CARLA: Adjusted common average referencing for cortico-cortical evoked potential data

Harvey Huang[*,a], **Gabriela Ojeda Valencia**[b], **Nicholas M. Gregg**[c], **Gamaleldin M. Osman**[c,f], **Morgan N. Montoya**[b], **Gregory A. Worrell**[b,c], **Kai J. Miller**[b,d], **Dora Hermes**[*,b,c,e]

[a]Mayo Clinic Medical Scientist Training Program, Rochester, MN;

[b]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN;

[c]Department of Neurology, Mayo Clinic, Rochester, MN;

[d]Department of Neurologic Surgery, Mayo Clinic, Rochester, MN;

[e]Department of Radiology, Mayo Clinic, Rochester, MN, 55901.

[f]Division of Child Neurology, Department of Pediatrics, McGovern Medical School at UTHealth, Houston, TX.

Abstract

Human brain connectivity can be mapped by single pulse electrical stimulation during intracranial EEG measurements. The raw cortico-cortical evoked potentials (CCEP) are often contaminated by noise. Common average referencing (CAR) removes common noise and preserves response shapes but can introduce bias from responsive channels. We address this issue with an adjusted, adaptive CAR algorithm termed "CAR by Least Anticorrelation (CARLA)".

CARLA was tested on simulated CCEP data and real CCEP data collected from four human participants. In CARLA, the channels are ordered by increasing mean cross-trial covariance, and iteratively added to the common average until anticorrelation between any single channel and all re-referenced channels reaches a minimum, as a measure of shared noise.

We simulated CCEP data with true responses in 0 to 45 of 50 total channels. We quantified CARLA's error and found that it erroneously included 0 (median) truly responsive channels in

[*]Corresponding: huang.harvey@mayo.edu & hermes.dora@mayo.edu.

the common average with 42 responsive channels, and erroneously excluded 2.5 (median) unresponsive channels at all responsiveness levels. On real CCEP data, signal quality was quantified with the mean R^2 between all pairs of channels, which represents inter-channel dependency and is low for well-referenced data. CARLA re-referencing produced significantly lower mean R^2 than standard CAR, CAR using a fixed bottom quartile of channels by covariance, and no re-referencing.

CARLA minimizes bias in re-referenced CCEP data by adaptively selecting the optimal subset of non-responsive channels. It showed high specificity and sensitivity on simulated CCEP data and lowered inter-channel dependency compared to CAR on real CCEP data.

Keywords

Cortico-cortical evoked potential; Intracranial EEG; Stereo EEG; Single pulse electrical stimulation; Common average reference; Re-referencing

1. Introduction

1.1. Offline re-referencing of intracranial EEG data

Intracranial brain stimulation is used increasingly to map effective connectivity in the human brain (Friston, 1994; Keller et al., 2014). A fundamental understanding of how electrical stimulation spreads through brain networks can be acquired by applying single pulse stimulation to select pairs of intracranial EEG (iEEG) electrodes and measuring the evoked potentials at others (Araki et al., 2015; Keller et al., 2014; Kundu et al., 2020). The measurement electrodes may lie on the convexity of the brain surface from electrocorticography (ECoG) or in deeper structures from stereoelectroencephalography (stereoEEG, sEEG). Single pulse electrical stimulation is performed using subsets of these electrodes, most often in anode-cathode pairs, in cortical and non-cortical structures such as white matter and thalamus. The voltage peaks and troughs captured by the measurement electrodes, termed cortico-cortical evoked potentials (CCEPs), quantify synchronous signal propagation downstream from the stimulation site and are a macroscopic analog to local field potentials (Logothetis et al., 2010; Silverstein et al., 2020). Accurately characterizing the amplitudes and shapes of these CCEPs can provide valuable information on connectivity between stimulation and measurement areas (Barbosa et al., 2022; Huang et al., 2023; Krieg, 2017; Kundu et al., 2020; Matsumoto et al., 2004, 2007; Valencia et al., 2023).

Raw iEEG data at each channel are recorded as the amplified potential difference between each measurement electrode and a reference electrode, which is chosen *a priori* by the clinician or researcher. The reference electrode is either external, in which case it is located on the skin or scalp, or internal, in which case it is another iEEG electrode that ideally picks up as little neural activity as possible. In both situations, the amplified iEEG data is prone to contain broadband and periodic noise (e.g., 50 or 60 Hz "line noise") (Mercier et al., 2022; Nunez & Srinivasan, 2006). The amplitude of this noise can be several orders of magnitude greater than the physiological signal of interest. Furthermore, the time-varying potential differences picked up by the reference electrode are synchronously introduced to

all channels. In general, these factors mean that the signal quality of iEEG data can be substantially improved by offline re-referencing prior to interpretation.

There is no single perfect re-referencing method for iEEG data, as different signal features are better preserved by different references. Local re-referencing methods such as bipolar and Laplacian, which are calculated as the differences between spatially adjacent sets of electrodes, attenuate signal features that are shared between neighboring electrodes to magnify highly focal signal features such as broadband activity (Dickey et al., 2022; Li et al., 2018; Mercier et al., 2022; Shirhatti et al., 2016). In the case of CCEP data, large amplitude deflections can be distributed across multiple neighboring electrodes and are often of greatest interest to quantify. Local re-referencing methods are therefore inadequate in this context because they can markedly reduce the amplitude of CCEPs, distort temporal profiles, and introduce phase reversals (Arnulfo et al., 2015; Shirhatti et al., 2016). A less severe re-referencing method for CCEP data is the common average reference (CAR), in which the mean signal across all channels approximates the common noise and is subtracted from each channel. One major disadvantage to this approach is that signals from highly responsive channels may be smeared into all other channels and introduce bias. Given these potential issues with both local re-referencing methods and CAR, many CCEP studies opt out of re-referencing altogether. Recently, a few modified referencing methods based on CAR have been proposed. One proposal is to use only channels corresponding to white matter electrodes, with the assumption that responses are negligible at white matter electrodes (Arnulfo et al., 2015; Uher et al., 2020). This assumption, however, does not always hold true (Mercier et al., 2017; Uher et al., 2020), and the number of white matter electrodes available can vary depending on the clinical plan. Using anatomical data to inform iEEG data preprocessing also requires accurate coregistration and segmentation of imaging data. Another proposal is to only use channels with low-variance signals as a reference. This approach requires determining which channels qualify as "low-variance", which can differ by dataset and by stimulation site within the same dataset. In this work, we center on the concept of low variance channels to develop a data-driven re-referencing algorithm, which adaptively optimizes which channels to include in the common average based on the input CCEP data. We demonstrate that our algorithm effectively separates responsive from nonresponsive channels on simulated CCEP data and performs well on real CCEP data recorded from four human subjects, as quantified by a reduction in cross-channel explained variance.

1.2   Theoretical components of cortico-cortical evoked potential data

The goal of common average referencing is to improve the signal of the evoked potential relative to the noise, without introducing bias. We first use simulated CCEP data to introduce the theoretical CCEP components of interest (Figure 1). The raw time series present at a single channel is the sum of a transient stimulation artifact, an evoked potential (when present), individual broadband noise, and common noise. The evoked potential is a set of consistent stimulation-locked voltage deflections across trials. The individual broadband noise is unique to each channel and trial and typically follows a $1/f^X$ power spectrum, where X may vary by experimental condition and equipment (Bédard et al., 2006; Miller et al., 2009). This "power-law" distribution is thought to arise from the sum of simple neuronal

processes, such as temporal integration and exponential decay of post-synaptic currents, and low-pass filtering by tissue, which are asynchronous and not stimulation-driven. The common noise is shared across all channels but unique in time to each trial; it is the sum of common periodic line noise ("mains hum", at 50 Hz or 60 Hz, + harmonics) and common broadband $1/f^X$ noise that is recorded and distributed by the original reference electrode.

The optimal common average referencing procedure should maximally attenuate the common noise (both periodic and broadband components) but minimally introduce bias from responsive channels. The individual broadband noise unique to each channel is not removed by re-referencing; rather, it can be mitigated by averaging the signal across multiple trials.

2. Materials and Methods

2.1. Ethics statement

The experiments in this study were conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Mayo Clinic (IRB #15–006530), which also authorizes sharing of the data. Each patient/representative voluntarily provided independent written informed consent/assent to participate in this study.

2.2. Analytic framework

The CAR is prone to introducing bias from highly responsive channels into all other channels. Variations on CAR exclude responsive channels based on anatomical position or signal properties. We propose here a variation on CAR that involves calculating the signal variance on a predetermined time interval (i.e., between 10 and 300 ms) for all channels, and then including only a subset of channels with the lowest variance in the common average. Signal variance is equivalent to the energy of the mean-centered signal on the time interval of interest, and is therefore related to response strength. To determine how many channels should be included in the (adjusted) common average, a percentile threshold can be chosen arbitrarily or by visual inspection of plotted signals, as an upper bound on variance. If the threshold is too lenient (e.g., 95% of all channels by lowest variance included in the common average), the common average risks including bias from responsive channels when the stimulation site projects to many channels. If the threshold is too strict (e.g., 10% of all channels by lowest variance included), there may be non-negligible dependencies between the common average and the individual noise of each channel used to create it (Box 1).

Our algorithm optimizes the number of channels to include in the adjusted common average, adaptively based on the signal features in the input data set. We considered the following set of criteria in developing this algorithm:

1. Logically similar to the rationale used in visual inspection of signals

2. Depends only on the CCEP data, not on other information such as anatomical location

3. Effective across a wide range of cases containing sparse to widespread channel responses

4. Minimal dependency on statistical thresholds and user-input parameters

2.3. Algorithmic optimization of channels in common average

The proposed algorithm, which we refer to as CAR by Least Anticorrelation (CARLA), is described below and a single-trial example is shown in Figure 2. In brief, the goal is to include the n channels with the lowest signal variance or covariance to construct the adjusted common average, on a per-trial basis, such that there is the least amount of anticorrelation between the re-referenced channels and any single channel that makes up the common average. CARLA is best applied after data have been preprocessed to remove channels and trials containing large artifacts (see section 2.5).

1. *Input signals*: Denote the input to be V_{input}, a 3-dimensional matrix of N channels by T time points by K trials conducted for the same experimental condition (e.g., same stimulation site).

2. *Essential preprocessing*: We filter V_{input} with notch filters at 60 Hz and its first two harmonics (120 Hz, 180 Hz), and denote the filtered signals V (Figure 2A). Filtering is done to attenuate line noise, which is often of high amplitude, such that signal variance (energy) and cross-channel correlations in V are more directly reflective of the stimulation-locked responses. V will be used to determine the optimal subset of channels, but the final re-referencing will be performed on the unfiltered input, V_{input} (Step 7).

3. *Ordering channels to include in the common average*: All channels are ranked in order of increasing mean covariance across all pairs of trials (self-covariance excluded) in V on a responsive time interval. Covariance is a good estimator of both signal strength and reliability across trials, with an expected value of 0 when trials are uncorrelated. Variance is used in place of covariance in the single-trial situation ($K = 1$). This step results in the less responsive channels being ordered earlier than the more responsive channels.

   a. *CCEP-specific parameter*: We chose the response interval to be 10 ms to 300 ms post-stimulation, as this window reasonably reflects early, direct responses (e.g., N1) that are typically contained in the first 100 ms, as well as later, indirect responses that typically begin well before 300 ms (e.g., N2). The first 10 ms are omitted avoid the stimulation artifact. This interval may be adjusted based on prior estimates of response duration in the user's dataset.

4. *Anticorrelation as the statistic of interest*: Some degree of anticorrelation is expected to exist between the common average referenced channels and each original channel (prior to re-referencing) that makes up the common average. This is because re-referencing distributes the individual noise of each channel, multiplied by $-1/N$, into all other channels. If a subset of n nonresponsive channels is used to construct the common average, the anticorrelation between any individual channel and all other re-referenced channels decreases in magnitude as n increases, because the proportion of each individual channel in the common average decreases $\propto 1/n$. However, when a channel with a large

response that deviates strongly from the common noise, such as a CCEP, is incorporated into the common average, it introduces a bias into the re-referenced channels that manifests as a strong anticorrelation between itself and all other re-referenced channels without a similar response morphology. CARLA optimizes the number of channels to include in the common average by minimizing this anticorrelation statistic.

5. *Iterative calculation of the statistic*: For $n = 2, 3, \ldots N$, the n channels with the lowest-ranked mean (co)variance are iteratively taken to form subset U_n, $U_n \subseteq V$. At each iteration, the common average is calculated from U_n, separately for each trial, and subtracted from U_n to yield the re-referenced $U_{n,CAR}$ (Figure 2B). Then for $i = 1 \ldots n$, $\bar{z}_{i,n}$ denotes the average Fisher z-transformed Pearson's r between the ith channel in U_n (before re-referencing) and all other channels in $U_{n,CAR}$ on the response interval. $\bar{z}_{i,n}$ therefore quantifies the extent to which the ith channel would influence all other channels in $U_{n,CAR}$ if U_n were used to make the adjusted common average. At each n, the most negative $\bar{z}_{i,n}$ over all $i = 1, 2, \ldots n$, $\min(\bar{z}_{i,n})$, belongs to the most globally anticorrelated channel in U_n, and is designated ζ. When $K > 1$, mean ζ and its 95% confidence interval are estimated across trials by bootstrapping.

6. *Optimization criteria*: The optimal common average can be constructed from the first n channels ordered by lowest (co)variance, at n corresponding to the least negative value (global maximum) in ζ (Figure 2C). This occurs when there is the least anticorrelation between any single channel in U_n and all others in $U_{n,CAR}$. Alternatively, a more sensitive n may be identified at the first local maximum in ζ prior to a significant decrease, where statistical significance is assessed by bootstrapping the difference between pairs of ζ from trials (only possible when $K > 1$). This stopping criterion is explained in greater detail in the results section below, and it better identifies the smallest subset of channels before the inclusion of any responsive channels.

7. *Output signals*: The final common average is calculated, per trial, as the mean time series across the optimal n channels with the lowest (co)variance from the unfiltered V_{input}. The common average is subtracted from V_{input} to produce the re-referenced signals.

Figure 2B shows that when too few channels are used to construct the common average (e.g., $n = 2$), re-referenced signals show strong anticorrelation with any individual channel making up the common average. When a channel with high variance is incorporated into the common average (e.g., $n = 5$), re-referenced signals show strong anticorrelation with the high-variance channel due to introduced bias. The optimal common average based on minimizing ζ, at $n = 4$ in this case, includes all nonresponsive channels and excludes highly responsive channels. Intuitively, this cutoff co-occurs with a shoulder in the sorted channel variances (Figure 2D).

Note also from Figure 2B that ζ at each n often belongs to the nth and most recently added channel ($i = n$) in U_n. Since the nth channel has the highest variance out of all channels in

U_n (or covariance across trials when $K > 1$), it is most likely to have the greatest influence on the common average. However, if the nth channel is anticorrelated with earlier responsive channels, it may paradoxically exhibit a positive correlation with the re-referenced channels on average, resulting in spuriously positive $\bar{z}_{i,n}$, $i = n$. This motivates the necessity of finding $\min(\bar{z}_{i,n})$ out of all possible channels at each iteration of U_n to designate as ζ.

2.4. Construction of simulated CCEP data

CARLA was first tested on simulated CCEP data, where we varied the number of channels containing true responses. Simulated CCEP trials were created at 4800 Hz for each responsive channel by summing the following components (see Figure 1): an evoked potential, an individual broadband noise unique to each trial and channel, a common noise shared across channels but unique to each trial, and a transient stimulation artifact at the beginning of the signal. Trials for nonresponsive channels were created by summing all noise and stimulation artifact components only, without evoked potential. The simple simulation procedure is described sequentially below. Note that our goal here was to generate the necessary components to test CARLA, rather than to provide the best biophysical approximation of real CCEPs.

Each evoked potential, $P(t)$, was modeled as a sum of two causal sinusoids, each enveloped by a difference of two exponential decays (Figure 3):

$$P(t) = A \times [S_1(t) + S_2(t)], t \geq 0$$
$$\text{where } S_1(t) = \left(e^{-t/\tau_1} - e^{-t/\tau_2}\right) \times sin(2\pi f_1 t - \phi_1)$$
$$\text{and } S_2(t) = \left(e^{-t/\tau_3} - e^{-t/\tau_4}\right) \times sin(2\pi f_2 t - \phi_2).$$

t is the time from stimulation, in seconds. A is a scalar amplitude, sampled uniformly at random between 80 and 120. τ_1 and τ_3 are time constants that control how quickly each envelope decays, sampled uniformly at random between 0.01 and 0.03 and between 0.06 and 0.14 respectively. τ_2 and τ_4 determine the rise time of each envelope and were held constant at 0.005 and 0.025, respectively. f_1 and f_2 are the frequencies of each sinusoid in Hz, sampled uniformly at random between 8 and 12 and between 1 and 3, respectively. Finally, ϕ_1 and ϕ_2 are the phases of each sinusoid, sampled uniformly at random between 0 and 2π (all possible phases). The intervals used for the time constants and frequencies were determined empirically from standard CCEP characteristics. S_1 captures a fast, direct response, with a mean half-period of 50 ms similar to the CCEP N1 deflection. S_2, the indirect response, has half-period and exponential time constants that are 5 times slower than S_1, on average, each of which with also more jitter. The resulting evoked potentials are diverse in waveform, characteristic of those recorded at different layers of cortex by sEEG (Huang et al., 2023; Miller et al., 2021).

The individual broadband $1/f^X$ noise unique to each channel and trial was modeled as Brown noise ($X = 2$) by cumulatively summing independent samples from a standard normal distribution, multiplied by a gain factor. This gain factor was 0.4 for the main analysis, but was increased stepwise when investigating the effect of decreasing signal-to-noise ratio (in greater detail below). The time series was then high pass-filtered with a Butterworth filter

above a −3 dB cutoff frequency of 0.5 Hz by forward-reverse filtering. This filtering step is the same as applied to our real CCEP data to remove low-frequency drift.

The common noise for each trial consisted of two subcomponents summed together. The first subcomponent is periodic line noise, $L(t)$, that is the sum of a 60 Hz sinusoid plus two out-of-phase harmonics at 120 Hz and 180 Hz:

$$L(t) = A_1 S_1(t) + A_2 S_2(t) + A_3 S_3(t),$$
$$\text{where } S_1(t) = sin(2\pi 60t - \phi_1),$$
$$S_2(t) = sin(2\pi 120t - \phi_2),$$
$$\text{and } S_3(t) = sin(2\pi 180t - \phi_3).$$

t is time from stimulation, in seconds. A_1, A_2, and A_3 are scalar amplitudes equal to 8, 2, and 1, respectively, representing decreasing contributions from the two harmonics. ϕ_1, ϕ_2, and ϕ_3 are phases each sampled uniformly at random between 0 and 2π. The second subcomponent is Brown noise constructed the same way as the individual broadband noise above, corresponding to noisy fluctuations recorded by the reference electrode.

Finally, a sharp transient stimulation artifact, $Q(t)$, was also added between 0 and 2 ms post-stimulation, as a fast constant-phase sinusoid described by:

$$Q(t) = A \times sin(2\pi 600t), 0 \le t < 0.002.$$

t is time from stimulation, in seconds, and A is a scalar amplitude sampled uniformly at random between 47 and 53. This artifact was included for completeness but did not affect the results since it was well before the analyzed response interval of [10, 300 ms].

Simulated CCEP data with 50 channels and 12 trials were created at 46 levels of responsiveness, where 0 (0%) to 45 (90%) of all channels contained true responses. 30 random sets of CCEPs were simulated at each level of responsiveness. The performance of CARLA was evaluated on these simulated data by quantifying the number of errors in CARLA's output. For each simulated set, responsive channels missed (RCM) indicate responsive channels erroneously included in the common average and nonresponsive channels missed (NCM) indicate nonresponsive channels erroneously excluded from the common average. Sensitivity to detect responsive channels refers to the fraction of all truly responsive channels correctly excluded from the common average. Conversely, specificity to detect responsive channels refers to the fraction of all truly nonresponsive channels included in the common average. To test the reliability of the method on small numbers of channels, we repeated the analysis with fewer total channels (25, 20, 15, and 10), while evaluating accuracy with the same percentages of all channels responsive. To investigate the impact of lower response signal-to-noise ratio (SNR) on method reliability, we also repeated the analysis (with 50 total channels) at decreasing levels of trial SNR. To do so, evoked potential $P(t)$ amplitudes were maintained while both broadband noise elements (the individual Brown noise and the Brown noise component of the common noise) were incrementally increased in amplitude. The periodic line noise was not changed because it can be largely attenuated by filtering regardless of amplitude. For each individual trial, SNR was quantified as the

ratio between the power of $P(t)$ and the power of the summed broadband noise on the response window (10 to 300 ms).

2.5. Collection and preprocessing of real CCEP data

iEEG voltage data were measured from 4 human subjects (2 male, 2 female, see Table 1) who had been implanted with sEEG electrodes for epilepsy monitoring. Recorded data were digitized at 4800 Hz on a G.Tec G.HIAmp biosignal amplifier, and then high pass-filtered above 0.5 Hz by forward-reverse filtering with a second-order Butterworth filter (MATLAB filtfilt). Data were originally referenced to an sEEG electrode in the white matter.

Electrode pairs were stimulated 12 times (trials) each with a single biphasic pulse of 200 μs pulse width and 6 mA amplitude every 3–5 s, using the G.Tec G.Estim PRO electrical stimulator. Stimulation sites were excluded from analysis if they overlapped seizure onset zones, per physician records. Measurement electrodes were excluded from analysis if they were located outside of the brain or if they contained consistent artifacts throughout an experimental run. Table 1 lists the number of measurement electrodes and the number of stimulation sites after the above criteria have been applied. Then for each individual stimulation site analyzed, measurement electrodes were excluded for those trials if they were part of the stimulation pair or located on the same lead and within 2 electrodes of the stimulated pair (inter-electrode distance was 3.5 mm, on average). Finally, all trials were visually inspected; for each individual stimulation site, measurement electrodes were excluded when they contained artifact or excessive epileptiform activity in any trial, and single trials were excluded altogether when the artifact or epileptiform activity was present across many measurement electrodes. This resulted in a minimum of 10 trials included in subsequent analysis for two stimulation sites in subject 3, and 11 or 12 trials included for all other stimulation sites.

2.6. Quality metric of referencing on real CCEP data

In contrast to simulated CCEP data, the true responsiveness of channels in real CCEP data is unknown, especially before establishing an optimal reference. Therefore, we quantified the quality of re-referenced signals using the mean cross-channel coefficient of determination (R^2), and we used this metric to compare referencing performance across different versions of CAR. In well-referenced CCEP data, the average cross-channel R^2 should be relatively low as there are minimal synchronous interdependencies between nonresponsive channels in the data. In poorly referenced data, the average R^2 is inflated by positive correlations due to the common noise, as well as by negative correlations between responsive channels and the negative bias that they introduce into all other channels.

To calculate the R^2, CCEPs were first averaged across trials at each channel. For each pair of channels, the mean time series at one channel, on the response interval between 10 and 300 ms, was used to predict the time series at the other channel using a linear model:

$$X_i(t) = \beta_1 + \beta_2 X_j(t),$$

Where $X_i(t)$ and $X_j(t)$ are the mean CCEP time series at the ith and j^{th} channels, respectively. The R^2 was calculated and averaged over all pairs of i and j, $i \neq j$, to produce a single value for each stimulation site. We calculated the average R^2 for all stimulation sites in all subjects, for five separate referencing strategies: without re-referencing (monopolar hardware-referenced), standard CAR using all channels, a naive low-variance CAR using the bottom 25% channels by mean cross-trial covariance, a second naive low-variance CAR using the bottom 50% channels by mean cross-trial covariance, and CARLA. To ensure that the results were not trivially driven by line noise, we applied notch filters to the re-referenced data, at 60, 120, and 180 Hz, prior to calculating R^2. We conducted independent Wilcoxon signed-rank tests to detect significant differences in mean R^2 pairwise between each of the initial four strategies and CARLA. The familywise error rate was controlled with Bonferroni correction across the four tests.

2.7. Electrode localization and visualization

Subject preoperative T1 MRIs were transformed into AC-PC space through affine transformations and trilinear voxel interpolation, such that the mid-sagittal plane lay on the y-z plane with anterior and posterior commissures on the y-axis (Huang et al., 2021). sEEG electrodes were localized from the postoperative CT scans and coregistered to the T1 MRIs (Hermes et al., 2010). This allowed for visualization of recording electrodes and stimulation sites on individual subject T1 MRI slices, in AC-PC space.

Stimulation of different tissue types may result in different optima detected by CARLA. To test this possibility, the subject T1 MRIs were segmented using the autosegmentation algorithm in Freesurfer 7 (Dale et al., 1999). Gyral and sulcal labels were generated for each subject's pial surface, during autosegmentation, by aligning surface topology to the Destrieux cortical atlas. Each stimulation site was assigned the cortical or subcortical label corresponding to the most frequent voxel label within a 3 mm radius of the linearly interpolated center position between the stimulated electrode pair. To understand whether stimulation sites in different tissue types result in different thresholds on the number of common average channels, we used a one-way ANOVA to compare the mean fraction of channels included in the common average across cortical gray matter, subcortical gray matter, and white matter.

2.8. Data and code availability

The data that support the findings of this study are available in Brain Imaging Data Structure (BIDS) format on OpenNeuro: https://openneuro.org/datasets/ds004977/versions/1.2.0. The code used to generate all results and figures is available on GitHub: https://github.com/hharveygit/CARLA_JNM. All simulated results can be reproduced with code alone prior to data release.

3. Results

3.1. Performance of CARLA on simulated CCEPs

We simulated sets of CCEP data with different percentages of channels containing true responses and tested the ability of CARLA to exclude responsive channels from the common

average (Figure 4). Each simulated set contained 50 channels and 12 stimulation trials, with between 0 and 45 channels containing true responses. 30 unique sets were simulated at each level of responsiveness. We identified the optimal common average using two methods. The first method, labeled "Global Optimum", was to simply choose the n at the global maximum (least negative) in ζ, the mean correlation statistic. The second method, labeled "First-Peak Optimum" was to choose the n at the first local maximum in ζ before a significant decrease. A significant decrease was defined algorithmically as follows. The bootstrapped values for ζ at the first local maximum are pairwise subtracted from the bootstrapped values for ζ at the trough in n before the next greater ζ (See right-most example in Figure 4A) to yield bootstrapped differences. A left-tailed 95% confidence interval in difference is estimated, and the decrease is considered statistically significant if this confidence interval did not overlap 0. Local maxima are evaluated in order of increasing n until the first significant one is detected. The global maximum is used by default if ζ increases monotonically or if no significant decreases are encountered at any local maximum. We set a floor for optimal n at 10% of all channels with this approach, to avoid unstable fluctuations in local maxima when n is low. Alternatively, it may be valid to configure the floor to be an absolute number of channels (e.g., 10 channels).

Figure 4A shows example plots of ζ corresponding to single simulated datasets with 0%, 20%, 40%, 60%, and 80% of all channels containing responses. Figure 4B shows the simulated dataset matching Figure 4A, with responsive channels labeled in red. These examples match the median performance, across 30 sets, at each level of responsiveness. The optimal subset of channels, U_n, to use for CAR are labeled by arrowheads in the colors matching the two methods described above. For each of the two methods, we quantified the error, where an RCM indicates a responsive channel erroneously included in the common average and an NCM indicates a nonresponsive channel erroneously excluded from the common average. Minimizing RCM is relatively more important than minimizing NCM, because the primary objective is to avoid introducing responsive channels into the common average. When the percentage of total channels responsive is low (i.e., below 60%), the two methods generally produced the same result, producing optimal n at the global maximum before the inclusion of responsive channels. With higher percentages of responsive channels, it became increasingly more likely for an early local maximum to be surpassed by the addition of more responsive channels. The addition of many more responsive channels, which are uncorrelated with each other on average, compensates for and divides the anticorrelations inflicted by any individual responsive channel. At 80% responsiveness, the global optimum produces a median common average with nearly all channels included. On the other hand, the first-peak optimum correctly stops at the 20% of channels with lowest covariance, right before the first responsive channel. RCM and NCM using each method across 30 simulated sets at each level of responsiveness are summarized with boxplots in Figure 4C. The median RCM remained 0 when up to 84% of total channels were responsive using the first-peak optimum, but only up to 68% using the global optimum. NCM remained low throughout all levels of responsiveness for both methods, with a maximum median value of 2.5 (out of 50 channels). Sensitivity and specificity to detect responsive channels are plotted in a similar format in Figure 4D. The mean sensitivity remains near 1 (perfect) up to

80% responsiveness for the first-peak optimum method. We focus on the first-peak optimum throughout the rest of the analyses due to its greater sensitivity to detect responsive channels.

We tested whether CARLA would function appropriately when only a small number of simulated channels are present (Figure S1). As long as the percent of responsive channels remained at or below 60% of all channels, CARLA succeeded in detecting them (median and 75th percentile RCM = 0). When there were fewer than 20 total channels and 80% of them were responsive, many responsive channels were missed. Overall, this suggests that a minimum of 4 nonresponsive channels was needed for CARLA to accurately detect responsive channels, provided that responses were sufficiently distinct. NCM remained low notwithstanding a small number of channels.

We also tested CARLA's reliability when the simulated data were constructed with incrementally lower SNR (Figure S2), quantified as the ratio between the evoked potential power and the broadband noise power on the response window, per trial. At the group level, each SNR condition was labeled according to the geometric mean SNR (σ SNR) across all simulated channels and trials, since SNR appeared to be log-normally distributed. The highest SNR condition (σ SNR = 2.42) corresponded to simulated data used in the other analyses throughout the manuscript. Here, we found that sensitivity to detect responsive channels decreased with lower SNR, primarily when a large fraction of channels was responsive. For simulated datasets with at most half of all channels responsive, median RCM remained zero down to the lowest σ SNR of 0.79. NCM remained low at all SNR levels. Together, these results indicate that CARLA performs well even when there are relatively few channels or low SNR.

3.2. CARLA results on real CCEP data

We next applied CARLA to real CCEP data where true responsiveness is unknown. Results from one stimulation site in subject 1 are presented in Figure 5. The global optimum and first-peak optimum occur at the same n = 154 of 206 total channels (Figure 5B). The optimal n coincides with a shoulder in the ordered covariances (Figure 5C), and the channels included in the common average appear visually less responsive than those that are not. ζ decreases precipitously at the end, which reflects the bias that would be introduced into the re-referenced signals from the last ~20 channels with visible responses of large amplitude. Although notch filters alone can remove a significant amount of periodic line noise that is shared across channels, the signal quality is improved substantially by re-referencing, especially for individual trials (Figure 5F). No obvious bias is introduced into channels identified as nonresponsive by CARLA (E.g., channel "i" in Figure 5F). In contrast, a slight negative deflection is visibly introduced across channels when the same data is re-referenced by standard CAR instead (Figure S3).

Figure S4 summarizes the results from CARLA applied to CCEP data from a different stimulation site in subject 1, in the inferior temporal sulcus, as well as from stimulation sites in three other subjects. Similar to the performance on simulated data, CARLA identified a large range of stimulation responsiveness, according to the optimal percentile threshold, from an optimal n of 56/108 channels (52%) in subject 4 to 195/205 channels (95%) in subject 1. Notably, CARLA identified a higher optimal n for the inferior temporal sulcus

stimulation site than the white matter stimulation site in subject 1. This coincides with the visually apparent difference in global CCEP excitability between these two stimulation sites, with the white matter stimulation site producing a greater number of high-amplitude evoked potentials across measurement electrodes.

3.3. CARLA in the presence of a global signal

The common noise term discussed so far is present across all channels but unique in time to each trial. An assumption has been that there is no globally correlated structure across all channels and trials, which is generally the case. This assumption is challenged, however, when the reference electrode shows a modest response to stimulation. This can arise when the reference electrode is close enough to tissue affected by stimulation, which may be difficult to avoid entirely when an experiment includes many stimulation sites across the brain. For example, reference electrodes in the white matter that are unresponsive to stimulation in the cortex could show weak responses during thalamic stimulation due to the thalamus's widespread outputs. This occurred at a thalamic stimulation site in subject 1 and described in the results below.

Therefore, we introduce an additional conceptual component of the CCEP, termed the global signal, which is stimulation-locked and consistent across all channels and trials, when present (Figure S5). The global signal is visible in the mean signal across trials of most or all channels (Figure S5B). Its presence limits the functionality of a common average that simply excludes responsive channels with a statistically significant response (e.g., compared to a baseline interval), as these methods would spuriously exclude most or all channels from the common average.

CARLA makes no assumptions about the overall responsiveness of all channels and is well-suited to re-referencing these data. We present below the effect of CARLA on simulated and real CCEP data that contain a global signal component (Figure 6). In the simulated data, the global signal was modeled in the same way as the evoked potential (see Methods, above), but with an amplitude, A, that was four times lower (sampled uniformly at random between 20 and 30). This amplitude range was chosen to approximate real CCEP data, in which a global signal, when present, would be generally of much lower amplitude than the evoked potentials of interest when the reference electrode has been selected reasonably well.

Figure 6A presents an example simulated data set, containing a global signal, with 50 channels and 12 trials. 25 channels contained true responses in addition to the global signal (red). On this simulated data set, CARLA identified an optimal subset of 24 nonresponsive channels to use for re-referencing, for both optima (Figure 6A). Due to the global signal, responsive channels might rank earlier (lower mean cross-trial covariance) than nonresponsive channels, and the frequency of this occurrence depends partially on the relative amplitude of the global signal. Overall, across 30 simulated sets at each level of responsiveness from 0 (0%) to 45 (90%) truly responsive channels, the first-peak optimum performance of CARLA remained stable and comparable to simulated data without a global signal. The median RCM remained 0 up to 86% responsive channels, highlighting the robust sensitivity despite a global signal. The NCM was marginally higher at all levels of

responsiveness than data without a global signal, with a maximum median NCM equal to 6, when 1 channel was responsive.

CARLA was applied to real CCEP data that visibly contained a global signal, resulting from a thalamic stimulation site in subject 1 and a hardware reference electrode in the white matter close to responsive gray matter tissue (Figures 6C–F). In this case, an identical deflection in the first 50 ms can be seen across almost all channels, but only a small subset of channels shows truly unique responses. On this data set, CARLA identifies an optimal subset of 181 out of 206 recording channels as nonresponsive, appropriately including most channels with the global signal in its construction of the common average. Channels identified by CARLA as nonresponsive have the common signal effectively attenuated by re-referencing (Figure 6F, channel labeled "i"). Interestingly, a few channels do not contain the global signal before re-referencing. Upon closer inspection, these electrodes were all located in close physical proximity to the hardware reference electrode. It was likely that their recorded signals were similar to that of the reference electrode and were therefore attenuated by the hardware reference. For instance, the channel marked "ii" (Figures 6E–F) was immediately adjacent to the reference electrode on the same sEEG lead, located 3.50 mm away (Figure 6C). Re-referencing re-introduces the negative global signal as a true response in such channels. Overall, the signal quality is improved substantially by CARLA, as was the case in the data without a global signal.

3.4. Size of the optimal common average across all stimulation sites

We calculated the optimal n identified by CARLA on all stimulation sites in all subjects (Figure 7). Some of these stimulation sites produced a visible global signal across all measurement channels while others did not. In subject 1, we observed that the cortical stimulation site (Figure S4) resulted in fewer responsive channels identified by CARLA than the white matter stimulation site (Figure 5). Thus, we hypothesized that the degree of responsiveness might depend on the type of tissue in which the stimulation sites were located, and so we grouped the stimulation sites into three major tissue types: cortical gray, subcortical gray, and white matter. Cortical gray included all stimulation sites located in the gyri or sulci of the neocortex. Subcortical gray included all stimulation sites located in the thalamus, amygdala, and hippocampus. White matter included stimulation sites in the white matter only. The size of the optimal common average, expressed as a percent of all measurement channels analyzed for each stimulation site, ranged widely from 11% to 100%. The algorithmic floor of 10% imposed by the first-peak optimum was never incurred. The mean percentage did not differ significantly between tissue types within any individual subject (ANOVA, $p > 0.05$). The mean percentages across all stimulation sites in subjects 1 through 4 were 75%, 80%, 62%, and 65%, respectively.

We compared the signal quality of CCEPs re-referenced by CARLA to four other similar referencing strategies, as quantified by the mean R^2 between all pairs of channels (Figure 8). Data were notch-filtered at 60, 120, and 180 Hz after re-referencing but before calculating R^2 to mitigate the contribution of periodic line noise. In well-referenced data, the mean cross-channel R^2 is expected to be low as there are few interdependencies between nonresponsive channels. Figure 8A shows example R^2 matrices from stimulation site 1 in

subject 1, which show the R^2 between all pairs of channels for each of the five referencing strategies. Without re-referencing, most channels are highly predictive of each other due to the large amount of common noise. CCEPs re-referenced by a standard CAR, where all measurement channels are used to construct the common average, show pairwise R^2 values that are substantially decreased over no re-referencing, but these R^2 values are on average still visibly greater than those from CCEPs re-referenced by the adjusted CARs. This is attributable to common bias introduced by responsive channels in the standard CAR (e.g., Figure S3). Figure 8B summarizes the mean cross-channel R^2, averaged over each matrix, for the 82 stimulation sites in all subjects for the five referencing strategies. CARLA produced significantly lower mean cross-channel R^2 than the no re-referencing, standard CAR, and bottom 25% CAR strategies (pairwise Wilcoxon signed-rank tests, $p < 0.001$, Bonferroni-corrected).

CARLA did not differ significantly from the bottom 50% CAR strategy, which suggests that a naive CAR using the bottom 50% of measurement channels by cross-trial covariance might be comparably effective to CARLA. However, this group-level similarity in average R^2 may depend on the data used and may be suboptimal for individual stimulation sites. Indeed, many stimulation sites showed an optimal common average size less than 50% of measurement channels (Figure 7). On the other hand, all but one stimulation site resulted in an optimal common average size greater than 25% of measurement channels. Since the ζ plots rapidly approached plateau for most stimulation sites (e.g., see Figures 5, S4), a conservative naive threshold of 25% may be reasonable. This threshold would prevent the introduction of bias from responsive channels in the vast majority of cases, at the cost of modestly increased inter-channel dependency.

4. Discussion

CAR is an effective offline re-referencing method that can improve signal quality in iEEG data, while preserving signal features that are distributed across local neighbor electrodes. CAR is somewhat uncommon in CCEP studies, given the potential introduction of bias from responsive channels into all other channels. In this paper, we have addressed this major shortcoming, by introducing CARLA, an adjusted CAR. CARLA constructs a common average using a low-variance subset of measurement channels, and it adapts the size of this subset by minimizing anticorrelation between any single channel and all other re-referenced channels. CARLA reduced noise without introducing bias in both simulated and recorded CCEP signals.

Throughout the manuscript, we have presented CARLA as it relates to optimizing outputs from individual stimulation sites. More generally, we do not prescribe a one-size-fits-all approach to using CARLA for all forms of CCEP analysis. Indeed, there are various ways to implement the base algorithm depending on the nature of the research question, and we illustrate several of these in Table 2, below. For instance, when comparing signals across multiple stimulation sites, it may be key to control for the channels or number of channels used in re-referencing.

There are also multiple possible ways to rank channels for inclusion in CARLA. We chose to use the mean pairwise covariance between trials (when $K > 1$), rather than the simpler mean variance across trials. We argue that this feature has several advantages, in general. Most importantly, the covariance between trials quantifies both average signal strength as well as reliability, which helps to prevent reliable responses, even of low amplitude, from being included in the common average. Furthermore, there are $(n^2 - n)/2$ values of pairwise covariance to average for n trials, compared to n values of variance, which results in a more reliable mean estimator for ranking. However, if it is desirable to pool multiple stimulation sites or experimental conditions before re-referencing (i.e., Table 2, implementation 4), the mean variance may be chosen instead as a configuration parameter because it does not assume identical CCEP shape across trials. Overall, the two metrics converge, as average covariance equals average variance in the ideal case that all trials are identical with zero noise.

CARLA employs a rather complex set of steps in developing the optimization statistic, ζ. These steps were designed to maximize the robustness of the overall method. For each subset of n channels U_n to be considered as a common average, $\bar{z}_{i,n}$ is calculated as the average z-transformed Pearson's correlation between the ith channel in U_n and all other channels in U_n after re-referencing. Only correlations between channels within U_n are calculated to avoid spurious positive or negative correlations with responsive channels that have not yet been considered. In other words, calculating correlations within U_n ensures that all channels considered are only weakly anticorrelated with each other before U_n has expanded to contain responsive channels. CARLA finds the minimum $\bar{z}_{i,n}$ from all channels in U_n to assign as ζ, rather than taking $\bar{z}$ calculated from the newest channel added to U_n, to maintain stability in ζ as n increases. The minimum $\bar{z}_{i,n}$ is robust to spurious positive correlations between a newly-added responsive channel that is anticorrelated with a previously responsive channel (and thus positively correlated to the re-referenced signals, on average), which occurs when sEEG electrodes cross signal dipoles in the laminar cortex (Buzsáki et al., 2012; Huang et al., 2023; Mitzdorf, 1985). An example of this can be seen in Figure 5D, where many responsive channels are visibly anticorrelated with each other. Indeed, this approach permits CARLA to be agnostic to response shape. Although we tested CARLA on sEEG data, CARLA would in principle function robustly in the EcoG setting as well, where cortical evoked potential shapes are expected to be more well-preserved across measurement channels on the cortical surface. The output of CARLA is a simple percentile threshold on channels ranked by (co)variance per stimulation site. This output simplicity permits a single threshold to be chosen across all stimulation sites if desired *post-hoc* (i.e., Table 2, implementation 5). Indeed, we provided justification for using fixed percentile thresholds of 25% or 50%. Finally, CARLA does not require extensive user fine-tuning of parameters, as the only adjustable parameters are the response interval and the significance threshold to detect a first-peak optimum.

It is not the goal of CARLA to completely minimize inter-channel dependency. Mean cross-channel R^2 was used in this analysis as a signal quality metric to compare CCEPs re-referenced by CARLA to those without re-referencing or re-referenced by other forms of CAR. Although CARLA yielded the lowest mean R^2 out of these methods, it would be

inadequate to determine the optimal common average as simply as that which minimizes mean R^2. This is because responsive channels are often highly correlated with each other in CCEP data, and the goal of re-referencing is not to completely eliminate all correlative structure between channels. Physiologic correlations between responsive channels may further increase in different brain states such as sleep or anesthesia (Zelmann et al., 2023). Local re-referencing methods would likely yield even lower mean R^2 (Li et al., 2018), but this is not ideal precisely because they eliminate signal features common to electrode neighbors.

CARLA presents several advantages over other possible re-referencing strategies on CCEP data. First, CARLA does not require a complete absence of stimulation-locked activity in channels to be included in the common average. This allows CARLA to function on CCEP data containing some amount of unwanted global signal or stimulation artifact. Moreover, defining which channels are responsive in an absolute sense is a statistical problem that requires additional user-defined significance thresholds and possibly long periods of baseline data to compare against. Alternatively, one might attempt to determine the optimal threshold by finding a shoulder in the ranked channel covariances alone, such as that in Figure 5C. This would not be trivial to generalize to all data sets, as the ranked covariances can take on different (e.g., sigmoidal) shapes and increase with varying slope. Some referencing methods employ dimensionality reduction techniques such as principal and independent component analysis (Alexander et al., 2019; Michelmann et al., 2018; Uher et al., 2020). These methods may produce unstable solutions that differ significantly for similar input data and require careful inspection of desired components. Average-based references like CARLA tend to exhibit greater stability and automation. CARLA is also indifferent to baseline offsets in the data, as both the covariance used to rank channels and the Pearson correlation used for optimization are calculated on mean-centered values. Finally, in contrast to white matter referencing methods, CARLA is entirely iEEG data-driven and does not require anatomical data. We note, however, that CARLA requires both some nonresponsive channels and a minimum total number of channels to be present (Figures 4, S1). In our simulated data, accuracy remained adequate with at least 10 total channels, 4 of which nonresponsive, but this may depend on the SNR of a given dataset. With few channels present, alternative, informed referencing strategies such as by known white matter electrodes may in fact confer greater benefit.

This method is optimized for evoked potentials in single pulse stimulation data, and it may not be optimal for other desired signal features, such as oscillations and broadband power changes. However, it is advantageous in that data re-referenced by CARLA can then be easily re-referenced again by local referencing methods (e.g., bipolar) with no loss of information; the subtracted common average cancels out between neighboring electrodes. This permits multiple signal features to be efficiently extracted in series on a linear pipeline. One assumption in both CARLA and local referencing methods is that all measurement electrodes have identical impedance, as otherwise the common noise may be differentially amplified across electrodes. In our data this assumption is generally well met, and we exclude channels with visibly different amplitudes before preprocessing. If this assumption is not met, the data might be better referenced by a scalable noise term using a regression-based approach. Noise may be common to subgroups of measurement electrodes, such as

those connected to a single connector box (e.g., Natus breakout box or G.Tec G.HEADbox). In these circumstances, CARLA might be more appropriately performed at the subgroup level rather than across all measurement electrodes at once (Table 2, implementation 2). The overall accuracy of CARLA can be seen to depend on the SNR of CCEPs (Figure S2). This may be due to multiple factors. First, the relative ranking of channels by responsiveness is impaired because the pairwise trial covariance is less reliable. Second, bootstrapped estimates of the mean signal and ζ are noisier across resamples (Section 2.3, step 5). These factors may be mitigated by increasing the number of trials collected. Similarly, if a global signal with comparable amplitude to evoked potential responses is present, the relative ranking of channels by covariance can be less reliable. This could result in responsive channels ranked earlier in the construction of U_n and a smaller optimal n that misses nonresponsive channels (more NCMs). This potential limitation is mitigated by careful selection of the reference electrode to be as electrophysiologically neutral as possible during data collection.

Finally, as CARLA operates fundamentally by separating nonresponsive channels from responsive ones, it may perhaps be useful as a standalone tool for analyzing neural data. We briefly examined whether the spread of CCEPs differed between stimulation sites in different tissue types (Figure 7). Previous studies have found stimulation in white matter to produce larger amplitude CCEPs than in gray matter (Parmigiani et al., 2022; Paulk et al., 2022). Although we did not find a significant difference in our limited analysis, further validation and focus may show promise in such contexts.

In conclusion, CARLA is an adjustable common average re-referencing method that improves the signal quality of evoked potentials in CCEP data. We have demonstrated that CARLA accurately excludes truly responsive channels in simulated CCEP data, improves signal quality in real CCEP data collected from four human subjects, and remains functional in the presence of an unwanted global signal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

Alexander DM, Ball T, Schulze-Bonhage A, & van Leeuwen C. (2019). Large-scale cortical travelling waves predict localized future cortical signals. PLOS Computational Biology, 15(11), e1007316. 10.1371/journal.pcbi.1007316 [PubMed: 31730613]

Araki K, Terada K, Usui K, Usui N, Araki Y, Baba K, Matsuda K, Tottori T, & Inoue Y (2015). Bidirectional neural connectivity between basal temporal and posterior language areas in humans. Clinical Neurophysiology, 126(4), 682–688. 10.1016/j.clinph.2014.07.020 [PubMed: 25190148]

Arnulfo G, Hirvonen J, Nobili L, Palva S, & Palva JM (2015). Phase and amplitude correlations in resting-state activity in human stereotactical EEG recordings. NeuroImage, 112, 114–127. 10.1016/j.neuroimage.2015.02.031 [PubMed: 25721426]

Barbosa DAN, Gattas S, Salgado JS, Marie Kuijper F, Wang AR, Huang Y, Kakusa B, Leuze C, Luczak A, Rapp P, Malenka RC, Miller KJ, Heifets BD, McNab JA, & Halpern CH (2022). A hedonic orexigenic subnetwork within the human hippocampus. Research Square. 10.21203/rs.3.rs-1315996/v1

Bédard C, Kröger H, & Destexhe A (2006). Does the 1 / f Frequency Scaling of Brain Signals Reflect Self-Organized Critical States? Physical Review Letters, 97(11), 118102. 10.1103/PhysRevLett.97.118102 [PubMed: 17025932]

Buzsáki G, Anastassiou CA, & Koch C (2012). The origin of extracellular fields and currents—EEG, ECoG, LFP and spikes. Nature Reviews Neuroscience, 13(6), 407–420. 10.1038/nrn3241 [PubMed: 22595786]

Dale AM, Fischl B, & Sereno MI (1999). Cortical surface-based analysis: I. Segmentation and surface reconstruction. Neuroimage, 9(2), 179–194. [PubMed: 9931268]

Dickey AS, Alwaki A, Kheder A, Willie JT, Drane DL, & Pedersen NP (2022). The Referential Montage Inadequately Localizes Cortico-cortical Evoked Potentials in SEEG. Journal of Clinical Neurophysiology : Official Publication of the American Electroencephalographic Society, 39(5), 412–418. 10.1097/WNP.0000000000000792 [PubMed: 33337663]

van Drongelen W. (2018). Signal Processing for Neuroscientists. Academic Press.

Friston KJ (1994). Functional and effective connectivity in neuroimaging: A synthesis. Human Brain Mapping, 2(1–2), 56–78. 10.1002/hbm.460020107

Hermes D, Miller KJ, Noordmans HJ, Vansteensel MJ, & Ramsey NF (2010). Automated electrocorticographic electrode localization on individually rendered brain surfaces. Journal of Neuroscience Methods, 185(2), 293–298. [PubMed: 19836416]

Huang H, Gregg NM, Valencia GO, Brinkmann BH, Lundstrom BN, Worrell GA, Miller KJ, & Hermes D (2023). Electrical Stimulation of Temporal and Limbic Circuitry Produces Distinct Responses in Human Ventral Temporal Cortex. Journal of Neuroscience, 43(24), 4434–4447. 10.1523/JNEUROSCI.1325-22.2023 [PubMed: 37188514]

Huang H, Valencia GO, Hermes D, & Miller KJ (2021). A canonical visualization tool for SEEG electrodes. 2021 43rd Annual International Conference of the IEEE Engineering in Medicine Biology Society (EMBC), 6175–6178. 10.1109/EMBC46164.2021.9630724

Keller CJ, Honey CJ, Mégevand P, Entz L, Ulbert I, & Mehta AD (2014). Mapping human brain networks with cortico-cortical evoked potentials. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1653), 20130528. 10.1098/rstb.2013.0528

Krieg J (2017). Discrimination of a medial functional module within the temporal lobe using an effective connectivity model: A CCEP study. 13.

Kundu B, Davis TS, Philip B, Smith EH, Arain A, Peters A, Newman B, Butson CR, & Rolston JD (2020). A systematic exploration of parameters affecting evoked intracranial potentials in patients with epilepsy. Brain Stimulation, 13(5), 1232–1244. 10.1016/j.brs.2020.06.002 [PubMed: 32504827]

Li G, Jiang S, Paraskevopoulou SE, Wang M, Xu Y, Wu Z, Chen L, Zhang D, & Schalk G (2018). Optimal referencing for stereo-electroencephalographic (SEEG) recordings. NeuroImage, 183, 327–335. 10.1016/j.neuroimage.2018.08.020 [PubMed: 30121338]

Logothetis NK, Augath M, Murayama Y, Rauch A, Sultan F, Goense J, Oeltermann A, & Merkle H (2010). The effects of electrical microstimulation on cortical signal propagation. Nature Neuroscience, 13(10), 1283–1291. 10.1038/nn.2631 [PubMed: 20818384]

Matsumoto R, Nair DR, LaPresto E, Bingaman W, Shibasaki H, & Lüders HO (2007). Functional connectivity in human cortical motor system: A cortico-cortical evoked potential study. Brain, 130(1), 181–197. 10.1093/brain/awl257 [PubMed: 17046857]

Matsumoto R, Nair DR, LaPresto E, Najm I, Bingaman W, Shibasaki H, & Lüders HO (2004). Functional connectivity in the human language system: A cortico-cortical evoked potential study. Brain, 127(10), 2316–2330. 10.1093/brain/awh246 [PubMed: 15269116]

Mercier MR, Bickel S, Megevand P, Groppe DM, Schroeder CE, Mehta AD, & Lado FA (2017). Evaluation of cortical local field potential diffusion in stereotactic electro-encephalography recordings: A glimpse on white matter signal. NeuroImage, 147, 219–232. 10.1016/j.neuroimage.2016.08.037 [PubMed: 27554533]

Mercier MR, Dubarry A-S, Tadel F, Avanzini P, Axmacher N, Cellier D, Vecchio MD, Hamilton LS, Hermes D, Kahana MJ, Knight RT, Llorens A, Megevand P, Melloni L, Miller KJ, Piai V, Puce A, Ramsey NF, Schwiedrzik CM, … Oostenveld R (2022). Advances in human intracranial electroencephalography research, guidelines and good practices. NeuroImage, 260, 119438. 10.1016/j.neuroimage.2022.119438 [PubMed: 35792291]

Michelmann S, Treder MS, Griffiths B, Kerrén C, Roux F, Wimber M, Rollings D, Sawlani V, Chelvarajah R, Gollwitzer S, Kreiselmeyer G, Hamer H, Bowman H, Staresina B, & Hanslmayr S (2018). Data-driven re-referencing of intracranial EEG based on independent component analysis (ICA). Journal of Neuroscience Methods, 307, 125–137. 10.1016/j.jneumeth.2018.06.021 [PubMed: 29960028]

Miller KJ, Müller K-R, & Hermes D (2021). Basis profile curve identification to understand electrical stimulation effects in human brain networks. PLOS Computational Biology, 17(9), e1008710. 10.1371/journal.pcbi.1008710 [PubMed: 34473701]

Miller KJ, Sorensen LB, Ojemann JG, & den Nijs M (2009). Power-Law Scaling in the Brain Surface Electric Potential. PLOS Computational Biology, 5(12), e1000609. 10.1371/journal.pcbi.1000609 [PubMed: 20019800]

Mitzdorf U (1985). Current source-density method and application in cat cerebral cortex: Investigation of evoked potentials and EEG phenomena. Physiological Reviews, 65(1), 37–100. 10.1152/physrev.1985.65.1.37 [PubMed: 3880898]

Nunez PL, & Srinivasan R (2006). Electric Fields of the Brain: The Neurophysics of EEG. Oxford University Press.

Parmigiani S, Mikulan E, Russo S, Sarasso S, Zauli FM, Rubino A, Cattani A, Fecchio M, Giampiccolo D, Lanzone J, D'Orio P, Del Vecchio M, Avanzini P, Nobili L, Sartori I, Massimini M, & Pigorini A (2022). Simultaneous stereo-EEG and high-density scalp EEG recordings to study the effects of intracerebral stimulation parameters. Brain Stimulation, 15(3), 664–675. 10.1016/j.brs.2022.04.007 [PubMed: 35421585]

Paulk AC, Zelmann R, Crocker B, Widge AS, Dougherty DD, Eskandar EN, Weisholtz DS, Richardson RM, Cosgrove GR, Williams ZM, & Cash SS (2022). Local and distant cortical responses to single pulse intracranial stimulation in the human brain are differentially modulated by specific stimulation parameters. Brain Stimulation, 15(2), 491–508. 10.1016/j.brs.2022.02.017 [PubMed: 35247646]

Shirhatti V, Borthakur A, & Ray S (2016). Effect of Reference Scheme on Power and Phase of the Local Field Potential. Neural Computation, 28(5), 882–913. 10.1162/NECO_a_00827 [PubMed: 26942748]

Silverstein BH, Asano E, Sugiura A, Sonoda M, Lee M-H, & Jeong J-W (2020). Dynamic tractography: Integrating cortico-cortical evoked potentials and diffusion imaging. NeuroImage, 215, 116763. 10.1016/j.neuroimage.2020.116763 [PubMed: 32294537]

Uher D, Klimes P, Cimbalnik J, Roman R, Pail M, Brazdil M, & Jurak P (2020). Stereo-electroencephalography (SEEG) reference based on low-variance signals. 2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC), 204–207. 10.1109/EMBC44109.2020.9175734

Valencia GO, Gregg NM, Huang H, Lundstrom BN, Brinkmann BH, Attia TP, Gompel JJV, Bernstein MA, In M-H, Huston J, Worrell GA, Miller KJ, & Hermes D (2023). Signatures of Electrical Stimulation Driven Network Interactions in the Human Limbic System. Journal of Neuroscience, 43(39), 6697–6711. 10.1523/JNEUROSCI.2201-22.2023 [PubMed: 37620159]

Zelmann R, Paulk AC, Tian F, Balanza Villegas GA, Dezha Peralta J, Crocker B, Cosgrove GR, Richardson RM, Williams ZM, Dougherty DD, Purdon PL, & Cash SS (2023). Differential

cortical network engagement during states of un/consciousness in humans. Neuron, 111(21), 3479–3495.e6. 10.1016/j.neuron.2023.08.007 [PubMed: 37659409]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Signal quality in intracranial EEG data can be improved by offline re-referencing.

- Existing re-referencing methods may introduce bias or eliminate shared responses.

- Our new technique, CARLA, is optimized to reduce noise while minimizing bias.

Box 1.

Theoretical basis to optimize the number of channels in a common average.

Let S be the true common noise across all channels. S can be thought of in this context as a common "signal" of interest, and the goal of CAR is to best estimate S from a sample of channels and subtract it from all channels. S is estimated by averaging over n channels. Even if the n channels are not responsive, they each contribute S plus channel-specific noise to the common average. Assuming simply random noise with 0 mean that is statistically independent across the n channels, the signal-to-noise ratio of the common average is proportional to $\sqrt{n}$ (Drongelen, 2018). Therefore, S is best estimated by increasing the number of channels used to construct the common average, up to the point when evoked signals of interest from responsive channels would be introduced into the common average as a systematic bias. Thus, the problem of determining the correct number of channels to use for CAR is suited for optimization.
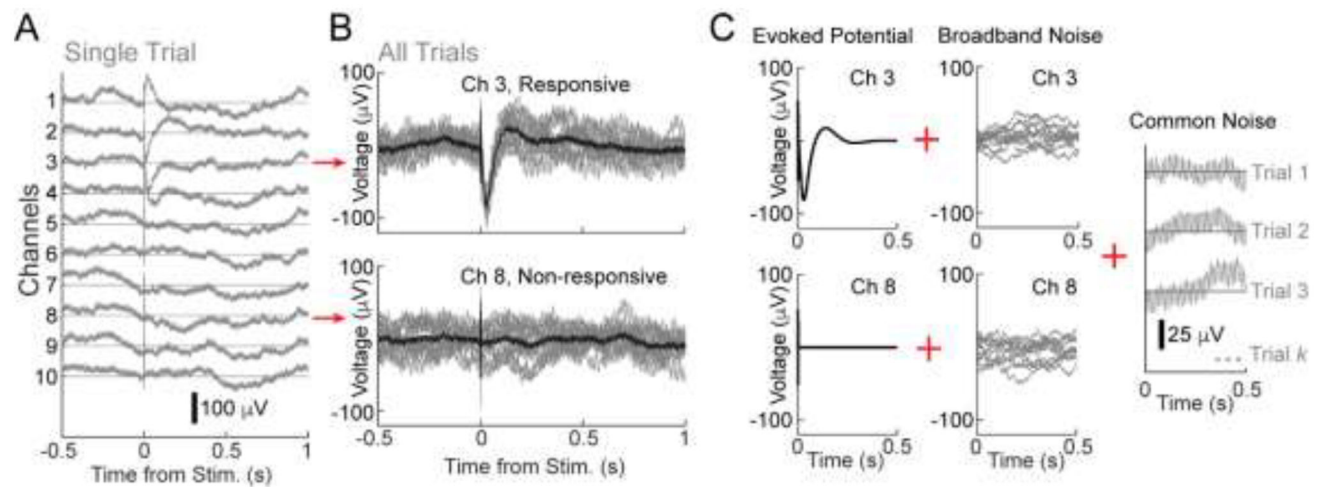
Figure 1. Theoretical components of the CCEP data.
A, Simulated CCEP data for 10 channels at one trial. Channels 1 through 4 contain true, distinct evoked potential responses, while channels 5 through 10 are nonresponsive. **B**, 12 individual trials shown for channels 3 and 8 in gray, with the mean across all trials in black. **C**, The three components of the CCEP data shown for channels 3 and 8. This includes a responsive evoked potential in channel 3 but not 8, individual broadband noise (Brown noise) unique to each channel, and common noise (60 Hz + harmonics + Brown noise) shared across channels. The stimulation artifact is between 0 and 2 ms and depicted here at the onset of the evoked potential.
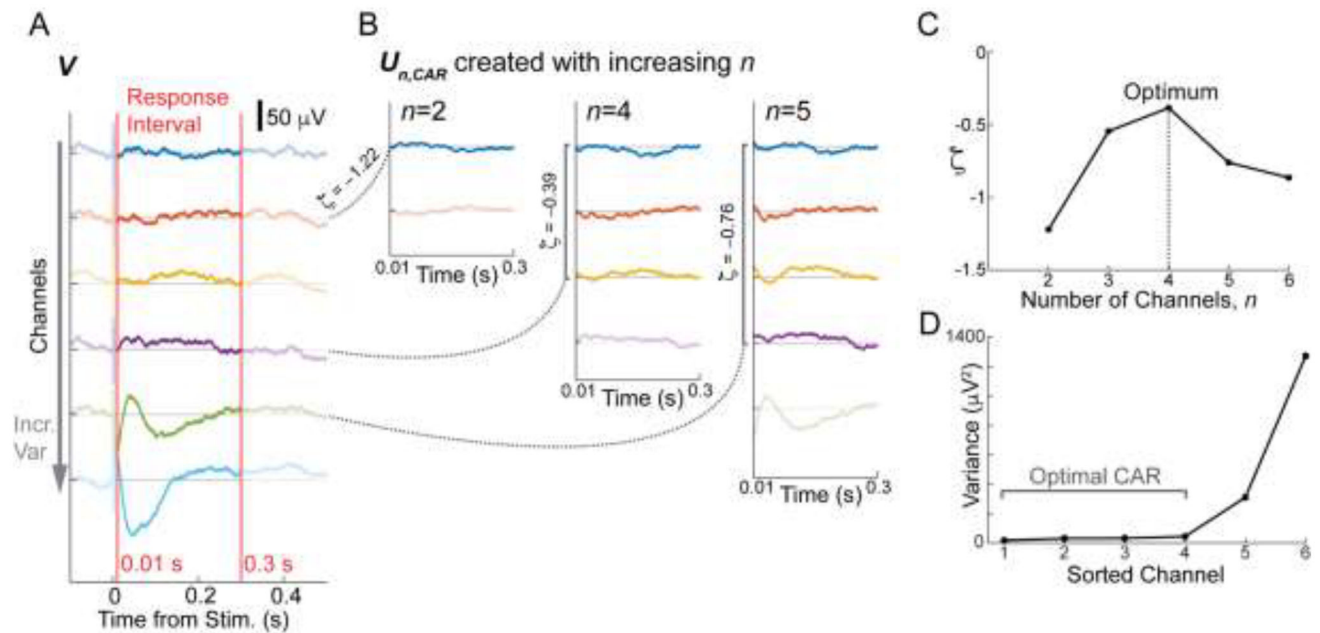
Figure 2. Demonstration of CARLA on a simulated single-trial example.

A, Simulated single-trial CCEPs are sorted in order of increasing variance on the response interval between 10 and 300 ms post-stimulation. Each channel contains a unique individual broadband noise, and all channels contain a shared common broadband noise. Line noise has been attenuated. Channels 1 through 4 contain no true responses, while channels 5 and 6 have distinct, true evoked responses added. **B**, Subset U_n is created iteratively with increasing n (shown here for $n = 2$, 4, and 5 only) and re-referenced by its common average to yield $U_{n,CAR}$. Calculation of ζ, the most negative mean correlation of any single channel in U_n to all others in $U_{n,CAR}$, is shown for $n = 2$, 4, and 5. **C**, The optimal number of channels, n, used to construct the adjusted common average occurs when ζ takes on its least negative value, here at $n = 4$. **D**, Variance on the response interval for each of the sorted channels.
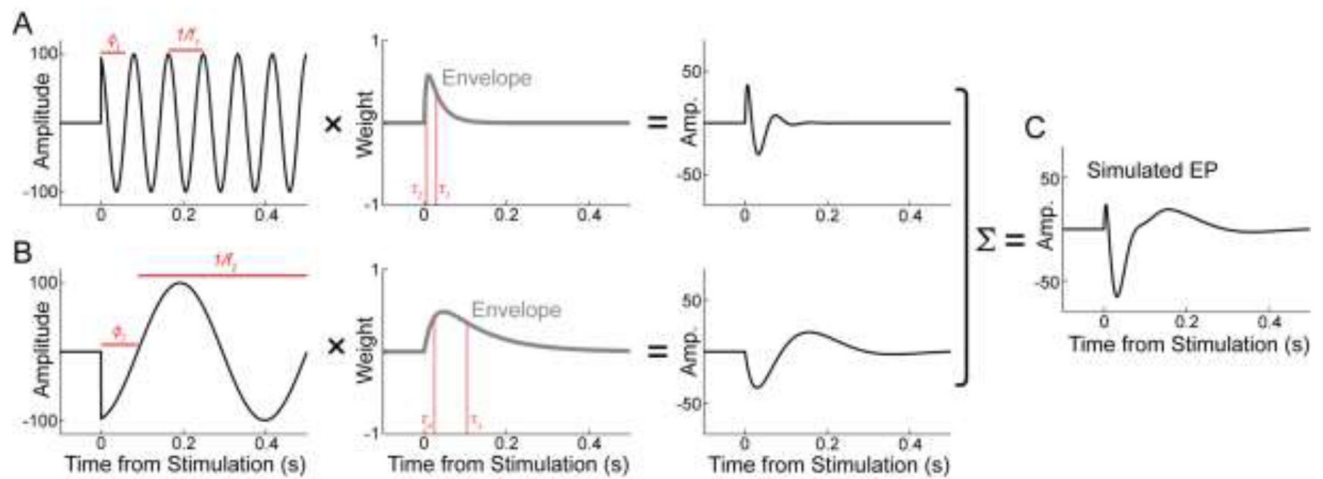
Figure 3. Example construction of simulated evoked potentials.
Each simulated evoked potential, **C**, was constructed by summing two causal sinusoids of random phases, each weighted by an envelope that was a difference of exponential decays. **A**, The enveloped sinusoid with higher frequency and shorter exponential time constants simulates a faster, direct response. **B**, The enveloped sinusoid with lower frequency and longer exponential time constants simulates a slower, indirect response.
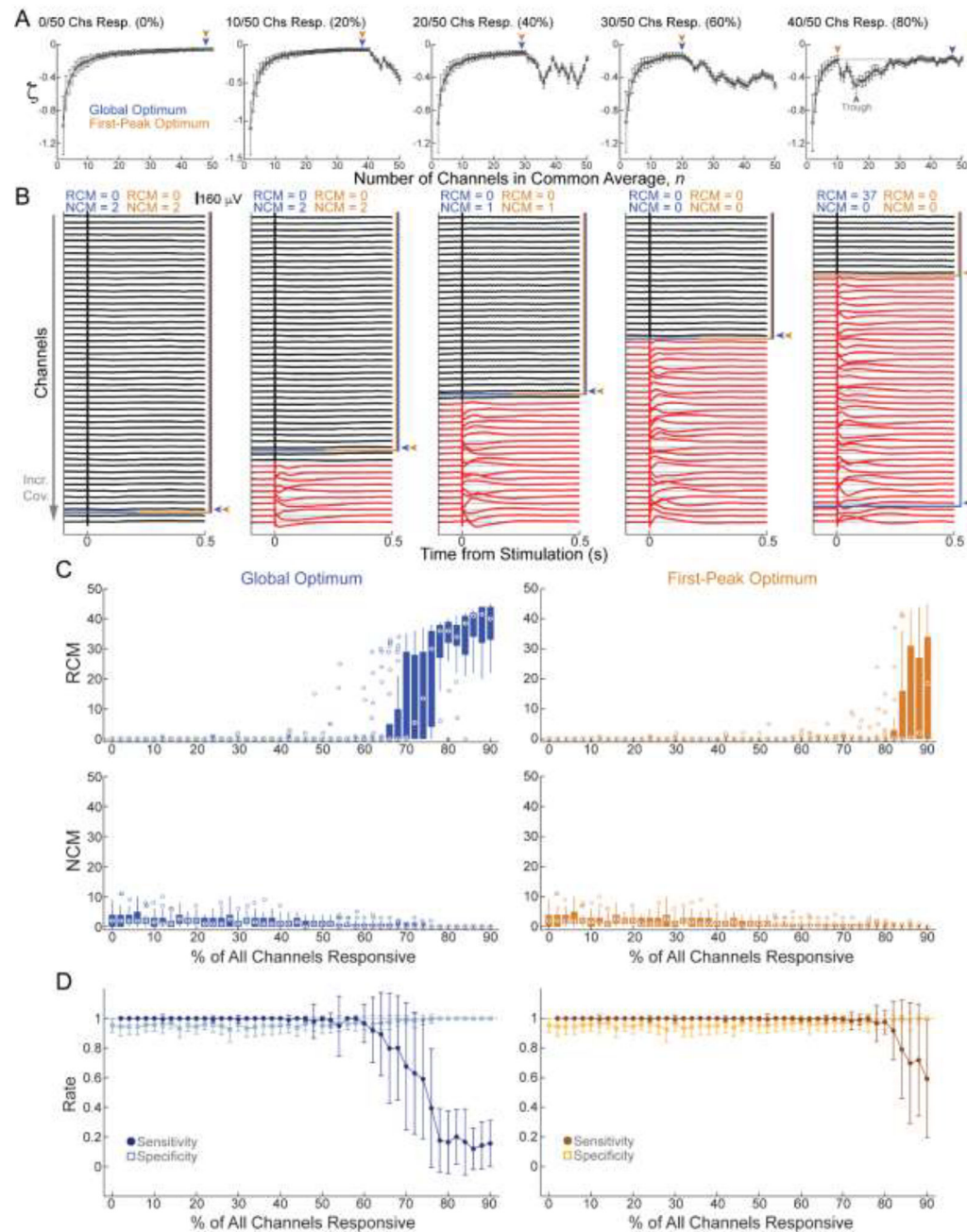
Figure 4. CARLA correctly separates responsive from nonresponsive channels to include in the common average across a range of responsiveness in simulated CCEP data.

A, Plots of ζ vs. n for example sets of simulated channels across quintiles of total channel responsiveness. The optimal number of channels to include in the common average, determined by the global optimum and the first-peak optimum, are labeled with blue and orange arrowheads, respectively. **B**, The mean signal across trials for each channel, corresponding to plots in A, sorted top-down by increasing mean cross-trial covariance. Signals in black contain no true response whereas signals in red contain a true response. The optimal U_n used for the construction of the common average is labeled by the colored brackets (and the matching arrowhead at n), where blue and orange correspond to the

global optimum and first-peak optimum methods, respectively. **C**, Boxplots showing the RCM (top) and NCM (bottom) for each method, across 30 simulated sets at each level of responsiveness. **D**, Mean sensitivity and specificity to detect responsive channels across the 30 simulated sets at each level of responsiveness. Error bars show standard deviation across sets.
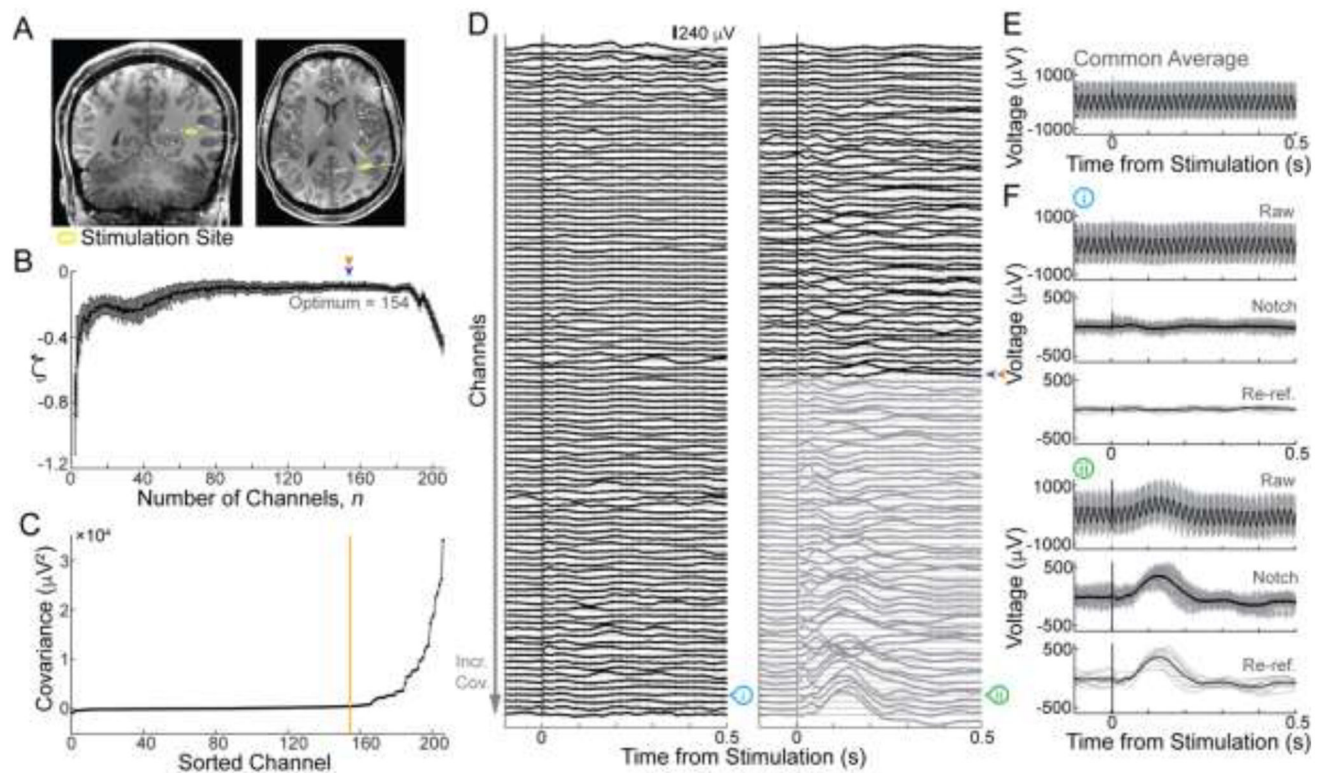
Figure 5. CARLA constructs optimal common average from visually nonresponsive channels in real CCEP data.

A, Coronal and axial T1-weighted MRI slices for subject 1 with all electrodes within 4 mm of each slice overlaid. The stimulation site, located in the white matter, is labeled in yellow. **B**, Plot of ζ vs. *n*. The global optimum (blue arrowhead) equals the first-peak optimum (orange arrowhead) and occurs at 154 out of 206 total recording channels. **C**, Mean cross-trial covariance for all channels sorted in increasing order, matching B. The orange vertical line is at the optimum *n*. **D**, Mean signal across the 12 trials for all channels, before re-referencing, plotted in order of increasing cross-trial covariance. Line noise was removed by notch filters at 60, 120, and 180 Hz to improve visibility. Black denotes the optimal subset of channels to be included in the common average and gray denotes channels to be excluded from the common average. **E**, The common average created from the optimal subset (no notch filter applied). Gray lines are individual trials, and the black line is the mean across trials. **F**, The channels labeled "i" (light blue) and "ii" (green) in **D**, before any processing (Raw), with line noise removed (Notch), and after re-referencing by CARLA, without line noise removal (Re-ref.).
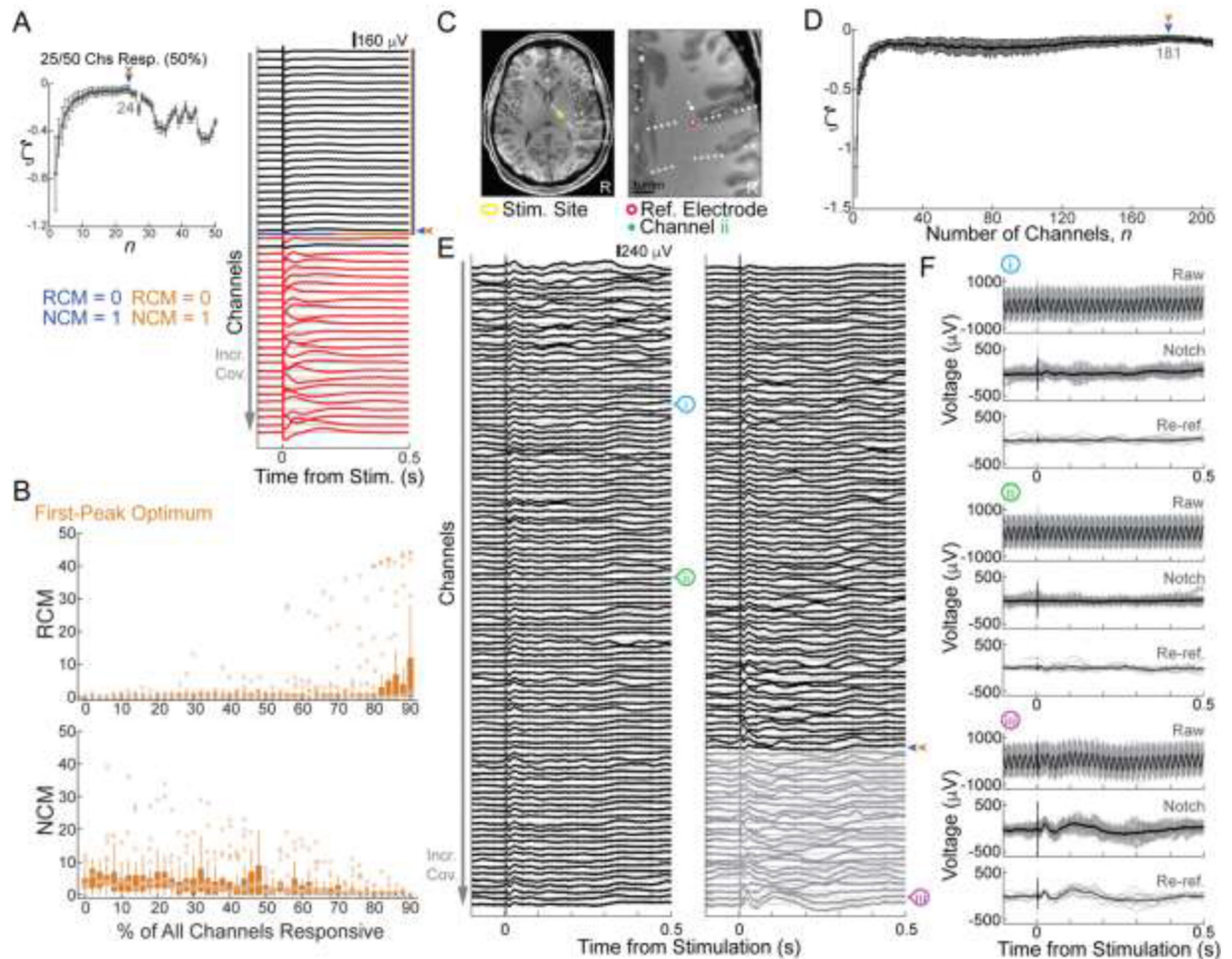
Figure 6. CARLA identifies nonresponsive channels to include in the common average in simulated and real CCEP data, in the presence of a weaker global signal.

A, Plot of ζ vs. n (left) and the mean signal across trials for each channel, sorted top-down in increasing trial covariance (right), for a simulated set of CCEPs where 25 of 50 total channels are responsive. All channels, including nonresponsive ones, contain a low-amplitude global signal that is consistent across trials. Channels in black contain no true response while channels in red contain a true response. The optimal number of channels to include in the common average, determined by the global optimum and the first-peak optimum, are labeled with blue and orange arrowheads, respectively, and are identical in this case. **B**, Boxplots showing the RCM (top) and NCM (bottom) for the first-peak optimum, across 30 simulated sets at each level of responsiveness. A unique global signal is present for each simulated iteration. **C**, Axial T1-weighted MRI slices for subject 1, highlighting the thalamic stimulation site (left) and the hardware reference electrode (right, zoomed-in slice). The green electrode corresponds to channel "ii" in **E-F**. All electrodes within 4 mm of each slice are overlaid. **D**, Plot of ζ vs. n. The global optimum (blue arrowhead) equals the first-peak optimum (orange arrowhead) and occurs at 181 out of 206 total recording channels. **E**, Mean signal across trials for all channels, before re-referencing, plotted in order

of increasing cross-trial covariance. Line noise was removed by notch filters at 60, 120, and 180 Hz to improve visibility. Black denotes the optimal subset of channels to be included in the common average and gray denotes channels to be excluded from the common average. A global signal can be seen in nearly all channels. **F**, The channels labeled "i" (blue), "ii" (green), and "iii" (purple) in **E**, before any processing (Raw), with line noise removed (Notch), and after re-referencing by CARLA, without line noise removal (Re-ref.).
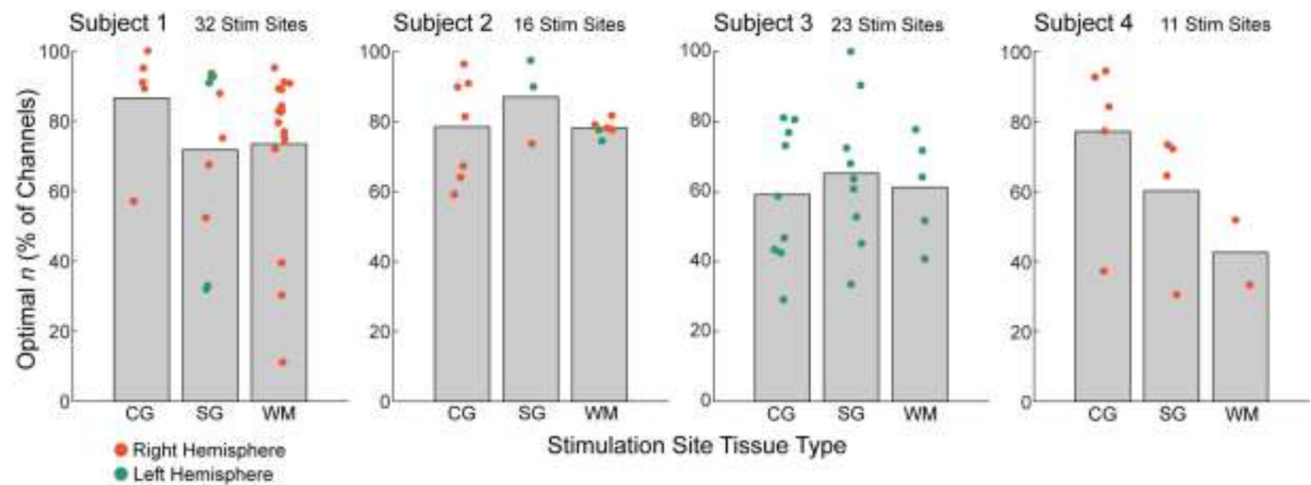
Figure 7. Optimal common average size calculated by CARLA across all stimulation sites, using the first-peak optimum.

All stimulation sites in each subject (filled circles) were colored by hemisphere and grouped by tissue type, where CG = "Cortical Gray", SG = "Subcortical Gray", and WM = "White Matter". Bars show mean across stimulation sites in each tissue type. Tissue type means did not significantly differ within each subject (ANOVA, $p > 0.05$).
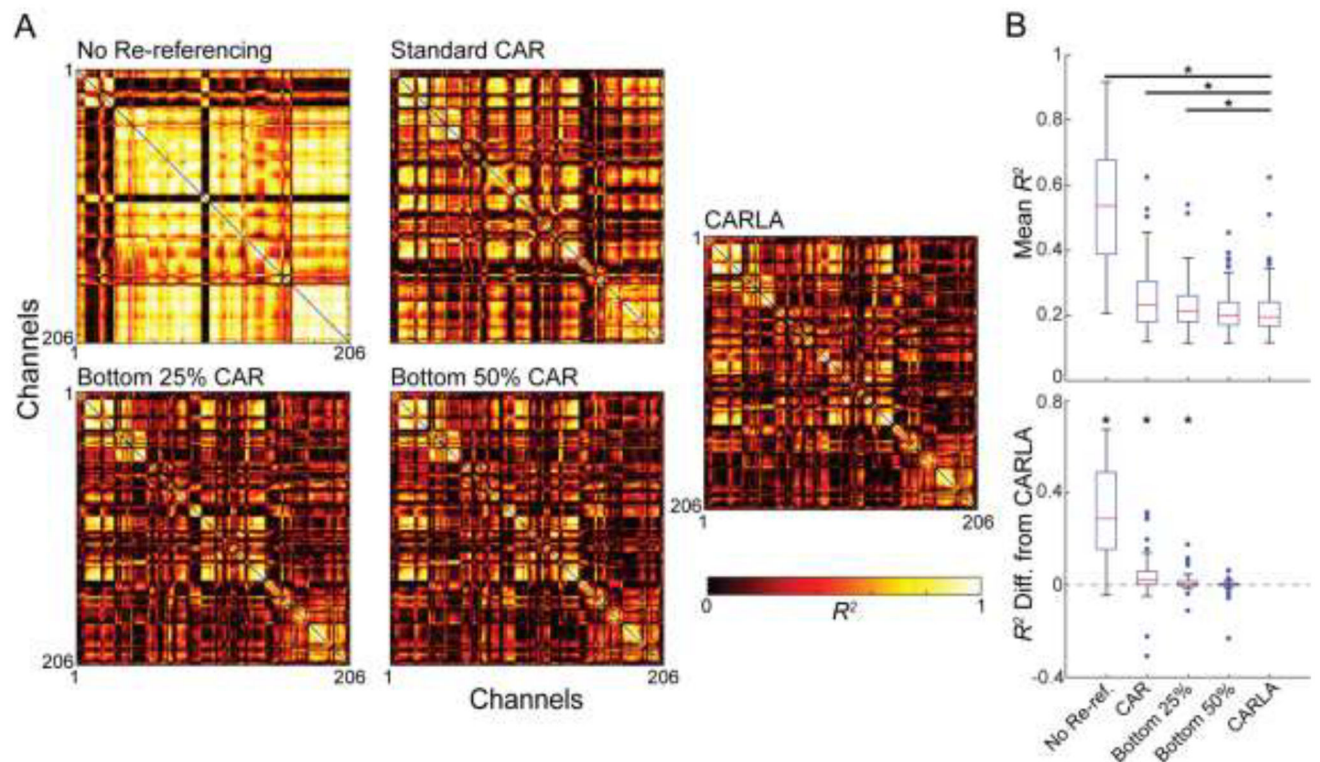
Figure 8. Cross-channel predictability for data referenced by different common average strategies.

A, R^2 matrix for all channel pairs in subject 1, from stimulation site 1 (same as in Figure 5), for each of the five referencing strategies. **B**, (Top) Mean R^2 for the five referencing methods, across all stimulation sites in all subjects. Red lines depict medians. (Bottom) Mean pairwise R^2 differences between each referencing strategy and CARLA. Asterisks (*) indicate significant differences by pairwise Wilcoxon signed-rank tests (Bonferroni-corrected $p < 0.001$). The only statistical comparisons performed were between each (non-CARLA) strategy and CARLA.

Table 1.

Subject Demographics

Subject ID	Age	Sex	Hemisphere(s) implanted	No. of measurement electrodes	No. of stimulation sites
1	18	M	Bilateral	213	32
2	19	M	Bilateral	204	16
3	19	F	Left	190	23
4	16	F	Right	114	11

A list of age, sex, implanted hemisphere(s), number of measurement sEEG electrodes, and number of stimulation sites for each subject.

Table 2.

Alternative Ways to Apply CARLA

Modified CARLA implementation	Example scenarios for usage	Advantages	Disadvantages
(1) CARLA applied individually for each experimental condition (as described in manuscript).	Comparison of CCEP shapes measured from different channels due to one stimulation site. Calculation of CCEP summary statistics such as peak height, latency, or area under the curve.	Optimal quantification of true response shape with least combined interference from other channels in terms of noise or bias.	Inadequate when comparing time series across experimental conditions, as signals end up with different final levels of residual noise and introduced bias after re-referencing, depending on the number and identity of channels used to construct the adjusted common averages in each condition.
(2) CARLA applied individually for each experimental condition, separately for subgroups of channels.	Same as (1), but with substantial differences in amplitude or quality of noise between subgroups of channels, due to headbox-specific effects.	Prevents contamination of differential noise across subgroups of channels. Computationally faster because CARLA has quadratic time complexity with respect to the number of input channels.	The channels in each subgroup may end up with different levels of residual noise and introduced bias due to differential re-referencing, similar to the disadvantage in comparing across conditions as described in (1).
(3) CARLA used to determine the optimal set of re-referencing channels for each experimental condition, with the final adjusted common average constructed from the common channels (set intersection) across all conditions.	Statistical comparison of CCEP time points produced by different stimulation sites or during different behavioral states (e.g., sleep vs. awake).	Residual noise and introduced bias are controlled between experimental conditions in re-referenced signals.	Greater remaining broadband noise amplitude in the adjusted common average than optimal because fewer channels are used to construct it. No guarantee that the intersection yields enough channels for re-referencing, especially if multiple experimental conditions are present.
(4) CARLA applied to all trials together, pooled across experimental conditions, with variance instead of covariance as channel ranking metric.	Comparison of signals across many experimental conditions that differ slightly from each other (e.g., titration of stimulation current) but which are expected to yield qualitatively similar CCEP outputs.	Residual noise and introduced bias are controlled between experimental conditions in re-referenced signals. Computationally faster than (3) and guaranteed to find an optimal adjusted common average, regardless of the number of conditions.	Ranking by variance instead of covariance may fail to detect low-amplitude but reliable responses. If signals are sufficiently different across conditions, the bootstrapped calculation of ζ across trials may be inaccurate. Channels with responses in a small number of trials may be erroneously included in the common average.
(5) All channels ranked by increasing response strength for each experimental condition, and a fixed percentile threshold (e.g., 25%) of lowest ranked channels is used to construct the adjusted common average for each condition. An optimal global percentile threshold may be first estimated by applying CARLA to all or a subset of conditions.	Same as (3); or a large number of stimulation sites are present and data needs to be preprocessed quickly.	Assuming all channels have similar noise amplitudes, the amplitude of residual noise would be controlled for between experimental conditions in re-referenced signals. Permits each condition to re-reference using its own optimal subset of channels. Computationally fast if the percentile threshold is not estimated from all conditions.	The single percentile threshold may not be close to optimal for all experimental conditions. Because the identities of channels used to re-reference each condition is different, minor differences in introduced bias may still be present between conditions.

Alternative re-referencing strategies using CARLA, example scenarios of when they may be appropriate, and their general advantages and disadvantages.