Syllabus for the research master course

Experimentation II: Neuroscientific Research Methods

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1 Technical Glossary

10/20 system An international convention for the montage and naming of electrodes over the scalp. The system is based on the principle that the distance from inion to nasion and from ear to ear can be divided into pieces of 10 or 20%. In the 10/20 system, the first one or two characters relate to the underlying cortical area (from nasion to inion: Fp, F, FC, C, CP, P, OP and O for fronto-polar, frontal, central, parietal, occipital or combinations thereof, and T for temporal). The additional number or character is related to the distance from the midline, with z meaning 'zero' because these electrodes are on the midline. Odd numbers represent electrode positions over the left hemisphere. Even numbers represent positions over the right hemisphere. For example, C3 is a position over the left central hemisphere, on 40% of the distance from the midline to the ears. The 10/20 system defines insufficient electrode positions for a cap with more than 64 electrodes.

AC Alternating Current: the type of electrical potential that switches positive and negative poles with a certain frequency. The energy supply in Europe uses 50 Hz AC, the energy supply in the USA uses 60 Hz AC.

Active electrode An electrode that is integrated with an amplifier, as in the BioSemi system. This technique minimizes interference from electromagnetic sources during the transport of the signal, because an amplified, and thus larger potential, travels through a wire. As a result, the contribution of the same amount of noise to the sum of noise + signal is much smaller.

Aliasing Incorrect recording of a nonexisting low frequency in a time series, caused by the presence of frequencies above the Nyquist frequency. For example, if a helicopter rotor rotates with 21 Hz, and it is filmed with 20 Hz, the wing seems to move very slowly. That is, every time a film frame is shot, the wing has rotated exactly one full circle (which would look like standing still) plus 1/20th of a circle, so that replaying the film reveals a nonexisting 1 Hz speed. In physiological recordings, similar phenomena can occur, with samples instead of film frames. For example, high frequency muscle activity can be misclassified as lower frequency EEG. To prevent aliasing, BioSemi applies an analog filter at 1/3 of the sample frequency.

Alpha The most prominent frequency range of activity in the EEG: 8 – 12 Hz. Alpha power increases when people close their eyes or become drowsy.

Ampère Unit of current (symbol: A)

Amplitude The size of a signal.
In case of EEG/ERP: the voltage. Peak amplitude = the maximum voltage observed in a positive peak or minimum voltage in a negative peak.
In case of BOLD: the % signal change

Analog The opposite of digital; analog information is not yet classified in discrete values and can contain detail that is lost upon digitization

Artifact Any recording that does not have the source of interest; e.g., eye movement can induce activity that mimics brain activity

Band Section of the spectrum. Subset of frequencies present in the EEG. The typical distinction is made between the delta band (1.5-4 Hz), theta band (4-8 Hz), alpha band (8-12 Hz), beta band (12-30 Hz) and gamma band (30-60 Hz), each of which has a different function in the brain.

Baseline	The amount of activity to which experimental activity is compared. The baseline is assumed to represent default brain activity prior to the time of task presentation. If baseline activity changes substantially over time, it is important to choose a baseline period that is close to the experimental period. In ERP recordings, the mean amplitude in a window of at least 100 ms that precedes task-related brain processes, such as evoked by stimulus presentation, is treated as the baseline.
Bipolar	As recorded between two electrical poles. In electrophysiological recordings: the potential difference between two electrode positions that are both relevant for the recorded activity (e.g., on both sides of a muscle, the heart, or the eyes).
BOLD signal	Blood Oxygenation Level Dependent signal. The change of the ratio of oxy-hemoglobine and deoxy-hemoglobine over time, as recorded with fMRI, which is related to the supply of oxygen for neural activity.
Bridge	In electrical circuits: a low resistance connection that occurs if two positions that are usually electrically separated are unintentionally connected by a conductive gel or metal
Calibration	In some amplifier systems (not BioSemi) it is necessary to record a very accurate potential (e.g., exactly 50 μV) along with every recording session to test the exact relationship between digital values and potentials. This procedure is known as calibration.
Canthus	The bone on the outer edge of the eye socket
Cardiogram	The display of heart-related activity over time
Channel	The information recorded on one electrode or a derivation from multiple electrodes
Charge	Add electrical potential, as in a re-usable battery
CMS	Common mode sense = an electrode from which activity is used as an approximation of common mode activity
Coherence	Enduring synchronicity between signals emitted by different brain areas. Coherence can exist for signals that are out of phase. Coherence is often interpreted as a reflection of communication between brain areas.
Common mode	The activity that is shared by all electrodes. In EEG recordings, the common mode is unlikely to have its origin in the brain. It is more likely to be caused by interference from large scale electromagnetic field in the lab onto the wires and electrodes
Component	A latent element of the ERP
Conduct	To let electricity pass through
CNV	Contingent Negative Variation. A slowly increasing negative potential in the EEG in anticipation of the presentation of a cued stimulus.
CSD map	Current Source Density map: a method for displaying EEG activity from the entire scalp based on local differences in voltage. This results in a reference-independent picture of the location of dominant activity. This technique is a display version of what a Laplacian derivation does.
Current	Streaming electricity (symbol I), expressed in Ampère
Cut-off frequency	The frequency above or below which the activity will be filtered out. In sophisticated filters there is a gradual reduction of power around the cut-off frequency.
DC	Direct Current: the type of electrical potential that keeps the same positive and negative poles over time. DC is provided for example by batteries.

DC recording	A recording that does not compensate for previous values of electrophysiological signals, so that true potential differences can be quantified. This is important for slowly responding body signals such as blood pressure, in which the absolute value of the amplitude is informative.
Digital	The opposite of analog; digital information is classified in a format that is ready for computer storage
Digitize	Change an analog signal into a digital signal, which is always associated with loss of precision of amplitude and time information
Drift	In electrical circuits: gradual increase or decrease of potential, usually due to poor grounding
DRL	Driven right leg: an obsolete name for an electrode connection in an electrical circuit that feeds back small amounts of electricity to compensate for drift. This function is comparable to grounding.
ECG	= EKG = Electro-cardiogram: the display of electrical heart-related activity over time
EEG	= Electro-encephalogram: the display of electrical brain-related activity over time
Electrode	A device for transmitting electrical activity
EMG	= Electro-myogram: the display of electrical muscle-related activity over time
EOG	= Electro-oculogram: the display of electrical eye-related activity over time
Epoch	= segment; a time window or period. In ERP recordings: the window that is cut from the EEG
ERN	= Error-Related Negativity: a relative negative amplitude occurring after participants detect an error.
ERP	= Event-Related Potential. Averaged EEG in a consistent time relation to an event such as a stimulus or response
Fourier	Fourier transformation is a class of mathematical techniques that translate a time series into a frequency spectrum. This procedure is used before filtering in the frequency domain (e.g., rejecting all frequencies above 30 Hz). Because digital time series are not continuous, neither are the spectra. That is, a frequency spectrum of a digital time series works in discrete steps of for example 1 Hz. This step size is determined by the Nyquist frequency and the length of the epoch to be transformed. A Fourier transformation can be reversed from the frequency domain to a time series without loss of information.
Frequency	Speed by which alternating activity such as components of the EEG repeat, expressed in Hertz (= times per second)
Frequency domain	An alternative way to represent electrophysiological data, showing amplitude or power as a function of frequencies. Time domain information can be translated into frequency domain information by Fourier transformation.
Gradient	Amount of change, the slope of a function. For example: change in amplitude per time unit.
Ground	In electrical circuits: a solid body or mass where electrical excess can move to.
GSR	= SCR = Galvanic Skin Response = Skin Conductance Response = the display of electrical resistance of the skin over time
Hertz	Unit of frequency (symbol Hz)
Impedance	= Resistence = Tendency of a material or body to obstruct electric current (symbol R), expressed in Ohm
Inion	The bump on the back of the head above the softer tissue
Jitter	Variance in latency of an activity. For ERP recordings, jitter usually refers to the variable timing of an ERP component due to natural (brain) or artificial (PC clock) causes. Jitter causes the

	average waveform to be more widespread and with a smaller amplitude than single-trial waveforms. Jitter of time intervals between stimuli is deliberately used in EEG and fMRI research to reduce the (unwanted) contribution of signals evoked by previous stimuli on the signal of the current stimulus.
Laplacian	Alternative way to reference an EEG signal. Around each relevant electrode, three more are placed in a triangle. The average signal measured with the surrounding electrodes is then subtracted from the original signal. The result is an EEG with very local information, which provides a better impression of the density of activity.
Latency	Moment of occurrence of an activity since a reference time point such as stimulus onset. For example, the onset latency of an ERP peak is the time between stimulus onset and the first signs for the occurrence of the ERP peak
Lead	= Channel; for example the information of one electrode
LRP	= Lateralized Readiness Potential: a derived ERP signal that shows the preference for actions with one hand over actions with another hand prior to performing an overt action
LSB	Least significant bit: the step size by which digital information is recorded
Macro	A series of instructions that can be run by a single command from inside programs to achieve one or multiple goals. Macro's can be written for Brain Vision Analyzer to perform operations that are not directly available from the menu.
Mastoid	The bald area just behind the ear shell
Microvolt	(symbol μV) 1 μV = 0.000001 V = 10^{-6} V
Midline	The line from inion to nasion, which runs over the division between the two cerebral hemispheres.
Millivolt	(symbol mV) 1 mV = 0.001 V = 10^{-3} V
Monopolar	Recorded with a combination of a relevant channel and an irrelevant reference channel. For example, EEG signals are usually recorded monopolarly, with an electrode over a silent area serving as reference (e.g., the mastoids, the ears, the tip of the nose).
N1, N2, N400	Names for ERP waves with a negative amplitude and a peak latency around 100, 200 and 400 ms after stimulus onset. Sometimes ordinal numbers are used, sometimes the approximate latencies are used. Because the initial peaks in the ERP are approximately 100 ms apart, N2 and N200 are two ways to refer to the same peak or underlying component.
Nasion	The dip between the eyes above the nose
Noise	The opposite of signal; activity that is recorded unintended and that obscures the signal. In ERP calculations, random noise is averaged out as more trials are included in the average. The contribution of random noise is reduced by a factor $\sqrt{n}$, where n = the number of trials
Notch filter	A filter that is specifically designed to filter out the interference from the AC power supply at 50Hz or 60Hz during or after recording. Luck warns against the use of a notch filter.
NanoVolt	(symbol nV) 1 nV = 0.000000001 V = 10^{-12} V
Nyquist frequency	The highest frequency in an electrophysiological signal that can be identified. This frequency is limited by the sample frequency. The Nyquist frequency is the sample frequency divided by two. Higher frequencies in the original signal will lead to aliasing.
Offset	Absolute amplitude level around which further modulation takes place. The offset is brought back to zero if low frequencies are filtered out, and not in case of DC recording.
Ohm	Unit of resistance (symbol Ω)

Ohm's law	Physical law that describes the relationship between electrical potential, current and resistance. V = I x R. Electrical potential is positively related to current and resistance. Also, I = V/R; current is inversely related to resistance. High resistance leads to low current.
Oscillation	Repetitive activity according to a sine-wave pattern
P1, P2, P3, P300	Names for ERP waves with a positive amplitude and a peak latency around 100, 200 and 300 ms after stimulus onset. Sometimes ordinal numbers are used, sometimes the approximate latencies are used. Because the initial peaks in the ERP are approximately 100 ms apart, P3 and P300 are two ways to refer to the same peak or underlying component.
Peak	A marked increase and subsequent decrease of electrode potential relative to the baseline ERP activity. A peak is sometimes the manifestation of a component.
Phase	The position in the sine-wave cycle, expressed in angles. To remain visible after averaging EEG to an ERP, oscillations evoked in different trials have to be in phase. That is, they should be positive and negative at more or less consistent latencies across trials.
Potential	Strength of the ability for electricity to stream between two poles if connected (symbol E, U, or V), expressed in Volts. If a battery runs empty, it loses electrical potential.
Power	The amount of energy in an electrophysiological signal, calculated by squaring the amplitude. Usually the power is calculated per frequency band (e.g. alpha power).
Reference	The point relative to which electrical potential is calculated. Without a reference it is impossible to express an electrical potential.
Resistance	Tendency of a material or body to obstruct electric current (symbol R), expressed in Ohm
Sample	Recording of single unit, such as the potential of the EEG on one time point on one electrode
Segment	= Epoch; a time window or period. In ERP recordings: the window that is cut from the EEG
Signal	Activity that the recordings are intended to pick up
SNR	Signal to noise ratio = the quality of the signal, expressed as the size of the signal relative to the size of the noise
Spectrum	Separation of a signal into its constituent frequencies. For example, a prism can turn white light into a spectrum of light with different frequencies and colors. EEG can be transformed into a display of the power of separate EEG frequencies.
Topography	The way a waveform is distributed over the scalp
Time constant	The time it takes before a constant electrical potential difference is suppressed by a filter to 1/e times the original amplitude, expressed in seconds. A time constant is another way to describe a high-pass filter. It removes constant potentials and irrelevant slow activity from the recordings, which is beneficial to compensate for drift and avoid clipping of the digitized signal.
Time domain	The default way of displaying electrophysiological data, with amplitude as a function of time.
Volt	Unit of electrical potential (symbol V)
Voltage map	A method for displaying EEG activity from the entire scalp using the recorded voltages. This results in a reference-dependent picture of activity.

2 Data Acquisition

EEG Lab Rules

The following rules apply for anyone working in SB12, the EEG lab:

- Psychophysiological data acquisition in SB12 is only permitted to researchers who have successfully followed the practical meetings (2x2 hours) included in the course Experimentation 2: Neuroscientific research methods. Alternatively, an equivalent individual training can be followed with a researcher who already has experience performing psychophysiological research in SB12.
- As part of the institutional rules set by the Ethics Committee of Psychology (http://www.fsw.leidenuniv.nl/psychologie/organisatie/commissies/commissie-ethiek-psychologie-cep.html), psychophysiological data acquisition needs to adhere to the Guidelines for Hygienic Testing (which can also be found in Experimentation 2 syllabus, Chapter 4).
- Before a researcher starts working in SB12 for the first time, he or she has to notify the lab coordinator Kerwin Olfers, and specify who will provide supervision and what kind of training he or she has received.
- The financing units of the different lab setups have priority in scheduling their own research, monitored by the unit representative. That is for EEG1 and EEG3: Cognitive Psychology (Kerwin Olfers), for EEG2: Clinical Psychology (Sandy Overgauw), for EEG4: Social Psychology (Daan Scheepers), and for EEG5: Education and Child studies (Arnout Korneef). Developmental Psychology has an EEG lab in SB39 (Melle van der Molen).
- Time slots that are not used by the financing units can be distributed over researchers who need additional research time. To monitor a fair distribution, use of spare capacity has to be reported to the lab coordinator Kerwin Olfers.
- Supervisors carry the responsibility for the research activities of their students. This requires sufficient guidance and technical support, as well as adherence to the lab rules. Support from the technicians (Maureen Meekel and Elio Sjak-Shie) should only be sought if the problem cannot be solved by the supervisor.
- The lab coordinator Kerwin Olfers should be informed about issues that apply to the lab as a whole, such as supplies. The unit representatives should be informed about issues that are specific to one setup.
- Changes to the configuration of software or hardware are not allowed without permission of the unit representative or lab coordinator.

General Remarks about Psychophysiological Recordings

Safety, Ethics and Hygiene

Being tested can be rather annoying. This is the case for behavioural studies, and even more so for physiological studies. Empathize that the participant may feel like s/he is undergoing a medical examination, and this entails extra responsibilities from the experimenter. Note that if a participant is feeling well, this will benefit the quality of the data. Avoid expressing uncertainty about the work you do: uncertainty will give participants the feeling that the situation is unsafe.

The participant is often volunteering not to benefit science, but to earn money or credit. Participants in physiological experiments deserve to be paid more than usual.

Just as in all experiments, as long as the identity of the participant is not hidden, data should never be accessible to other people than the experimenter. Therefore, you should keep questionnaires, informed consent forms etc. stored in dedicated folders that cannot be accessed by others. Use participant codes to prevent identification.

Cardiography sometimes requires that electrodes are attached under clothes. The experimenter is responsible for guarding the privacy of the participant. The rule is that electrodes under clothes of female participants should be applied by female experimenters.

Because the experimenter and the participant have direct contact and because for EEG recording the experimenter even scratches the skin, the current and following participants need to be protected against all possible sources of contamination. This is why all materials that come in contact with the treated skin directly (e.g., a blunt needle, the electrodes) or indirectly (e.g., the syringe, the electrode cap, the gloves) are disposed or disinfected after each participant. **Faculty guidelines for hygienic testing should be studied and followed up**. These guidelines are enclosed in this syllabus.

All participants should sign an informed consent form, containing
- information about what is expected from the participant
- experiment duration and reward
- the fact that participation is voluntary and that the participant is allowed to leave the experiment without providing an explanation
- the fact that as psychophysiologists we are not capable of detecting neural deficiencies
- the fact that participants agree with the use and publication of their (anonymized) data
- the fact that the methods used comply with high safety regulations and that no danger is known
- name and telephone number of the responsible researcher

NOTE: If the measures could possibly be judged to be deficient (e.g., irregularities in ECG, blood or DNA samples), the participant has to agree in advance to the transfer of this information to his/her general practitioner. The doctor's name should be registered in advance.

First Aid

In rare occasions, participants faint before or during a recording session. This is not caused by malfunctioning equipment, because that is very safe. It is most likely caused by participants' emotional response to the situation, (comparable to when people see blood or needles). To prevent fainting, it is always important to guard the quality of the climate. Fainting happens sooner if the temperature is elevated and if there is insufficient fresh air. Furthermore, show mastery over the techniques. Hesitation can be interpreted as a sign that the situation is unsafe. Also, the experimenter should prevent sudden threats (e.g., showing a needle without proper warning) and should avoid ambiguous remarks about physiological signals (e.g., "your signal is not OK", "we will monitor the electricity on your brain").

In the event that participants faint, lay the person down flat on the ground with the head to the side and the legs slightly lifted, and call the FSW Aid Service (telephone 3600 / 3701). The first aid room is 0A02.

Illustrated Guide to EEG Recording

Electrode types

A B C

Figure 1: Two types of electrodes. A) Separate electrodes do not have a conductive pin, but a flat round area to record electrical potentials. They are used for all applications that are not included in the electrode cap configuration, such as the positions around the eyes (EOG), at muscles (EMG), or as the reference at the mastoid positions. B) Bundled electrodes have a conductive pin because they can be plugged into an electrode cap. C) An electrode bundle contains wires for 32 electrodes.

All BioSemi electrodes are Ag/AgCl sintered. That is, the surface contains tiny balls of silver/silver chloride, which have excellent conductive properties. Furthermore, the BioSemi electrodes include a miniature amplifier. The microvolt range of brain potentials is amplified on the spot to the millivolt range, which is a way to reduce the impact of electromagnetic interference that appears on the way between the electrode and the digitizer due to the presence of electrical equipment such as the monitor. Because of the amplification the electrodes are called active electrodes. Older amplifier systems make use of passive electrodes, in which the microvolt range of brain potentials are amplified to larger ranges after the signal is conducted through a wire to the amplifier. The use of passive electrodes requires slightly different protocols, which are not described in this syllabus.

Important note: The BioSemi electrodes are expensive and fragile because they contain micro-electronics! Do not pull, twist, or fold the wires and do not scratch the electrode surface. Hold the electrodes only by their plastic body. Do not let electrode surfaces come in contact with metal and avoid aggressive and abrasive chemicals. Do not use more tape on the wires than strictly necessary: the glue seems to make the wires porous.

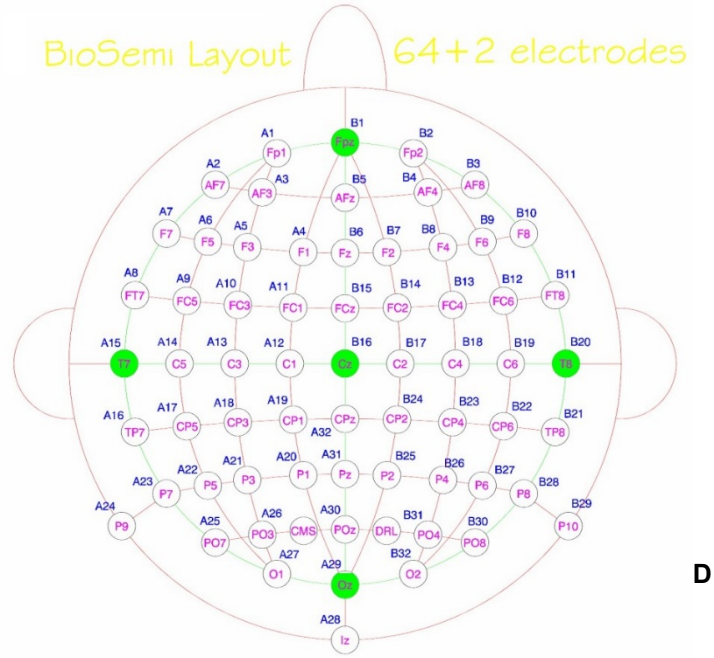
Separate electrodes

A B C D

Figure 2: A) Separate electrodes are attached to the skin with a round sticker. Place it around the brown-grey conductive area, and let the brown lip face outward to facilitate removing the white sticker cover. B) Signa gel, an electrode gel, is a highly conductive gel on water basis that is used to make optimal contact between the skin and the electrode. For separate electrodes, it is injected as a single drop in the conductive electrode cup within the sticker ring. C) The syringe and blunt needle are used to inject the gel in the electrode cup before you attach it to the face. To avoid having air bubbles in the syringe, you can put a new syringe on the tube of electrode paste, and then squeeze the tube while you suck it into the syringe. Fill the syringe with electrode gel before the needle first comes in contact with the skin and never let the gel tube get into contact with a used needle or syringe. After you fill the syringe, you can screw on the needle. **Do not touch the electrode surface with the needle or any other metal: it will cause damage and reduce the quality of the signal**. D) Before attaching separate electrodes, you should reduce the impedance (=electrical resistance) of the skin by removing grease and dead skin cells from the upper skin layer. This can be done with NuPrep on a cotton tip or with alcohol swaps. NuPrep is an abrasive substance. It is gently rubbed over the skin with a cotton tip. Note that particularly the skin below the eyes is sensitive. Alternatively, wipe the skin with an alcohol swap (but be careful not to spill alcohol too close to the eyes!). Immediately after you prepare the skin, attach the separate electrode (refer for example to a birthmark to remember the exact spot), and add a piece of Leukopor tape to keep the electrode from falling off. An optimal combination is to use only alcohol around the eyes, and only NuPrep for electrodes behind the ears. Eye movement recordings have a strong signal to noise ratio, so they can be obtained without scrubbing the participant's facial skin. In contrast, the EEG signal behind the ears is weak and needs to be recorded with precision to serve as a reference for other EEG electrodes, so this justifies the scrubbing with NuPrep.

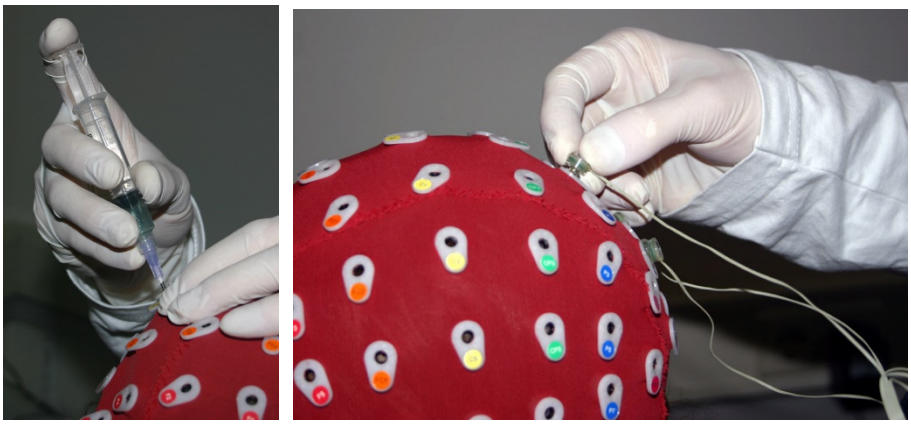
Electrode caps

A B C



D

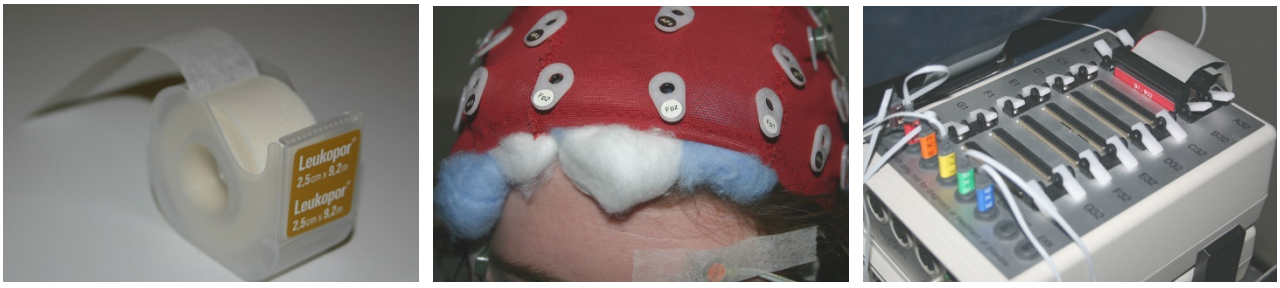
Figure 3: A) For different circumferences of the head, there are different sizes of electrode caps, as indicated on a tag on the backside. Note that there are electrode caps for 128 electrodes (labels starting with A, B, C and D) and caps for 64 electrodes (labels starting with A, C, P, F, O, and T). Select the cap with the correct number of holes. For adults, you can use yellow (50 – 54 cm), red (54 – 58 cm), or blue (58 – 62 cm). In case of doubt, a tight cap is better, but only if the participant feels comfortable enough with it. If people have thick, curly hair and wear a cap that is somewhat oversized, the electrodes are pushed outward, away from the skin. This will make it difficult to conduct the signal. A tight cap can help to pull the electrodes close to the skin.
B) Measure the circumference of the head with a measurement tape over the horizontal plane along the forehead and *inion*. The inion is the lowest point of the skull over the midline, right above the neck muscles. C) Attach the Velcro straps to the rings of the cap, preferably with the flat side inward. For some people, the straps are too short to fit comfortably under the chin without a (black) extension strap. Give the cap its initial position over the head and close the Velcro straps, with the size-tag on the backside. Let the ears stick out of the cap through the holes. D) Measure the distance from the inion to the **nasion** (the pit between the eyes). The Cz electrode should be located exactly at 50% of this distance. To move the cap, use both hands and make the entire cap move, rather than just one hole. Measure the distance from one ear to the other, on symmetrical points. Again, the Cz electrode should be located exactly at 50% of this distance. If Cz is in the correct position in both directions, the only way the cap can be misplaced is if it is rotated. This is something you have to do on visual impression.



A B

Figure 4: A) For preparing the skin underneath the electrode cap, you should use the blunt needle, mounted on a syringe. The blunt needle should be held with two fingers and be lowered until the skin. Move it back and forth without adding pressure. However, while you move it around over the skin, you should feel some resistance to ensure that the needle moves. It is possible, and therefore necessary, to scratch without causing pain. Finding the optimal way to scratch the skin requires practice. One function of the needle is to move away the hair; another is to reduce the impedance of at least a small piece of skin. Many novice EEG researchers move the needle while keeping the needle on the same place on the skin, which of course has little effect. Moving the needle approximately ten times is enough if you can feel the needle scratch over the skin. If it turns out not to be enough, there is an opportunity for improvement later on. After scratching the skin, you should add gel. This should be enough gel to make good contact by filling all the space between the skin and the electrode - not more, not less. While the gel is injected in the hole, press the surrounding plastic to the skin. If you don't do this, there will be too much gel under the cap and it will create low resistance electrical bridges between adjacent electrode positions, which disrupt location and voltage information. B) Electrodes are attached to the cap by plugging them into the holes. Regardless of which electrode positions you want to record, it is always necessary to attach electrodes CMS (common mode sense) and DRL (driven right leg). These are together used as the initial reference and as ground for recording voltages.

After attaching the electrodes



A B C

Figure 5: A) Paper tape can be used to fix the positions of separate electrodes and to avoid wires pulling when the participant moves his/her head. Attach all the wires to the shoulder with 20 cm tolerance. B) if you don't record from frontal electrode cap positions, do the participant a favour, and put some cotton balls underneath the elastic and frontal electrode holes to prevent a red stripe in the face. C) the separate electrodes should be plugged into positions EX1 – EX8. **Keep electrode gel away from the connectors.** Electrode bundles should be plugged into the bundle sockets. After you add a bit of pressure, the white handles will rise. To remove the bundle plug afterwards, push the white handles and the plug will be released.

Recording with BioSemi

A **B** **C**

Figure 6: A) For a 32-channel setup, you should use one electrode bundle and plug it in socket A. In a 64-channel setup, you can use up to two bundles of 32 channels each and plug them into socket A and B. In a 128-channel setup, you can use up to four bundles (A-D). The two-electrode bundle with CMS and DRL should be plugged in on the front side of the BioSemi box. If the impedance of CMS or DRL is not sufficiently low or if one of these electrodes is broken or not connected, the brain potentials of all other electrodes are useless. A blue light in front of the box will indicate whether CMS and DRL are working appropriately (continuous light) or not (flashing light). It is recommended to do this before plugging in the remaining electrodes. Even if the blue light is not flashing, the impedance of these channels may be too high. If you monitor the electrophysiological signal and all channels contain too much noise, the best thing to do is first check CMS and DRL. DRL compensates for shifts in overall electrical potentials (it allows for a weak return current). Recorded signals are the electrical potential differences between each electrode and the CMS electrode. Because CMS picks up a lot of the same interference as other electrodes (such as 50Hz from the monitor), this interference will not show up in the potential differences. It is recommended to subtract a different reference time series from the data later, consistent with common practice in the literature. References that are used most often are Cz, the average of the mastoids, or the average of all electrodes. Cz may be an appropriate reference if you are interested in signals over the temporal lobe. The average mastoids are most often used because there are relatively few important ERP components arising from that area. The average of all electrodes is the optimal reference, but only if you have recorded from enough electrodes that are placed evenly around the scalp (ideally, even below the ears).
B) The BioSemi box runs on rechargeable batteries and can be recharged by connecting it to the black box. Check the BioSemi battery and change it if necessary. This is the meaning of the indicators on the BioSemi box:

- Battery empty: the power light cannot be switched on
- Battery low: the indicator is lit.
- Battery full: power light can be switched on and the battery low indicator is off
- Charger power is on: green light

The charger has different indicators:

- Battery is full: green light
- Battery is almost full: yellow light
- Battery is too empty: red light

C) The response buttons are connected to the digitizer. Eight different responses can be recorded as a time series independently. Together with the stimulus code information that is transferred from the stimulus PC during stimulus presentation, the response code information is recorded as an extra status channel.

The Recording Setup

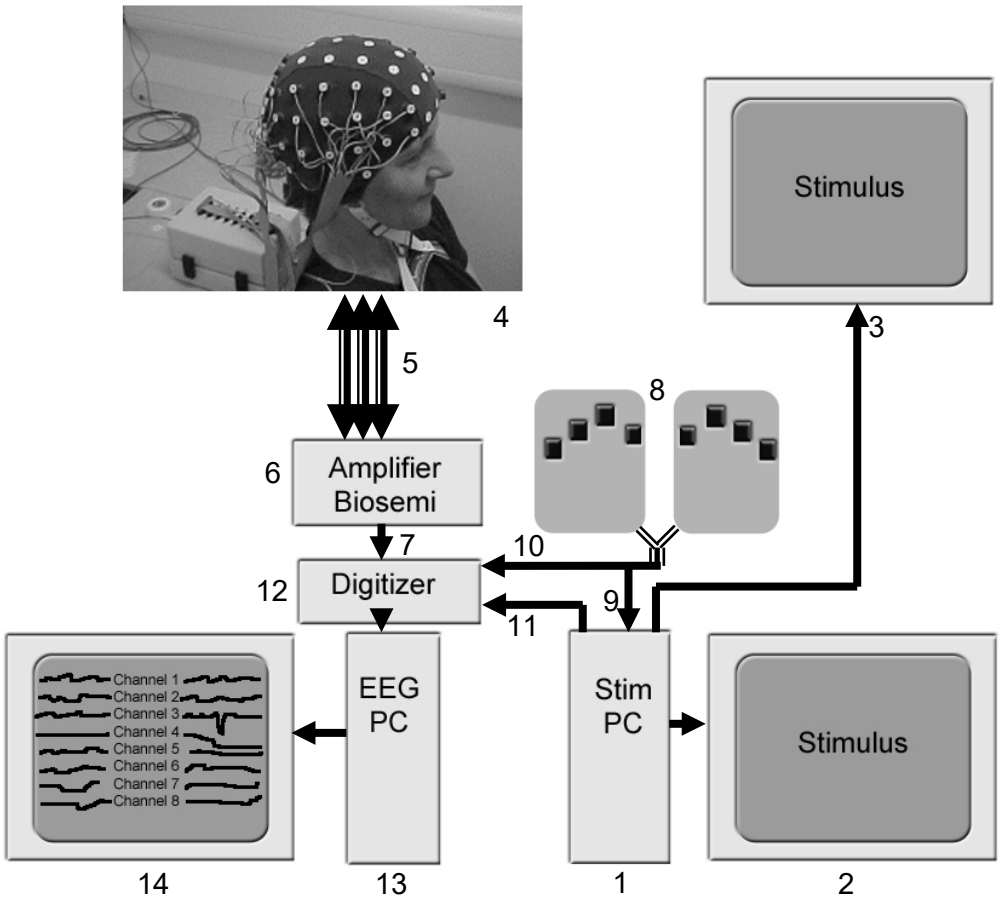


Figure 7: The stimulus PC (1) shows the task, usually programmed in E-prime, to two monitors; one in the experimenter room (2) and one in the recording chamber (3). The participant (4) performs the task and his/her EEG is being picked up by electrodes. The weak electrophysiological signal is amplified locally by the BioSemi amplifier Active Two (6). The amplified signal then passes back through bundles of electrode wires (5) to the BioSemi box, which translates the electrical signal to an optical signal that is transmitted through an optical wire (7) to the digitizer (12), which in turn determines the digital 24-bit equivalent of a voltage for every channel for a fixed number of times per second, that is the sampling rate, between 128 and 2048. Responses pressed by the participant on the response boxes (8) are transmitted to the stimulus PC (9) through the E-prime SR-box, while real-time information about up to eight responses is fed as response code pulses to the digitizer (10). In the meantime, the stimulus PC feeds stimulus code pulses to the digitizer to indicate the exact moment of stimulus presentation (11). The digitizer records the stimulus code pulses and the response code pulses together as one status channel. The EEG PC (13) writes the information that is digitized on the hard disk, and simultaneously displays the time series on the EEG monitor (14) using the application ActiView.

The PCs are optimally configured for stimulus presentation and recordings.

Safety and Signal Quality

There are several precautions in this setup for the safety of the participant and the quality of the data.
First, the participant is not connected to any high voltage. The electrical circuit for moving the dentist chair is switched off and the response buttons run on low voltages. Moreover, all the 220V connections share a safety fuse. Thus, even the most unlikely risks are prevented, because a participant with well conducting wires and gel attached to the scalp should never be exposed to strong electrical currents.
Second, the setup uses as little alternating current (AC) as possible, because AC is picked up by the electrodes as noise. The lights in SB12 use a high frequency to avoid a dominant source of 50Hz. The amplifier uses a battery (direct current = DC). The main remaining source of AC is the monitor. Some older labs are shielded against inflow of electro-magnetic fields by a metal cage, known as a Faraday cage. With the BioSemi active electrode system, this is not recommended.

ActiView

EEG Data inspection and data recording is controlled and monitored with the application ActiView.

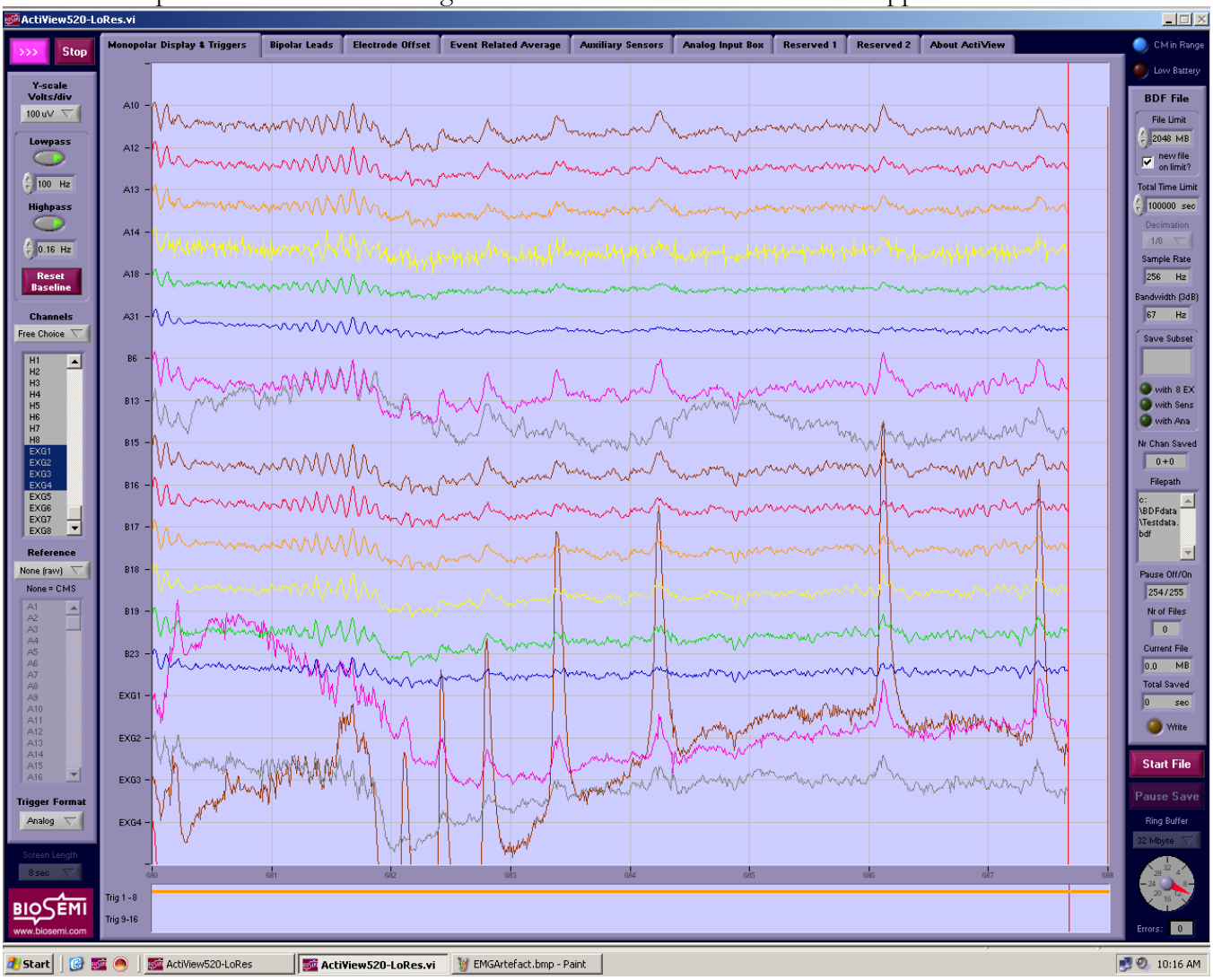


Figure 8: In this screen shot of ActiView you see the time series of 18 monopolar channels. Monopolar channels contain the signal of one electrode referenced to a common reference. Bipolar channels contain the difference between signals of two electrodes. The left column settings specify how the signals are **displayed**; the right column settings determine how it is **recorded**.

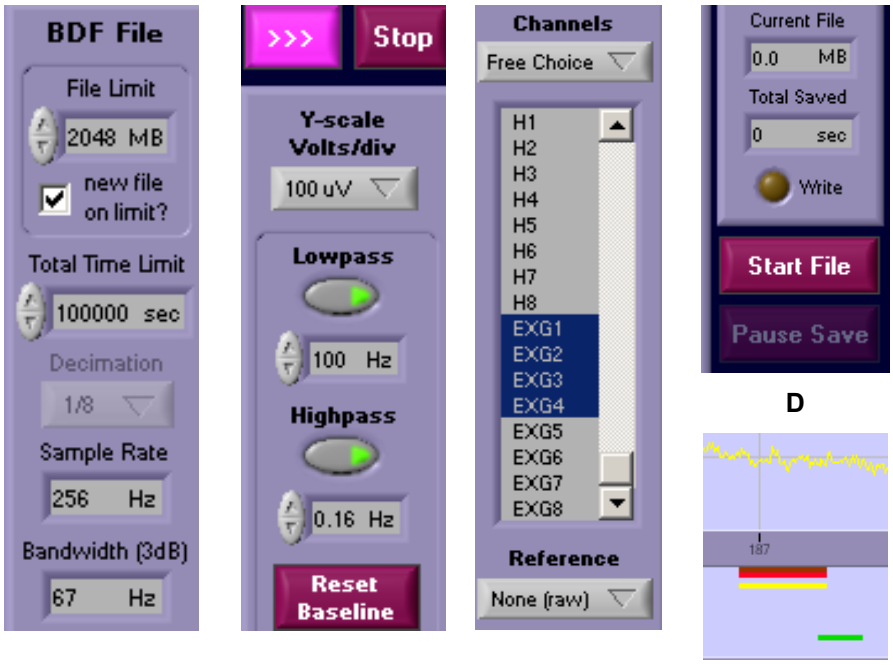


Figure 9: A) The top-right part of the screen has to be set before you start displaying and recording. The maximum sample rate of the BioSemi system is 2048 Hz; that is 2048 recordings per second, per electrode. If you do not change the sample rate, you will end up with 1 CD worth of data per participant. For most cognitive ERP applications, you can restrict the sample rate to 256 Hz. Do this by selecting Decimation 1/8. A rule of thumb is that with a sample rate X, you can distinguish EEG contributions up to frequency X/4. Thus, with a sample rate of 256 Hz, you will have access to the spectrum from 0-64Hz. B) The top-left part is used for starting and stopping the display of signals. The Y-scale can be adjusted to fit in the largest signals. During preparation you may prefer a low resolution (> 50 μV/division). During recording of EEG 50 μV/division is usually a convenient display. Some hardware filtering is already applied by BioSemi to prevent aliasing. The rest of the filtering has to be done by software after recording. It is possible to filter the *displayed* data without affecting the recording. This comes handy to keep the signal within the required range. A highpass filter removes slow drifts. Resetting the baseline is hardly ever necessary. ActiView resets the 'pen' of each channel to the initial position when the pen returns to the left of the screen. C) To select channels for display, select the option Free Choice, and then click each required channel while holding the Control key. A reference to CMS is recommended. To do this, select None (raw). D) To start recording, begin with the button start file and specify which channels to record under which name. Then it may seem as if recording has already started. However, as long as the file size displayed in Current File is not rising, you are not actually recording. Press *Paused* to start recording. E) At the bottom of the screen, you see the time series for Trig1-8 and Trig9-16. These lines code when each of the stimulus and response bits is ON. For example, when the participant presses response 1, Trig 9 will shortly be ON. If the stimulus PC presents a stimulus with code "11", the channels Trig1, Trig2 and Trig4 will be ON for some time, indicating the code $2^0 + 2^1 + 2^3 = 11$. Thus, the stimulus code can have values between 1 and 255. Responses are coded separately so that multiple responses (also pedals and voicekeys) can be recorded with temporal overlap as separate events.

Signal quality

EEG signals relevant for experimental psychology consist of contributions between 0.1 Hz (one cycle in 10 seconds) and 40 Hz (40 cycles per second). Frequencies higher than 40 Hz exist in the brain, but in the EEG recordings they are primarily caused by artifacts, such as muscle activity or a bad connection. A bad connection can be caused by a defective electrode, insufficient gel, or high skin impedance. If a channel has a bad connection, other electromagnetic sources than the brain contribute more to the recorded signal. An extra tool for checking the quality of the signal is the measurement of electrode offset, displayed on the third tab of ActiView's screen. Strive for offsets between -20 and +20 mV for all the channels on which you record.

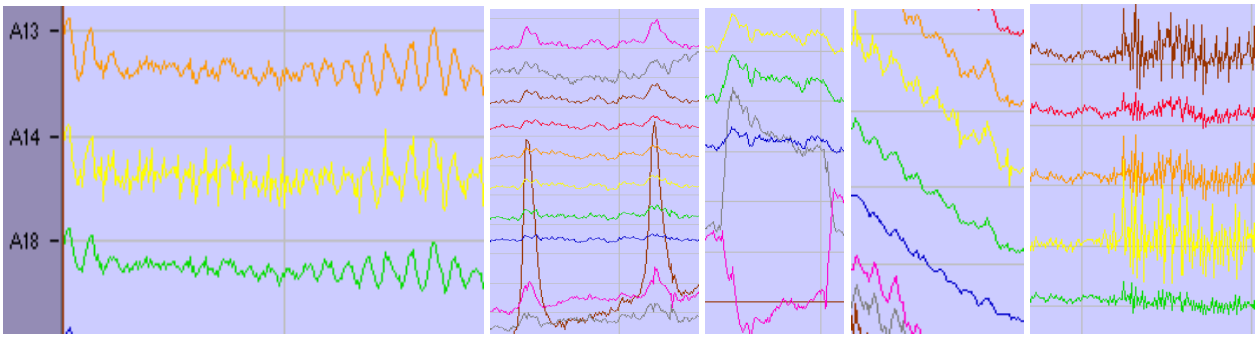
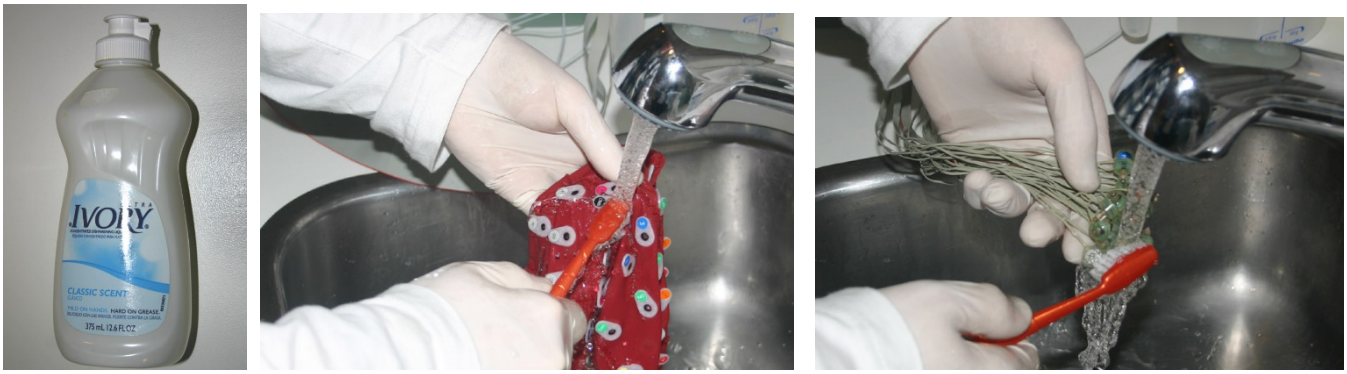


Figure 10: A) This part of the signal shows a lot of alpha activity (8-12 Hz). It appears when the participant feels sleepy or closes the eyes. It is often not related to the task, and should therefore be minimized by keeping the participant alert. In many cases providing coffee is beneficial for the signal, although the use of coffee may be prohibited in some experiments. The signal on channel A14 shows high frequency activity in addition to the alpha waves. These spikes are not part of EEG, but are caused by a poor connection. B) In this picture, there are two dominant peaks in the EOG channels, caused by eye blinks. It can clearly be seen that the EEG channels, particularly the ones closer to the eyes, are strongly affected by the blinks. This illustrates that it is essential for the quality of the EEG to reduce the number of eye blinks in the relevant epochs (time windows). You can instruct the participant not to blink too often, and if necessary to blink between trials. It may seem good to have the participant blink immediately after the response, but this has several unwanted side effects. C) The effect of horizontal eye movement on EOG and EEG. The lower channels (EOG) show opposite potential changes that last for some time before the reverse change occurs. This is a clear case of horizontal eye movement. The channels on top (EEG) show clear artifacts caused by this horizontal eye movement. Horizontal EOG artifacts become a problem if longer words or sentences are presented as stimuli. In some paradigms participants need to focus their eyes on the center of the display. Horizontal EOG can then be used to monitor adherence to this instruction and to select trials later on. D) A clear case of drift. If electrodes are attached correctly and the participant sits still, this should never occur. However, if one of the electrodes gets loose, or if the participant moves, skin potentials will occur. Small displacements of the electrodes change the local impedance of the skin under the electrode. This can then cause low frequency currents in the skin, which cannot always be distinguished from cortical activity. E) Muscle artifacts. The initial part of the signal is free of high frequency activity, and suddenly several channels show high frequency activity. This can happen for example if the neck muscles, tongue or cheek muscles (yawning or chewing gum) are active.

In general, if the signal quality is bad for all electrodes, you should start by improving the connection of CMS and DRL. In most cases that is the source of problem occurring simultaneously on all channels. Furthermore, if DRL and CMS are very bad, the signal will exceed the voltage range that can still be digitized. If this happens, you will see the blue light on the BioSemi box flashing rather than stay on.

Cleaning afterwards



A B C

Figure 11: A) Ivory detergent is a regular dish washing soap that is recommended for initial cleaning of electrodes after use, because it is not abrasive (i.e., it does not scratch off the sensitive surface of the electrode). B) A wooden stick comes handy to remove the gel from all corners of the cap. Use the soap in combination with a soft toothbrush to further remove residues of electrode gel from the cap and the electrodes before disinfection. C) Do not let the electrodes get in contact with metal. You can use a plastic box with some detergent to save water and to keep electrodes away from the metal in the kitchen sink.

A B C D

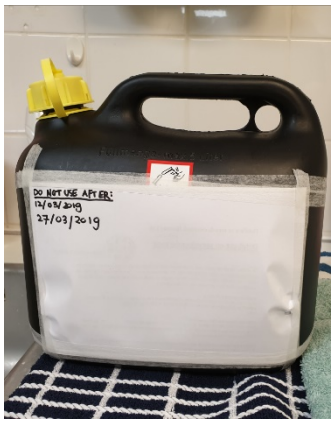
Figure 12: In the kitchen, you can find dedicated containers for cleaning and disposing materials. (A) Used needles should be put in a needle container. (B) Incidin is the disinfectant that we use for cleaning electrodes and electrode caps after use. **Make sure you have read the instructions** (next page) before you use it, **NEVER USE UNDILUTED**. (C & D) Always wear a labcoat, eye-protection and new gloves when handeling Incidin.

Incidin Plus ® EEG Cleaning Procedure

A

B

C



D

E

- Make sure to protect your own skin against the aggressive chemicals by wearing (new) gloves, eye-protection and wearing a labcoat. Do not handle Incidin solutions with bare arms.
- Undiluted Incidin can be available in bottles (A) or in 20ml packages (B). However, we always use **diluted (2%)** solutions for cleaning.
- A one-day use preparation of 2% Incidin solution is kept in the round plastic container (C). The label on the wall next to it will state when the solution was prepared. Keep the container closed whenever possible. If the solution is less than a day old, place the rinsed electrodes and electrode cap in the solution for **15 minutes** – not longer, not shorter. Keep as much of the cables as possible out of the solution, submerging only the electrodes themselves and a few centimetres of cables at most. The Velcro-straps should not be disinfected. After removing the electrode and cap from the solution, keep it under running water for some time to remove the aggressive chemicals that might otherwise come in contact with the next participant's hair or skin.
- If the solution in the round container is older than one day, do not use it, instead dispose of the solution in the sink, make sure to rinse the sink with water afterwards.
- To prepare new cleaning solution for one day: poor 500 ml of solution from the black container (D) into the round plastic container. Diluted Incidin can be kept for up to 21 days in the black container. Check the label on the black container to make sure the solution is not too old.
- If the black container is empty or if the solution is too old, dispose of it in the sink and prepare a new batch in the container by adding 80ml of undiluted Incidin (from packets or bottle) to 4 litres of water. Make sure to update the label on the container to indicate the last date that the new solution can be used.
- Make sure all the containers (including Incidin bottles) are always closed properly after use.

First aid measures for exposure to Incidin:

- In case of any complaints or symptom's consult a doctor.
- If fumes are inhaled, seek fresh air. In case of lingering complaint/symptoms, consult a doctor.
- In case of direct contact with skin: immediately rinse with water (15 minutes). Take off contaminated clothing. Consult a doctor. (P303, P361, P353)
- In case of contact with eyes: immediately rinse excessively with water (at least 15 minutes), including under the eyelids. Remove eye contacts if possible. Use the eye-washer on the wall (E). Consult a doctor and/or intoxication centre immediately. (P305, P351, P338)
- If swallowed: rinse mouth with water (NOT milk), do NOT induce vomiting, medical treatment needed immediately. (P310)

National intoxication center	**(0) 030-2748888 and 3701**
First Aid via reception FSW	**3701**

For external numbers called from a university phone: start with 0
For local numbers called with a cell phone: start with 071-527….

EEG Recording: Quick Checklist for the Default Protocol in SB12

Preparing a participant for the recording of EEG can take between 30 and 60 minutes before recording can start. Because this is unpleasant for the participant, it is a good idea to prepare as much as possible before the participant arrives. However, before you start working with this checklist, first study the details in the more elaborate illustrated guide.

General preparation

- Have an information sheet and an informed consent form ready for reading and signing.
- Have a log-sheet ready for recording background information of the participant.
- Clean up the recording room.
- First turn on the monitors, then start the PCs .
- At the EEG PC; open the program ActiView.
- At the stimulus PC; open the task to be presented first (usually in E-prime).
- Check the BioSemi battery and change it if necessary.

EEG preparation

- Make space for the EEG accessories near the chair of the participant.
- Lay down a sheet of paper towel to catch garbage and gel.
- Gather the following disposable accessories.
    - a syringe filled with electrode gel
    - a blunt needle
    - alcohol swaps, or cotton tips + abrasive such as NuPrep.
    - gloves of the appropriate size
    - soft tissues (for the face) and paper towels (for other purposes)
    - paper tape
    - stickers for the separate electrodes
- Cut ten pieces of paper tape, with a length of 8cm each.
- Gather the following reusable accessories.
    - a measurement tape
    - the electrode cap
    - Velcro chin straps (two white ones, and a black one)
    - the separate electrodes and electrode bundles

After the participant arrives

- Welcome the participant, and make him/her feel comfortable.
    - take the coat, bag.
    - let the cell phone be switched off.
    - be friendly and calm.
    - provide enough information.
- Ask the participant to read the information and sign the informed consent form.
- The participant can fill out any other form or questionnaire while the experimenter is busy attaching electrodes.
- Put on gloves of the appropriate size.

Separate electrodes

For each electrode:

- **Treat electrodes with great care. They are expensive and fragile. Be particularly careful with the connection point of the wire.**
- Attach the ring sticker with the extension facing outwards.

- Inject electrode paste below the sticker. **Do not touch the electrode surface with the needle: it will cause damage**.
- Keep one or two pieces of paper tape ready.
- Prepare the skin.
- Attach the electrode on the same spot.
- Make the wire point to the ear or the shoulder. Lead the EOG wires over the ears.
- Immediately support the electrode with a piece of paper tape.
- Avoid creating a white blob in the visual field of the participant.
- Release the tension from the wire and attach it to the shoulder.
- Connect the electrode to the BioSemi amplifier box.

Placing the electrode cap

- Measure the circumference of the head and select the appropriate cap.
- Attach the Velcro straps to the rings of the cap.
- Give the cap its initial position, close the Velcro straps. Let the ears stick out of the cap.
- Make sure Cz is located in the middle and the cap is positioned straight.
- Consider placing cotton balls above the eyes.

Connecting the electrode cap

- Prepare the skin with a blunt needle.
- Add gel in the electrode holder while avoiding bridges.
- Plug in the electrodes, starting with only CMS and DRL.

Inspecting the signal

- Switch the BioSemi box on.
- Check whether the blue light stays on. If not, the connection of CMS and DRL is insufficient. If the blue light stays on, the rest of the electrodes can be plugged in.
- Start ActiView.
- Set up the configuration for inspection and recording.

Starting the experiment

- During the experiment and while touching computers, take off the gloves.
- First start the recording, then start the task.
- Keep track of the signal quality and do not hesitate to improve the EEG signal between task blocks.
    - Ask the participant to comply with eye blink and motion instructions.
    - Correct defects and bad connections.

After recording

- Put on new gloves again.
- Handle the participant with priority.
- Remove electrodes, provide tissues and skin lotion.
- If the participant want to wash his/her hair: explain the shower/tap, provide towel, shampoo, drier, and mirror.
- Handle exit questions.
- Handle the participant's payment.
- Show the participant the way out and thank him/her.
- Turn off the BioSemi box.
- Carefully remove tape from the wires and cap.

- Wash the gel from electrodes and cap.
- Disinfect the electrodes and cap.
- Discard garbage.
- Dispose the needle in a dedicated container.
- Mind the time for disinfection to prevent chemical damage to cap and electrodes.
- Tidy the room for future use.
- Shut down PCs, leave the monitors on, and turn off the light.

Troubleshooting during EEG data acquisition

General

The time of a lab coordinator and technicians is scarce: we are not a helpdesk. Therefore, always try to solve the problem yourself first or with the help of your supervisor, before calling the lab coordinator or the technician for help. Having said this, we understand that interrupting an experiment is undesirable. Furthermore, it is important that problems are identified and solved: do not leave a problem for others to encounter later.

Medical troubles: First Aid

See the telephone listing in the back (Appendix D) for the most suited numbers.
If you have had eye/mouth/skin contact with Incidin, always use lots of water. See Appendix B for details.

BioSemi acquisition box does not show a constant blue light

This is an indication that the incoming signal exceeds the digitizable range, resulting in useless recordings. This can be the result of

- Any of the electrodes has a defect and causes a short circuit. The simplest to find out which, is to unplug (from the BioSemi box) bundles and electrodes one by one, until only CMS and DRL are left, and check during which change the light becomes normal again. Do not discard the electrode immediately: defects often occur temporarily because the electrode has not dried long enough.
- CMS or DRL is not properly connected, or is broken. Make sure you used enough gel for these electrodes. Are the electrodes not pushed away from the scalp by curly hair? If this does not help, try a different set of CMS and DRL.

Mark which electrode showed the defect. If an electrode repetitively yields problems, inform the lab coordinator.

Signal has a bad quality

If the signal of all channels is bad:

- most likely, the problem is in CMS. Because it serves as the reference for other channels, the noise of CMS is visible in all monopolar channels.

If the signal of only one channel is bad, and the signal of other channels is good:

- There may be insufficient gel under the electrode, for example due to an air bubble. Add gel and try it again. The effect of gel will improve yet.
- Try to replace the electrode and adjust the ActiView label if necessary. Sometimes electrodes have not dried enough and cause interference. If the problem is solved, please mark the defect in the logbook of the electrode bundle. For your own records: make note of the electrode placements you eventually used during recording.

Battery is running low

There is quite some time left between the first warning and a battery that is really too low. Wait for a convenient moment to interrupt the recording session, for example a break between experimental blocks. Pause the recording and the task, replace the battery by a better one, wait for a minute to get the signal settled again, and resume the recording and task.
Batteries that are empty should be charged again right away, so there should always be a spare battery. Do not take batteries from the other setups without notifying the lab coordinator.
If a battery runs empty too fast, notify the lab coordinator.

Supplies are running low

The lab coordinator keeps track of disposables, such as gloves, tissues, Incidin, etc. He depends on your warning signals to be on time with new shipment orders.
Cotton towels, if offered, are picked up on Wednesday morning and returned a week later. Notify the lab coordinator if the supply is getting low or the laundry bag is piling up. Note: cotton towels are a service for the subject to wash his/her hair and face. **For all other purposes, use paper towels**.

3 ERP Data analysis

Introduction

Simply put, event-related potentials (ERPs) are obtained by averaging pieces of clean EEG that are evoked by a stimulus or response event. This short description conceals a world of methodology. We will keep it simple and analyze data of an Eriksen flanker task. This task is chosen based on the following criteria:

- The ERP components and the experimental results have clear psychological interpretations
- The paradigm is well known and widely applied
- The data are not too complicated to analyse

The experiment illustrates a number of dissimilar techniques that are representative of ERP research. It provides an introduction in the use of Brain Vision Analyzer version 2 (BVA), the selection of analytic techniques, and the interpretation of data.

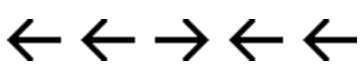
ERP analysis involves a lot of choices, some of which are based on tradition, some on reasoning about human physiology. It is difficult to oversee the consequences of deviating from standard procedures. For those of you who want to continue working with ERP, it is worth studying the guidelines listed in

T.W. Picton, S. Bentin, P. Berg, E. Donchin, S.A. Hillyard, R. Johnson, Jr., G.A. Miller, W. Ritter, D.S. Ruchkin, M.D. Rugg, and M.J. Taylor (2000). Guidelines for using human event-related potentials to study cognition: Recording standards and publication criteria. *Psychophysiology*, *37*, 127-152. http://sprweb.org/articles/Picton00.pdf

Eriksen experiment

Background

The Eriksen experiment is a conflict task. It belongs to the same family of paradigms as the Stroop and Simon task. Participants are instructed to respond as fast as possible to the direction of the central stimulus in an array like this by pressing a response button with the corresponding index finger (in this case the right index finger).



Flanking stimuli around the target are irrelevant, but can prime the correct response (congruent), or the opposite response (incongruent). Immediately after stimulus presentation, the attentional spotlight (the area of stimulus information that is selected for further processing) has not yet been narrowed, so the dominant information derives from the flankers, which may point at the wrong response. As the spotlight is narrowed, the support for the correct response increases. In the ERP you are likely to see initial signs of motor preparation for the hand indicated by the flankers (LRP) and signs of error detection processes (ERN). In addition, the ERP will show large components such as the P300. Prior to the P300, there is a weak negative component that is sensitive to the presence of conflict in the decision processes (N200). We will see to what extent these waves can be identified and quantified. Some of these ERP peaks will be analysed in the following.

Experiment

The experiment started with a practice phase in order to stabilize the reaction time. The experimental phase consisted of eight blocks of 48 trials each. Trials were selected randomly from conditions 1-4.

Condition code	Correct response	Congruence	Phase
S1 = >>>>>	R 16 = right	Congruent	Experimental
S2 = <<><<	R 16 = right	Incongruent	Experimental
S3 = <<<<<	R 8 = left	Congruent	Experimental
S4 = >><>>	R 8 = left	Incongruent	Experimental
S11 = >>>>>	R 16 = right	Congruent	Practice
S12 = <<><<	R 16 = right	Incongruent	Practice
S13 = <<<<<	R 8 = left	Congruent	Practice
S14 = >><>>	R 8 = left	Incongruent	Practice
R 8 = left	Response onset Left		
R 16 = right	Response onset Right		

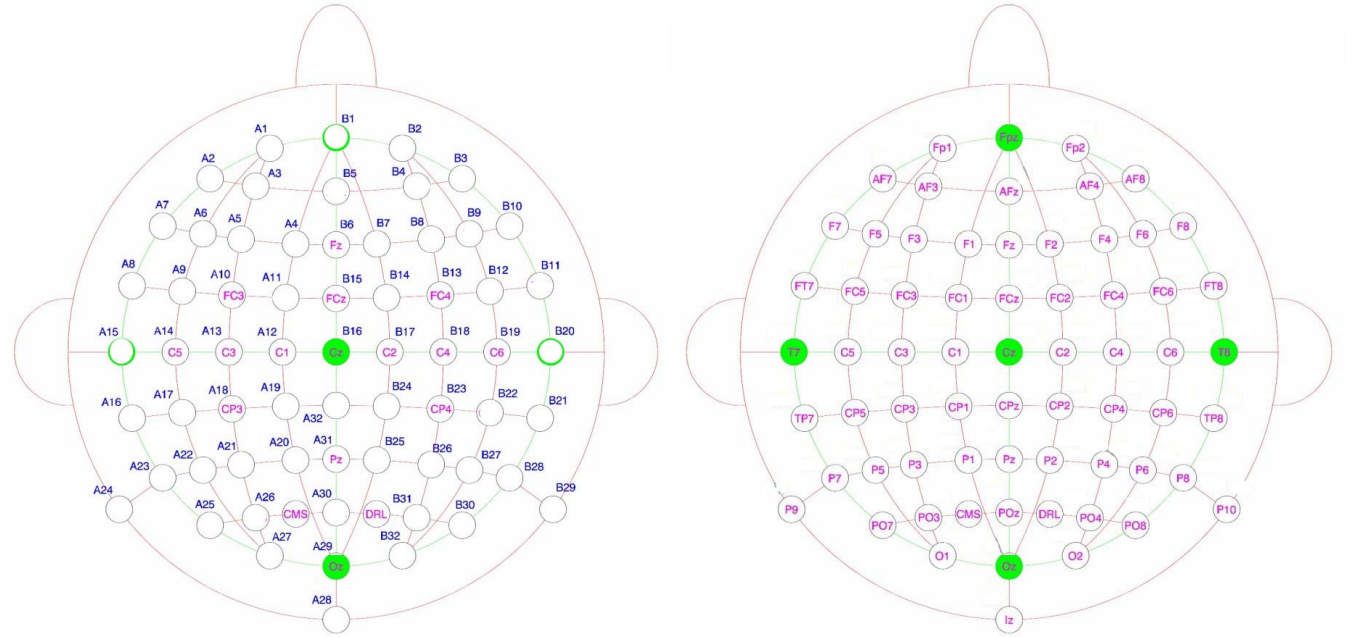
Codes (in blue) that can occur in the status channel, and their interpretation

EEG recordings: Formal description

Recordings have been made for nine participants with the following electrodes. This is a formal description of the recording method, as it would be reported in an experimental paper. Refer to the glossary for details.

Channel names: The BioSemi names and the corresponding names according to the 10/10 or 10/20 convention (http://en.wikipedia.org/wiki/10-20_system_(EEG))

A10	FC3	B6	Fz	EXG1	Left Horizontal Eye
A12	C1	B13	FC4	EXG2	Right Horizontal Eye
A13	C3	B15	FCz	EXG3	Upper Vertical Left Eye
A14	C5	B16	Cz	EXG4	Lower Vertical Left Eye
A18	CP3	B17	C2	EXG5	Left Mastoid
A31	Pz	B18	C4	EXG6	Right Mastoid
		B19	C6	Status	8-bits Stimulus +
		B23	CP4		8-bits Response



Left: The electrodes used in the experiment (pink), and their corresponding BioSemi codes (blue).
Right: The labels of the 10/10 system (pink), an extension of the 10/20 system.

The electrical potentials were pre-amplified at the electrode and were digitized at 256 Hz by a BioSemi Active Two system to 24-bit values with a full range of 524 mV, and therefore with a least significant bit of 31 nV. BioSemi always applies an analog (hardware) filter to pass only the frequencies between 0 and ¼ x sample frequency (i.e. 0-64 Hz). This is done to prevent aliasing. A combination of CMS and DRL was used as reference and for grounding. All recordings were monopolar. The skin impedance for electrodes in the electrode cap was reduced by scratching the skin with a blunt needle. Impedance for the external electrodes was reduced by wiping the skin with alcohol.

Working with BVA vs. other EEG software

BVA is a program for analyzing EEG, ERP and other time series data. The program is selected because of its clarity, speed, and flexibility. FSW owns 16 licenses for BVA. FSW also owns a license for the program BESA, which is specialized in EEG/ERP source analysis. BESA is not so flexible in the basic steps of calculating ERPs. Other software that is worth mentioning is the NeuroScan software, which is rather expensive, but in other respects comparable to BVA. EEGLAB is open source (=free) software, but it requires knowledge of programming in Matlab.

Getting started

BVA provides online support and a user manual via the menu item "Help".

TASK Open BVA 

- When you start working on a data set, you have to create a new workspace: select *File* → *New Workspace* … A workspace defines where you want to read and write your data, and can thereby contain all relevant information for one experiment, or for a subset of analyses.
- Create or select the folders where Raw data are held (by the user), where the History files are held (by BVA), and to where data such as amplitude value tables, and graphs will be exported. Do this for the Eriksen experiment. The raw data are files with the extension .BDF. If you have permission to write on a hard disk, it is strongly recommended to work with raw data files that are on the hard disk, because the speed of analyzing hundreds of megabytes from a CD player or a network share is substantially lower.
- Save the workspace under a meaningful name (e.g. "Eriksen").
- If you want to use macros, you can define the default location of macros by choosing *Configuration* → *Set folder for workfiles*. Using macros in BVA will not be explained in this course, however. If you are interested in writing macros, you can obtain a macro cookbook from the BVA website.

You now see in the left pane of the display a list of all readable files that have been found in the Raw Files folder that you have defined in the workspace. If this pane shows no data files, copy or move the files you want to analyze to the Raw Files folder and choose *File* → *Refresh*. Each data file is represented in the left panel as a book icon.

TASK Click on the + sign next to one of the data file icons. The book opens and shows the Raw Data. Double click on the Raw Data node (a node is an icon in the hierarchy of a data file), to see the data in the main window.

You can now see the recorded time series in the main window. Below the data, you see a bar with markers for all events that have been coded so far, such as stimuli (in red) and responses (in blue). Later on in the analyses, pink markers will be added for identified artifacts. Below the marker bar, there is an indicator of the current position within the testing session.
Scrolling through the data can be done in three ways:

- Press an arrow ◄or ► in the bottom left corner for scrolling one page backward or forward
- Press an arrow |◄or ►| to scroll one second backward or forward
- Click on the marker bar to indicate where you want to go. The same can be done by dragging the blue indicator to the desired position.

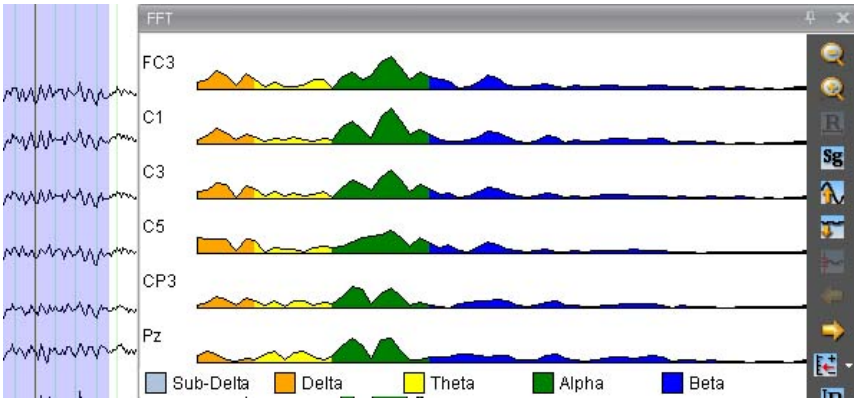
TASK Explore the data and try to see whether you can recognize eye blinks, eye movements, muscle artifacts and alpha rhythm.

When you perform transformations on the data, the **raw data are never lost.** Instead, BVA stores the entire processing sequence in a history file, visible as a hierarchical display of processing steps that you can expand or close, just as in Windows Explorer and E-prime. BVA calculates the current data only for the window that you are watching, not for the remainder of the recording session. Calculation for an entire data set will only happen when it is really necessary, for example for averaging.

For each node (processing step) you can:

- Change the label (press F2 and type the new label)
- Check the operation that has been performed (right-click and select Operations Info)
- Copy it to a different position in the hierarchy (drag and drop to the desired position)
- Remove it if is not acceptable
- Add new operations

A node can have more than one branch. After you run an operation on the data in a node, select the same data again (double click!) and perform another operation, both operations will form separate branches.



If you select a piece of data and right-click on it, you can choose between a few options to perform on the selected data. Fast Fourier transformation (FFT) is one of them: it is a translation of a time series into a spectrum. Each time series, even a square wave, consists of a unique combination of pure sine waves of different frequencies, the spectrum. You can assume that most of the frequencies in the spectrum correspond to the frequencies produced by brain activities underlying the EEG. Different frequencies have different meanings. Frequencies are usually classified in bands, each with a different meaning. For example slow activity in the delta band (below 4 Hz) is strongly present during sleep, whereas activity in the beta and gamma band (12 – 30 Hz) appears during mentally engaging activities. The FFT window has its own buttons for increasing/decreasing amplitudes and selecting a narrower or wider spectral band.
If you run an FFT on what you perceive as an artifact, you can check which frequencies are present in those data. If there is a dominant 50Hz component, your recordings were not sufficiently shielded against the electrical fields induced by power supply and electrical equipment such as the monitor.

Step 1: Know your data, know your procedures

Throughout data analysis, there is one step that needs to take place again and again; visual data inspection. Despite the fact that BVA is an advanced program, it only does what you tell it to do. The researcher remains responsible for the choices made. A default list of analysis steps is likely to yield incorrect results if you do not check the data and the results of each step. If you apply a filter, check whether the settings are optimal. If you correct for ocular artifacts, check if the eye blinks were indeed removed from EEG. If you encounter noisy data, try whether you can correct it. For each of these steps, you have to be aware of the consequences. For example, if you first apply a strong low-pass filter to EOG and then try to remove the contribution of

ocular artifacts to the EEG, the filter will disrupt the ocular correction. If you average data before you remove artifacts, it is too late to do so afterwards. It requires some experience with EEG and time series analysis to understand which procedures and which data pattern are acceptable.
While you are optimizing the settings for your data analysis, you may need to iterate through the data set as the settings that seem acceptable for one participant may not be acceptable for a different participant due to excessive artifacts or missing data. It is commonly expected that you apply the same settings to the data of all participants. If participants have unusually poor quality data, you may need to reject the participant's data altogether, rather than apply inappropriate settings to all data.

Step 2: Reorganizing the data

The current data set has only a default, temporary monopolar reference, the labels are not informative, and several channels contain data that are not required for further processing. This calls for some reorganization.

Step 2a: Re-referencing

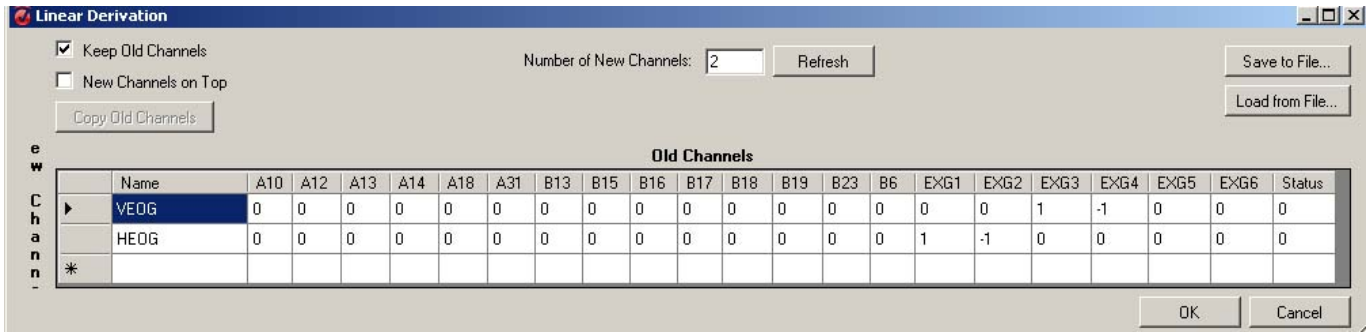
The data have been recorded in the program ActiView as "Raw". This means that because no reference was defined, all potentials were calculated by default relative to the electrode CMS. CMS is not an optimal reference, because it is placed close to the midline, and especially close to the electrode position Pz, where the P300 is typically recorded. Better is to use mastoids as the reference. However, if you are interested in lateralization of signals, you should have a reference that is neutral with regard to the left-right dimension. Therefore, the average of the signals recorded over the left and right mastoids will be subtracted from all the signals to obtain re-referenced signals. Because the mastoids are on opposite sides of the brain, dipoles that cause a positive potential over one mastoid are likely to produce a negative potential over the other mastoid, so the average mastoid is indeed rather neutral. Re-referencing involves only a subtraction of the time series on the new reference channel(s) from that of the other channels.

TASK In the menu, choose *Transformations* → *Channel Preprocessing* → *New Reference*. Select EXG 5 and EXG 6 as the new reference channels and have their signals subtracted from all EEG channels (i.e. the channels with names A* and B*). After you finish this, the processing hierarchy shows a new icon "New Reference". To make it more informative, you can rename the node to "ReRefAvMastoids". If you forgot the details of a processing step, you can always right click on a node and select Operation Info to get an overview.

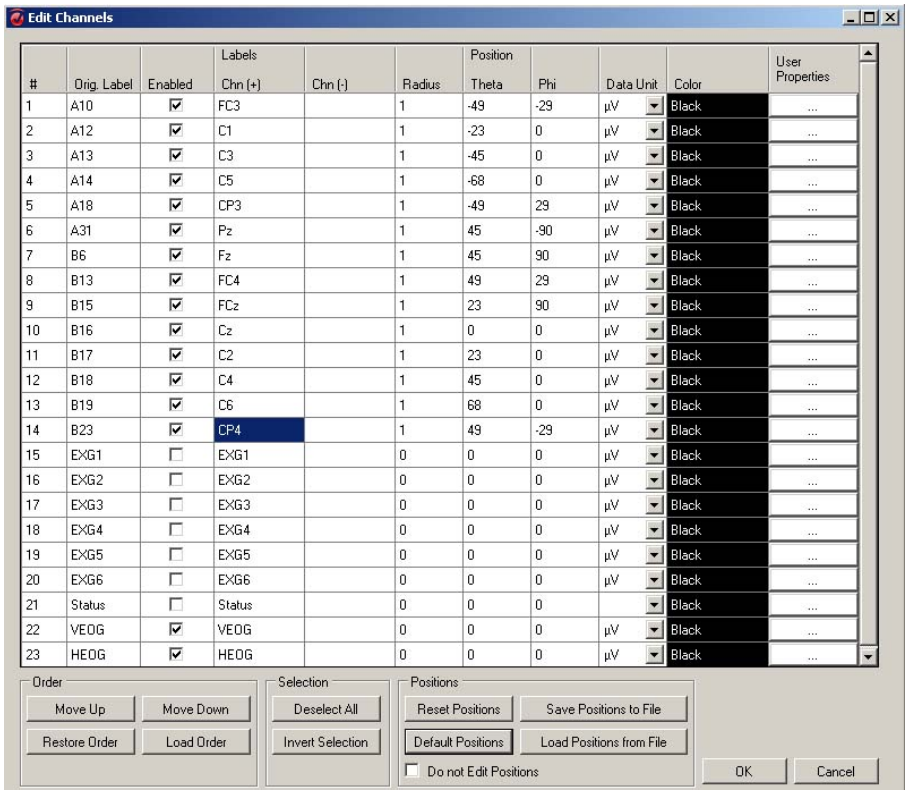
If EEG has been recorded over a large number of electrodes, covering all sides of the scalp, the use of a reference to the average of all EEG electrodes is a good alternative. Average references are particularly popular if source localization is practiced. Note, however, that each reference procedure has consequences for which parts of the EEG remain visible and which do not. For example, if you are interested in the EEG just above the ears, subtracting average mastoid activity may discard relevant information.

TASK The rereferencing is a step that needs to be performed for every participant. To save time, BVA allows you to drag-and-drop an identical processing step to a different data set. Open the data of a different participant (press +) and drag-and-drop the rereferencing node to the raw data of this participant. Just as with a single node, you can also drag-and-drop a series of processing steps, by picking up the first (highest) node in the hierarchy. All the subsequent processing steps are copied along with the step you drag.

Step 2b: Defining Bipolar EOG channels



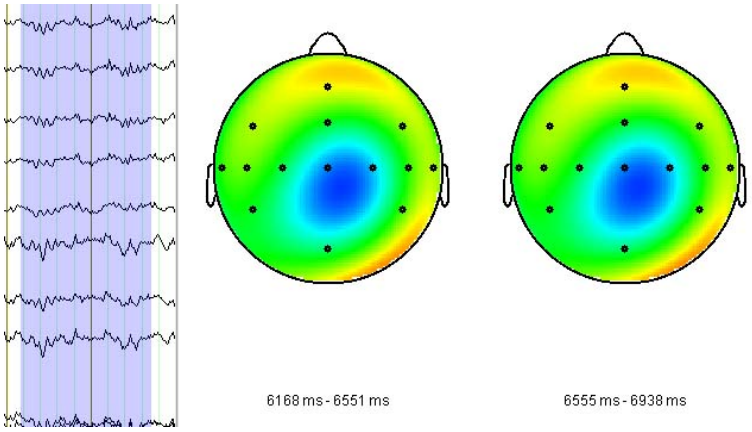
TASK Calculate two new signals from the four eye electrodes by pairing the two horizontal and the two vertical electrodes. For the eye electrodes, the most telling information is contained in a bipolar derivation, because shared activity without an ocular origin is subtracted out. This irrelevant activity includes noise at the reference channels and distant EEG sources that affect both EOG electrodes in the same way. Create a bipolar signal by selecting *Transformations* → *Linear derivation*…
Choose Number of New Channels: 2, and click Refresh. You will now see two rows of numbers that can be used for calculating a new channel. Because HEOG = EXG1 – EXG2, you should enter the numbers and 1 and -1 in the column under EXG1 and EXG2. Do the same with EXG3 and EXG4 to derive VEOG.



Step 2c: Editing channel displays

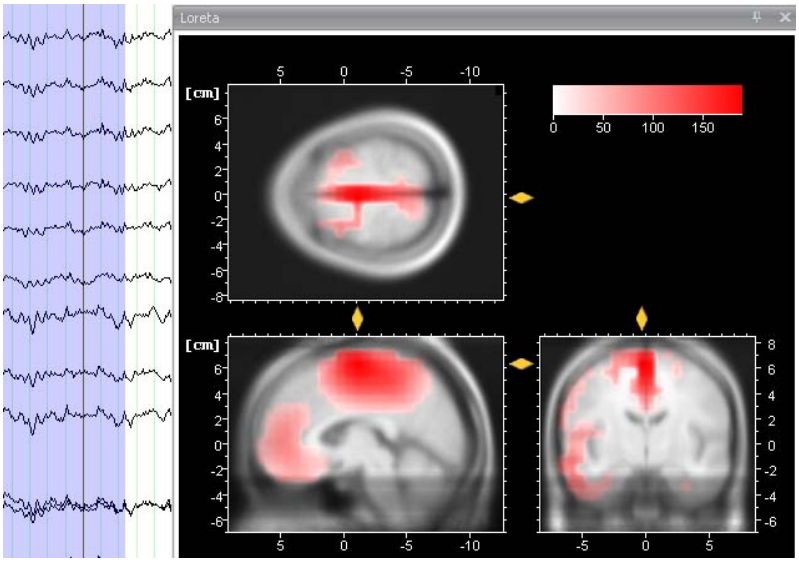
TASK Labels like A31 are not very informative about the actual position of the electrode, so we change these now. Choose *Transformations* → *Edit channels*…
This interface allows you to specify which channels should be enabled or disabled, and how they should be called. At this stage, the Status channel and EXG channels can be removed. For EEG channels, replace the BioSemi labels (e.g., A31) to the 10/20 name (e.g., Pz). Now that you have specified universal names, BVA understands where these electrodes were located on the scalp. Select the button 'Default positions' to automatically fill out the coordinates.

Note; If you copy this 'edit channels' step to other participants, make sure that the data of this participant were recorded with the same original labels. Otherwise, the relabeling will go wrong.



With the electrode positions defined by the 10/20 system, you can now watch not only the time series and FFT, but also two kinds of map displays. Select a piece of the time series that looks interesting, and right click it. A Current Source Density (CSD) map shows local differences in voltage relative to their surrounding. The image will show the activity in the time window you have selected. Note that BVA also displays activity outside the grid of the electrodes (the black dots). However, while potentials in between two electrode positions (interpolation) can be estimated quite reliably, activity outside the electrode grid (extrapolation) is not interpretable.

Another possibility using electrode coordinates is a Loreta source localization estimate of the brain potentials displayed. Note that with the number of electrodes used in this data set, source localization is not reliable, especially not with raw EEG. Source localization will not be discussed in this syllabus.



Step 3: Steps towards removing ocular artifacts

You have probably seen the strong contribution of ocular artifacts to EEG. We would now like to remove artifacts caused by eye movements and eye blinks. To do this, we want to use a convenient regression-based algorithm developed by Gratton and Coles (step 3b). However, before we can do this, we have to be sure that the offset of the data is close enough to zero to make the regression approach work properly. This will be done by inspecting the data (Step 3a) and filtering.

The correlation between each channel's activity and that of EOG channel activity is used to calculate how much an EOG artifact contributes to the recorded EEG for a given channel. This contribution can subsequently be predicted from the recorded EOG data and be subtracted from each EEG channel.

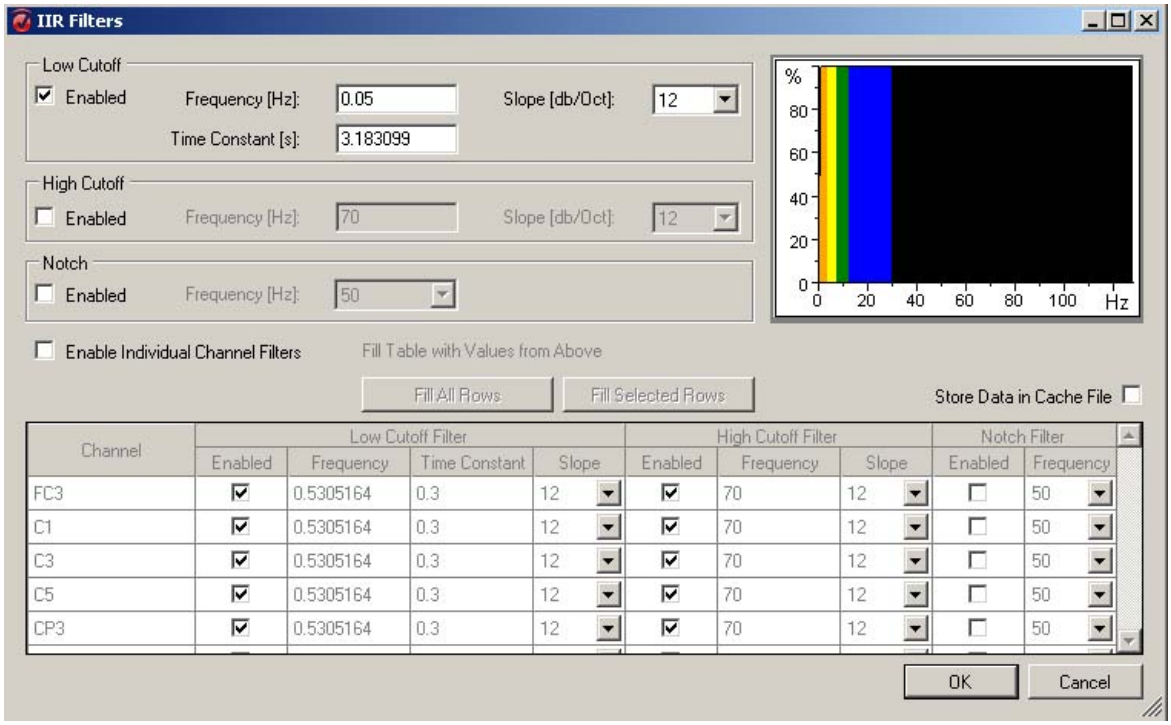
There are a few drawbacks to the Gratton & Coles procedure:

- The EOG channel also records some frontal cortex EEG activity. By treating this as ocular artifacts, relevant signals are removed from the data.
- Different ocular artifacts have different effects on EEG. In the ideal case each kind of ocular artifact should be treated separately. However, BVA's implementation of the Gratton & Coles method only distinguishes between eye blinks and other ocular artifacts.
- The noise on the EOG channels is used along with true EOG when EEG is corrected. This leads to some additional noise on the corrected EEG channel.

Despite these limitations, we will use the Gratton & Coles procedure. It does a reasonable job and superior alternatives are too complicated for this course. These alternatives would involve an Independent Component Analysis of all the data to extract the dominant ocular contribution to all the data.

Step 3a: High pass filtering

Before we can perform EOG regression on the data, it is important to realize that the data now have a substantial offset. That is, the data are not centered around zero V, but they can center around 10 mV for one channel and around -10 mV for another as a result of static electrical potentials building up around the skin. You can inspect the data to determine the offset on a number of places in the recording session. Hold the mouse over a signal until you see an X appear. Now watch for the absolute value of the amplitude in the lower right corner. Without any filtering, this amplitude may amount to thousands of μV. This is the result of our DC recording, which does not remove any drift. These data will give an inaccurate impression of EOG amplitudes on a given time point, and therefore they will serve as a poor basis for EOG correction. Therefore, we should run a high pass (=low cutoff) filter on the data to remove the drift from the EOG and EEG channels.



TASK Select *Transformations* → *Data Filtering* → *IIR Filters* … and specify your filter. If you have really clean data, a cutoff frequency of 0.01 Hz would be possible, but if the participants in your population were moving a lot (children), or if the electrodes made poor contact, a more rigorous rejection of drift may be required, with levels like 0.1 Hz. The less low frequencies you reject, the better, especially if you are interested in slow ERP components.

The shape of the filter can be adjusted at different levels. The setting 12 dB/octave is a relatively modest filter, going from all to none filtering over a long section of the spectrum. The drawback is that very low frequencies will sometimes not be entirely rejected. The other extreme is 48 dB/octave, which is a filter with a rather steep function, resulting in strong suppression of the low frequencies. The drawback of a steep filter function is that it will add nonexisting frequencies to the spectrum. When these frequencies are translated back to a waveform, you can find waves that were previously not present (and that are not of a biological origin), particularly around the cut off frequency. We choose the intermediate filter function; 24 dB/octave. At this point in the analysis it is not yet necessary to remove high frequencies, because these high frequencies can be helpful in estimating the relationship between true EOG and EEG.

TASK Run the filter for a few different parameters: 0.01 Hz or 0.1 Hz and 12/24/48 dB/octave. Watch the data before and after the filtering. Decide which result looks best based on the offset and the shape of the EEG.

Because BVA plots the data relative to the amplitude occurring at the beginning of a displayed time-window, it seems as if there was no drift in the original data, so you will not see big changes. However, if you look at the true potentials, the data now remain substantially closer to an offset of 0 mV and this is a requirement for estimating the relationship between EOG and EEG.

Step 3b: Detection of residual drift

If the high-pass filter has done its work properly, you should now have reduced all the offset values to less than 1 mV. You can check by hand, but you can also test this for the entire data set by looking for extreme amplitudes. If after high-pass filtering with appropriate parameters the signal still reaches absolute amplitude values outside the range of -1 to +1 mV, this is a sign of other problems than mere drift.

TASK Look at the filtered data in data file Eriksen002. The data look fine in most parts of the file, but now scroll to the range around time 13:15. There is a sudden drop in amplitude, probably due to electrodes coming off or losing contact.

Notice that when the offset becomes really high, there are spikes in the data with an exact frequency of 1/second.
This is a signal that the amplifier creates when the data go out of the range of the BioSemi recorder. Usually this happens if CMS/DRL were not making proper contact, or if any of the electrodes created a short circuit. This period should of course not be included in the estimation of ocular contributions to EEG.

TASK Select *Transformations* → *Raw Data Inspector*… This function is identical to *Transformations* → *Artifact Rejection* …, except that the former works on continuous data, while the latter works on segmented data. We will run the rejection in automatic mode. Detect artifacts of the type Amplitude for the EEG channels and HEOG with the settings -200 μV to +200 μV and a window of 200 ms around the artifacts. This will identify and reject amplitudes that are larger than any electrophysiological signal. Note that this is a very lenient artifact criterion. More stringent criteria can be applied to the data later. The periods marked as bad will automatically be excluded when the regression parameter for ocular artifact correction is calculated. Now run Raw Data Inspector again, but only for VEOG and with a more lenient criterion (-800 μV to +800 μV). This needs to be done separately because eye blinks have substantially larger amplitudes than EEG and stable horizontal EOG. At the same time, normal eye blinks need to be included in the data for ocular artifact correction.

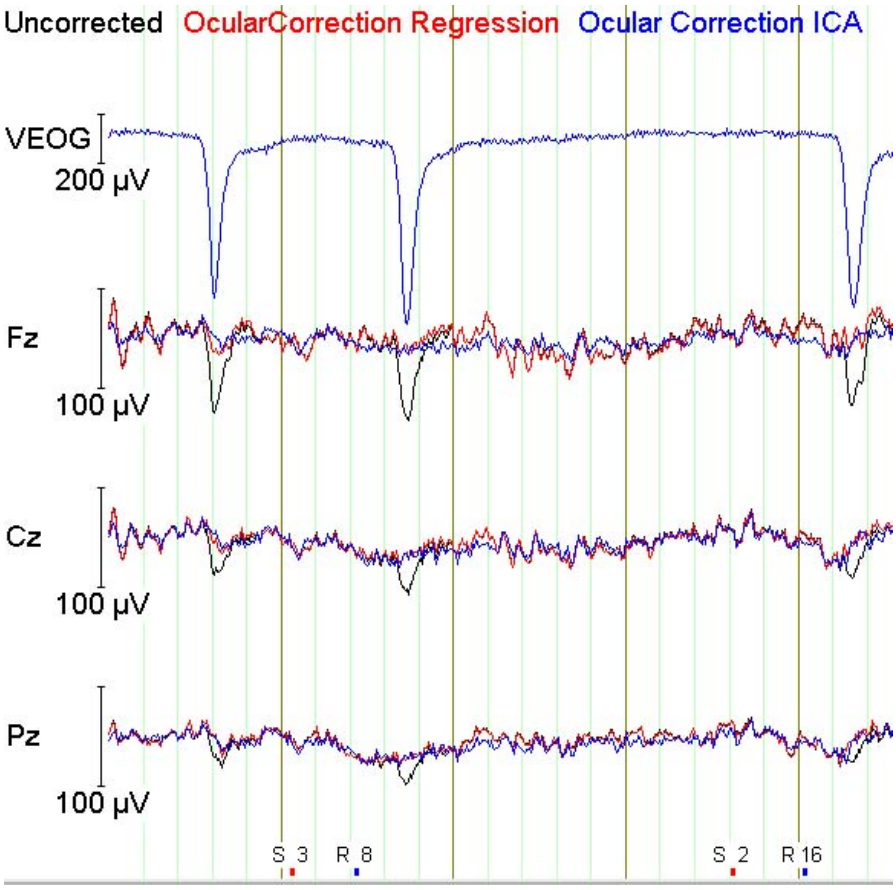
Step 3c: Ocular artifact correction

We are now ready to estimate and remove the contribution of ocular activity to EEG. This is done almost automatically.

TASK Select *Transformations* → *Ocular Correction*… You only have to specify which channels represent the EOG and which represent the EEG. Select VEOG and HEOG and mark Common Reference, which is a way to indicate that the signals of VEOG and HEOG can be used in their current form (the bipolar derivation has previously been calculated already).

The bipolar derivations of VEOG and HEOG are used as predictors of EEG. The size of the correlation between EEG and EOG is used as a correction parameter. The predicted contribution of ocular activity to the EEG recordings can thereby be subtracted.
We correct all the EEG channels, but not the EOG channels. We later want to see how large the original EOG was in the averages of the accepted trials.

You can now inspect what the ocular artifact correction has done for the data.



TASK Drag the data node from before the ocular correction into the window that shows the data after correction. You should now see two lines: one with and one without the eye artifacts. Walk through the data to see whether all eye artifacts were correctly removed. You will see that occasionally there is over- or undercompensation, but that is better than doing nothing.

In the adjacent figure with three lines, you can see that at least on visual inspection, the correction by Independent Component Analysis and by regression are equally successful in removing ocular artifacts.

Step 4: Artifact rejection

While ocular artifacts have a known source and can be corrected, artifacts due to chewing gum, tense neck or biting muscles, bad electrodes and others can not be corrected – they have to be rejected. That is, the affected periods will be marked as bad, and during averaging you can choose not to include the trials with bad periods. Settings for artifact criteria do not have a universal validity: in one data set a rejection criterion is allowed to be high, for example because it looks too much like the signal of interest, in others it needs to be low to isolate a small peak that otherwise drowns in noise. The same can be said for the window that is considered bad. It seems safe to mark a window of 100 ms before and after the artifact as bad, but there may be situations that call for a longer window duration.

TASK Select *Transformations* → *Raw Data Inspection* … We will run the rejection in automatic mode. This means that the processing node you create here can be applied to other data sets without needing to through a BVA dialogue. The drawback of running raw data inspection automatically is that it is tempting not to check whether this step worked as intended.
There are four classes of artifact criteria predefined in BVA:

- Gradient: if there is a sudden change in amplitude, this is not likely to be caused by true brain activity. One of the largest ERP components, the P300 shows a rise of 30 µV in 100 ms, but background EEG is larger and should not always be marked as bad. You can try for example the setting 50 µV /sample when you use 256 Hz. Of course this should be adjusted if you use a different sample rate.
- Min-Max: not all large amplitude changes take place within one sample. Therefore, a moving window can be used to detect large changes with a somewhat shallower slope. Try for example a maximum change in amplitude of 100 µV in a window of 200 ms.
- Amplitude: if you are not recording DC, the offset will return to zero, and large absolute values of the amplitude are an indication of excessive drift. We have already inspected the data for such drifts prior

to ocular correction, so if the settings then were acceptable, there is no need to repeat that. Moreover, the same goal is already achieved by the Min-Max setting.

- Low activity: there can be a defect in the battery or other hardware, which may result in unrealistic low amplitude activity. The default setting is to mark as bad the activity that remains within a range of 0.5 μV in a window of 100 ms.

NOTE 1: especially if you want to compare between groups, it is important to use the same artifact criteria for all participants. However, exceptions are possible if you can justify them to critical reviewers.
NOTE 2: there is no need to apply the same artifact criteria to all the channels. For example, one channel may be more prone to artifacts than others because it is closer to the chewing muscles. The criteria may also depend on your paradigm. If the experiment required that participants always focused their eyes on the middle of the screen, you can apply a very strict artifact criterion for HEOG.
NOTE 3: you don't have to mark artifacts in all the channels if these channels are not used for further analyses. There is no need to reject useful data.

After running raw data inspection, you should inspect whether your criteria were not too lenient and not too strict. You should find an optimum between losing too much data and accepting too much noise. Finding optimal settings across multiple participants may take some time, but it is worth the investment.
You can iterate the artifact scans with different settings for different channels. For example, it is possible to reject horizontal EOG with a stringent criterion, because ERPs are meaningful only if participants focus on the stimulus. Remember that artefacts are usually restricted to some of the channels. For subsequent processing steps, you may be better off to edit out channels that are not important, otherwise more trials will be excluded than really necessary. For example, if you are interested in an ERP peak over Pz, you can consider performing the analyses with only Pz and the eye electrodes.

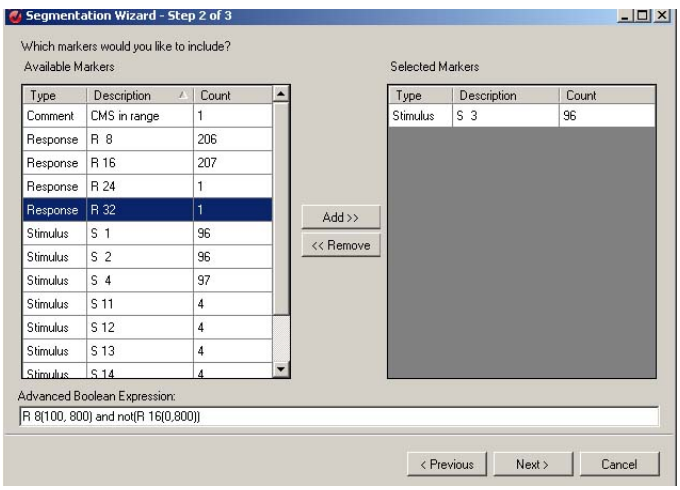
Step 5: Low-pass filtering

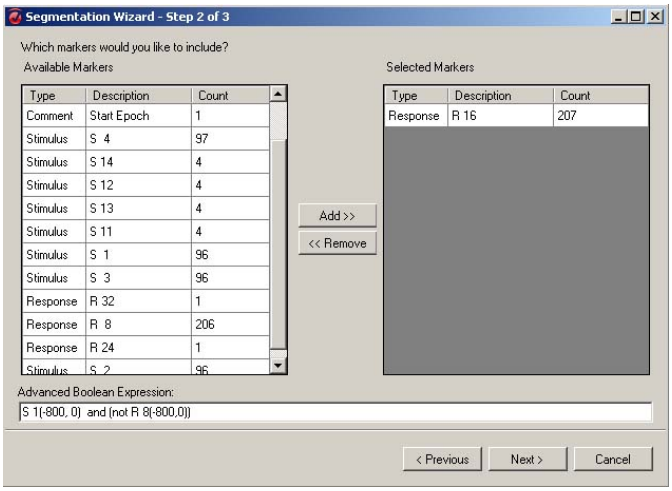
Now that all the artifacts have been corrected or marked, we also apply a low-pass filter to the EEG data. There is some debate about the preferred filter settings. Some experts (e.g. Steve Luck) recommend not to filter more than is strictly necessary for removing artifacts. After all, filters with a smooth gain function always affect the amplitude from low to high frequencies, and some of the lower frequencies (e.g. 10 Hz) may be relevant. In contrast, BVA's technical expert advices to, if possible, select a cut off frequency below 10 Hz in order to reduce alpha, which is the dominant background EEG contribution. You can estimate which frequency the interesting peaks have, for example 2-4 Hz for the P3 and 10 Hz or higher for the N1. These characteristics also determine the optimal filtering.
If you are interested in early visual potentials with 20 Hz characteristics, it would be rather silly to cut off frequencies above 10 Hz.

TASK Select *Transformations* → *Data Filtering* → *IIR Filters*… and specify the high cutoff = low pass filter. Do not filter EOG channels. Run the filtering several times on the same data and compare the results.

Step 6: Segmenting

We have performed all the previous operations on one long continuous time series and this was for good reasons. However, now is the time to extract segments around the relevant events. Remember that it is possible to perform either stimulus-locked averaging or response-locked averaging. We will practice with both. We usually need to segment for each condition separately. In this case, we have four experimental conditions by which segments need to be extracted. For the S1, the correct response was R5. This means that we want to extract a window around S1 or around R5, and specify additional criteria to ensure that only accurate responses were included.





TASK Select *Transformations* → *Segmentation* … In the first step, you can choose to use the stimulus markers as the basis for segmentation (that is, select "Create new segments based on marker position") and that intermediate results should be saved to a temporary file (that is "Cache data to a temporary file"). In the second step, select the marker that will be the constant time point (e.g., S3) and specify in the Advanced Boolean Expression which responses are allowed or required. In the example, R 8 should follow 100-800 ms after the S-marker and R 16 (the incorrect response) should not appear in the window 0-800. This will ensure error-free trials. In the third step you have to specify the window relative to the marker. Select -200 to +800 ms for the reasons explained below. Accept minor adjustments of these latencies by the program.

If you are ready with four stimulus-locked segmentations, calculate the response locked segmentation. To do so, you should select R-markers first in step 2, and further specify in the Advanced Boolean Expression which S-marker is required or accepted prior to the R-marker. For these response-locked segmentations, choose a segment from -1000 to + 600 ms.

To choose the segmentation window, you need to be aware of the reaction times you can expect, and the need to have a neutral baseline period in later steps. Given that the reaction times on an Eriksen task are usually below 500 ms, it is safe to segment up to 800 ms after stimulus presentation and take for granted that trials with RT > 800 ms are rejected. This is even better because unusually slow trials will contribute unusual EEG to the average. A period of 200 ms prior to the stimulus is just enough to serve as a baseline. A baseline is a time period in which no event-related brain processes are taking place, so that it can serve as the best approximation of background activity, which later needs to be subtracted from the average ERP. For response-locked averages, the period of 200 ms preceding the response is clearly not neutral, so you would need a much earlier baseline window.

Step 7: Averaging

We now have data that are clean of artifacts, contain no errors, and are segmented and organized by condition. This means that we can calculate the average waveforms.

TASK Select *Transformations* → *Average.*

The result should look like a real ERP. A good check is always to find the P300, which has a positive potential, is large over Pz and reaches its peak after 400 ms in stimulus-locked averages following a relevant and meaningful stimulus.

TASK After averaging, select *Transformations* → *Baseline* and define the baseline from -200 to 0 ms.

It is recommended to use a baseline of at least 100 ms duration, and as close as possible preceding event related brain processes, but not overlapping with it. A baseline duration of 200 ms is better, because it contains at least two full cycles of alpha activity, which is the most dominant background EEG source.

Step 8: LRP: Lateralized readiness potential

Because responses were to be made with the left or right hand, unpredictably and in equal probabilities, no lateralization of motor activity is expected on average as long as participants have not decided about left-hand and right-hand responses. As soon as preference emerges, however, this could lead to lateralized motor activity. The LRP can be used as a real time ERP component that isolates bias for the correct or incorrect response, while rejecting virtually all the ERP contributions that do not vary systematically with the direction of the response. LRPs can be calculated as the activation over the hemisphere contralateral to the correct response minus the activation over the hemisphere ipsilateral to the correct response. Prior to a right-hand response, the left primary motor cortex is more active (=more negative) than the right primary motor cortex and for left-hand responses this is reversed. The activity of the primary motor cortex is strongest over electrode positions C3 (left hemisphere) and C4 (right hemisphere) in case of hand responses.
The LRP calculation should be performed separately for the left-hand and the right-hand conditions before the left-hand and right-hand conditions are merged. That is why we have differentiated condition codes (and ERP calculations) for left and right-hand conditions. In BVA, this so-called double subtraction is not very visible, but it is applied. The LRP should be derived from pairs of conditions, i.e. S1 and S3 together are used to calculate the LRP for congruent trials and S2 and S4 together are used to calculate the LRP for incongruent trials. On congruent trials, the LRP is expected to start at baseline level, and then deviate from the baseline in the direction of the correct response (= negative potential). On incongruent trials, the LRP is expected to deviate in the direction of the incorrect response (= positive potential) before switching towards the correct direction.
Just as ERP, the LRP can be calculated synchronized (=locked) to the stimulus onset, or to the response onset. Depending on the locking event, the average LRP will be more sensitive to motor activity evoked by the stimulus presentation, or to the activity surrounding the execution of a response, so the two versions will not look identical.

TASK If you have selected the stimulus-locked average of S1 and select *Transformations* → *Result Evaluation* → *LRP*, you get the chance to select the average that should serve as the mirror image of the current average: the average of S3. Specify which channels should be subtracted. The true LRP channels are C3 vs. C4, located over the primary motor cortex, but now that you are busy, you can also calculate it for some other pairs, such as CP3 and CP4; and FC3 and FC4, and inspect which "LRP" is largest. Drag the LRP for incongruent conditions on top of the LRP for congruent conditions. You can repeat the calculation for the response-locked averages. What can you tell about the latency of decisions, and what about the direction of decisions? Does the LRP look better over C3 and C4, or over other positions? Which LRP looks better, the stimulus-locked or the response-locked LRP?

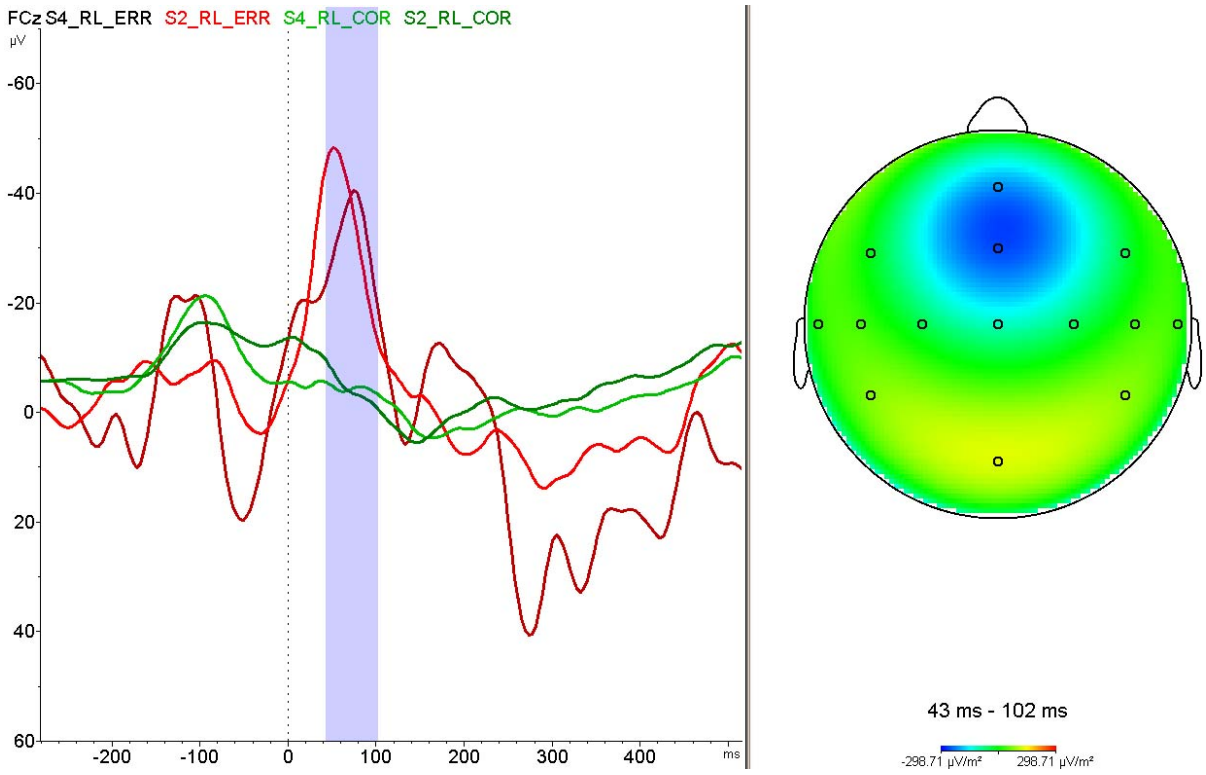
Step 9: ERN: Error-related negativity

In the Eriksen task, participants are likely to commit more than 10% errors. This may allow us to compare the brain activity immediately following an incorrect response with that following a correct response. You are likely to find an increased negativity over the fronto-central midline on error trials, which is interpreted as the brain's response to detecting an error.

If you want to calculate an ERN, you have to have enough error trials without bad sections. There are probably not so many participants who fulfil this criterion, but you can try. The only thing you have to do is segment (Step 6) again locked to the response, but using the incorrect response as a criterion. The resulting segments should be averaged again. Depending on whether there are enough error trials available, you may choose to combine conditions (Step 10).

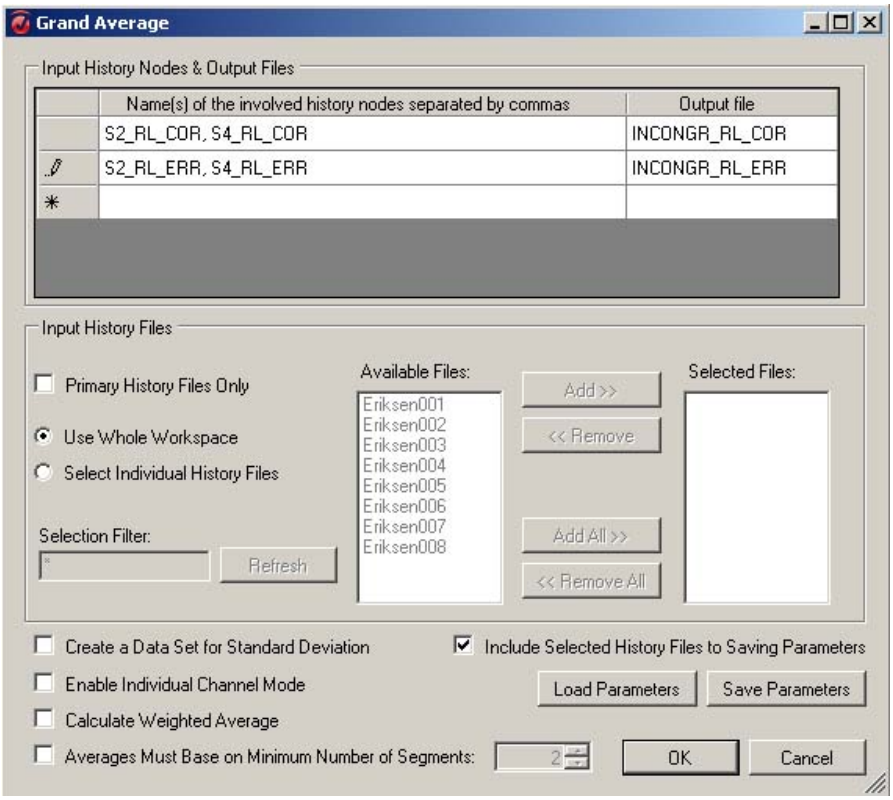
TASK Calculate the response-locked averages, separately for correct and for incorrect responses and for each condition. This may yield the message "no segments found" for incorrect responses on congruent conditions, because some participants simply do not err on a congruent Eriksen stimulus. At least for the incongruent condition, it should be possible to display in the same window the ERPs for errors and correct responses. Pay attention the differences. Despite the fact that these averages are based on a small number of trials, the difference is consistent with the literature. That is, there is a strong negative potential on error trials

in comparison with correct trials, in the first 100 ms following the response. This is the ERN. Select the peak, and create a CSD map. Usually, the ERN has the largest negativity over its source, the anterior cingulate cortex, that is at electrode FCz.

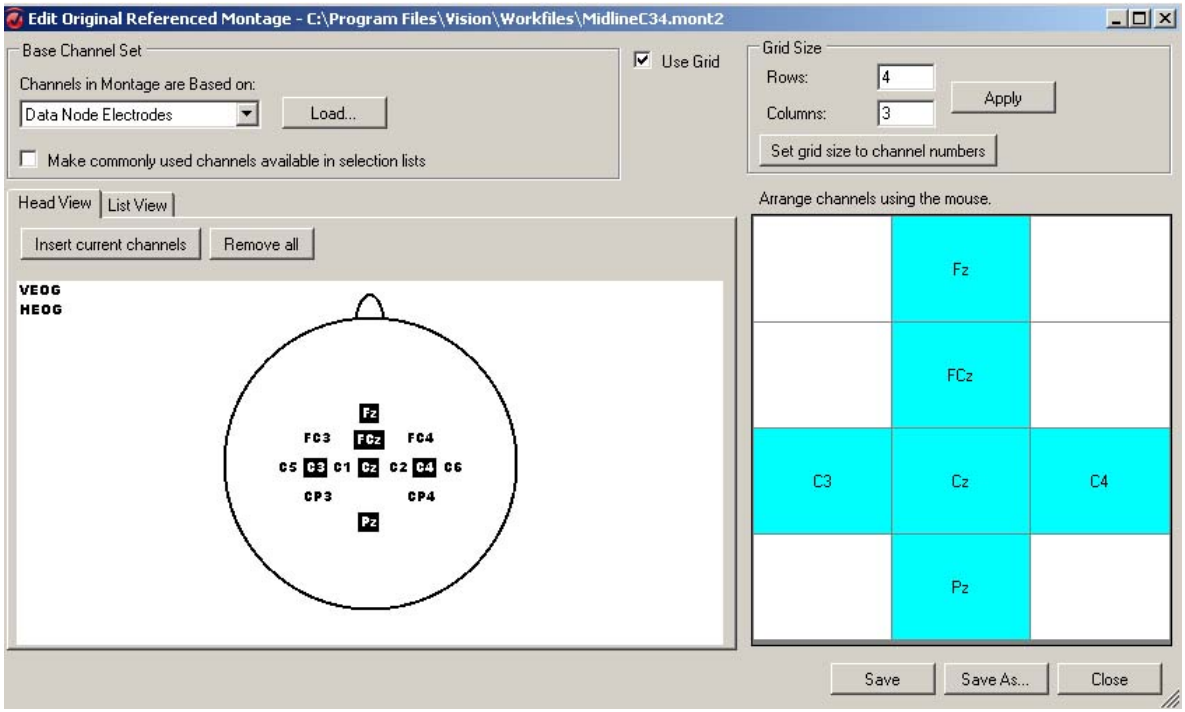


Step 10: Combining conditions

Average ERPs can be combined (pooling is a more common name than combining, but this name has a different meaning in BVA). In the Eriksen task, incongruent conditions can be combined for conditions 2 and 4, and those for congruent conditions can be combined for conditions 1 and 3. Combining conditions, also known as calculating a Grand Average, involves calculating the *average of the averages* of conditions. It is important to consider the consequences of combining two conditions of which one contains more trials than another. If condition A is noisy and B is not, the combined ERP may have a worse signal-to-noise ratio than the ERP of condition B separately. In some cases, this might be reason to choose for a weighted average, which means that the number of trials underlying each average is taken into account in combining the conditions, so that the result always has less noise than the averages of each condition. This is acceptable if there are no reasons to expect different ERPs for the conditions you combine, for

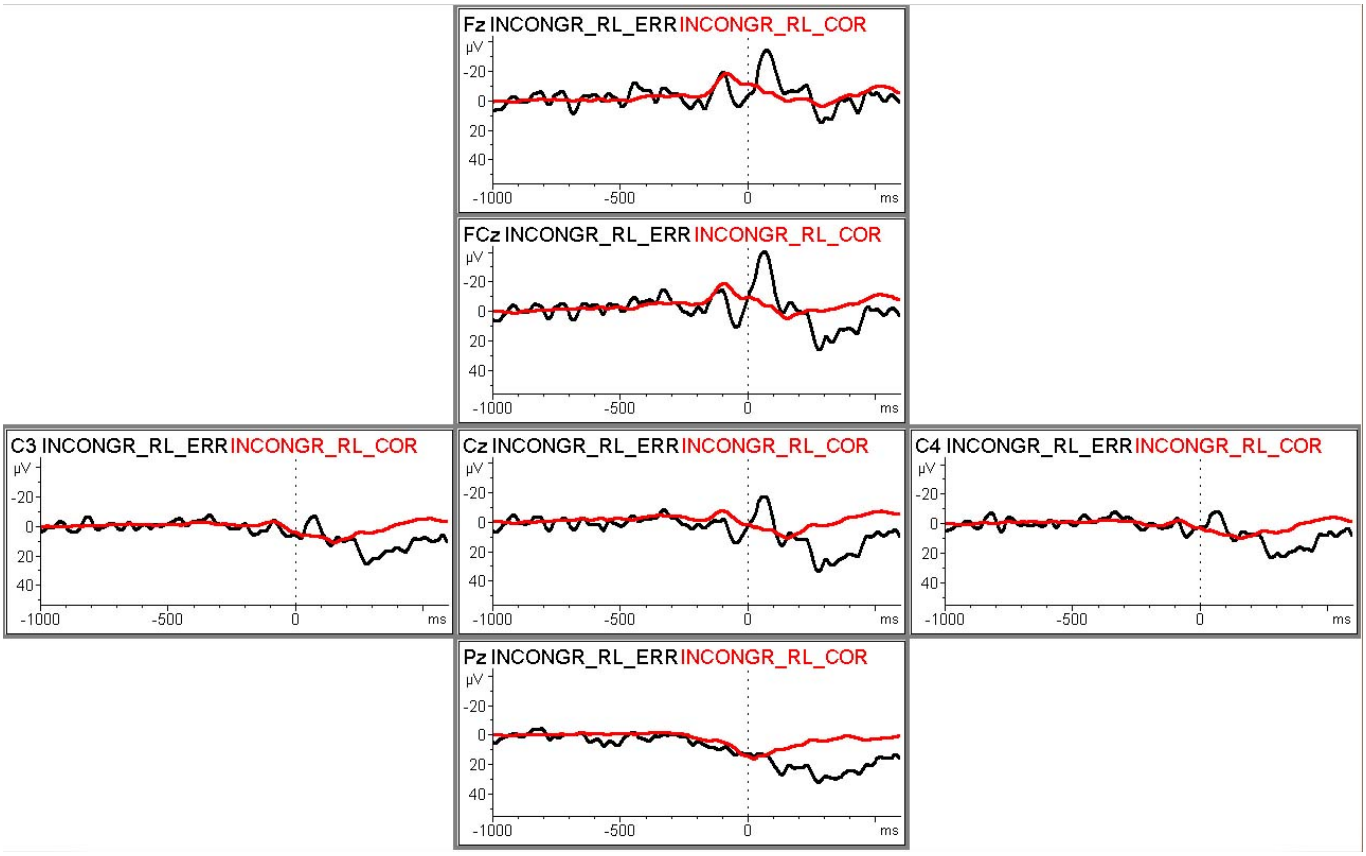


example if you have first distinguished between the first and second block of trials and later decide to bring all trials together again.
There are situations in which a weighted average is not appropriate, and non-weighted averaging is required. Imagine that you are interested in lateralization of ERP components involved in language (independent of motor processes). You decide to combine conditions with left-hand (80 trials) and right-hand responses (60 trials). In this case, both conditions should contribute equally to the grand average; otherwise the condition with left-hand responses will contribute a dominant right-ward lateralization of motor potentials, which incorrectly suggest right-ward lateralization of linguistic processes.



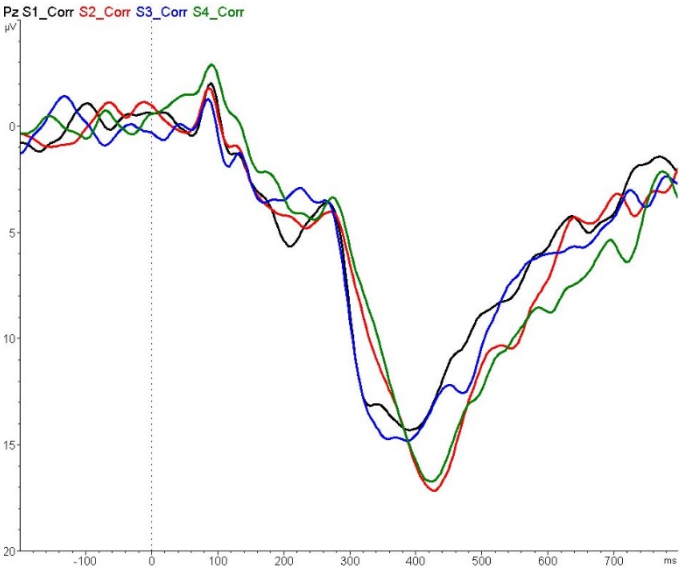
TASK Select *Transformations* → *Result Evaluation* → *Grand Average*. Calculate the non-weighted grand average for the two congruent conditions. Do the same for the two incongruent conditions. If you have data for multiple participants, each with the same history node name, combine the data for each participant by selecting the relevant files. The result will show up under the tap *Secondary* in the left window.
To optimize the view, right-click on the channel label and select *Settings of grid view*. Play with the settings, particularly with the tab *Overlay* and its options on line styles and labels in order to obtain a view that would be appropriate for a power point presentation. Journals usually do not allow for pixel-based images, and instead demand vector-based images, which can be created in Excel.

You can build your favourite display montage, that is the selection and layout of channels. Choose *Display* → *Create Montage* to select which channels to add to the montage, and in what column/row configuration. After saving this montage, choose *Display* → *Display Montage* → <*the name you gave it*>.



Step 11: Export

Eventually, you always have to derive a dependent variable from the data, as the input for statistical comparisons. In case of ERP components this is usually an amplitude or a latency. If a dependent variable is defined by a distinct peak, such as the P300, you first need to detect where the peak is, by using *Transformation* → *Result Evaluation* → *Peak Detection*. In the case of the P300, we know that there is a version of the P300 (P3b) that is a positive maximum over Pz between 300 and 500 ms following relevant stimulus presentation.



TASK Detect the P300 for the stimulus-locked ERPs of the four conditions. You will notice that the P300, which is interpreted as an index of the relative duration of stimulus categorization, is earlier in congruent than in incongruent conditions. This indicates that some of the confusion induced by the incongruent arrows is caused before the level of stimulus categorization. We have seen in the incorrect lateralization of the LRP that there is also some confusion at the motor level.

TASK There may also be a difference between congruent and incongruent conditions in the amplitude of the P300. The amplitude could be scored by the peak amplitude, or by a mean amplitude in a window surrounding the peak. Note that an area measure conveys the same information as the mean amplitude for calculations with the same window width. Now export the peak amplitude, peak latency, and mean amplitude in a window of choice around the peak for statistical analysis with *Area Information* or *Peak Information*, under *Export*. Check in the export folder that you have defined in your workspace whether you can read the data you produced into Excel.

TASK Look at the figure displaying four ERPs at Pz, where a low-pass filter at 20 Hz had been used. Imagine what would be the consequence for the peak latency, the peak amplitude, and the mean amplitude (area) if the filtering had been performed at 8 Hz. What does this imply for the reliability of peak latency, peak amplitude and mean amplitude?

TASK In addition to dependent variables, you may also want to export entire time series. These can then be processed in ways that BVA does not allow. For example, you can create better graphs in Excel, or apply more advanced data analyses in EEGLAB, a MatLab-based software package. To export data, choose *Export* → *Generic Data Export*. Choose *Multiplexed* as the data orientation: this will put adjacent samples in columns in the output table, while *Vectorized* would put it in rows. Apply this step to the average congruent and the incongruent ERPs. Read the data into Excel.
Insert a new column A with the time information: in cell A1 type

```
Time (ms)
```

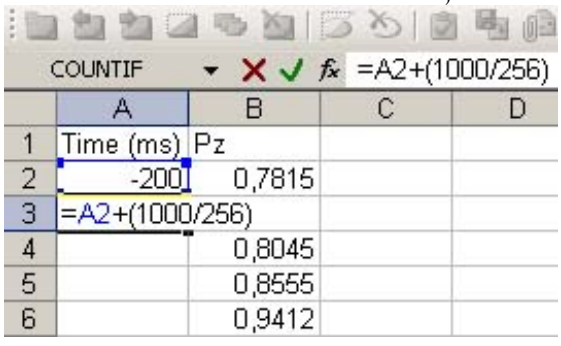
If the ERP started at -200 ms go to cell A2 and type

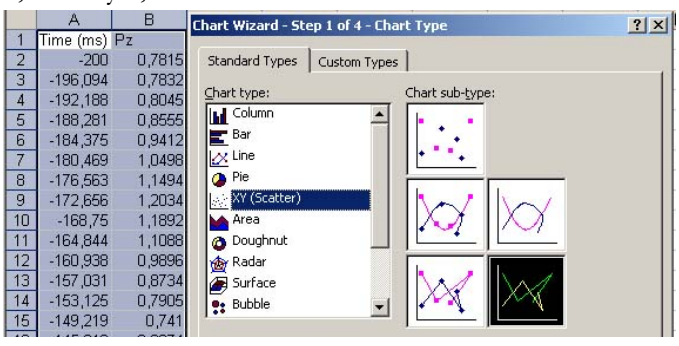
```
-200
```

In cell A3 type

```
=A2+(1000/256)
```

This last step is a calculation of the timing of the second sample: every sample obtained with a sample frequency of 256Hz comes (1000/256) ms after the previous sample. Now copy the same formula of A3 to the remainder of the column, and the timing of each new sample will be calculated relative to the previous. To copy the formula, you can also select cell A3:A257 and press Control-D, a key combination for Fill Down.
If all the data you want to graph are present in the table, select the column with the timing and the columns with ERP time series and let Excel create a graph from the scatterplot type. Once the graph is created, you can double click each element to adjust font size, line style, etcetera.



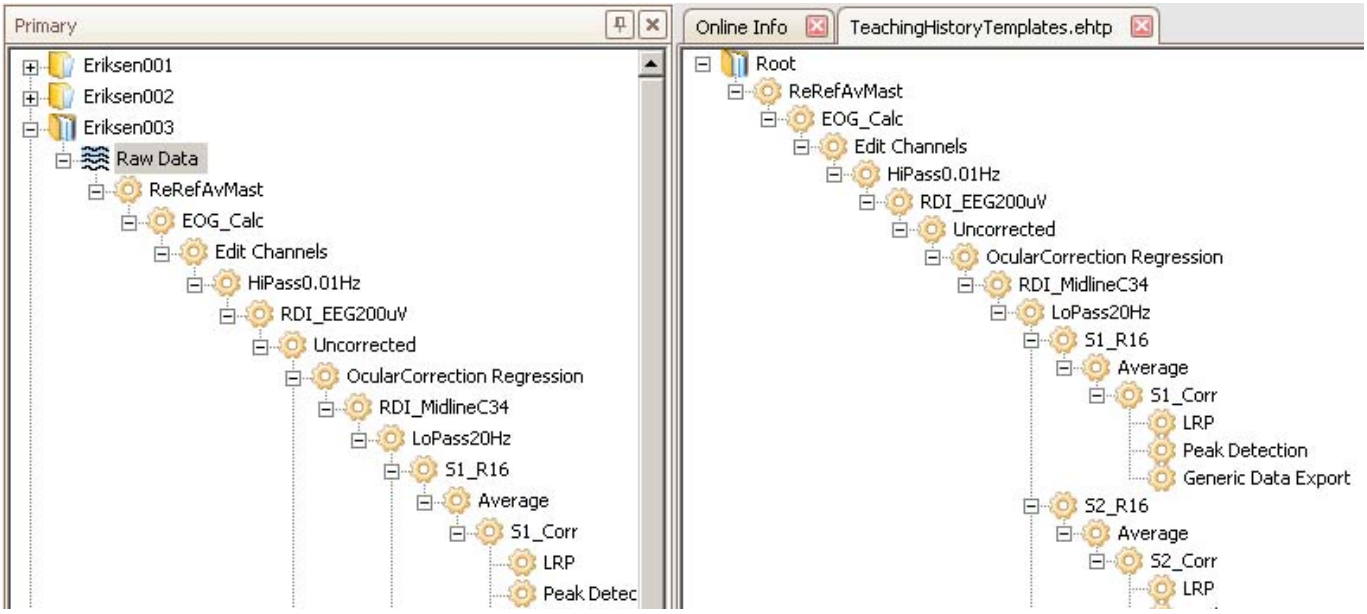


Step 12: History template

The analyses have now been done for one participant. To perform the same operations for the other participants, you can drag the entire history branch after raw data onto the raw data of another participant. However, the more elegant solution is to save the branch as a history template.

TASK Select *History Template* → *New*. A dummy history root will appear in the right window. Now drag the history branch you created for participant Eriksen003 onto the root icon in the right window panel. Then save the history template. Next select *History Template* → *Apply to History File(s)*. In this case the starting point to apply the history template is the root, that is the Raw Data node of each participant's file you select. In other occasions, you may want to apply a template only to a subset of the data, to a later processing level of

data that are already partially analyzed, or to grand averages. In those cases, you select Choose Data Set to define the starting point for the template.
Note that writing dependent variables to an export file is something that can best be done after the data of all participants have been preprocessed, so that relevant information can be extracted in one command applied to all included participants, resulting in one output file.



Closing words

You have practiced with a few representative ERP data analysis techniques. By applying these techniques adapted to the specifics of your paradigm, you can obtain the input data for statistical analyses in SPSS or other tools. Do not forget that the sequence of steps presented here involved many choices, which need to be made again with every new data set.

In addition to the techniques discussed here, BVA provides tools for EEG spectral analysis, as well as tools for the analysis of eye blink rate (EOG), muscle activity (EMG) and other time series analyses.

4 GUIDELINES FOR HYGIENIC TESTING

THE PREVENTION OF INFECTIONS DURING PSYCHOPHYSIOLOGICAL RESEARCH

PREFACE

Despite the small scale at which psychophysiological research is conducted within the Faculty of Social Sciences, this kind of research requires specific guidelines because of the many respects in which it differs from nonpsychophysiological research. The difference between physiological and nonphysiological research is often strikingly reflected by remarks of participating subjects, like "Gosh, this place looks just like a hospital..." Such an observation is correct in that psychophysiological research often involves procedures that have a medical appearance: attaching electrodes, sampling of blood or saliva, measuring blood pressure, etc.

Although it is true beyond doubt that chances of an infection during psychophysiological experiments are smaller than in medical settings, one can think of several arguments for placing more emphasis on a hygienic way of working.

1) Physiological research often involves action in which physical contact between the subject (S) and the experimenter (E) takes place.

2) Often, instruments are used that come into direct contact with the S (blunt or sharp needles, electrodes, etc.)

3) Research generally takes place at the same location for all Ss and is performed by the same E with the same instruments.

It readily follows from these three arguments that relatively harmless infections such as the flue or a cold can easily spread from S to E and from E to S. This, however, is in essence not different from other psychological research. The whole idea changes when you realize that:

4) Extraction of body fluids (blood, saliva, etc.) from the S can be performed on purpose, or can happen unintentionally (e.g., by damage to the skin).

Physiological research thus involves the possibility that participants (S as well as E) come into contact with body fluids of other people either through direct contact or through contact with instruments (of any kind). It should be clear that the transmission of dangerous infections such as Hepatitis-B or HIV (AIDS) is not precluded. Finally, it can be remarked that:

5) During many physiological experiments, chemicals (alcohol, collodium, acetone, disinfectants, etc.) are used, all of which are volatile and many of which are stupefying, toxic or corrosive.

6) All aspects of research (recruitment of Ss, preparations, the experiment itself, actions and conversations after the experiment) need to be structured in such a way that the mental well-being of the subject is not endangered.

In the following, a number of recommendations are made concerning the hygienical procedures in psychophysiological research. The recommendations are based on the guidelines for the health care of the Health council of the Min. of Public health and Environmental Hygiene (1977) and the Centres of Disease Control of the US. Dept. of Health and Human Services (1987). In addition, they closely resemble the recommendations in the 'SPR ad hoc committee on the prevention of disease transmission', titled: 'Guidelines for reducing the risk of disease transmission in the psychophysiology laboratory' (Putnam, Johnson & Roth, 1992). A compromise was aimed for between the stringent demands in the health care sector, on the one hand, and the everyday routine of psychophysiological research, on the other hand. The resulting guidelines compensate for lack of knowledge of hygienic conduct. If the guideline is to have any chance of success, a change in mentality among the people involved will have to take place. If one individual chooses to work under the assumption that "it won't come to difficulties", setting of guidelines is useless - despite the effort of a majority.

Original version: July 1995. Guido Band, Winni Hofman, Guido Valk, Janneke van der Velde, Oscar Winter, Amsterdam
Revised version: June 1998, December 1999, Guido Band, Amsterdam
Revised and updated for FSW Leiden: January 2003, Guido Band, Leiden.
Revised and English version: March 2006, Tristan Lavender and Guido Band, Leiden.
Revised English version: January 2009, Guido Band, Leiden.

GUIDELINES

1. General

1.1. The following guidelines apply, inside the laboratory, to:
- all units that are used for psychophysiological research,
- all people who participate in psychophysiological research,
- all people who enter units meant for psychophysiological research (researchers, students, technical personnel, cleaning personnel, etc.).

1.2. A number of guidelines serve as **recommendations** (marked **R**). Other guidelines serve as **prescriptions** (marked **P**). They have to be executed without exception. It is not permissible to obey some prescriptions and not to obey others. Note: other people use the same units and equipment!

1.3. Act on the assumption that *all* subjects and experimenters may be infected.

1.4. When research involves a reasonable chance of blood-to-blood contact, a hepatitis vaccination is recommended (**R**).

1.5. In supervisor-student situations, the final responsibility for safety and hygiene lies with the supervisor. He/she should care for the student's awareness of these guidelines and should, in addition, see to it that they are, in fact, being complied with.

1.6. The board of the Faculty of Social Sciences has the following responsibilities:
- Keeping the guidelines up to date
- Advising about central laboratory supplies
- Checking the practical attainability of the given recommendations
- Bringing the guidelines to the attention of students and personnel
- Supplying supplementary advice/information, also to individual researchers
- Supervising compliance to the guidelines

2. People

2.1. Postpone the experiment in case of a flue, heavy cold or other infection of the experimenter or the subject (see argument 1, 2 and 3) (**R**).

2.2. Pay attention to good general bodily and clothing hygiene while conducting research (**R**).

2.3. Always wear (disposable) gloves during physiological activities/preparations:
a) cleaning or abrading the subject's skin (**P**)
b) attaching electrodes to the clean/abraded skin (**P**)
c) removing electrodes (**P**)
d) all actions involving blood, sperm or vaginal juices (**P**)
e) working with disinfectants (**P**)
f) in case of clearly visible wounds or irritation of the skin of S or E (**P**).

- It is recommended to wear gloves with *all* physiological activities/preparations.
- Wearing gloves reduces but does not preclude totally the possibility of blood contact.
- As noted in argument 4, there is always a chance of blood-to-blood contact.
- Use fresh gloves for each subject and dispose of old gloves immediately after use.
- For taking off gloves, hold the edge and strip it off in one movement. The glove will automatically go inside-out. Never take off the glove by pulling the fingers, so as to avoid contact with any infected surface.
- Always wash your hands right after you take off the gloves.
- Blood contact can occur even when (red) blood is not visible.
- Check whether chemicals can damage gloves before using them.

2.4. The simplest act of disinfection is to wash your hands. This should happen (independent of the use of gloves):
- preceding physiological acts (**P**)
- following physiological acts (**P**)
- after going to the lavatory (**P**)
- after sneezing, coughing or wiping your nose (use paper tissues!) (**R**)
- after unforeseen contact with blood or other body fluids (**P**)
Gloves can be damaged. Therefore, still wash your hands before and after you wear them.

2.5. Rings should preferably not be worn during research (**R**)
They make it harder to wash the hands.
They increase the chance of damage to gloves.

2.6. Nails should be kept short during the period of research (**R**)
to make and keep hands clean.
to avoid damage to gloves.

3. Equipment

3.1. Prepare the experiment as much as possible, so that a minimum of lockers, knobs, bottles and jars have to be touched during the physiological preparation (**R**).

3.2. If possible, make use of disposable materials and throw them away (after one-time use) in the proper containers (**P**). Consult Kerwin Olfers (tel. 3803) for questions

a) Blue BI-waste container

Not part of the standard equipment. Necessary when disposable materials with increased risk of infection are used, such as materials containing blood or sperm. In case of doubt, contact Paul Barnhorn, amanuensis at the Gorlaeus laboratory, tel: 071-5274609

b) Needle container

Because needles have a high risk of infection, all used needles should be deposited in a special container. Needle containers can be ordered from the central storehouse of the Gorlaeus laboratory or from the LUMC. Store the containers in a safe place. Take full containers to the LUMC.

c) Chemicals container (usually green)

Used disinfectant is collected in a chemicals container. These containers are replaced from time to time. To have a container replaced outside this schedule, ask Kerwin Olfers (tel. 3830).

d) Garbage cans

For garbage that does not require separate disposal.

3.3. Durable tools should be disinfected (if necessary sterilized) per subject. (P) (see Appendix B)

If instruments have been or will come in contact with the body without reaching the blood stream and without trespassing mucous membranes, there is a legal prescription to reach 'high-level disinfection'. This implies that all viruses, fungi and bacteria should be killed. If the blood stream is reached or a mucous membrane is trespassed, the prescription is to also kill bacterial traces, so that full sterility is reached. For high-level disinfection, we make use of Incidin Plus, with the active ingredient glucoprotamine. This substance is dissolved in water. To-be-disinfected materials should be submerged in the solution during 15 minutes (based on a 2% solution).
- Durable needles cannot be disinfected with glucoprotamine. Use disposable needles instead.
- Collect to-be-disinfected materials on disposable paper. If you use a durable container to collect materials, this container should also be disinfected.
- Clean all material with lukewarm water and appropriate soap (e.g. Ivory) prior to disinfection. If you use brushes or reusable gloves, these should also be disinfected, or be used exclusively prior to disinfection.
- If full sterility is required, all steps that involve the use of water should be performed with distilled water.
- Never use reusable towels or other cloths in the disinfection procedure. These are excellent breeding grounds for bacteria.

3.4. If beds are used for the experiment:
- use cotton blankets. These can go into the laundry (per subject) at 90 ºC, just as all other bedclothes.
- use clean bedclothes for each new subject (including blankets!)
- supply pillows and mattresses with a washable cover and clean this per subject.

3.5. Towels, washing-gloves, etc. that are provided for the subject should be cleaned per subject.

4. Research units

4.1. For physiological preparations/actions (attaching electrodes, extracting blood/saliva, centrifuging, disinfecting, pipetting, etc.) make maximum use of the general research unit that is meant for that purpose (R). In this way, all possible infections are restricted to one place that can be suitably cleaned.

4.2 In case certain preparation need to be performed in places other than those named under 4.1 (e.g. the experimental setup), these should be restricted to the subject-side of the unit. The researcher should keep the laboratory-coordinator informed about the kind of actions that are performed in that room and the precautions that other users (technical personnel, colleagues, etc.) should take (P).

4.3. Units where physiological preparations are performed cannot be used for the preparation of meals or the consumption of edibles.(P).

4.4. Units where physiological preparations take place should be cleaned on a daily basis (e.g. with a bleach solution). (P) This constitutes:
- wiping the floor
- wiping furniture and other horizontal surfaces with a wet cloth

4.5. Units where physiological activities take place should be checked daily as to whether:
- the paper towel role needs to be changed.
- the soap dispenser needs a refill.
- waste containers need to be emptied.

4.6. Units where physiological preparations are performed should have sufficient air conditioning.

4.7. Smoking is forbidden in the psychophysiological laboratory. (P)
Research takes place in public locations, to which national prescriptions apply.
Research involves volatile and other inflammable substances.
Some subjects are non-smokers.

5. Actions

5.1. Invasive actions (such as extraction of blood) can only be performed by authorized personnel.

5.2. While working with organic substances or chemicals, the working surface should be covered with absorbent paper (**P**).

5.3. Each experimenter should inform him/herself **by consulting the manual** about the proper use, safety and cleaning prescriptions of equipment or substances prior to their use. (**P**)

5.4. While working with disinfectant, safety glasses should be used.

6. Laboratory facilities

6.1. Laboratories in the FSW (faculty of social sciences) building in which physiological actions are performed should possess a central room with the following specifications (P).
- All electrical wiring should be in secured electrical supply groups
- If chemical vapors are produced in the recording procedure, there should be a fume-cupboard with a separate ventilation channel
- If chemicals with risk for the eyes are used, there should be safety glasses present
- There should be a kitchen sink with taps for hot and cold water
- There should be a soap dispenser and a paper towel dispenser
- All dedicated waste containers (see 3.2) should be present
- There should be a first aid kit
- There should be a telephone with a list of emergency telephone numbers (see appendix D)
- The tap should have a flexible hose so that the eyes can be flushed in case of emergency
- The floor covering should be easy to clean
- If blood samples need to be stored, there should be a dedicated freezer
- If chemical substances need to be cooled there should be a dedicated refrigerator

6.2. Rooms in the FSW building in which physiological recordings take place should have the following characteristics (P):
- All electrical wiring should be in secured electrical supply groups
- There is a climate control system that is sufficient for the research requirements
- The recording chamber should be near a central room as described in 6.1
- The floor covering should be easy to clean

6.3. For research taking place outside the FSW building, there are no separate guidelines. Research taking place under the responsibility of FSW but outside the FSW building should follow the same rules. If this is not possible in the opted location, permission for the study should be obtained from the ethics committee of FSW. Research in the LUMC should comply with LUMC regulations.

LITERATURE

1. Advies inzake herziene richtlijnen ter preventie en bestrijding van ziekenhuisinfecties. (Advise concerning revised guidelines for prevention and abatement of hospital infections) Min. van Volksgezondheid en Milieuhygiene, 's-Gravenhage (1977).(Ministry of Public Health and Environmental Hygiene in The Hague)

2. Recommendations for the prevention of HIV transmission in health-care settings. Centers for Disease Control (1987). *Morbidity and Mortality Weekly Report*, *36*, *(suppl 2S)*, *Is-18s*.

3. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. Centres for Disease Control (1988). *Morbidity and Mortality Weekly Report*, *37*, *377-388*.

4. Extracts from “Electrode maintenance and infection control in the EEG laboratory” [A.M.Grass & E.R.Grass]. *In: Regan, D. Human brain electrophysiology*. New York: Elsevier (1989).

5. Information brochure CIDEX'". Johnson & Johnson Medical BV

6. Admission decree CIDEXPI. Ministerie van Welzijn. Volksgezondheid en Cultuur (1991). (Ministry of Public Health and Culture)

7. Putnam, L.E., Johnson, R.Jr. & Roth, W.T. (1992) *Guidelines for reducing the risk of disease*

transmission in the psychophysiology laboratory. (SPR ad hoc committee report).

8. Huth, S., Yo, Z-J., Yu, T., & Crawford, L. (2000). Factors to consider when selecting a high-level disinfectant. http://www.metrex.com/TechInfo/FactorsToConsider.pdf

APPENDIX A: WORKING SAFELY WITH CHEMICAL SUBSTANCES

The term ‘Chemical substances’ applies to all substances that are used during physiological research: alcohol, acetone, electrode paste, disinfectant, collodion, etc.

1. Ensure that labels on all bottles, jars, cups, etc. are clear and accurate.

2. Always check the label of a substance before use, mainly to make sure that the right substance is being used, but also find out whether any precautions have to be taken (when in doubt, read the manual)

3. Bottles, jars, cups and boxes should be kept closed as much as possible, for both economical and health care reasons, and to avoid vapors in the eyes (which is most unwelcome, e.g. in visual tasks).

4. Never smell bottles directly, but rather wave some of the odor towards the nose.

5. Always care for good ventilation.

6. Never use a bottle or jar for substances other than those indicated on the label.

7. When a substance touches the skin immediately flush it off with lots of water for safety.

8. When a substance comes into eyes, immediately flush it out with lots of water. Never rub! Bend the plastic hose on the faucet upward and use it as a shower for the eyes. Always use cold water and arrange the flow with the free hand. Flush each eye in turn and pause after some time to avoid hypothermia of the eye. Notify a doctor if necessary (LUMC First Aid: (0)5262025 / (0)5262320)

9. In case of poisoning, contact:
   - LUMC First Aid: (0)5262025 / (0)5262320
   - National intoxication centre: (0)030-2748888

10. Never smoke in rooms where chemical substances are used and be careful with open fire. Most substances that are used in psychophysiological research are (highly) inflammable.

11. Never flush chemicals down the drain. Instead, store them in the particular refuse container for the particular (class of) substances (see 3.2). The color of the container indicates which class of substances can safely be disposed in it. Information about the color coding can be asked for at the university Arbo- and Environmental services. To order a container, contact Jan de Koning (room 0A13, tel 3615) or Paul Barnhorn (Gorlaeus, room 46, tel. 4609). Store the container in a safe place. To have full containers replaced outside the regular schedule, ask Kerwin Olfers (tel. 3803)

12. Always transport chemicals in a well closed container, provided with a clear label.

APPENDIX B: USER MANUAL FOR INCIDIN PLUS

1. Always wear safety glasses, safety gloves and an apron or lab-coat when using Incidin PLUS. Incidin PLUS is corrosive and can damage skin, eyes and clothes. (H332, H314, H400, R22, R20, R34, R50)

2. Dilute Incidin PLUS to 2% (1 part Incidin PLUS with 49 parts cold water) to prepare a disinfection liquid. Preparing a total of 500 ml daily usually suffices.

4. Replace the disinfection liquid in the round container every day. Try to keep the container with disinfection liquid closed.

3. Every new stock dilution of Incidin PLUS is stored in the black jerry can. Use 80 ml of undiluted Incidin and 4 liters of cold water. A new dilution can be used up to 21 days after preparation. Write the last date that the new solution can be used on the label.

5. Make sure that all materials in need of disinfection are being rinsed first to remove the gel.

6. All parts that might have been in contact with blood, sperm, excrement, or other body fluids need to be submerged in the disinfection liquid for 1 hour. This does not apply to EEG electrodes (see point 8).

7. Incidin PLUS is suitable for 'Electrocaps', syringes and electrodes. For all other materials please make sure that Incidin PLUS is a suitable disinfectant.

8. Do not leave any material submerged in the disinfection liquid longer than appropriate as this can corrode the materials. When diluted to 2%, the incubation time is 15 minutes.

9. Diluted 2% solutions can be disposed of through the normal water drain system (i.e. sink). Make sure to rinse with water thoroughly afterwards.

10. Rinse all materials that have been disinfected with Incidin PLUS thoroughly with streaming water before they can make contact with the skin.

11. This disinfection method is appropriate against infection with HIV and Hepatitis-B.

12. Make sure that Incidin PLUS, the diluted disinfection liquid and the disinfection bath are always stored in a closed container.

13. The efficacious ingredient in Incidin PLUS is **GLUCOPROTAMINE** (25% - 30%)

14. Incidin PLUS is in accordance with DGHM and DVV

17. Treatment of electrocaps after each measurement:
- Remove the Velcro straps (sticky cloth). These do not need to be disinfected.
- Remove the electrodes.
- First remove all remaining gel thoroughly with cotton tips and a soft toothbrush under running water. Ivory soap is appropriate in this stage.
- Use Incidin diluted liquid for 15 minutes to disinfect the cap, the electrodes, and syringe.
- Always thoroughly rinse with water afterwards to prevent skin irritation.
- When necessary use the hair-dryer to speed-dry the materials. Make sure the temperature of the materials does not exceed 40 degrees C.

Risks: serious burn wounds and eye-damage (H314), harmful when inhaled (H332), very toxic to organisms in water (H400).

First aid measures for exposure to Incidin:

- In case of any complaints or symptom's consult a doctor.
- If fumes are inhaled, seek fresh air. In case of lingering complaint/symptoms, consult a doctor.
- In case of direct contact with skin: immediately rinse with water (15 minutes). Take off contaminated clothing. Consult a doctor. (P303, P361, P353)
- In case of contact with eyes: immediately rinse excessively with water (at least 15 minutes), including under the eyelids. Remove eye contacts if possible. Use the eye-washer on the wall (E). Consult a doctor and/or intoxication centre immediately. (P305, P351, P338)
- If swallowed: rinse mouth with water (NOT milk), do NOT induce vomiting, medical treatment needed immediately. (P310)

National intoxication center **(0) 030-2748888 and 3701**
First Aid via reception FSW **3701**

For external numbers called from a university phone: start with 0
For local numbers called with a cell phone: start with 071-527….

Import of Incidin Plus for The Netherlands:
Ecolab BV, Iepenhoeve 7a+7b, 3438 MR, MN Nieuwegein, tel: 030-6082222
NLCustomerServices@Ecolab.com

Ecolab emergency phone number: +31852085762

APPENDIX C: WASHING AND CARING FOR YOUR HANDS

1. Preferably, use warm water.

2. Bring wet hands in proper contact with soap from the dispenser.

3. Rinse hands with lots of water.

4. Dry wet hands with paper disposable towels.
   It is important to have dry hands, for they contain less bacteria.

5. Close the tap with the same paper towel.

6. Regularly treat hands with cream.
   By washing hands regularly, chaps may arise. In these chaps, micro-organisms can settle that are hard to wash out.

7. Keep nails clean and short. If necessary, use a nail brush in addition to washing hands.

Note Normal liquid soap can be used, preferably “Unicura”. Use of additional disinfectants for hands is not necessary.

APPENDIX D: IMPORTANT TELEPHONE NUMBERS

Alarm in case of life-threatening situations (our local FSW first aid will help and call other services)	3701
Alarm, other calamities FSW	3701
National intoxication center	(0) 030-2748888 and 3701
First Aid via reception FSW	3701
In case of prick incidents	(0) 071-5263643
In case of prick incidents outside office hours	(0) 071-5299418
Reception FSW	3600
Disposal of chemical garbage	3198
Internal Arbo- and Environmental Services UL	3198
Safety and Environment UL	3198
Ecolab (import of Incidin plus)	(0) 030-6082222
SOLO (research and lab technicians)	5693
Roel van Dooren (EEG lab coordinator)	6869

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