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Common Median Referencing for Improved Action Potential Detection with Multielectrode Arrays

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Abstract—**Referencing is frequently used to remove common-mode signals from multielectrode data, in both freely moving animals and *in vitro* preparations. For action potential (AP) detection, referencing by subtracting the common average signal has been shown to increase AP signal-to-noise ratio (SNR). This method fails, however, when large transients occur on individual electrodes, as occurs during electrical stimulation or with large APs during spontaneous recordings. To deal with these cases, we propose using the common median as a reference. The common median has an improved SNR for AP detection (leading to more isolated single units and more detected APs per unit) and, unlike common average referencing, does not generate spurious APs when processing large single-electrode transients.**

I. Introduction

Noise in multielectrode recordings has several origins, e.g., thermal noise due to electrode impedance, electromagnetic interference from nearby electronics, and biological signals that are not of interest to the investigator. This noise hinders our ability to detect signals of interest, such as action potentials (APs) or local field potentials (LFPs). Referencing (i.e., subtracting one time-varying signal from another) is one approach to dealing with such noise, functioning by removing common-mode signals (e.g., biological noise, 50/60 Hz noise) that are shared across the electrode and reference [1]. Frequent choices of reference in freely moving animals are low-impedance skull screws, stainless steel wires, or a high-impedance microelectrode, carefully selected so as not to actively record single cells (which would otherwise show up on the referenced channels with inverted polarity).

It was recently reported that using the average signal across microelectrode channels was superior to alternative references in terms of noise reduction [2]. While useful, the average reference has undesirable properties, namely that large signals on a single channel will skew the average toward outlying values. These large values then pollute the referenced channels, leading to spurious AP detections or large baseline shifts.

These problems become acute when conducting experiments involving microelectrode stimulation, a well-used experimental paradigm [3-7]. Stimulation pulses are typically on the order of 100 mV – 10 V, which is 10^3-$10^5\times$ as large as a typical extracellular AP. Recording electronics typically do not amplify linearly in this regime, but the signals on the stimulating electrode nevertheless dominate any computed average.

As an alternative to common average referencing, we propose common median referencing. The median is less susceptible to influence from outliers, as compared to the mean, yet is statistically equivalent when the inter-channel variability is Gaussian. When non-Gaussian, the median provides a better approximation of the distribution's center.

II. Methods

A. Surgery

All work with animals was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Emory University Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats (350-450 g) were anesthetized with isoflurane, several anchoring skull screws were implanted, and a craniotomy was drilled over the right dorsal hippocampus. After removing the dura, a 16-channel microwire array with two rows of 8 electrodes (row 1, 4 mm long; row 2, 2.8 mm long) was carefully lowered into craniotomy, with the longer row of the array targeted to the CA3 region, and the shorter row to CA1. Proper depth (usually 3-4 mm ventral to pia) was determined by monitoring electrophysiological recordings during implantation, using our lab's custom recording hardware and software, the NeuroRighter system [8]. The craniotomy was then sealed with dental acrylic and the rat was allowed to recover for 5-8 days before recordings began.

B. Recording

Rats were tethered to a 100× gain recording headstage and a custom-built stimulator [8], but otherwise freely mobile in a Plexiglas enclosure. Extracellular signals, acquired at 25 kHz, were band-pass filtered from 1-9000 Hz in hardware. In software, the signals were split into two streams: spikes (filtered from 500-5000 Hz) and LFPs (1-500 Hz,

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downsampled to 2 kHz). Data was stored for offline analysis, which was conducted using MATLAB r2008. Spikes were detected as threshold crossings of ≥5× RMS, unless otherwise specified. Spikes were sorted using superparamagnetic clustering across wavelet coefficients with the Wave_clus software [9].

III. Results

We first describe theoretical results for signal contamination and attenuation with common average referencing, then present empirical data to illustrate the advantages of common median referencing over average referencing in practice.

A. Theoretical Contamination and Attenuation

Each signal s_i contributes $1/N$th of the average reference signal's amplitude, where N is the number of electrodes averaged. Two effects are noted. First, each s_i is attenuated by a factor of $1 - 1/N$, since its contribution to the average is now being subtracted from itself. Second, the inverse of the signal will now be present on all channels with an attenuation of $1/N$ (Fig. 1).

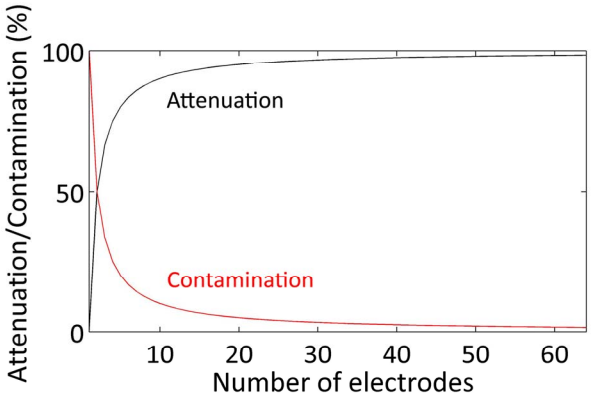

Fig. 1. Theoretical attenuation (black) and contamination (red) of channels with common average referencing.

These results show that, for example, using a 10-electrode array and common average reference, that a 100 µV AP would be attenuated to 90 µV, and that a "phantom" 10 µV AP would show up on every other channel.

B. Spontaneous Experimental Data

Is the worry of contamination well founded, or are the spurious spikes too attenuated to be detected as APs? Empirical data suggests that the worry of induced spurious spikes is real. For example, a recording from an anesthetized rat hippocampus, using 16 electrodes, shows threshold-crossing contamination when large APs are detected (Fig. 2). That is, with common average referencing, the "bleed-through" of an AP on one channel causes a spurious action potential on all other channels. These spurious APs cross the 3.5× standard deviation threshold, from Ludwig et al. [2], in 11/16 channels, and the 5× threshold in 1/16 channels (not including the channel of the actual AP in either case). This same type of contamination occurred 22 times in 5 sample minutes of recording. These problems do not occur with median referencing (0 times in 5 minutes).

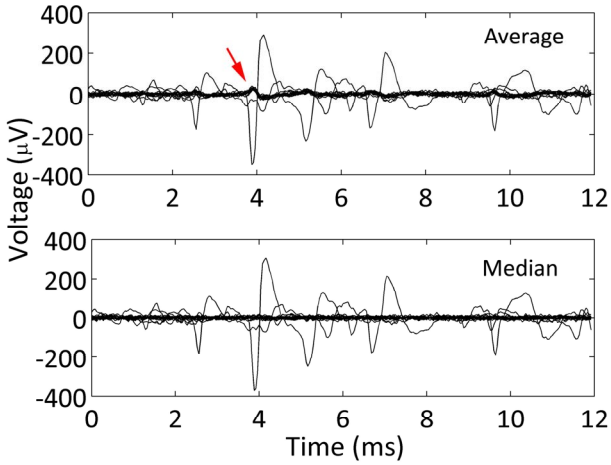

Fig. 2. Contaminated traces with common average referencing vs. common median referencing. A large action potential on a single channel dominates the mean, creating spurious APs on all other channels (red arrow) when using average referencing (top). These problems do not occur with median referencing (bottom).

C. Stimulation Data

While the spurious spikes are problematic in spontaneous data, a more notable problem arises with electrical stimulation. When delivering a stimulus pulse to a single electrode, the resulting artifact is referred to all other channels when using common average referencing (Fig. 3).

The data shown in fig. 3 is broadband filtered (1-9000 Hz). If purely interested in APs, a much tighter band-pass would likely be used (e.g., 500-5000 Hz), in which case the prolonged baseline shift would be less notable. In either case, however, common median referencing is impervious to these contamination artifacts.

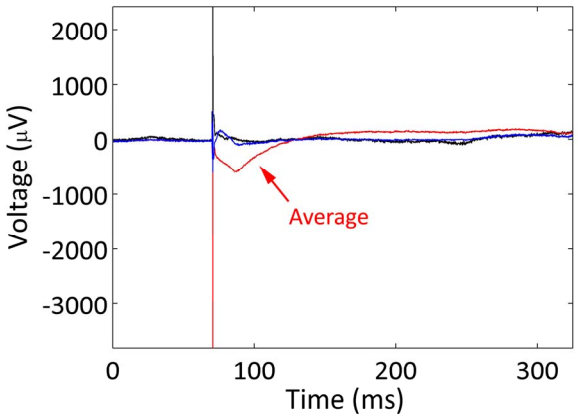

Fig. 3. Stimulus artifact contamination. The original trace (black) shows minimal baseline shift following the stimulation pulse. The common average reference (red trace) reflects the long baseline shift from the stimulating electrode. Common median referencing (blue trace) avoids this problem.

D. Noise Reduction

To assay the effectiveness of the two referencing modes (average vs. median), we computed the RMS noise value for referenced band-pass filtered signals (500-9000 Hz), suitable for detecting APs. The data (duration of 2 minutes) was

acquired from 16 electrodes in the hippocampus of a freely moving rat.

Common average referencing had a mean RMS of 6.1 ± 0.5 μV across electrodes, common median referencing 6.2 ± 0.6 μV (compared to 7.3 ± 0.6 μV for unreferenced data). The median reference RMS value is significantly greater (by 0.1 μV) than the average reference RMS ($P < 0.01$, Wilcoxon sign-rank test). However, it should be recalled that with median referencing there is no statistical attenuation of signals. Thus, while the RMS noise for each channel using median referencing is higher than that of average referencing, the SNR is still greater with median referencing. As an example, given the above empirical RMS values from 16 electrodes, an AP with 100 μV amplitude will be attenuated to 94 μV using common average referencing (or 15× the RMS noise level. For median referencing, the same AP (unattenuated) is 16× the RMS noise level.

E. Detection Performance

Using 5 minutes of spontaneous recordings from three animals (16-channel arrays in the dorsal hippocampus; see Methods), we compared the effectiveness of common average and common median referencing. The data was referenced separately according to both methods, APs were detected (using 5× the RMS threshold of the referenced data, specific to each channel and each referencing method), and APs were sorted with superparamagnetic clustering of the wavelet decomposition [9].

With common median referencing, 0.66 additional well-sorted units were detected per dataset, on average (Fig. 4). Additionally, across sorted units, there was an average increase of 10% in the number of detected APs (Fig. 4). In no cases did common median referencing perform worse than common average referencing.

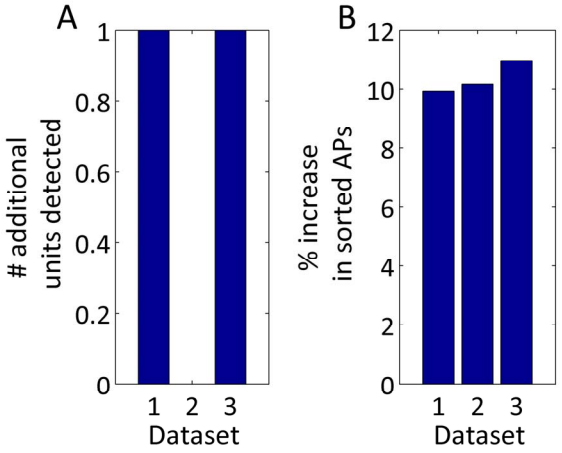

Fig. 4. AP detection is improved with common median referencing vs. common average referencing. (A) Additional units are detected with spike sorting in 2/3 datasets, using common median referencing. (B) More APs are detected from sorted units with common median referencing as compared to common average referencing.

IV. Discussion

Multielectrode recordings have provided useful insights into normal and pathological brain function [10]. Biophysical and electromagnetic noise sources, however, are a constant nuisance that obscure target neural signals. A simple method for reducing correlated noise (common-mode noise) is digital or analog referencing [1]. Such referencing often selects a single electrode with low activity as a representation of background noise. This method is highly sensitive, however, to any uncorrelated noise on the reference channel, such as APs, stimulus artifacts, or other localized biological transients. In the field of electroencephalography (EEG), common average references have routinely been used as one means of preventing uncorrelated noise from affecting referenced channels [1]. This method was recently applied to multi-microelectrode arrays to improve the detection of APs, with an identical rationale [2].

The mean of a statistical sample, especially when there is a small sample size, is dominated by any occurring outliers. For multielectrode neural recordings, this translates to large APs from one electrode appearing on referenced electrodes (Fig. 2) or baseline shifts from stimulus artifacts contaminating signals from other electrodes (Fig. 3). The median provides a more stable representation of a distribution's central tendency that is less affected by large transients on a few channels.

While the median provides an improved estimate of the common signal, the RMS noise of this estimate is higher for the median referenced signals than for the average referenced signals. This is expected, in fact, since for most probability distributions, the absolute value of the median is guaranteed to be less than or equal to the mean [11, 12]. Therefore, since the referencing signal has lower power, the referenced signal will have a slightly higher power than the comparable averaged referenced signal. This higher power will lead to an improved SNR of the median referencing scheme as compared to the average referencing scheme, even before accounting for the average referencing scheme's signal attenuation. Indeed, when analyzing data from multiple animals, common median referencing resulted in more isolated single units and more APs per unit than common average referencing (Fig. 4).

While a common median reference is readily computed in real-time (e.g., we routinely use it during 64-channel recordings with our NeuroRighter software [8]), it nevertheless takes longer to calculate than the simpler common average reference. When scaling to very high channel counts (e.g., 1000s to tens of 1000s of electrodes [13]), computationally simpler methods might be advantageous. Yet robust statistics are still crucial for high performance. In these cases, hybrid methods might be used (e.g., removing outliers, then computing a common average [14]) that preserve the spirit of a robust statistic [15], but are less computationally expensive.

V. Conclusion

Empirically and theoretically, median referencing leads to a higher SNR for APs, and prevents false positive detections of spurious APs or other transients (e.g., stimulus artifacts). The method is simple and easily computed in real-time during data acquisition.

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