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Laplacian reference is optimal for steady-state visual evoked potentials

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Abstract

Steady-state visual evoked potentials (SSVEPs) are widely used in human neuroscience studies and applications such as brain-computer interfaces. Surprisingly, no previous study has systematically evaluated different reference methods for SSVEP analysis, despite that signal reference is crucial for the proper assessment of neural activities. In the present study, using four datasets from our previous SSVEP studies (1–3) and three public datasets from other studies (4–6), we compared four reference methods: monopolar reference, common average reference, averaged-mastoids reference, and Laplacian reference. The quality of the resulting SSVEP signals was compared in terms of both signal-to-noise ratios (SNRs) and reliability. The results showed that Laplacian reference, which uses signals at the maximally activated electrode after subtracting the average of the nearby electrodes to reduce common noise, gave rise to the highest SNRs. Furthermore, the Laplacian reference resulted in SSVEP signals that were highly reliable across recording sessions or trials. These results suggest that Laplacian reference is optimal for SSVEP studies and applications. Laplacian reference is especially advantageous for SSVEP experiments where short preparation time is preferred, since it requires only data from the maximally activated electrode and a few surrounding electrodes.

Keywords

Referencing method; Laplacian reference; steady-state visual evoked potential; Signal quality

New and Noteworthy

The present study provides a comprehensive evaluation of the use of different reference methods for steady-state visual evoked potentials, and has found that Laplacian reference increases signal-to-noise ratios and enhances reliabilities of SSVEP signals. Thus, the results suggest that Laplacian reference is optimal for SSVEP analysis.

Introduction

Steady state visual evoked potentials (SSVEPs) are oscillatory brain responses in the visual cortex, elicited by viewing visual stimuli that are modulated periodically as a function of time (7–11). SSVEP responses confine to narrowband peaks at the stimulation frequency and its harmonics. Due to high signal-noise-ratio (SNR) and robustness to artifacts, SSVEPs have been widely employed in human sensory and cognitive neuroscience, clinical applications, and brain-computer interface (BCI) designs (for review, see 12, 13).

Signal referencing for EEG data analysis is crucial to eliminate common background noise. A number of studies have tested the effect of using different reference methods on event-related potential (ERP) studies (for recent reviews, see 14, 15). The advantages and limitations of each reference method for ERPs have been investigated and discussed extensively. However, no previous study has comprehensively evaluated reference methods for SSVEP signals.

Several reference methods have been used in previous SSVEP studies. We searched SSVEP studies from 4 representative journals (*NeuroImage, Journal of Neuroscience, Neuropsychologia, Journal of Vision*) over the past 10 years, and counted the reference methods these studies used (a total of 114 studies as searched on Sept 2022; see Table 1 in https://doi.org/10.6084/m9.figshare.23690046.v1). The most commonly used methods are: the common average (65%), monopolar (16%), and averaged-mastoids (14%).

The common average reference is used in most SSVEP studies. This is not surprising since the common average is the most widely used reference for EEG studies in general (15). Common average reference is based on the assumption that the whole-head scalp electrical activity over a dipole in the layered spherical surface is zero (16), and it is regarded as a high-pass spatial filter that can eliminate the DC component of the spatial frequency spectrum at a fixed time (17). Based on this assumption, common average is considered the “gold standard” when the following preconditions

are met: (1) a large number of electrodes are recorded (e.g., 64 or 128), and (2) the electrodes are uniformly distributed over the entirety of the head (18–20). However, for SSVEP studies, there are lots of situations where only a few electrodes are recorded, since SSVEP responses are typically confined to the electrodes above a single sensory cortex (for review, see 12). By recording a small number of electrodes, the preparation time for experiments is much reduced, which is especially advantageous for studies involving special subjects such as children or clinical patients. In these studies, the monopolar reference is often used, i.e., referring to a single electrode (e.g., 22–24). It is, however, unknown whether the use of monopolar reference sacrifices signal quality to a certain degree, compared with other reference methods such as the common average reference. Another method, the averaged-mastoids reference, has also been used in previous SSVEP studies (see Table 1 in Appendix). Even though it has been found that averaged-mastoids would seriously bias the EEG power and distort the field maps for ERPs (24–26), it remains unknown how averaged-mastoids reference affects SSVEP signals.

The Laplacian reference has been used in SSVEP studies, but only rarely (20, 27–35). The Laplacian is a mathematical function, named after the French mathematician Pierre-Simon de Laplace (1749–1827), which exhibits positive values in the center and negative values in the surround. It estimates the second spatial derivative of the electric potential distribution on the scalp and is utilized in EEG recordings to estimate the brain generators of scalp electrical activity (36). Laplacian, similar to current source density (CSD) and scalp current density (SCD), are methods aimed at identifying the neuronal generator of scalp EEG. While Laplacian and SCD are based on the spherical shell head model (37–40), CSD is based on a linear volume-conduction model (38, for review about CSD, see 34, 39, 40). In practice, the measured EEG potentials have low spatial resolution and are spatially correlated, and the CSD/SCD estimate is approximately linearly related to the Laplacian estimate. Laplacian is calculated by the difference between the potential at one center electrode and the averaged potential of its nearest neighbors (36). For SSVEPs, Laplacian reference uses signals from the

maximal activated electrode, typically in medial occipital region at Oz (12), and subtracts the average of several neighboring electrodes. Mackay et al. (2003) found that using Laplacian reference in the analysis allowed for the detection of significant SSVEP signals based on shorter epochs compared to monopolar reference, suggesting that Laplacian reference may increase the signal-to-noise ratio (SNR) for SSVEPs. Laplacian reference has also been found to be the optimal reference for studying oscillatory activities in stereo-electroencephalography (44). Since Laplacian reference is known to be sensitive to shallow local sources (40, 45), and the origin of SSVEPs is typically confined to a single sensory cortex (for review, see 12), it suggests that Laplacian reference may be a suitable choice for SSVEPs. However, it remains to be tested whether Laplacian reference is also the optimal reference method compared to others such as the common average reference and averaged-mastoids reference.

In summary, several different reference methods have been used in previous SSVEP studies, which suggests a need for a comprehensive assessment on these reference methods to address the issues as outlined above. Therefore, in the present study, we re-analyzed four SSVEP datasets from our previous publications (1–3), as well as three public datasets from other studies (4–6), to evaluate the effect of reference methods on the quality of SSVEP signals. On top of the most common quality metric, i.e., the SNR, we also calculated a reliability index as a measurement of the signal consistency across recording sessions or trials. The rationale is that EEG signals capturing neural representations faithfully should be highly reproducible across trials (46, 47). Our results suggest that SSVEP signals using Laplacian reference have the highest SNR and best reliability, compared with other reference methods (i.e., monopolar reference, common average reference, the averaged-mastoids reference).

Methods

Datasets

We re-analyzed SSVEP data from 4 experiments in our previous publications (1–3), and also public data from 3 other studies (4–6). The studies are briefly described here, more details can be found in the published articles.

Dataset #1

The *dataset #1* was from the previous study (2), which is publicly available at: https://zenodo.org/record/808197. Twenty-five observers (15 females and 10 males) participated in the experiment. They either fixated at the screen center or executed smooth pursuit eye movements to a moving target, against a full-screen background that was counter-phase flickering at 7.5 Hz (7.5 reversals per second) to evoke SSVEPs. Here, we analyzed data from both the fixation and the pursuit condition together. Each trial lasted 150 seconds, and each observer underwent 8 trials in total. An EEG system (Brain Products, Munich, Germany) recorded EEG signals according to the international 10–20 system with 32 channels (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T7, T8, P7, P8, Fz, Pz, Oz, FC1, FC2, CP1, CP2, FC5, FC6, CP5, CP6, TP9, TP10, HLeo, Veo, HReo), at a sampling rate of 1,000 Hz. The ground electrode was placed at the AFz, and the online reference at the Cz.

Dataset #2

The *dataset #2* was from the first experiment of the study (1), which is publicly available at: https://zenodo.org/record/817545. The observers (N=12) either fixated at the screen center or executed smooth pursuit eye movements to an array of flickering targets, which were moving across the screen back and forth. Here, we analyzed data from both the fixation and the pursuit condition. The left and right side of a black-and-white checkerboard (8.15°×8.15°) were pattern-reversal flickering at 6.7 Hz or 7.5 Hz (balanced across trials). Each trial lasted 150s, and each observer conducted 4 trials. EEG recording was the same as *dataset #1*.

Dataset #3 and #4

The *dataset #3* and *#4* was from the first and second experiments of the study (3), which is publicly available at: https://zenodo.org/record/2636083. During the experiment, observers passively viewed a flickering filled circle at center of the screen. In Experiment 1 (N = 8), the circle flickering at 8 Hz for 52 seconds. The circle was presented at two physical viewing distances (40 cm and 80 cm). The size of the circle was changed every 5 seconds except the first size was presented for 7 seconds (the first 2 seconds after stimulus onset would be excluded from analysis to avoid abrupt visual responses). The diameter changed from 1° to 10°, or from 10° to 1° (balanced across trials), at a step of 1°. The EEG was recorded same as in *dataset #1*. In Experiment 2 (N = 8), the filled circle flickering at 8 Hz or 30 Hz at the center visual field. The size of the circle changed from 2° to 8° (or from 8° to 2°, balanced across trials) at a step of 1° at every 5 seconds, with the exception of the first size presented for 7 seconds. Each trial last for 37 seconds. The number of trials was 24. EEG signals were recorded from 32 active electrodes (actiCAP, Brain Products) according to the international 10–20 system, sampling rate at 5000 Hz. The ground electrode was placed at FPz, and the on-line reference electrode at FCz location.

Dataset #5

The *dataset #5* was from a study (5) which is publicly available at: https://osf.io/x9zr8/. We re-analyzed the data of 16 healthy control participants (there were 19 participants in total; we did not include 3 of them since we were not able to decode the EEG markers in their data). The stimuli were sine-wave gratings flickering at 4 Hz between 0 and their nominal contrast (i.e., 0, 1.5, 6, 24, and 96%). We analyzed data from all conditions together and took their average SSVEPs response. There were 8 blocks in total, with 25 trials in each block. Each trial lasted for 12 seconds. EEG data were recorded by ANT Neuro system according to the international 10-20 system with a 64-channel Waveguard cap, sampled at 1,000 Hz. The ground electrode was placed at AFz, and the EEG signals in each channel were referenced to the whole-head average.

Dataset #6

The *dataset #6* from a study (6) which is publicly available at: https://osf.io/y4n5k/. We analyzed the data of 99 healthy control participants (there were 100 participants in total; sub 43 was excluded due to having a number of bad channels that three times the standard deviation away from the mean). The stimuli were sinewave gratings flickering at 7 Hz between 0% contrast and their nominal Michelson contrast (7 contrast conditions, vary from 0, and 2-64% in logarithmic steps). We analyzed data from all conditions together and took their average SSVEPs response. There were 4 blocks in total, with 40 trials in each block. Each trial lasted for 11 seconds. EEG recording was the same as *dataset #5*.

Dataset #7

The *dataset #7* was from a recent study (4), which is publicly available at: https://osf.io/e62wu/. Participants (N = 12) fixated at a central marker, which was surrounded by a cluster of 20 sinusoidal grating patches. All target stimuli sinusoidally flickering at 5 Hz between 0% contrast and their nominal Michelson contrast (0, 6, 12, 24, 48, or 96%). We analyzed data from all conditions together and took their average SSVEPs response. There were 12 blocks, with 42 trials in each block. Each trial lasted for 11 seconds. EEG recording was the same as *dataset #5*.

Reference methods

Our EEG data were recorded using an online reference at Cz, FCz, or the whole-head average. Here we evaluated 4 additional (re-)reference methods: monopolar reference, common average reference, averaged-mastoids reference, and Laplacian reference. Since SSVEP responses in *datasets #1* to *#7* were all maximal at the occipital electrode Oz, our analyses focus on Oz only.

For monopolar reference, we re-referenced the signals at Oz to Fz in *dataset #1* to *#3*, and Cz for *dataset #4* to *#7*. For common average reference, the signals at Oz were re-referenced to the average of all 29 channels (not including the 3 EOG electrodes) in *dataset #1* to *#3*, all 32 channels in *dataset #4*, and all 64 channels in *dataset #5* to *#7*. For averaged-mastoids reference, we re-referenced the signals to the average signal of

TP9 and TP10 in *dataset #1* to *#4*, and the average of M1 and M2 in *dataset #5* to *#7*. For Laplacian reference, we re-referenced the signal from central electrode to the average of 5-9 nearest neighbor electrodes. The differentiation grid of a standard 10–10 montage with 67-channels for Hjorth Laplacian were shown in Figure 5 in (43), and Eq. (1) in (48) was used for the calculation. We re-referenced the signals from Oz against the average of 7 or 9 parietal-occipital electrodes. That is, 7 (i.e., O1, O2, P3, P4, P7, P8, Pz) in *dataset #1* to *#4*, and 9 (i.e., O1, O2, PO3, PO4, PO5, PO6, PO7, PO8, POz) in *dataset #5* to *#7*.

SSVEP analyses

The analyses for EEG signals were carried out using EEGLAB toolbox (49) and customized scripts in MATLAB. For all datasets, we detected the noisy channels and perform interpolation using functions from the EEGLAB plugin clean_rawdata() (http://sccn.ucsd.edu/wiki/Plugin_list_process). Firstly, we employed clean_flatlines() to detect channels that have no signal variation for a duration longer than 5 seconds. Then, for the remaining channels, we eliminated slow-wave drifts using clean_drifts() (forward-backward filter with a transition band of 0.5-1 Hz and stop-band attenuation of 80 dB). Secondly, we used clean_channels() to remove the channels with excessive line noise. This algorithm extracted line noise (signals above 50 Hz) from the raw EEG signal and calculated the noise-to-signal ratio for each channel. The noise-to-signal ratio was computed as the median absolute deviation (MAD) of the difference between the raw EEG and the line noise, divided by the MAD of the line noise. Channels with a z-transformed noise-to-signal ratio above 4 were identified as bad channels. For the removed channels, we applied eeg_interp() from EEGLAB to perform spherical interpolation. The average number of noisy channels across *datasets #1* to *#7* was 1.7.

The repetitions of conditions vary across datasets. In *dataset #1* and *#2*, each observer only has one single 150-s trial for every condition, while in *dataset #3* to *#7*, there were more than 2 trials in each condition. In *dataset #3-7*, the EEG signals from each condition and each individual observer were first averaged in the time domain. This averaging process was performed to increase the signal-to-noise ratio by reducing

non-phase-locked EEG noise that is not synchronized with the stimulation (e.g., 5, 47). Subsequently, the EEG signals for each condition were cut out into 5-s epochs, resulting in 30 epochs in *dataset #1* and *#2*, 10 epochs in *dataset #3*, 7 epochs in *dataset #4*, and 2 epochs in *dataset #5* to *#7*. All epochs were first de-trended by removing the linear fit (51). Then we zero-padded the signal to 10 s to get a frequency resolution of 0.1 Hz (e.g., 1, 49). We applied fast-Fourier transform (*fft*.m in MATLAB) to obtain the amplitude spectrum in each epoch. The amplitudes of all epochs in each condition were then averaged for further analysis.

SSVEP signal quality metrics

To evaluate the quality of SSVEP signals after applying the 4 reference methods, we calculated the signal-noise-ratio (SNR) and reliability of SSVEP responses in all 7 datasets.

Signal-noise-ratio (SNR)

The SNR is a metric widely used to evaluate SSVEP signals. In our analysis, we calculated SNR using the EEG amplitude at the target frequency (e.g., 7.5Hz) divided by the average noise at 10 adjacent frequency bins (e.g., 6.9, 7.0, 7.1, 7.2, 7.3, 7.7, 7.8, 7.9, 8.0, 8.1 Hz, two immediately adjacent bins were not included to avoid frequency leakage of target signals due to zero-padding). Since ratio data such as SNRs tend to be skewed rather than normally distributed, we used log-transformed SNR values in statistical tests.

Reliability

Reliability shows the estimated inter-item reliability of measurement obtained from repeated items, typically obtained by calculating the correlation between one half of the measurement and the other half (53). In our analyses, we calculated the correlation between SSVEP responses obtained after splitting EEG epochs or trials within the same condition.

The SSVEP responses were calculated by summing the amplitudes at all harmonics below 45Hz (54). At each stimulation frequency (e.g., 7.5Hz), we subtracted from the

peak amplitude the mean amplitude of the 10 nearby bins (e.g., 6.9, 7.0, 7.1, 7.2, 7.3, 7.7, 7.8, 7.9, 8.0, 8.1 Hz, two immediately adjacent bins were not included due to frequency leakage after zero-padding), in order to remove the baseline background noise.

For all datasets, we split the epochs into odd and even subsets and then calculated the mean SSVEP amplitude for odd and even subsets of each observer. The cross-observer Pearson correlation was computed between the two subsets for each condition. The reliability score reported in the result was the average of correlation coefficients across all conditions. With all conditions, the reliability was computed between two EEG recordings each with a duration of 600 seconds, 300 seconds, 200 seconds, 280 seconds, 1,200 seconds, 880 seconds, and 2,772 seconds, for each subject, in *dataset #1* to *#7*, respectively.

Results

The present study evaluated the effect of using different reference methods on SSVEP data across 7 different existing datasets. Overall, we found that Laplacian reference can effectively reduce broadband EEG noise, and enhance the signal-to-noise ratio and reliability of SSVEP responses.

Laplacian referencing reduces broadband noise

The effect of different reference methods on EEG signals from *dataset #1* is illustrated in Figure 1. Figure 1A and 1B shows identical EEG epochs from a sample observer after monopolar reference and Laplacian reference, respectively. Epochs with Laplacian reference show less noise. Figure 1C shows average spectrums of all observers for 4 reference methods. The broadband noise in EEG signals is lowest when using Laplacian reference, compared to the other three reference methods. The averaged-mastoids reference is not as effective as common average or Laplacian in term of reducing noise, and even slightly increases broadband noise at high frequency above 30Hz. Figure 1D depicts the topographic distribution and reveals the occipital origin of SSVEP responses. Left panel displays the topography of SSVEP amplitudes obtained from the raw data, while right panel shows the topography of SSVEP amplitudes after applying the Laplacian reference on every electrode. The distribution of SSVEP responses in the topography from the raw data is more dispersed compared to the Laplacian referenced data. Thus, Laplacian reference is useful to remove broad noise and enhance the clarity of the SSVEPs responses' origin.

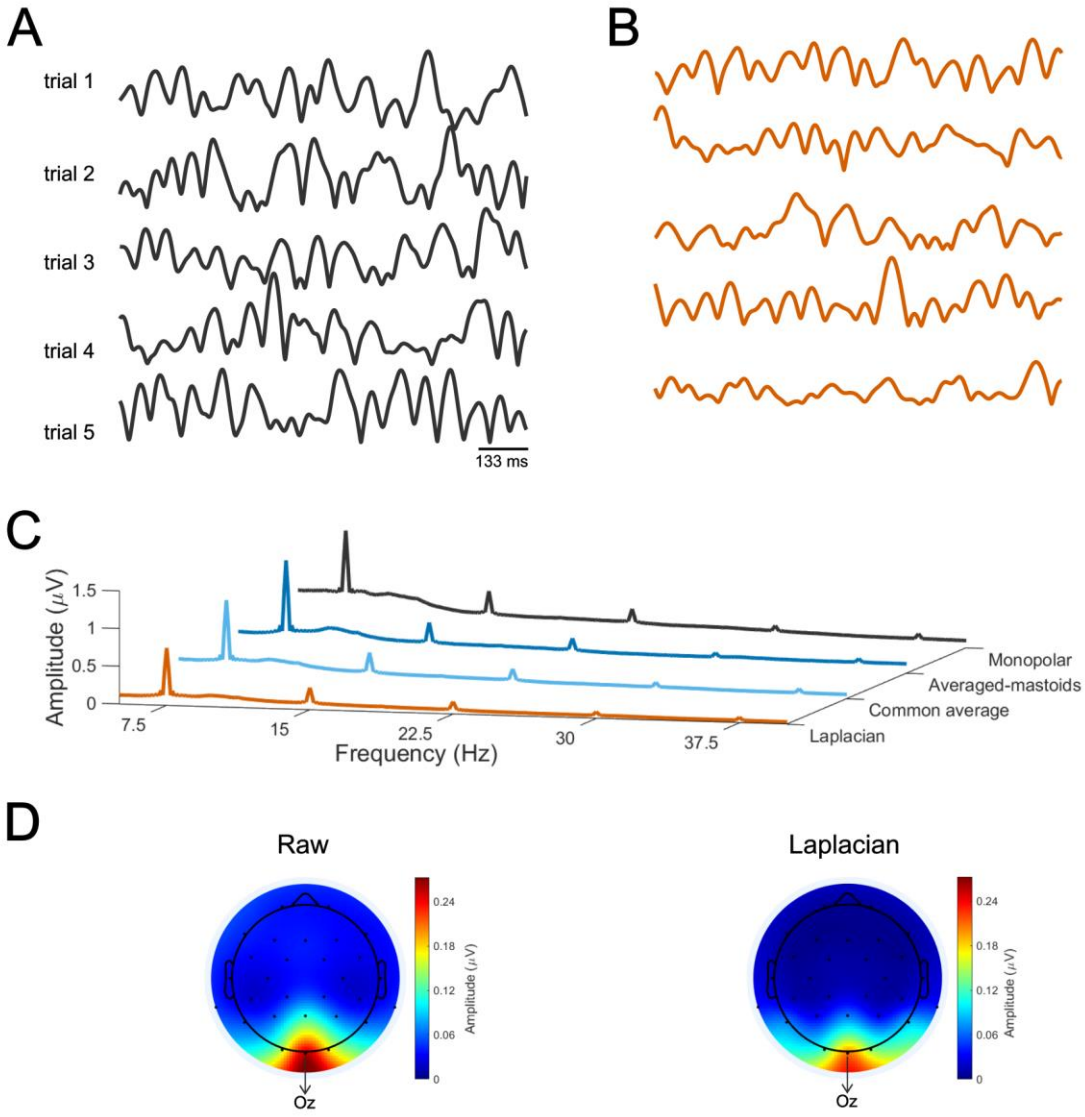

Figure 1. Effect of reference methods on EEG signals in *dataset #1* (A-D). ***A*** and ***B*** shows example EEG epochs at Oz electrode from a sample subject, with common average reference (A) and Laplacian reference (B). ***C*** shows average spectra of all subjects for the 4 reference methods. ***D*** represents the topographic plots that illustrates the occipital origin of these SSVEP responses. Left panel displays the topography of SSVEP amplitudes obtained from the raw data, while right panel shows the topography of SSVEP amplitudes after applying the Laplacian reference on every electrode. Overall, Laplacian reference reduces broadband noise the most, followed by common average, the averaged-mastoids, and monopolar reference.

Laplacian referencing increase SNRs

To assess the effect of reference methods on the quality of SSVEP responses, we computed the SNRs at the stimulation frequency and its harmonics. Figure 2 shows the result of *dataset #1*. Common average reference outperforms monopolar reference at all

harmonics. The averaged-mastoids reference increases the SNR at fundamental frequency, while at higher harmonics, the SNRs of the averaged-mastoids reference are not as good as that of the monopolar reference. Importantly, Laplacian reference resulted in the highest SNRs at stimulation frequency as well as harmonics. We conducted a 2 (type of reference method: Laplacian reference vs. common average reference) × 5 (harmonics: 1st to 5th) repeated-measure ANOVA and simple effects analysis on log-transformed SNRs. There were main effects for reference method, $F(1, 24) = 81.11$, $p < .001$, $\eta_p^2 = 0.782$. The pairwise comparison results revealed that the Laplacian reference has significantly higher SNRs in the 1st to 5th harmonics (all Ps < .01).

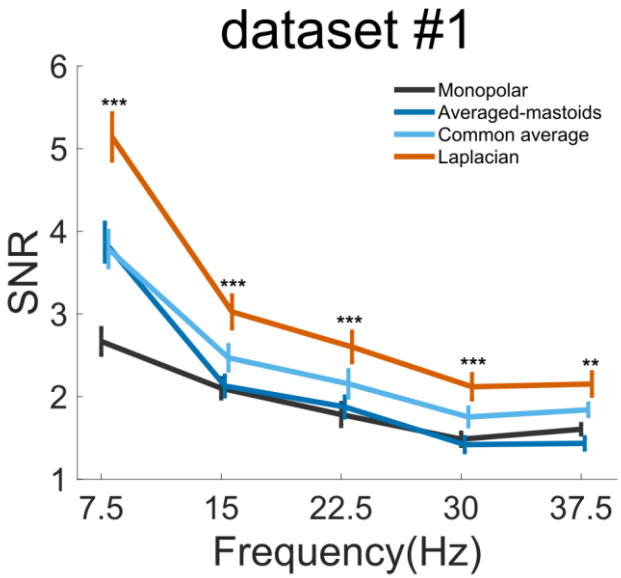

Figure 2. SNRs of SSVEP responses at the fundamental frequency (7.5 Hz) and higher harmonics in *dataset #1*. Laplacian reference results in the highest SNRs. Error bars represent standard errors across observers. Asterisks denote the significance of the difference between SNRs of SSVEP responses for common average reference and Laplacian reference, established using paired t-tests: *** for $p < .001$, ** for $p < .01$.

Next, we calculated SNRs in *dataset #1* to *#4*. Figure 3 shows the results. The SNR values here are the average of all harmonics below 45Hz. We conducted one-way ANOVA and pairwise comparisons on log-transformed SNRs in *dataset #1-4*, respectively. It is worth noticing that in *dataset #1* to *#4*, Laplacian reference resulted in the highest SNRs, which were significantly higher than monopolar reference and averaged-mastoids reference in *dataset #1* to *#4* (all Ps < .01). Since monopolar

reference is widely used for SSVEP studies (see Introduction), one interesting question is whether the use of monopolar reference sacrifices the signal quality compared with common average or averaged-mastoids reference. The statistical results on log-transformed SNRs show that, compared to monopolar reference, common average reference results in higher SNRs in *dataset #1* to *#4* (all Ps < .001); and the averaged-mastoids reference also has higher SNRs in *dataset #1* and *#2* (Ps < .05), but not in *dataset #3* and *#4* (Ps > .4). Therefore, despite of the convenience, the use of monopolar reference does have the disadvantage of lower SNRs compared with common average reference and Laplacian reference.

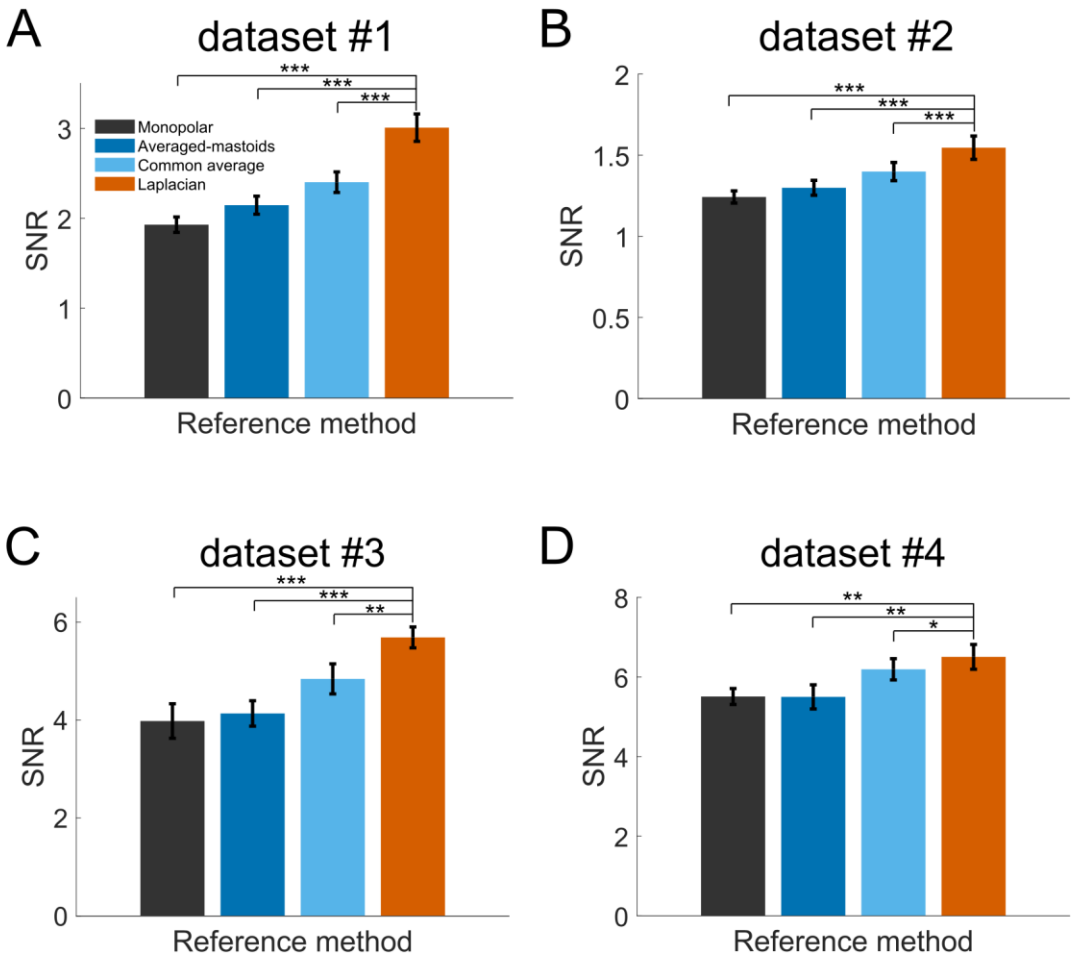

Figure 3. SNRs for 4 reference methods in *dataset #1 to #4*. Laplacian reference led to higher SNRs compared to other 3 reference methods in all 4 datasets. Error bars represent standard errors across observers. Asterisks denote the significance of the difference between SNR of SSVEP responses for different referencing methods, established using paired t-tests: *** for $p < .001$, ** for $p < .01$, * for $p < .05$.

Does Laplacian reference increase SNRs for all observers? Here, we further

examined SNR results in individual observers. In Figure 4, each dot represents data of a single observer, with *x* axis representing the SNR values using common average reference, and *y* axis the SNR values using Laplacian reference. Note that most observers fell above the diagonal line, indicating higher SNR with Laplacian reference compared to common average reference. This result suggests that Laplacian reference has a tendency to boost SNRs across observers.

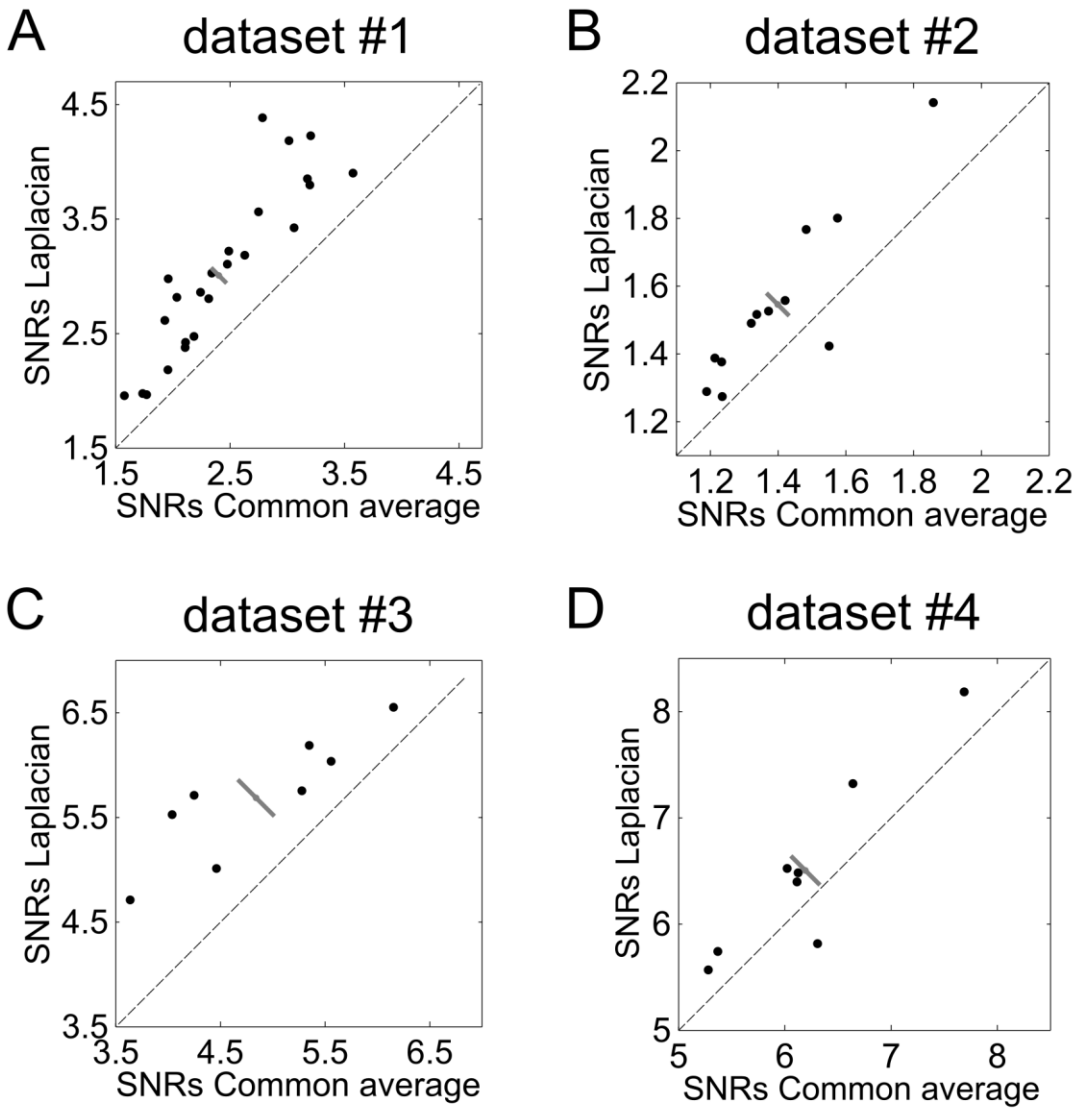

Figure 4. SNRs of SSVEPs in individual observer after common average and Laplacian reference in *dataset #1* to *#4* (A-D). Each filled circle represents data of a single observer. Most of the data points fell above the diagonal line, indicating higher SNR for the Laplacian reference than common average reference. Black bars denote 95% confidence intervals of the mean along the negative-slope diagonal line.

Laplacian referencing increases reliability

We assessed the reliability of SSVEP responses after applying each of the reference methods. The rationale was that high-quality physiological signals should be also highly reproducible across trials (epochs), given identical visual stimulations. Figure 5 shows an illustration for the calculation of reliability index. We calculated the cross-observer correlation between SSVEP amplitudes in the odd/even subsets of epochs of the same condition. The illustration shows an example for the calculation. In this case, Laplacian reference results in more reliable SSVEPs than common average reference with the same raw EEG data.

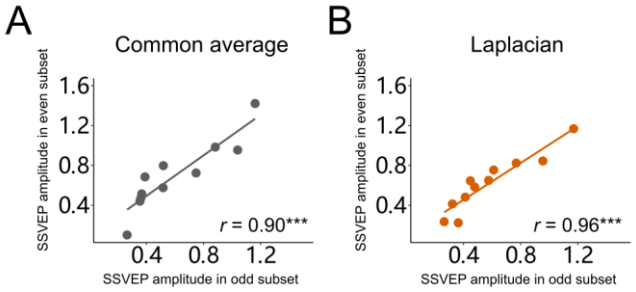

Figure 5. An illustration for the calculation of reliability. ***A*** shows the correlation of SSVEP amplitudes in odd and even subset of epochs from the same condition with common average reference (*dataset #2*). ***B*** shows the same data with Laplacian reference. Filled circles denote individual observers. SSVEPs are more reliable (i.e., more consistent across trials given identical stimulations) using Laplacian reference. Asterisks denote the significance of the coefficient, established using Pearson correlation: *** for $p < .001$.

The average reliability scores after applying each of the reference methods in *dataset #1* to *#4* are shown in Figure 6. Most of the reliability indices were high. This means that the quality of SSVEP responses in our datasets were excellent overall. Importantly, SSVEP signals using Laplacian reference show the highest reliability in *dataset #1* to *#4*, with reliability values of 0.993, 0.952, 0.988, and 0.993, respectively.

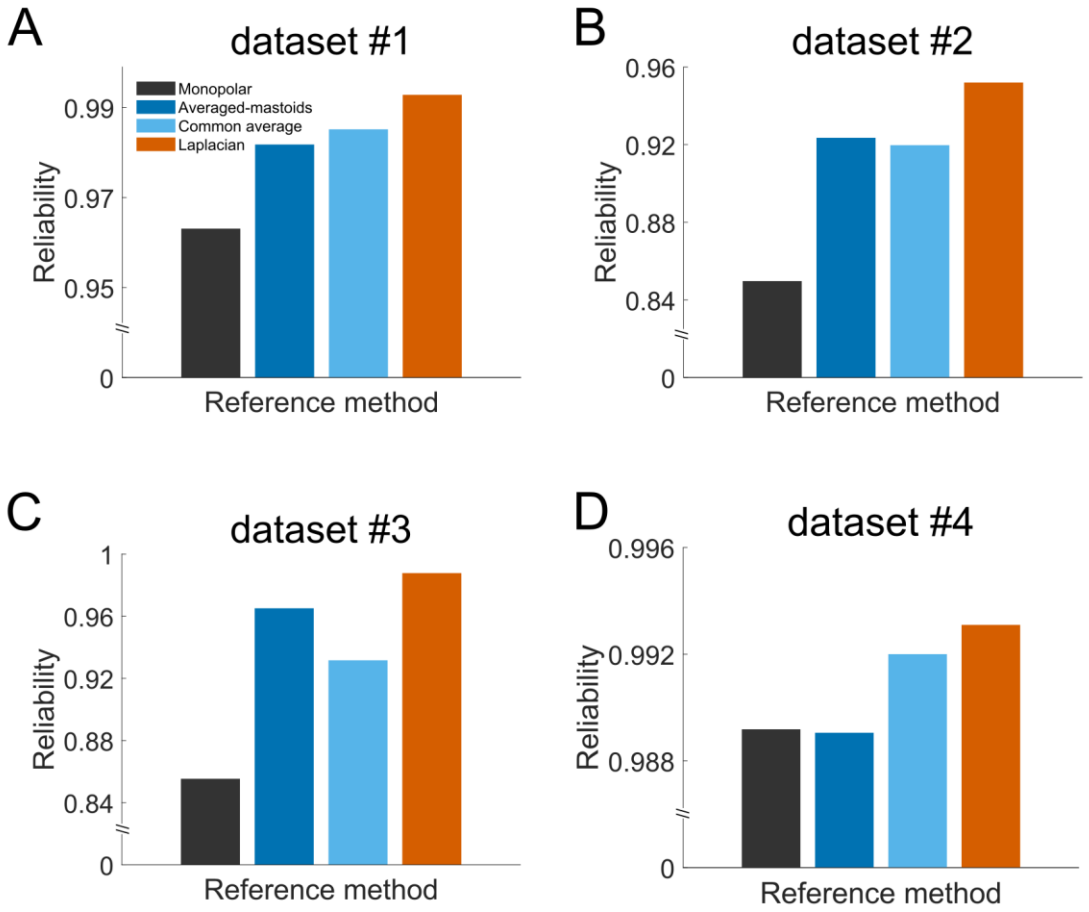

Figure 6. Split-half reliability for 4 reference methods in *dataset #1* to *#4*. SSVEP signals using Laplacian reference showed the highest reliability in *dataset #1* to *#4*.

The findings can be replicated in public datasets

A further question is how general these findings are, given that we have analyzed data from our own previous studies. Therefore, we tried to find out SSVEP studies which have made their data public, and obtain *dataset #5*, *#6*, and *#7* to extend our results. Figure 7 shows the SNRs and reliability after applying each reference method in *dataset #5* to *#7*. We conducted one-way ANOVA and pairwise comparisons on log-transformed SNRs in *dataset #5* to *#7*, respectively. SNRs with Laplacian reference are significantly higher than other reference methods in these 3 datasets (all Ps < .01). In addition, split-half reliabilities with Laplacian reference are the highest in *dataset #5* to *#7*. Therefore, with public datasets, we could replicate the finding that the Laplacian reference results in better SSVEP signals compared to other methods.

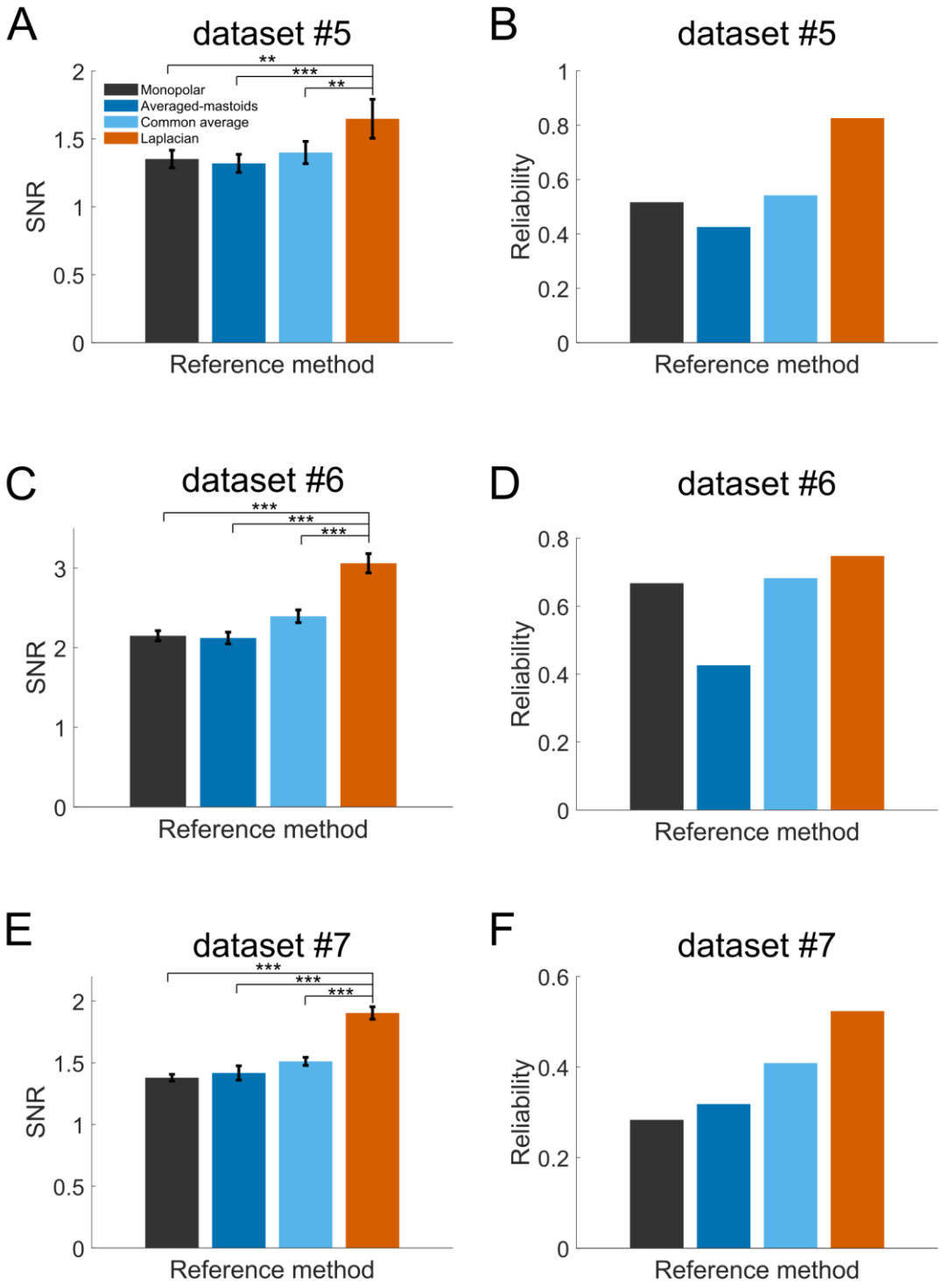

Figure 7. SNRs (*left*) and reliability (*right*) for 4 reference methods in *dataset #5* to *#7*. A whole-head average reference was used in the original analysis in these 3 datasets. SSVEP signals using Laplacian reference show the highest SNRs and reliability. Error bars represent standard errors across observers. Asterisks denote the significance level: *** for $p < .001$, ** for $p < .01$.

Laplacian referencing increase SNRs as a function of contrast

In *dataset #5* to *#7*, which included measurements of full contrast response

functions, we further evaluated the effect of reference methods on SNRs in the full contrast response function for the target-only condition (i.e., the contrast of mask is 0). Figure 8 shows the results. At low contrasts (i.e., 0%, 1.5%, and 2%), the SSVEP responses were weak, and the SNRs were close to 1. The 4 reference methods yielded similar SNRs in this range. We selected contrast levels which had significant SNRs (determined by one-sample t-tests on common average referenced data, SNRs > 1, $p < .05$) to examine whether the Laplacian reference outperforms common average reference in terms of SNRs across these contrasts. We conducted a 2 (type of reference method: Laplacian reference vs. common average reference) × n (contrast levels, 2, 6, and 5 in *dataset #5-7*, respectively) repeated-measure ANOVA and pairwise comparisons on log-transformed SNRs in *dataset #5* to *#7*. There were main effects for reference method in these 3 datasets: $F(1, 15) = 4.56$, $p = .050$, $\eta_p^2 = 0.233$; $F(1, 98) = 71.65$, $p < .001$, $\eta_p^2 = 0.422$; $F(1, 11) = 19.95$, $p < .001$, $\eta_p^2 = 0.645$. The statistical results revealed that Laplacian reference led to significant higher SNRs than common average reference at median to high contrast (i.e., 4%, 8%, 12%, 16%, 24%, 32%, 48%, 64%, and 96%), as depicted in Figure 8. These results suggest that the use of the Laplacian reference offers benefits in terms of SNRs across a broad range of contrast levels.

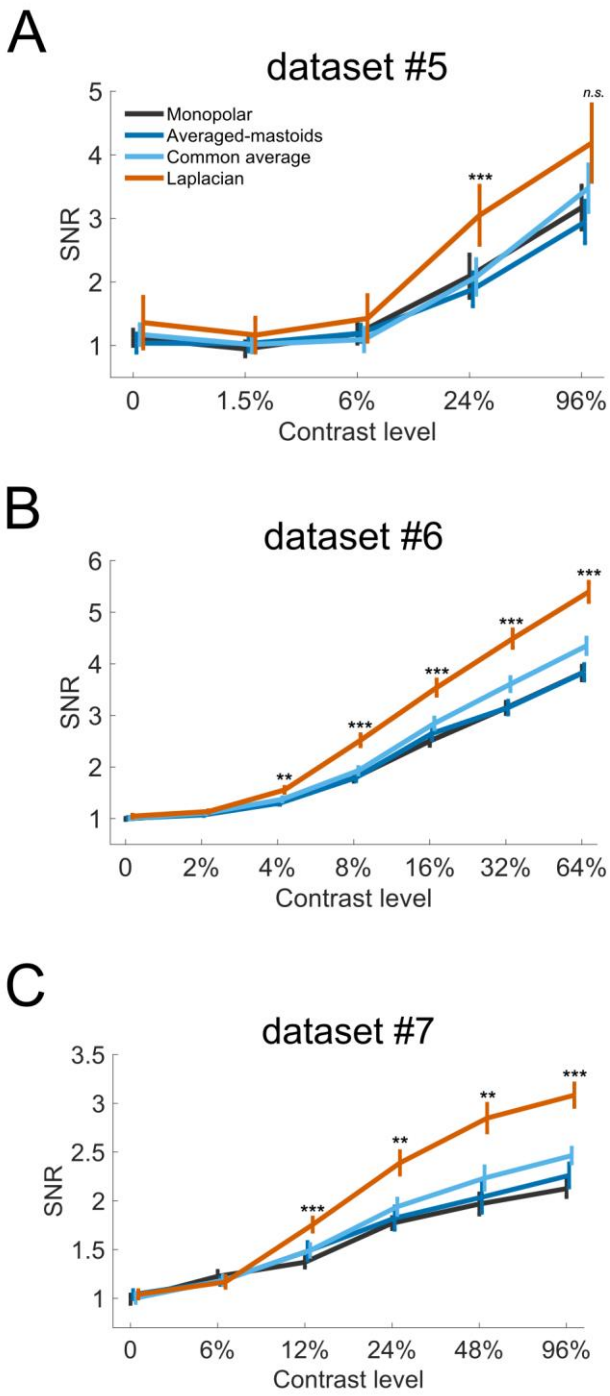

Figure 8. The SNRs as a function of contrast using 4 reference methods in *dataset #5* to *#7* at the target only condition (i.e., the contrast of mask is 0). Error bars represent standard errors across observers. Asterisks denote the significance of the difference between SNRs of SSVEP responses for common average reference and Laplacian reference, established using paired t-tests: *** for $p < .001$, ** for $p < .01$, *n.s.* for $p > .05$.

Optimal stimulation durations for different referencing methods

Here, we asked a further question about the optimal stimulation duration for

SSVEP studies. Since SNRs of SSVEP signals increase with longer stimulation durations, the optimal stimulation duration to achieve a certain SNR would differ for different reference methods. That is, given that Laplacian reference would result in higher SNRs, it would require shorter stimulation durations than other reference methods. We analyzed the *dataset #1* by using different lengths of epochs. Figure 9 shows SNR values as a function of epoch durations. Overall, SNRs increase with longer epochs. To achieve a certain level of SNR (e.g., 5), Laplacian reference requires an epoch length of 18 seconds, whereas other reference methods need 1.5 to 3 times longer. Therefore, by using Laplacian reference, SSVEP experiments could afford to use shorter stimulation durations.

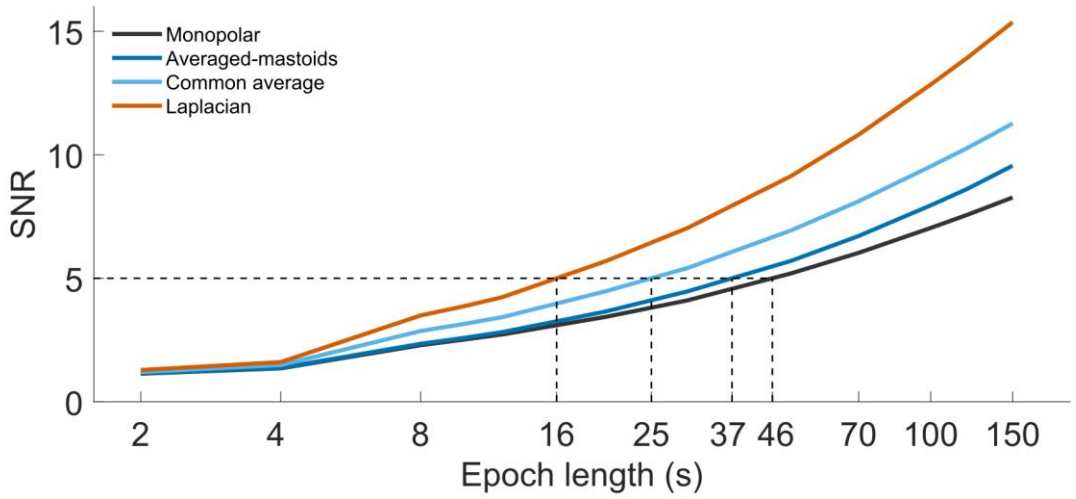

Figure 9. SNRs as a function of epoch lengths, for 4 reference methods in *dataset #1*. SNRs increase with longer epochs. To achieve a certain SNR, Laplacian reference requires the shortest epoch.

Discussion

The present study provides a comprehensive evaluation on the use of different reference methods for SSVEPs. Across 4 datasets from our own studies and 3 public datasets, we consistently found that Laplacian reference resulted in SSVEP signals that have lowest broadband noise, highest SNRs, and best reliability. It suggests that Laplacian reference enhances signal qualities compared to other reference methods such as monopolar, averaged-mastoids, and common average reference. Thus, our results support the use of Laplacian reference for SSVEP studies.

One of the major advantages of SSVEPs is that it provides a neural response with high SNRs, at a pre-defined narrowband frequency and at a known small brain region (usually at occipital electrodes, especially Oz). A large number of previous studies have taken advantage of this, and recorded only a few or even a single occipital electrode (e.g., 21, 22, 31, 32, 52). Preparation time for EEG setups would, therefore, be much reduced, which is especially important for experiments or applications involving special subjects such as children or clinic patients. In most of these cases, monopolar reference (referring to Cz or FCz) is used. Based on our current results, monopolar reference does sacrifice signal quality compared with the other 3 methods in terms of the level of noise, SNRs and reliability (Figure 1, 2, 3, 6, 7, 8, and 9). Our results indicate that Laplacian reference has the best performance. In term of preparation time, Laplacian reference is similar to monopolar reference, as only a few occipital electrodes are required (the maximal activated electrode and a few nearby electrodes). But the resulted SSVEP signals with Laplacian reference are better in all the quality metrics we have accessed. The SNRs as a function of contrast of the 4 reference methods demonstrates that utilizing the Laplacian reference can provide advantages in terms of SNRs across a wide range of contrast levels (Figure 8). Furthermore, by using Laplacian reference, SSVEP experiments can afford to use shorter stimulation durations (Figure 9), which could reduce recording time required or increase the experiment conditions one can test. Thus, based on our current results, Laplacian reference method is strongly recommended for SSVEP studies and applications.

We evaluated only 4 reference methods that are commonly used and are relatively simple to implement. There are other more sophisticated methods for SSVEPs, that has been used in certain situations, for example the Reliable Components Analysis (47) and the rhythmic entrainment source separation method (57). These methods require data from all EEG electrodes over the head, and have also other requirements or assumptions. For example, the rhythmic entrainment source separation was developed based on the assumption that steady-state activity is spectrally and spatially stationary over time, and also requires high-quality data with many time points. The Reliable Components Analysis decomposes all-channel EEG data into a small number of reliable components by maximizing trial-to-trial consistency, which requires dozens of homogeneous and phase-locked trials. If these requirements are satisfied, the Reliable Components Analysis could be a better choice than Laplacian reference.

As SSVEPs are widely used for BCI designs, previous studies in the field of BCI have also tried to optimize the reference method in order to improve the recognition accuracy for SSVEP signals. A large number of optimization methods that combines data from multiple EEG channels with various algorithms have been proposed (see 54, 55 for recent progresses and reviews). In terms of detecting SSVEP signals at certain frequency as in BCI applications, these methods would in principle result in higher detecting accuracy compared to a reference method as simple as the Laplacian. For example, the Generated Reference Filter method has been shown to provide higher accuracy than Laplacian and common average reference (58). For the spatial filter like common average reference and Laplacian reference methods, the classification results from Laplacian reference outperforms common average reference (60). However, stimuli used in neuroscience research are very different from BCI research. Testing these optimization algorithms is outside the scope of the current study. Future studies are needed to examine whether these algorithms developed in the BCI field also benefit SSVEP studies in neuroscience research.

The averaged-mastoids reference has been used more often in some old studies. However, it has been known for decades that averaged-mastoids reference would

seriously bias the EEG power and distort the field maps (24–26), and is thus not recommended for ERP studies. Here, we found that the averaged-mastoids reference is better than monopolar reference for SSVEP signals in most of the cases, but does not perform as well as the common average or Laplacian reference (Figure 1, 2, 3, 6, 7, 8, and 9). There seems to be no reason to use the averaged-mastoids reference in future SSVEP studies. The Laplacian is by all means the better choice.

It seems there is no well-accepted principle to the numbers of nearest neighbor electrodes for Laplacian reference in SSVEPs. Previous studies in SSVEPs took the averaged EEG signals from 2 to 9 electrodes surrounding the central electrode for Laplacian reference (e.g., 20, 28–32). Traditionally, the Laplacian operator is represented in orthogonal coordinates (36). However, because the Laplacian operator takes the potential difference between the central electrode and the mean of the surrounding electrodes, triangular and hexagonal arrays exist in addition to orthogonal arrays (61, 62). Usually, the number of nearest neighbors for Hjorth Laplacian estimate is 3-5, while any number of 'nearest' neighbors can be defined, up to the total number of recording sites minus one (43, 48). Based on these findings, we recommend to take the potential difference between the central electrode and the average of 5-9 nearest neighbor electrodes for Laplacian reference in SSVEPs studies (Figure 5 in (40) shows the differentiation grid of a standard 10–10 montage with 67-channels).

The focus of the present study was on the effect of Laplacian reference on SSVEP signals. However, it is a valid question whether Laplacian reference should be used universally for all EEG techniques, including ERPs and spontaneous oscillations. If a study were to analyze SSVEPs, transient ERPs, and spontaneous oscillations together, what reference method should be used? While a definitive answer to this question goes beyond the scope of the present study, we believe that the choice between different reference methods depends on the specific application scenario. Each reference method has its advantages and limitations, which make them suitable for different scenarios. Laplacian reference is sensitive to shallow local sources but insensitive to distributed

deep sources (40, 45). This in principle makes it a preferred reference method for certain ERP components and spontaneous oscillations that are generated by local shallow brain regions. This also explains why Laplacian reference is particularly good for SSVEPs, which are known to be mostly locally generated in a single sensory cortex (e.g., 1, 2, 21, 59–62). In future work, it would be beneficial to summarize the most recommended reference method for each EEG component based on the signal origin.

Overall, Laplacian reference provides high-quality SSVEP data and requires only several recording electrodes. Besides, Laplacian reference can be applied at every electrode on the whole head (Figure 1D). Since SSVEPs are not confined in occipital electrodes in other SSVEP paradigms, i.e., periodic stimulations with complex stimuli such as faces or words would elicit responses at other brain areas anterior towards the temporal lobe (e.g., 47, 63, 64), Laplacian reference to the whole head can help find the maximal activated electrode and facilitate the following analysis. However, in the case of recording a limited number of electrodes, the maximal activated electrode should to be known before setting up recording electrodes at the maximal and nearby locations in the use of Laplacian reference. In some situations, researchers might choose a cluster of electrodes, instead of a single electrode with maximal responses, for analysis. Does Laplacian referencing outperform other referencing methods in this case? We compared SNR values when the cluster of O1/Oz/O2 was used, and found that Laplacian referencing led to highest SNRs compared to other reference methods as well. Therefore, the current finding can be generalized to the situation where a cluster of electrodes is used.

Conclusion

The present study provides an empirical assessment on reference methods for SSVEP studies and analyses. Based on quality metrics of SNRs and reliability, the use of Laplacian reference is highly recommended. Laplacian reference is especially

573 advantageous in certain studies or applications where short preparation time is favored,
574 since it only requires data from the maximal activated electrode and a few surrounding
575 electrodes.
576

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