Description: Students will take this laboratory as an introduction to the other physiology laboratories in which they will use the knowledge and skills acquired. The course presents an introduction to the acquisition of bioelectrical signals.

I. Content

- Main concept in biomedical signals. Signal Types, signal statistics.
- Spectral analysis.
- Practical issues when recording biomedical signals.
- Example of Biomedical signals. The human EEG.
- Sensors (electrodes) and interfaces.

II. Concept Map

```
Signals
  Deterministic
  Stochastic

Amplification
Filtering

Increase gain
Removal of Artifacts
Avoid saturation
Remove undesired frequencies
Signal to Noise Ratio

Analog signal
  AD conversion
  Sampling frequency
  Samplig Range
  Nyquist Frequency
  Aliasing

Digital Signal
  Increase gain
  Removal of Artifacts
  Avoid saturation
  Digital filtering

Storage (e.g. paper)

Signal Statistics
  Amplitude
  Frequency

Computer storage

Signal Statistics
  Amplitude
  Frequency
```
III. Learning Outcomes

- Identify the main concepts in biomedical signal acquisition.
- Classify the biomedical signals.
- Produce report and interpret different signal statistics.
- Identify potential sources of noise when acquiring a biomedical signal.
- Develop a strategy to solve a 'noise problem' with the signal acquisition.
- Produce measurements of the frequency components of a signal.
- Produce an EEG signal and measure its frequency components as a function of the behavioral state of the subject (i.e., eyes closed, eyes opened).
- Produce auditory ERP and measure amplitude and latency.

IV. Instructional Methods

- Pre-laboratory lecture
- Laboratory

V. Material

Notes on the virtual lab (and on WebCT): http://www.medicine.mcgill.ca/physio/vlab

Books:
- *Signals and Systems*. Oppenheim, A. Willsky AS with Ian T. Young.
- *Clinical Neurophysiology*. Misulis KE, Head, TC.

Notes on Signal Acquisition

**Signals**: Are functions of one or more in dependent variables and typically contain information about the behavior or nature of some phenomenon. Systems usually respond to particular signals by producing other signals.

Electrical brain activity => Power Lab => voltage variations in time

<table>
<thead>
<tr>
<th>Signal</th>
<th>System</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information</td>
<td>in a signal is contained in a pattern of variations of some form. E.g. the human vocal mechanism produces speech by creating fluctuations in acoustic pressure.</td>
<td></td>
</tr>
</tbody>
</table>

**Deterministic Signals**: A signal is deterministic if it is exactly predictable for the time span of interest. Deterministic signals can be described by mathematical models, e.g., a sinusoidal signal is described by: \( V(t) = A \cdot \sin(\omega t) \), where \( V(t) \) is the signal over time. ‘A’ is the amplitude and \( \omega = 2\pi f \) (\( f \) = frequency of the signal).

**Stochastic or Random Signals**: A signal whose value has some element of chance associated with it, therefore it cannot be predicted exactly. Consequently, statistical properties and probabilities must be used to describe stochastic signals.

**Usually**, biological signals often have both deterministic and stochastic components.

**Desired Signal**: A signal that it is not corrupted by noise.

**Signal Amplitude Statistics**: A number of statistics may be used as a measure of the location or "center" of a random signal

*The mean* is the average amplitude of the signal over time.

*The median* is the value at which half of the observations in the sample have values smaller than the median and half have values larger than the median. The median is often used as a measure of the "center" of a signal because it is less sensitive to outliers.

*The mode* is the most frequently occurring value of the signal.

*Maximal and minimal amplitude* are the maximal and minimal maximal values of the signal during a given time interval.

*Range*: The range or *peak-to-peak amplitude* is the difference between the minimum and maximum values of a signal.
**Noise:** Any unwanted signal that modifies the desired signal. It could have multiple sources.

**Signal to Noise Ratio (SNR):** It is a measurement of the amplitude of variance of the signal relative to the variance of the noise. The higher the SNR, the better you can distinguish your signal from the noise.

**Noise sources:** Any discussion of filtering for noise reduction would be incomplete without some discussion of noise. Let’s start by defining some common types of noise.

**Thermal noise** – the random motion of atoms generates this random, uniformly distributed noise. Thermal Noise is present everywhere and has a nearly constant Power Spectral Density (PSD).

**Interference** – imposition of an unwanted signal from an external source on the signal of interest.

**Aliasing** – an artifact of the acquisition process, specifically sampling (see Nyquist rate).

**Sampling noise** – Another artifact of the acquisition process, Sampling Noise occurs when you digitize a continuous signal with an A/D converter that has a finite number of steps. It is interesting to note that you can dither (add white noise) your signal to reduce the overall sampling noise.

**Narrowband/broadband** – two general categories of noise. Narrowband noise confines itself to a relatively small portion of the overall signal bandwidth as defined by Nyquist. Broadband noise occupies a significant portion of the Nyquist bandwidth. For example, 60-Hz hum is narrowband because it typically limits itself to a 60 Hz component. Thermal noise is definitely broadband because its PSD is constant, meaning that it distributes its energy over nearly the entire spectrum.

**Waveform:** The representation of a signal as a plot of amplitude versus time

**Continuous time signals:** The independent variable is continuous, the signals are defined for a continuum of values of the independent variable X(t).

**Discrete time signals:** Only defined at discrete times, the independent variable takes on only a discrete set of values X(n).

A discrete time signal may represent a phenomenon for which the independent variable is inherently discrete (e.g., amount of calories per day on a diet). On the other hands, a discrete signal may represent successive samples of an underlying phenomenon for which the independent variable is continuous (e.g., a visual image capture by a digital camera is made of individual pixels that can assume different colors).

An analog signal is a continuous time signal.

A digital signal is a discrete time signal.

**Analog-digital converters (ADC):** It is a system that inputs an analog electrical signal such as voltage or current and outputs a binary number (0 or 1).

The computer’s ADC allows an electrical signal to be sampled and converted into a digital signal, which is then sent within the computer for further processing. The ADC samples the analogue voltage at its input at a point in time and converts it into a 16-digit binary number. Since each digit of a binary number can take one of the two values 0 or 1, a 16-bit (bit = binary digit) number can take one of \(2^{16}=65536\) values, representing the integers from 0 to 65535. This integer number is then sent to the computer.

When the hardware gain is set to 1, your ADC converts voltages over a range of ±10volts (a 20 volt range). In this case the A/D conversion of +10 (10 000 millivolts (mV)) and -10 volts (-10 000 millivolts (mV)) would be:
Voltage | Binary value | Decimal number sent by the ADC to computer
--- | --- | ---
-10 000 mV | 0000000000000000 | 0
+10 000 mV | 1111111111111111 | 65535

The input voltage range within -10 000 to +10 000 mV is divided into 65536 levels (the integers values 0 - 65535), with each level being 20 000 mV/65535 = 0.305 mV wide. An input voltage lying within one of these 0.305 mV-wide ranges is converted into a specific binary number: for example, any voltage lying in the range from -10 000 to -9 999.695 will be converted into the binary number 0000000000000000, while any voltage in the range between 9 999.695 and 10 000 mvolts will be converted into the binary number 1111111111111111, which is equivalent to the decimal number 65535.

It is important to keep the input signal within the input voltage range of the ADC. If the input voltage exceeds the ±10 volt range, a 16-bit binary number with an equivalent decimal value of 65535 is still returned to the computer. The computer would thus interpret the voltage being sensed to be +10 000 mV, which would be an error. This error is called saturation of the ADC. However, the input signal to the ADC should also span as much of the ADC input voltage range as possible, without saturating the ADC, since this increases the signal resolution (Fig. 1).

For example, if the signal to be recorded is much smaller than ±10 000 mV, say ±5 000 mV, then the range over which the board operates should be decreased. By changing the hardware gain from 10 000 mV (10 V) to 5 V, the operating range of the board is changed from ±10 000 mV to ±5 000 mV. This allows the experimenter to record the ±2 V signal with a significant improvement in signal resolution (2 times greater). This occurs because the minimum resolvable voltage would be 10 000mV/65535 or 0.152 mV versus 0.305 mV when the board's operating range was set to ±10 volts.

**Signal sampling:** The process of obtaining a sequence of instantaneous values of a particular signal characteristic, usually at regular time intervals.

**Sampling frequency:** The sampling frequency is the frequency at which the ADC samples the analogue signal (usually in number of samples per second, (Hz)).

**Sampling Period:** The reciprocal of the sampling frequency, i.e., the interval between corresponding points on two successive sampling pulses of the sampling signal.

**Sampling Range:** The range between the minimal and maximal values at which you will sample the signal (e.g., if you sample between -10 V and +10 V the sampling range is 20V)

**Offset:** A fluctuation in the baseline value of the signal.

**Gain and amplification:** It is the factor by which you multiply your signal. If a gain is 1, the signal remains unchanged, if the gain is higher than 1, the signal is amplified, if the gain is lower than 1, the signal is reduced.

**Amplitude saturation:** It occurs when the intensity of a signal exceeds the values within the sampling range. For example if we acquire a signal which intensity is +20V and we are sampling between -5V and +5V. It produces a distortion of the signal, i.e., over the interval in which the signal reaches the +20V, the output of our ADC will be +5V.
**Spectral analysis:** Is the process of decomposing a signal in different frequency components and plot the intensity of each component as a function of its frequency.

**Fourier analysis:** It is a mathematical technique that allows us to perform a spectral analysis on the recorded signal.

**Nyquist interval:** The maximum time interval between equally spaced samples of a signal that will enable the signal waveform to be completely determined. The Nyquist interval is equal to the reciprocal of twice the highest frequency component of the sampled signal. In practice, when analog signals are sampled for the purpose of digital transmission or other processing, the sampling rate must be more frequent than that defined by Nyquist's theorem, because of quantization error introduced by the digitizing process. The required sampling rate is determined by the accuracy of the digitizing process. To provide a safety factor to guard against information loss, it is usual to sample at five to ten times the highest expected frequency rather than the minimum two times.

**Nyquist Sampling Rate:** Is the value of the sampling frequency equal to twice the maximal frequency of the signal we are acquiring.

**Filters:**
A biological signal can be broken down into fundamental frequencies, with each frequency having its own intensity. Display of the intensities at all frequencies is a power spectrum. Usually we are interested in signals of a particular frequency range or bandwidth. The bandwidth is determined by filters, which are devices that alter the frequency composition of the signal.

*Ideal Frequency-selective filter:* Is a filter that exactly passes signals at one frequency and completely rejects the rest.

There are three types of filter:
- Low frequency or in old terminology high pass. Filters low frequencies
- High frequency or in old terminology low pass. Filters high frequencies.
- Notch filter. Filters one frequency, usually 60 Hz from the power lines.

**Real filters** or **hardware filters** alter the frequency composition of the signal. It means after filtering the signal, we cannot recover the frequencies that have been filtered.

**Digital filters** change the frequency of the signal by performing calculations on the data. It means you can record all the frequency components of your signal and by digitally filtering it, eliminate the unwanted frequencies. You can still recover the filtered frequencies if you keep a record of the original signal.

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**Notes on EEG**

The electroencephalogram (EEG) is a recording of the electrical activity of the brain from the scalp. The recorded waveforms reflect the cortical electrical activity.

**Signal Intensity:** EEG activity is quite small, measured in microvolts (µV).

**Signal frequency:** The main frequencies of the human EEG waves are:
- **Delta:** has a frequency of 3 Hz or below. It tends to be the highest in amplitude and the slowest waves. It is normal as the dominant rhythm in infants up to one year and in stages 3 and 4 of sleep. It may occur focally with subcortical lesions and in general distribution with diffuse lesions, metabolic encephalopathy hydrocephalus or deep midline lesions. It is usually most prominent frontally in adults (e.g. FIRD - Frontal Intermittent Rhythmic Delta) and posteriorly in children e.g. OIRDA - Occipital Intermittent Rhythmic Delta).
- **Theta:** has a frequency of 3.5 to 7.5 Hz and is classified as "slow" activity. It is perfectly normal in children up to 13 years and in sleep but abnormal in awake adults. It can be seen as a manifestation of focal subcortical lesions; it can also be seen in generalized distribution in diffuse disorders such as metabolic encephalopathy or some instances of hydrocephalus
- **Alpha:** has a frequency between 7.5 and 13 Hz. Is usually best seen in the posterior regions of the head on each side, being higher in amplitude on the dominant side. It appears when closing the eyes and relaxing, and disappears when opening the eyes or alerting by any mechanism (thinking, calculating). It is the major rhythm seen in normal relaxed adults. It is present during most of life especially after the thirteenth year.
• **Beta**: Beta activity is 'fast' activity. It has a frequency of 14 Hz and up to 20 Hz. It is usually seen on both sides in symmetrical distribution and is most evident frontally. It is accentuated by sedative-hypnotic drugs especially the benzodiazepines and the barbiturates. It may be absent or reduced in areas of cortical damage. It is generally regarded as a normal rhythm. It is the dominant rhythm in patients who are alert or anxious or who have their eyes open. See figure 2 below.

![EEG waves](image.png)

**Figure 2. EEG waves**

**Variables Used in the Classification of EEG Activity**

*Frequency*: Frequency refers to rhythmic repetitive EEG activity (in Hz). The frequency of EEG activity can have different properties including:

- **Rhythmic**: EEG activity consisting of waves of approximately constant frequency.
- **Arrhythmic**: EEG activity in which no stable rhythms are present.
- **Dysrhythmic**: Rhythms and/or patterns of EEG activity that characteristically appear in patient groups or can be rarely seen in healthy subjects.

*Voltage*: Voltage refers to the average voltage or peak voltage of EEG activity. Values are dependent, in part, on the recording technique. Descriptive terms associated with EEG voltage include:

1. **Attenuation** (synonyms: suppression, depression). Reduction of amplitude of EEG activity resulting from decreased voltage. When activity is attenuated by stimulation, it is said to have been "blocked" or to show `blocking."
2. **Hypersynchrony**: Seen as an increase in voltage and regularity of rhythmic activity, often within the alpha, beta or theta range. The term implies an increase in the number of neural elements contributing to the rhythm (Note: term is not used in interpretative sense but as descriptor of change in the EEG).
3. **Paroxysmal**: Activity that emerges from background with a rapid onset, reaching (usually) quite high voltage and ending with an abrupt return to lower voltage activity. Though the term does not directly imply abnormality, much abnormal activity is paroxysmal.

*Morphology*: Morphology refers to the shape of the waveform. The shape of a wave or an EEG pattern is determined by the frequencies that combine to make up the waveform and by their phase and voltage relationships. Wave patterns can be described as being:

- **Monomorphic**: Distinct EEG activity appearing to be composed of one dominant activity.
- **Polymorphic**: Distinct EEG activity composed of multiple frequencies that combine to form a complex waveform.
- **Sinusoidal**: Waves resembling sine waves. Monomorphic activity usually is sinusoidal.
- **Transient**: An isolated wave or pattern that is distinctly different from background activity.
  (a) **Spike**: a transient with a pointed peak and a duration from 20 to under 70msec.
  (b) **Sharp wave**: a transient with a pointed peak and duration of 70-200msec.

*Synchrony*: Synchrony refers to the simultaneous appearance of rhythmic or morphologically distinct patterns over different regions of the head, either on the same side (unilateral) or on both sides (bilateral).

*Periodicity*: Periodicity refers to the distribution of patterns or elements in time (e.g., the appearance of a particular EEG activity at more or less regular intervals). The activity may be generalized, focal,
or lateralized.

**EEG Electrodes**: Small metal discs usually made of stainless steel, tin, gold or silver covered with a silver chloride coating. They are placed on the scalp in special positions. These positions are specified using the International 10/20 System (see Fig. 3). Each electrode site is labeled with a letter and a number. The letter refers to the area of brain underlying the electrode e.g. F - Frontal lobe and T - Temporal lobe. Even numbers denote the right side of the head and odd numbers the left side of the head.

**Electrode Gel**: Acts as a malleable extension of the electrode, so that the movement of the electrodes leads is less likely to produce artifacts. The gel maximizes skin contact and allows for a low-resistance recording through the skin.

**Impedance**: A measure of the impediment to the flow of alternating current, measured in ohms at a given frequency. Larger numbers mean higher resistance to current flow. The higher the impedance of the electrode, the smaller the amplitude of the EEG signal. In EEG studies should be at least 100 ohms or less and no more than 5 kohm.

**Electrode positioning (10/20 system)**: The standardized placement of scalp electrodes for a classical EEG recording has become common since the adoption of the International 10-20 system. The essence of this system is the distance in percentages of the 10-20 range between Nasion - Inion and fixed points. These points are marked as the frontal pole (Fp), central (C), parietal (P), occipital (O), and temporal (T). The midline electrodes are marked with a subscript z, which stands for zero. The odd numbers are used as subscript for points over the left hemisphere, and the even numbers over the right (see Fig. 3).

![Figure 3. 10/20 System of electrode placement](image)

**EEG Montages**: Montage means the placement of the electrodes. The EEG can be monitored with either a bipolar montage or a referential one. Bipolar means that you have two electrodes per one channel, so you have a reference electrode for each channel. The referential montage means that you have a common reference electrode for all the channels.

**EEG artifacts**: The biggest challenge with monitoring EEG is artifact recognition and elimination. There are patient related artifacts (e.g. movement, sweating, ECG, eye movements) and technical artifacts (50/60Hz artifact, cable movements, electrode paste related), which have to be handled differently. There are some tools for finding the artifacts. For example, FEMG and impedance measurements can be used for indicating contaminated signal. By looking at different parameters on a monitor, other interference may be found.

**Differential amplifier**: It is the key to electrophysiological equipment. It magnifies the difference between two inputs. An unwanted signal that is common to the two inputs will be subtracted.

The **standard filtering settings** for routine EEG are:
- Low frequency Filter: 1Hz and High Frequency Filter 50-70Hz
In all the laboratory sessions concerned with the acquisition of electrophysiological data, you will use a complete system – connected to a computer via a special port (USB) - which acquires, amplifies and transforms an analogue signal into a digital signal.

In this first part, you will perform exercises using a function/waveform generator, connected via a "BNC" connector to one and/or two channels of the Powerlab unit. You will set the frequency and intensity (amplitude) of the signal produced by the generator (as specified in the following pages and in your lab report) and be asked to acquire this signal using the acquisition software. Concepts such as sampling rate and range, amplification gain, hardware filtering, saturation and aliasing will be explored.

Equipment

- Computerized data acquisition device

- Waveform/function generator:

Your waveform generator may look like the one below. Locate the frequency selection knobs (multiplication factor and Hz), the offset adjustment, the waveform (sinusoidal, triangular etc…) and its amplitude adjustment knobs.
Software

From the desktop, double-click on LabChart 7; the Powerlab 4/25T acquisition system should be already turned on and be recognized by the software. The welcome Centre panel appears (figure 1.3); double-click on the exercise1_sinewave file under “My Settings” tab.

![LabChart Welcome Center](image)

Figure 1.3 - Welcome Centre

The exercise1_sinewave displays default settings which must be configured for the specific tasks you need to perform.

a) Identify the waveform generator: it should be set to produce a sine wave.

**Task:** adjust the generator to produce a 20 Hz, 10V (peak to peak) signal. The amplitude of the signal will only be visible when the signal is acquired by the Powerlab device and displayed through its software.

b) Locate the connection from the waveform generator to the inputs of the Powerlab 4/25T unit:

Input 1: channel 1
Input 2: channel 2

c) From the top menu, select “Setup” > “Channel Settings…”
d) Study the Channel Settings menu (figure 1.5) and make sure you understand which parameters you need to modify in order to acquire simultaneously on two channels a 20Hz, 10V (pp) sine wave. When the appropriate parameters are set, click ok to return to the “Chart View” window (questions to think about: what is the minimum theoretical sampling rate needed to acquire the 20 Hz waveform, according to the Nyquist theorem? What happens if you select a lower than minimum theoretical sampling rate? What would changing the sampling range do to the resolution of the incoming signal; in which case is this change necessary?)

Task: On Channel 1: set the sampling rate to the minimum theoretical sampling rate according to the Nyquist rate.
On Channel 2: set the sampling rate to 20 Hz. Answer the questions in your lab report.
7 – Under “Calculation”: different signal computations can be made:

Two features are of interest:

“Arithmetic...” permits you to do calculations on the entire contents of a channel. Amplification of the signal (gain) is achieved if the channel is multiplied by a factor of 2 or more.

“Digital filter...”: the options are Low-pass, High-pass, Notch etc...

Once the appropriate parameters are set, click ok to return to the “Chart View” window as well as the Spectrum view window (figure 1.9). Two graphs are featured in the Spectrum View. The top graph shows the Power Spectral Density (PSD), while the bottom graph displays the Spectrogram plot.

Task: Click “Start” to acquire the signal; make sure you acquire at least 30 seconds of data and stop. To select (highlight) both channels, double-click below the x-axis (time). The spectrum of the data in both channels will appear. Maximize the plot by clicking on the right-most icon of the spectrum window; you may need to adjust the abscissa (Hz) (figure 1.10).

Print-out the PSD view. Answer the questions in your lab report. Complete the other exercises detailed in your lab report; refer to the menus above as well as to the Waveform Acquisition Appendix to be able to navigate through the software.
Figure 1.10- Spectrum view details: maximize the PSD pane and adjust the horizontal scale.

The image below (figure 1.11) is an example of data acquired and displayed in the “Chart view” menu: a 10 Hz sine wave acquired simultaneously on two channels with different sampling rates (Channel 1: 100Hz and channel 2: 10 Hz). The channels display the amplitude of the signal versus time (time domain). The lower part of the image shows the spectrum view (PSD pane only) of the same signal (superimposed channels): this is the result of a spectral analysis which decomposed the signal into different frequency components and plotted the intensity of each component versus the frequency (frequency domain).

Figure 1.11- Example of an acquired sine wave under different acquisition parameters and its associated spectrum (PSD plot).
Waveform Acquisition Appendix

a) General software organization:

b) Accessing the Welcome Centre:
c) **Program toolbar:**

- **Comments tool:** right-click on the data point where you wish to locate the comment, choose “Add Comment…”. This can be done either on the Chart View or Zoom View (see below).

- **Zoom tool (magnifying glass) and printing:** Click and drag (highlight) the data points you want to zoom in and click on the zoom tool. **Do not forget to add a comment to identify your work for printing purposes:** right-click on a data point and choose Add comment… then print zoom window (use landscape arrangement for printing).

- **Spectrum View tool (Open the Spectrum Window by selecting Window > Spectrum):**

  A spectrum is a representation of data based on the frequency distribution of its component sine waves. LabChart provides methods for generating, displaying, analyzing and printing spectra. Spectra indicate the strength of the various frequencies in a time-varying waveform. This may make apparent significant frequencies in a waveform that would not otherwise be easily observed. It could be used, for example, to break down an EEG waveform into its various components: beta waves, alpha waves, theta waves and delta waves. Spectrum works in “real-time”, so you see the results as you sample, and after sampling is finished on pre-recorded data.
**d) Measurements (use the Zoom View):**

Locate the marker M in the lower-left corner. Drag the marker along your waveform to the one point you want to measure from. Release the mouse button to drop the marker. The read-out from the waveform cursor will now be displayed as relative time $\Delta s$ and amplitude $\Delta V$ from that of the Marker point.

**e) Displaying your data in the Chart View window:**

- The vertical Amplitude axis on the left of the window for each channel indicates the amplitude of the recorded waveform.

- Click the compression-selection button to display a pop-up menu with a list of available compressions.
Experiment 2 - Recording EEG waves

The aim of this session is to provide an introduction to the electroencephalogram and to explore the electrical activity of the brain. In this second part you will record electroencephalograms from a volunteer, look at interfering signals, and examine the effects of visual activity on alpha waves.

Background

The cerebral cortex contains large numbers of neurons. Activity of these neurons is to some extent synchronized in regular firing rhythms (‘brain waves’). Electrodes placed in pairs on the scalp can pick up variations in electrical potential that derive from this underlying cortical activity. EEG signals are affected by the state of arousal of the cerebral cortex, and show characteristic changes in different stages of sleep. Electroencephalography is also used in the diagnosis of epilepsies and the diagnosis of brain death.

EEG recording is technically difficult, mainly because of the small size of the voltage signals (typically 50 µV peak-to-peak). The signals are small because the recording electrodes are separated from the brain’s surface by the scalp, the skull and a layer of cerebrospinal fluid. A specially designed amplifier, such as the Bio Amplifier front-end, is essential. It is also important to use electrodes made of the right material, and to connect them properly. Even with these precautions, recordings may be spoiled by a range of unwanted interfering influences, known as ‘artifacts’.

You will record EEG activity with two electrodes: a frontal electrode on the forehead, and an occipital electrode on the scalp at the back of the head (Fig 2.2). A third (ground or earth) electrode is also attached, to reduce electrical interference. In clinical EEG, it is usual to record many channels of activity from multiple recording electrodes placed in an array over the head.

Setup and Required Equipment

Five-lead Shielded Bio Amp Cable &
Three snap-connect Shielded Lead Wires with EEG Flat Electrodes
Electrode paste
Alcohol swabs
Paper ruler

Software: Close the previous files and if the Welcome Centre is not open, click on the Top menu File > Welcome Centre. Double-click on the EEG_settings file under “My Settings” tab.

Chart View: Channels 1 and 2 are hidden at the top; Channel 3 occupies most of the display and is named “EEG (channel 3)”. Below the Chart View, you can recognize the PSD and the spectrogram panes.

1. From the Channel 3 (EEG) Channel drop-down menu, choose BioAmp. Ensure that the settings are as shown in Figure 2.1. The settings should be:
   - Sampling rate: 400 Hz
   - Range: 200 µV (suggested)
   - Low-pass filter: 50 Hz; High pass filter: 1Hz; 60 Hz notch and mains filter ticked

2. Click the OK button to return to the Chart View window.
Exercise 2A: recognizing artifacts

Objectives
To examine some of the artifacts that can contaminate an EEG record.

Task: With the electrodes inside the beaker with alcohol, record the signal for 5 seconds, and then hold the electrodes up by their cables like an antenna; record for another 10 seconds and stop. Double-click under the time axis to select the traces: print the PSD plot (spectrum view). Answer the questions in the lab report.

Now, connect the electrodes to the subject.

Task: Generate a print out of the artifacts you are about to record. Indicate their frequencies (using the spectrum analysis tool) and print the frequency histograms. Answer the questions in your lab report.

Subject preparation
It is preferable for the volunteer to have washed his/her hair the night before, or the morning of the experiment.

3. Attach the occipital EEG Flat electrode:
   a) Measure with the paper ruler the distance between the nasion and inion of your subject, and the circumference of the head as indicated in the diagrams below (fig 2.3 and 2.4).
   b) Then affix the negative electrode at 10% from the inion and at 10% from the midline to the right side:
      • Part the hair and wipe that area of the scalp with an alcohol pad and dry.
      • Squeeze some electrode paste onto the concave (hollow side) of the electrode and press the electrode on the skin.
4. Attach the frontal EEG Flat electrode: at 10% from the midline on the right side, after wiping that area with an alcohol pad and adding electrode paste to the electrode.

5. Attach the earth (ground) EEG Flat electrode to the forehead of the volunteer in the same manner as the frontal electrode, but on other side of the midline.

6. Get the volunteer to sit and relax.

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*Figure 2.2. The equipment setup for this experiment, showing the placement of EEG flat electrodes on the head of the subject.*

*Figure 2.3: place the paper ruler to measure nasion to inion distances, along the midline.*
Procedure
Remember to ensure that the volunteer is relaxed and sits still except when instructed otherwise.

1. Click the Start button to start Chart recording. Press on the 'blinking' function key while the volunteer blinks repeatedly. After 5–10 seconds, click the Stop button (stop Chart recording).

2. Click the Start button. Press on “EMG activity” function key while the subject raises his/her eyebrows and holds that position.

Analysis
- Examine the vertical scale at the left of the Chart window, and note the positions corresponding to +50 µV and −50 µV. True EEG signals rarely exceed these limits.

- Use the scroll bar at the bottom of the Chart window to review the recordings. You will probably find large signals outside the ±50 µV range. Such large signals are artifacts. If you see such artifacts, check the electrode connections, and if necessary, remove and re-attach any connections that seem of dubious quality.

There are three common causes of artifacts such as those you have recorded: electromyographic (EMG) activity in muscles of the face or scalp, mechanical movement of electrodes, especially the occipital one, whose attachment is made insecure by hair; and potentials arising from rotation of the eyes, called electro-oculographic or EOG signals.

Figure 2.4: place the paper ruler to measure distances laterally.
Exercise 2B: Alpha waves in the EEG

Objectives
To examine alpha waves (alpha rhythm) in the EEG, and the effect of opening the eyes.

Task: Generate a print out of the data collected when the eyes are shut versus when the eyes are open. Indicate the main frequencies (using the Spectrum View windows) and print the PSD plot generated from a selection of alpha waves. Answer the questions in your lab report.

Procedure
1. Ensure that the subject is relaxed and has both eyes closed.
2. Click the Start button in the Chart View window to start Chart recording, and click on “Eyes shut” function key.
3. After about ten seconds, ask the subject to open both eyes. Immediately click on “Eyes open” function key.
4. After about ten seconds, ask the subject to shut both eyes. Immediately press the “Eyes shut” function key to annotate your record.
5. Repeat steps 3 and 4 twice, to give you three sets of results. Your EEG data should resemble Figure 2.5. Adjust the vertical scale in the Amplitude axis so that the trace fills a little more of the channel, if you prefer.

Figure 2.5 - An EEG, viewed with a 2:1 horizontal compression. Alpha waves show as fine oscillations that stop when the eyes are opened.

Analysis
- Use the View buttons in the Chart window to change the horizontal compression to 2:1. This stretches the data out, and makes it easier to see alpha wave activity.
- Use the scroll bar to review those parts of your recording that were made with the subject’s eyes shut, looking for alpha waves. You can recognize these by their amplitude (usually less than 50 µV, although it can be quite variable from subject to subject) and their timing. Each cycle of an alpha wave should last almost exactly 0.1 s.

If you cannot find any alpha waves, check that you are examining records taken with the subject’s eyes shut. If you still cannot find signs of alpha activity, or if your records consist mainly of large-
amplitude artifacts, you may need to re-attach one or more electrodes, following the instructions given in ‘Subject preparation’ section above. Note however that some otherwise normal subjects may not exhibit alpha wave activity. If this seems to be the case, then try a different subject.

- Use the View buttons in the Chart window to change the horizontal compression, if need be. Drag across a few alpha waves, in an ‘eyes shut’ part of the recording. The Spectrum window (top) displays the frequency content of the selected data (Figure 2.6). The spectrogram (bottom) is a false-colour plot (i.e. 3-dimensional plot) of spectral power, frequency and time. The spectrogram displays spectral power as a coded colour against time and frequency.

![Figure 2.6. The spectrum view of an EEG, showing alpha wave activity in the range 8–13 Hz.](image)
Experiment 3 – Recording late Event Related Potentials (ERP) following auditory stimuli

Basic concepts of Event related potentials

Event related potentials constitute an example of deterministic signals. They consist of voltage changes in one EEG segment (epoch) which are time-locked to a stimulus presentation or a specific event. The electrical activity generated by a single stimulus presentation is too weak to be detected from the mixed electrical activity that forms the EEG. For this reason, it is necessary to employ techniques to extract this ERP waveform from the background EEG.

The same principles of filtering and artifacts detection previously explained apply for ERP recordings and recognition. In addition to these, the most used technique to solve the problem of ERP detection is to average the electrical activity over several similar trials. The brain’s response following a certain stimulus in a certain task is assumed to be the same from trial to trial provided all other conditions remain the same. The changes in voltage dependent on stimulus presentation will occur at similar fixed time in a deterministic way trial after trial, while the background EEG unrelated to the stimulus varies randomly. The result of the averaging will be an increase in ERP signal, with marked reduction in noise (almost zero). Any replicable change in voltage specifically linked to a stimulus presentation and associated with a functional stage of information processing or to an anatomic generator is called an ERP component.

ERPs are classified according to the nature of the stimulus: visual, somato-sensory, and auditory; they can also be classified according to the latency at which their components occur after stimulus presentation: short latency (<100msec) and long latency (>100msec) potentials. The shorter latency components are generated during the sensory stimulus processing stages (exogenous components). The longer latency components represent the cortical processing stages, which are less determined by the physical features of the stimulus (endogenous components).

<table>
<thead>
<tr>
<th>Exogenous potentials</th>
<th>Endogenous potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Depend on physical features of the sensory stimulus.</td>
<td>- Do not depend on physical features of sensory stimulus. They can be evoked, just with stimulus expectancy, even in the absence of stimulus.</td>
</tr>
<tr>
<td>- Do not depend on the subjects’ level of consciousness.</td>
<td>- Can change depending on the level of attention, its relevancy during the task and resources required for stimulus processing.</td>
</tr>
<tr>
<td>- Are not influenced by cognition processes.</td>
<td>- Related to cognition processing.</td>
</tr>
</tbody>
</table>

The classification into early or late components ERPs is useful in practical terms, however it is more theoretical than realistic since ERPs generation is a continuous process.

In this session the stimulus used to evoke the responses is auditory, so the responses will be auditory related potentials.

Early auditory related potentials include five positive waves that occur during the first 10 msec after stimulus presentation and are labeled from I to V according to their order of appearance. They are very stable in shape, amplitude and latency in subjects with no hearing impairment. It is well proven that these components are generated as a result of the activation of brain stem nuclei of the auditory pathway during auditory stimuli information processing. Due to their stereotyped behaviour, even during sleep and unconsciousness states, these potentials have been very helpful as an objective functional evaluation of auditory system in newborns and psychological deafness.
**Long latency potentials** are referred to those components that appear after 100 msec of stimulus presentation and are thought to represent cortical information processing. They are affected by level of attention, stimulus significance, task relevance and stimulus processing requirements.

We are going to record P100 (first positive [P] component appearing 100 ms after the stimulus) using auditory stimuli, although this component can also be evoked visually; the most important factor is that the stimulus must be unpredictable in time.

This kind of potential has been used for psychophysical assessment in patients with cognitive and attention disorders such as Alzheimer’s dementia, schizophrenia, and speech disorders.

The aim of this lab is to record late latency auditory related potentials.

**Variables analyzed from ERPs**

**Absolute latency**: is the time interval between stimulus presentation and the point of maximal value (peak) of a defined component. It is expressed in milliseconds and represents the time taken by the stimulus information to generate the component.

**Relative latency (inter-peak latency)**: is the time interval between two components and measures the conduction of the impulse between two generators.

**Amplitude**: vertical distance measured from the trough to the maximal peak (negative or positive). It expresses information about the size of the neuron population and its activation synchrony during the component generation.

**Duration**: Time interval from the beginning of the voltage change to its return to the baseline. It is also a measurement of the synchronous activation of neurons involved in the component generation. Longer durations indicate less synchronous neuronal activation.

**Setup and Required Equipment**

Audio monitor  
Five-lead Shielded Bio Amp Cable &  
Three snap-connect Shielded Lead Wires with EEG Flat Electrodes  
Electrode paste  
Alcohol swabs  
Paper ruler

**Software**

Close all previous files or documents. From the Labchart Welcome Centre, double-click on random_pulseERP under “My Settings” tab.

**Equipment setup**

*Figure 3.1: Setup connections.* A voltage delivered by the stimulator inside Powerlab is amplified by the audio monitor, and converted as an audible “click” through the head phones.
Subject preparation

In order to record ERP, the electrode connected to the negative terminal is placed in Cz(-), the positive in the ipsilateral mastoid process and the grounding electrode in Fp2 (earth). Refer to figure 3.2, below.

Figure 3.2: positioning the electrodes for ERP

1- Attach the (-) electrode to Cz:
   a) Measure with the paper ruler the distance between de nasion and inion of the subject and the circumference of the head as indicated in the diagram above.
   b) Then affix the negative electrode in the midline just at the half way point of the distance between de nasion and inion (Cz).
      • Part the hair and wipe that area of scalp with an alcohol pad and dry.
      • If necessary, add more electrode paste onto the concave (hollow side) of the electrode and press the electrode on the skin.

2- Attach the (+) electrode to the ipsilateral mastoid process following a similar procedure to that of electrode Cz.

3- Attach the earth (ground) electrode in Fp2 (above nasion, 10% of the distance between the nasion and inion).

Scope Settings for long latency ERP recordings

A voltage delivered by the stimulator inside Powerlab is amplified by the audio monitor, and converted as an audible “click” through the head phones; by pressing start in the acquisition display, a segment of EEG which follows the audio stimulus is recorded.

Software settings:

The file random_pulseERP contains the default settings permitting you to use the software not only as a “chart” (as in previous exercises) but also as a “scope” which allows the averaging of signals 500 msec before and 500 msec after the random stimulus delivery.
Two windows appear: on the left (fig 3.3), the recordings are displayed in the “Scope View”:

- **Channel 1** displays the stimuli (stimulus marker)
- **Channel 2** counts the events recorded on Channel 1
- **Channel 3** records the EEG traces.
  - Range: 200µV
  - Acquisition rate: 1000Hz
  - Low-pass filter: 50Hz
  - High-pass filter: 1 Hz
  - 60 Hz notch and mains filter selected.
- **The time frame**: 500 msec before and after the stimulus presentation.
- **On the left end side**: the number of collected (and averaged) pages is recorded.

**Fig 3.3 Scope view**: the scope is set to averaging based on the event mode calculated on Channel 2.

Cyclic measurements (which count the stimuli represented on Channel 1).

On the right (fig 3.4), the “Chart View” of the same recording is displayed:

The Chart View displays the recordings from channels 1, 2 and 3 for a time frame of 5 minutes.

**Fig 3.4 Chart View**
The sound stimulus is delivered in a random fashion through the following menu (Fig 3.5 and 3.6): from “Setup > Stimulator…”

![Fig 3.5 Setting up the stimulator](image)

The expression which delivers the stimulus (pulse) is:

\[
\text{pulse}(5,1,(2+\text{random}*2),0,1)
\]

The arguments are:

- **Pulse**(amplitude(V), width, initial delay, gap between pulses, # of pulses)

The sound amplifier should already be turned on.

**Experiment 3A: Control task**

1. Ask the subject to close their eyes and to plug their ears with their fingers as best as they can without disturbing the electrode on the mastoid process. Set the volume button of the sound amplifier to zero. This way, the subject will not perceive any auditory stimulus delivery. **Make sure that the subject does not hear anything!**

2. Click on the “Start” button to record 5 minutes of data. Once the “Start” button is pressed, a sweep of 500 msec (or page) is generated around the stimulus delivered randomly.
3. After 5 minutes, stop the recording. Right-click on a page on the left-most column of the scope display to “Select All Pages” and click on the average tool on top.

![Page averaging tool](image1.png)

Fig 3.7 Averaging pages of data

4. Select the averaged contents of channel 3 on the Scope window (see fig 3.8) as well as the other two channels by double-clicking under the time axis and use the zoom tool. Stack all three channels.

![Fig 3.8](image2.png)

5. Measure latency and amplitude using the marker M, bottom left corner and print a zoom view of the three channels.

6. **Save** this recording as “experiment3a_bench#” in the PHGY212 folder on the desk top. Close the file.

**Exercise 3B: Randomized single auditory stimulus presentation**

Open a new file, keeping the settings from the file you just made.

This time the subject will perceive the random stimuli through the headphones once the “Start” button is pressed: **adjust the volume of the amplifier to mid-range.**

Ask the subject to close their eyes and to focus on the auditory impulses that he/she listens to. **Make sure that the head phones do not disturb the electrodes.**
Every recording generates a screen (sweep) which is saved as a page. After 5 minutes, stop the recording. You should have around 100 pages.

**Task:** Average the pages (as you did previously), zoom on the averaged data and make measurements of latency and amplitude (see fig 3.9 and 3.10). Save the file as “experiment3B_bench#”. Answer the questions in your lab report.

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**Fig 3.9**

**Fig 3.10**