Baby EEG
Protocol for testing and analysis

Contents

1. Introduction

This protocol was written for researchers, research assistants and students who are planning to run EEG experiments with infants in the UiL OTS Babylab. It is an extensive document which includes both the *general* protocol for testing, as well as a comprehensive step-by-step guide to analysing your EEG data.
NOTE: The step-by-step analysis does not apply for all experiments! Researchers must adapt and modify the analysis when needed. This protocol does *not* focus on how to design an EEG experiment.

Throughout the entire protocol, we use an oddball paradigm experiment as an example. The aim of this research project was to investigate whether 4-month-olds and 12-month-olds could discriminate between differences in pitch in different modalities. The two modalities that were investigated were music and lexical tones (in Standard Mandarin Chinese). Also, the ability to discriminate between different native vowels was investigated. For this, we looked at the 'Mismatch Negativity' component (referred to as 'MMN' in this document), which exhibits as an amplitude difference between the ERP to a deviant stimulus (the 'oddball') and the ERP to a standard stimulus. Therefore, this analysis overview is most suitable for researchers who want to compare ERPs following different types of stimuli. Please note that different research questions may need a different approach to the analysis.

A reference is made to ready-to-use MATLAB scripts several times throughout the analysis section. If you wish to use one of these scripts, please contact Ao Chen (a.chen@uu.nl). Note that you still need to adapt these scripts to make them fit for your own experiment and stimuli.

Should you have any questions or suggestions, please contact us by sending an email to:
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2. A preliminary remark on how to treat electrodes with care

Before you start your first testing day, make sure you have read and understood this section carefully. Should you have any questions, please do not hesitate to ask!

Electrodes are very fragile and an entire electrode bundle may become completely useless once only one of the electrodes dies. The lifespan of electrodes can be shortened drastically when handled without care. Therefore, it is important to keep a few things in mind when working with electrodes.

- **NEVER let the electrodes touch metal.** Always remove all of your jewellery before preparing the cap and cleaning the electrodes. Also, make sure your participant isn't wearing any metal hairclips.
- When preparing the cap, let the bundles hang around your neck to prevent the ends from touching the floor.
- When cleaning the electrodes, make sure that the water is lukewarm. The gel will dissolve more easily when the water isn't too cold, but too hot water will cause the electrodes to break faster.
- Never let the electrodes lay in the water for too long; when you put them into the water, clean and (gently) dry them immediately.
- NEVER let the other end of the bundle (which connects to the amplifier) touch water.
- NEVER let the electrodes sit under running water.
- Always clean the electrodes as soon as possible, before the gel gets the chance to dry.
- Be sure that no gel is left behind on the electrodes.
- Always clean the electrodes gently, using a soft brush. Don't push or rub too hard.

3. The standard testing procedure

This section offers you a detailed description of how to run an EEG experiment with infants. In the lab, we make use of BioSemi equipment, and all experiments should be written in Presentation. Before you can start testing, make sure you have:
- Approval of the ethics committee
- Designed your experiment. **NOTE:** Make sure that all triggers are sent at the precisely right time from Presentation to Actiview!
- Scheduled your participants
- Reserved the EEG lab. There are two EEG labs at Janskerkhof 13: room K.13 and room K.02.

Note that a brief checklist of the entire procedure can be found here.

3.1 The Babylab waiting area

At the beginning of your testing day, make sure you open up the waiting area (room K.01) and turn on the light. Check if there is still water in the watering can, the kettle and the Nespresso machine, and if the plant needs watering. Also make sure that the room looks clean and tidy. You can open the waiting area and the EEG lab with the same key.
General instructions for correct use of the waiting area can be found here. **NOTE**: this document is password protected, please contact Desiree Capel for the login details.

3.2 The control room

The control room is where the test leader will be during the experiment.

3.2.1 The set-up (K.13)

IMAGE 3.1

The control room in K.13 contains the following equipment:
- Two computers ('EEG1' for recording and 'EEG2' for presenting; circled in yellow in IMAGE 3.1). You can find labelled stickers indicating which is which on the computers and screens.
- Two screens for the left computer (numbered '1' and '2' in IMAGE 3.1)
- Two screens for the right computer (numbered '3' and '4' in IMAGE 3.1)
- One webcam monitor (numbered '5' in IMAGE 3.1)
- One Tangent amplifier (circled in green in IMAGE 3.1)
- One audio switch box (circled in blue in IMAGE 3.1; enlarged in IMAGE 3.3)
- One video switch box (circled in red in IMAGE 3.1; enlarged in IMAGE 3.3)
- Two remote controls (IMAGE 3.2)

3.2.1.1 Preparation
- Turn on both computers and all screens.

IMAGE 3.2

- Turn on the webcam monitor ('5' in IMAGE 3.1) by pressing the power button on the Philips remote control (circled in purple in IMAGE 3.2, the power button is circled in blue).
- Use the remote control circled in yellow in IMAGE 3.2 to turn on the camera in the test room. Set it to the right position by using the arrow buttons circled in red. Watch the webcam monitor from the door while adjusting the webcam position to immediately check whether the position is right.
- Turn on the Tangent amplifier by pressing the power button on the left hand side.

IMAGE 3.3

- Set the audio switch box to 'T' (Tangent amplifier; circled in **green** in IMAGE   3 .3).
- Set the video switch box to the correct screen by clicking either one of the two buttons circled in **purple** in IMAGE   3 .3. The left button is to select screen number 1 (in IMAGE   3 .1), the right button is to select screen number 3 (IMAGE   3 .1). The green light, circled in **yellow** in IMAGE   3 .3, indicates which screen is selected. The selected screen will be on the screen in the test room, and will be visible for the participant.
    - If the child needs to watch a DVD during the experiment, insert the DVD into the computer on the left hand side. Select screen number 1 using the video switch.
    - If the experiment contains visual stimuli in Presentation, select screen number 3. If the child needs to watch a DVD with sound, also select screen number 3 and insert the DVD into the computer on the right hand side.

3.2.2   The set-up (K.02)

IMAGE 3.4

The control room in K.02 contains the following equipment:
- Two computers (one for presenting the stimuli, circled in **red** in IMAGE   3 .4, and one for recording, circled in **green** in IMAGE   3 .4). You can find labelled stickers indicating which is which on the computers and screens.
- Headphones for masking the researcher (circled in **yellow** in IMAGE   3 .4).
- A microphone for talking to the parent in the test room (circled in **blue** in IMAGE   3 .4).
- A kitchen block, containing electrode bundles, electrode gel, caps and other EEG materials, and material for cleaning all equipment.

3.2.2.1 Preparation
- Turn on both computers. Make sure they start up in the right mode (when using Zep, start Kubuntu/when using Presentation, start Windows).
- On the Recording PC: start the program OBS Studio. Check the position of the webcam.
- If the child needs to watch a DVD at some part during the experiment: insert it into the Presentation PC. Open the DVD in VLC Media Player and drag it to the correct screen in the test room.

3.3 The test room

The test room is where the participant will be during the entire experiment.

3.3.1   The set-up (K.13)

The test room in K.13 (IMAGE 3.5) contains the following equipment:
-   A high chair for children
-   A chair for adults
-   A table, covered with soft material to mute the sound of toys that are smashed onto the table
-   One monitor for the presentation of visual stimuli
-   Two speakers for the presentation of auditory stimuli
-   A webcam (circled in **blue** in IMAGE 3.5)
-   A plastic cabinet (circled in **green** in IMAGE 3.5), containing the BioSemi amplifier, head caps, electrode stickers, cotton pads and alcohol
-   A kitchen block, containing material for cleaning all equipment, electrode bundles (circled in **red** in IMAGE 3.5), electrode gel and spare materials.
-   An air humidifier (circled in **yellow** in IMAGE 3.5, enlarged in IMAGE 3.10)
-   A decibel meter (next to the **orange** arrow in IMAGE 3.5, enlarged in IMAGE 3.8)

IMAGE 3.5

3.3.2   The set-up (K.02)

The test room in K.02 (image 3.6) contains the following equipment:
-   A high chair for children
-   A chair for adults
-   A table
-   Three monitors for the presentation of visual stimuli
-   Two speakers for the presentation of auditory stimuli
-   A webcam (circled in **red** in image 3.6)
-   A pair of headphones for masking the parent (circled in **green** in image 3.6)
-   The BioSemi amplifier (circled in **blue** in image 3.6)
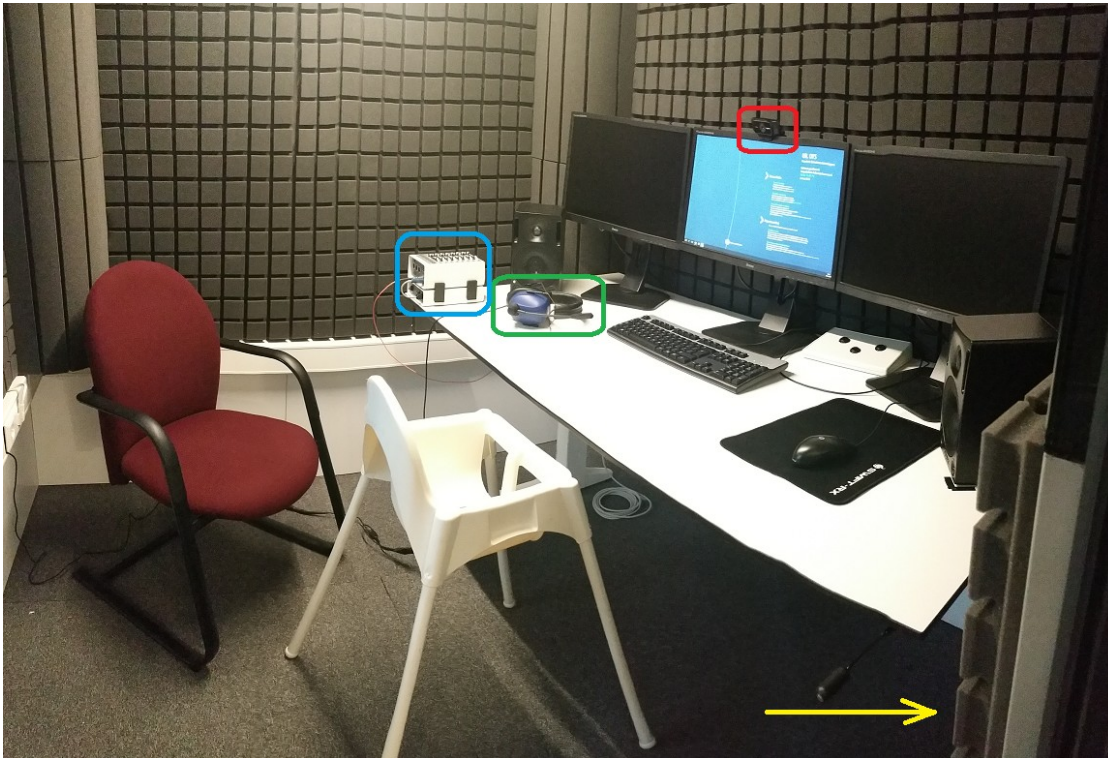-   A ventilator (indicated with the **yellow** arrow in image 3.6)

IMAGE 3.6

3.3.3   General preparation

From the plastic cabinet next to the table (K.13) or the kitchen block in the control room (K.02), get out the following items:
- Electrode stickers ('**1**' in IMAGE 3 .7)
- Cotton pads ('**2**')
- Alcohol ('**3**')
- Electrode gel ('**4**')
- A syringe ('**5**')

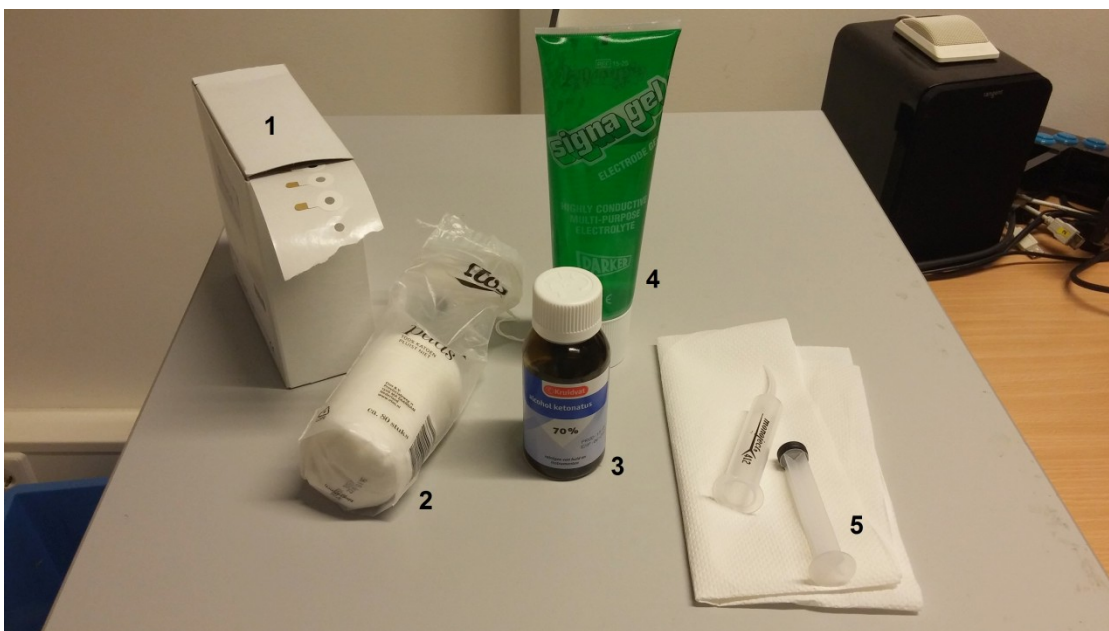Place all items on top of the cabinet/table in the test room.

IMAGE 3.7

Next, consider whether you want the child to sit in the baby chair, or whether you want it to be on the parent's lap, for instance if it's too young to sit down independently. Place the chairs according to your decision. Make sure that the child is seated right in front of the screen, in the middle of the two speakers. When testing in K.02, turn on the two speakers and the display(s) that you're going to use by pushing the on/off-buttons. Keep the other displays turned off. When testing in K.13, check if the table is covered with the towel.

Once everything is prepared, start the experiment on the Presentation (K.02) or EEG2 (K.13) computer and check the sound level. There is a decibel meter on the shelf in the test room in K.13. Hold the tip of the decibel meter (circled in **yellow** in IMAGE 3 .8) at the place where the child's ear will be during the experiment, and turn on the device using the switch circled in **red** in IMAGE 3 .8. You will see a number starting around 110 on the screen of the device, which will drop slowly until it keeps on circling around a certain number. This is the current sound level. The desirable sound level is approximately 65 decibel. If it is too high or too low, change the sound level in your experiment. For more information on how to use the decibel meter, please visit the UiL OTS labs website.
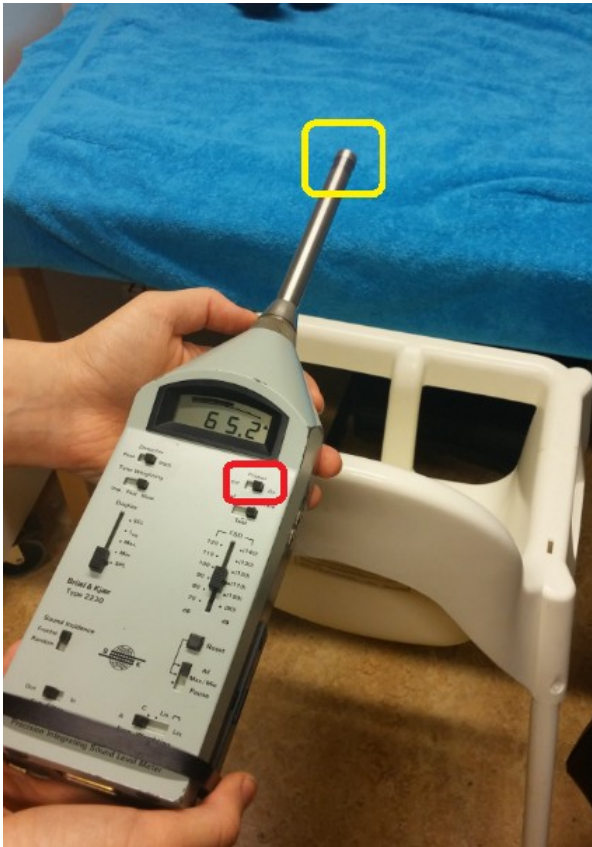

IMAGE 3.8

3.3.3.1 Changing the sound level in Presentation

If, after checking the sound level with the decibel meter, you find that the sound level is too high or too low, there are two ways to change the volume when using Presentation. Depending on your settings in Presentation, you could do it in either one the following manners.
One way to change the sound level, is to change the volume of the Presentation/EEG2 computer in the lower right corner of your screen (as you usually change the volume). This will work, as long as you haven't installed a fixed sound level in your Presentation experiment. If you notice that the volume automatically jumps back to the original sound level after restarting the experiment, you need to adjust the volume in Presentation itself.

To do this, open up your experiment and go to the tab 'Settings' (circled in **green** in IMAGE 3.9). On the left hand side, go to 'Audio'. You will see the audio settings appear. Underneath 'Volume', you will probably see that the checkbox in front of 'Set Device and Application Volume Device Volume' is checked (circled in **blue** in IMAGE 3.9). Adjust the sound level by typing in a higher or lower number between 0.0 and 1.0 in the square circled in **red** and click 'Apply now'. Restart the experiment and check the sound level again. You should now see that the volume has changed.
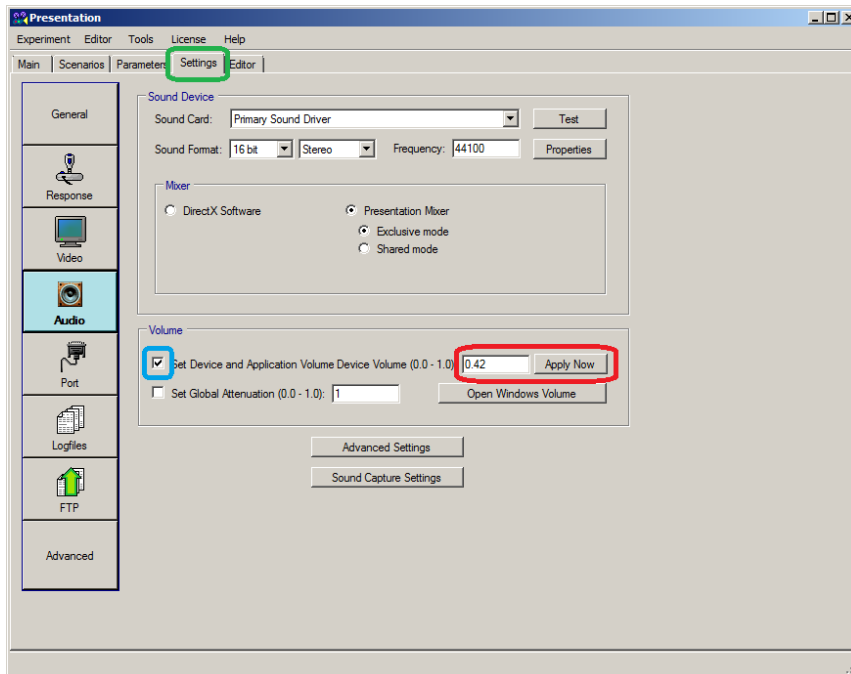

IMAGE 3.9

3.3.4   Air circulation

3.3.4.1 Room K.13

When testing in K.13, you'll find an air humidifier next to the kitchen block (IMAGE 3.10). This device secures a pleasant air humidity inside the test room, to make sure that participants don't blink their eyes too much.

Before you start testing, remove the entire grey part from the white part by lifting it up. Fill up the white box with water until the water level reaches 'max' (you can check the water level by looking at the indicator circled in **red** in IMAGE 3.10). Put the grey part back on again, plug the humidifier in and press 'power' (circled in **green**).
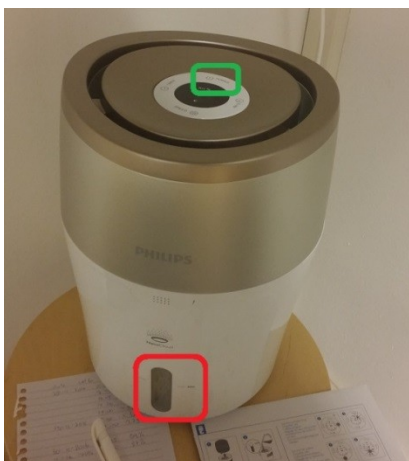

IMAGE 3.10

3.3.4.2 Room K.02

Before testing in K.02, make sure you refresh the air with help of the ventilator. Underneath the table, you will find a small ventilator attached to the wall (image 3.11). Press the on-button (circled in **red** in image 3.11) to start the air flow. Turn it off when your experiment starts to reduce the noise.

IMAGE 3.11

3.4 Receiving the participant and preparing the cap

After you have prepared the control and test room, it is time to receive the parent and the baby in the waiting area. In the waiting area, shortly explain what is going to happen and let them sign the informed consent form. Take them to the EEG lab. Once they are seated, you can start the preparation of the cap.

3.4.1   Apply gel on the baby's wrist

Before doing anything at all, apply a little bit of gel on the baby's wrist using the syringe. Tell the parent that you're doing this to make sure there won't be an allergic reaction to the gel. You can show them that the syringe won't hurt the child, by tapping the tip of the syringe onto your own palm. Tell the parent that the chances of getting an allergic reaction are extremely small, due to the fact that the gel is basically only a combination of water and salt.

3.4.2   Explain the procedure

While giving the baby's skin a chance to react to the gel, explain the procedure to the parent. Mention the following things:
- What the experiment is about
- A brief explanation of the EEG procedure
- The duration of the actual experiment
- What the child is and isn't allowed to do (e.g. play with the toys)
- Tell the parent **not to speak** during the experiment
- Also tell the parent to **try to prevent the baby from pulling the cap or the electrodes** as good as they can.
- Ask the parent to **switch their phone to airplane mode**, otherwise its signals will interfere with the EEG.
- Let them know that they are being watched through the webcam, and whether there will be a video recording of the experiment.

- If they want to stop the experiment, for whatever reason, they can wave to the camera.
- If the baby becomes too restless, you can stop at any time.
- They can wash their baby's head at the end of the experiment.

After the explanation, check the baby's wrist once more. If the skin hasn't reacted to the gel, proceed with the preparation. If there seems to be an allergic reaction, quit the experiment.

3.4.3   Applying the mastoid electrodes

Before placing the cap on the child's head, you have to apply the 'mastoid electrodes'. These electrodes are also indicated by the names 'EXG1' and 'EXG2' (or EX1 and EX2; see IMAGE 3.12). These are two separate electrodes, and are not part of a larger bundle.

The mastoid electrodes have to be placed behind the ears of the infant, on the 'mastoid'. This is the most bony area behind the ear, where presumably no (or few) signals from the brain are received. In an EEG experiment, all electrodes on the left hand side are usually indicated by an odd number, and all electrodes on the right hand side are usually indicated by an even number. This means that **EXG1** has to be placed behind the **left** ear, and **EXG2** has to be placed behind the **right** ear of the child.
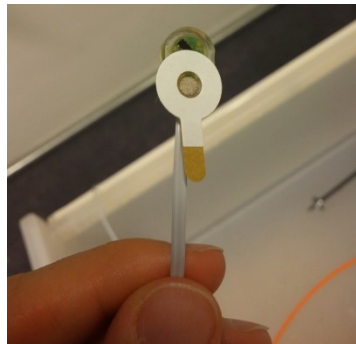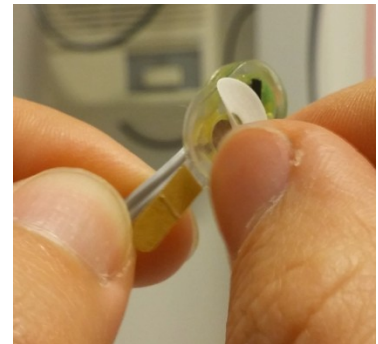

IMAGE 3.12

IMAGE 3.13

IMAGE 3.14

When applying the mastoid electrodes:
- First, put a little bit of alcohol onto a cotton pad (numbered '**2**' and '**3**' in IMAGE 3.7).
- Degrease the area behind the child's ears by rubbing it gently with the cotton pad with alcohol.
- Take one of the electrode stickers (numbered '**1**' in IMAGE 3.7).
- Stick it onto the electrode (as in IMAGE 3.13).
- Remove the white protection layer (as in IMAGE 3.14).
- Add a little drop of gel in the circle in the middle (as in IMAGE 3.15). **Do not forget to do this!** It will hurt the child if you forget to add gel. However, also make sure you don't add too much gel.
- Try to feel with your fingers where the mastoid is located and stick the electrode onto the mastoid (IMAGE 3.16).
- Insert the other end of the electrode into the BioSemi amplifier (circled in blue in IMAGE 3.20
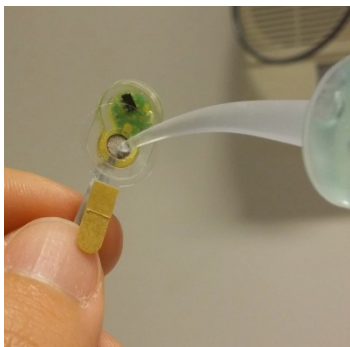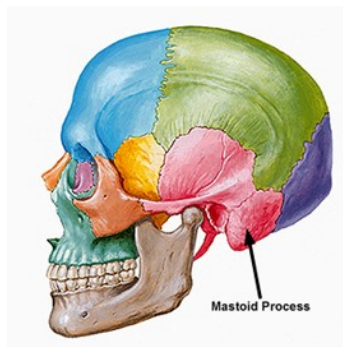- Watch out that the child doesn't pull off the electrode right away!

IMAGE 3.15

IMAGE 3.16

Mastoid Process

NOTE: You can also prepare the cap first, and try to squeeze the mastoid electrodes underneath the cap afterwards to make sure the child cannot pull them off right away. A disadvantage of this method is that it's harder to find the right location of the mastoids when the cap is already o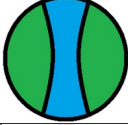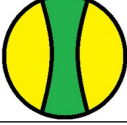n the head. The importance of the mastoid electrodes also depends on whether you will use the linked left and right mastoid as reference for offline processing. Average reference may work fine. If you use the average as a reference, you don't necessarily need the electrodes on the left and right mastoid (for more information about referencing your data, read this section).

3.4.4   Preparing the cap

After you have adjusted the mastoid electrodes, take out a cap in a size that fits the baby's head circumference. There are several cap sizes available, that can be distinguished by colour.
The available cap sizes in the Babylab are the following:

Colour		Head circumference
Brown		34-38 cm
Pink/brown		36-40 cm
Light blue/pink		40-44 cm
Light blue		42-46 cm
Green/light blue		44-48 cm
Green		46-50 cm
Yellow/green		48-52 cm

Which size you need, can primarily be based on the age of the infant. If you have an indication of the average head circumference of the age group you're testing, you won't have to measure every infant's head beforehand. There are plenty of online guides which predict the infant's head size, for instance this one, which also allows you to make a prediction based on gender. It is still recommended to measure the head though, because not every child is the same. To measure the head of the child you can use the yellow measuring tape from one of the plastic cabinets (K.13) or from the kitchen cabinet (K.02).

After you've decided which cap to use, try to put on the cap in one go. Do it fast and try to avoid having to adjust the position of the cap too much, because it might upset the baby. Make sure that Cz is right on top of the head, in the exact middle between the ears and between the tip of the nose

and the back of the head. The Fp-electrodes should be in the front; the O-electrodes should be at the back of the head. Take the ears through the little holes (only if it is possible) and fasten the cap with the Velcro.

Next, fill up the syringe with gel and put a little bit of gel into each electrode (IMAGE 3.17). Make sure the gel touches the scalp, so that a good connection can be made, but be gentle with the baby's head.

IMAGE 3.17

IMAGE 3.18

Take one of the 32-electrode bundles (indicated with only a letter and no numbers at the beginning of the strap). Insert all electrodes into the corresponding holes in the cap, indicated by letters, numbers and colours (IMAGE 3.18). Start with the electrodes closest to the BioSemi amplifier, and end with the ones furthest away to avoid the electrodes from getting tangled. When looking at the electrode bundle, you will see that one side has a red edge (indicated with an arrow in IMAGE 3.19). By beginning with this side, you will start with the electrodes on the left hand side (odd numbers). By beginning with the other side of the bundle, you will start with the electrodes on the right hand side of the cap (even numbers).

After you've inserted all electrodes into the cap, insert the other edge of the bundle into 'A1' on the BioSemi amplifier (circled in green in IMAGE 3.20). Press until the plastic white 'wings' on either side come up. Turn on the amplifier by pressing the button circled in red in IMAGE 3.20. A blue light, as circled in yellow in IMAGE 3.20, should start to burn continuously. If the light flickers, this means that the electrodes CMS and DRL aren't attached properly and may

IMAGE 3.19

need more gel. Keep on adding more gel and pressing the cap to the head until the light stops flickering.
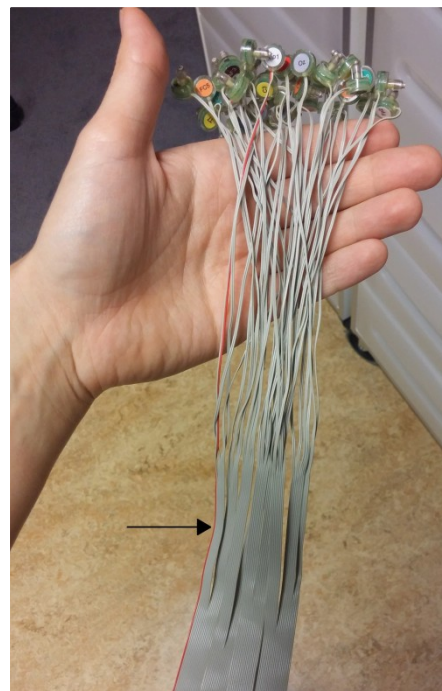
NOTE: Good signal from CMS and DRL is very important. If these two are not well connected, your signal will be messy and not usable!
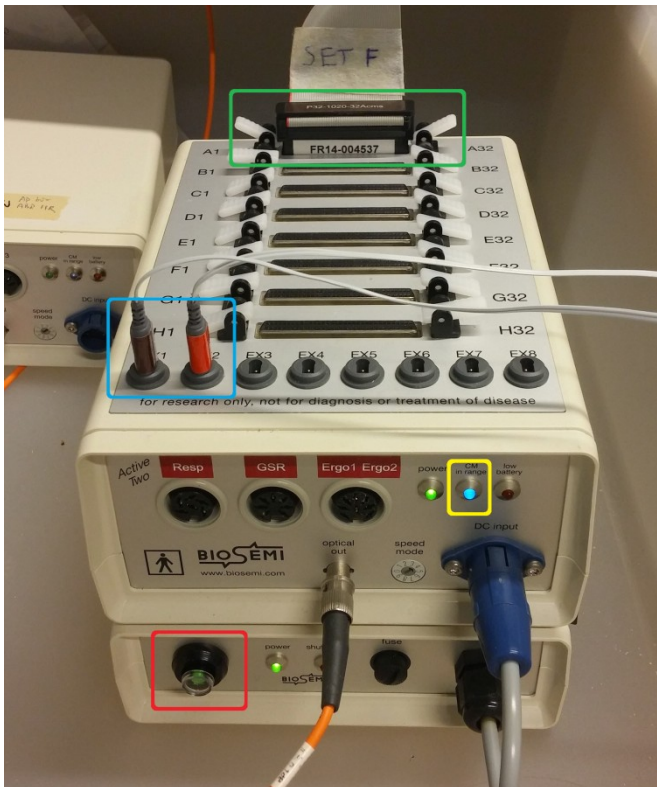
IMAGE 3.20

3.4.5 Preparing the cap without participant

When testing an infant, there are two ways to prepare the cap: directly on the infant's head (as described above), or by preparing it **before the participant arrives**. Both methods have several advantages and disadvantages. Preparing directly on the participant's head allows for using a better cap size, because you can choose the cap size after you've seen the baby's head, but will require a lot of patience and cooperation from the infant as you adjust the electrodes one by one on its head. Preparing before the appointment is a faster, but also more sensitive option as you won't know the baby's head size or amount of hair before the appointment. This may result in having to prepare directly on the infant's head after all, if there are too many errors to start the experiment. Also, note that after a certain age, the signal will become too noisy when preparing the cap before the appointment, due to the development of a thicker skin. Read this section to see which method works best for which age group.

If you decide to prepare the cap before the participant arrives, you can use a small ball (e.g. from Action) on a **non-metal** object (such as a porcelain teapot) as a substitute for the infant's head. Place the cap **inside out** on the ball (as in IMAGE 3 .21). Insert gel into all holes using the syringe (as in IMAGE 3 .22). Fill the holes all the way to the edge, to make sure a decent connection can be made.

Next, turn the cap inside out again (IMAGE 3 .23). Take out the 32-electrode bundle and insert all electrodes in the corresponding holes (IMAGE 3 .24). Start with the side that will be closest to the BioSemi amplifier.

IMAGE 3.21

IMAGE 3.22

IMAGE 3.23

IMAGE 3.24

Turn the cap inside out again, and add a little more gel to all electrodes in the same way as in IMAGE 3 .22. Leave the cap like this until the participant has arrived.

After you have received the parent and the baby, follow the first part of the procedure as described above (apply the gel on the wrist, explain the procedure, apply the mastoid electrodes). After you're finished, adjust the prepared cap onto the baby's head in one go. Do it as fast and precise as possible to try to prevent the baby from getting upset. Also, make sure that the position of the cap is immediately right, as adjusting it may cause a connection between electrodes because of the gel. Make sure that Cz is right on top of the head, in the exact middle. The Fp-electrodes should be in the front; the O-electrodes should be at the back of the head. Take the ears through the little holes and fasten the cap with the Velcro. Stick the other end of the electrode bundle into the BioSemi amplifier (circled in **green** in IMAGE 3 .20). Turn on the amplifier and check if the blue light isn't flickering (circled in **yellow** in IMAGE 3 .20).

3.5 Checking the signal

3.5.1 CMS and DRL

After all the electrodes are adjusted properly, it is time to check the signal. The first check is the blue light on the BioSemi amplifier (circled in **yellow** in IMAGE 3 .20). If this light is flickering, it indicates that the electrodes CMS and DRL are badly connected. These electrodes do not give you data, but form a feedback loop which acts as a reference and reduces the noise. Without a good CMS/DRL

connection, your EEG measurements will be useless. Therefore, always make sure the blue light on the BioSemi is stable.

3.5.2 ActiView

After making sure that the CMS and DRL are working properly, it is time to start ActiView. Open up ActiView on the EEG1 (K.13)/Recording (K.02) computer. Before you start recording, make sure you have loaded the **correct configuration file**. A pop-up will open automatically in which you can select the correct configuration file. Go to 'Desktop', click 'Configuration file' and select 'Infant_32channels.cfg'.

On your monopolar display, press 'start' (circled in yellow in IMAGE 3.25). You will find that for each selected electrode, the signal is displayed on the screen (circled in red). Set the reference to Cz, by selecting 'Free choice' under 'Reference' (circled in green in IMAGE 3.25) and selecting Cz in the list underneath. Check the signal for each electrode. The flatter the signal, the better. If you notice that one or more signals are very noisy, try adding more gel to that particular electrode, but not too much as you don't want two electrodes to be connected through the gel. If you find that all signals are extremely noisy, add more gel to CMS and DRL or press the cap a little firmer onto the head with your hands. If the overall signal is better when you don't select Cz as your reference, consider adding more gel to Cz. If you've tried to add gel to an electrode for a maximum of three times without seeing a significant improvement, remove the electrode from your display. You can do this by selecting 'Free Choice' under 'Channels' (circled in orange in IMAGE 3.25, enlarged in IMAGE 3.26), and deselect the particular electrode by pressing CTRL and selecting the channel from the list underneath. The data for this channel will still be saved, even though it is removed from the display.
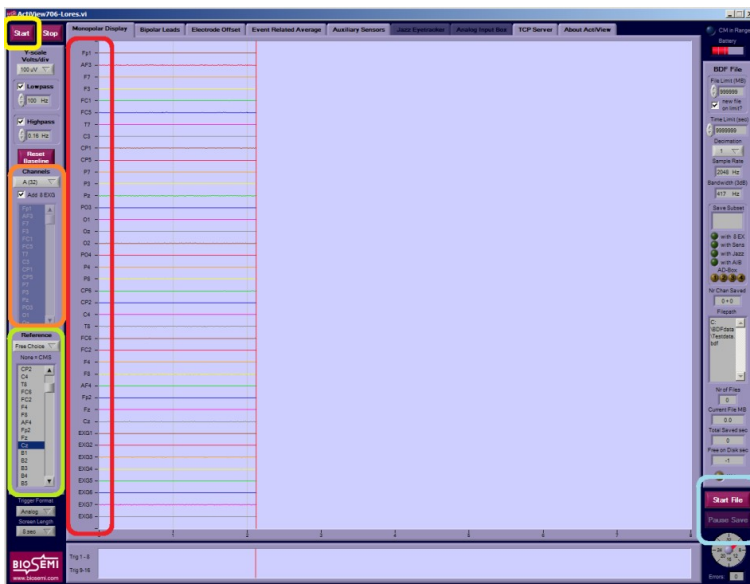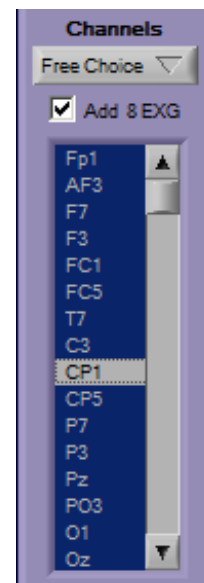

IMAGE 3.25

IMAGE 3.26

While checking the signal, also look at the impedance by clicking 'Electrode offset' in the toolbar. Set the displayed range to 50 mV (circled in yellow in IMAGE 3.27). You should see that the bars turn to blue. All bars should be within the range of +25 mV and -25 mV. The closer to zero, the better the signal. If one of the bars is above +25 mV or below -25 mV (for instance the bars circled in red in IMAGE 3.27), consider adding more gel to the corresponding electrode. If you see that one of the bars is very unstable, also consider adding more gel to that particular electrode or remove it from the display.

NOTE: it may be difficult to get all electrodes with good signal when testing infants. Researchers are usually only interested in some electrodes rather than all. For instance, MMN is mostly visible at

frontal-central electrodes. Hence, if the electrodes at the parietal and occipital locations are noisy, it is not worth it to spend a lot of time adjusting these electrodes, as children very easily get annoyed by people working on their head. Researchers need to be aware of the trade-off between a very good signal and crying babies. In addition, if you use left and right mastoids as reference, a few noisy electrodes can be tolerated, but if you want to use average reference in offline processing, decent signal from all electrodes is needed, otherwise your reference will not be accurate.
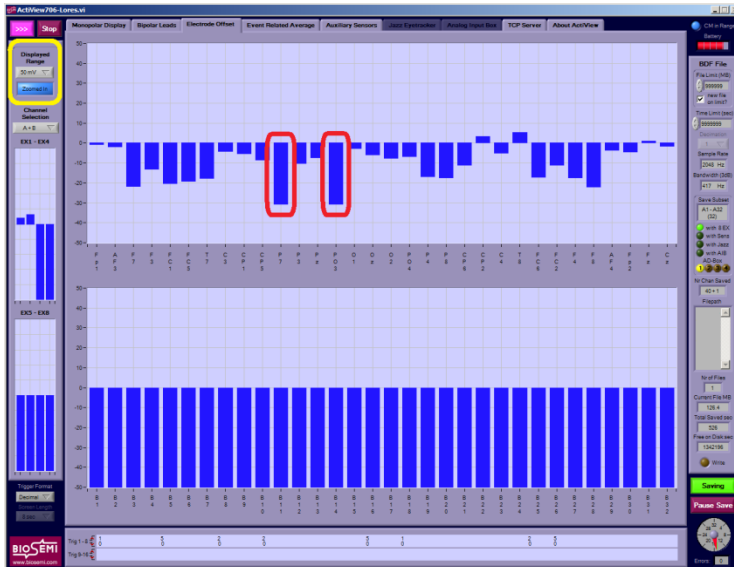

IMAGE 3.27

3.6 Testing

3.6.1   Start recording (EEG1/Recording computer)

After you have checked the signal and everything is looking as good as possible, it is time to start testing. Close the door between the test room and the control room. **NOTE:** When testing in K.02, make sure you push the door handle **upwards** after closing it to secure the cabin is soundproof!

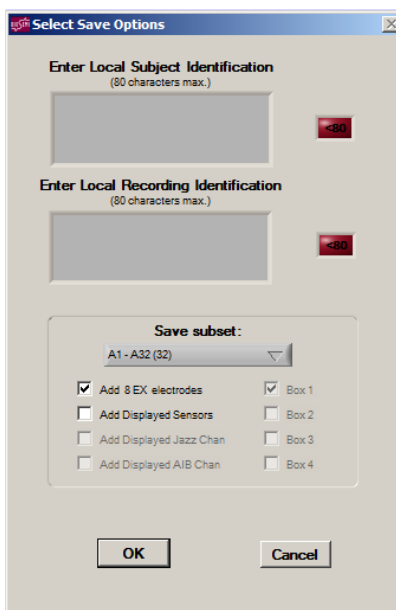Press 'Start File' in the bottom right corner of ActiView (circled in **blue** in IMAGE 3.25). A dialog pops up in which you have to type the participant number (IMAGE 3.28). Type your participant number in the box under both 'Enter Local Subject Identification' and 'Enter Local Recording Identification'. Press 'OK'. After this, you will be able to select a folder in which you want to save the recording, type the same participant number again as the file name. Click "OK".

NOTE: After you've pressed OK, you are **not** recording yet! Click 'Pause Save' at the right bottom corner of the screen when you want to start your recording. The buttons should look like the ones in IMAGE 3.29, otherwise you are not recording!

IMAGE 3.28

IMAGE 3.29

3.6.2   Start experiment (EEG2/Presentation computer)

Once you've made sure that you are actually recording, start your experiment in Presentation on the EEG2 computer. Click 'run', type in the participant number and press 'enter'. Keep on pressing 'enter' until the sound starts playing.

Return to the ActiView screen and check whether the triggers are sent. You can see this in the window circled in **red** in IMAGE 3.30. A colour or number should appear (depending on your settings) every time a stimulus is played. You can adjust the settings by selecting something else in the window circled in **green** in IMAGE 3.30. This will not affect your data, but will only change your current view. **If you do not see any triggers appear, stop the experiment and call lab support.** Without triggers being sent, no offline processing can be performed, so you are recording for nothing!

You can always stop the experiment in Presentation halfway by pressing the Esc button. This will stop the entire block you were running. If a block has ended, press 'enter' a few times again to proceed on to the next block. It won't be possible to pause a block and continue later. However, you can restart a block by starting the experiment again and skip the previous blocks (press 'enter' and 'esc' right after each other when starting a new block). Keep in mind that the log files from your experiment will overwrite themselves when restarting the experiment. If you want to keep those, make sure to copy them before you restart your experiment.
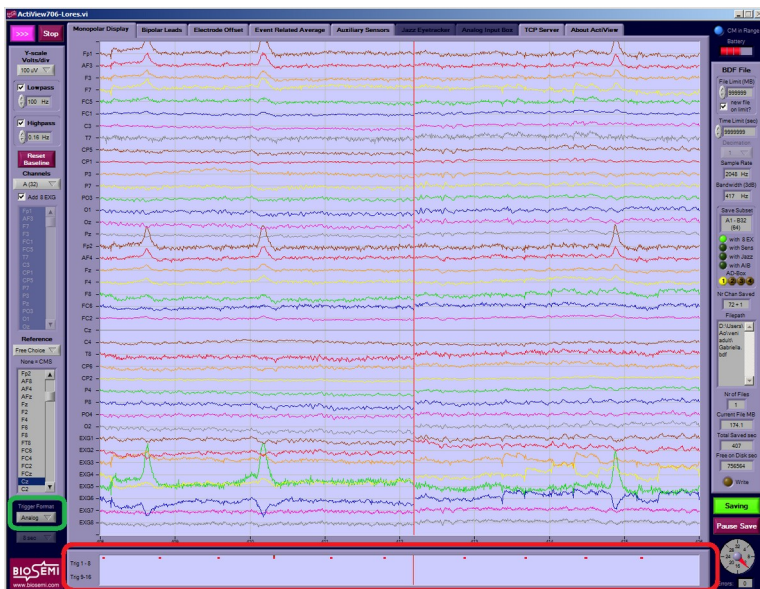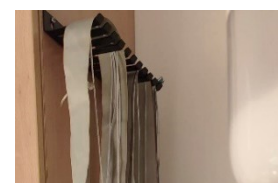

IMAGE 3.30

Once the recording has finished, press "pause save" at the right bottom of the screen, and then press "stop" at the up left of the screen.

3.7 Cleaning

After you completed the testing phase, remove the cap from the infant's head and take off the mastoid electrodes. Guide the parent and child to the washing room and show them the towels and shampoo, or give them a Zwitsal paper cloth if they don't want to wash the hairs immediately (this is usually enough cleaning for the youngest children without many hairs). Before you say goodbye to the parent and child, take them back to the waiting area and give them a gift (usually a book that's appropriate for the child's age) and reimburse travel expenses (if applicable). For more information about this, read the waiting area protocol. Also, give the parent the opportunity to ask any questions they may have.

Afterwards, fill the two plastic washtubs with **lukewarm** water (IMAGE 3.31). Remove the electrodes from the cap and gently place them into the water. Do not let the electrodes sit under running

water! Use the softest toothbrush to gently clean the electrodes. Pat dry them with a piece of paper towel. Hang the bundle back to it's place and put the end with the electrodes back in the bag to prevent them getting (sun)light;   image 3 .32   image 3 .32).

Place the cap in the other washtub. Use the other brush to remove the gel from the holes in the cap. Hang the cap up to dry on one of the hooks above the sink.

Fill the syringe a few times with lukewarm water and press it empty to remove the gel in the tip.
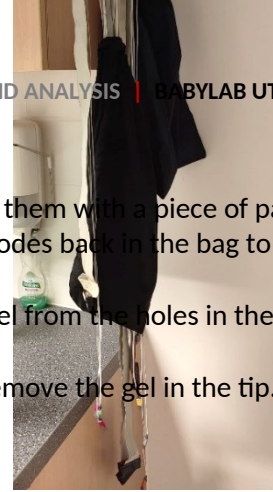
IMAGE 3.31   **IMAGE 3.32**

3.8 Replacing the battery

After a few appointments, you may get an error message in ActiView stating that your battery is low. There is always an extra battery in the control room, on the right hand side of your desk. Here, you will also find the charger.

The battery of the BioSemi amplifier is the large block underneath the block in which you plug in the electrode bundles (IMAGE 3 .). First, remove the cable circled in **red** in IMAGE 3 . by pulling it out. Then remove the cable circled in **green**, by (gently) pushing and turning it. Disconnect the battery from the amplifier by clicking all four black locks sideways and up. Take the battery to the control room.
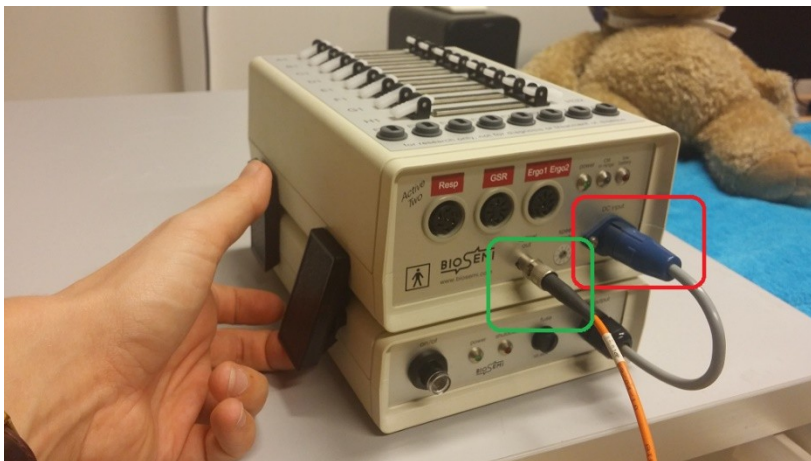

IMAGE 3.33

In the control room, you'll find a spare battery and the charger (IMAGE 3.). Connect the empty battery to the charger by connecting the blue parts (circled in **green** in IMAGE 3.) and plug the charger into the socket. **Don't forget to unplug it once the battery is fully charged again!** Take the spare battery back to the amplifier and connect it to the amplifier by adjusting the two cables and the four black locks again.

IMAGE 3.34

4. Guidelines for testing different age groups

Testing infants may be quite challenging and approaches can differ widely as infants grow older. Therefore, it is always necessary to search for the correct approach when starting an experiment with a new age group. So far, we have personally experienced what it's like to test 4-, 12- and 20-month-old infants. In this chapter, we'd like to share our experience and provide certain guidelines on how to deal with these age groups. Note that no matter the age group, a bubble blower will make all children happy. Use this as your first choice munition in any bad situation.

4.1 How to test 4-month-olds

At 4 months, infants have just gained control over their heads and cannot sit down properly yet. Therefore, they have to sit on their parent's lap during the experiment instead of in a high chair. A problem with this, however, is that infants tend to lean against their parent's stomach, which may cause a lot of noise in the signal. Ask the parent explicitly to prevent this as much as possible. Because their motor skills haven't quite developed yet, you won't have to worry about them wanting to run away from the table. Instead, there's a big chance that the child will cry during the experiment. If it does, try distracting the child, for instance with a bubble blower. If this doesn't work, check with the parent whether it may need something. The child may be hungry or may need a diaper change, which are problems that can easily be solved after which you can continue the experiment. If the child is tired, however, it will be more difficult to continue the experiment and you may want to consider aborting it.

As far as the preparation of the cap is concerned, our experience is that the preparation before the appointment works best for 4-month-olds. Preparation directly on the infant's head can take quite a long time, and 4-month-olds tend to get restless pretty quickly. Also, the signal of the pre-prepared cap is quite similar as to the one that's prepared immediately onto the infant's head. Preparing the cap before the appointment therefore saves a lot of time and frustration for the child. You can apply the mastoid electrodes first, and then put the prepared cap onto the head in one go. Make sure you immediately get the position right, as readjusting it may cause bridges between two electrodes.
Best cap size: blue/pink

4.2 How to test 12-month-olds

At 12 months, infants have gained a lot more motor skills already and some may have even started walking a little bit. This can be pretty cute, but the consequence of this is that they also want to move around and explore during your experiment, whereas you really want them to sit still. Make sure there is enough distraction for them as you put the cap onto their heads. You could for instance play Dora the Explorer on the screen, or have toys on the table for them to play with. If the infants aren't allowed to play during the experiment, however, be careful when providing them with toys, as they may get upset when you take the toys away from them before the experiment starts. Instead, choose to play something fun on the screen.

For this age group, it also works best to prepare the cap in advance, to make sure they don't have to sit still for a long time as you prepare the cap onto their heads. Be careful though with children who already have a lot of hair, as it may be more difficult to get a clean signal this way.
Best cap size: blue

4.3 How to test 20-month-olds

At 20 months, infants can already walk and they have started talking a little bit. You will notice that these children also have become much more aware of what is happening to them, and differences in character will be clearly visible. The word 'no' is especially well-known, and they won't think twice about using it whenever they can. At this age, it is far more important to talk to them directly as

opposed to the younger infants. Whereas you could still put the cap onto their heads without saying anything when they were just 12 months old, they now demand an explanation and a certain degree of autonomy from you. Below, you will find an overview of the tricks we used while testing 20-month-olds:

- Make the EEG caps look more child friendly, for instance by giving all the stuffed animals in the room an EEG cap as well.
- Decorate two caps of the correct size with child friendly figures, for instance flowers and stars, and let them choose which cap they want to put on their head. Make them believe they have a choice in the matter.
- As soon as the child walks into the room, point your attention directly to the child and explain to the child that all their stuffed animal friends wear beautiful caps, and they want to play a game with the child, but then the child gets to wear a cap too. Explain to them that they can choose which cap they want. Always be enthusiastic and point out all the nice things about wearing a cap.
- If the child is very shy or actively refuses to make a decision, take more time for this part and try to involve the parent. For instance, ask the parent if they also want to wear a cap, or put one on your own head as well to show the child it isn't harmful. Another thing you can do is let the child choose if either you or the parent should put the cap onto the child's head. Make sure you instruct the parent on how the cap should be placed.
- Once you have tricked the child into wearing the cap, first clean their skin behind the ears with alcohol before you proceed to put the cap onto their head. As soon as you have put the cap on, start a video with children's songs to distract the child immediately. Our experience is that they will totally forget that they have something on their head as soon as they hear the songs.
- While the infant is listening to the children's songs, you can put a little bit of gel onto the child's wrist, explain to the parent what you are going to do and afterwards check the child's wrist again for any allergic reactions. If there aren't any, you can start the preparation of the cap by inserting the gel into the holes.

Note that preparing the cap before the appointment does **not** work for this age group, as their scalp has become thicker and they have gained a lot more hair. Therefore, more distraction is needed to keep them from getting upset while undergoing the preparation.

Best cap size: green/blue for girls, green for boys
-

5. Analysis

5.1 Getting started

5.1.1   Downloads

After you have tested enough participants, it is time to analyse your data. Before you start, make sure you've installed the following programmes on your computer:

- The EEG analysis server (ask ICT & Media for the download and for adding you as a user to the server)
- EEGLAB (download)
- The following plugins:
    - BIOSIG data import (download)
    - Grandaverage (download)
    - ERPLAB (download)

Once ICT & Media has given you access to the EEG analysis server, open the server by inserting your username ('SOLISCOM\[your solis-ID]') and solis password. While in the server, download EEGLAB. Save the file in your personal user folder on the D: drive. **NOTE:** Always save it in this folder while in the EEG analysis server, otherwise you won't be able to access it!

After downloading EEGLAB, install the other plugins. Save the files in the following folder: 'Computer/D:/Users/[your solis-ID]/eeglab13_6_5b[1]/plugins/'. Make sure you unzip the ERPLAB and grandaverage plugin before saving it in the 'plugin' folder. Your directory should now look similar to **IMAGE 5 .32.**
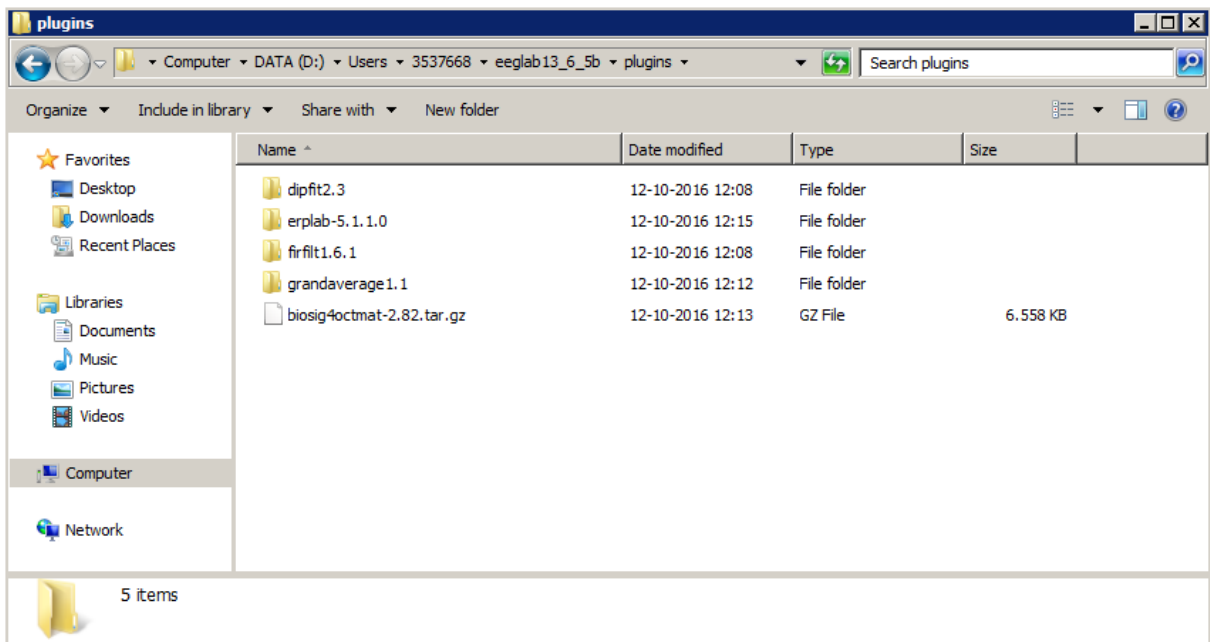
IMAGE 5.32

5.1.2   Copy your data to the server

Copy your raw data to your user folder (D: drive) on the analysis server. From here, you will do all the analysis.

---

[1] This could have a different name if you're using a different version of EEGLAB

5.1.3   Starting EEGLAB

Next, open MATLAB, which is already installed on the EEG analysis server. Click 'Set Path' in the toolbar (circled **red** in IMAGE 5 .33).
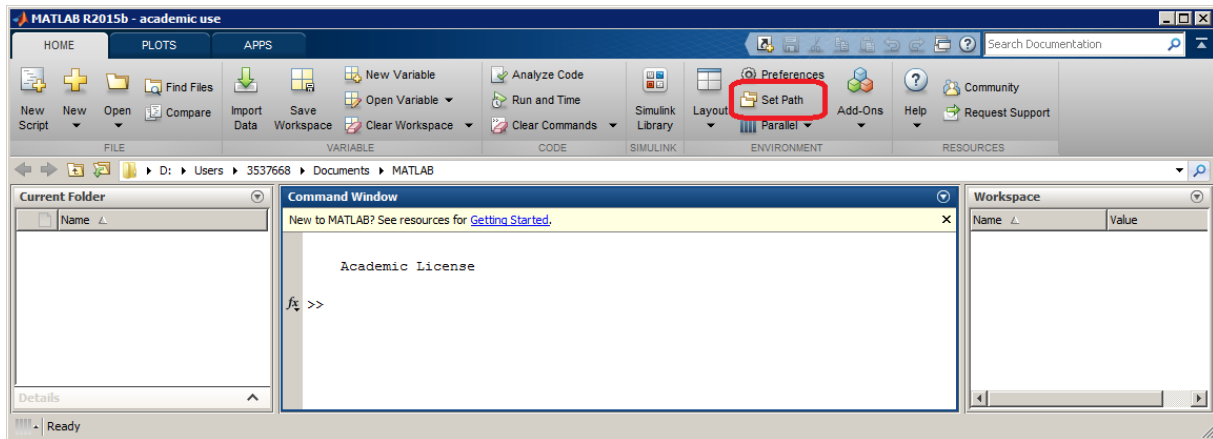
IMAGE 5.33

Click 'Add Folder' (circled in **red** in IMAGE 5 .34). Set the path to the folder where you saved EEGLAB (circled in **green**). Click 'Close' (circled in **blue**). When asked to save the path, click 'no', as saving is not possible.

NOTE: because saving isn't possible, you have to do this again every time you start MATLAB.
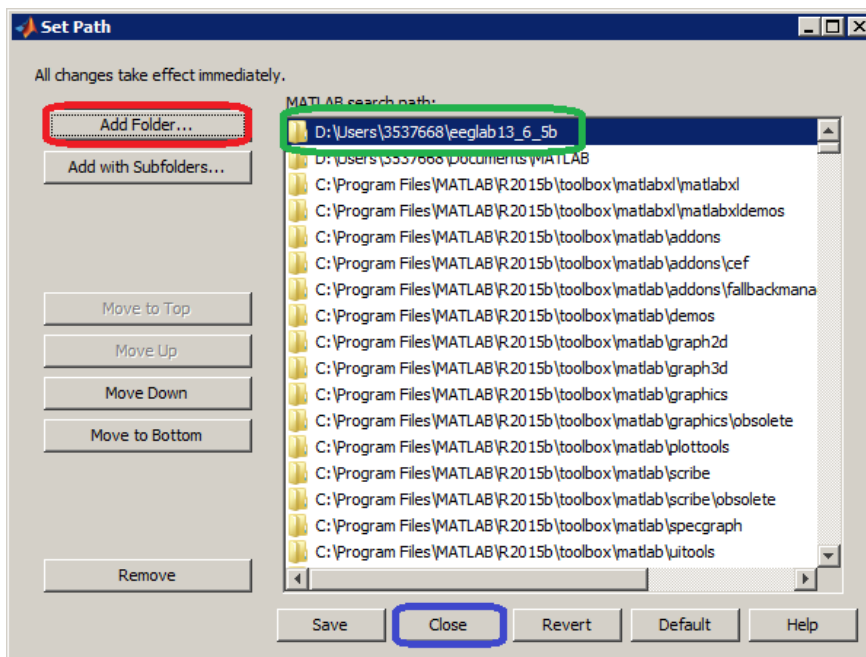
IMAGE 5.34

After setting the path, type 'eeglab' into the command window (circled in **red** in IMAGE 5 .35). Press 'enter'.
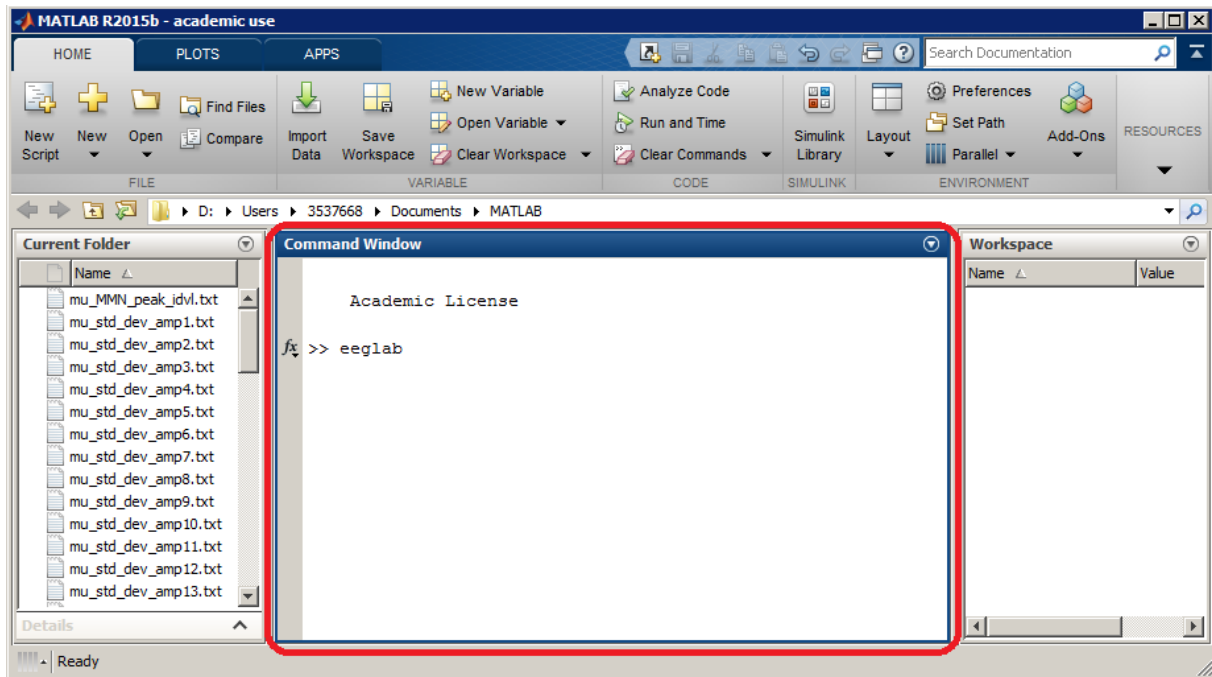
IMAGE 5.35

A new window opens, similar to the one in IMAGE 5.36. You are now ready to start analysing!
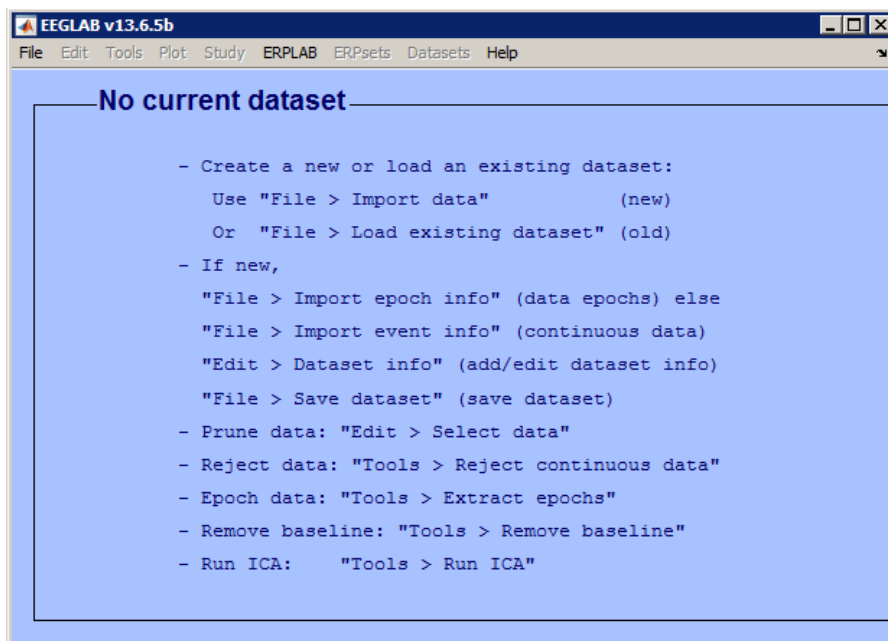
IMAGE 5.36

5.2 Epoching

First of all, you need to upload your raw data. Import your data by using the BIOSIG toolbox, as seen in IMAGE 5.37. Go to the folder where you saved your raw data files and select one of the .bdf files.
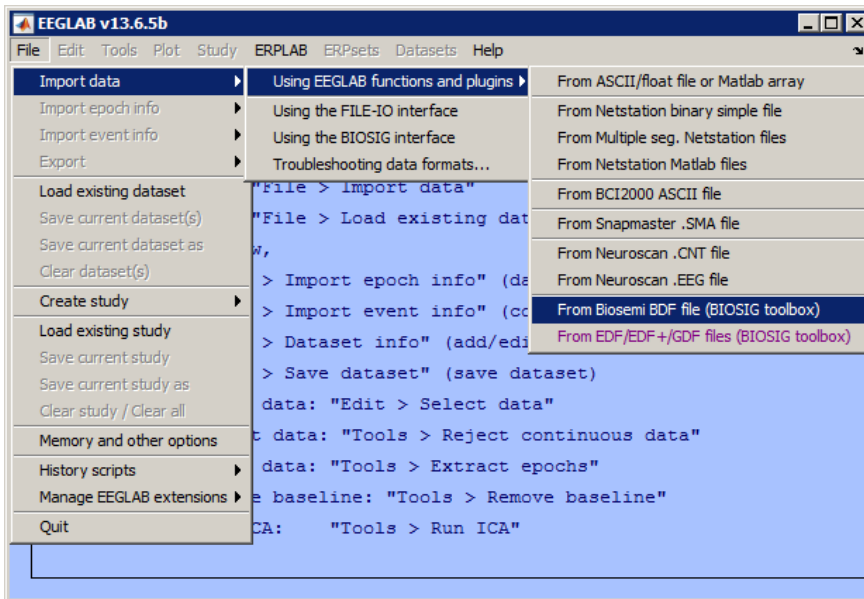
IMAGE 5.37

Another window opens, similar to the one in IMAGE 5.38. While uploading, set the reference to Cz, which is the electrode in the very middle of the cap. In MATLAB, all electrodes are numbered and referred to by their corresponding numbers. You can find an overview of the electrodes and their corresponding numbers here. You can see that the corresponding number to Cz is 32. Set the reference to Cz by typing '32' into the white box circled in **red** in IMAGE 5.38. Leave the rest as it is, and press 'OK'.
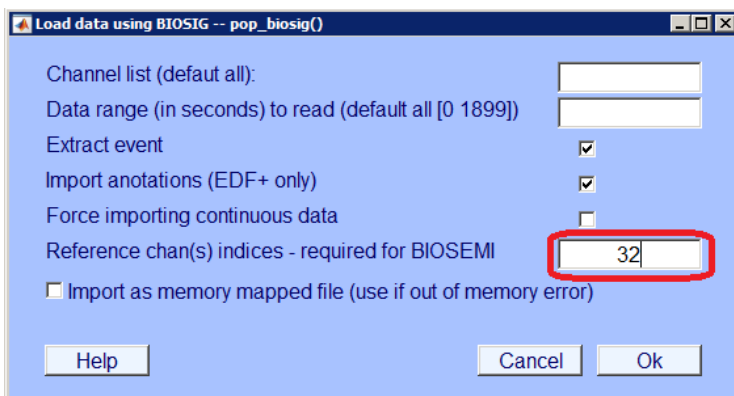
IMAGE 5.38

You will be asked to give up a name for your new dataset. In this example, we will use the fictive name ChildX (IMAGE 5.39).
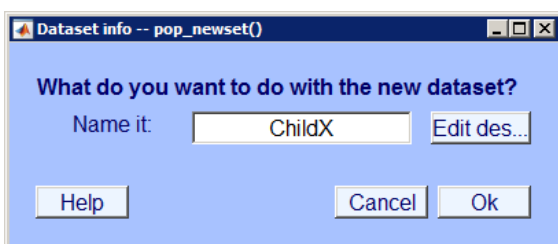
IMAGE 5.39

After uploading your dataset, you should see that there are 40 channels per frame (circled in **red** in IMAGE 5.40), and that the reference is set to Cz (circled in **green** in IMAGE 5.40).

30

NOTE: Do you see 72 channels per frame? Click [here](#) to find out what to do.
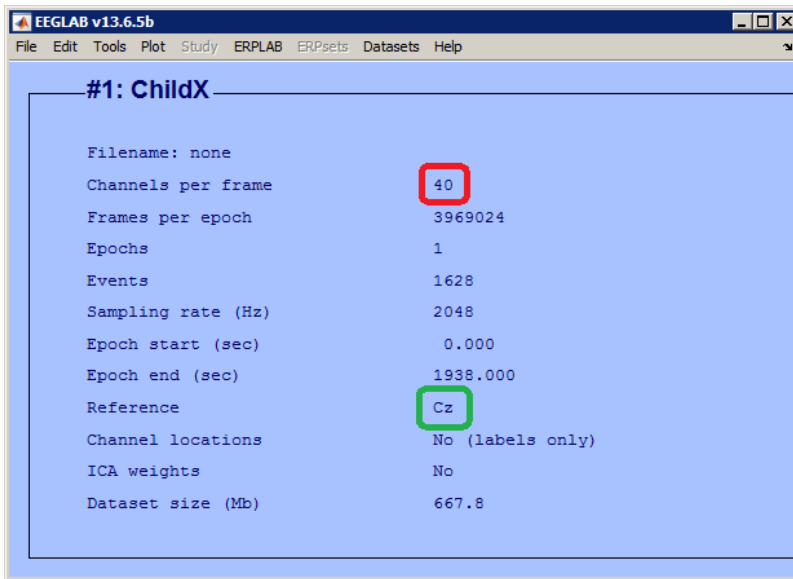
IMAGE 5.40

5.2.1 Filter the data

The next step is to filter your data. This is done in order to filter out the low and/or high-frequency artefacts in the data. We choose a bandpass filter to filter out all data below 0.3 and above 20Hz. Note that the type of filter and filter range depends on the component that you are interested in and the age of the children, and should be determined per experiment. Select 'Basic FIR filter (new, default)' as depicted in IMAGE 5.41. A dialog will open similar to IMAGE 5.42. Insert your lower edge and higher edge and click 'Ok'.
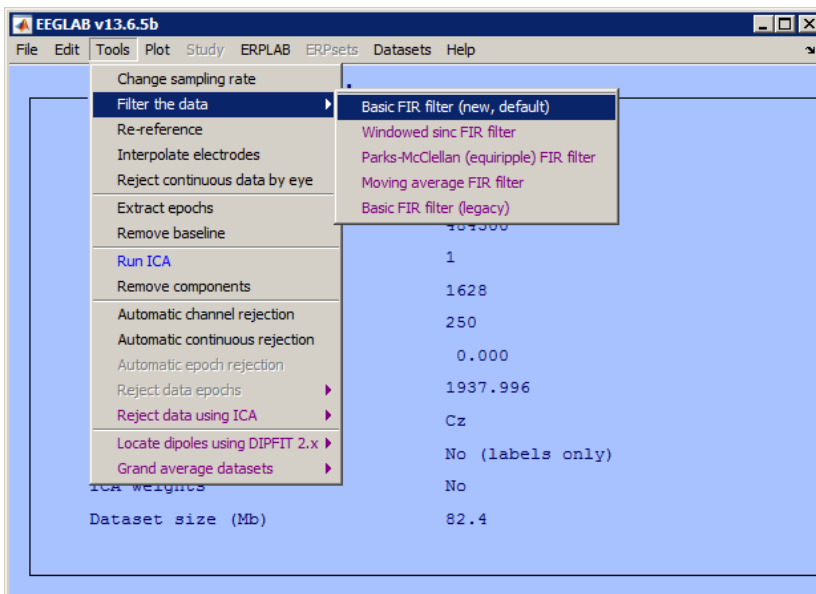
IMAGE 5.41
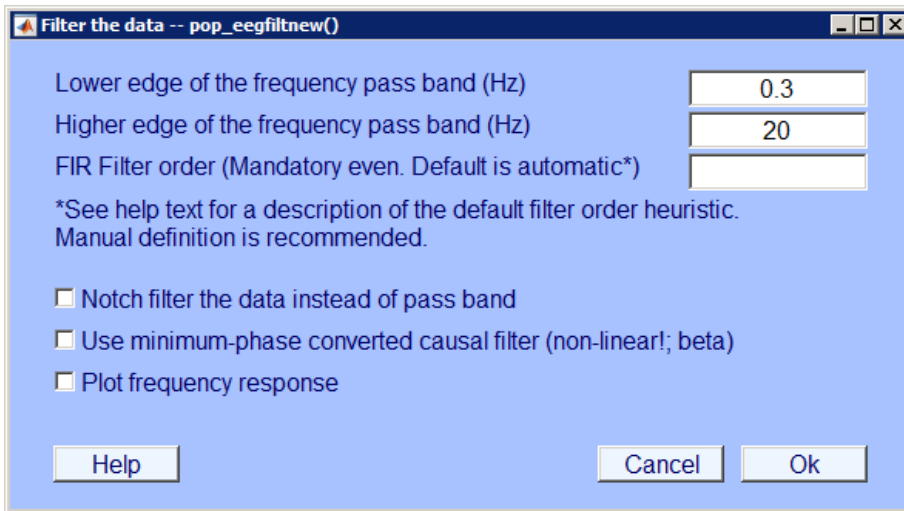
IMAGE 5.42

Name the new dataset as you prefer. A new dataset will open in the old dialog again. As you will notice, nothing has changed on the outside.

5.2.2   Change the sampling rate

The sampling rate of the data gives the number of samples per second. By default, the sampling rate of the BioSemi is set at 2048Hz. This accounts for extremely large datasets; as you can see in IMAGE 5 .40, the size of this dataset is 667.8 Mb. Therefore, it is necessary to change the sampling rate to a lower rate, but still keep it high enough to give you enough detail for your analysis. For our research, we would like to change the sampling rate to 250Hz. For your own research, this number may differ.

If you would like to change the sampling rate, go to 'Tools' and click on 'Change sampling rate' (IMAGE 5 .43).
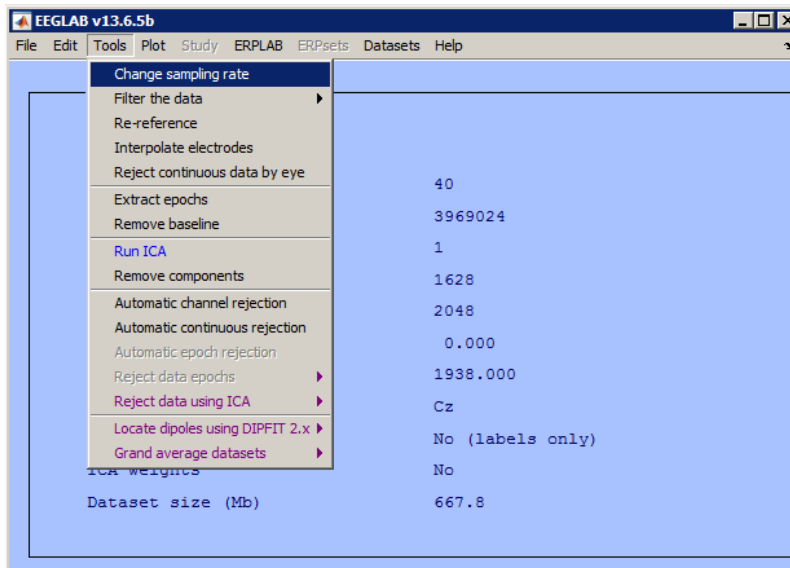
A window will open similar to IMAGE 5 .44. Insert your desired sampling rate in Hz and click 'OK'.
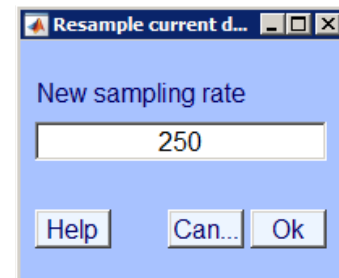
IMAGE 5.43
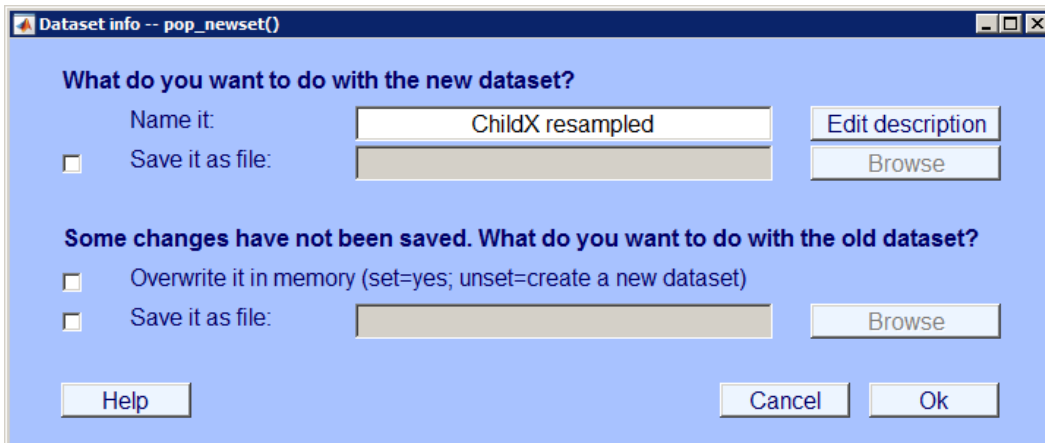
IMAGE 5.44

IMAGE 5.45

A window opens which will ask you to give a name to your new dataset (IMAGE 5.45). By default, EEGLAB adds 'resampled' after the previous name. You can either choose to accept this, change it to a different name, or change it to the previous name by removing 'resampled'. Click 'Ok'.

NOTE: Every time you change something to your dataset, a new dataset will be created and you will be asked to give up a name for your new dataset in the same way as in IMAGE 5.45. In order to remember what is different about each dataset, the advice is to always add something after the child's name which reminds you of the step you took in creating this dataset. Sometimes EEGLAB does this by default, but not always.
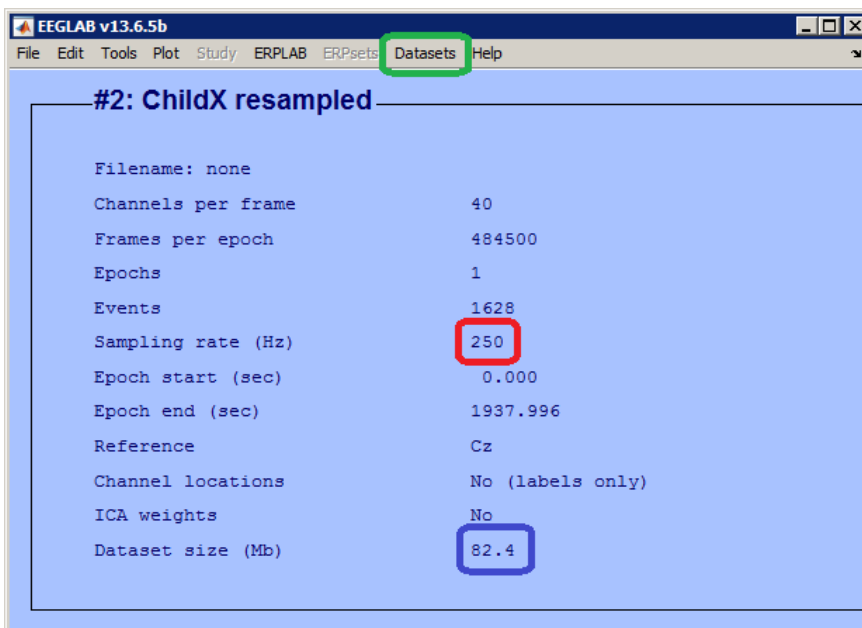
IMAGE 5.46

Your new dataset will open in the same dialog as your previous dataset (IMAGE 5.46). By clicking on 'Datasets' (circled in **green**) you can switch between datasets. You can see in your new dataset that the sampling rate has been changed to 250 (circled in **red**). Consequently, the size of your dataset has also become smaller (circled in **blue**).

5.2.3 Add channel locations

The next step is to add the channel locations to your dataset. Adding channel locations will give you the opportunity to plot your ERP data in the correct place in the scalp. You can add channel locations

by clicking 'Channel locations' via 'Edit' (IMAGE 5 .47). A new dialog will open as in IMAGE 5 .48. The correct file will already be loaded by default, so you shouldn't have to do anything. Click 'Ok'.
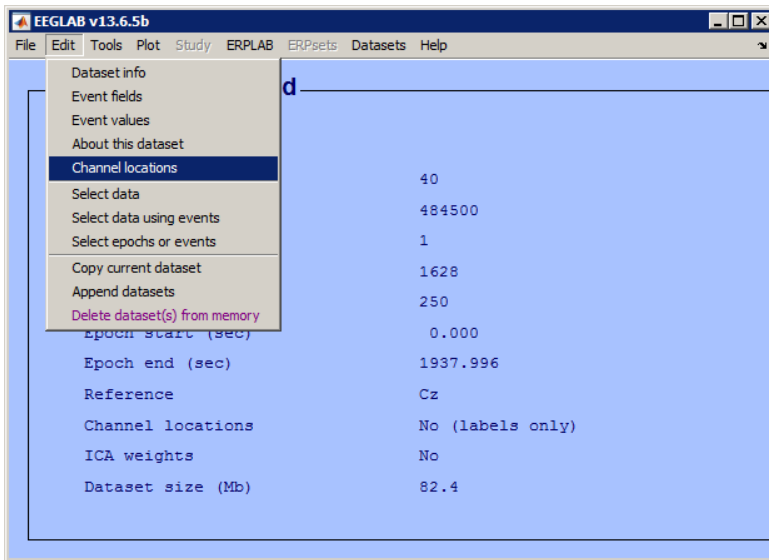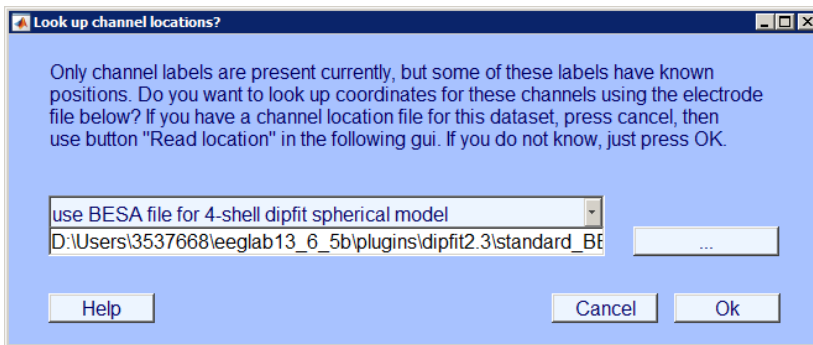

IMAGE 5.47

IMAGE 5.48

After clicking 'Ok', a new window will open similar to IMAGE 5 .49. You will see that all the information about the location of each channel is already inserted. Click 'Ok'.
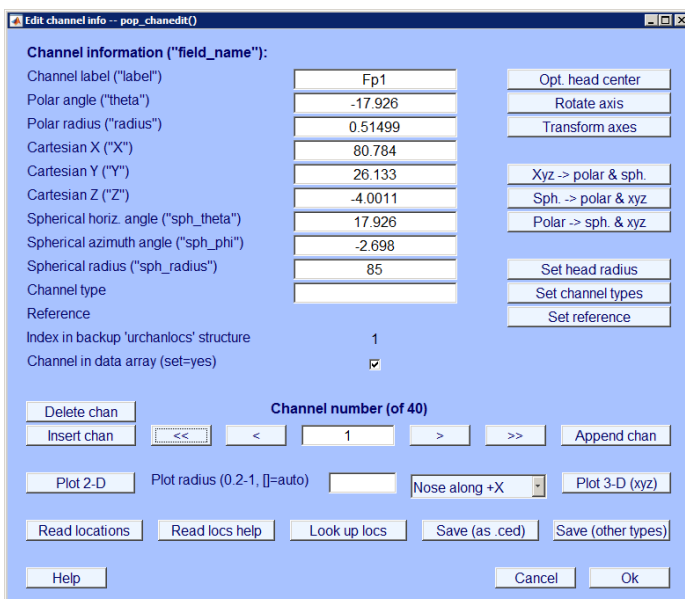

IMAGE 5.49

This time, no new dataset will be created. You will find that the channel locations have been added to the old dataset (circled in **red** in IMAGE 5.50), which you created after filtering the data. Consequently, another thing that has changed to your dataset is the reference. As you will find, the reference is now unknown (circled in **green** in IMAGE 5.50).
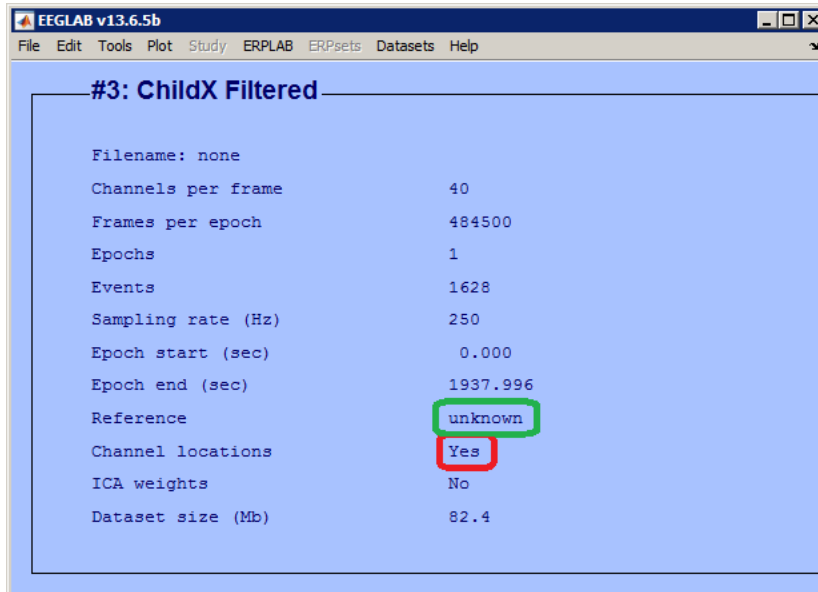

IMAGE 5.50

5.2.4 Select channels

So far, our dataset has consisted of 40 channels. These are the 32 channels which were placed on the cap, and EXG1-EXG8. As you remember, we only used EXG1 and EXG2 while recording (these were the electrodes that were placed on the mastoids), which means that the other six are empty. Therefore, you have to remove these empty channels from the dataset.

At this stage, you should also decide what you want to use as a reference. When referencing your data (at a later stage), you can either choose to use the mastoid electrodes (EXG1 and EXG2), or use the average of the 32 electrodes on the cap. Note that when choosing the mastoid electrodes as your reference, they need to be perfectly attached for **all** participants. As infants are more tempted to pull these electrodes off while testing, EXG1 and EXG2 are usually less reliable in infants compared to adults. Therefore, in our research, we will use the average of the 32 remaining electrodes as a reference instead. Because we do not need EXG1 and EXG2 for our data anymore, we should remove all EXG-electrodes and only select the 32 channels that were on the cap.

To (de)select channels, go to 'Edit' and click 'Select data' (as in IMAGE 5.51).
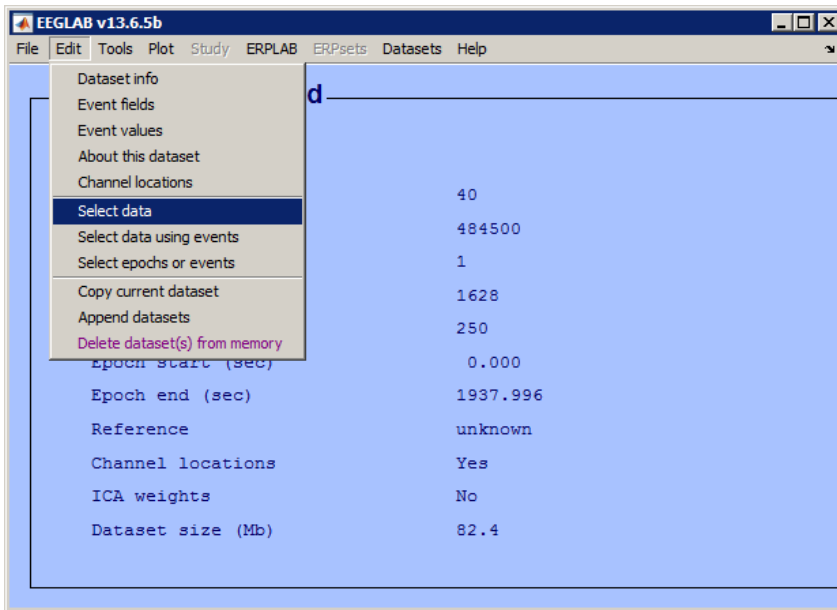
IMAGE 5.51

A new dialog opens which gives you several options for selecting data. Choose the last option, 'Channel range', to select the channels that you are interested in. There are several options for your input into the blank space:

- If you know the numbers that correspond to the electrodes, you can type in the individual numbers with spaces in between (e.g. 1 2 3 32).
- If you wish to select the whole range between two electrodes, you can type the first and last number with a colon (:) in between (circled in **blue** in IMAGE 5.52).
- If you do not know the corresponding numbers, you can select the channels by label by clicking the button circled in **red** in IMAGE 5.52.
- You can also decide to insert the electrodes that you want to **remove** from your set. You can select this option by ticking the checkbox circled in **green** in IMAGE 5.52. In this case, you only have to select EXG1-EXG8 (or 33:40), tick the box and press 'Ok'.
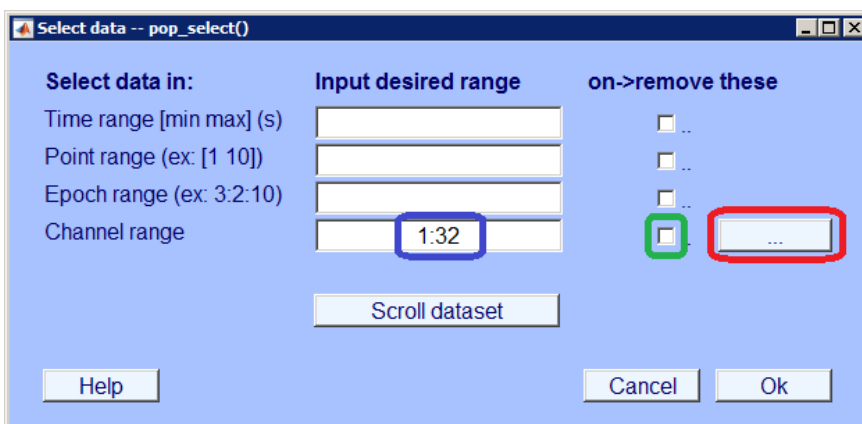

IMAGE 5.52

A new dataset will be created again. As you will find, it now contains only 32 channels (circled in **red** in IMAGE 5.53).
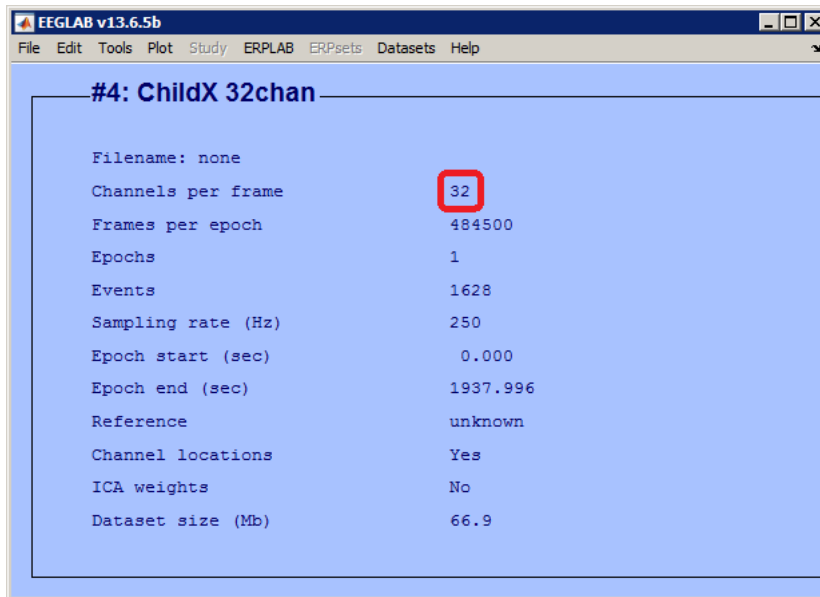
36

IMAGE 5.53

5.2.5   Epoch the data

By now it is time to epoch your data. To epoch means to break your large string of data into smaller strings, based on the timeframes in which the stimuli (or 'events', as EEGLAB calls them) occurred. As you can see, your dataset now contains only one epoch, which means that there is only one extremely large string of EEG data. An epoch usually begins a few milliseconds before the onset of the stimulus, and ends after the ERP response should have occurred. In this example, we choose our epochs to start 100ms before the onset of the stimulus and end 900ms after the onset of the stimulus.

In the experiment that is used as an example, there were 9 different types of stimuli:

Event number	Stimulus type
1	Lexical tone: standard that immediately follows a deviant
2	Lexical tone: standard that follows another standard
3	Lexical tone: deviant
4	Music: standard that immediately follows a deviant
5	Music: standard that follows another standard
6	Music: deviant
13	Vowel: standard that immediately follows a deviant
14	Vowel: standard that follows another standard
15	Vowel: deviant

Because of the uncertainty that the ERPs following the numbers 1, 4 and 13 (standard after deviant) really represent the ERPs following a standard stimulus, we are only interested in the numbers 2, 3, 5, 6, 14 and 15. Therefore, we will only extract the timeframes surrounding these stimuli from the large dataset.

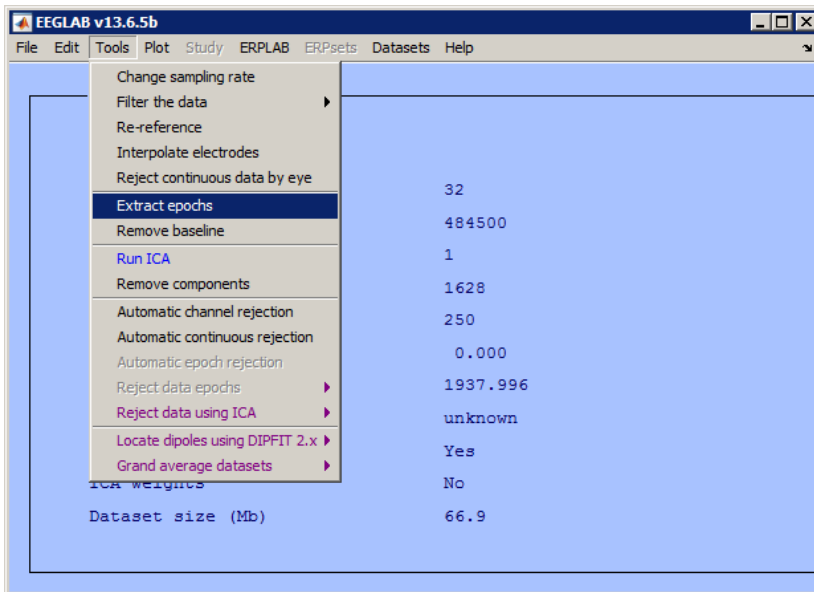To epoch your data, go to 'Tools' and click 'Extract epochs' (IMAGE 5.54).

IMAGE 5.54

A new dialog will open, similar to the one in IMAGE 5.55. Specify which events you want to select, either by typing in the corresponding numbers with spaces in between or by clicking the button circled in **red** in IMAGE 5.55. Also, specify what the timeframe should be. Because we want the epoch to start 100ms before the onset of the stimulus, and end 900ms after the onset of the stimulus, we typed '-0.1 0.9' in the blank space (circled in **green**). You can also immediately specify the name of your new dataset (circled in **blue**). Click 'Ok'.
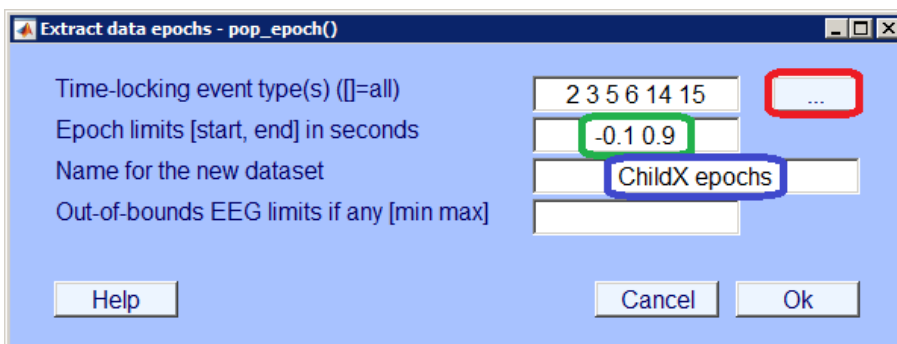
IMAGE 5.55

After the familiar window in which you will be asked to name and/or save your new dataset, a dialog similar to IMAGE 5.56 will pop up. Don't change anything. Click 'Ok'.
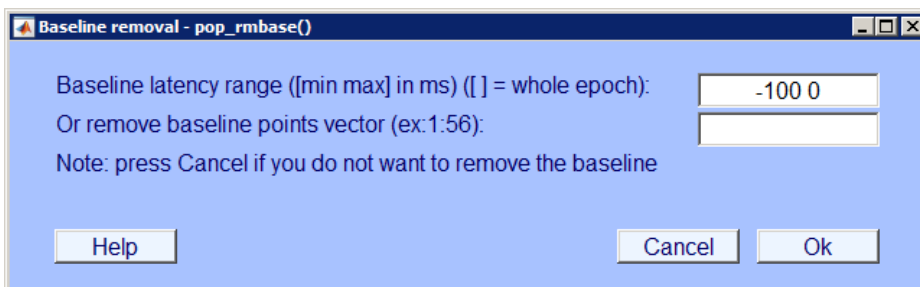
IMAGE 5.56

As you will see, your new dataset now consists of many more epochs (circled in **red** in IMAGE 5.57), which all start 100ms before the stimulus and end 896ms after the stimulus (circled in **green** in IMAGE 5.57).
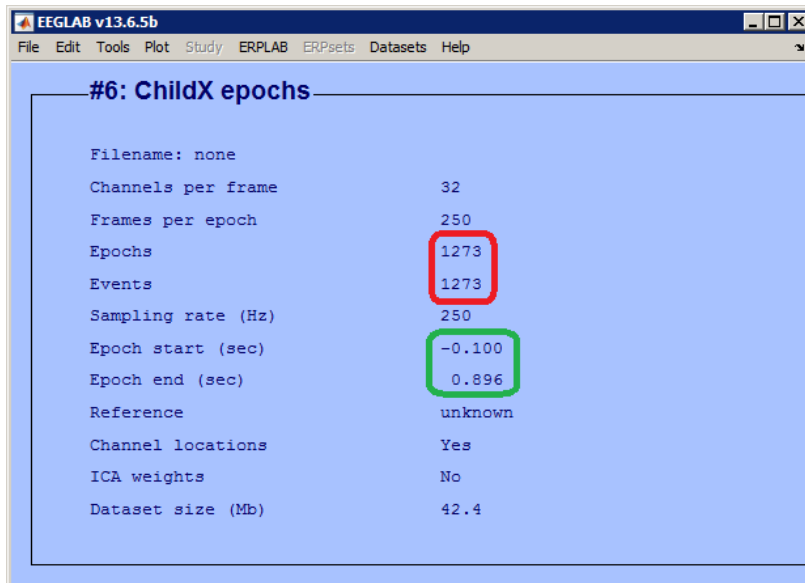
IMAGE 5.57

5.2.6 Set the reference

Finally, we have to reset the reference. When uploading the file, the reference had to be Cz, but now we set the reference either to the mastoid electrodes or to the average of all 32 electrodes on the cap. When selecting the channels, you have already made the decision to select or deselect the EXG1 and EXG2 electrodes. If you've kept these in your dataset, you can set the reference to the average of EXG1 and EXG2. If you removed these electrodes, you can set the reference to the average of the 32 remaining electrodes. In this example, we choose the last option.

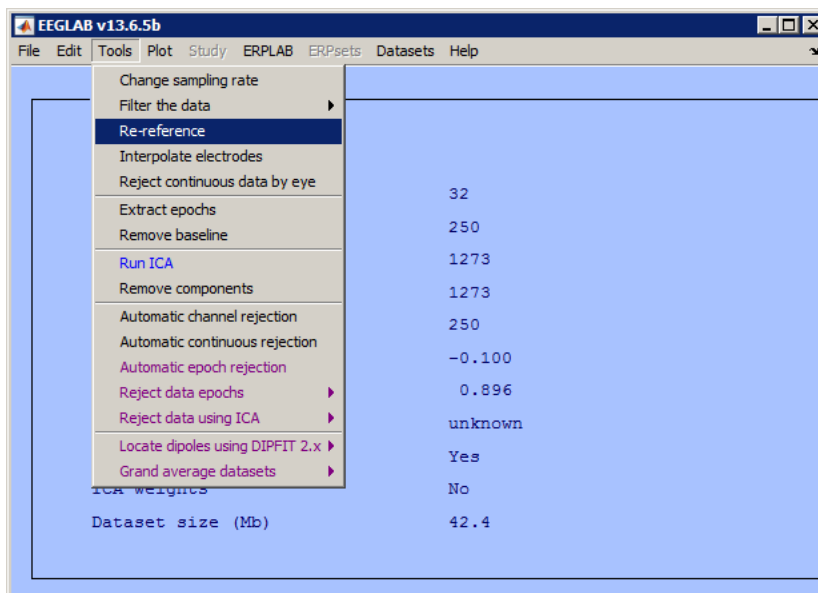In order to set the reference, go to 'Tools' in the toolbar and click 'Re-reference' (IMAGE 5 .58).

IMAGE 5.58

In order to set the reference to the average, tick the checkbox circled in **red** in IMAGE 5 .59 (this checkbox will already be ticked by default). Click 'Ok'.

NOTE: If you want to set the average to EXG1 and EXG2, tick the box 'Re-reference data to channel(s)' and type in the corresponding numbers, or select the mastoid electrodes from the list.

Next, select 'Retain old reference channels in data' to return Cz to your dataset, otherwise the recorded data from this channel will be lost.
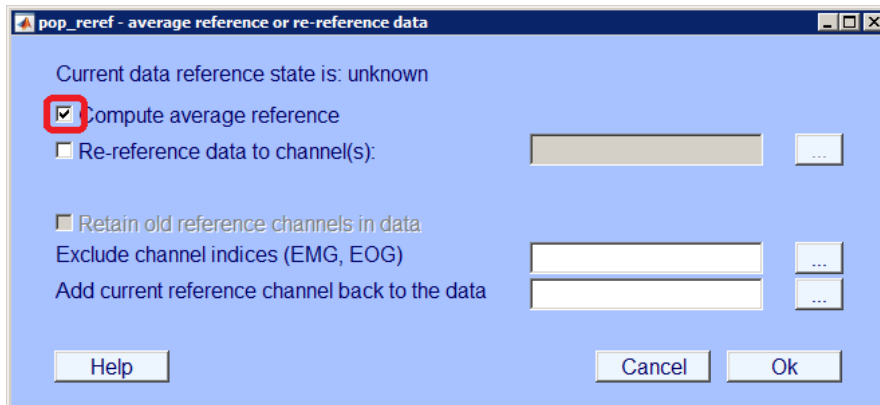

IMAGE 5.59

This time, **save** your new dataset under the name '[ChildX]_epochs' (**IMAGE 5.60**). It will be saved as a .set file, and you can upload it whenever you start MATLAB again to do the remaining analysis.

Up until here, all the analysis can be done with a script. You can either try to write your own script by clicking 'New script' in the MATLAB toolbar, or you can contact Ao Chen *to ask for a standardised script. Don't forget to adapt her script to your own experiment!*
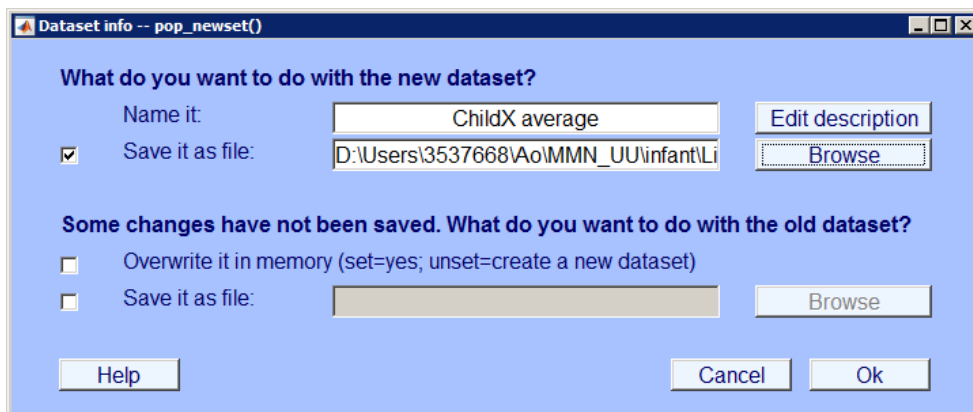

IMAGE 5.60

5.2.7 Problem: My dataset has 72 channels

If you uploaded a dataset, chances are that it contains 72 channels per frame instead of 40 (circled in **red** in **IMAGE 5.61**). This happens when you forgot to use the 32-channel configuration file when recording. Consequently, you'll find that the reference isn't set to Cz, but to CPz (circled in **green** in **IMAGE 5.61**). This is because the channels were incorrectly labelled due to the use of the wrong configuration file. This means that CPz holds the information of the Cz electrode, but is labelled differently.
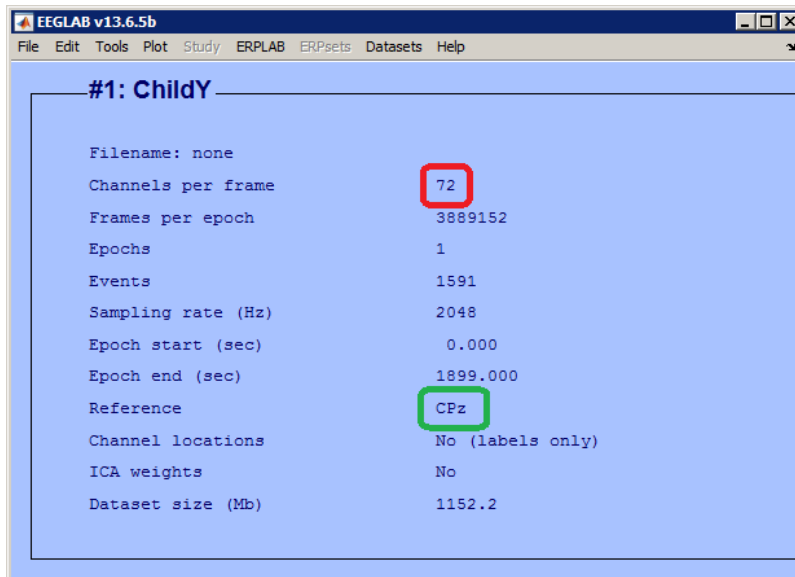
IMAGE 5.61

When you find that your dataset contains 72 channels, you will have to do a part of the abovementioned analysis slightly differently.

The first part of the analysis (change your sampling rate and filter your data) can be done in the same way as described above. After filtering, however, make sure you **first** select the channels you want to use. Remember here that the labels do not correspond to the actual data. Instead, only the first 34 labels actually contain data; the remaining 38 labels are empty. If you wish to use EXG1 and EXG2 as your reference, type '1:34' in the field behind 'Channel range'. If you wish to use the average as your reference, type '1:32' (circled in **red** in IMAGE 5 .62).
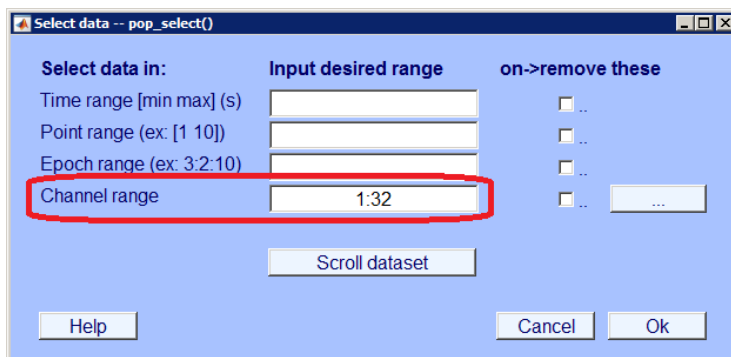

IMAGE 5.62

After selecting the number of channels you want to use, it is time to add the channel locations. Go to 'Channel locations' via 'Edit' (image 5 .63). A new dialog opens similar to IMAGE 5 .64. Click 'Ok'.
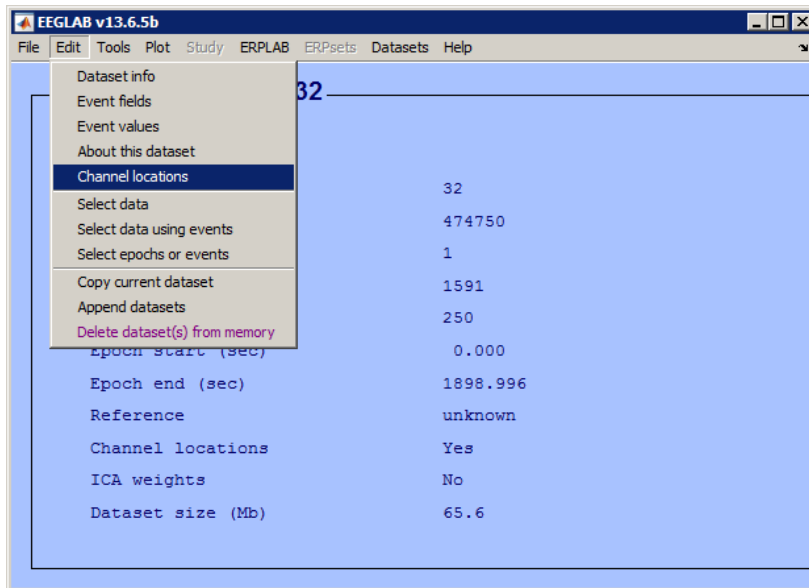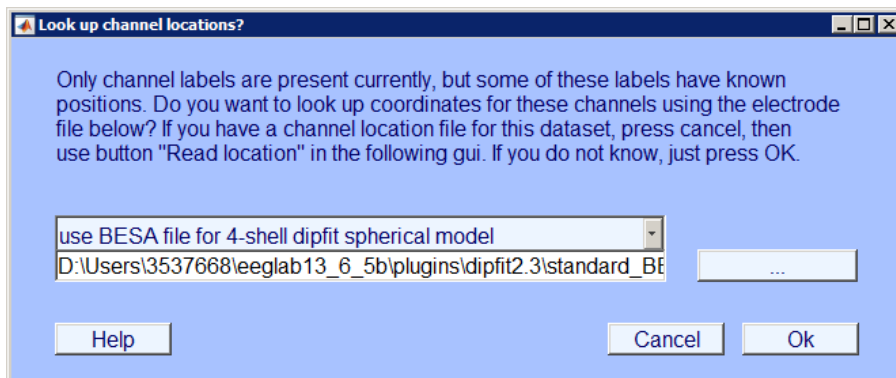
IMAGE 5.63

IMAGE 5.64

Next, a new dialog opens in which all the labels are already loaded together with their locations on the scalp (as in IMAGE 5.65). Remember, however, that these labels are **wrong** and therefore the locations are wrong as well. This means you have to change all the labels manually. You can do this by typing the correct label for each channel into the space circled in **red** in IMAGE 5.65 (you can find the label per channel here). Empty all the fields circled in **green**. You can see which channel number you're editing in the field circled in yellow. Go on to the next, or back to the previous channel by clicking the arrows circled in **blue**. Once you are done, click 'Ok'. Now, you have changed the labels, but you haven't added the channel locations yet. Go to 'channel locations' again (IMAGE 5.63) and click 'Ok' again in IMAGE 5.64. You will see that the same dialog opens again as in IMAGE 5.65, only now you will find the correct labels and location information when you scroll through the channels. You can also check this by comparing the numbers to this overview of the channel locations. Click 'Ok'.

After adding the channel locations, you can break up your data into epochs and set the reference as described above.
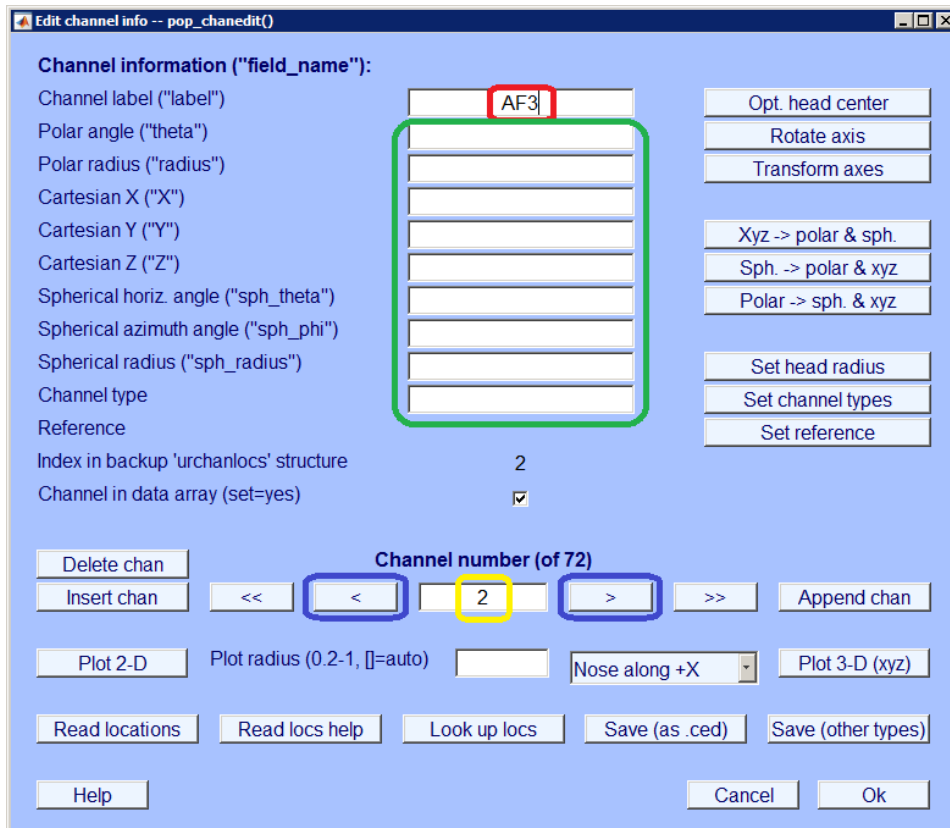
IMAGE 5.65

Finally, after setting the reference, you can check for mistakes you may have made in labelling by plotting the data. You can do this by clicking 'In scalp/rect. array' via 'Plot' and 'Channel ERPs' (IMAGE 5.66). A dialog opens similar to the one in IMAGE 5.67. Click 'Ok'. Then, another dialog opens which shows the average brainwave per channel. If everything went well, your channels should be located in the same way as in IMAGE 5.68. From now on, you can do the rest of the analysis in the same way as for the 40-channel data files.
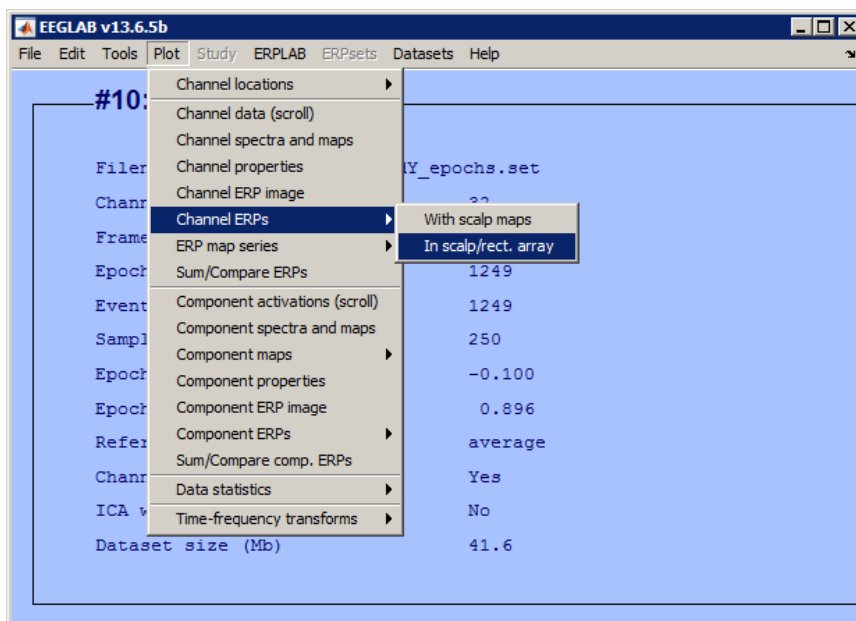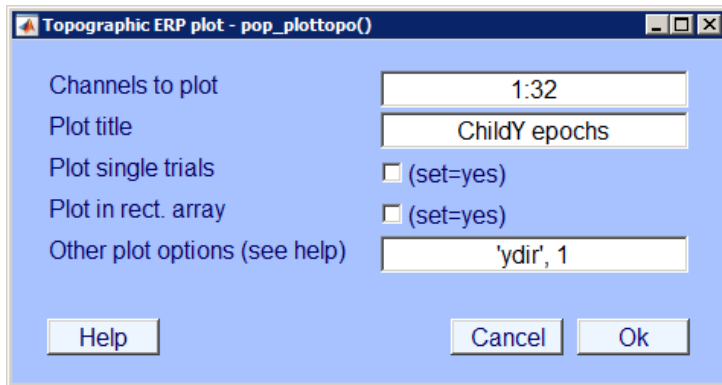

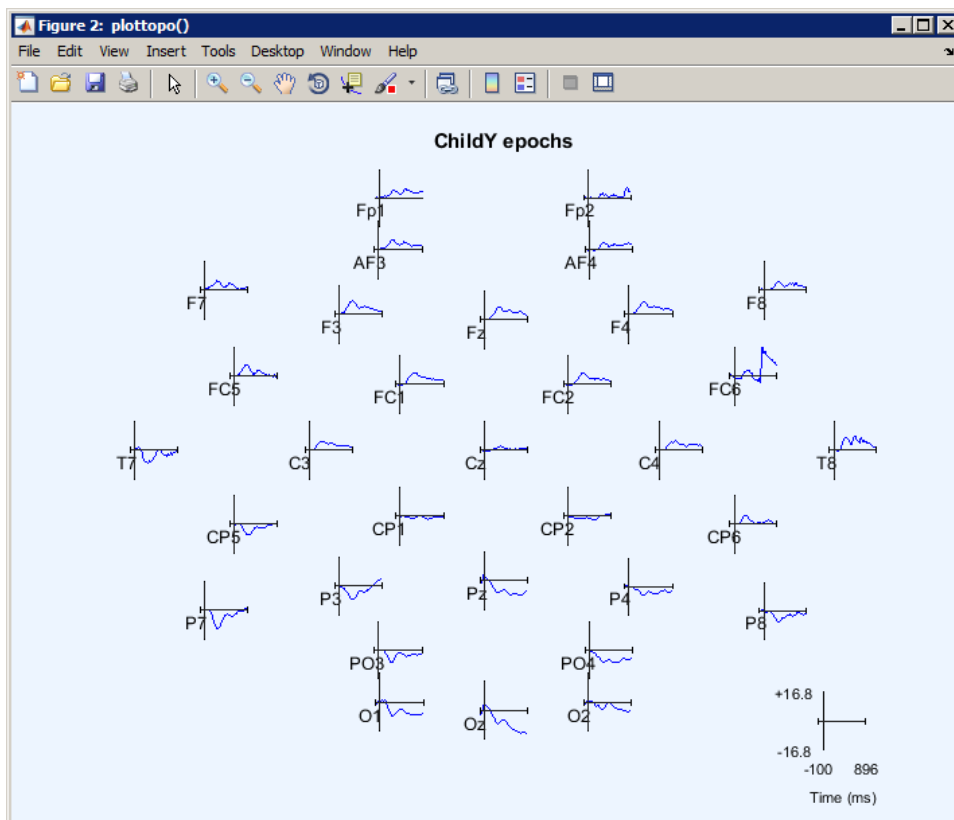**IMAGE 5.66**

IMAGE 5.67

IMAGE 5.68

Adding the channel locations manually is extremely time consuming, especially if there are more participants that have been tested with the wrong configuration file. Therefore, it is a lot easier to epoch your 72-channel data file with a script. You can either try to write your own script by clicking 'New script' in the MATLAB toolbar, or you can contact Ao Chen to ask for a standardised script. Don't forget to adapt her script to your own experiment!

5.3 Artefact reduction

After epoching your data files and saving them as .set files, it is time to remove the artefacts. If you do this step right after epoching, you can continue working in the same dataset. Otherwise, you can upload an epoched dataset by clicking 'Load existing dataset' via 'File' (**IMAGE 5.69**). Upload your '[ChildX]_epochs' set.
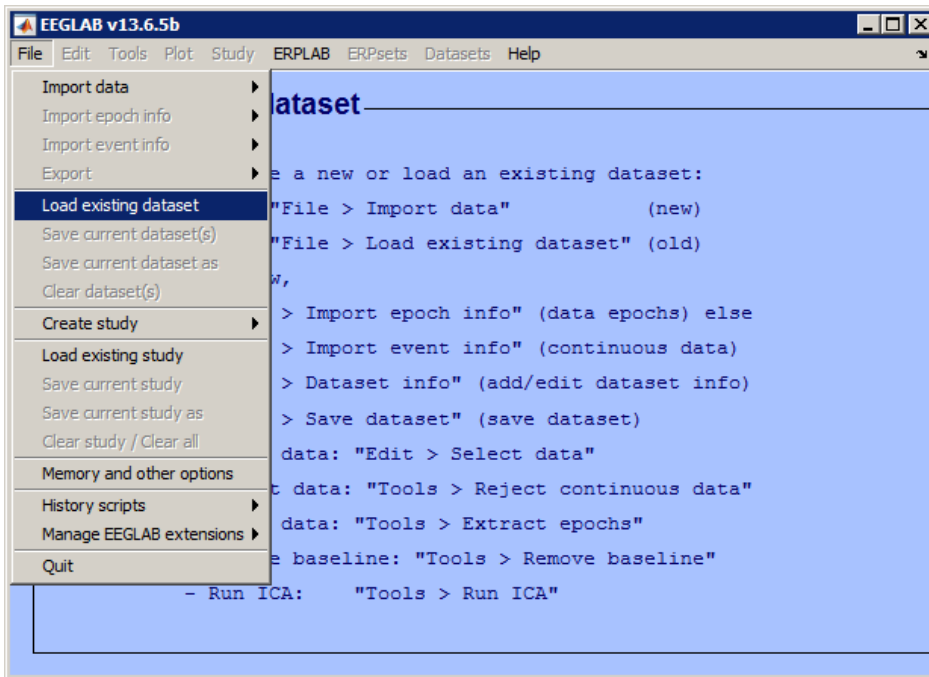
IMAGE 5.69

5.3.1 Plot your data

Your first step in removing the artefacts from your data is to plot your dataset. You can do this by going to 'Plot' in the toolbar, and click 'In scalp/rect. array' via 'Channel ERPs' (IMAGE 5.70).
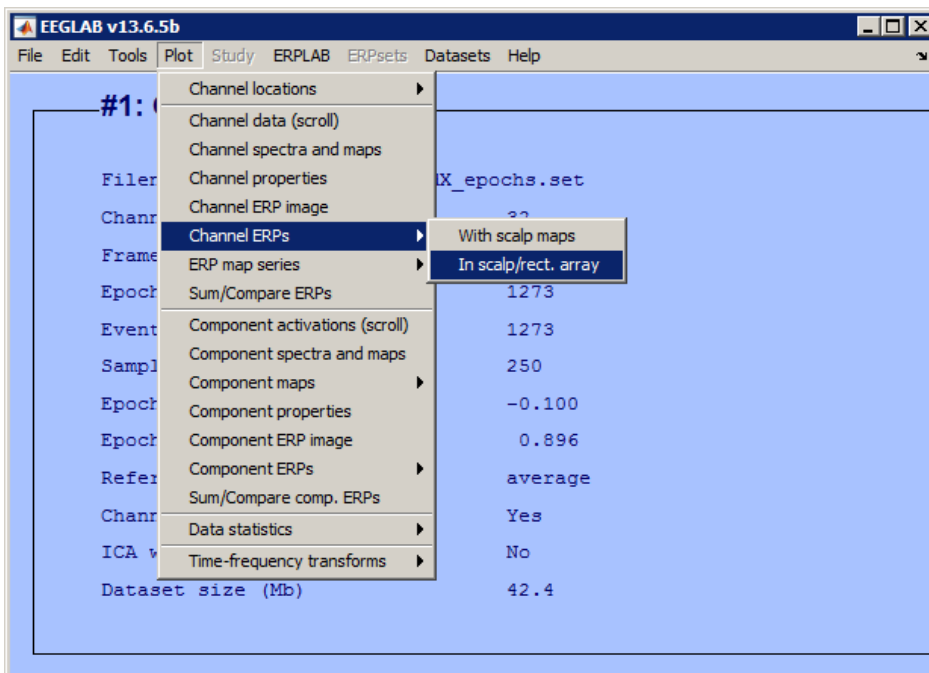

IMAGE 5.70

A dialog opens similar to the one in IMAGE 5.71. Click 'Ok'. Another dialog opens similar to the one in IMAGE 5.72, which shows the average brainwave per channel.
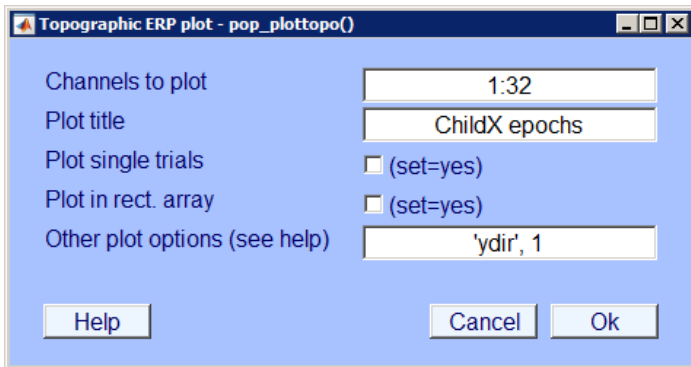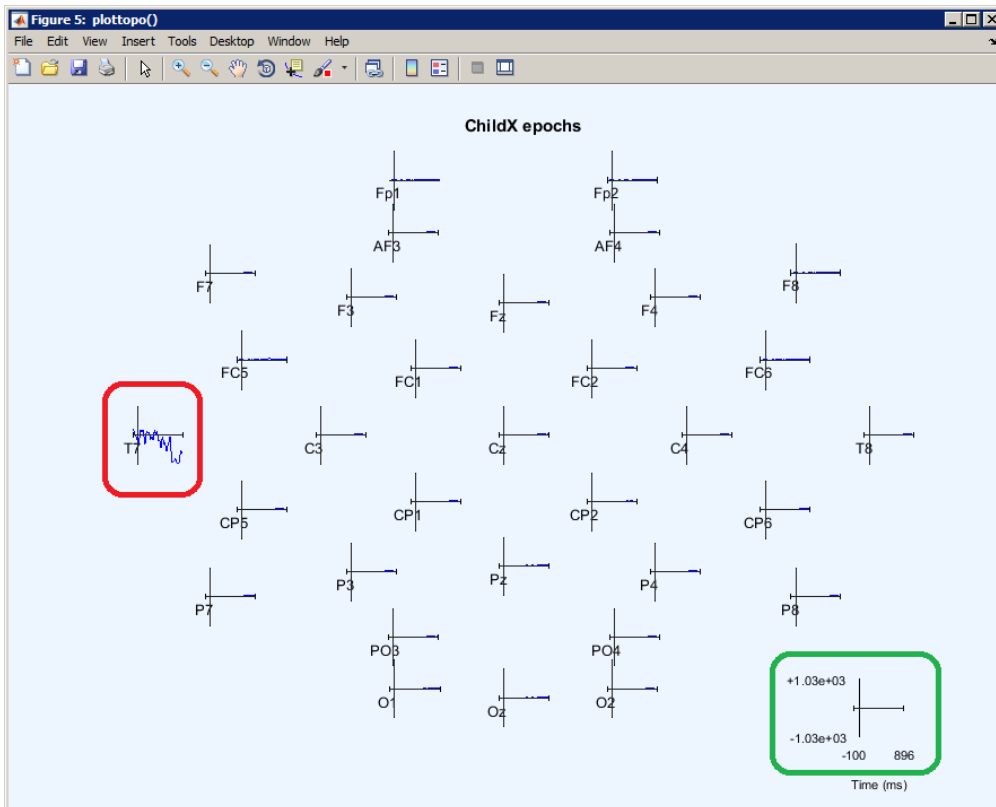
IMAGE 5.71

IMAGE 5.72

When looking at IMAGE 5.72, you will see that there is one wave (T7, circled in **red**) that looks quite different from the rest. This usually means that this channel is quite noisy compared to the other ones. Because this channel is so noisy, the y-axis of the scale circled in **green** is also extremely large. Ideally, you'll want the y-axis to be as low as possible (based on our experience, the range between -50 and +50 is usually good for further artefact reduction and averaging for infant data). Also, remember that we are using the average of all electrodes as a reference, which means that one noisy electrode affects the data of all other electrodes as well. This means that you'll have to interpolate the channel T7, to reduce the noise from your dataset.

5.3.2   Interpolate noisy channels

To interpolate means to replace the data of one channel with the average of the data of the surrounding channels. If you want to interpolate an electrode, click 'Interpolate electrodes' via 'Tools' (IMAGE 5.74).
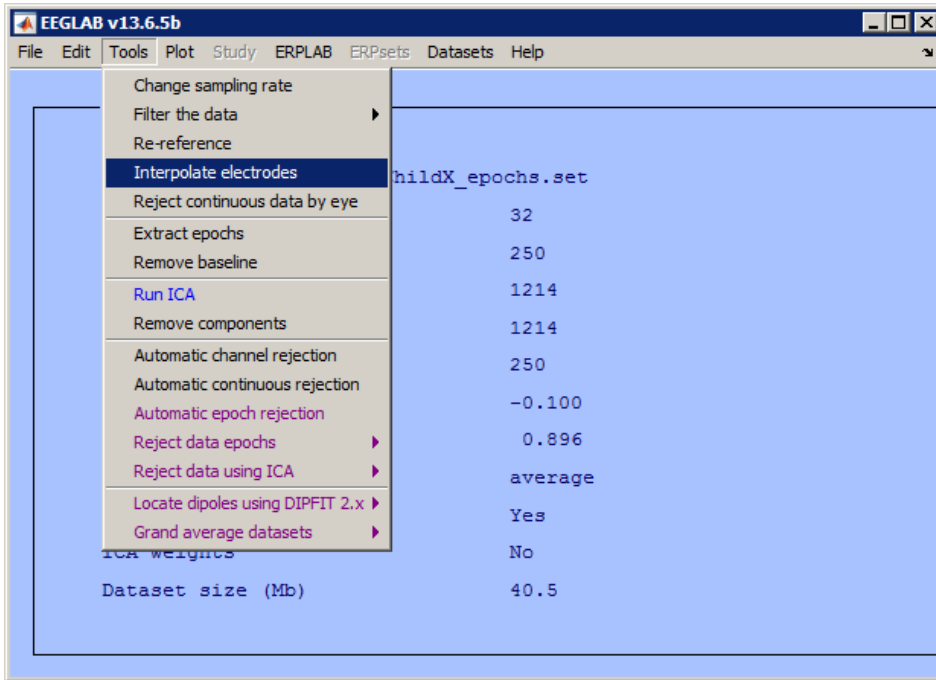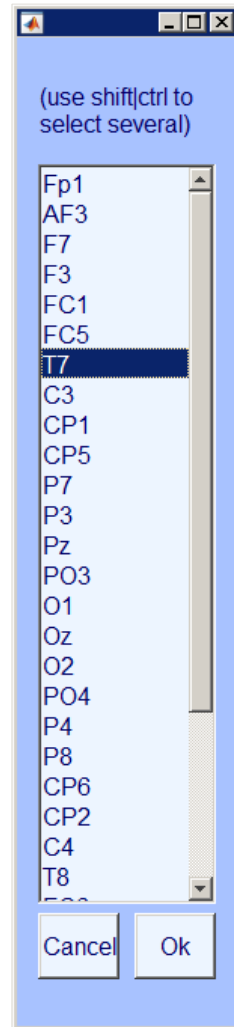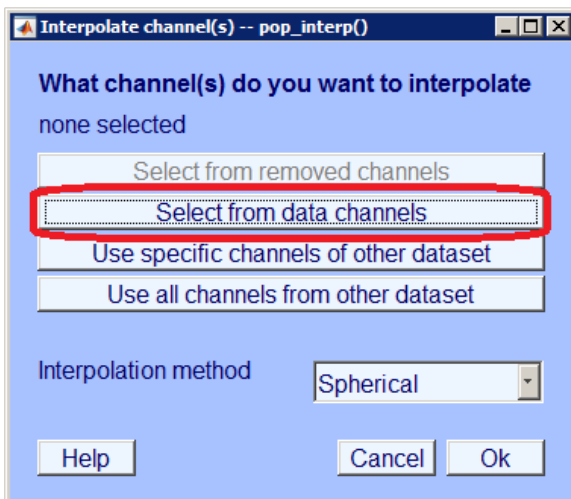
IMAGE 5.74

IMAGE 5.73

IMAGE 5.75

A popup opens similar to IMAGE 5.75. Click 'Select from data channels' (circled in **red** in IMAGE 5.75). A list containing all channels appears, similar to IMAGE 5.73. Select the channel(s) you wish to interpolate. Click 'Ok'. Then, click 'Ok' in the dialog in IMAGE 5.75. Name the new dataset as you wish.

After interpolating, plot your data again as described above. Hopefully, you will now find that all your channels look more similar to each other (as in IMAGE 5.76), and that your y-axis (circled in **green** in IMAGE 5.76) is a lot smaller than before. If, after interpolating, you find another electrode that looks very different from the rest, you can consider interpolating that electrode as well.

NOTE: We strongly recommend to get good signal when you prepare the cap before starting the experiment, rather than doing interpolation for many times offline. The less channels you have on the cap, the more the data will be distorted by interpolation. Some researchers prefer to identify and interpolate noisy channels visually by scrolling through the whole dataset, which is very time consuming for large dataset. However, such a way may provide more detail about the dataset, which may be desired for certain experiments. You need to figure out the best way for interpolation, and whether you want to interpolate in the first place, yourself.
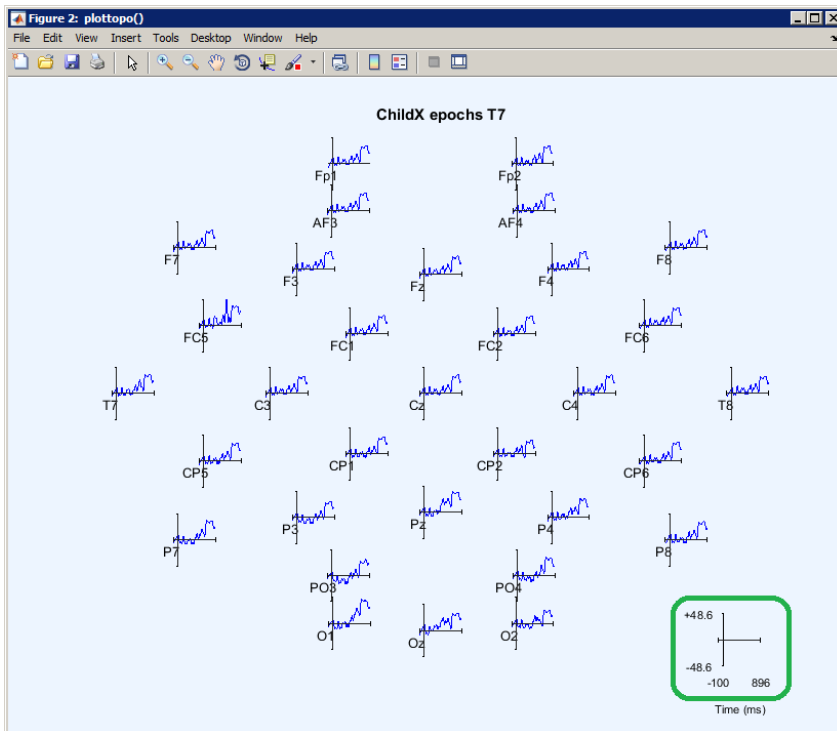
IMAGE 5.76

5.3.3 Reject extreme values

After interpolating based on your plot, it is time to look more closely to the extreme values per channel and trial. When removing artefacts based on extreme values, you get rid of the trials that contain noise. MATLAB does this by looking for each trial if one of the electrodes exceeds a certain artificial threshold. If so, it rejects the entire trial. This will clean up the noisy trials, and give you only the clean ones which you can use for the rest of your analysis.

To remove the artefacts, go to 'Reject data epochs' via 'Tools' and click 'Reject extreme values' (**IMAGE 5.77**).
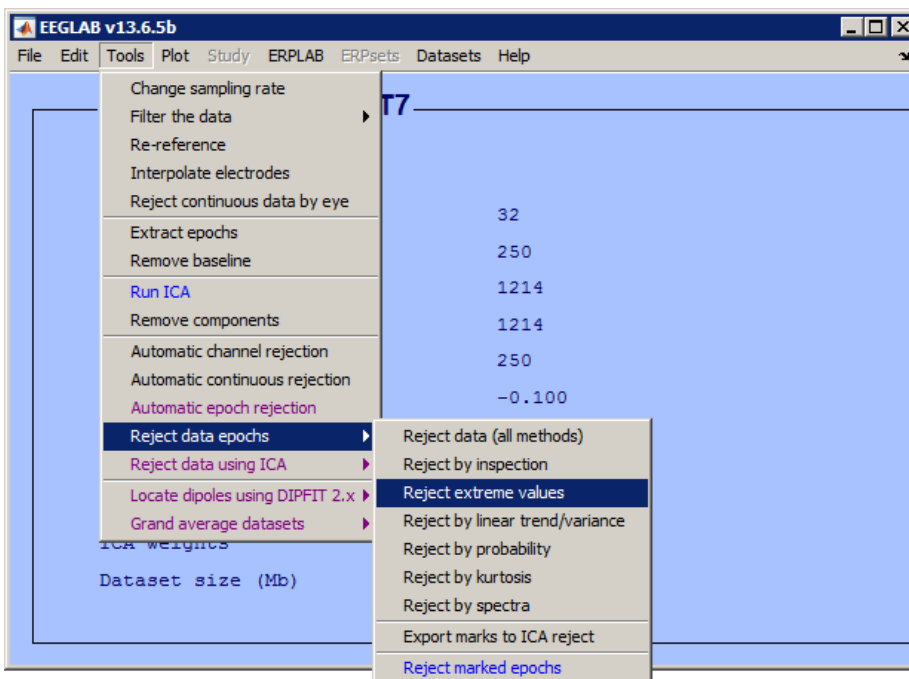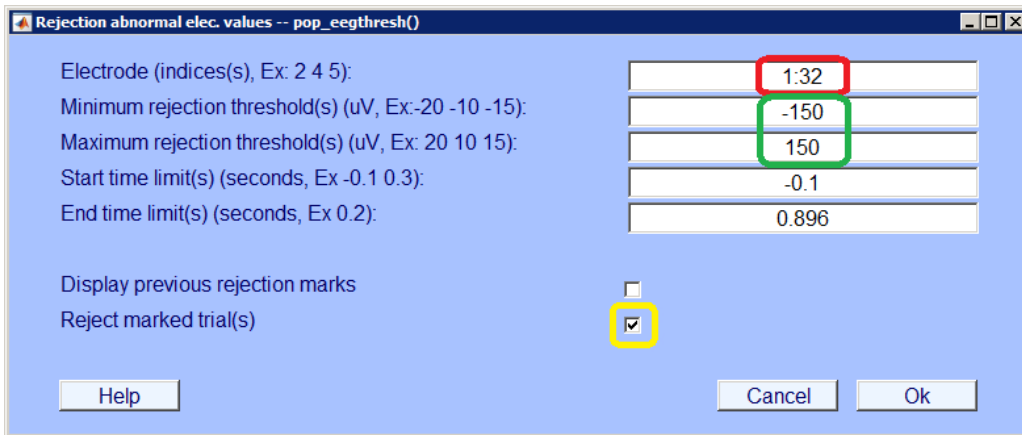

IMAGE 5.77

IMAGE 5.78

A dialog opens similar to IMAGE 5 .78. In the area circled in **red**, type in the numbers of the electrodes you want to look at. If you need all your electrodes to be without noise for your further analysis, select all electrodes (1:32). This will give you the cleanest data, but more rejected trials. If you only want to look at a few electrodes, you can also decide to reject trials based on only the surrounding electrodes. For instance, if you are mainly interested in the frontal electrodes, and you notice that in a lot of children the posterior electrodes are very noisy, you can decide to base the rejection of trials only on the frontal and central electrodes. This will probably give you fewer rejections. However, your overall EEG data will be less clean.

Next, decide what you want to use as a minimum and maximum threshold (circled in **green** in IMAGE 5 .78). Per trial, MATLAB will look if there is an electrode that exceeds this threshold, and if there is, the entire trial will be rejected. For our analysis, we will use +150 and -150 as our thresholds. You may decide to do otherwise.

Tick the box circled in **yellow** in IMAGE 5 .78. This does not mean that the noisy trials will be removed immediately, but it will give you the opportunity to remove them after scrolling through the rejected trials. Then, click 'Ok'.
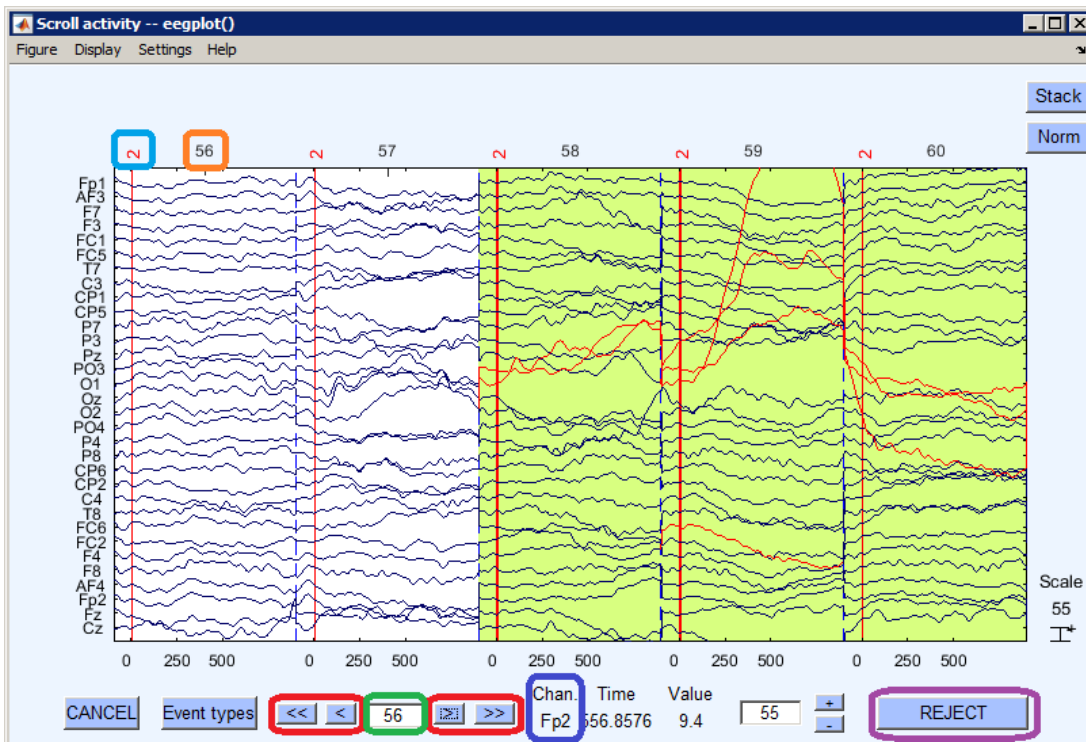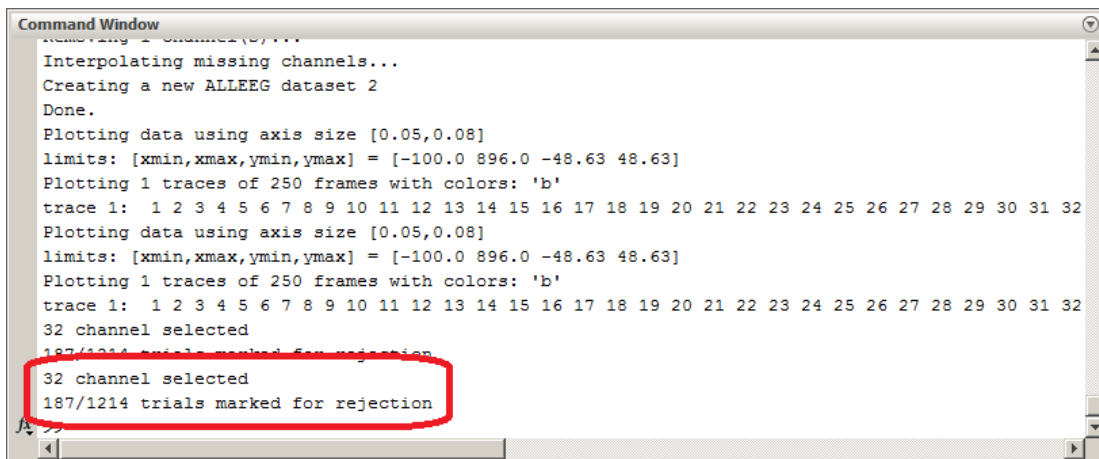

IMAGE 5.79

In IMAGE 5.79, you can see the data after looking for extreme values. In here, you find the signal of all selected electrodes per trial. The trial number is indicated by the number circled in **orange**, and the trials are separated by a dashed blue line. The red line indicates the onset of the stimulus, and the red number circled in **light blue** indicates the stimulus type. In this image, you see that some trials are shaded in green and some are white. The green ones indicate rejected trials. The red signals within the green trials indicate the electrodes that exceeded the threshold in that particular trial, which account for the rejection of that trial. Trial 58, for example, is rejected because both PO3 and O1 exceeded the threshold here. Which electrode belongs to which signal may be difficult to see sometimes, but if you go to the signal line with your cursor, the label will appear in the area circled in **dark blue**. You can scroll through the trials by clicking the arrows circled in **red**, or by typing a trial number in the area circled in **green**. By scrolling through the dataset, you can find out if there is a particular electrode that accounts for many rejected trials. For instance, if you see that PO3 and O1 are the cause for many of the rejections, you can consider interpolating these two channels as described above. If you look at the rejected trials again after interpolating, you can see if it helped you get more clean trials. If the difference is negligible, however, use the original data for those electrodes.

IMAGE 5.80

If you go back to your command window in MATLAB, you can see exactly how many trials are rejected (circled in **red** in IMAGE 5.80). Ideally this number is as small as possible, but as children tend to move a lot, there will always be rejected trials. Decide how many accepted trials are necessary for your further analysis. Based on this, you can either decide to use a less strict rejection criterion (for instance by rejecting trials based on only a part of the electrodes), or exclude the participant, if the number of accepted trials is too low. **NOTE:** your rejection criterion has to be the same for all participants!

If you are happy with the number of rejected trials, and no other electrodes need to be interpolated, click 'Reject' (circled in **purple** in IMAGE 5.79). Save your new dataset as '[ChildX]_artfree.set'.

Unfortunately, this part cannot be done with help of a script, as you need to decide for each individual dataset which electrode(s) you need to interpolate. This will be different for every dataset, and for every dataset the criteria for interpolation may be different as well. Try to find a good balance between interpolating electrodes in order to get less rejected trials, and preserving your original data as good as possible. This is may be difficult sometimes and is usually primarily based on experience.

5.4 Grand averages

5.4.1 Separate according to stimulus type

Next up is to extract the different stimulus types from your total dataset. In our research, we used 9 different types of stimuli, of which 3 were removed earlier when extracting the epochs. Therefore, 6 stimulus types still remain for our further analysis:

Event number	Stimulus type
2	Lexical tone: standard that follows another standard
3	Lexical tone: deviant
5	Music: standard that follows another standard
6	Music: deviant
14	Vowel: standard that follows another standard
15	Vowel: deviant

These six stimulus types are now still combined into one large dataset. To compare the ERPs for different stimulus types, however, it is necessary to break the large dataset into smaller datasets which contain only one single stimulus type.

To extract all epochs with the same stimulus type from the larger dataset, go to 'Select epochs or events' via 'Edit' (IMAGE 5.81).
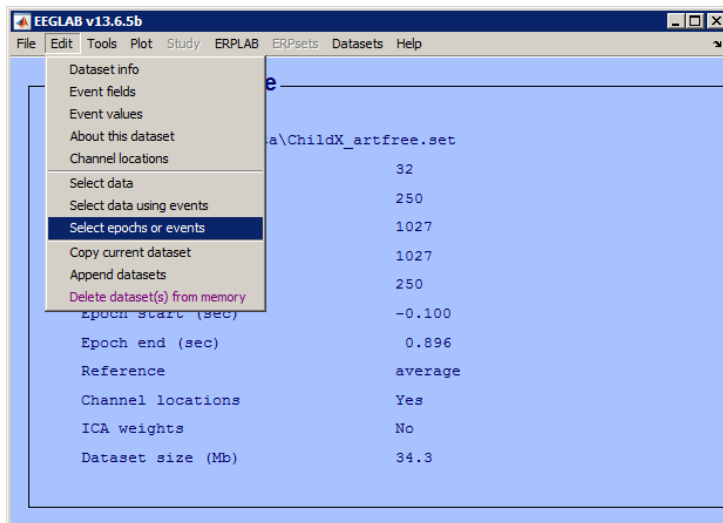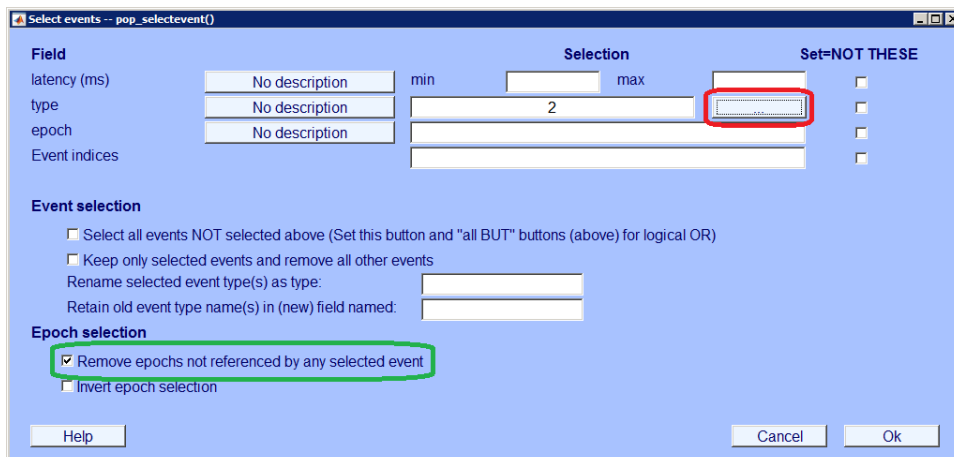

IMAGE 5.81

IMAGE 5.82

A window pops up similar to IMAGE 5.82. Select the event number you want to extract from the dataset by clicking the button circled in **red** in IMAGE 5.82 or by typing the event number in the blank next to it. Click 'Remove epochs not referenced by any selected event' (circled in **green** in IMAGE 5.82). Click 'Ok'. A warning pops up like in IMAGE 5.83. Click 'Ok'.
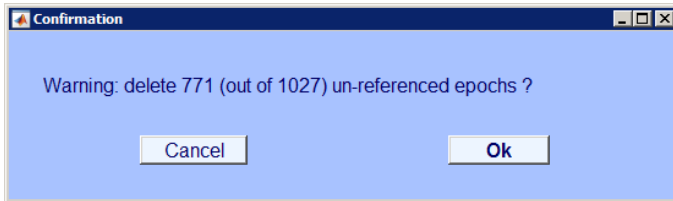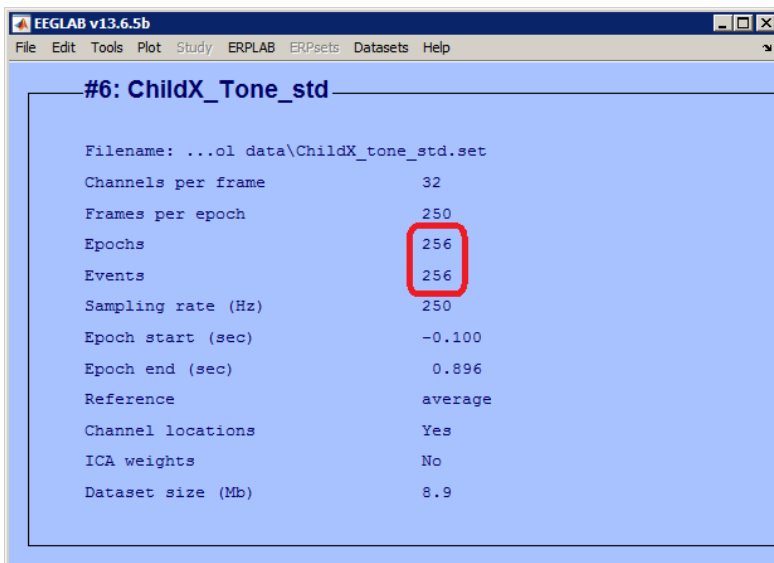

IMAGE 5.83

IMAGE 5.84

Add something to your new dataset's name to remind you of the stimulus type it contains. You will see that the number of events has changed drastically: ours has changed from 1027 accepted epochs to 256 epochs (circled in **red** in IMAGE 5.84). This means that there are 256 accepted trials containing the standard lexical tone (event number 2).
Repeat this step for all of your stimulus types. In our research, we will eventually end up with six different datasets for each participant.

This step may take a long time when done manually. It can also quite easily be done with a script. Write your own script, or contact Ao Chen if you want to make use of a standardised script. Note that you still have to adapt her script to your own experiment!

5.4.2   Concatenate into grand averages
Once you have completed all steps described above for every participant, you will have a nice overview of the amount of accepted trials per stimulus type per participant. Decide what the minimum amount of accepted trials per person per condition should be, and in- or exclude your participants based on this amount.

Once you have decided who to include and who to exclude, you can make a grand average file per stimulus type. Go to 'Grand average datasets' via 'Tools' and click 'Grand average datasets from files' (IMAGE 5.85).
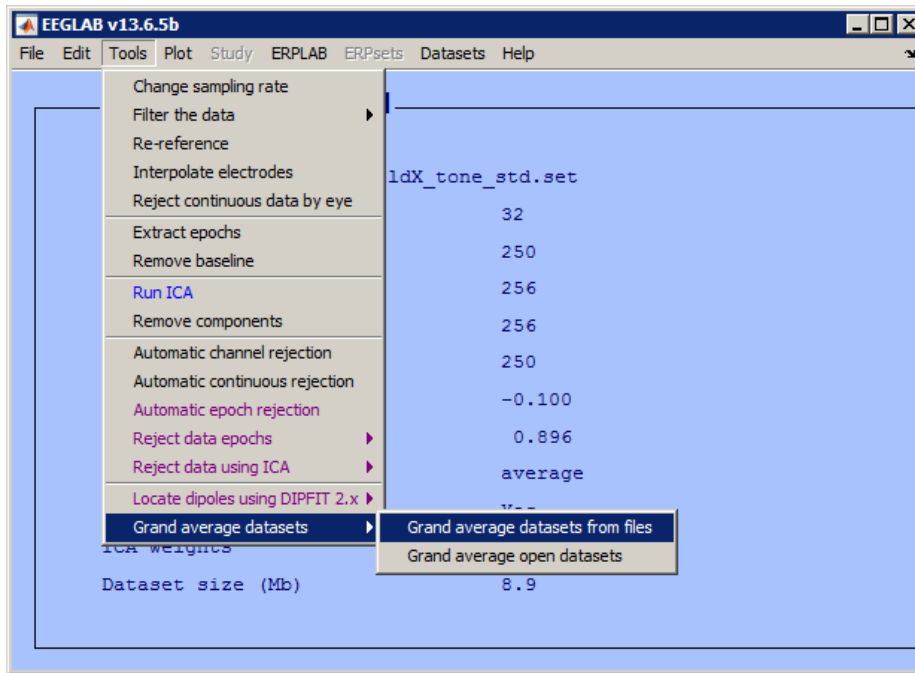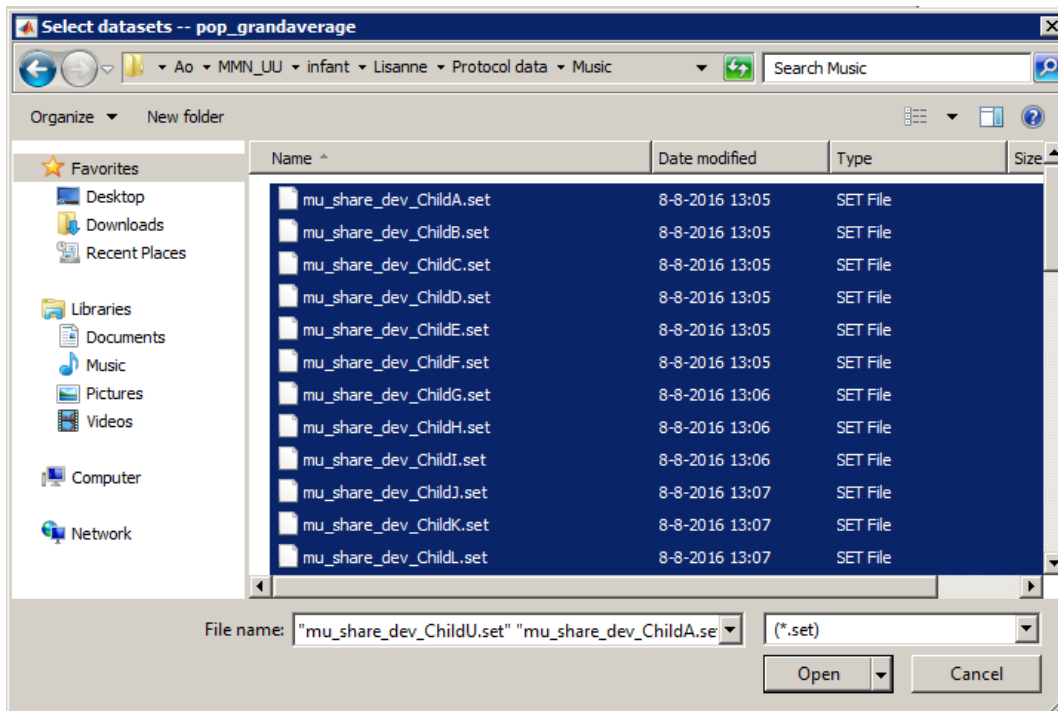
IMAGE 5.85

IMAGE 5.86

Next, select all included datasets from the same stimulus type (IMAGE 5.86). Click 'Open'. A new dialog opens as in IMAGE 5.87. Name your grand average file as you wish, but make sure you remember which data it contains. Also, save the new dataset as a file.
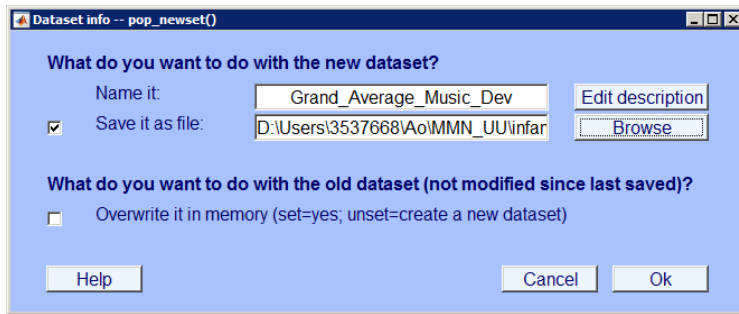
IMAGE 5.87

A new dataset will open, in which you will see that the number of epochs is now the same as the number of included participants (circled in **red** in IMAGE 5.88). This is because in the previous step, the average ERP was calculated for every participant, after which all averages were combined to make up the grand average. Every participant therefore now represents one epoch. Repeat the same step for all your stimulus types.
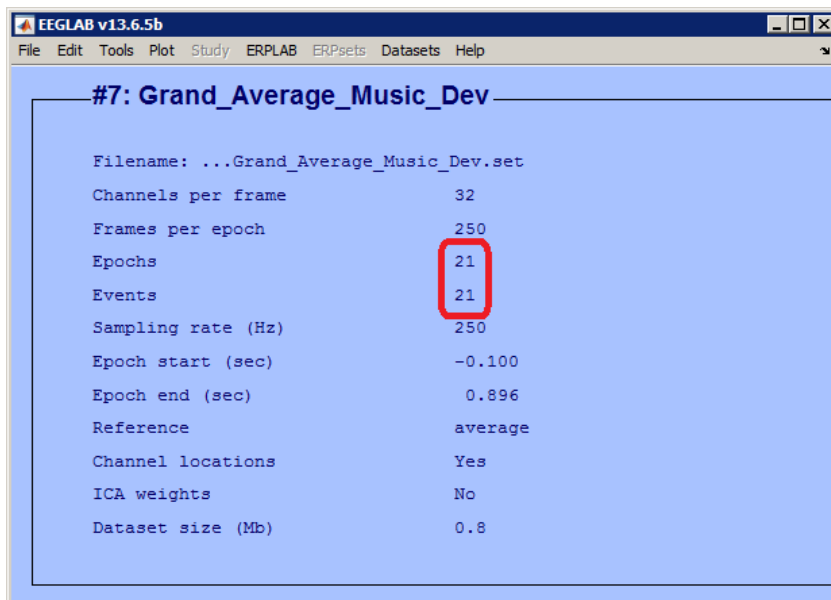
IMAGE 5.88

5.5 Plotting graphs

After converting your data into grand averages, you can have a first look at your results by plotting two (or more) stimulus types against each other. In our research, we are interested in the difference between ERPs following a standard and a deviant musical stimulus. Therefore, we have made a grand average of all accepted ERPs following the standard stimulus, and one for all the accepted ERPs following the deviant stimulus.

First of all, make sure you have loaded all the grand average datasets that you want to plot or compare in EEGLAB. Next, click 'Sum/Compare ERPs' via 'Plot' in the toolbar (IMAGE 5.89). A window opens, similar to IMAGE 5.90, in which you can define several options for your plot.

IMAGE 5.89

IMAGE 5.90

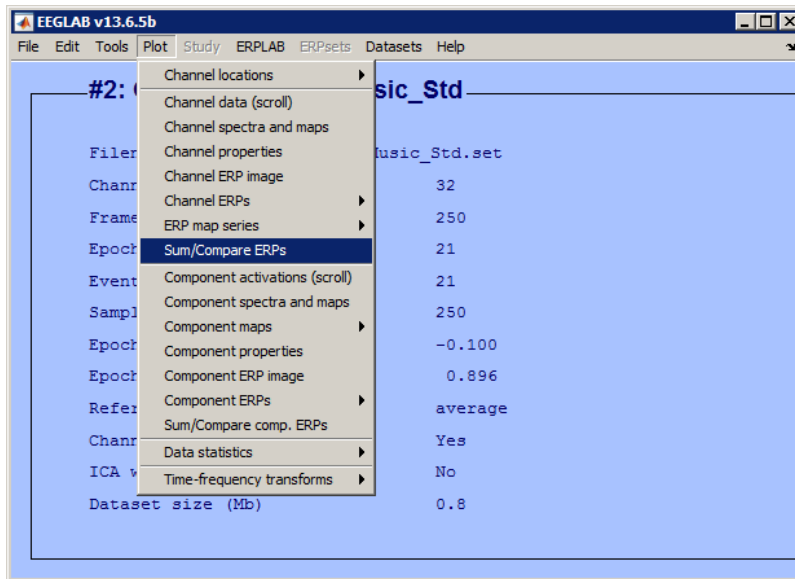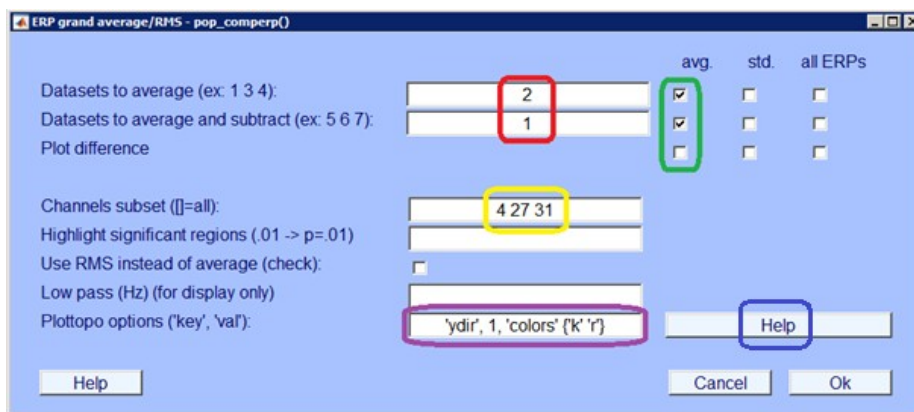In the areas circled in **red** in IMAGE 5.90, insert the numbers of the datasets you want to compare against each other. If you are not sure which number belongs to which dataset, you can go to 'Datasets' in the EEGLAB toolbar and look up the numbers (circled in **red** in IMAGE 5.91). The dataset number is also indicated in the area circled in **green** in IMAGE 5.91.

In the area circled in **green** in IMAGE 5.90, indicate what kind of data you want to see in the plot. You can choose to either see the average of both datasets plotted against each other, or plot the difference between the two (or show all three waves together by ticking all three boxes). Other possibilities are to plot the standard deviation (instead of the average), or plot all ERPs within one dataset.

In the area circled in **yellow** in IMAGE 5.90, indicate which channels you want to plot. If you don't insert anything here, all 32 channels will be visible in your plot. Because we are only interested in F3, Fz and F4, we type the <u>corresponding numbers</u> here so that only these channels will be visible.

In the area circled in **purple** in IMAGE 5.90, you can insert a few things that will change the look of your plot, such as direction and colour. By default, there will be *'ydir', -1* typed here. This means that your y-axis will have positive values down and negative values up. By removing the minus sign (as seen in IMAGE 5.90), your y-axis will be turned upside down, so that positive values are up and negative values are down. Also, by default the colours of your two plotlines will be green and blue, which can be hard to distinguish. By typing *'colors' {'k' 'r'}* – or any other <u>letters that resemble certain colours</u> – the colours will be changed, in this case to black (k) and red (r). There are more options to restyle your plot. An overview of all of these options can be found in the 'Help' button (circled in **blue** in IMAGE 5.90). You can play with these options and see which look satisfies your needs.

IMAGE 5.91

IMAGE 5.92
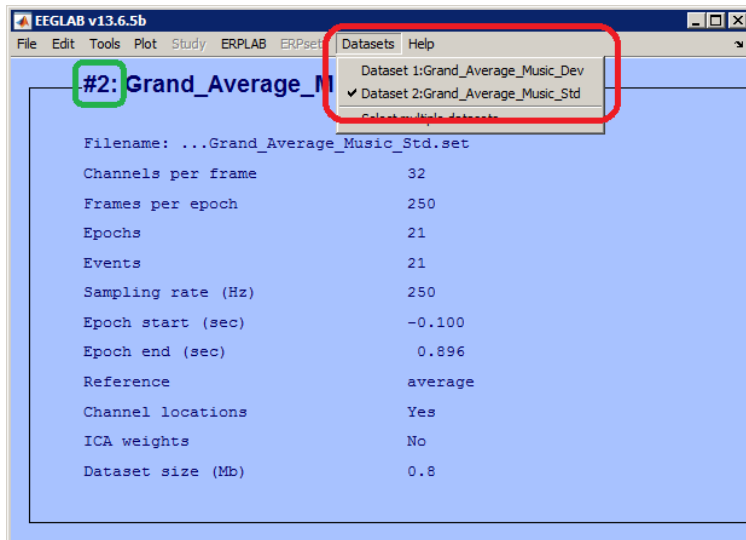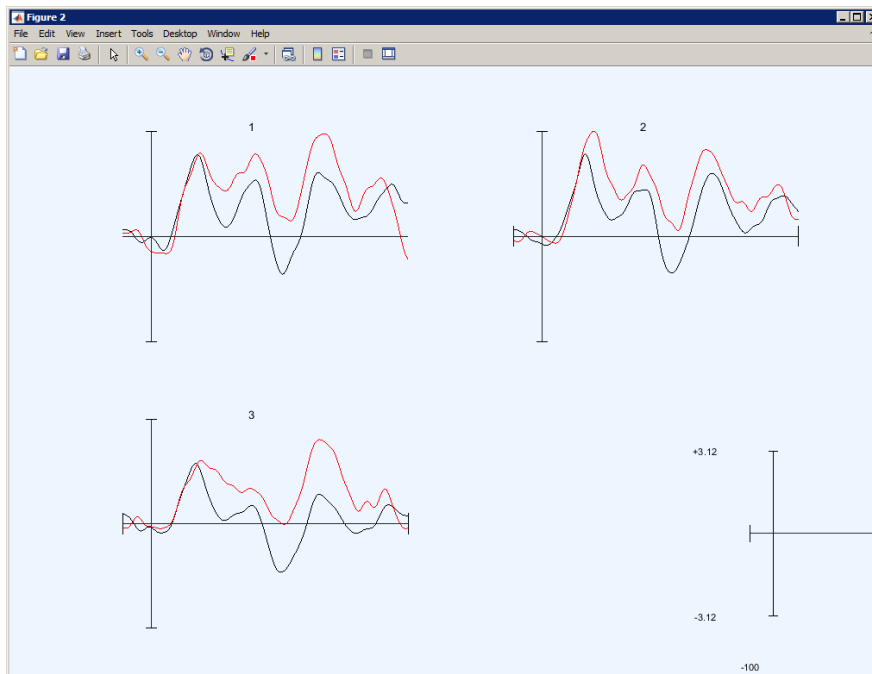
After you have clicked 'Ok', you can finally see the output of your research in a graph (IMAGE 5.92). If you have selected only certain channels to display, these channels will be indicated by numbers in the order you typed them in IMAGE 5.90 instead of their labels. You can click on one of these channels to enlarge them and to see which colour represents which stimulus type (IMAGE 5.93).
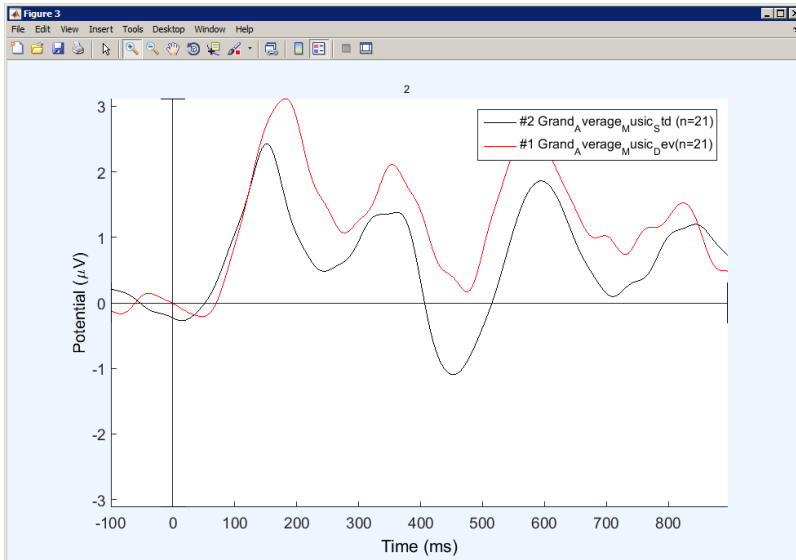
IMAGE 5.93

5.6 ERPLAB: Prepare your data

Once you have plotted your data, you finally have a first raw impression of your research outcome. However, statistics are needed to conclude whether the differences between ERPs are significant. In order to do statistics, you need to use the plugin ERPLAB.

5.6.1   Create binfile

First of all, open Notepad in Windows and create a .txt file in which you specify the different 'bins' in your output. A 'bin' contains averaged ERP waveforms for every channel, which are based on a certain type of data or event. State the bin number, the bin label and the event number as indicated in the .set files. Use the format as shown in IMAGE 5.94. A more elaborate description of possible bin formats can be found on the website of erplab.
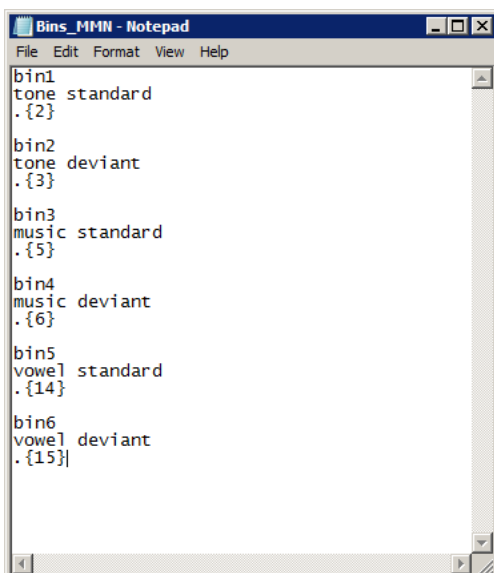
IMAGE 5.94

5.6.2   Convert artefact free files to ERPsets

In order to use ERPLAB, you first need to convert your datasets into .erp files. For the conversion, we will use the artefact free datasets that you saved underlined earlier. Open EEGLAB in MATLAB and load an existing artefact free file. Next, go to 'ERPLAB', click 'Utilities' and then click 'Convert an epoched dataset into a continuous one' (IMAGE 5.95). You will probably see a warning in yellow saying that you are about to concatenate an epoched dataset. Click 'Yes'.
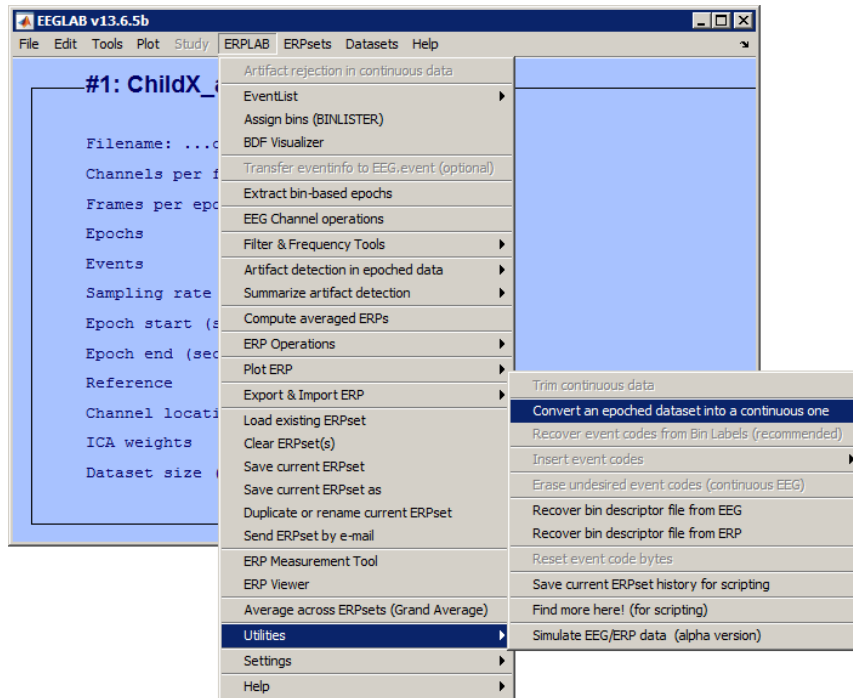
IMAGE 5.95

After creating a continuous dataset, you have to create an event list. Go to 'Create EEG EVENTLIST' via 'ERPLAB' and 'Eventlist' (IMAGE 5.96). You may see a yellow warning pop up which asks you to change the labels of your events. Ignore this for now and click 'Continue'.
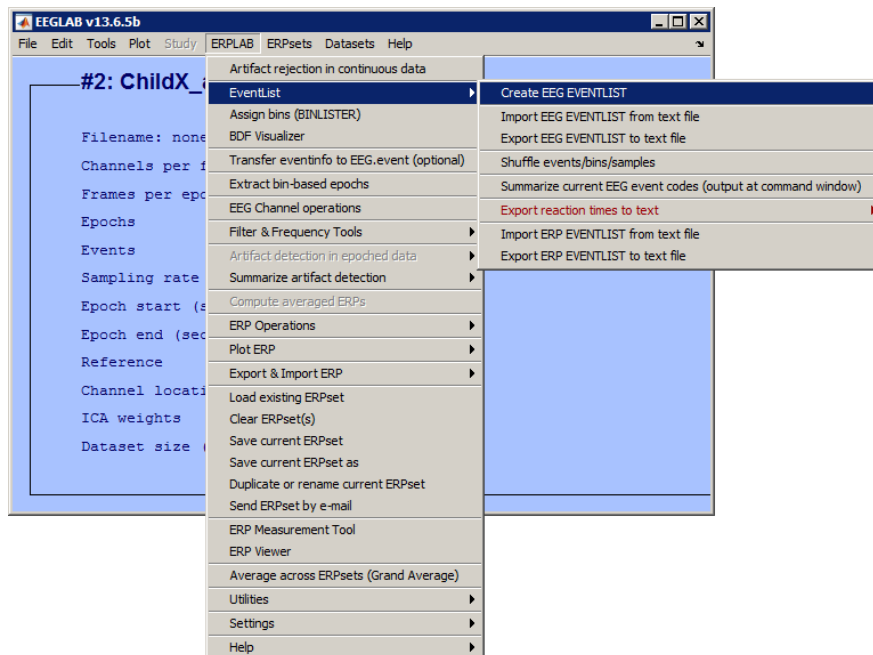
IMAGE 5.96
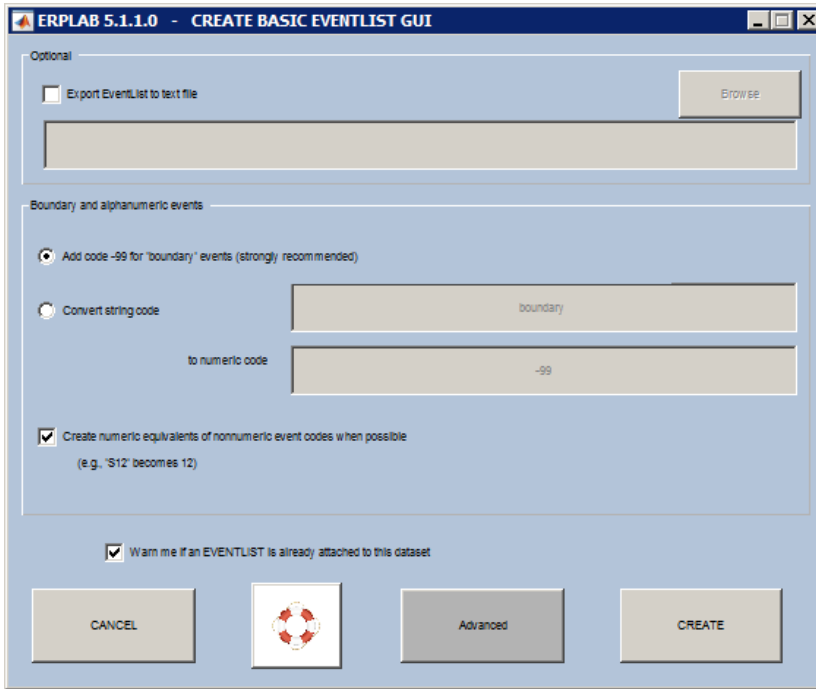
IMAGE 5.97

A pop-up opens, similar to IMAGE 5.97. Tick the boxes 'Add code -99 for boundary events' and 'Create numeric equivalents of nonnumeric event codes when possible', as shown in IMAGE 5.97. Click 'Create'.

Next, you need to assign the bins to your new dataset. Go to 'ERPLAB' and click 'Assign bins (BINLISTER)' (IMAGE 5.98).
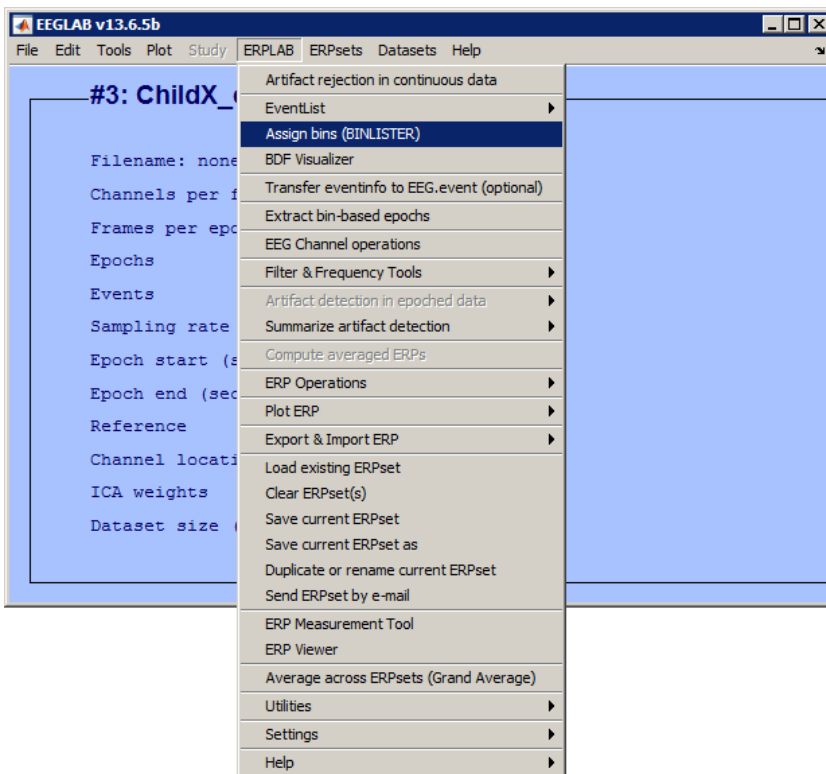
IMAGE 5.98
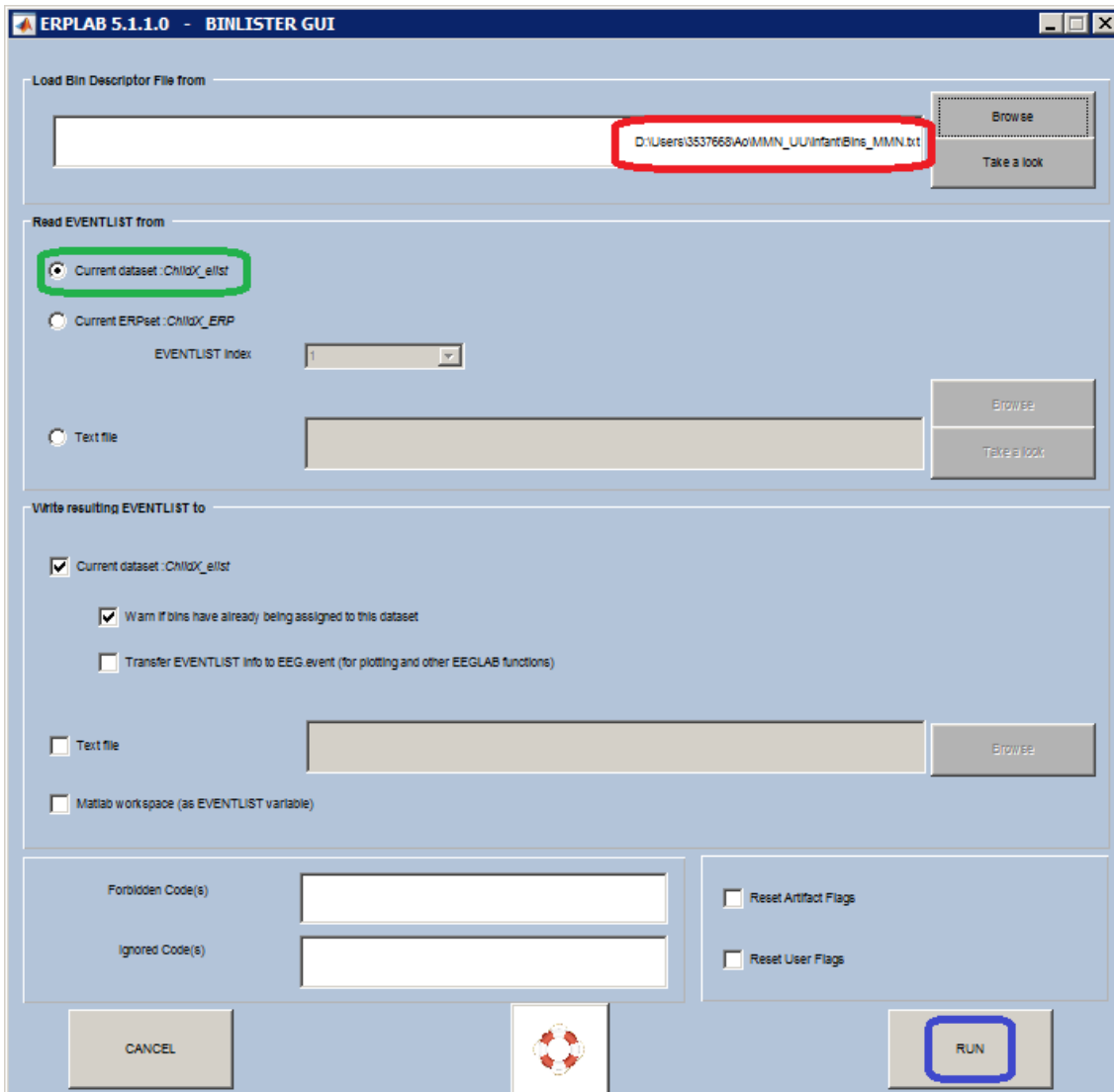
IMAGE 5.99

A window opens similar to IMAGE 5.99. In the area circled in **red**, load the .txt file in which you specified your bins. The box 'Current dataset' (circled in **green**) should be ticked. Click 'run' (circled in **blue**).

Now, you can epoch your data again. Go to ERPLAB in the EEGLAB toolbar and click 'Extract bin-based epochs' (IMAGE 5.100). A new dialog opens, similar to IMAGE 5.101. Specify the time range (in milliseconds) of your epochs (circled in **red** in IMAGE 5.101). Click 'run'.
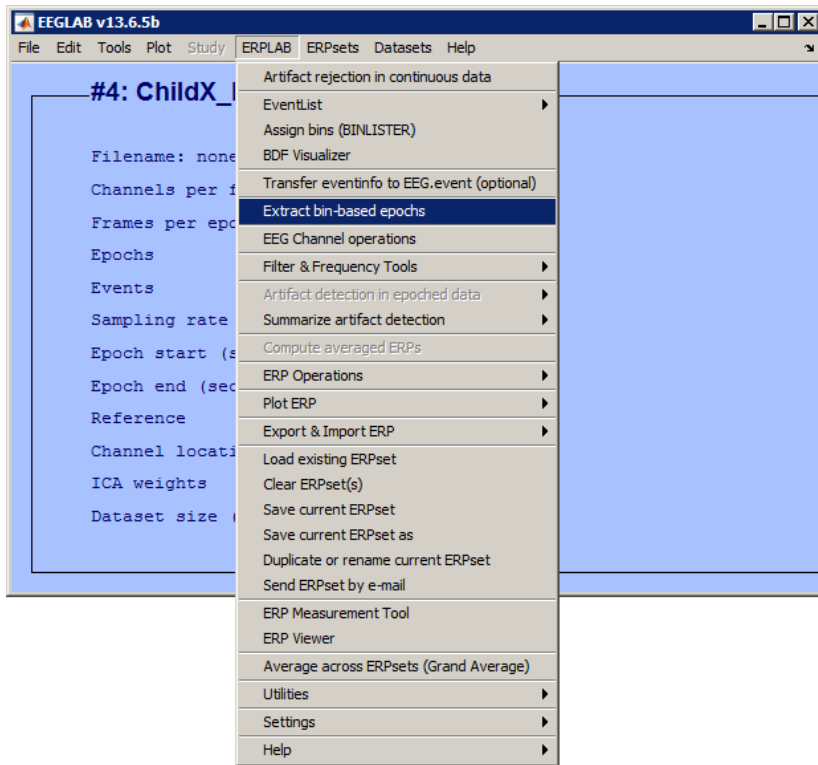
IMAGE 5.100
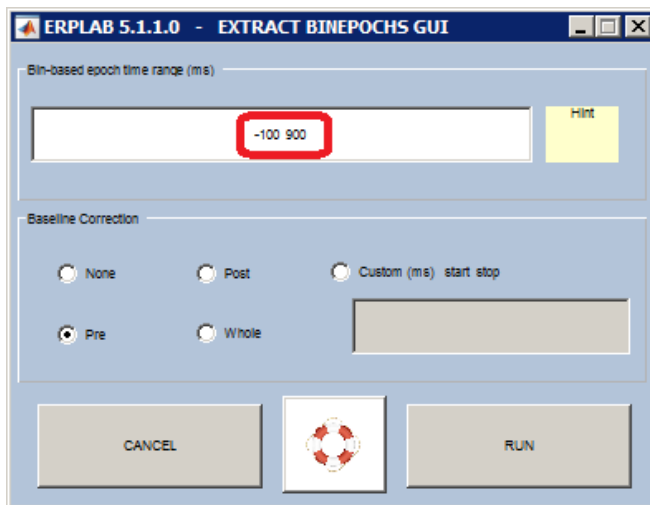
IMAGE 5.101

Now, the last thing you have to do is to compute the averaged ERPs, which will finally turn your dataset into an ERPset. Go to 'ERPLAB' in the toolbar and click 'Compute averaged ERPs' (IMAGE 5.102). A dialog similar to IMAGE 5.103 will pop up. Make sure the correct number of the to-be-averaged dataset is written in the blank circled in **green** in IMAGE 5.103 (in this case, number '5', as seen in the title of the dialog in IMAGE 5.102). Also, tick the boxes 'include ALL epochs (ignore artefact detections)' and 'include standard error of the mean' (both circled in **red** in IMAGE 5.103). Click 'run'.
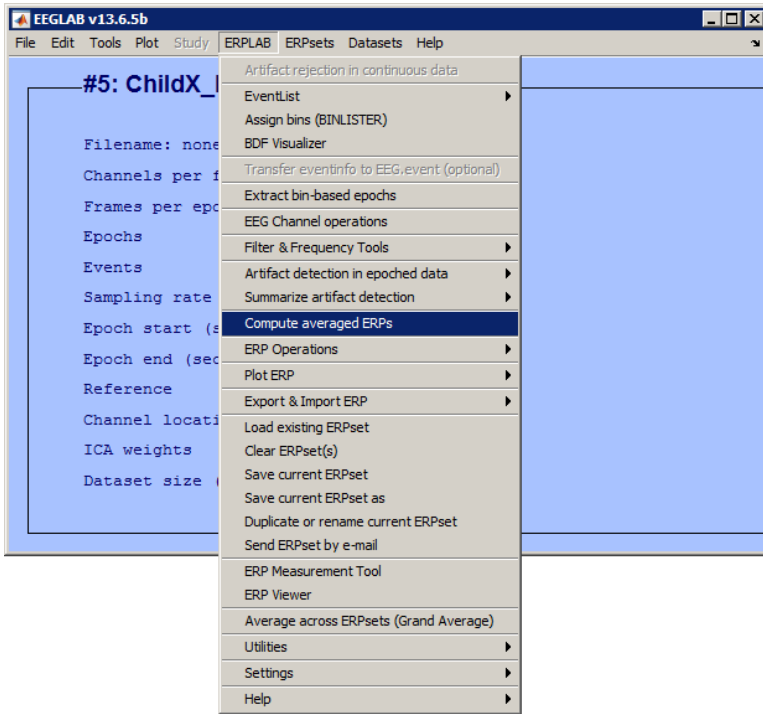
IMAGE 5.102
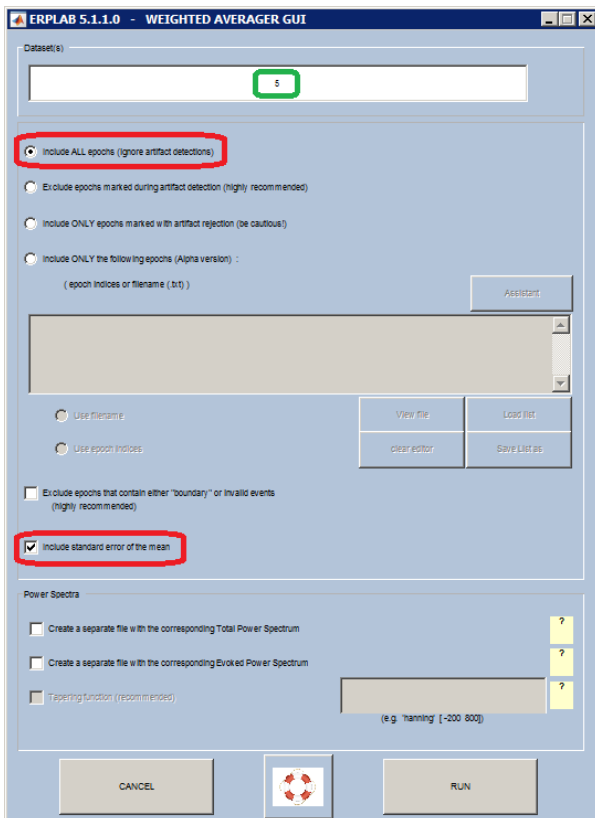
IMAGE 5.103

A dialog will pop up similar to IMAGE 5.104. This window allows you to name and save your new ERPset. Click 'create a new erpset' (circled in **red** in IMAGE 5.104) and type in the name of your new ERPset in the blank. Below, click 'save ERP as' (circled in **green**), click 'browse' and save your new ERPset in your data folder. Click 'Ok'. Your artefact free dataset is now converted into an .erp file.

IMAGE 5.104

This step can easily be done with a script, which will save you a lot of time. Write your own script, or contact Ao Chen if you want to make use of a standardised script. Note that you still have to adapt her script to your own experiment!

5.6.3 Add difference waves to bins

As described earlier, our ERPsets now contain six bins: the standard and deviant stimuli of the lexical tones, the standard and deviant stimuli of the musical melodies, and the standard and deviant stimuli of the vowels. However, we are interested in the *differences* between the standards and deviants. Therefore, we have to add another bin to the file which calculates the difference wave for every modality. In our case, this means three extra bins: one for the lexical tones, one for the musical melodies and one for the vowels.

To add new bins to your ERPset, go to 'ERPLAB' in the toolbar, go to 'ERP operations' and click 'ERP bin operations' (IMAGE 5.105).
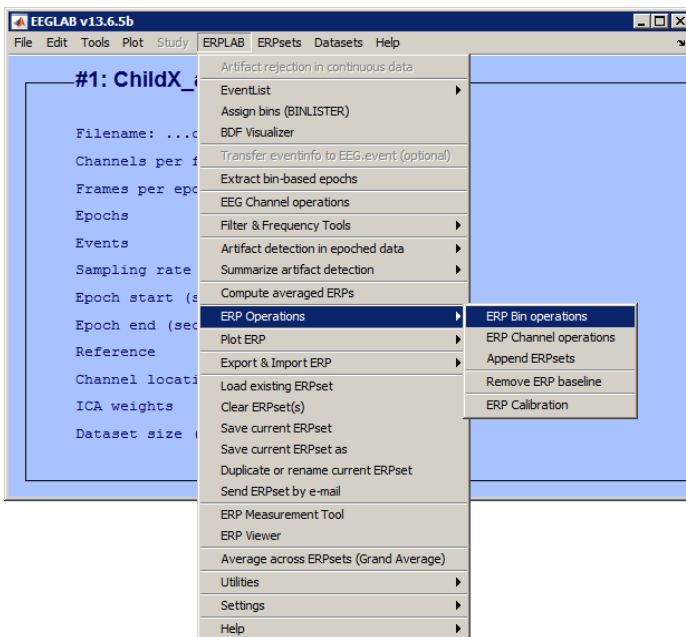
IMAGE 5.105
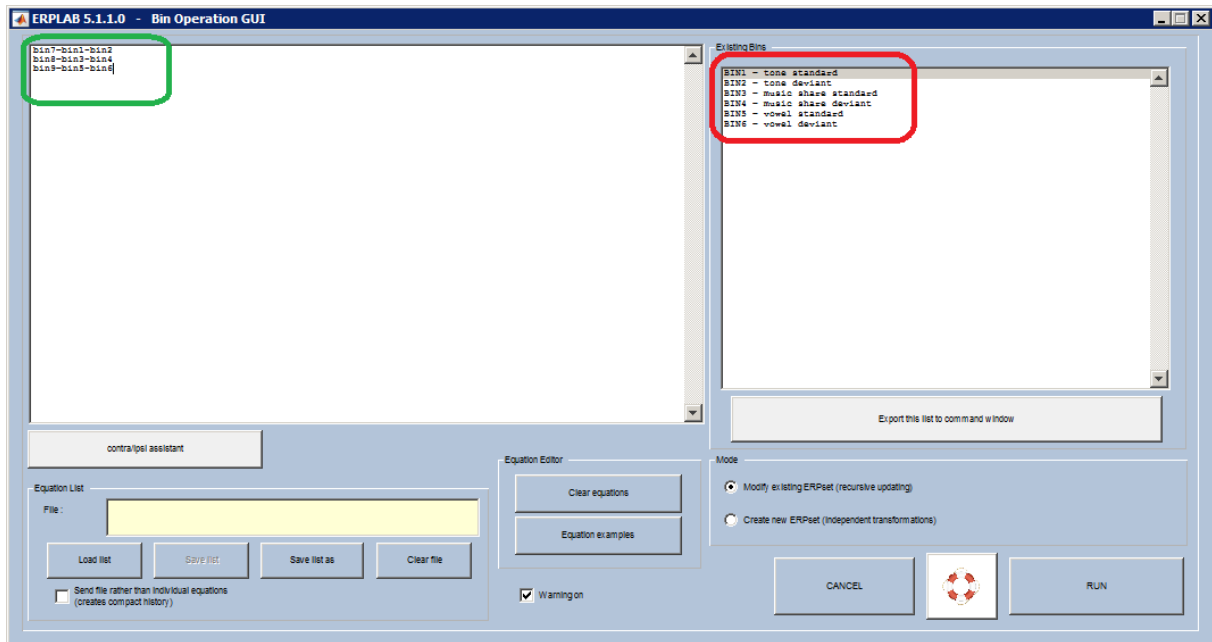
IMAGE 5.106

A window opens similar to IMAGE 5.106. In the area circled in **red**, you will see a description of your existing bins and their corresponding numbers. In the area circled in **green**, write down the equations for your new bins, specifying the new bin number and the correct label for your new bin. In our case, we write down the following:

bin7=bin1-bin2 label tone MMN
bin8=bin3-bin4 label music MMN
bin9=bin5-bin6 label vowel MMN

Click 'run'. Be careful: after you've done this adaption, you won't be asked to save your new ERPset, but the adaptation won't be saved automatically either. Save your new ERPset by going to 'ERPLAB' in the EEGLAB toolbar and clicking 'Save current ERPset as'.
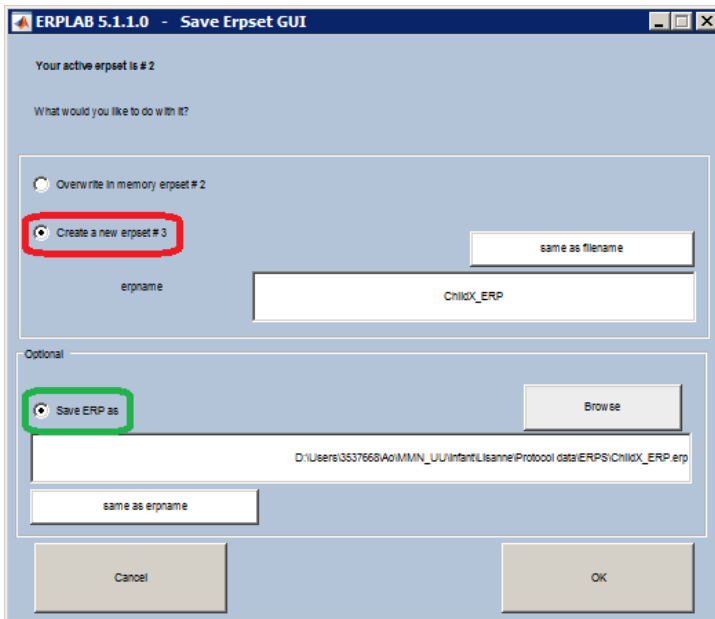
This step can easily be done with a script, which will save you a lot of time. Write your own script, or contact <u>Ao Chen</u> if you want to make use of a standardised script. Note that you still have to adapt her script to your own experiment!

5.6.4 Compute ERP grand averages

Once you have converted all your datasets into ERPsets and added new bins for the difference waves, you can compute the grand averages again. Go to 'ERPLAB' and click 'Average across ERPsets (Grand Average)' (IMAGE 5.107).
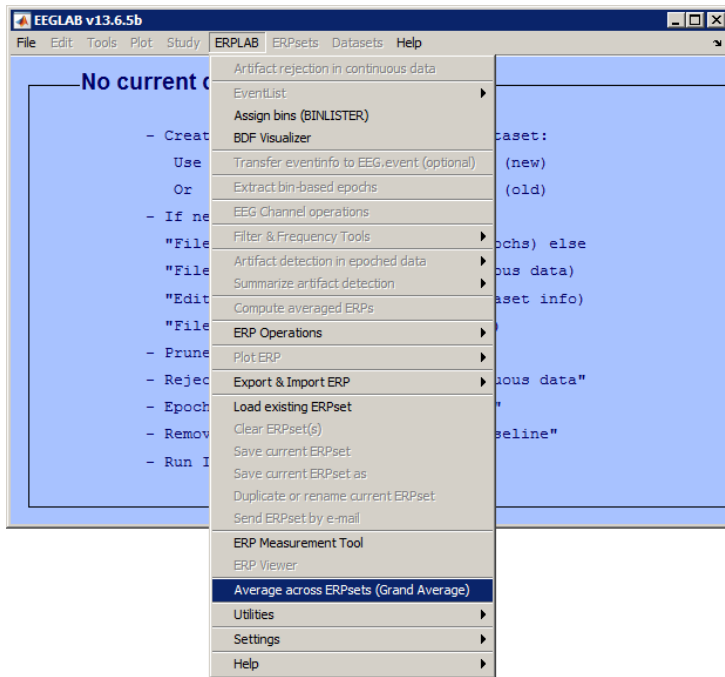
IMAGE 5.107

A window pops up similar to **IMAGE 5 .108**. Click 'Add ERPset' (circled in **red**), and select all the .erp files that you created in the previous step. After you have clicked 'open', you will see the filenames appear in the large white blank (circled in **blue**). This is your list of included participants. Click 'Save List as' (circled in **green**) to save it as a .txt file and give your participant list a name. In future steps, you can now easily upload your list by clicking 'load list' instead of selecting all participants again. Finally, click 'run' (circled in **yellow**).
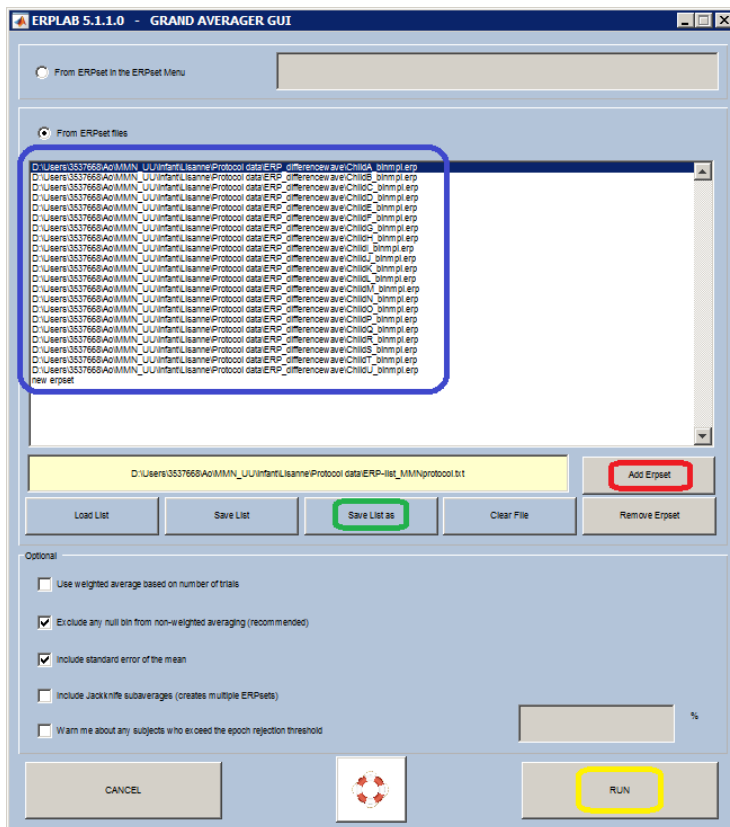
IMAGE 5.108
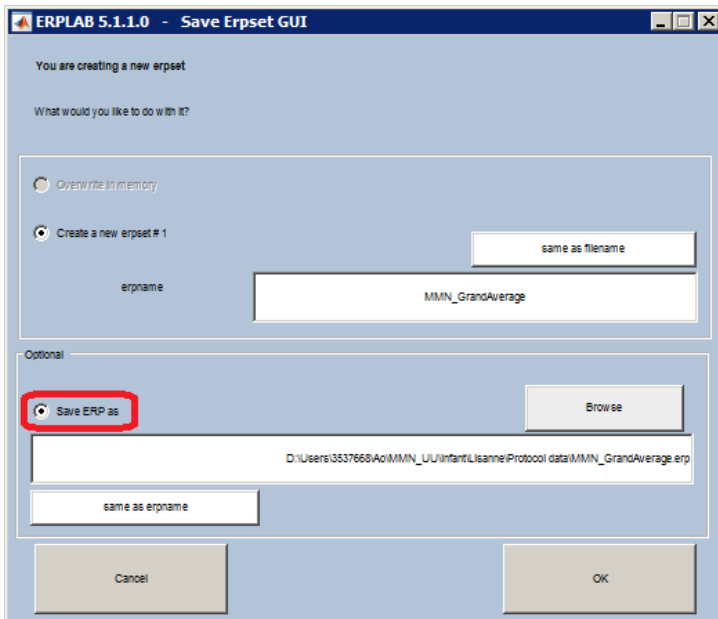
IMAGE 5.109

In the next window that pops up, tick 'create a new erpset' and give it a suitable name. Also, tick 'save ERP as' (circled in **red** in IMAGE 5 .109), click 'browse' and save your new .erp file in your analysis folder. Click 'Ok'. You have now created a grand average erpset.

With your new erpset, you can plot your difference waves again. Go to 'Plot ERP' in 'ERPLAB' and click 'Plot ERP waveforms' (IMAGE 5 .110).
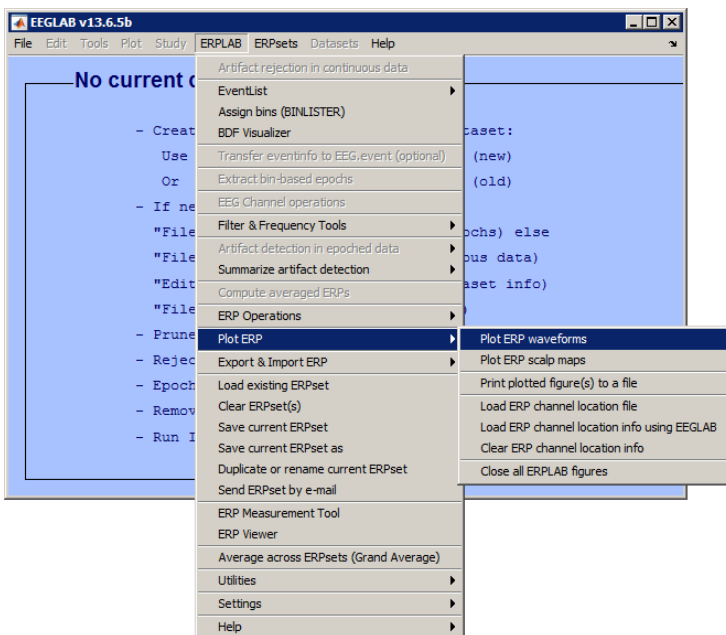
IMAGE 5.110

A dialog pops up similar to IMAGE 5 .111. In the area circled in **red**, indicate the bins you want to plot. Type the corresponding bin number to your difference wave in the large blank (in this example, we are going to plot the difference wave for the vowel ERPs, which is bin number 9). In the area circled in **green**, type the channels you want to plot. Indicate the channels in which you expect the highest effect, and therefore want to use for your final analysis. Make sure the number circled in **yellow** matches the number of the erpset you want to plot. If you don't know the number, you can look it up

in the EEGLAB toolbar (IMAGE 5 .112). You can also select a different erpset in the toolbar before you start plotting.

When you've specified everything you need for your graph in IMAGE 5 .111, click 'Plot' (circled in **blue**).
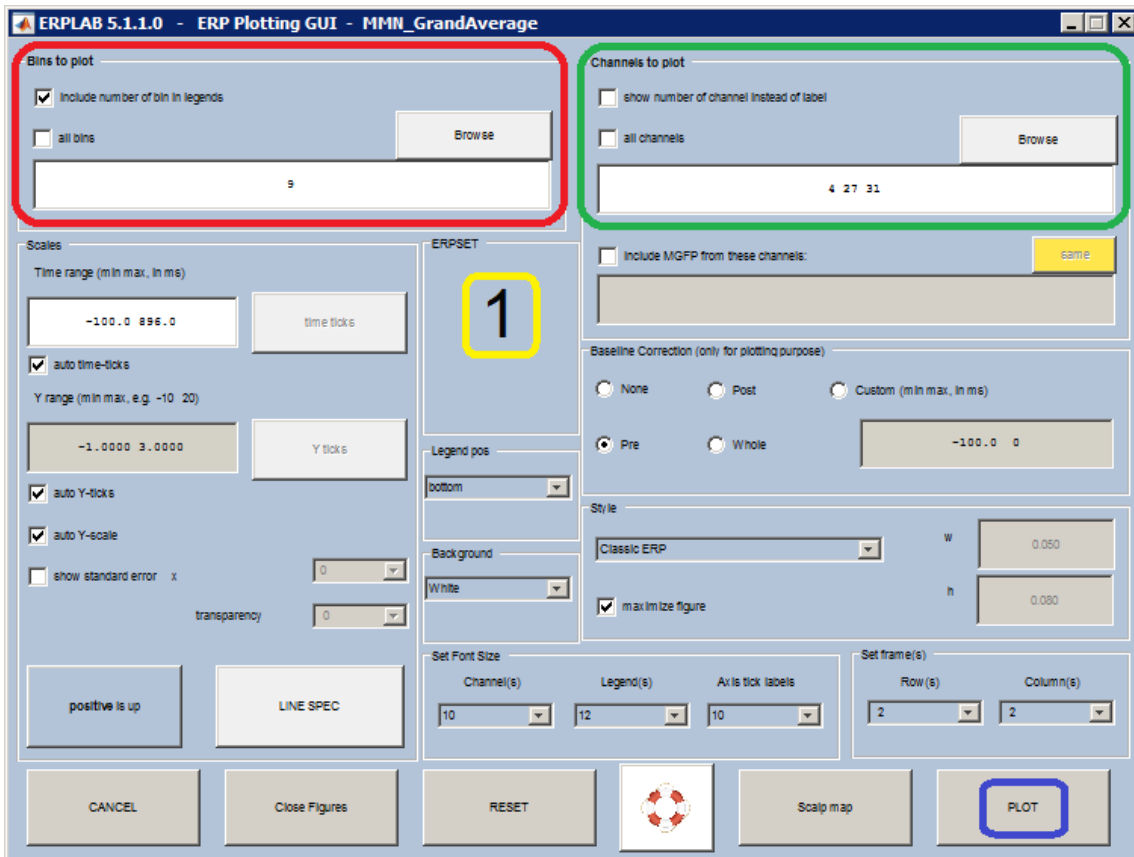
IMAGE 5.111
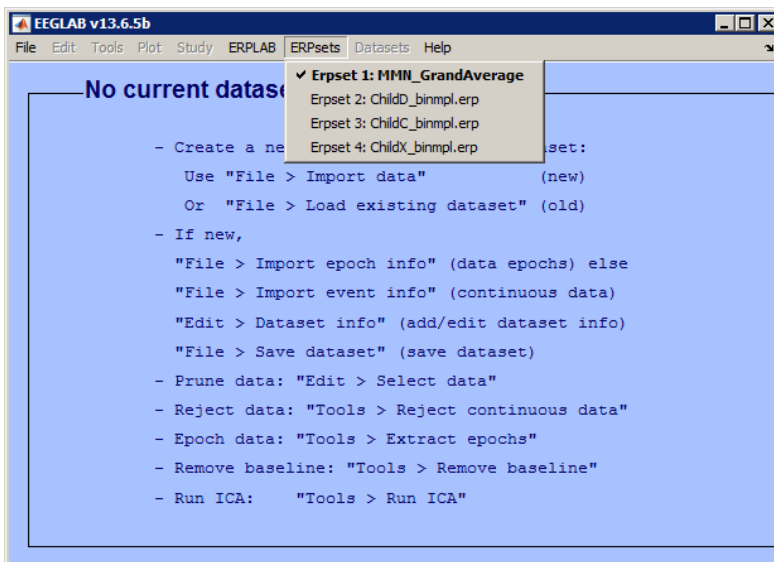
IMAGE 5.112

The plots will finally be displayed as in IMAGE 5 .113. When doing EEG experiments on adults, you will probably find one clear negative peak somewhere around 150ms, but in infants, it is unknown where to find the peak. As you see in IMAGE 5 .113, the difference wave is mostly positive and stretches over a long period of time. Therefore, the analysis will be slightly different, depending on the looks of your plot.
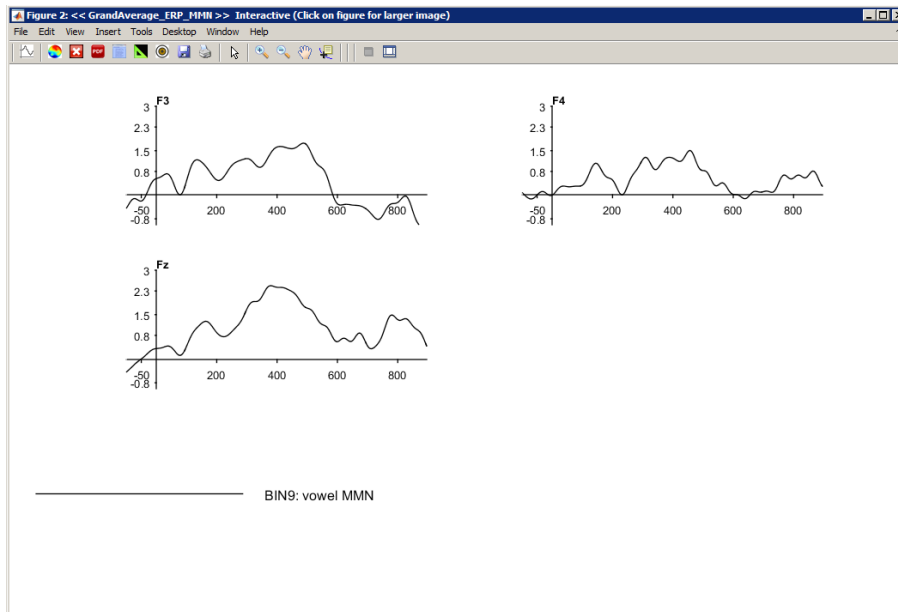
IMAGE 5.113

5.7 ERPLAB: Final analysis

The analysis from here afterwards is only suggestive for the sample dataset. Statistical analysis of infant ERP data can be very tricky, search through literatures and think carefully how you want to perform the statistical analysis. The following part may not be appropriate for your own dataset!

When doing the final analysis, there are two possible ways, depending on the looks of your difference wave. If your plot shows a clear MMN peak, you can calculate the individual latencies and amplitudes of the peak. However, infant data usually doesn't show a clear peak, but rather a very diffuse difference wave. In that case, you have to measure the differences in amplitude in sliding windows.

5.7.1   My plot shows a clear MMN peak

Suppose you have plotted your difference wave in ERPLAB, and you find a clear peak in your plot (as in IMAGE 5.114, indicated by the red arrow). This is your MMN peak, on which you want to do your final analysis.
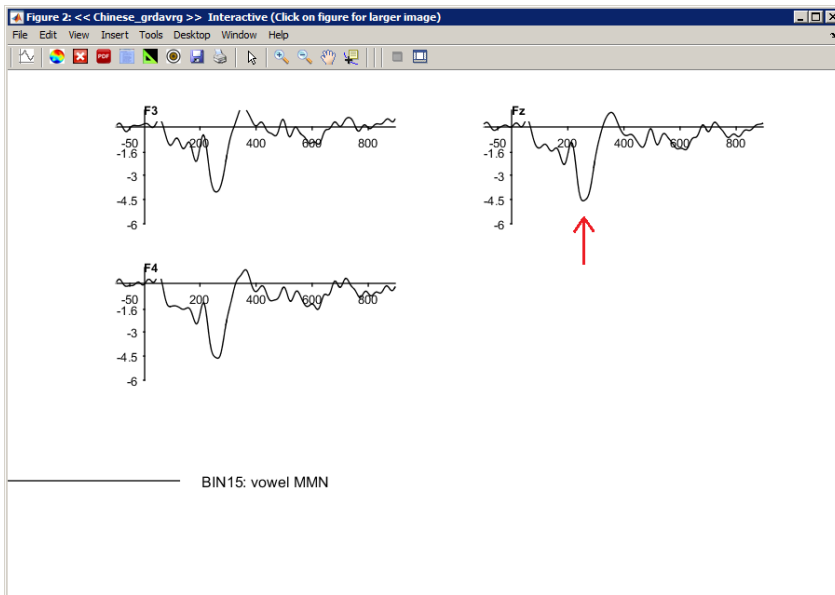
IMAGE 5.114

5.7.1.1 Find the peak latency of the grand average

First, we need to calculate the latency of the MMN peak on the difference wave. Go to the ERP measurement tool in ERPLAB (IMAGE 5 .115)
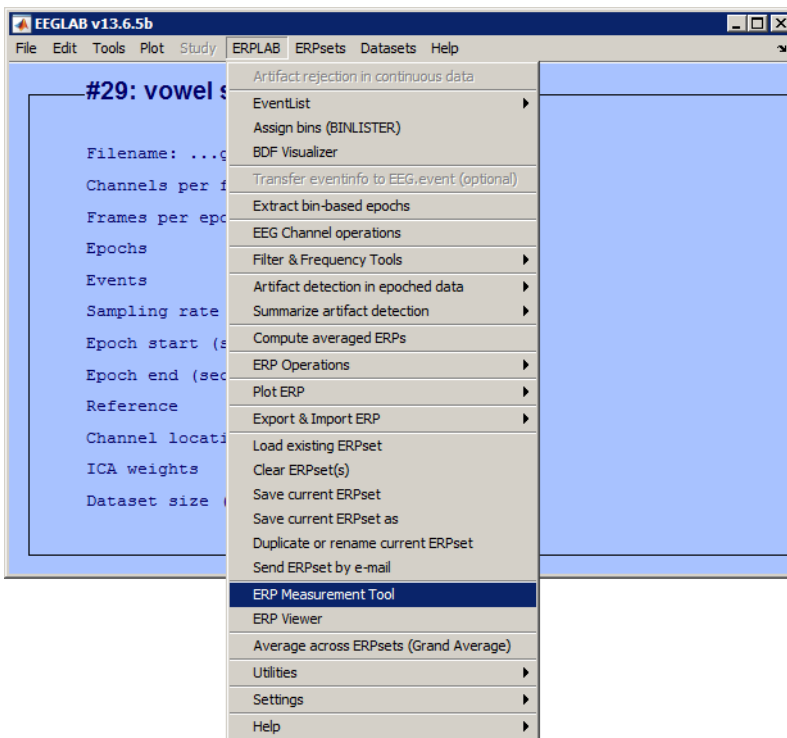
IMAGE 5.115

In the window that pops up, you can specify what kind of data you want. In the large blank on the left hand side, load the grand average .erp file you created <u>earlier</u> to plot your data. You can do this by clicking 'add ERPset' (circled in **red** in IMAGE 5 .116). You may see a yellow warning pop up asking you if you want to add this directory to your path. Click 'yes'. Adding erpsets creates a new list. Click 'save list as' (circled in **green**) to save it, otherwise it will not run.

Note: Now you have two lists: one includes all individual .erp files, and one contains only the grand average .erp file. Be sure not to mix them up!

In the area circled in **blue** in IMAGE 5.116, you can state what you want to know about this list. In this case, we want to know the peak latency of the difference wave, so we select 'peak latency'. In the area circled in **yellow**, indicate whether you would like to know the latency of the most positive or the most negative peak. As seen in IMAGE 5.114, we have a negative peak, so we select 'negative'. In the area circled in **orange**, indicate the bin number of the difference wave you are analysing. In this dataset (which is actually an adult dataset), the bin number for the vowel difference wave is 15. Below, state one channel on which you expect the difference wave. Because Fz is in between F3 and F4, we choose to look at Fz (circled in **purple**). In the area circled in **pink**, indicate two latencies that surround the MMN peak, so that the ERP measurement tool will find the most negative peak in between these two latencies. Choose these latencies based on your plot. As you can see in IMAGE 5.114, our peak is somewhere between 200 and 300 ms, which is why we indicate '200 300' in this blank. Finally, indicate where and how you want to save the output in the area circled in **brown** in IMAGE 5.116.
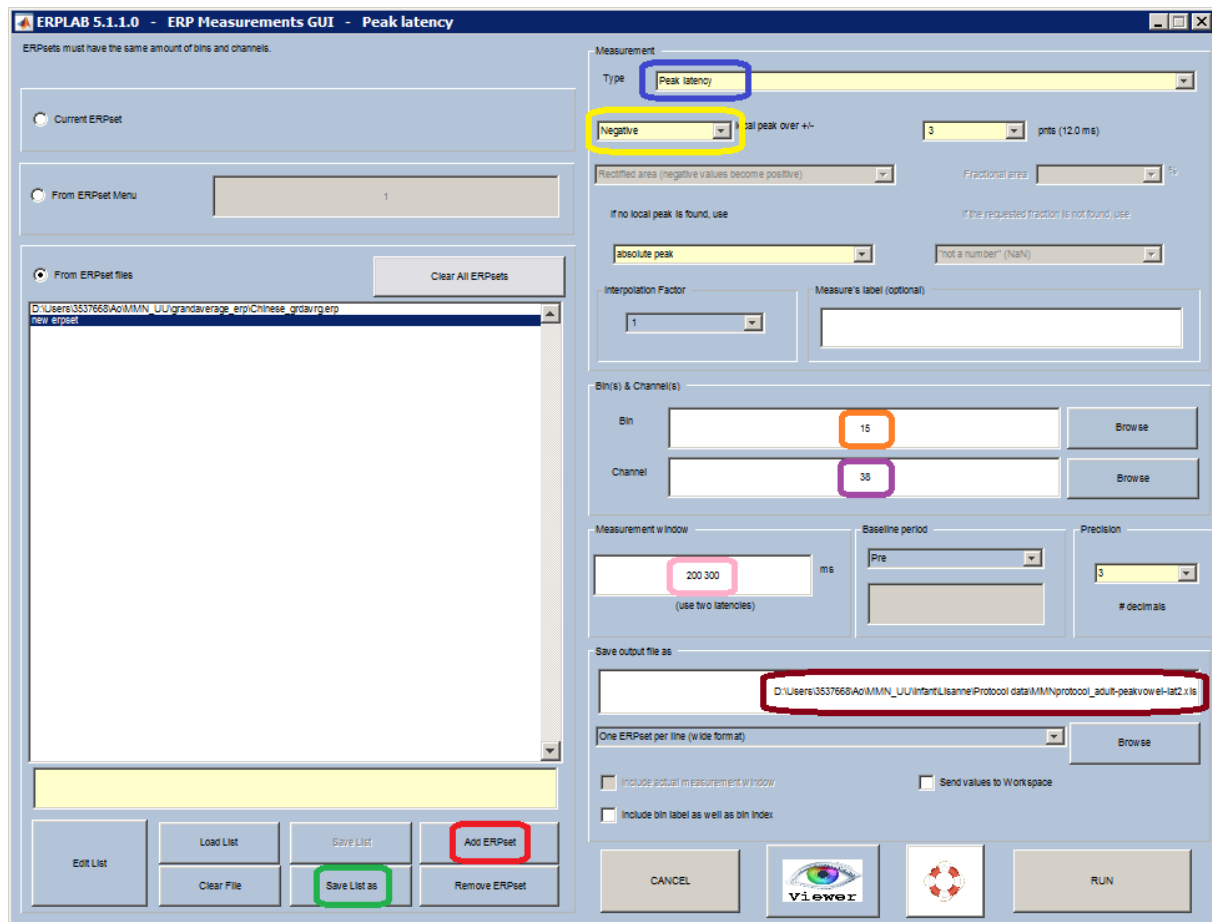
IMAGE 5.116

After you click 'run', your output will be saved as a .txt file as indicated in the area circled in **brown** in IMAGE 5.116. Go to the folder in which you saved the output, and open the .txt file. If everything went well, it will resemble IMAGE 5.117. In this file, you can see that the latency of the MMN peak is 252.009 milliseconds.
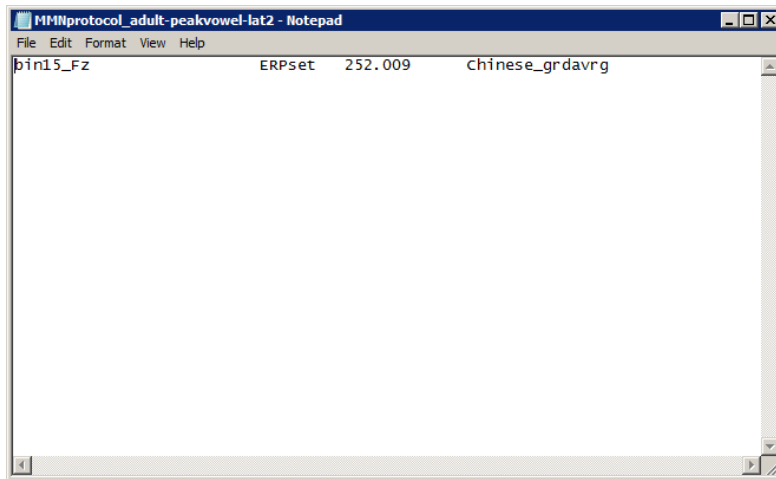
IMAGE 5.117

5.7.1.2 Find the individual peak latencies

Now that we know the latency of the MMN peak in the grand average file, we can look for the individual peak latencies. Because there is such a clear peak on the grand average difference wave, we assume that the individual peaks must be somewhere around this latency as well. Open the ERP measurement tool again, and edit the following things:

- Click 'load list' (circled in **red** in IMAGE 5.118) and select the list of the individual .erp files that you created earlier when making your grand average file. You will see that all the individual erpsets pop up in the large blank on the left hand side.
- In the area circled in **green**, indicate the latencies ±25ms around the grand average peak latency (in our case, these are 227 and 277ms). We expect all peak latencies to be within this range. You can also choose to use a different range, for instance ±20ms.
- In the area circled in **blue**, choose a different name to save your new output file, otherwise the old file will be overwritten.

Click 'run'.
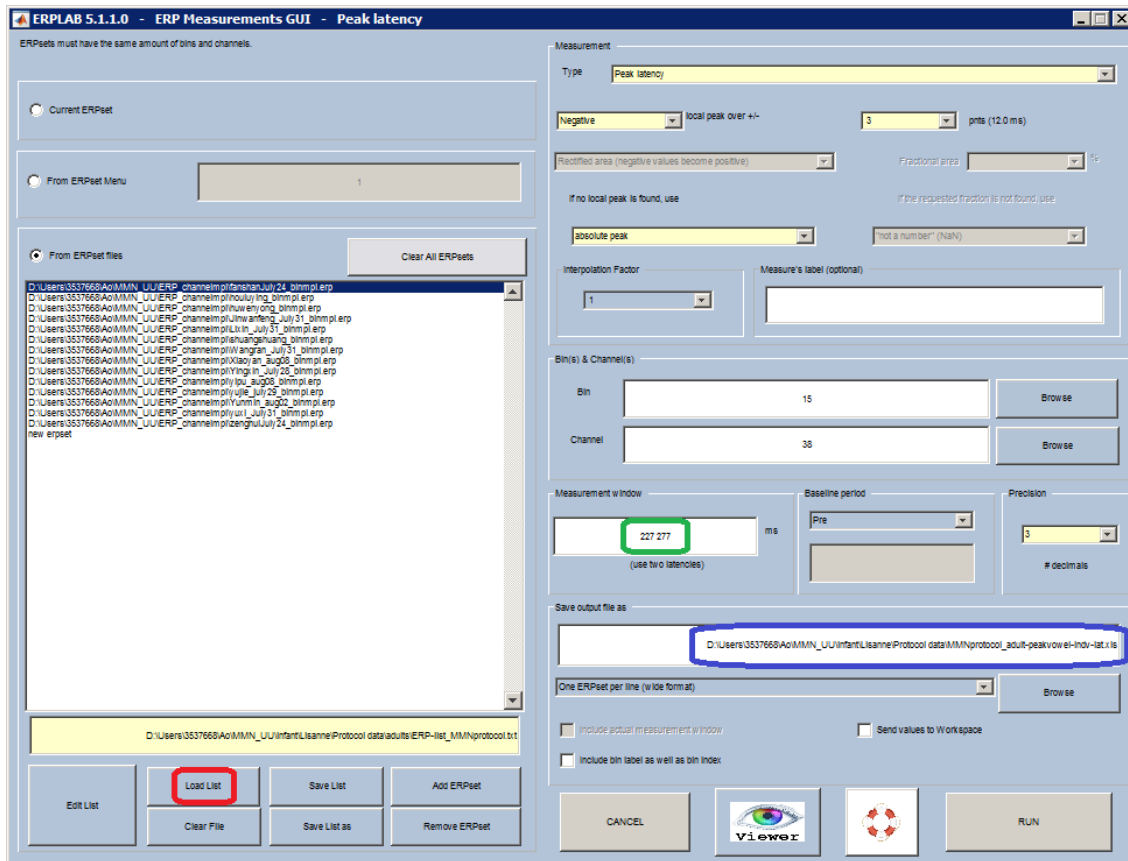
IMAGE 5.118

In the new output file, you see that all data is written next to each other which makes it look cluttered. Select and copy the entire content of the .txt file, minimize the analysis server window (as it does not support Excel), and open up Excel on your own computer. Paste the data into Excel (IMAGE 5.119). Now, you can easily see each individual peak latency. You can see that every individual peak latency lies within the two latencies we specified in the ERP measurement tool.
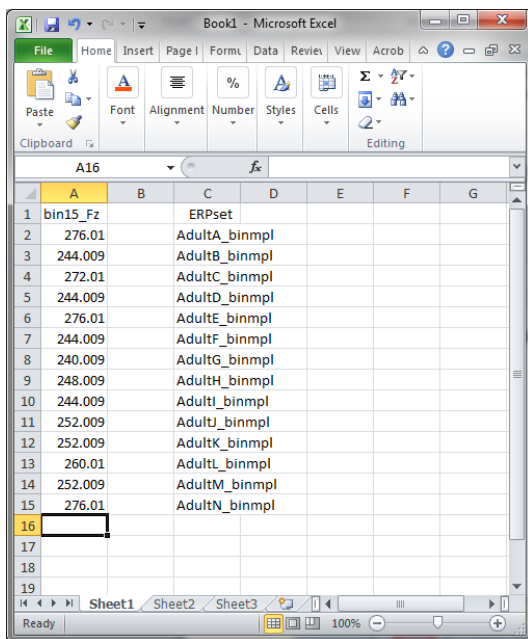
IMAGE 5.119

When comparing different groups, make sure you compute a separate grand average file for each individual group, and find the peak latencies per group. This could mean that you have different time windows per group in which you search for the individual peak latencies. Finally, you can compare the individual peak latencies by doing t-tests in SPSS, to see if there is a significant difference in latency between the groups.

5.7.1.3 Find the individual peak amplitudes

Now, we're going to look at the amplitudes of the standard and deviant waveforms, as we want to know whether these differ significantly. Open the ERP measurement tool again (IMAGE 5 .120).

Edit the following:
- In the area circled in **red** (IMAGE 5 .120), select 'mean amplitude between two fixed latencies'.
- In the area circled in **green**, indicate the bin numbers of the standard and deviant waveforms.
- In the area circled in **blue**, indicate the channels that you're interested in again (in our case, F3, Fz and F4).
- In the area circled in **yellow**, insert yet another name for your output file.

Click 'run'.
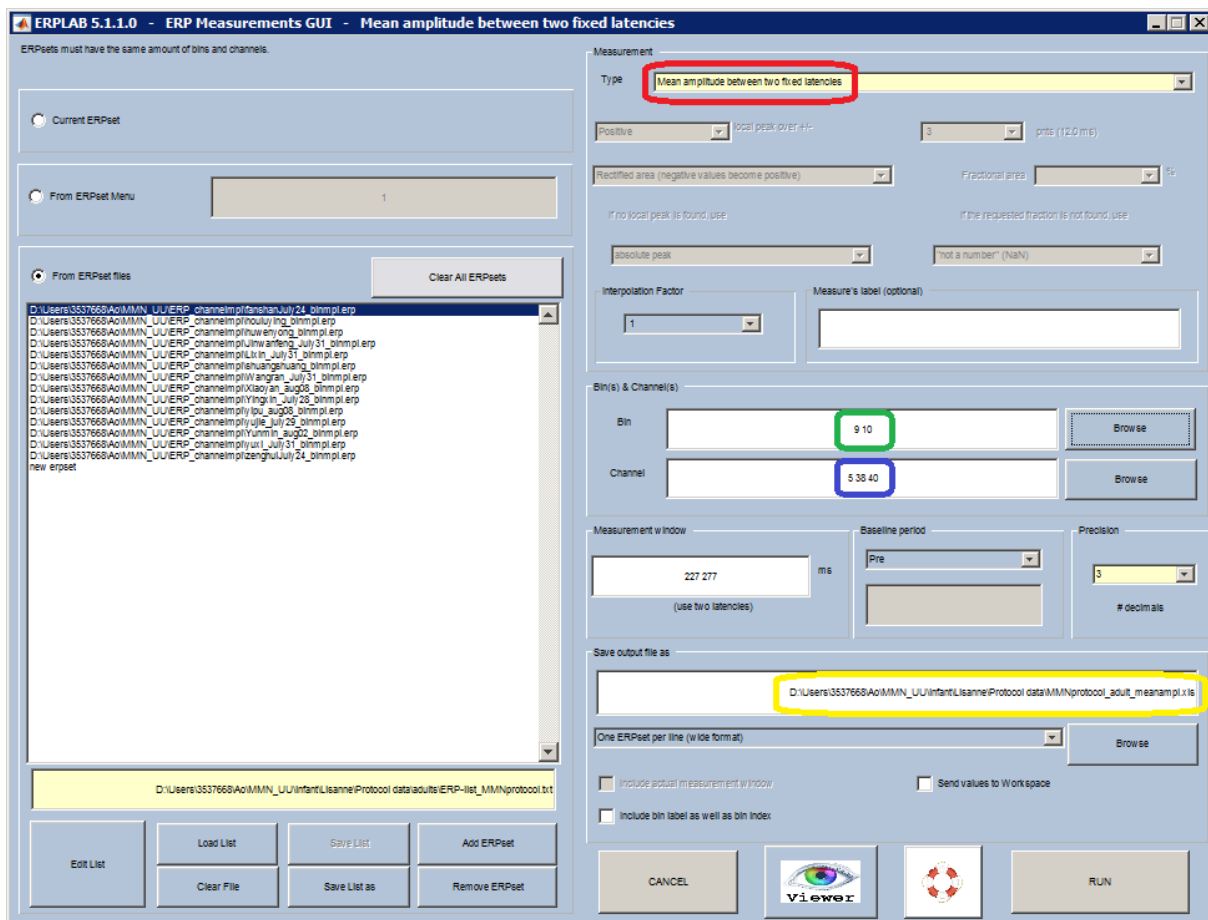
IMAGE 5.120

Copy your data from the .txt file again and paste it into Excel (IMAGE 5 .121). You can now copy everything to SPSS and run the rest of the analysis, by doing t-tests comparing 'bin9_F3' to 'bin10_F3', 'bin9_Fz' to 'bin10_Fz' and 'bin9_F4' to 'bin10_F4' (bin9 being the standard and bin10 the deviant).

IMAGE 5.121

5.7.2   My plot shows a diffuse difference wave

When testing infants, you are more likely to find a very diffuse difference wave, as shown in the plot in IMAGE 5.82. When finding a plot like this, it is hard to decide where the peak is, even though a clear difference wave is visible. Therefore, we will analyse the entire waveform using sliding windows.

5.7.2.1 Measure amplitudes in sliding windows

Because we cannot find a clear peak, we will skip the steps for finding the peak latency as described in the example above. Instead, we will cut the waveform into small timeframes of 50ms (you can also choose different timeframes, such as 100ms), and measure the mean amplitude of the standard and deviant per timeframe.

Open the ERP measurement tool (IMAGE 5 .115). A window will open up similar to IMAGE 5 .122. Click 'load list' (circled in **red**), and load the participant list that you created earlier when computing your grand average file. The list will show up in the large blank on the left hand side.
In the area circled in **green**, select 'mean amplitude between two fixed latencies', as you want to know the mean amplitude of the standard and deviant wave per participant per timeframe. In the area circled in **blue**, indicate the bin numbers for the standard and deviant. In the area below, indicate the channels you want to analyse (we choose F3, Fz and F4; circled in **orange**). In the area circled in **purple**, indicate the timeframe of the first window. In our case, we'd like to analyse the waveform between 100 and 600 in sliding windows of 50ms each, so our first timeframe will be 100-150ms. In the area circled in **yellow**, indicate where and how you want to save your output file. Make sure to give this file a different name each time you analyse a new timeframe, otherwise the old file will be overwritten. Click 'run'.
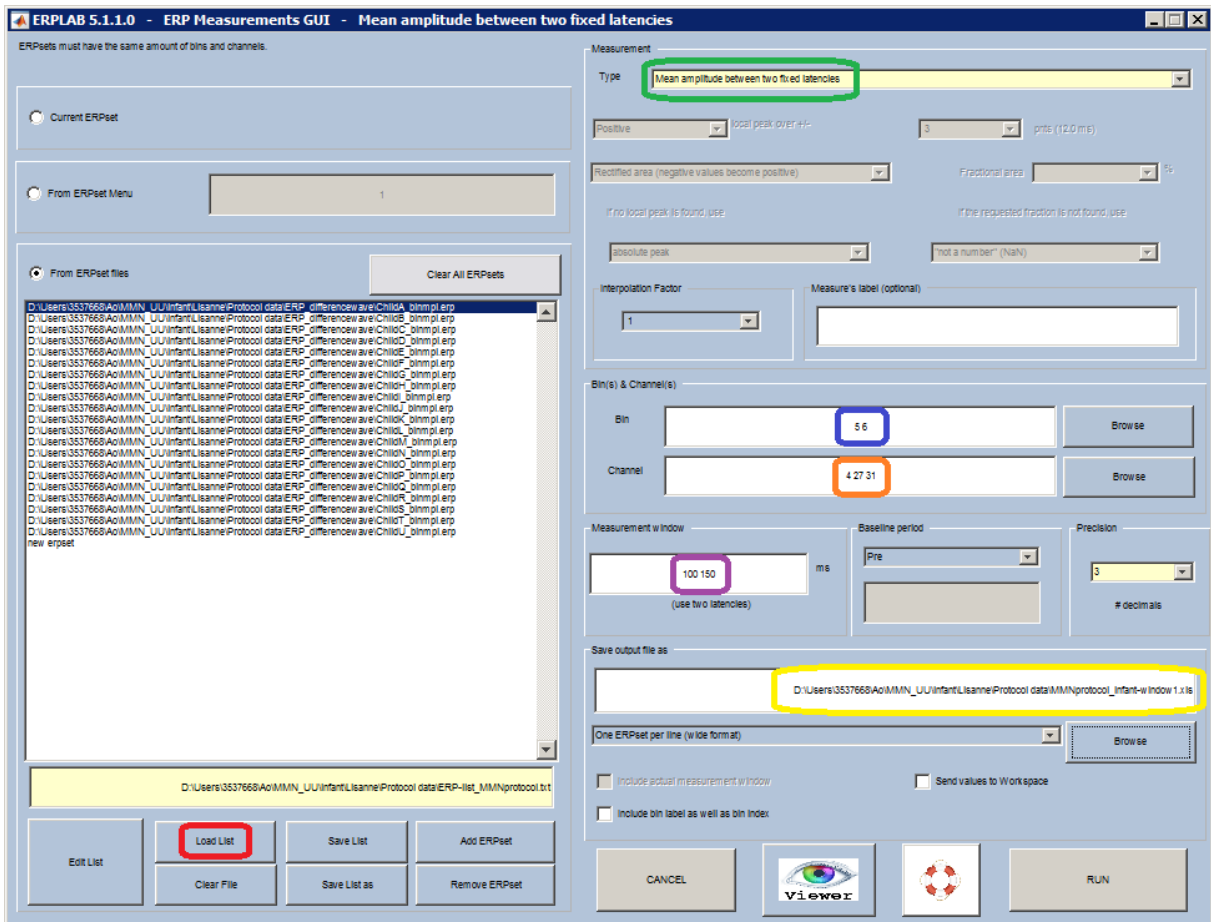
IMAGE 5.122

Your output file will be saved as a .txt file, in the folder indicated in the blank circled in **yellow** in IMAGE 5.122. When you open up this file, you will find that your data is all written next to each other, instead of in columns (IMAGE 5.123). Therefore, you have to select and copy the entire content of the .txt file, minimize the analysis server window (as it does not support Excel), open up Excel on your own computer and paste the data into Excel. Now, you can easily see the mean amplitudes per participant per channel for the first timeframe. Repeat these steps for every timeframe (150-200, 200-250, 250-300, and so on), and copy all data into Excel. Eventually, your Excel file will look similar to the one in IMAGE 5.124.

IMAGE 5.123

IMAGE 5.124

Once you have all the data for all timeframes, you can compare the amplitudes for the standard and deviant waves per channel and timeframe. This means you compare all amplitude values for the standard stimulus on F3 in the first timeframe, with all amplitude values for the deviant stimulus on F3 in the first timeframe (circled in **red** in IMAGE 5 .124), and so on, by using t-tests in SPSS. This will give you p-values for every channel and every window, so you can see exactly where the difference between standard and deviant is significant, and whether the MMN is lateralised or not.

*This final step will take a huge amount of time, so we recommend you to write a script for this. While writing a script, you can immediately include the t-test analyses, so you will get a nice overview showing you the outcomes of the t-tests per timeframe, channel and stimulus type (**IMAGE   5 .125**; description of the values is written below). Try to write your own script, or contact Ao Chen if you want to make use of the script she wrote for this experiment. Note that you still have to adapt her script to your own experiment, and that this script requires more programming skills than the previous scripts!*

resultt_mu ✕ resultt_vowel ✕

14x10 double

	1	2	3	4	5	6	7	8	9	10	11
1	20	0	-1.6064	0.1239	0	-1.6261	0.1196	0	-1.2748	0.2170	
2	20	0	-1.0564	0.3034	0	-1.6598	0.1125	0	-1.1627	0.2587	
3	20	0	-0.8641	0.3978	0	-1.3030	0.2074	0	-0.2881	0.7763	
4	20	0	-1.4099	0.1739	0	-1.8049	0.0862	0	-1.0503	0.3061	
5	20	0	-1.1133	0.2788	1	-2.3022	0.0322	0	-1.2125	0.2394	
6	20	0	-1.4075	0.1746	1	-3.0089	0.0069	0	-1.3476	0.1929	
7	20	0	-1.7961	0.0876	1	-2.8901	0.0091	0	-1.7006	0.1045	
8	20	0	-1.9709	0.0627	1	-2.1421	0.0447	0	-1.6003	0.1252	
9	20	0	-1.2862	0.2131	0	-1.5023	0.1486	0	-0.7897	0.4390	
10	20	0	-0.3081	0.7612	0	-0.9173	0.3699	0	-0.4134	0.6837	
11	20	0	0.2866	0.7774	0	-0.7552	0.4590	0	0.0650	0.9488	
12	20	0	0.3382	0.7388	0	-0.8702	0.3945	0	-0.1079	0.9152	
13	20	0	0.6722	0.5092	0	-0.6209	0.5417	0	-0.1923	0.8494	
14	20	0	0.4024	0.6916	0	-1.5431	0.1385	0	-0.7956	0.4356	
15											
16											
17											
18											

IMAGE 5.125

Statistics of the comparison between the standard and the deviant stimulus for the vowel condition:
Column 1 = df
Column 2 = significance between std and dev on F3; 0 = not significant, 1 = significant
Column 3 = t-value for std-dev on F3
Column 4 = p-value for std-dev on F3
Column 5 = significance between std and dev on Fz
Column 6 = t-value for std-dev on Fz
Column 7 = p-value for std-dev on Fz
Column 8 = significance between std and dev on F4
Column 9 = t-value for std-dev on F4
Column 10 = p-value for std-dev on F4

6. Appendixes

6.1 General testing checklist K.13

Preparation

In the control room:
- Turn on computer EEG1 (Recording) and EEG2 (Presentation), and turn on all the screens.
- Select an infant movie to play (if applicable). Set the video switch to the correct screen.
- Turn on the Philips monitor.
- Turn on the camera in the test room.
- Turn on the Tangent amplifier (under the Philips monitor).
- Set the audio switch to Tangent amplifier.
- Before a baby is in the lab, run the experiment once to check whether sound and visual stimuli work well.

In the test room:
- Put the sound absorbing towel on top of the table
- Put the toys on the table (if applicable)
- Put gel into syringe
- Get one baby EEG cap
- Get the 32 Channel electrodes bundle
- Turn on the air humidifier
- Check the sound level using the audiometer. A loudness of 65 dB is recommended for the sound stimuli.
- **Optional:** prepare the cap in advance

Receive the parent and child
- Welcome the parent and the baby in the waiting area. Take them to the lab
- Put a little bit of gel on the baby's wrist.
- Explain to the parent what the experiment is about and what is expected of them/what they can expect. Tell the parents:
  - Don't let the baby pull the hat or the electrodes.
  - Don't talk during the experiment.
  - If the baby gets fuzzy, you can stop at any time.
- Check the baby's wrist for allergic reactions. If absent, continue with the experiment.

Start the experiment

In the test room:
- Clean the skin behind the baby's ear with a cotton pad with alcohol.
- Put EXG1 on the left mastoid and EXG2 on the right mastoid, connect these two electrodes to the amplifier in the drawer.
- Put on the cap.
- Put gel into all electrodes.
- Connect electrodes to the corresponding holes in the cap and to the amplifier.
- Turn on the amplifier and check the blue light.

In the control room:
- Click the BioSemi icon on desktop in the control room.

- Click "start" on the BioSemi window, you should see all the electrodes now.
- Check impedance: go to tab "electrode offset", set the scale on the left to 50mv, and all the blue bars should be in the range ±25mv.
- Set reference to Cz: go to the tab "monopolar display". On the left of the screen, click "Free choice" under "Reference", and use the menu below to select Cz.
- Click "Start File" at the right bottom of the BioSemi window.
- Type in the participant's information and save in your data folder.
- **Click "pause save", otherwise you are not recording!**
- **Open OBS Studio and click 'Start recording'.**
- On EEG2, open the experiment file, click run.
- Fill in the name of the participant in the pop up window "Enter Subject Name/ID", in the same format as in ActiView (EEG1).
- Use Enter key on the keyboard to start experiment. Use Esc if you want to abort the experiment.
- **On EEG1, check whether triggers are sent. If not, call lab support.**

After the experiment
- Ask if the parent wants to wash their baby's head.
- Collect consent form from the parent.
- Give the baby the gift.
- Reimburse travel expenses.
- Ask for remaining questions.

Cleaning
- Clean the cap and electrodes
- Always use lukewarm water
- **Never let electrodes sit under running water!**
- Put away the toys and the cushion (if applicable)
- Fill in the experiment report

6.2 Corresponding numbers to electrodes (32 channels)

Number	Channel
1	Fp1
2	AF3
3	F7
4	F3
5	FC1
6	FC5
7	T7
8	C3
9	CP1
10	CP5
11	P7
12	P3
13	Pz
14	PO3
15	O1
16	Oz
17	O2
18	PO4
19	P4
20	P8
21	CP6
22	CP2
23	C4
24	T8
25	FC6
26	FC2
27	F4
28	F8
29	AF4
30	Fp2
31	Fz
32	Cz

6.3 Channel locations (manual input)

#	Label	Theta	Radius	X	Y	Z	Sph_theta	Sph_phi	Sph_radius	Urchan
1	Fp1	-17.9260	0.5150	80.7840	26.1330	-4.0011	17.9260	-2.6980	85	1
2	AF3	-22.4610	0.4211	76.1528	31.4828	20.8468	22.4610	14.1970	85	2
3	F7	-53.9130	0.5281	49.8714	68.4233	-7.4895	53.9130	-5.0550	85	3
4	F3	-39.9470	0.3446	57.5511	48.2004	39.8697	39.9470	27.9730	85	4
5	FC1	-44.9250	0.1812	32.4362	32.3514	71.5981	44.9250	57.3870	85	5
6	FC5	-69.3320	0.4082	28.7628	76.2474	24.1669	69.3320	16.5180	85	6
7	T7	-90	0.5332	5.1765e-15	84.5385	-8.8451	90	-5.9730	85	7
8	C3	-90	0.2667	3.8681e-15	63.1713	56.8717	90	41.9960	85	8
9	CP1	-135.0750	0.1812	-32.4362	32.3514	71.5981	135.0750	57.3870	85	9
10	CP5	-110.6680	0.4082	-28.7628	76.2474	24.1669	110.6680	16.5180	85	10
11	P7	-126.0870	0.5281	-49.8714	68.4233	-7.4895	126.0870	-5.0550	85	11
12	P3	-140.0530	0.3446	-57.5511	48.2004	39.8697	140.0530	27.9730	85	12
13	Pz	180	0.2534	-60.7385	-7.4383e-15	59.4629	-180	44.3920	85	13
14	PO3	-157.5390	0.4211	-76.1528	31.4828	20.8468	157.5390	14.1970	85	14
15	O1	-162.0740	0.5150	-80.7840	26.1330	-4.0011	162.0740	-2.6980	85	15
16	Oz	180	0.5067	-84.9812	-1.0407e-15	-1.7860	-180	-1.2040	85	16
17	O2	162.0740	0.5150	-80.7840	-26.1330	-4.0011	-162.0740	-2.6980	85	17
18	PO4	157.5390	0.4211	-76.1528	-31.4828	20.8468	-157.5390	14.1970	85	18
19	P4	140.1030	0.3445	-57.5840	-48.1428	39.8920	-140.1030	27.9900	85	19
20	P8	126.1330	0.5281	-49.9265	-68.3836	-7.4851	-126.1330	-5.0520	85	20
21	CP6	110.6680	0.4082	-28.7628	-76.2474	24.1669	-110.6680	16.5180	85	21
22	CP2	135.0750	0.1812	-32.4362	-32.3514	71.5981	-135.0750	57.3870	85	22
23	C4	90	0.2667	3.8679e-15	-63.1673	56.8761	-90	42	85	23
24	T8	90	0.5332	5.1765e-15	-84.5385	-8.8451	-90	-5.9730	85	24
25	FC6	69.3320	0.4082	28.7628	-76.2474	24.1669	-69.3320	16.5180	85	25
26	FC2	44.9250	0.1812	32.4362	-32.3514	71.5981	-44.9250	57.3870	85	26
27	F4	39.8970	0.3445	57.5840	-48.1426	39.8920	-39.8970	27.9900	85	27
28	F8	53.8670	0.5281	49.9265	-68.3836	-7.4851	-53.8670	-5.0520	85	28
29	AF4	22.4610	0.4211	76.1528	-31.4828	20.8468	-22.4610	14.1970	85	29
30	Fp2	17.9260	0.5150	80.7840	-26.1330	-4.0011	-17.9260	-2.6980	85	30
31	Fz	0	0.2534	60.7385	0	59.4629	0	44.3920	85	31
32	Cz	0	0	5.2047e-15	0	85	0	90	85	32
33	EXG1									33
34	EXG2									34