The role of inflammation on the functionality of intracortical microelectrodes

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Abstract

\textit{Objective}. Neuroinflammation has long been associated with the performance decline of intracortical microelectrodes (IMEs). Consequently, several strategies, including the use of anti-inflammatoryatories, have been employed to mitigate the inflammation surrounding IMEs. However, these strategies have had limited success towards achieving a chronically viable cortical neural interface, questioning the efficacy of anti-inflammatoryatory approach. \textit{Approach}. Herein, we conducted a systematic study in rats implanted with functional devices by modulating inflammation via systemic injection of lipopolysaccharide (LPS), dexamethasone (DEX), a combination of both, or none to assess the degree of inflammation on device functionality. We hypothesized that implanted rats treated with LPS will have a negative impact, and rats treated with DEX will have a positive impact on functionality IMEs and histological outcome. \textit{Main results}. Contrary to our hypothesis, we did not observe adverse effects in recording metrics among different groups with LPS and/or DEX treatment despite alterations in initial pro-inflammatory markers. We also did not observe any functional benefit of anti-inflammatory treatment. Regardless of the treatment conditions, the recording quality degraded at chronic time points. In end-point histology, implanted rats that received LPS had significantly lower NeuN density and higher levels of CD68 surrounding the implant site, indicative of the pro-inflammatory effect of LPS, which, however, contradicted with the recorded results. \textit{Significance}. Collectively, our results suggest that acute inflammatory events may not be the key driver for functional degradation of IMEs. Future intervention strategies geared towards improving the functional longevity of intracortical devices may benefit using multi-modal approaches rather than a single approach, such as controlling the initial inflammatory response.

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(Some figures may appear in colour only in the online journal)

Introduction

Intracortical microelectrodes (IMEs) are brain-implanted micro-devices that can be used to survey cortical regions for a wide variety of applications. They are useful as a basic science tool to monitor changes in neuronal behavior and also offer a potential therapeutic option for patients with debilitating neurological conditions [1–3]. Unfortunately, the lack of functional reliability of IMEs has been a major setback for long-term experiments and clinical translation. Ideally, once implanted, IMEs should remain functional for many years; however, preclinical studies to date have provided evidence that the functionality of IMEs degrades over time as manifested by a decrease in the number of functional sites, a drop in signal-to-noise ratio, and an increase in the magnitude of the impedance at 1 kHz as a function of time [4–9].

Though the overall stability of an implanted device is governed by many factors [10–13] the above mentioned electrophysiological changes have been associated with the underlying changes in biology surrounding an implanted device [5, 14–16]. Specifically, the decline in recording quality is attributed to a local loss of neuronal population [16], whereas an increase in impedance is associated with the formation of the glial sheath, which is primarily composed of activated microglia, macrophages, and reactive astrocytes surrounding an implanted device [14–16]. Neuroinflammation, a complex biochemical cascade comprised of multiple cell types including activated microglia/macrophages, reactive astrocytes, and neurons, has been associated with the performance decline of chronically implanted IMEs [17, 18]. As a result, numerous biochemical intervention strategies have been employed to mitigate cellular response and improve neuronal health surrounding an implanted device. However, most of the studies are conducted using electrically, inactive devices and thus are limited to histological assessment of the nearby tissue [19–25]. With growing evidence that histological findings may not necessarily correlate with functionality [11, 26, 27], it is unclear how histological improvements using various mitigation approaches aimed at reducing neuroinflammation manifest to IME functionality. Furthermore, determining the impact of inflammation on performance of IMEs is crucial for future intervention strategies and eventually towards the development of a chronically viable neural interface.

In this report, we sought to characterize the degree of inflammation on the functionality of IMEs. We hypothesized that an increased inflammation leads to an early failure of a brain-implanted microelectrodes. To test our hypothesis, we conducted a comprehensive study modulating inflammation with lipopolysaccharide (LPS) and/or dexamethasone (DEX) and collected multi-parametric datasets from rats implanted with functional microelectrodes. LPS, a bacterial endotoxin and a cell wall component of gram negative bacteria, binds to toll-like receptor 4 (TLR4) present on immune cells, including brain resident microglia [28, 29] and initiates an inflammatory cascade by secreting pro-inflammatory molecules [30]. DEX, a glucocorticoid agonist, is a known anti-inflammatory drug that has been previously reported to mitigate cellular response to brain-implanted microdevices [19, 20]. Longitudinal (biochemical and electrical) and end-point histological datasets from implanted rats that received either LPS, DEX, both, or none, were compared between groups to (a) further tease apart the impact of inflammation, and (b) determine the effect of an anti-inflammatory treatment on device functionality.

Materials and methods

Microelectrode implantation

All animal work was conducted following Institutional Animal Care and Use Committee guidelines at the University of Florida. 3–5 month-old male Sprague Dawley rats (Charles River, Wilmington, MA) were used for this study (N = 4–5 per group). To minimize surgeon-related variability, the same surgeon performed all surgeries. Following aseptic techniques, single shank silicon microelectrode arrays (Item number: A1 × 16-3mm-50-703-HZ16) purchased from NeuroNexus Technologies (Ann Arbor, MI) were implanted in the primary motor cortex (M1), approximately 2 mm anterior to bregma and 2 mm lateral to midline. Prior to implantation, microelectrodes were sterilized using ethylene oxide and rinsed with sterile saline. Rats were anesthetized using 3% isoflurane (Zoetis, Parsipany, NJ) and maintained at 1%–1.5% in O2 during surgery. Eye ointment was applied to prevent the eyes from drying. After the hair was clipped from the surgical site, rats were placed in stereotaxic frame and cleaned with three alternating washes of chlorohexidine and ethanol. A water circulating heating pad was used to maintain the body temperature (37 °C), and vitals were recorded using a pulse oximeter (Kent Scientific, Torrington, CT). After a midline incision was made, the underlying tissue was removed to access the skull. A craniotomy was created over M1 and burr holes were drilled using a micro drill to place bone screws for grounding and securing the head cap. After a dural slit was made, an automated micro-insertion system (Physik Instrumente, Karlsruhe, Germany) was used to implant a microelectrode array to a depth of 1.5 mm at an insertion speed of 20 mm s−1. Kwik-Sil (World Precision Instruments, Sarasota, FL) was used to cover the craniotomy and secure the implant. Exposed wires and skull were secured using dental acrylic (Fusio Liquid Dentin, Orange, CA). The skin was sutured, and a triple antibiotic (Actavis, NC) was applied to the wound site to prevent infection. For rats receiving LPS, LPS from Escherichia coli strain...
055: B5, Sigma Aldrich) was dissolved in ultrapure water and 5 mg kg\(^{-1}\) was injected intraperitoneally immediately following microelectrode implantation. For rats receiving DEX, 0.2 mg kg\(^{-1}\) of sterile DEX (Clipper Distributing Company, St. Joseph, MO) was injected subcutaneously a day before implantation and until day five post implantation. In all animals, meloxicam (1 mg kg\(^{-1}\)) (Norbrook, United Kingdom) was administered subcutaneously prior to surgery and until 2 d after surgery to relieve pain and distress. The experimental timeline of treatment administration and data collection is detailed in figure 1.

**Serum collection and cytokine analysis**

Blood samples were collected from the saphenous vein at multiple time points as outlined in figure 1. Blood was clotted for 30 min at room temperature (RT) and centrifuged at 2000 g. Separated serum samples from all animals were stored at \(-20^\circ\)C until analyzed. Multiplex plates, pre-coated with capture antibodies for pro-inflammatory cytokines, were used to measure circulating levels following the manufacturer’s protocol (Meso Scale Discovery, Gaithersburg, MD). In brief, the plates were blocked with blocking solution for 1 h and washed with wash buffer three times. Then, serum samples and calibrators were placed in a 96-well plate and incubated for 2 h with gentle shaking at RT. The plates were then washed with wash solution, and detection antibodies were added to each well. After incubating for 2 h with shaking, the plates were washed with wash buffer, and 150 \(\mu\)l of read buffer was added to each well. The plates were read immediately using an Image Sector 2400 (Meso Scale Discovery, Gaithersburg, MD). Serum samples and calibration samples (used to generate the standard curve) were run in duplicates and the average of two samples was used for quantification. A standard curve generated using serially diluted calibration solutions for each cytokine was back-fit to determine the concentration of serum samples. Post-treatment levels of keratinocyte derived chemokine/growth related oncogene (KCGRO) also known as CXCL1, tumor necrosis factor-alpha (TNF-\(\alpha\)), and interleukin-1beta (IL-1\(\beta\)) at each time point were normalized to baseline measurements (pre-treatment and/or pre-implant levels) to compute the fold change. As cytokine levels did not follow a normal distribution, a log transformation of the fold change was computed across all datasets before running statistical tests.

**Electrical measurements and post-processing of electrophysiological recordings**

The timeline of electrical measurements (electrophysiological recordings and electrochemical impedance spectroscopy (EIS)) is included in figure 1. Electrical measurements and post-processing of electrophysiological recordings were performed similarly to previously described methods with slight modifications [9]. In brief, electrophysiological recordings from awake, freely moving rats were obtained for 5 min using a Tucker-Davis Technology (TDT) signal acquisition system; PZ5 Neurodigitizer connected to an RZD Bioamp Processor (TDT, Alachua, FL). Raw data was collected and stored using a custom-written script in MATLAB (MathWorks, Natick, MA). Following electrophysiological recordings, an Autolab PGSTAT128N (Metrohm Autolab B.V., Utrecht, The Netherlands) was used to assess EIS. Impedance spectra for each site was collected by applying 15 sinusoidal waves over logarithmically spaced frequencies from 10 Hz to 30 kHz. The 1 kHz impedance magnitude interpolated from the measured spectrum is reported in this study.

Post-processing of electrophysiological recordings was conducted as previously described [9]. Briefly, raw neural recording data were filtered at 300 Hz – 3 kHz and an amplitude threshold of 4 times the noise level \(\sigma_N\) (=median(abs(signal))/0.6745 was set for spike detection. Movement related artifacts across multiple channels were discarded as previously described [9]. Spike sorting was carried out using a commercially available software, Plexon Offline Sorter V3 (Dallas, TX). Spikes were sorted in the 3D principal component domain with scanning \(k\)-means clustering, and units with firing rate equal to or greater than 0.5 Hz (>150 single units in 5 min recording session) were considered as single units [9]. Sorted units were visually inspected to further ensure the validity of sorted units. Signal-to-noise ratio (SNR) of the sorted unit was computed by dividing the peak-to-peak amplitude by two times the noise level (\(\sigma_N\)), and SNRs above 2.0 were reported in this study.

**Tissue processing and immunohistochemistry**

At four weeks post-implantation, the rats were transcardially perfused with 1 × phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) solution. After decapitation, the heads were fixed in PFA at 4 \(^\circ\)C overnight and washed with PBS. Next, the devices were carefully explanted, and the
extracted brains were cryoprotected in 30% sucrose solution and then flash frozen in 2-methylbutane at −40 °C (Sigma Aldrich, M32631-500 ML St. Louis, MO). The brains were then sectioned into 20 µm thick horizontal sections and stored at −80 °C until staining. Prior to staining, slices were brought to RT and rehydrated in PBS. Slices were blocked in wash solution (4% vol/vol normal goat serum, 0.3% Triton-X in HEPES buffered hanks solution (HBHIS)) for 2 h then incubated in primary antibodies; mouse anti-rat CD68 (MAB1435, EMD Millipore, Burlington, MA) for activated microglia/macrophages, chicken anti-GFAP (NBP1-05198, Novus Biologicals, Littleton, CO), and rabbit anti-NeuN (ABN78, EMD Millipore, Burlington, MA) at 4 °C overnight. Slides were then washed three times with wash solution to remove unbound primary antibodies and incubated with secondary antibodies; Alexa fluor 488 goat anti-mouse (A11029, Life Technologies, Carlsbad, CA), Alexa fluor 555 goat anti-chicken (A21437, Life Technologies, Carlsbad, CA), and Alexa fluor 633 goat anti-rabbit (A21103, Life Technologies, Carlsbad, CA) at RT for 2 h. Slices were again washed with PBS three times to remove unbound secondary antibodies. 4′,6-diamidino-2-phenylindole (DAPI) was added to the first wash as a counter-stain. Stained slides were cover-slipped using Fluoro-gel (Electron Microscopy Sciences, Hatfield, PA) and stored in a dark container at 4 °C until imaged.

Confocal microscopy and quantitative histology

Histological images were acquired with a plan-Apochromat 10× (Numerical Aperture: 0.45) objective using a laser scanning confocal microscope Zeiss LSM 710 (Zeiss, Germany). Four laser lines (405/488/555/633 nm) were used; images were collected by sequential excitation of 405/555 followed by 488/633 lines. Laser settings for each line were held constant for all images. To account for possible depth-related variability [31, 32] analyzed sections were taken approximately from the same depth for all the animals. A minimum of three sections, per animal, spanning the depth of approximately 700–1050 µm from the surface of the cortex were used to obtain the average values for each animal. For CD68 and GFAP markers, MINUTE v1.5 software was used as in previous publications [33–35] to calculate the average fluorescence intensity as a function of distance. The quantification of neuronal density was performed using an open source software, FUJI [36] and MATLAB. Images were thresholded, and open holes were filled and watershed to separate fused cells before counting. Neuronal counts were binned into 10 µm distance using custom written MATLAB script. Fluorescence intensity profiles (for GFAP and CD68) and neuronal cell counts were normalized to a distant area (at least 400 µm away from implant site) and binned into 50 (up to 200 µm) and 100 µm (200–400 µm) intervals for quantification.

Scanning electron microscopy (SEM)

Explanted devices were soaked in PBS containing 0.3% triton with gentle rocking to remove debris. Devices were rinsed in distilled water and were dried with 100% ethanol. Devices were then mounted on conductive carbon tabs, sputter coated with gold/pallidum for 120 s, and imaged using a Hitachi SU5000 Schottky Field-Emission SEM (Tokyo, Japan).

Statistics

Statistical analysis was performed in SPSS version 24 (IBM Corporation). Normality was assessed using Shapiro–Wilk test. A two-way ANOVA was run by treating groups and either time (longitudinal data-sets) or bin size (for end-point histology) as two independent variables and one outcome variable as the dependent variable. Furthermore, one-way ANOVA was performed to test the differences between subjects by either blocking time or bin size. The homogeneity of variances was confirmed using Levenne’s test. Following ANOVA, either Tukey HSD (for equal variances) or Games-Howell (for unequal variances) post hoc tests were used. Significance levels were determined at an alpha value of 0.05. Graphs were plotted using MATLAB.

Results

Biochemical analysis

To confirm the effect of pro- and anti-inflammatory treatment, we compared changes in the circulating levels of the pro-inflammatory molecules; KCGRO, TNF-α, and IL-1β. Post-treatment and post-implant levels of each pro-inflammatory molecule were normalized to pre-treatment levels, and differences across four groups at each time-point were compared. At day 1 following implantation and LPS injection, we observed an increased level of pro-inflammatory markers in implanted rats treated with LPS compared to rats that received the ‘Implant only’ and/or ‘Implant + DEX’ (figure 2). Specifically, at 1 d post implant (DPI), KCGRO levels were significantly higher in groups that received LPS compared to rats that received DEX only. The ‘Implant only’ group had significantly lower levels of KCGRO as compared to ‘Implant + LPS + DEX’ group. Though not statistically significant, the ‘Implant only’ group had lower KCGRO levels as compared to the ‘Implant + LPS’ group (figure 2(a)). TNF-α levels were significantly higher in rats that received LPS as compared to rats with the ‘Implant only’ and the ‘Implant + DEX’ at 1 DPI (figure 2(b)). IL-1β levels were also significantly higher in the ‘Implant + LPS + DEX’ group compared to the ‘Implant + DEX’ and the ‘Implant only’ group. Though not statistically significant, the mean fold change for 1L-1β levels were higher in the ‘Implant + LPS’ group as compared to the ‘Implant + DEX’ and the ‘Implant only’ group (figure 2(c)). At 3 DPI, the ‘Implant + LPS + DEX’ groups had significantly higher levels of KCGRO compared to the ‘Implant only’ and the ‘Implant + DEX’ group. Implanted rats that received DEX only had lower systemic levels of KCGRO and TNF-α levels, though not statistically significant than ‘Implant only’ group. A table with comparison outcomes between different groups at acute time points is presented in figure 2(d).
Recording quality

To determine the recording quality between groups we compared: (a) the fraction of active sites (sites capable of recording identifiable units), (b) the SNR of active sites, and (c) the median noise floor. There was a decrease in overall recording quality as a function of time in all implanted rats irrespective of the treatment condition. We observed a decrease in fraction of active sites as a function of time (figure 3(a)). The percentage drop in active sites was dramatic; with greater than 30% active sites from the first few days to mostly below 15% or less after the first week. Animals in the ‘Implant + DEX’ group had the lowest fraction of active sites, and we did not observe any active sites after 14 d in animals in this group.

Figure 2. Change in systemic levels of cytokines in rats post-implantation with each treatment. Mean fold change in levels of keratinocyte derived chemokine/growth related oncogene (KCGRO) (a), interleukin-1 beta (IL-1β) (b), and tumor necrosis factor-alpha (TNF-α) (c) at different days post implant. (d) Table displaying p-values (post hoc Tukey test) for comparison between groups. *denotes that the mean difference is significant at alpha level of 0.05 (N = 4–5). Error bars represent standard error of mean.
SNR of the active sites is a commonly used metric to compare the recording quality across different groups. We ran two-way ANOVA to see if there was an overall treatment effect in which we found that ‘Implant + LPS + DEX’ group was significantly higher than Implant + LPS (p < 0.05) and Implant + DEX group (p < 0.05) (figure 3(b)). No statistically significant differences among other groups were observed. No significant interaction effect was also observed between groups and days post implant. We also used one-way ANOVA to compare the effect of treatment among different groups at a given time point. No statistically significant differences among groups was observed at any day during the first 28 days post implant.

Figure 3. Comparison of recording quality across four different groups. (a) The fraction of active sites (sites capable of recording identifiable units) decreases as a function of time across all groups. (b) Signal-to-noise ratio (SNR) of active sites for four different groups. Overall, ‘Implant + LPS + DEX’ had significantly higher SNR compared to ‘Implant + LPS’ and ‘Implant + DEX’ group (Two-way ANOVA, p < 0.05). However, no significant differences were observed among groups at any time-point (one-way ANOVA, p < 0.05). (N = 3–5). Error bars represent standard error of mean.

Figure 4. Comparison of median noise level across four different groups. No statistically significant differences were observed except at 2 d post implant, where the ‘Implant + LPS + DEX’ was significantly higher than the ‘Implant + LPS’. *denotes p < 0.05 with one-way ANOVA followed by post hoc Games-Howell (for unequal variances) (N = 3–5). Error bars represent standard error of mean.
week. Since very few sites were able to record identifiable units past the first week, post hoc test was ran only on first-week data sets.

We observed an initial increase in noise during the first week that later stabilized across all groups (figure 4). The ‘Implant + LPS’ group had lower noise levels as compared to other groups (until day seven); however, it was not statistically significant except at 2 d post implant. At 2 d post implant, the noise level in the ‘Implant + LPS + DEX’ group was significantly higher than the ‘Implant + LPS’ group.

**Magnitude of 1 kHz impedance**

The 1 kHz impedance magnitude is of considerable interest since 1 kHz is the fundamental frequency of most action potentials. The 1 kHz impedance magnitude was higher at later time points in comparison to initial days post implant (figure 5) for all groups. For the first week, there was a linear increase in the 1 kHz impedance magnitude that plateaued afterwards (mostly between 1.2–1.5 MΩ) across all groups. Using two-way ANOVA, no statistically significant differences were observed among groups.

**Quantitative histology**

**Neuronal density.** Immunostaining with NeuN antibody, a marker for neuronal nuclei, has been used as a standard to assess neuronal health surrounding the implanted device. As evident in figure 6, neuronal density near the implant, especially at 0–50 µm, is 60% or lower compared to distant areas across all groups. First, two-way ANOVA was run to identify differences among groups followed by post hoc Tukey test.
The ‘Implant + LPS’ group was significantly lower than the ‘Implant only’ group. Though not statistically significant, the ‘Implant + LPS’ group was lower than the ‘Implant + DEX’ ($p = 0.062$) and the ‘Implant + LPS + DEX’ ($p = 0.086$). No interaction between groups and bin size was observed. Next, one-way ANOVA was performed at each binned interval; however, no statistically significant differences among groups were observed.

Activated macrophages/microglia. Cluster differentiation 68 (CD68) is a commonly used marker to assess the activation state of macrophages and microglia. CD68 levels were elevated and highly variable near the implant site as indicated by large error bars across all groups (figure 7). As evident in figure 7(b), CD68 intensity was higher for the ‘Implant + LPS’ group out to 200 $\mu$m away from the implant site, and intensity was lower for the ‘Implant + DEX’ group out to 100 $\mu$m from implant site. Using two-way ANOVA, however, we did not observe any statistically significant differences among different groups.

Reactive astrocytes. The level of glial fibrillary acidic protein (GFAP), an intermediate filament protein present in astrocyte, is elevated during gliosis. As evident in figure 8, the fluorescence intensity of GFAP was higher up to approximately 200 $\mu$m away from the implant site in all groups. Qualitatively, GFAP response seems to be confined and lower in the ‘Implant + DEX’ groups as reported in previous qualitative study [19]; however, we did not observe statistically significant differences among groups (figure 8).

SEM images. Previous studies have reported the mechanical failure of silicon devices implanted for time points greater than 160 d [12]. Using SEM, we examined explanted devices to observe any morphological changes. Figure 9 shows the representative SEM images of an explanted device from a
rat that received LPS. We did not observe any cracks and/or delamination on any of the devices that we imaged ($n = 9/20$, at-least two per group).

Discussion

The objective of this study was to elucidate the effect of inflammation on the functionality of IMEs. Though neuroinflammation has long been considered to negatively influence the recording quality, to date, no studies have investigated the cause-and-effect relationship between inflammation and recording quality. We report here the results obtained from a comprehensive study where we modulated inflammation by systemic injection of either LPS or DEX or a combination of both and compared biochemical, electrical, and histological metrics from rats implanted with single-shank, silicon microelectrode for four weeks. Our results demonstrate that changes in initial inflammatory milieu may not be as critical to improve chronic functionality and attempts to manage this initial phase alone may not enough to create a chronically, viable microelectrode.

To investigate the impact of managing initial inflammation on the functionality of brain-implanted microelectrodes, we administered LPS and DEX via systemic route to bi-directionally modulate the inflammation. The opposing effects of the LPS and/or DEX treatments that we observed acutely in circulating levels of pro-inflammatory markers and end-point histology confirm our approach to modulate inflammation. At acute time points, implanted rats treated with LPS had higher levels of pro-inflammatory markers whereas implanted rats treated with DEX alone had the lowest levels of pro-inflammatory markers. Though systemic response following LPS administration subsides quickly, several studies have reported the effect of LPS in the brain to last longer [30, 37, 38]. For example, at 7 days following a single dose of systemic injection of LPS, Zamanian et al., demonstrated increased Iba1 and GFAP immunoreactivity in the cortex, an indication of an increased inflammation [37]. At four weeks, we neither observed any differences among groups at the distant site (at-least 400 µm away from implant site) nor did we see differences in the contralateral hemisphere in microglial and astroglial activation, similar to what has been previously reported following four weeks after LPS exposure [38]. It is possible that the stimulant delivered to the implant site via systemic route may not have been enough to alter the result past acute time points suggesting that future studies should consider alternative strategies to deliver stimulants for a prolonged time, preferably via local delivery methods. In ‘Implant + DEX’ group, though not statistically significant, we observed lowered CD68 and GFAP responses at the implant site suggesting DEX’s anti-inflammatory effect, which is in agreement with previous reports on the effect of DEX on GFAP response [19, 20]. Interestingly, we noticed the highest levels of pro-inflammatory markers in the group of implanted rats that received both LPS and DEX (though not significantly higher than LPS alone). Elevated levels of inflammatory markers in ‘Implant + LPS + DEX’ group could be either due to insufficient amount of DEX to bring down the acute effect of LPS or a combined effect of both the pro-and anti-inflammatory substance as the effect of initial spike in inflammatory cytokines did not manifest in the end-point histology results. Local assessment of biochemical markers may have provided a better explanation on the relation between biochemical and histological changes at the device-tissue interface [18, 39, 40].

The electrophysiological results suggest that the initial amplifications in pro-inflammatory markers do not have an adverse effect on the recording quality. During the first week, neither the fraction of active sites nor SNRs were reflective of changing levels of pro-inflammatory markers. Despite higher levels of pro-inflammatory markers in LPS treated groups, we did not observe a dramatic reduction in recording quality in comparison to the groups that did not receive LPS. As evident in figure 2, the recording quality was best for the group that had the highest systemic levels of pro-inflammatory cytokines i.e. the ‘Implant + LPS + DEX’ group. On the other hand, despite having the lowest levels of pro-inflammatory markers, the DEX alone treated animals, did not show any functional improvements in terms of both SNR and fraction of active sites. One interesting observation in our study was that the implanted animals treated with LPS alone were lethargic and less mobile during the first week. Perhaps less activity may have resulted in a lower noise floor during the first week as sickness behavior is common in animals treated with LPS [30]. We also observed lower neuronal count in the ‘Implant + LPS’ group compared to other groups, which may have attributed to the lower noise floor seen on LPS alone treated groups as lower neuronal density has previously been shown to produce lower biological noise [41]. Lower neuronal counts were seen only at the implant site, and we did not observe differences between groups in the neuronal count at distant sites (400 µm or away) or the contralateral site. Even though LPS does not
directly cross the blood brain barrier (BBB) [29]. BBB is compromised at the implant site and LPS may have perpetuated the inflammatory response as indicated by increased CD68 activity from phagocytic macrophages/microglia. This could have resulted in lower neuronal count at the implant site in the ‘Implant + LPS’ group. It was, however, difficult to make conclusions regarding the functional consequence of lower neuronal counts in LPS treated animals as we had very few sites capable of recording single units from all groups despite having better neuronal health compared to ‘Implant + LPS’ group. With end-point histological data, it was not possible to associate changes in electrical metrics with histological outcomes surrounding an implanted device.

The 1 kHz impedance magnitude is a routinely used metric to assess the quality of the device-tissue interface and has been shown to correlate with the formation of the glial sheath around an implanted device [5]. We did not see any significant differences in the impedance magnitude at 1 kHz among groups throughout the course of our study. Perhaps this is to be expected, since the GFAP and CD68 labels were not significantly different for the groups in the region surrounding the device. The 1 kHz impedance magnitude was similar across groups despite altered levels of pro-inflammatory markers at acute time points. During the first week, we noticed that LPS alone treated animals had lower 1 kHz impedance, which could be due to excessive swelling and edema from the LPS. Emerging *in vivo* testbeds allowing simultaneous collection of longitudinal changes in electrochemical and *in vivo* tissue dynamics [42] may help better explain these results. At long-term time points, we did not observe any significant difference for the 1 kHz impedance magnitude or the levels of CD68 and GFAP, primary constituents of the glial sheath. In confirmation with our findings, previous reports on accelerated aging showed little or no change in 1 kHz magnitude impedance in devices similar to the ones used in our study [43].

Our results are not completely surprising given the inconsistency and unsatisfactory results often observed in many intervention approaches including biochemical approaches [44, 45]. DEX has been widely used in the field of neural implants via systemic and local means and has been reported to decrease reactive astrocytes and improve neuronal health [20, 46] without unknown functional consequence in terms of recording quality. In our study, despite slight improvements in histological observations (though not statistically significant), we did not observe any functional benefits of systemic DEX treatment in terms of recording quality. Though single unit activity and SNR of active sites were not reported, a recent study in which a controlled, local release of DEX was employed, also did not observe any differences in impedance magnitude compared to the control group [45]. Finally, the results obtained from this study where we studied the role of inflammation (one of the biological factors) and other studies aimed at examining the effect of a single factor, i.e. placement of sites [9] or flexibility [35], explains the multi-dimensionality of the problem. Though we can rule out the material failure aspect in our study (as evident in figure 9), we cannot neglect the possibility of other biological factors, such as micro-motion induced injury thereby damaging a nearby larger blood vessel [47, 48]. Cellular response to implanted devices is highly dynamic and varies from animal to animal, as observed in our study (figure 10) and reported by other studies as well [35, 49]. The limited insight offered by end-point histology is a major challenge in determining causality between inflammation and neuronal behavior. Longitudinal *in vivo* imaging studies [42], in conjunction with novel transgenic mouse models [50], may help to capture such phenomenon.

**Conclusions**

In this study, we utilized a biochemical approach to understand the impact of inflammation on the functionality of single-shank silicon microelectrodes. The results from our comprehensive study suggest that attempts to manage initial inflammation alone is not enough to improve the functionality of brain-implanted microdevices and future mitigation studies aimed at increasing the functional longevity of brain-implanted devices may benefit using a combinational approach and should be carried out using functional devices.

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