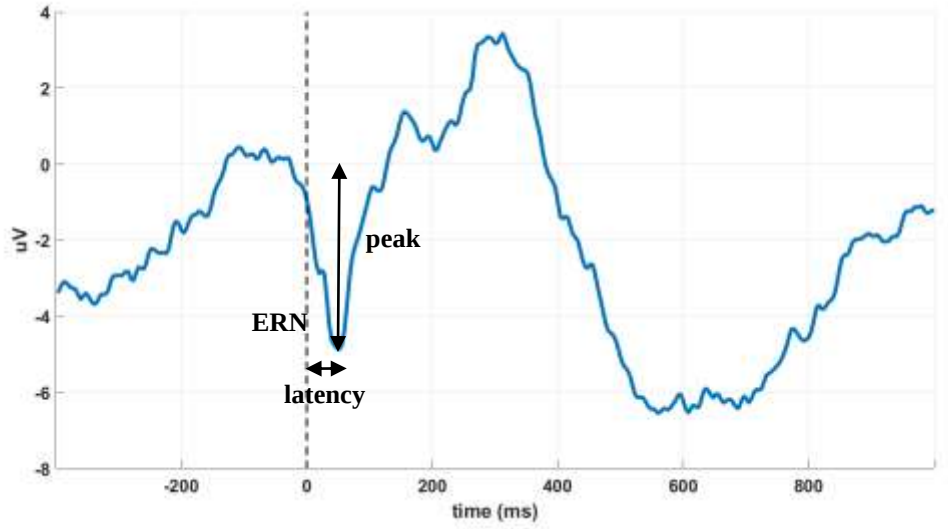
The P2 was identified as the most positive deflection in the 150-250 time range following stimulus presentation in correctly-responded epochs in the stimulus-locked averages. The P3 was identified as the most positive deflection, also in the stimulus-locked ERPs, between 250 and 600 ms following stimulus presentation.

Grand mean average of 9 subjects (stimulus locked)



The ERN was identified in the response-locked averages as the negative deflection in the ERP appearing between 0 and 100 ms following a choice error. The ERN was quantified as the peak voltage in the 0-100 ms post-error time window relative to the preceding trough (trough-to-peak amplitude).

Grand mean average of 9 subjects (response locked)



3.5 Number of trials/repetitions

Plot in the same figure and subplot the first five Stimulus-locked EEG epochs overlapped using 5 different colour traces to analyze easily the similarity between them. Using several subplots in the figure repeat it separately for the Fz, Cz and Pz channels and considering congruent and incongruent stimulus also separately. Are you able to distinguish the P2, N2 and P3 components clearly?

Then, plot in another figure and in the same subplot five Response-locked EEG epochs overlapped using five different colours to analyze easily the similarity between them. Using several subplots in the figure repeat it separately for the Fz, Cz and Pz channels considering both congruent and incongruent stimulus. Are you able to distinguish the ERN component clearly?

Afterwards, calculate the average Stimulus-locked epoch considering the first ten, the first twenty, the first thirty, the first forty and all the epochs available. Plot in the same figure and subplot all five averages with different colours in order to evaluate visually the effect of averaging a highest number of repetitions/epochs. Using several subplots in the figure repeat it separately for the Fz, Cz and Pz channels and considering congruent and incongruent stimuli also separately.

Next, repeat it the same but considering average Response-locked epoch and using subplots in the figure for the Fz, Cz and Pz channels separately considering both congruent and incongruent stimuli. Review the ERN component.

Interesting features from the ERPs are peak or amplitude and latency as described above. The purpose of the next exercise is to evaluate the influence of the number of repetitions/epochs on these features calculated from the average epoch. In order to do that, calculate both features in the average EEG epoch considering different number of epochs: from 5 to the total epochs increasing in a step of five. This is, averaging from the first epoch to the fifth, from the first one to the tenth, from the first to the fifteenth and so on until averaging all available epochs. Then from each average calculate the features from both P3 (using Stimulus-locked epochs and only incongruent stimuli) and ERN (using Response-locked epochs) components. To show the results, build a figure overlapping in the same subplot:

- The P3 amplitude as a function of number of repetitions/epochs averaged and overlapping the traces for Fz, Cz and Pz
- The ERN amplitude as a function of number of repetitions/epochs averaged and overlapping the traces for Fz, Cz and Pz
- The P3 latency as a function of number of repetitions/epochs averaged and overlapping the traces for Fz, Cz and Pz
- The ERN latency as a function of number of repetitions/epochs averaged and overlapping the traces for Fz, Cz and Pz

Thus, a figure with four subplots is generated. Analyze the results and find the minimum number (approx) of epochs should be considered for averaging to find a “stable feature value” from then. Are the values obtained logical quantitatively?

3.6 Epochs alignment

Functions *promedioStimulusLockedv2.m* and *promedioResponseLockedv2.m* are the same as the ones above but with an extra input parameter sigma. These functions calculate the same *TableSIG* but the time instant when the Stimulus or the Response are locked for extracting the epoch are changed a little bit by adding a random value following a normal gaussian distribution with zero mean and standard deviation sigma. The sigma input parameter is in samples (remember that sampling frequency is 250 Hz).
Then, calculate the *tableSIG* considering a misalignment with sigma=10 samples and also sigma=20 samples. Repeat it using both functions. Finally, show the results in a figure overlapping the three average epochs with perfect alignment, and both misalignments. Considering only averaging all epochs (both congruent and incongruent stimuli separately for the Stimulus-locked average) and show also in different subplots for Fz, Cz, and Pz separately. Repeat the same procedure with the ERN (combining both congruent and incongruent stimuli. Try to conclude if the effect of the misalignment ca be significant to modify the ERPs

3.7 Topographic study

In this section, we will try to localize the P3 and ERN on the scalp. For this purpose we will use the function *draw_topogram* already known from the Lab session 3. The P3 and ERN peaks must be calculated from the average of all available epochs in the 19 EEG channels taken into account in this function (consider absolute values in the case of ERN component).
Thus, three topograms must be plotted in one figure:

- P3 peaks (in average of all trials) distribution over the 19 channels for all congruent stimuli
- P3 peaks (in average of all trials) distribution over the 19 channels for all incongruent stimuli
- ERN peaks (in average of all trials) distribution over the 19 channels for all correction responses

Observe and comment differences between them (if there are)

4. LAB EXERCISE 2

EEG signals recorded from MUNR students at the ETSEIB with the OpenBCI equipment are available at the Atena Virtual Campus. They were recorded during the cognitive task of visual short term memory in Lab 2 session. The whole data from the four students are provided but several bad EEG channels and artifacts affected the data quality in student1, student 3 and student 4. We propose to use the EEG signals recorded from student 2 to analyse the event related potential P300.

The variables inside each Matlab file are the following:

- *eegmu2*: an array with the fourteen EEG channels per columns (see Lab 2 guide)
- *fs*: sampling frequency in samples/second (Hz)
- *marks*: events in each trial along the recording associated with the following code numbering:
    - 9 for the fixation cross,
    - 3 for the first image

    - 4 for the second image when they are congruent (objects from both images are the same) or
    - 5 for the second image when they are incongruent (objects from both images are different)
    - 1 for correct response from the subject
    - 0 for incorrect response from the subject
- *marksamples*: the time instants (in samples, that is, integers) when the event from the vector "marks" appears.

Code your own Matlab routine to calculate and plot the average stimulus-locked EEG epoch in Fz, Cz and Pz channels for congruent, incongruent and joint (both congruent and incongruent) stimulus. For this purpose, consider the following aspects:

- An epoch starts 100 ms before the second image appears and ends one second after this stimulus of second image
- Baseline correction in each trial/repetition must be performed subtracting the mean activity between 100 and 0 ms prior to stimulus presentation of second image.
- Smooth the average EEG epoch by low pass filtering with a cut-off frequency of 7 Hz
- Consider only the trials/repetitions with a correct answer from the subject.

The amplitude/peak of the ERP P3 in each of the 14 EEG channels is calculated with the maximum of the average EEG epoch between 250 ms and 400 ms after the stimulus of the second image. Then, the P3 must be localized on the scalp. For this purpose, we will use the function *draw_topogram2* which has been adapted to this case of 14 EEG channels with the same order as presented in the Lab 2 session guide. Three topograms should be obtained considering congruent, incongruent and joint stimulus.

Finally, other EEG-non related features such as the response time (interval between the second image and the motor response) or % or correct responses in congruent and incongruent cases can be calculated to analyse the subject's performance during the experiment. Obtain this response time in mean and std as well as the % of correct reponses not only in student 2 but also the others because they are not related to the EEG signals quality.