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A Novel μECoG Electrode Interface for Comparison of Local and Common Averaged Referenced Signals

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Abstract

Micro-electrocorticography (μECoG) is a minimally invasive neural interface that allows for recording from the surface of the brain with high spatial and temporal resolution [1], [2]. However, discerning multi-unit and local field potential (LFP) activity with high signal to noise ratio (SNR) is challenging. Here we describe a novel μECoG design to compare the effect of referencing recordings to a local reference electrode and common average reffigerencing (CAR). The high-pass filtering effect and the increase in evoked signal to noise ratio (ESNR) can be seen after re-referencing for both types of referencing. In a preliminary analysis, re-referencing the μECoG signals has the ability to increase recording performance at high densities in the auditory cortex. This design can be applied to both in-house and commercially fabricated electrodes.

Index terms—

electrocorticography; micro-ECoG; local reference; spatial filtering; common average referencing

I. Introduction

Micro-electrocorticography (μECoG) is a neural interface method that captures high-resolution neural activity from the surface of the brain while minimizing invasiveness typically seen with penetrating recording electrodes [3], [4]. However, improving the reliability, signal to noise ratio and spatial precision of the recorded neural activity is a continued challenge in the field [2], [5]. The highly spatially localized element of the LFP, along with the desire to record multi-unit potentials, drives the inquiry as to whether or not locally re-referencing recordings from high density arrays to a nearby local reference electrode will increase the signal quality of neuronal surface recording [3], [5], [6].

In this study, we fabricated a novel 59-channel μECoG electrode that features a local reference electrode surrounding the main recording electrodes. The reference electrode was designed to surround almost the entirety of the recording region of the electrode array to reduce unequal spatial influence on the recorded local signal. Previous work has shown the feasibility and reliability of using low-cost 61-channel μECoG electrodes to chronically record evoked potentials from the rodent auditory cortex [7]. This electrode design with a

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built-in local reference allows for real-time recording of broader cortical activity during recording sessions, enabling later re-referencing of the channels to reduce global noise and spatially refine the recorded signals. The locally re-referenced signals were compared to signals re-referenced using Common Average Referencing (CAR) of the recording contacts, an established referencing technique used in EEG and some μECoG recordings [8]. The raw signals both before and after re-referencing were analyzed to determine the effect of the re-referencing procedures on the shape of the waveforms over time. The raw data and ESNR of the polyimide (PI) fabricated electrode was compared to a commercially-fabricated 61 channel electrode from Dyconex used in previous acute and chronic studies[9].

We report that the re-referencing of the main recording contacts using a local reference and CAR increases the ESNR of both the Dyconex and the PI electrode. Utilization of the local reference electrode also allows for improved signal recording capabilities in tone-based auditory recordings while still retaining essential waveform characteristics of evoked auditory responses. Therefore, utilizing a local reference electrode is a promising option for reducing global noise, improving the localization of recorded signals, and improving SNR.

II. Materials and Methods

A. Flexible μECoG Local Reference Electrode Fabrication

A 60-channel μECoG electrode array was fabricated using micro-fabrication methods within a cleanroom environment at the Shared Materials Instrumentation Facility at Duke University. The full array was composed of 59 contacts with 150 μm diameter and inter-electrode pitch of 406 μm and a local reference electrode of total length of 14.4 mm and width of 150 μm surrounding the contacts for optional re-referencing (Figure 1b). The Dyconex electrode array used for comparison is an array with a liquid crystal polymer (LCP) substrate base, with electrode contact sizes of 229 μm in diameter with interelectrode pitch of 406 μm, as shown in Figure 1a.

The fabrication process of the electrode is shown in Figure 1c. The base electrode substrate consisted of a 25 um Kapton Polyimide sheet (Fralock, Inc., Valencia, CA), which was manually laminated onto a glass slide coated with cured polydimethylsiloxane (PDMS) (Dow Corning, Midland, MI). Using an e-beam metal evaporator (CHA Industries E-Beam), 20 nm of Cr and 250 nm of Au were deposited onto the Kapton PI layer. S1813 positive photoresist (Shipley Microposit) was used as a positive mask to wet-etch the Au and Cr layers (Gold Etch TFA, Cr Etchant 9057; Transene, Danvers, MA). Next, a 6um-layer of PI 2611 (HD Microsystems, Parlin, NJ) was spun onto the surface of the array and cured at 260°C. Once cured, AZ P4620 photoresist was used as a positive mask to etch through the polyimide layer using a Trion Phantom II reactive ion etcher (RIE). The electrodes were then removed from the glass substrate and impedance tested in saline solution using a NanoZ system (Plexon, Inc., Dallas, TX) at 1 kHz frequency.

B. Surgery Protocol

All animal procedures were performed in accordance with National Institutes of Health standards and were conducted under a protocol approved by the Duke University

Institutional Animal Care and Use Committee. A surgical protocol was developed for placement of this electrode in rats. Experiments were carried out in a sound-attenuated chamber. Female Sprague Dawley rats age 4–6 months were anesthetized using ketamine. The head was secured in a custom head-holder orbital clamp that left the ears unobstructed. A longitudinal incision was made along the midline to expose the skull. The right temporalis muscle was reflected and a 6 × 6 mm craniotomy was made on the right temporal skull to expose the brain. A sterilized electrode array was placed epidurally over the core auditory cortex using vascular landmarks. Recordings of impedances and evoked responses to stimulus clicks and tones (outlined in the recording section below) gave information to optimize electrode placement. A bone screw was inserted into the skull and connected to the recording system ground and reference. The bone screw was also electrically connected to the orbital clamp to increase the ground electrode surface area.

C. Recording Protocol

Recordings were carried out in a sound-attenuated chamber. Acoustic stimuli were generated using a multifunctional data acquisition system (PXI-6289, National Instruments) and custom MATLAB code. The free-field speaker (Mackie CR3, LOUD℠ Audio, LLC) was calibrated to have <1% harmonic distortion and flat output in the frequency range used.

Responses to tone pips of 13 frequencies (0.5–32 kHz, 0.5 octave spacing, 50 ms in duration, 2 ms cosine-squared at eight sound pressure levels (SPLs, 0–70 dB SPL, 10 dB SPL steps) were recorded to reconstruct frequency intensity response areas. Each tone was repeated 30 times for each loudness level. Responses to brief broadband click stimuli (0.2 ms in duration, 70 dB SPL, 1.25 Hz, 120 repetitions) were recorded as well as in vivo impedance for each electrode.

D. Data Analysis

Acquired neural signals were analyzed using methods previously described by our group [2]. Data was initially re-referenced, and then filtered using a bandpass filter from 2–200 Hz and notch filters at 60, 120, and 180 Hz to remove 60 Hz noise. Raw impedance data were analyzed using MATLAB.

III. Results

The effect of re-referencing on tone-evoked responses was quantified. The ESNR was computed for each channel of the electrode array at the frequency at which that channel had the greatest response [2]. For the fabricated PI electrode, one of the 59 contacts was not exposed for long-term encapsulation reliability study purposes and was therefore excluded from analysis.

A. In-Vitro and In-Vivo Impedance Measurement

The Dyconex electrode array had an *in-vitro* impedance of 21.97 +/− 2.98 kΩ at 1 kHz frequency. The fabricated PI electrode had an *in-vitro* impedance of 257.22 +/− 40.66 kΩ at 1 kHz frequency for the recording contacts alone. The reference electrode was measured to have an *in-vitro* impedance of less than 1kΩ. The *in-vivo* impedance of the Dyconex array was 21.88 +/− 17.79kΩ. The *in-vivo* impedance of the PI array recording contacts was 146.05 +/− 7.45 kΩ, with the local reference electrode having an *in-vivo* impedance of 1.30kΩ.

B. Analysis of Raw Data

The effect of re-referencing using the local reference electrode and the common average of all the electrodes was compared in Figure 2a. Evoked responses were observed following tone and click stimuli, with evoked responses from click stimuli being shown in Figure 2. For the locally re-referenced electrode data, the amplitude of the signals decreased but clear peaks in the data were discernable. For the common average referenced electrode data, some the evoked potential peaks are slightly discernable, with the overall signal being lower in amplitude.

C. Average Evoked Response at Best Frequency

The average evoked response over all tone trials was plotted for each recording contact at the tone frequency for which it had the greatest evoked response, as described in [2]. Figure 3 shows the average evoked response for the raw signals and the common average referenced signals of the Dyconex electrode as well as the raw signal, locally re-referenced, and common average re-referenced signals for the PI electrode. The evoked potentials are clearly visible in the signals as acquired. In both devices, the amplitude of the evoked response was reduced after common average re-referencing. However, the baseline amplitude prior to the onset of the stimulus was also reduced. The PI electrode also showed decreased amplitude evoked responses after re-referencing using the local reference, but to a lesser extent.

D. Evoked SNR Analysis

An analysis of normalized ESNR was conducted between the Dyconex array data and the PI array data normalized to pre-stimulus baseline for each data group [2]. The baseline period was defined as 100 ms before the stimulus. The evoked SNR was calculated by comparing the signal in the stimulus response window of 125 ms after the stimulus to baseline period. The overall evoked SNR shown in Figure 4 shows the ESNR normalized to the baseline for each electrode channel at its best frequency based on response. The locally re-referenced PI array had a highly significant increase in ESNR over the raw data, with a highly significant increase in ESNR also seen in the CAR data for the PI array. There was also a significant decrease in ESNR seen between the raw and CAR data of the Dyconex electrode array. This indicates that the local reference electrode can effectively identify and reduce global background noise shared across recording contacts.

IV. Discussion

This work provided an initial quantification of the effects of local and common average re-referencing through a novel μECoG electrode design. By including a reference electrode in the perimeter of the recording contacts, spatially broad global signals and noise can be filtered out while concurrently recording from the main recording contacts of the array. This is important due to the fact that the local signal recorded by a μECoG is highly correlated; using CAR, which is typically used in EEG with more spatially dispersed electrodes, may not isolate the LFP as effectively as a local reference electrode. This work also demonstrated the usage of an in-house fabricated μECoG array, which has shown comparable or improved performance to commercially-fabricated electrodes.

We demonstrated that both local and common average re-referencing can improve the SNR of the signals while still retaining the waveform characteristics of the raw signal. The PI electrode showed an increase in ESNR after re-referencing. The unexpected reduction in ESNR seen in the Dyconex array after CAR will be investigated further with future repeated acute recordings. The choice of referencing is an extremely influential component of a recording setup and later data analysis [10]. This work shows the re-referencing has the potential to increase device performance for μECoG recordings.

V. Conclusion

The results of this work indicate that re-referencing of the neural signals acquired by μECoG electrodes indeed improves certain metrics of signal quality. This work establishes a path into further investigating the effects of local re-referencing on signal metrics. We have also shown that we can fabricate a μECoG array interface using cleanroom facilities that can be reliably used for acute recordings. Future work will analyze the effect of re-referencing on awake rat brain activity from chronically implanted arrays.

Acknowledgments

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References

[1]. Viventi J et al., “Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity in vivo.,” Nat. Neurosci, vol. 14, no. 12, pp. 1599–605, 2011. [PubMed: 22081157]

[2]. Insanally M et al., “A low-cost, multiplexed μECoG system for high-density recordings in freely moving rodents.,” J. Neural Eng, vol. 13, no. 2, p. 26030, 4 2016.

[3]. Chang EF, “Towards large-scale, human-based, mesoscopic neurotechnologies.,” Neuron, vol. 86, no. 1, pp. 68–78, 2015. [PubMed: 25856487]

[4]. Yu KJ et al., “Bioresorbable silicon electronics for transient spatiotemporal mapping of electrical activity from the cerebral cortex.,” Nat. Mater, vol. 15, no. 7, pp. 782–791, 7 2016. [PubMed: 27088236]

[5]. Kajikawa Y and Schroeder CE, “How Local Is the Local Field Potential?,” Neuron, vol. 72, no. 5, pp. 847–858, 12 2011. [PubMed: 22153379]

[6]. Khodagholy D et al., “NeuroGrid: recording action potentials from the surface of the brain,” Nat. Neurosci, vol. 18, no. 2, pp. 310–315, 12 2014. [PubMed: 25531570]

[7]. Woods V et al., “A low-cost, 61-channel μECoG array for use in rodents,” in 2015 7th International IEEE/EMBS Conference on Neural Engineering (NER), 2015, pp. 573–576.

[8]. Slutzky MW et al., “Optimal spacing of surface electrode arrays for brain–machine interface applications,” J. Neural Eng. J. Neural Engq, vol. 7, pp. 26004–9, 260AD.

[9]. Palopoli-Trojani K, Woods V, Chiang C-H, Trumpis M, and Viventi J, "In vitro assessment of long-term reliability of low-cost μECoG arrays," in 2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2016, vol. 2016, pp. 4503–4506.

[10]. Dien J, "Issues in the application of the average reference: Review, critiques, and recommendations," Behav. Res. Methods. Instruments. Comput, vol. 30, no. l, pp. 34–43, 1998.

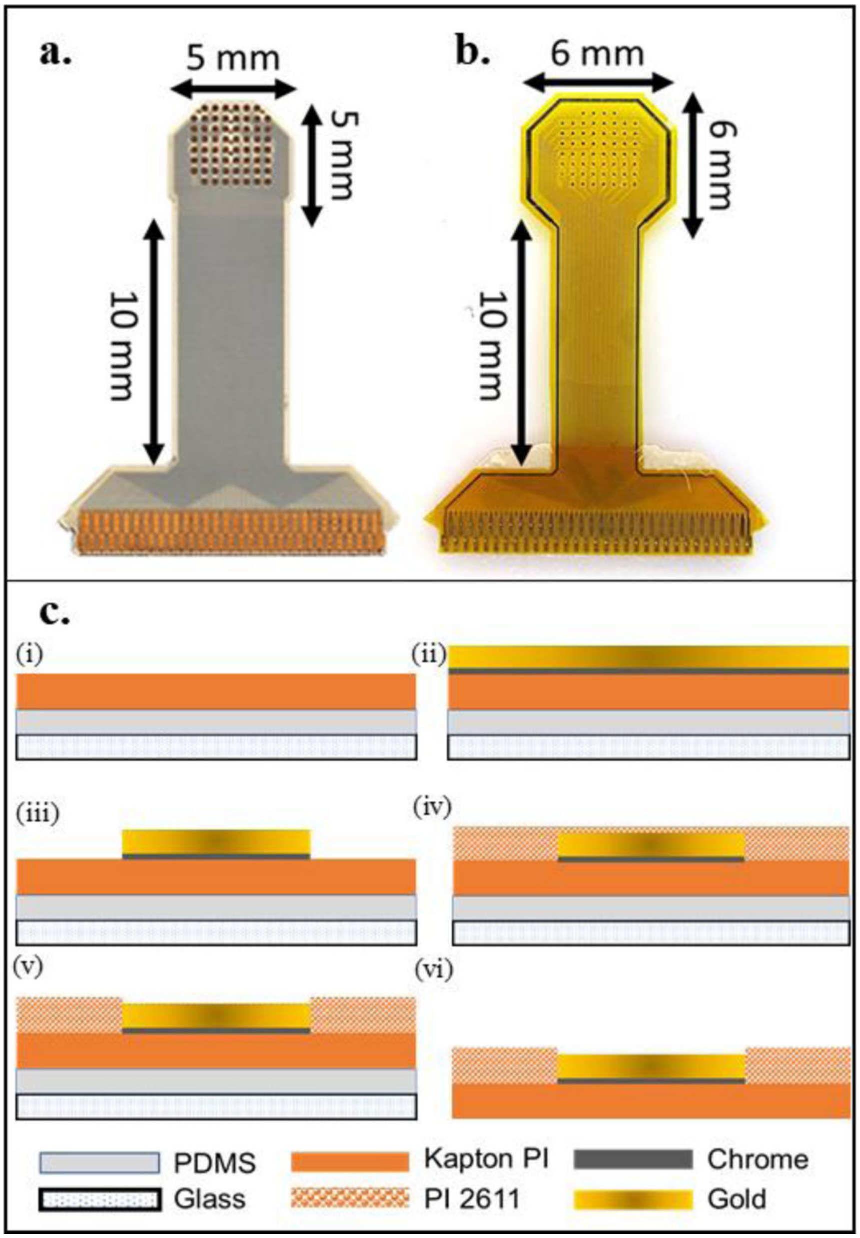

Figure 1.
(a) A standard Dyconex electrode array with 61 recording contacts. (b) The fabricated polyimide (PI) μECoG electrode array with the local reference around the 59 recording contacts. (c) The fabrication process of the PI electrode: (i) lamination of the Kapton PI layer onto the glass substrate; (ii) deposition of chrome and gold onto the PI layer; (iii) wet-etch patterning of the metal layer; (iv) deposition of PI 2611 on the top of the array; (v) dry-etch patterning of the cured PI 2611 layer; (vi) removal of the electrode array from the glass substrate.

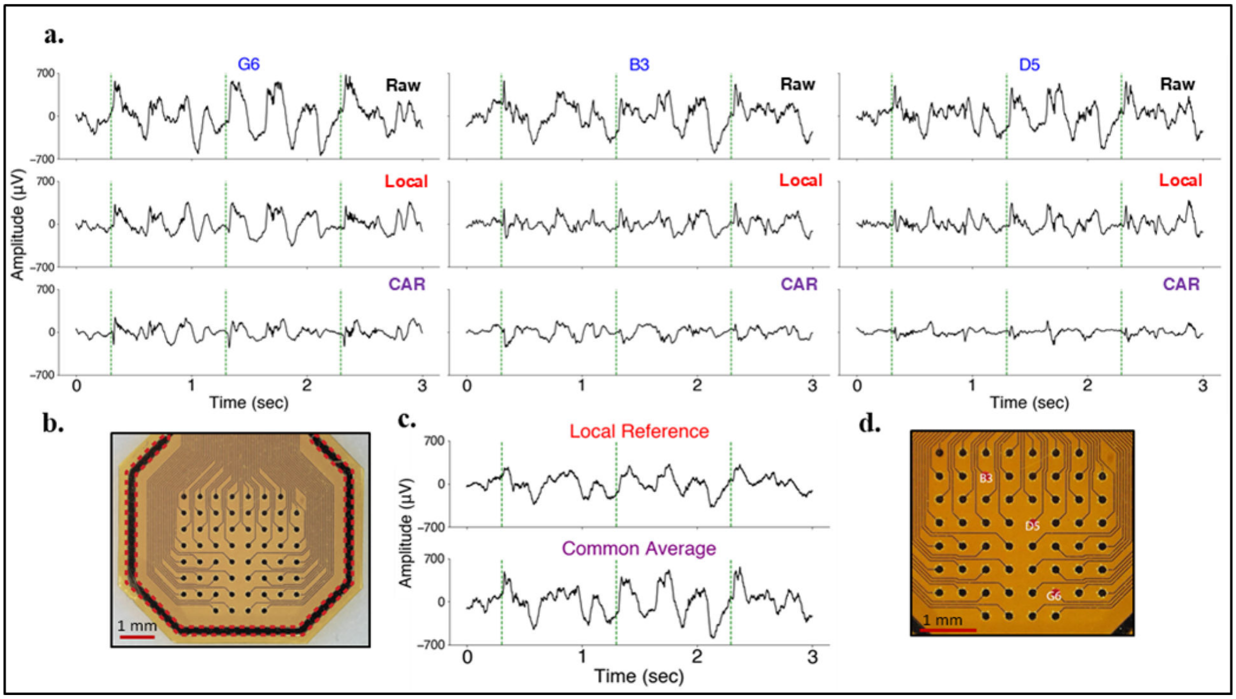

Figure 2.

Raw data taken over a three second period from specific contacts on the array. Each row shows the recording from the specific channel before and after re-referencing (referencing type indicated in the upper right corner). The green dashed line indicates when clicks were played to the rat during recording. Raw data (non-re-referenced), locally re-referenced raw data, and common average referenced data from contacts G6. B3. and D5. (b) The recording area of the PI array, with the local reference contact outlined in red dashes, (c) The raw data from the local reference electrode contact alone and the common averaged data from the same time, (d) The physical locations on the electrode arrav of the contacts shown above.

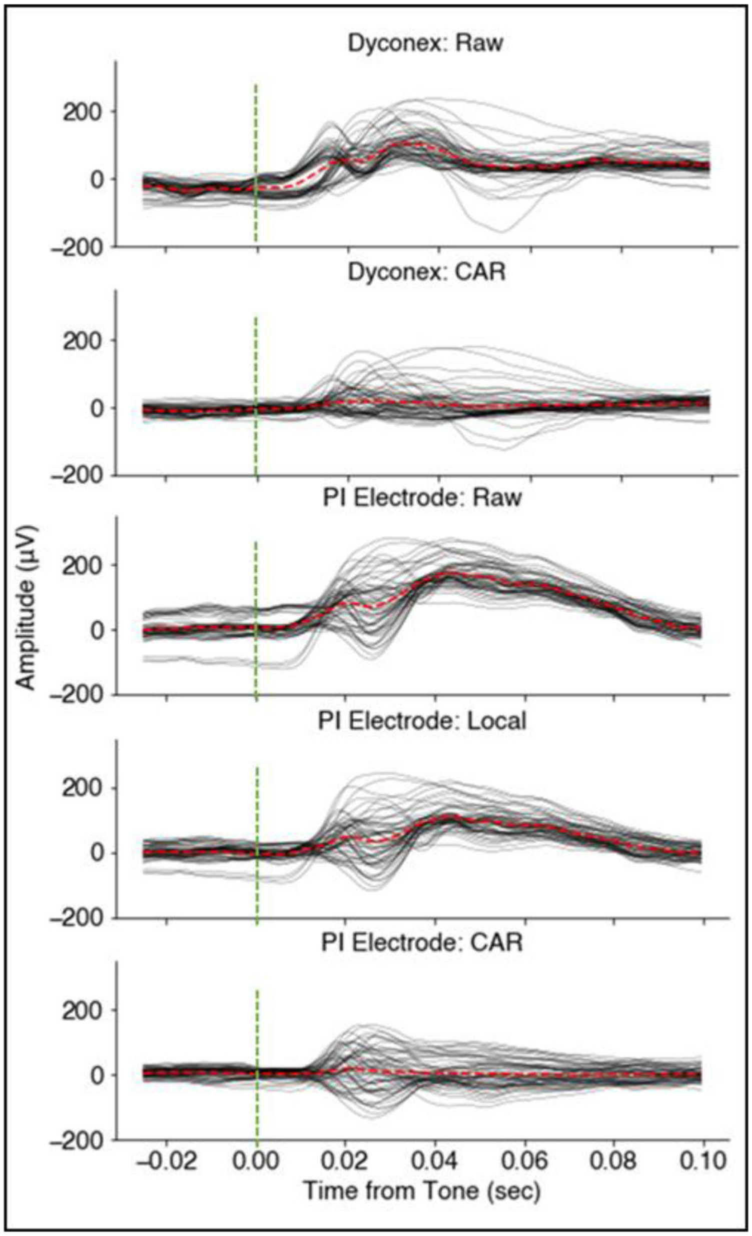

Figure 3.

The evoked potentials after a stimulus tone for each contact. These averages are for the best frequency for each of the contacts. The dashed ween line indicates when the stimulus tone was played. The dashed red [race is the average of all recording electrodes in the array.

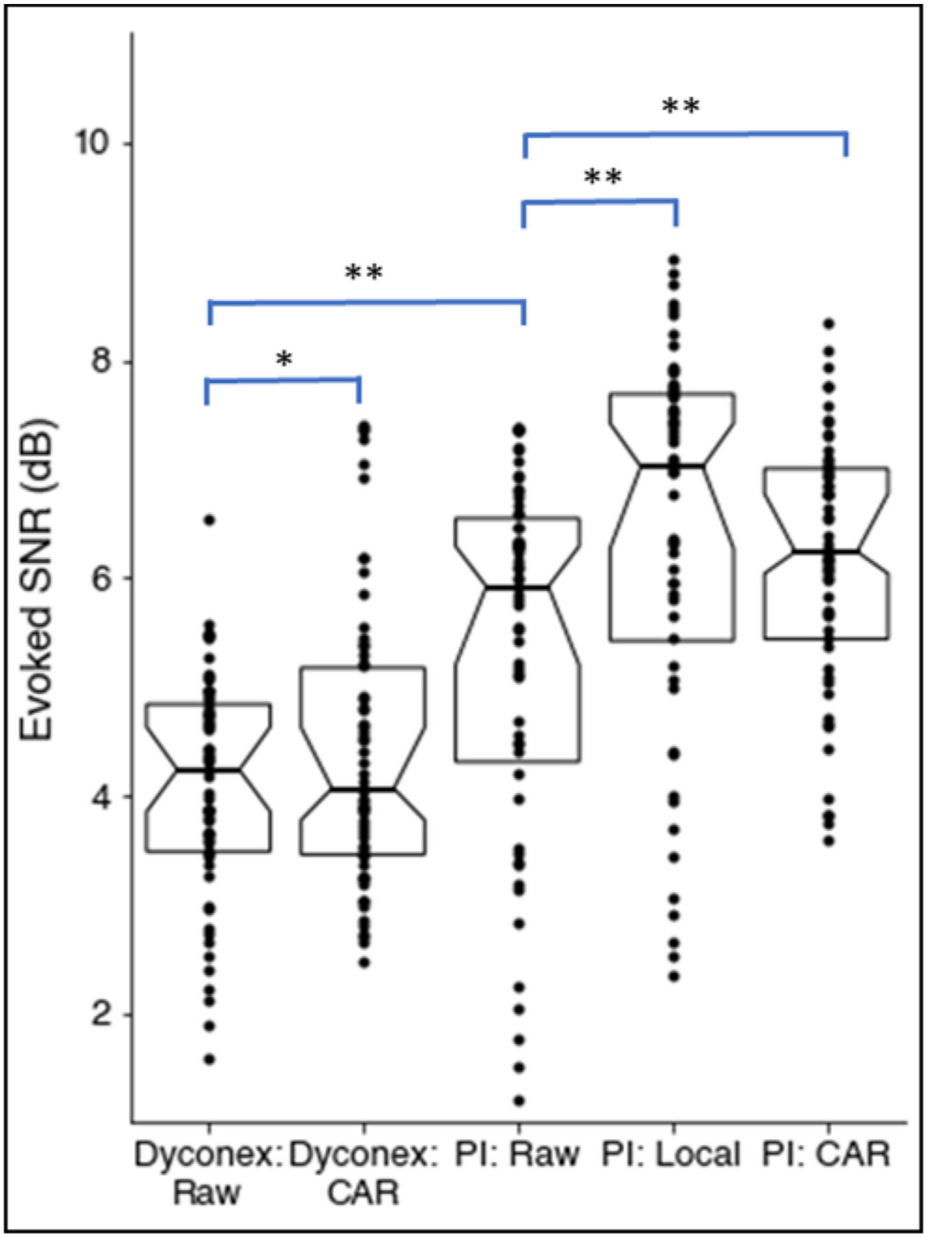

figure 4.

Evoked signal-to- noise ratio (ESKR) for the raw data and the re-reference data for both electrodes for the Dyconex and PI electrode irray. Significance values arep <0.05 (*)&p <0.001 (**).