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Blood brain barrier (BBB)-disruption in intracortical silicon microelectrode implants

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Abstract

Chronically implanted microelectrodes in the neural tissue elicit inflammatory responses that are time varying and have been shown to depend on multiple factors. Among these factors, blood brain barrier (BBB)-disruption has been hypothesized as one of the dominant factors resulting in electrode failure. A series of events that includes BBB and cell-membrane disruption occurs during electrode implantation that triggers multiple biochemical cascades responsible for microglial and astroglial activation, hemorrhage, edema, and release of pro-inflammatory neurotoxic cytokines that causes neuronal degeneration and dysfunction. Typically, microwire arrays and silicon probes are inserted slowly into the neural tissue whereas the silicon Utah MEAs (UMEA) are inserted at a high speed using a pneumatic inserter. In this work, we report the sequelae of electrode-implant induced cortical injury at various acute time points in UMEAs implanted in the brain tissue by quantifying the expression profile for key genes mediating the inflammatory response and tight junction (TJ) and adherens junction (AJ) proteins that form the BBB and are critical to the functioning of the BBB. Our results indicated upregulation of most pro-inflammatory genes relative to naïve controls for all time points. Expression levels for the genes that form the TJ and AJ were downregulated suggestive of BBB-dysfunction. Moreover, there was no significant difference between stab and implant groups suggesting the effects of UMEA insertion-related trauma in the brain tissue. Our results provide an insight into the physiological events related to neuroinflammation and BBB-disruption occurring at acute time-points following insertion of UMEAs.

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Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Keywords

microelectrode array; Utah arrays; blood brain barrier (BBB) disruption; neuroinflammation; electrode failure

1. Introduction

Neuroprosthetic technologies [1–5] require a reliable neural interface that allows recording of high quality neuronal recordings for long periods of time. However, intracortical electrodes [6–12] implanted chronically in the brain tissue are affected by multiple factors [13–24] and a complex time-varying interaction of these factors (mechanical, material, electrical, and biological) lead to temporal degradation in the recorded signal [13, 22, 24–27]. Electrode-implant induced injury leads to a series of events that includes strain and blood-brain barrier (BBB) and cell-membrane disruption. The BBB provides a physical and metabolic barrier in the brain that regulates cerebral homeostasis while protecting the central nervous system (CNS) from pathogens and toxic compounds [28, 29]. The damage leads to multiple biochemical cascades responsible for neuronal dysfunction and degeneration. Among these pathways, BBB disruption is one of the most dominant pathways resulting in hemorrhage, edema, ischemia, microglial activation, and the release of pro-inflammatory neurotoxic cytokines [30] and has been indicated as key determinant affecting the chronic electrode-tissue interface stability [18, 31]. A BBB breach results in the infiltration of neurotoxic factors and pro-inflammatory myeloid cells [32, 33] and reactive oxygen and nitrogen species leading to oxidative stress in the local tissue around the electrode sites [30]. To some extent, local microglia can deal with increased oxidative stress but this is not sufficient to handle the long-term infiltration of these species created by a chronic, non-healing wound. Moreover, prolonged oxidative stress results in excitotoxicity and adversely affects microglial cells by causing microglial dystrophy that contributes towards their premature demise [34, 35]. The resulting loss of important microglial neuroprotection adversely affects neuronal function and firing [35–37]. Saxena et al showed that electrodes that performed poorly chronically had a highly permeable BBB as compared to better performing electrodes that had better wound healing and smaller BBB breach [18]. Their study directly correlated electrode function with the extent of BBB breach and suggested that intracortical electrodes induce a chronic breach of the BBB, which in a feed-forward loop leads to chronic inflammation, neurodegeneration, and electrode failure.

While studies have been performed to relate histological data to electrode function [13, 18, 22, 38–42] and expression of IgG around electrode sites indicative of BBB leakage [39, 40] these studies do not provide an insight into the evolving molecular mechanisms related to the BBB disruption and dysfunction at the electrode tissue interface for the clinically relevant Utah microelectrode arrays (UMEAs). Within the CNS vasculature, the BBB is localized at the interface between the blood and the cerebral tissue and is composed of endothelial cells that are connected by an extensive network of complex tight junction (TJ) and adherens junction (AJ) proteins [43–48], which influence junction organization at the BBB [49]. Any change in this organization as a result of pathology or trauma results in the compromise of endothelial cell reactions at the BBB. Within the BBB, TJ proteins consist

primarily of the occludin, claudin, and zonula occludens family of proteins [29, 47], of which occludin decreases paracellular transport [50] and claudin-5 restricts the movement of large molecules across the BBB [51]. AJs, which consist of the cadherin and catenin family of proteins, are required for the correct organization of TJs [52], involved in signaling crosstalk [53], and affect overall vascular homeostasis [49]. The expression of these proteins is tightly regulated and is closely associated with changes in BBB permeability. Together, these TJ and AJ proteins maintain the integrity of the endothelial cell junction complex and thus, the integrity and permeability of the BBB.

Typically, microwire arrays and silicon probes are inserted slowly into the neural tissue using a micropositioner, whereas the UMEAs are inserted at a high speed using a pneumatic inserter. While the sequelae of electrode implantation injury has been described for microwire arrays [18, 27, 38, 40, 54] and silicon arrays [17, 21, 55], the foreign body response of the high-density UMEAs have been shown to be different from all the above described electrode types that are slowly inserted and create a single penetrating injury [39]. Furthermore, the molecular changes at the gene transcript level that occur during inflammatory response and BBB-disruption caused by the UMEA is not reported. In this study, we seek to understand and characterize the physiological events at acute time-points that occur at the electrode-tissue interface due to BBB-disruption following the fast insertion of the UMEAs. We evaluated the gene regulation at acute time-points of up to 72-hour post implantation in an adult rat model, specifically the inflammatory regulating cytokines and chemokines and genes that regulate the tight junction and adherens junction proteins in the BBB.

2. Methods

2.1 Overview and animal groups

All procedures were approved by the University of Miami Institutional Animal Care and Use Committee (IACUC). Adult, age, and weight (250–300 grams) matched male Sprague Dawley rats were used in this study. A total of $n=50$ animals were used in the study (Table 1). Animals were divided into “implant” and “stab” groups and compared to a group of unoperated controls ($n=10$). “Implant” group animals received the electrode array implant, which remained in the brain tissue until the animal reached its respective time-point. For the “stab” group, which was used as a control injury model for electrode insertion, the electrode array was implanted following the exact surgical procedure as the “implant” group; however, the array was manually withdrawn from the tissue 2 minutes after implantation. Both the implant and stab groups were examined at 6-hr, 24-hr, 48-hr, and 72-hr post-surgery ($n=5$ animals for each time-point for each group) to study the acute effects following electrode array implantation on inflammation and BBB-disruption. At their respective time-points, animals were deeply anesthetized and euthanized using an overdose of ketamine-xylazine cocktail. The head cap was removed and the electrode array was gently extracted from the tissue. A $4\text{mm} \times 4\text{mm} \times 2\text{mm}$ piece of tissue harboring the electrode was measured using a rat brain matrix and weighed, immediately flash-frozen in liquid nitrogen, and stored at -80°C . Age and weight matched unoperated control animals were euthanized in a similar manner, extracting a similar size of the brain tissue.

2.2 Surgical Procedure

A custom-made, non-functional UMEA consisting of 4×4 grid of 1mm long parylene-coated silicon shanks with Pt metallization that were 400µm apart was used. Detailed microelectrode surgical procedures described previously were followed [13, 22]. Animals were anesthetized using isoflurane and deep anesthesia was maintained during the surgical procedure. Briefly, a midline incision was made along the rostral-caudal axis on the rat's skull and the periosteum was scraped to expose the skull. Four stainless steel screws were drilled on to the cortex as supporting screws for the headstage assembly. A craniotomy (approximately 4×4mm) was drilled over the sensorimotor cortex (1mm lateral and 2mm posterior to the bregma). A 25-gauge needle angled at ~45° was used to pierce the dura and the dura was cut using a microscissor. The dura was reflected on the sides to expose the cortex. The UMEA was carefully placed on the cortical surface and a pneumatic inserter (Blackrock Microsystems, Inc, UT) was stereotactically positioned on top of the array. The UMEA was inserted into the cortex where the pneumatic inserter drops a weight inside the wand, which displaces the chuck at the bottom of the wand by 1mm to insert the electrode (Figure 1). After array insertion, the craniotomy was covered by a thin (~25µm) silastic sheet. Dental acrylic was then used to cover the craniotomy and the skull surface. For stab surgeries, similar implant procedures were followed. However, the UMEA was taken out manually 2 minutes after implantation and a similar headstage assembly was used.

2.3 Quantitative Real-Time PCR

Brain tissues were dissected in RNase-free phosphate buffered saline (PBS) before RNA isolation. A protocol was developed and followed for RNA extraction, reverse transcriptase, and cDNA amplification. Tissue samples were weighed and homogenized with 1ml of PureZol (BioRad, CA). The lysate sample was allowed to rest for 5 minutes at room temperature for complete dissociation of nucleoprotein complexes to occur. RNA purity and concentration was determined by the absorbance at 260nm and 280nm using Nano Drop ND-1000 (ThermoFisher Scientific). After extraction, 0.5µg RNA was used for reverse transcriptase using Superscript First-Strand Synthesis System. Collected cDNA was stored at -80°C until used for real-time quantitative PCR (qRT-PCR). A solution consisting of 10 µl of ssOAdvanced universal mix, 3 µl of cDNA, 1 µl of primer, and 6 µl of H₂O was used for each well on the PCR plate. Each gene was run in duplicate wells and GAPDH was used as the housekeeping gene for all samples. All experimental (implant and stab) tissue samples were normalized with respect to control tissue samples from unoperated animals, which served as the baseline values for gene transcription. All control and experimental samples were run in duplicate for each primer tested. The control and experimental sample C_q values were first normalized with respect to the sample GAPDH C_q values, producing C_q ($C_q = C_q(\text{a target gene}) - C_q(\text{a reference gene i.e. GAPDH})$). The C_q value was calculated by normalizing each experimental C_q to its control sample C_q value ($C_q = C_q(\text{a target gene}) - C_q(\text{a control sample})$). Finally, the relative fold changes in mRNA levels of genes with respect to control (unoperated) animals was calculated using the $2^{(-C_q)}$ method [56]. A twofold or higher change in gene expression was considered significant [57]. Table 2 provides a summary of the various transcripts used in this study.

2.4 Statistics

Fold change gene regulation values from each group (“stab” and “implant”) and time-points was tested for normality using the Shapiro-Wilk test before applying analysis of variance (ANOVA). For each gene (all time-points included), we combined groups across time and used 2-way ANOVA to determine whether there was a difference in the “stab” vs the “implant” groups or across time. If there was a significant difference ($p < 0.05$) in either row or column, one-way ANOVA followed by Tukey post hoc comparison test was used to find which groups were significantly different. No significant interaction was found for all genes across time and injury type.

3. Results

3.1 Acute signaling of inflammatory cytokines in UMEA implants

The CNS inflammatory response consists of several cytokines secreted by cells that are involved in cellular signaling after an injury. Cytokines stimulate the growth and differentiation of lymphocytes during activation as well as activate different effector cells to remove the source of inflammation [58]. White blood cells such as lymphocytes, monocytes, phagocytes, or macrophages respond to an injury by releasing cytokines. Cytokines are interesting from a therapeutic standpoint, where they can be used as either agents or targets for molecules that can act as antagonists to inhibit inflammation in a variety of CNS injury and diseases. We used qRT-PCR to determine the relative mRNA expression of specific cytokines that are involved in pro-and anti-inflammatory signaling at multiple acute time-points (6-hr, 24-hr, 48-hr, and 72-hr) after electrode implant surgery. Figure 2 shows the gene regulation for the pro-inflammatory cytokines TNF α , IL6, IL1 α , and IL1 β and the anti-inflammatory cytokines IL1Rn and Bcl2 at the various acute time-points. We found that the expression of these pro-inflammatory cytokines was most upregulated right after electrode implantation at 6-hr (Figure 2A). At all time-points, the mRNA transcript expression levels were upregulated for the cytokines TNF α , IL6, IL1 α (>5-fold relative to unimplanted controls) whereas the cytokines IL1 β , Cxcl1, and Cxcl2 showed expression values greater than 20-fold (Figures 2A and 3A–B) indicating electrode implant-induced injury. IL6 and IL1 β remained elevated at all time points (Figure 2A). TNF α and IL1 α showed transcription values significantly higher at 6-hr compared to the subsequent time points (Figure 2A). The anti-inflammatory cytokines IL1Rn and Bcl2 were monitored at similar time points (Figure 2B). IL1Rn remained upregulated at all time points but displayed significantly higher fold change values at 48-hr (2B). Bcl2 remained unchanged following injury (2B).

3.2 Acute signaling of inflammatory cytokines in stab vs implant injury model

We used a stab-injury model to study if there was a difference in the gene regulation of pro-and anti-inflammatory cytokines between animals where the electrode array was implanted for the duration of study and in stab group where the electrode was taken out after insertion. A comparison of the cytokine expression at various time-points is shown between “stab” and “implant” animal groups in Figure 3. There is a rapid upregulation of IL1 α right after surgery at 6-hr (>10-fold) in both groups after which the IL1 α levels reduced and remained similar for both groups (3C). IL1 β was consistently upregulated at all time-points for both groups (>20 fold) (3D). We also observed greater inter-animal variability in the IL1 β , Cxcl1,

and Cxcl2 expression (suggested by large standard deviation bars) compared to other markers. IL6 marker remained similar (no significant differences) for all time points and between the injury groups (3E). We observed a large increase in TNF α transcript at 6-hr for both groups. TNF α levels then decreased at the subsequent time-points tested. There was also no significant difference between TNF α expression levels between the stab and implant groups (3F). Chemokines from the CXC family, Cxcl1 and Cxcl2 play an important role in early recruitment of neutrophils after injury or during inflammation [58] and were compared for both injury models (3A–B). Cxcl1 was greatly expressed (>30-fold) at all time points with no difference between the stab and implant groups. At 6-hr, the expression levels for both groups were greater than 30-fold and remained elevated for the time-points tested. However, Cxcl1 decreased significantly ($p < 0.0332$) from the 48-hr to 72-hr time-point (3A). Cxcl2 expression levels varied considerably within animal groups but remained highly upregulated at all time-points. Interestingly, we observed that cytokine expression levels remained upregulated across all animals in the stab group. These results are similar to that observed in immunohistochemical expression of astroglial and microglial markers of injury where studies have shown no significant difference in the expression between stab and implant groups [39, 59]. These results also suggest that the initial injury that occurs as a result of implant, in this case a fast insertion of UMEA into the brain tissue, may be the cause of upregulation of pro-inflammatory factors. We speculate that the reason for some stab animals to have slightly higher expression (but not significantly different) of these markers compared to implant group may be due to a second injury that can occur to the tissue during withdrawal of the electrode array.

3.3 Acute signaling of BBB tight and adherens junction markers in UMEA implant injury model

We assessed the structural integrity of the BBB by the gene regulation of the TJ (Figure 4) and AJ (Figure 5) proteins in implanted animals relative to unimplanted animals. We specifically studied the expression of these TJs ((claudin (Cldn5), occludin (Ocln), zonula occludens (Zo-1 and Zo-2)) and AJs (cadherins (Cdh1 and Cdh5)) genes that are very tightly regulated and get downregulated after any injury to the brain tissue, indicative of degradation of these proteins and thus the barrier itself. The BBB is known for its highly regulated protein structure [29]; therefore, minute changes in expression levels relative to control values were considered as significant. Upon initial injury due to implant, claudin-5 and occludin were slightly downregulated relative to unimplanted animals where baseline expression level was 1 (Figure 4). The initial downregulation of these genes regulating the TJ proteins is indicative of the acute damage to the BBB structural integrity as a result of implant injury that increases the BBB-permeability and leads to barrier dysfunction. At the 24-hr time point, all the TJ genes, except for occludin, increased above baseline values after the initial injury, suggesting the BBB trying to restore itself after an injury. However, due to the presence of an electrode array in the brain tissue, which leads to persistent disruption of the BBB, we observed downregulation of all the TJ genes at later time-points except Zo-2 (Figure 4). We also monitored the expression of cadherin family of genes that regulate the AJ proteins. Cadherin-5 (Cdh-5) also known as vascular endothelial (VE)-cadherin, showed an increase in expression values upon injury peaking at 48-hr and significantly decreasing

($p < 0.03$) at 72-hr post-implant (Figure 5). Cadherin-1 (Cdh1), also known as epithelial (E)-cadherin, remained downregulated at all the time-points tested.

3.4 Acute signaling of BBB markers in stab vs implant injury model

We used the stab injury model to study how and if the BBB-disruption was different from the implant injury model (Figure 6). We observed a difference in expression of the vascular endothelial (VE)-cadherin, Cdh5, between the stab and implant groups where the stab animals had significantly ($p < 0.0332$) higher expression at its initial injury at 6-hr (Figure 6B). However, at the following time points, there was no significant difference in Cdh5 expression between the two groups and by 72-hr, Cdh5 levels returned to baseline values for both groups (Figure 6B). TJ proteins, specifically claudin-5 and occludin are responsible for regulating the paracellular transport across the BBB. Claudin-5 (Cldn5) was significantly ($p < 0.0001$) upregulated for the stab group at 6-hr whereas it was downregulated for the implant group suggestive of the degradation of these proteins begin to occur right after injury (Figure 6C). At 48-hr, we observed a significant difference ($p < 0.0002$) between the expression of Cldn5 between the two groups whereas there was no difference in expression levels at 24-hr and 72-hr. We observed a similar trend in the expression of occludin (Ocln) where the stab animals at 6-hr showed significantly higher expression ($p < 0.03$) compared to implant animals (Figure 6D). In stab animals, as the electrode array was withdrawn, barrier restoration process appears to be happening as early as the 6-hour time-point tested as the expression levels for Cldn5, Ocln, and Cdh5 regulating the TJ and AJ are significantly higher than the “implant” group at that time-point. During BBB restoration, increased expression of TJs such as occludin and claudin has been observed to improve homeostatic properties of the BBB [60]. However, this innate reparative mechanism is not enough to repair the barrier and this may have led to similar expression levels between “stab” and “implanted” groups at all later time-points tested. This also suggests that the presence of electrode array in the tissue was leading to a disrupted BBB. The difference in expression levels at these acute time-points illustrate that the degradation of these TJ and AJ proteins that are critical to the maintenance and functioning of the BBB occurs very early after injury. Furthermore, the expression levels become closer to the baseline levels at the longest time-point of 72-hr tested in this study. However, they are still down- and upregulated which suggests the degradation of these barrier forming proteins, ultimately resulting in BBB dysfunction.

3.5 Matrix metalloproteinases (MMPs) regulation in UMEA implants

We also observed changes in the gene regulation of matrix metalloproteinases (MMPs), which are known for their ability to degrade the extracellular matrix constituents [61, 62]. MMPs are a family of proteolytic enzymes that play a critical role in the pathophysiology following BBB-disruption [63–71]. There is a rapid upregulation of MMPs after brain injury; their upregulation is known to worsen BBB-permeability and inflammation as they cause the degradation of TJ and AJ proteins. Specifically, we studied the MMP-2 and MMP-9, which play a major role in the pathophysiology following barrier breakdown (Figure 7). At 24-hr, MMP-2 and MMP-9 were not significantly expressed after which there was a rapid upregulation of MMP-2 and MMP-9 ($p < 0.0332$). In stab animals (Figure 7A), MMP-2 expression was similar to the implant animals at 24-hr and 72-hr. This may be due

to the reason that MMP-2 has also been implicated for wound-healing [72] after injury and without the presence of an electrode array in the tissue, wound healing process may begin to occur early. On the contrary, MMP-9 is known to cleave matrix components and its upregulation degrades TJ and AJ proteins making the barrier more permeable. As expected, MMP-9 in general was higher for the implant group compared to the stab animals for the 48-hr and 72-hr time points (but not significant) (Figure 7B).

4. Discussion

In this study, we showed the molecular responses related to inflammation and BBB-disruption associated with the implant of a 4×4 UMEA in the rat cortex. Further, we hypothesized that fast insertion of this electrode array type primarily contributes towards the acute inflammatory response and BBB-disruption. For this, we compared the animals implanted with UMEA for the duration of the study with animals that had only stab wounds. To study inflammation, we evaluated the expression of the genes involved in pro-inflammatory response at multiple acute time-points. To study the BBB-breakdown, we studied the genes that regulate the tight junction (TJ) and adherens junction (AJ) proteins that form the BBB. We chose acute time points to show the evolution of genes involved in the inflammatory response and degradation of BBB right after injury. Our results indicate significant upregulation (>2 fold) in all pro-inflammatory regulating cytokines and chemokines tested in this study occurring as early as 6-hr post implantation. We also found degradation of TJ and AJ proteins that occur right after implant due to BBB-disruption. From a neuroprosthetics perspective, our results are indicative of the need for addressing the acute inflammation and BBB-disruption in cortical implants that may have implications toward managing chronic electrode-tissue response.

4.1 Tight and adherens junctions of the BBB after electrode-implant injury

The BBB is composed of endothelial cells that are connected by an extensive network of complex TJ and AJ proteins [43–48], which influence junction organization at the BBB [49] and regulate the barrier permeability [46, 47, 50, 51]. These TJs and AJs remain holstered into the vascular endothelial cells by supporting junctional proteins from the occludin family [29, 47]. Together, the TJ and AJ proteins are critical to maintaining the structural integrity and permeability of the BBB. The TJs of the BBB are the most apical surface of the junctional complex, which include TJs, AJs, and junction adhesion molecules (JAMs). The TJs are extremely critical for paracellular permeability across the BBB [46, 50, 51] and consist primarily of occludin, claudin, zonula occludens, and JAMs [29, 47] family of proteins. Of these, occludin is a regulatory protein which increases transendothelial resistance and decreases paracellular transport [50] [73] whereas claudin restricts the movement of large molecules across the barrier and forms the primary seal of the TJ [51] [74]. Among the claudin family of proteins, claudin-5 has been shown to be expressed primarily in the brain endothelial cells of the blood vessels and capillaries [75] and is a key mediator of BBB permeability [33, 76–78]. An initial downregulation of Occludin and Claudin-5 (Figure 4) indicates the initial degradation of these structural proteins of the BBB due to implant injury causing a significantly leaky-BBB. This could temporarily make the barrier more permeable allowing an increase in foreign molecules and pathogens to the brain

parenchyma resulting in BBB-dysfunction. Any alterations in the expression of TJs, such as those observed in our study with electrode implants, result in an increased paracellular permeability [79] and BBB-dysfunction, also commonly observed with a loss of Cldn5, Occludin and Zo-1 in several brain pathologies and neurodegenerative disorders [80–83]. In addition to the transmembrane proteins, the TJs are also made up of several cytoplasmic accessory proteins that contribute towards the structural integrity of the barrier. The zonula occludens family of proteins, Zo-1, Zo-2, and Zo-3 are scaffolding proteins of the membrane-associated guanylate kinase-like (MAGUK) family that do not access the intercellular domains of vascular endothelial cells as do claudin-5 and occludin [84]. They instead directly and indirectly holster Cldn5 and Occludin to actin of the cytoskeleton within endothelial cells of the BBB [85]. Zo-1 acts as an intracellular regulatory protein, while Zo-2 mimics Zo-1 functions [86]. A suppression of Zo-1 and Zo-2, such as that observed early after injury (at 6-hr, Figure 4) has been suggested to alter their engagement with TJs and produce a more permeable barrier [87, 88].

The AJs lie closer to the basal lamina of the BBB and assist in cell-to-cell adhesion [52]. They consist of the cadherin and catenin family of proteins that are required for the correct organization of TJs [52], involved in signaling crosstalk [53], and affect overall vascular homeostasis [49]. Cdh1 downregulation across all time points (Figure 5) could further make the BBB more permeable due to the disruption of Cdh-1 adhesion. Vascular cadherin (VE-cadherin or Cdh5) is needed for endothelial integrity in quiescent as well as activated state such as during inflammation [89]. Under quiescent condition, such as that of an adult endothelium, junctional proteins restrain cell migration and proliferation, which otherwise is seen to be enhanced during angiogenesis [52, 90, 91]. An increase in Cdh5 after implant injury suggests angiogenesis where new blood vessels are being formed right after implant (Figure 5) as implant induced injury ruptures and damages the blood vessels. However, the Cdh5 was observed to be return to baseline at the 72-hr time-point consistent with other junctional proteins suggestive of persistent damage by the electrodes. Similarly, the genes regulating the TJs (Cldn5, Occludin, Zo-1 and Zo-2) and AJs (Cdh1 and Cdh5) upregulated after the initial injury suggesting they are trying to restore its scaffolding functions (Figure 4). However, this upregulation was uniformly lost across all the genes at the longest time-point of 72-hr evaluated in this study where the presence of indwelling electrodes in the brain tissue causes persistent BBB-disruption.

4.2 Effects of inflammatory cytokines on the BBB

The BBB, which consists of the brain microvascular endothelial cells (BMVEC) together with the extracellular matrix, astrocytes, pericytes, microglia, and neurons forms the neurovascular unit that is critical for maintaining homeostasis and functioning of the central nervous system [86, 92]. During an injury, neurological disorders or other CNS pathologies, BBB-dysfunction has been associated with significantly elevated levels of pro-inflammatory cytokines such as the TNF α , IL1 β , and IL6 that are released from the injury sites [93–97]. There are several mechanisms that have been proposed to explain the opening of the BBB related to pro-inflammatory cytokines. *In vitro* [98–101] and *in vivo* [102, 103] studies have shown the permeabilizing capability of TNF α on BMVECs as well the effect of TNF α and IL6 on BMVECs to cause decreased mRNA and protein expression of TJ proteins Occludin,

Cldn5, and Zo-1 [104–106] and AJ proteins (Cdh1, Cdh5) [107]. For instance, bradykinin, which is a mediator of inflammation can activate nuclear factor- κ B (NF- κ B) in astrocytes to stimulate production and release of IL-6 [108] which then directly acts on the endothelium. This also leads to the release of TNF α and IL1 β by the astrocytes that act on the endothelium [109], which all have the ability to make the BBB more permeable. Additionally, TNF α released by microglia can act on endothelial endothelin 1 and indirectly lead to the release of IL1 β from the astrocytes [110]. Further, the microglia releases reactive oxygen species (ROS), which are directly capable of acting on brain endothelial cells causing TJ [111–113] and AJ [114–116] modification and MMP activation, all of which further alters BBB permeability to cause BBB-dysfunction [111, 117–125]. In this study, we observed marked upregulation in TNF α (~10-fold), IL1 β (~30-fold), and IL6 (~15-fold) (Figure 3A) at the 6-hr time-point post-implant, which was correlated with a downregulation of the TJ proteins claudin and occludin as well as Cdh1 (Figures 4 and 5). This is consistent with previous studies which have shown the effect of these inflammatory cytokines on the BBB-junction complexes ultimately leading to BBB-disruption and dysfunction. The anti-inflammatory cytokine IL1Rn upregulation positively correlated with the expression levels of IL1 β (Figure 2B). IL1Rn inhibits the signaling process of IL1 β [126] whereas Bcl2 positive correlation with TNF α and IL1 α suggests it helps inhibit some of the repercussions of having an excess of mRNA expression levels of TNF α and IL1 α .

4.3 Matrix Metalloproteinases (MMPs)-mediated BBB-disruption

BBB breach have been associated with a rapid upregulation of the MMPs after brain injury and play a critical role in the pathophysiology following an injury [63–68, 70, 71, 127]. MMPs, known for their ability to cleave extracellular matrix constituents, are involved in the degradation of TJ proteins and basal lamina proteins such as laminin and fibronectin, which results in further degrading and opening of the BBB [61, 62]. Expression of MMP-2, and –9 lead to the degradation of occludin and claudin-5, which are TJ proteins critical to BBB leakage and integrity [128–134]. The protein expression levels of these TJ proteins decrease right after brain injury indicating increased barrier permeability and BBB dysfunction. The time course of MMP upregulation, specifically MMP-9, correlates well with maximal BBB disruption [135, 136]. MMPs also act as mediators of leukocyte infiltration that amplifies secondary inflammatory cascade [137–139], directly modulate the formation of glial scar [140–142], and degrade myelin basic protein, which is a major component of the myelin sheath [143–146]. On the contrary, MMPs also play a role in wound healing and angiogenesis and are modulators of late recovery processes [71]. In this study, we observed MMP-9 to be rapidly upregulating in the first 72-hrs with peak values (~>20-fold) at 48-hr and 72-hr (Figure 7). MMP-2 and MMP-9 upregulation was not considered significant at the 6-hr and 24-hr period, however they increased rapidly after that (Figure 7). This suggests that initial breakdown of the BBB results in the degradation of the TJ and AJ proteins (Figures 5 and 6) where the MMPs likely do not play a role towards degrading these proteins. After initial downregulation of the TJ proteins, they tended to increase in an attempt to repair and close the BBB (after 6-hr time-point) at which point MMPs do not seem to play a role in BBB-disruption. However, by 48-hr period, MMP-9 was significantly upregulated, which was also correlated with all TJ and AJ genes becoming downregulated again suggesting the possible role of MMP-9 in causing secondary damage and degrading

the TJ proteins to cause further BBB-dysfunction. During this period, MMP-2 that has been implicated in wound healing [72] was upregulated at the 48-hr after which it reduced at the longest time-point of 72-hr evaluated in this study. Similar to previous inferences made with regard to genes regulating TJs and AJs, comparing these mRNA expression levels in implanted animals with stab group indicates that the presence of indwelling electrodes resulted in persistent upregulation of MMP-9, which is known to cause further BBB-disruption.

5. Conclusions

In summary, we showed the molecular responses and signaling changes in the cytokines involved in neuroinflammation and genes that regulate the tight and adherens junction proteins of the BBB, which are critical for maintaining the barrier functions of the BBB following UMEA implants in the rat cortex at acute time-points. The results also showed there was no difference in the regulation of genes, tested in this study, involved in inflammation between stab animals and animals with implanted arrays suggesting the effect of insertion on acute inflammation. An understanding of these molecular mechanisms at the electrode-tissue interface is important to the design of any intervention at the interface to minimize acute BBB-disruption.

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Figure 1. Surgical implantation of UMEA in rat cortex

(A) Craniotomy and the location of anchoring screws that was used in the implants. (B) The dura was resected and the UMEA was inserted into the brain tissue using a pneumatic inserter.

Figure 2. Gene regulation in implant group

A) Pro-inflammatory cytokine mRNA regulation in UMEA implanted animals relative to unoperated controls at 6-hr, 24-hr, 48-hr, and 72-hr post-surgery. Mean $\pm$ SD and significant difference if found is shown for each group (* $p < 0.0332$; ** $p < 0.0021$; *** $p < 0.0002$). B) Anti-inflammatory cytokine mRNA regulation for IL1Rn and Bcl2 in UMEA implanted animals relative to unoperated controls at 6-hr, 24-hr, 48-hr, and 72-hr post-surgery. Mean $\pm$ SD and significant difference if found is shown for each group (** $p < 0.0021$; *** $p < 0.0002$).

Figure 3. Comparison of gene regulation between implant and stab groups

Pro-inflammatory cytokine and chemokine mRNA regulation in UMEA implanted animals vs stab animals are shown. Fold change values shown are relative to unoperated control animals at 6-hr, 24-hr, 48-hr, and 72-hr time-points. Mean $\pm$ SD is shown for each group. Significant difference across implant (* $p < 0.0332$; ** $p < 0.0021$; *** $p < 0.0002$) and stab groups ($\dagger$ $p < 0.0332$) are shown. No significant difference in any transcript levels was found between the injury types (stab and implant groups).

Figure 4. Gene regulation of TJ proteins in implant group

mRNA expression of the genes regulating the tight junction (TJ) proteins that form the BBB. Fold change values shown are for the implant group animals relative to unoperated control animals at 6-hr, 24-hr, 48-hr, and 72-hr for UMEA implanted animals. Mean $\pm$ SD and significant difference if found is shown for each group (** $p < 0.0021$).

Figure 5. Gene regulation of AJ proteins in implant group

mRNA expression of the genes regulating the adherens junction (AJ) proteins that form the BBB. Fold change values shown are for the implant group animals relative to unoperated control animals at 6-hr, 24-hr, 48-hr, and 72-hr for UMEA implanted animals. Mean $\pm$ SD and significant difference if found is shown for each group (* $p < 0.0332$).

Figure 6. Comparison of gene regulation of TJ and AJ between implant and stab groups
mRNA regulation of tight (TJ) and adherens (AJ) junction proteins in UMEA implanted animals vs stab animals are shown. Fold change values shown are relative to unoperated control animals at 6-hr, 24-hr, 48-hr, and 72-hr time-points. Mean $\pm$ SD is shown for each group. Significant difference across time in implant (* $p < 0.0332$) and stab groups ($\dagger$ $p < 0.0332$; $\dagger\dagger$ $p < 0.0021$; $\dagger\dagger\dagger$ $p < 0.0002$) and between stab and implant-induced injury are shown (# $p < 0.0332$; ### $p < 0.0002$; #### $p < 0.0001$).

Figure 7. Comparison of MMP regulation between implant and stab groups
mRNA expression of the genes regulating matrix metalloproteinases, MMP-2 and MMP-9 in UMEA implanted animals vs stab animals. Fold change values shown are relative to unoperated control animals at 24-hr, 48-hr, and 72-hr for UMEA implanted animals. Mean $\pm$ SD is shown for each group. Significant difference across time in implant group (* $p < 0.0332$) and stab group († $p < 0.0332$; †† $p < 0.0021$; ††† $p < 0.0002$) and between stab and implant-induced injury are shown (# $p < 0.0332$).

Table 1

Summary of experimental animal groups

Animal Groups	6-hr	24-hr	48-hr	72-hr
Stab	5	5	5	5
Implant	5	5	5	5
Unimplanted controls	10			
Total				50

Table 2

Summary of transcripts used.

	Transcripts used
Neuroinflammation	TNF α , IL6, IL1 β , IL1 α , IL1Rn, Bcl2, Cxcl2, Cxcl1, MMP2, MMP9
Blood-brain barrier (BBB)	Ocln, Cldn5, Cdh1, Cdh5, Zo-1, Zo-2
Housekeeping gene	GAPDH