VEP Tutorial

The eye and the retina

The eyes are formed by several structures:

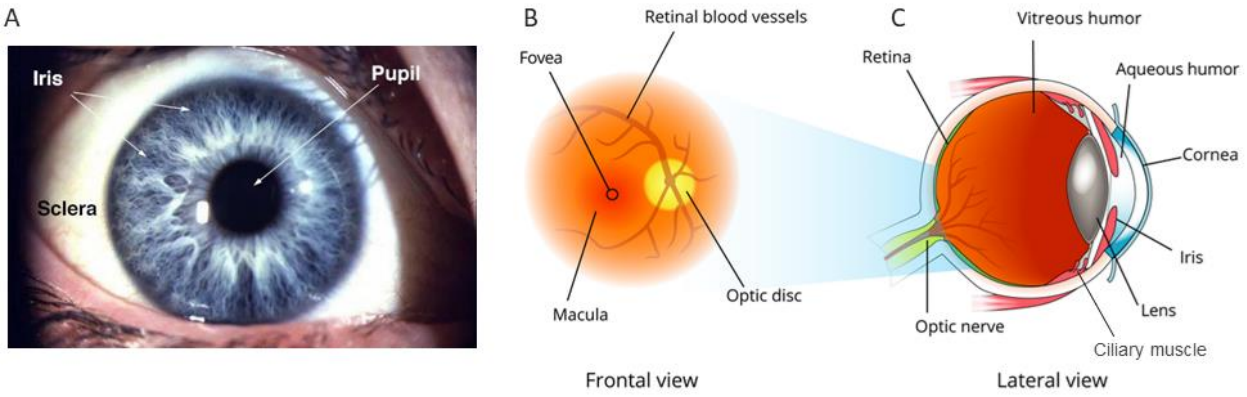


Figure 1. The human eye (From Webvision and Lt ADInstruments) A) Frontal view of the eye. B) View of the fundus seeing trough the pupil. C) Sagittal section of the eye ball.

1. A black-looking aperture, **the pupil,** that allows light to enter the eye (it appears dark because of the absorbing pigments in the retina) (Fig1A).
2. A colored circular muscle**, the iris,** which is beautifully pigmented giving us our eye's color (the central aperture of the iris is the pupil) (Fig. 1A). Circular and radial muscles control the size of the pupil so that more or less light, depending on conditions, is allowed to enter the eye. Eye color, or more correctly, iris color is due to variable amounts of eumelanin (brown/black melanin) and pheomelanin (red/yellow melanin) produced by melanocytes.
3. A transparent external surface**, the cornea,** that covers both the pupil and the iris (Fig 1A). This is the first and most powerful lens of the optical system of the eye and allows, together with **the crystalline lens** the production of a sharp image at the retinal photoreceptor level.
4. The "white of the eye"**, the sclera,** which forms part of the supporting wall of the eyeball. The sclera is continuous with the cornea.

The sagittal section of the eye (Fig 1C, right) also reveals **the lens** which is a transparent body located behind the iris. The lens is suspended by ligaments attached to the anterior portion of the ciliary body. The contraction or relaxation of these ligaments as a consequence of ciliary muscle actions, changes the shape of the lens, a process called accommodation that allows us to form a sharp image on the retina (Fig C). Light rays are focused through the transparent cornea and lens upon the retina. The central point for image focus (the visual axis) in the human retina is **the fovea** (Fig 1B). Here a maximally focused image initiates resolution of the finest detail and direct transmission of that detail to the brain for the higher operations needed for perception.

The retina is a thin multilayer consisting of several types of neuronal and glial cells. Much like the film of a camera, the retina is charged with the critical task of receiving, modulating and transmitting visual

stimuli from the external world to the optic nerve and, ultimately, the visual cortex of the brain. Adequate conveyance of visual signals depends largely upon the highly specialized anatomy of the retina.

The retina is a thin (0.5mm) transparent sheet of tissue divided into three layers containing five types of neurons (Fig.2). The "photoreceptor layer" on the outer surface of the eye, where the photoreceptor cells (rods and cones) allow the transduction of light signal into a nerve signal. The "intermediate layer" containing three types of neuron. The bipolar cells making direct links between the photoreceptors to the ganglion cells and the horizontal and amacrine cells both allowing lateral interactions in the retina. Finally, the "ganglion cell layers" on the inner side of the retinal, in which the ganglion cells transform the neuronal signal received from the intermediate cells layer into spike trains forming the output signal of the retinal (Fig.2).

When light enters the eye, it falls on the retina, which stimulates the receptors (rods and cones) (Fig.2). This results in depolarization and the generation of action potentials in the optic nerve. The optic nerve contains the ganglion cell axons (more than a million fibers in humans) running to the brain and, additionally, incoming blood vessels that open into the retina to vascularize the retinal layers and neurons (Fig. 2). A section of a portion of the retina reveals that the ganglion cells (the output neurons of the retina) lie innermost in the retina closest to the lens and front of the eye, and the photoreceptors (the rods and cones) lie outermost in the retina against the pigment epithelium and choroid. Light must, therefore, travel through the thickness of the retina before striking and activating the rods and cones (Fig. 2). Subsequently the absorption of photons by the visual pigment of the photoreceptors is translated into first a biochemical message and then an electrical message that can stimulate all the succeeding neurons of the retina.

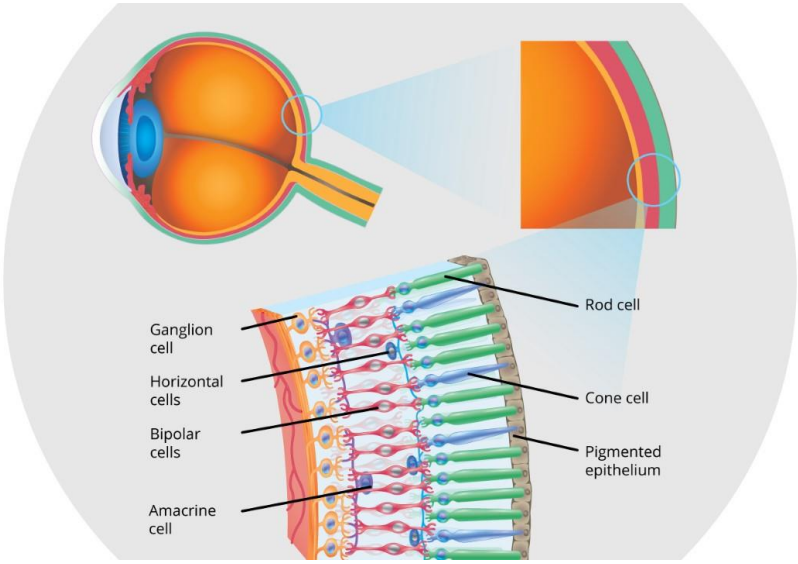


Figure 2. The retina cytoarchitecture (From Lt ADInstruments)

The retinal message concerning the photic input and some preliminary organization of the visual image into several forms of sensation are transmitted to the brain from the spiking discharge pattern of the ganglion cells. These action potentials pass through the anterior visual pathways (through the optic nerve, optic chiasm and optic tract) to connect the neurons of the thalamic Lateral Geniculate Nucleus (LGN) and

reach through the optic radiations the primary visual cortex in the occipital lobe of the cerebral cortex (Fig3). Here the visual information is processed through a complex system of neuronal connections. The flow of ions that result from this processing creates a unique pattern of varying voltages over both space and time. It is these changes in electrical activity that are able to be measured by recording electrodes attached to the scalp.

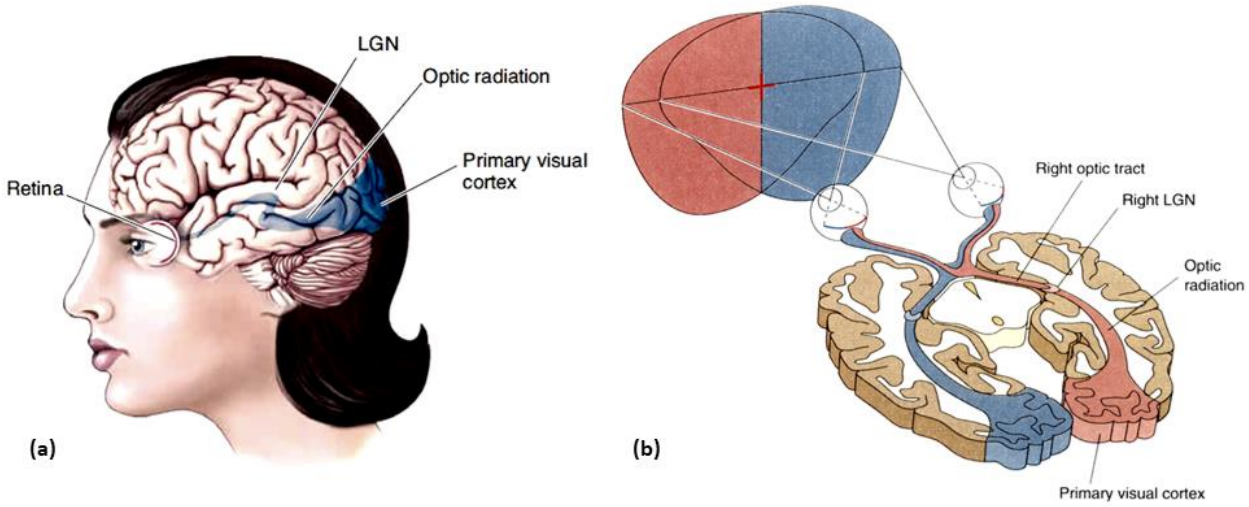


Figure 3. The retino-thalamo-cortical visual pathways (Bear et al. 2016 Neuroscience 4th edition)

Visual evoked potentials (VEP)

Evoked potentials are electrical activities that occur in the neural pathways and structures as a response to external electric or sensory stimulations (light, sound, smell, taste …etc.). They are thus different from the spontaneous potentials produced by brain and normally recorded as electroencephalograms (EEGs) at the surface of the skull. Evoked potentials are polyphasic waves that often present with an amplitude between 0.1-20 µA which are formed within 2-500 ms. The source of these activities is probably the summation of the action potentials generated by the afferent tracts and the electrical fields or activities of the synaptic discharges or post-synaptic potentials on those tracts. Understanding evoked potentials bears importance in terms of controlling the entire pathway from the stimulation point to the cortical areas, in other words, to the primary visual cortex.
By examining visual evoked potentials (VEP), we can find answers to many questions such as: Does the response against the stimulus reach intended destinations on time? Does the response show any loss of intensity? If there is a problem in the neural pathways, what is its exact location?

When a person receives a visual stimulus such as a flashlight, the EEG (electroencephalogram) record concerning the occipital region (particularly at the O1-P3 and O2-P4 derivations) demonstrates waves that are called “photic driving response” which form within about 150 ms and can be observed after any kind of light stimulation.

Because the ongoing spontaneous physiological activity recorded by EEG (and noise) is much greater in amplitude than the evoked potentials, signal averaging has to be used to extract the evoked potentials from the "background noise". For this reason, a number of repetitive sweeps need to be recorded (Fig 4).

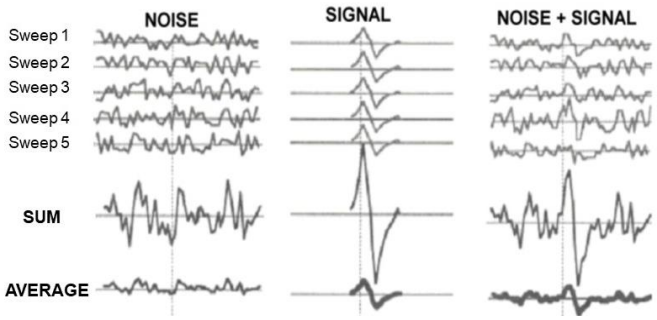


Figure 4. Averaging of several trials (Modified from 'Evoked Potentials', A. Akay)

As the evoked potential is time-locked to the stimulus (whereas the stimulus-independent EEG and noise, are not), the noise and other signals are cancelled out by the averaging. Therefore, the evoked potential emerges as averaging progresses. Most of the electrical activity seen is from the propagation of action potentials down axons. Therefore, the final tracing is the summed action potentials from many axons which were activated synchronously by the stimulus.

Types of VEP

There are two common ways of generating VEP in the lab and clinic: pattern reversal and flash. Flash testing is used when the subject (for example, an infant or child with hyperactivity) cannot focus on the pattern reversal for an extended period. Pattern reversal testing is primarily used because of its ability to detect minor visual pathway abnormality with much greater sensitivity and accuracy.

Flash

In the flash test, the subject sits about 1 m from the screen and flashes of light are shown every second. This is similar to watching a strobe light. The generated waveform induced by the flash stimulation (Fig5A) presents a first peak typically seen at 30 ms post-stimulus (wave I), but this waveform is much more variable between subjects. There is also a large positive peak near 100 ms, (wave IV).

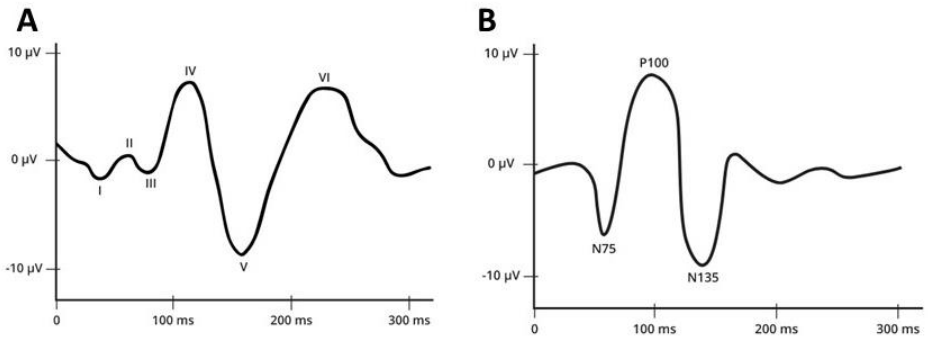


Figure 5. (A) A representative flash VEP. (B) An example of pattern reversal VEP (from Lt ADInstruments)

The nomenclature of the flash VEP waves is different to that of the pattern reversal VEP so they can be distinguished quickly and easily. The most common way to label flash VEP waveform peaks is to use

Roman numerals, but some researchers and clinicians also choose to distinguish between the positive and negative peaks using the nomenclature N1, P1, N2, P2, N3, and P3. These six major peaks occur in the first 250 ms. There are also peaks seen after this time frame, but they are considered rhythmic after-discharge and are not important.

Pattern reversal

Pattern reversal elicits the highest peak amplitude. Each pattern typically is shown on the screen for half a second, which means the reversal rate is 2/s. A checkerboard pattern is most used for this test. There are guidelines researchers and clinicians follow to create this pattern: the contrast between the checks must always be greater than 50%; the check size and field size depend on the subject's visual field; and the subject sits about 1 m away from the screen. The waveform is characterized by three peaks (Fig 5B). The first peak occurs around 75 ms post-stimulus and has a negative polarity; therefore, it is called N75. The second peak occurs around 100 ms and has a positive polarity. It is referred to as P100 and is the primary waveform researchers and clinicians look for. The third peak occurs around 135 ms and has a negative polarity. This peak is called N135. In clinical testing, the clinician usually covers one of the subject's eyes and completes the test using each eye individually.

P100 is in the normal range if the peak occurs between 90 ms and 117 ms. Women typically have shorter P100 latencies than men, but the difference is so small that the same P100 representation is used for both genders.

Origin and source of VEP

The neural generators of the waves of the visual evoked potential (VEP) (Fig 5) are not clearly defined. Research with multichannel scalp recordings, visual MRI activity and dipole modeling, supports the interpretation that the visual cortex is the source of the early components of the checkerboard-induced VEP (N75) prior to P100 (Slotnick, et al., 1999). The early phase of the P100 component with a peak around 95-110 ms, is likely generated in dorsal extra-striate cortex of the middle occipital gyrus. The later negative component (N175) is generated from several areas including a deep source in the parietal lobe (Di Russo et al., 2002). The VEPs induced by flash stimulation (Fig 5A) characterized by the classic triphasic pattern originated in thalamic area, visual cortex, inferior temporal cortex and posterior parietal cortex (Youssofzadeh et al., 2015).

Clinical use of VEP

Clinically, VEPs are used to assess the visual pathways and can help determine if there are lesions affecting the visual system. Such lesions include stroke, trauma, and tumors. VEPs can also be used to help diagnose multiple sclerosis, an autoimmune disorder which destroys the nerve fiber's myelin sheaths. This destruction prolongs the VEP latencies significantly as the demyelinated axons can no longer conduct action potentials efficiently, hence delaying the signal.

References:

- https://webvision.med.utah.edu/
- https://webvision.med.utah.edu/book/electrophysiology/visually-evoked-potentials/
- Evoked potentials, A. Akay, https://www.intechopen.com/books/electrophysiology-from-plants-to-heart/evoked-potentials
- Lt ADInstruments
- -Youssofzadeh et al., 2015, DOI: https://doi.org/10.1523/JNEUROSCI.2269-15.2015.

Experimental setup

The block diagram of the recording system is represented in Figure 6.

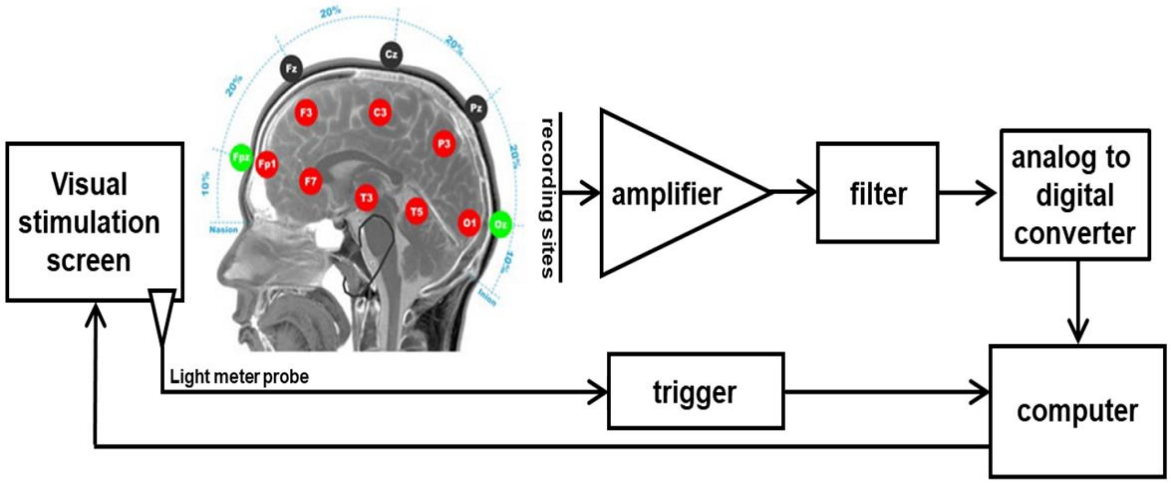


Figure 6. EEG cup electrode positions and experimental setup (Modified from 'Evoked Potentials', A. Akay)

Electrode positioning

- ✓ Remove any jewelry from the volunteer's head, neck, and ears.
- ✓ Measure the distance nasion-inion (figure 6, left) and mark 10% of this value up from the nasion.
- ✓ Wash the skin over the marks with the alcohol napkin. This helps to reduce the skin's resistance and ensures good electrical contact.

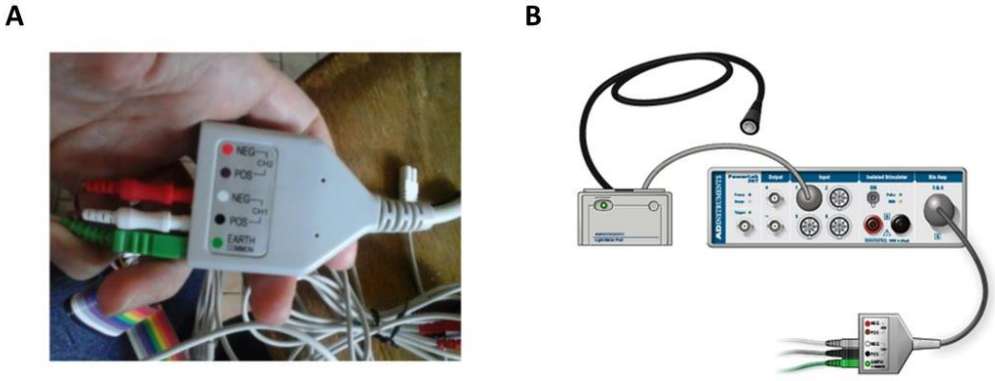


Figure 7. A) BioAMP amplifier. B) Analog to Digital (A/D) converter and Light-meter.

- ✓ Place the EEG cup with the Fp electrodes over the cross in the middle of the forehead. All the other electrodes will be in the standard positions for EEG (figure 6, left).
- ✓ Connect the Fp1 or Fp2 to the negative input and O1 or O2 to the positive input of CH1 BioAMP (Figure 7A).
- ✓ Connect the EEG cup to the harness.
- ✓ Squeeze a small amount of electrode gel onto the EEG cup at the level of recording electrodes.
- ✓ Place the earth electrode at the ear and put some gel in it. Connect this electrode to the 'earth' entry of the BioAMP amplifier (connection in green Fig7A).

- ✓ Before proceeding, check that all the electrodes are properly connected to the volunteer and the BioAmp cable, and make sure the cables and bandage are not in the volunteer's line of vision.

Recording setup

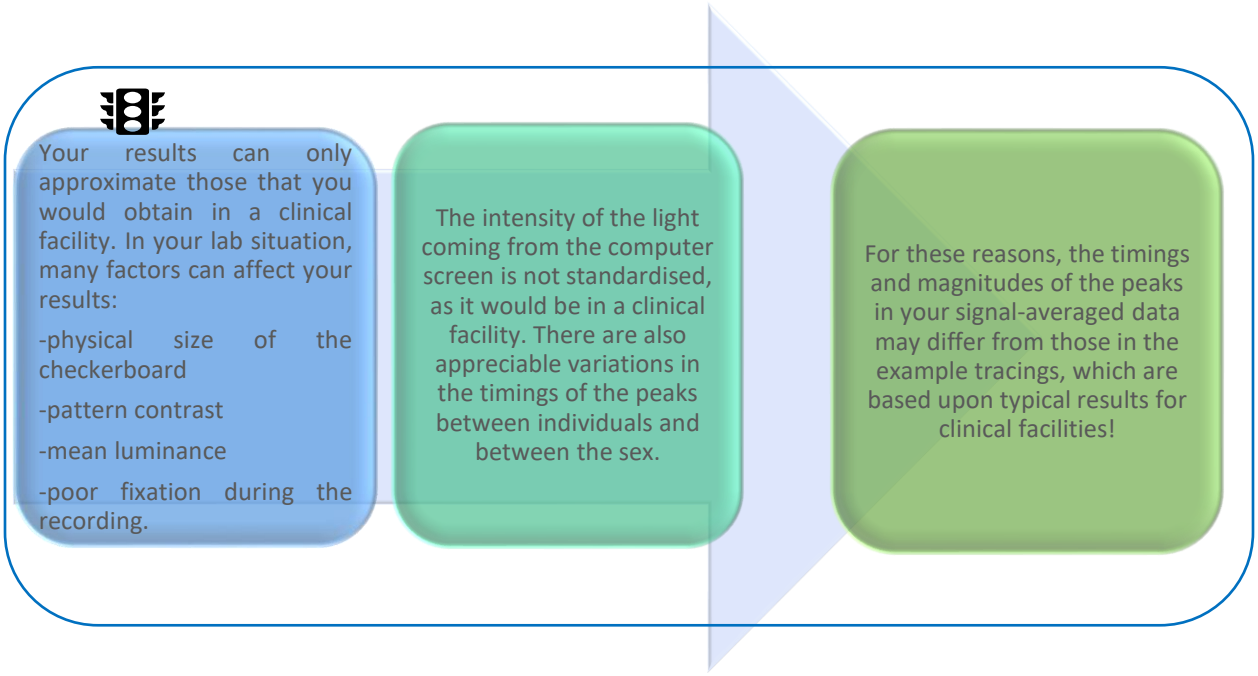
- ✓ Connect the Light Meter Pod to Input 1 on the front of the PowerLab (Fig 7B). Attach the light meter probe to the back of the Light Meter Pod.
- ✓ The person that does not carry the PEV electrodes, will position the light meter probe directly over the flashing dot at the bottom left corner of the screen.
- ✓ Open LabChart8-getting started-LabChart-Visual Evoked Potentials
- ✓ Set-up-Sampling-Trigger above: set **2.5 lux**. Close the window
- ✓ Ask the volunteer to sit in a relaxed position, about 0.8 m from the stimulation screen.
- ✓ Make sure the lighting conditions are the same on each side of the volunteer.
- ✓ Open the directory VEP-Setting files and select **'Flash exercice.htlm'**. Open it but do not start the video yet.
- ✓ Ask the volunteer to focus on the dot in the middle of the screen. The volunteer should stay focused on this point and try not to blink.
- ✓ Go back to LabChart et click on START, then immediately minimize the LabChart window and PLAY the flash video.
- ✓ This will produce a bright flash every 1 s and it has to be presented more than 100 times (>100 flashes).
- ✓ The Scope panel will begin recording only when it will be triggered by the light meter Pod.
- ✓ Then after recording more than 100 responses to flashes, stop the video by going immediately to labChart and STOP the acquisition of VEP.

REPEAT THE EXERCICE (3 TIMES)

- ✓ Now choose 'Checkerboard Exercise.html' in the Setting files. And follow the same instructions as for Flash patterns.
- ✓ Ask the volunteer to focus on the dot in the middle of the screen and stay focused on this point during the stimulation period and try not to blink at all (if possible).

REPEAT THE EXERCICE with smaller or larger checkerboard size (HF, MF and LF meaning respectively high, middle and low spatial frequency).

MOVE TO DATA ANALYSIS


Your results can only approximate those that you would obtain in a clinical facility. In your lab situation, many factors can affect your results:
-physical size of the checkerboard
-pattern contrast
-mean luminance
-poor fixation during the recording.
The intensity of the light coming from the computer screen is not standardised, as it would be in a clinical facility. There are also appreciable variations in the timings of the peaks between individuals and between the sex.
For these reasons, the timings and magnitudes of the peaks in your signal-averaged data may differ from those in the example tracings, which are based upon typical results for clinical facilities!