

Press Release

Support & Tips

Choosing your reference – and why it matters

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How does the reference affect my data? What happens during re-referencing? This article provides some useful information about referencing that might help you choosing the optimal reference for your data analysis.

Abstract

After introducing the general concept of (re-)referencing, some effects of the reference on the data are described and important factors for the choice of the reference are discussed. After presenting two commonly used reference schemes, this article describes how to re-reference your data with the *New Reference Transformation* in [BrainVision Analyzer 2](#).

1. Referencing during recording – the online reference

1.1. The relationship between ground, reference and scalp channels

EEG recordings measure differences in electrical potentials between two points (Voltages) and are usually expressed in units of microvolts (μV). This means the signal displayed at any channel is in fact the difference in electrical potential to some other recording site. Primarily, this other recording site is the **ground electrode**. As the name indicates, this electrode is connected to the ground circuit of the amplifier, and it thereby picks up electrical noise that does not reach the other scalp electrodes. Consequently, the voltage difference between ground and EEG scalp electrodes is also affected by this noise.

To eliminate this ground-related noise, an **online reference** is chosen out of the recorded EEG channels, which is used as “baseline” for all remaining EEG channels. This means the signal at the other EEG channels is expressed as the **difference in electrical potential to this reference** instead of to the ground. Relating the EEG channels to the reference cancels out the noise stemming from the ground circuit ($[\text{channel} - \text{ground}] - [\text{online reference} - \text{ground}] = \text{channel} - \text{online reference}$). Differential amplifiers amplify this (less noisy) voltage difference between EEG channels and reference. Therefore, when looking at your EEG data, keep in mind that the amplitude at any channel constitutes the difference in electrical potential to the chosen reference.

1.2. Which online reference should I use?

Many EEG caps have a pre-defined reference channel, for example at the position of FCz or Cz. In other cases, dedicated electrodes can be attached to the nose, earlobes or mastoids (the bones behind the ears) to serve as online reference. In general, it is recommended to use a **single channel as online reference** during recording (instead of e.g. physically linked mastoids). Otherwise neither the contributions nor the signal quality (impedances) of the different channels forming the online reference can be disentangled and controlled.

You will find that **the reference itself is not displayed as a channel** in your data. Recording the reference against itself (online reference – online reference) would mean amplifying and recording zero Volt differences. The online reference is hence not available as a channel with meaningful information. However, note that the choice of the online reference is not too decisive: you still have the option to choose a different reference during offline referencing (see [2.1](#)).

2. Re-referencing after recording – the offline reference

Changing the reference offline after recording is called re-referencing. The idea behind re-referencing is to express the voltage at the EEG scalp channels with respect to another, new reference. It can be composed of any recorded channel or an average of several channels. This ultimate reference for your data will also affect your analysis and should be chosen with care.

2.1. How does re-referencing work?

Before re-referencing, the new reference itself reflects the difference in electrical potential to the online reference (new reference – online reference). During re-referencing, the signal of the new reference is subtracted from each EEG channel. Afterwards, the voltage at these channels reflects the difference to the new reference and no longer to the online reference ($[\text{channel} - \text{online reference}] - [\text{new reference} - \text{online reference}] = \text{channel} - \text{new reference}$). In the same way, the new reference is subtracted from itself during re-referencing and thus reflects the new level of zero voltage.

The same process can in fact also be applied to the online reference, which – after the subtraction of the new reference – is simply the equivalent of the **negated new reference** ([online reference – online reference] – [new reference – online reference] = online reference – new reference). This way, the signal of the online reference, which has been implicitly contained in all other channels, can be regained with respect to a new reference. This can be useful if the online reference channel (for example Cz or FCz) is of relevance for your data analysis.

2.2. How does the choice of the reference affect the data?

The reference influences the **amplitude** at each EEG channel and time point: it defines the level of zero voltage and all other channels are expressed in relation to it. Channels that are located closely to the reference are thereby likely to be affected by similar electrical activity. Their difference in potential to the reference, i.e. their amplitude, will therefore be rather small. By contrast, channels that are distant from the reference are potentially also affected by other electrical sources (e.g. other brain activity or noise). They are more likely to display larger voltage differences to the distant reference, and thereby exhibit a larger amplitude. This way, the **spatial distance** to the reference can affect the **amplitude** of a channel, and potentially also the **level of noise** remaining in the signal after re-referencing. Alike the amplitude, also the **polarity** of the signal at different channels depends on their relationship to the reference, i.e. if their electrical potential is lower or higher in comparison. For these reasons, the offline reference is usually chosen to be **symmetric**. Otherwise, the voltage distribution on the head will likely be biased towards one hemisphere.

The **topography** of the EEG signal is shaped by the relative differences in voltage between the scalp channels. Re-referencing does not affect this distribution because the new reference is subtracted equally from all channels. However, given that the new reference determines the level of zero voltage, re-referencing can **shift the overall amplitude of the topography** to a higher or lower level.

2.3. Choosing the optimal offline reference

Several publications have discussed the effect of different referencing schemes on EEG recordings (e.g. Dien, 1998; Luck, 2104; Li et al., 2018). To date, there is no agreement on an “optimal reference”, however, several facts can inform your choice:

The reference should optimally be influenced by **the same noise as the other scalp channels** (stemming from the ground circuit, pulse, movements etc.). When subtracting the new reference, this noise is subtracted as well. Just be aware that also the reverse is true: if the reference contains noise that is not shared with the other scalp channels, this noise will be introduced into all other channels during re-referencing. For picking up comparable noise, the channel(s) ultimately forming the new reference should hence be **located closely enough** to the region of interest (on the head) and provide **good data quality** (i.e. low impedance).

At the same time, the reference should optimally pick up **as little signal of interest as possible**. This signal, too, would be subtracted during re-referencing which might reduce the effect you are looking for. For this reason, the reference should **not be located too closely** to the region of interest for your specific research question. You see that the choice of the reference hence entails a trade-off.

When choosing your reference, it is also important to consider the **comparability to other studies**. If study results are to be compared, they should also be referenced in the same way. It is thus useful to find out in other publications which referencing scheme is common for your specific research question or paradigm.

Figure 1 (below) illustrates how the choice of the reference may affect your data. The example data stems from an Oddball paradigm (N=8). The displayed signal at Cz was referenced to **A) FCz, B) an average of all 32 recorded channels and C) an average of two mastoid electrodes**. The black and blue curves display the event-related potentials (ERPs) in response to frequent and rare stimuli, the red curve reflects their difference.

The largest difference between the rare and frequent condition is expected to appear around 300 ms post-stimulus (P300) and to be located over central-parietal regions. You can see how the choice of the reference affects the signal time course at Cz, while the topographic distribution merely shifts in its overall amplitude (note that the topographies in B) and C) are based on one more channel because FCz was restored). For the detection of the P300 in this paradigm, the average mastoid reference would be preferable over the other two referencing schemes. This might of course differ for other ERPs or for your specific research question.

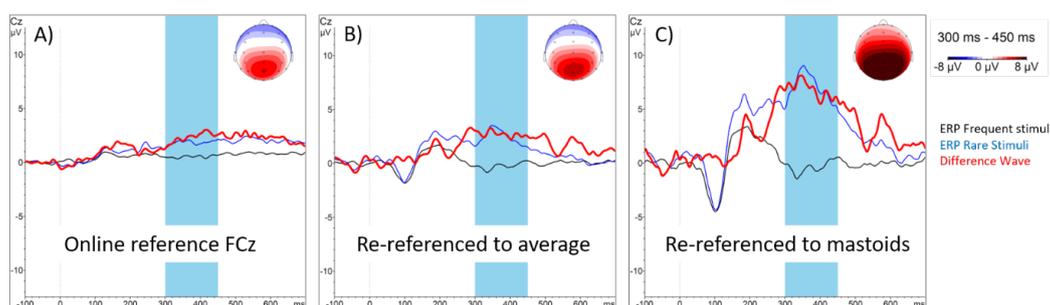


Figure 1: Event-related potentials (ERPs) from a visual and auditory Oddball task (N=8). Curves display ERPs in response to frequent (black) and rare (blue) stimuli. The maps reflect the topography of the difference wave (red) from 300 to 450 ms (interval marked in light blue)

2.4. What are commonly used referencing schemes?

As described above, the optimal reference always depends on the signal of interest. However, two commonly employed referencing schemes will be described in some more detail. For some alternative approaches please refer to the “Some more facts”-box on page 4.

Average mastoids

The average of the two mastoids is often used as offline reference, especially if the signal in central areas or along the midline is of interest. The electrodes on the mastoids are expected to pick up relatively little cortical activity from the top of the head. At the same time, they are located closely enough to the other scalp channels to pick up similar noise. Averaging across the left and right mastoid furthermore offers a symmetric reference that should not prefer one of the hemispheres. Dedicated mastoid electrodes can be placed behind the ears (often called M1 and M2 or A1 and A2) or the mastoids can be approximated by the electrode positions TP9 and TP10.

Average of all scalp channels (Common Average Reference)

When applying the so-called Common Average Reference, the new reference is the average electrical activity measured across all scalp channels. Re-referencing is achieved by creating an average of all scalp channels and subtracting the resulting signal from each channel. After re-referencing, the overall electrical activity (amplitude) across all channels will sum up to zero at each time point. When using this reference, amplitudes will be overall reduced, but each channel will contribute equally to the new reference.

Theoretically, the re-referenced signal should not be biased towards any specific location on the scalp. However, the head is normally not evenly covered by electrodes. Instead, electrodes are often placed more densely on the top of the head and do not cover the underside at all. If the signal on top of the head is overrepresented in the reference, the amplitudes in this region will be reduced (see 2.2.).

To minimize this bias, the average reference hence requires high coverage with at least 64 to 128 electrodes (Nunez, 2006). Optimally, electrodes should thereby be evenly spaced and cover over 50% of the head surface (Luck, 2014). Luck (2014) furthermore urges reporting of exactly which channels were included in the average reference to allow replicability and comparability to other studies.

3. The New Reference transformation

In BrainVision Analyzer 2, re-referencing can be achieved by applying the *New Reference* transformation. It can be found under *Transformations > Dataset Preprocessing > Channel Preprocessing > New Reference* in Analyzer 2.1.x or under *Transformations > Dataset Preprocessing > New Reference* in Analyzer 2.2.x. With this transformation, you can re-reference your signal in three steps.

Note: You can find an example for re-referencing to the mastoids in our Webinar “[Analysis of Event-Related Potentials](#)”.

3.1. The steps of the New Reference transformation

First dialog window: Select the channels that form the new reference

In the first dialog (Figure 2 A) you can select the channel(s) that should form the new reference. If several channels are selected, their average will constitute the new reference. If for example the mastoid channels M1 and M2 are selected, the new reference signal will constitute their averaged signal $(M1 + M2) / 2$.

The option ‘*Include Implicit Reference...*’ should only be selected if the (implicit) online reference channel is intended to be part of the new reference. One reason to use this option would be if one mastoid formed the online reference and you would like to re-reference to both. Another reason to include the online reference would be given when re-referencing to the average of all channels (see “Average of all scalp channels” in 2.4.). Even though the implicit reference won’t contribute any (non-zero) amplitude values to the average calculation, it will still increase the channel number in its denominator.

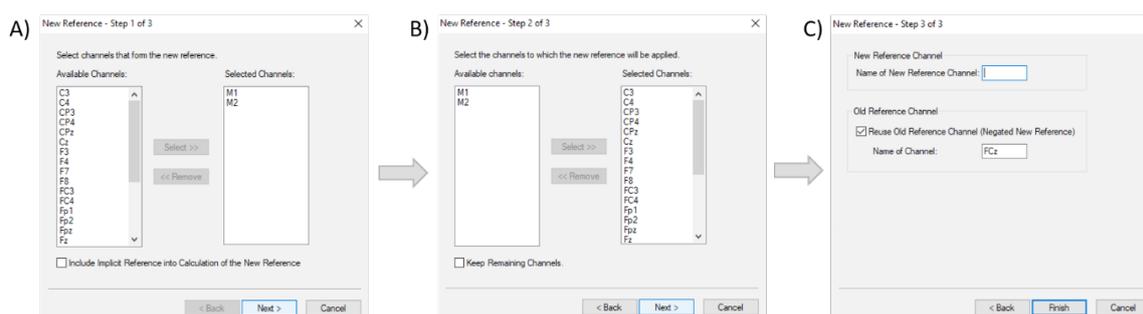


Figure 2: The dialogs of the New Reference transformation in Analyzer 2.

Second dialog window: Select the channels to which the new reference will be applied

In the second dialog (Figure 2 B), you can select which channels the new reference shall be applied to (i.e. from which channels it will be subtracted, see 2.1.). You will also have the option to keep channels that are not being re-referenced. Please note that usually all scalp channels should refer to the same reference (for exceptions see the “Some more facts”-box on the right). Otherwise you risk mixing different referencing schemes in transformations that include all scalp channels (such as topographic maps, ICA, connectivity calculations etc.).

Third dialog window: New and old reference channel

If the ‘Name of New Reference Channel’ option is selected in the third dialog (Figure 2 C), the chosen name will be added as a suffix to all re-referenced channels.

If the box ‘Reuse Old Reference Channel’ is ticked, you can reconstruct the signal of the online reference channel with respect to the new reference. The new reference is then subtracted from the online reference, which will after this step consist of the negated new reference (see 2.1.).

Caution: Note that in Analyzer 2 you also have the option to display your data with a specific referencing scheme in a montage. Please be aware that montages only constitute a display and do not actually re-reference your data.

3.2. Prerequisites: when to apply the New Reference transformation

The *New Reference* transformation can **theoretically be applied at any time**. As the subtraction of the new reference occurs point-wise, neighboring data points are not affected by the calculation. However, as stated above, changing the reference will also affect the amplitude and the time course of the signal. Following transformations can deliver different results, depending on the applied reference. When for example screening for artifacts, you would likely want the signal to be in its final state with its final referencing scheme. In most cases

it is therefore advisable to apply the *New Reference* transformation **early in the analysis pipeline** and to apply the subsequent analysis steps on the final referencing scheme.

— Some more facts —

There are **other specific referencing schemes** that are not discussed in detail in this article. For example, it is common to apply bipolar referencing to ocular channels and referencing to contralateral electrodes is commonly applied in sleep research. Other attempts have been made to find references that are as “neutral” as possible. The nose for example can be considered as a symmetric reference that picks up relatively little brain activity. However, a reference electrode on the nose might be distracting and difficult to attach in some cases. Another approach is the *Reference Electrode Standardization Technique* (REST), which references the signal to a theoretically neutral reference point in infinity (Yao, 2001).

In addition, there are **“reference-free”** ways to express EEG signal. One such option is the estimation of *Current Source Density* (CSD), which is also available as a transformation in Analyzer 2. CSD estimates the current flow out of the scalp at every location on the head. This measure does therefore, other than voltage, not require a reference point. Another Analyzer 2 transformation which works independently of applied referencing is the source localization approach **LORETA**.

Conclusion

Whichever reference you choose, it forms an important basis for your further data analysis and deserves some consideration. Hopefully, some of the information provided in this article will be helpful for your choice. If you would like to read more about referencing, you can find a selection of literature discussing this issue below. Should you need further support with referencing in BrainVision Analyzer 2, please don’t hesitate to contact us, the Brain Products **Scientific Support team**.

Literature

- Dien, J. (1998). “Issues in the application of the average reference: Review, critiques, and recommendations.” *Behavior Research Methods, Instruments, & Computers* 30(1): 34-43.
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