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Understanding the Inflammatory Tissue Reaction to Brain Implants To Improve Neurochemical Sensing Performance

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Abstract

Neurochemical sensing probes are a valuable diagnostic and therapeutic tool that can be used to study neurodegenerative diseases involving deficiencies in neurotransmitter signaling. However, implantation of these biosensors can elicit a harmful tissue response that alters the neurochemical environment within the brain. Transmission of chemical messengers via neurons is impeded by a barrier-forming glial scar that occurs within weeks after insertion followed by progressive neurodegeneration, attenuating signal sensitivity. Emerging research reveals that non-neuronal cells also influence the neurochemical milieu following injury both directly and indirectly. The reactivity of both microglia and astrocytes to inserted probes have been extensively studied in the past yet there remains other glial subtypes in the brain, such as oligodendrocytes and their precursors, the myelin structures they form, as well as vascular-bound pericytes, that have the potential to contribute significantly to the inflammation due to their responsibility to maintain tissue homeostasis. A brief overview of how tissue injury alters the neurochemical makeup followed by alternative potential targets of investigation and novel strategies to enhance the chemical sensing abilities of implantable probes will be discussed.

Graphical Abstract

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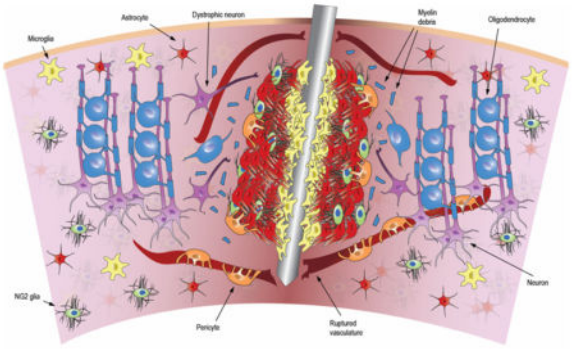
Notes

The authors declare no competing financial interest.



Keywords

Neurotransmitter signaling; biosensors; foreign body response; tissue injury; microelectrode; homeostasis

Abnormalities involving neurotransmitter exchange in the brain is the primary cause underlying neurological disorders such as Parkinson's disease, epilepsy, and depression. Investigators use implantable neurochemical sensors in order to monitor fluctuations of these signaling molecules in healthy and injured states as well as modulate chemical transmission within neural circuits to alleviate symptoms of neurodegenerative disease. However, the present state of implantable biosensors is still burdened by a reactive tissue response that dampens signal detection and may potentially alter neuron–neuron and neuron–glia activity. Device insertion injury can impact nearby cell function as well as neurochemical release and clearance profiles in the tissue, ultimately decreasing signal detection over time. Growing literature on implantable neural sensors focuses on elucidating the mechanisms of blood-brain barrier (BBB) disruption-induced activation of microglia and astrocytes which encapsulate the device and secrete degenerative proinflammatory factors that compromise neuronal viability. While microglia, astrocytes, and BBB cells are important mediators of neuronal function, there are a variety of other cell types within the parenchyma that share important physiological relationships with these cell types as well as each other. In order to develop a comprehensive understanding of the tissue reaction to inserted devices, other potential participants in the injury response must be considered. Expanding existing basic science knowledge on the inflammatory events leading up to device failure can improve the rate and success at which strategies are developed to improve the device–tissue integration of implantable sensors and enhance and sustain their chemical sensing capabilities.

ALTERED NEUROCHEMICAL SIGNALING DUE TO DEVICE IMPLANTATION INJURY

A detailed review on the biochemical pathways that result from device insertion has been previously published.[1] Upon insertion, glia cells become activated and switch from a ramified to transitional morphology, ceasing normal physiological activity in order to mediate the inflammatory response. The hydrophobic nature of most implantable probes encourages deposition of inflammatory plasma proteins onto the surface of the device due to

BBB disruption, which triggers the reactivity of microglia and astrocytes. Within the first 30 min, microglia extend processes and ensheath the device with lamellipodia, which begin to form a physical barrier for devices inhibiting the exchange of ions and large molecules with the chemical sensing surface. Astrocytes, which maintain the responsibility of regulating neurotransmitter activity in the extracellular environment through synapsing onto neuron–neuron connections, also respond by becoming hypertrophic and increase in density around the implant. Proinflammatory cytokines (interleukin 1-beta IL-1β, tumor necrosis factor alpha TNF-α), chemokines (monocyte chemotactic protein-1 MCP-1), and reactive oxygen and nitrogen species (ROS, RNS) produced from activated microglia introduces additional stress on neurons and other surrounding cells, leading to neuronal apoptotic cell death. Mechanical strain imparted on glia and neurons due to the stiff mechanical signature of most inserted devices can also contribute to cell loss leading to attenuation in signal sensitivity. Lastly, plasma proteins, which spill into the parenchyma from ruptured BBB during insertion, act on glial cells. In turn, glial cells upregulate and release proinflammatory factors that negatively regulate endothelial cells lining the vascular membrane.[1] For example, albumin, which is a major component of blood serum, acts on tumor growth factor beta (TGF-β) receptors in astrocytes leading to the weakening of endothelial cell–cell interactions and promoting BBB permeability. Injury to the BBB results in increased inflammation, increased mechanical strain from the influx of plasma proteins, decreases oxygen/nutrient delivery, and increased accumulation of neurotoxic waste products. These negatively impact the ability of neurons to drive action potentials, release neurotransmitters, and clear accumulated signaling molecules.

Interestingly, glial cells and other non-neuronal cells have receptors and transporters for many neurotransmitters that alter their functions as well as other nearby cell types.[1] This, in combination with the activation of multiple inflammatory pathways that exacerbate inflammation, eventually progress into a neurodegenerative response. The influence of these injuries on neurotransmitter release and parenchymal accumulation has been difficult to study with traditional neurochemical sensors. However, the emergence of neurotransmitter sensing fluorescent reporters (SnFRs) provides new avenues to study this phenomena.

Investigations involving stab wound studies demonstrate that tissue can heal after acute inflammation without the onset of glial scarring or neurodegeneration suggesting that the prevalence of a chronic inflammatory response is due to the physical presence of the implanted probe. Tight junctions between microglia and astrocytes during scar tissue formation act as a biochemical and mechanical barrier to growth factors essential for the survival or regeneration of neurons and prevent the diffusion of ions, solutes, and chemical signaling molecules secreted from surrounding neurons. Thus, preventing the diffusion of ions and charged solutes as well as neurochemical signals between neurons and the device surface impairs chemical sensing performance. Similarly, if the implanted probe impedes the repair of the BBB, delays in BBB repair lead to a prolonged mismatch in metabolic supply and demand. As a result, the cells and molecular machinery required for neurotransmitter release and uptake become impaired.

POTENTIAL UNINVESTIGATED CNS EFFECTORS OF NEUROCHEMICAL DYNAMICS FROM SENSOR IMPLANTATION INJURY

The performance of biosensors in the brain depends primarily on the state of neuronal health in the tissue. While neurons are the source of neurotransmitters, their overall health and activity can be greatly influenced by non-neuronal cells including microglia, astrocytes, and the BBB. The pathology of microglia, astrocytes, and neurons are well characterized in the context of ionic current sensing electrophysiological electrodes; however, how microglia and astrocytes impact neurotransmitter uptake and neurotransmitter release have yet to be extensively studied. This is of particular importance since most non-neuronal glia and neurovascular cells express receptors and transporters for neurotransmitters.[1] In addition, other potential CNS contributors to the dynamic neurochemical activity profiles in the brain such as oligodendrocyte lineage cells and pericytes are relatively understudied. Similar to other non-neuronal cells, oligodendrocyte lineage cells express several neurotransmitter receptors and transporters that influence their overall activity, and in turn, neurotransmitter detection (Figure 1).[1]

Oligodendrocyte precursors, known to differentiate into myelinating oligodendrocytes during development or in instances of demyelination, exist as a separate glial entity (also known as NG2 glia, which express the chondroitin sulfate proteoglycan NG2) and reside in the adult brain until death. NG2 glia have been implicated as participatory elements in the formation of the glial scar due to their intrinsic ability to migrate, proliferate, and differentiate into astrocytes and their characteristic release of axon-growth inhibitory molecules around an injury lesion. Indeed, NG2 glia react to inserted probes by extending processes and migrating toward the device, similar to microglia, however, on the scale of hours post-insertion as opposed to minutes (Figure 2A,B). Unique to glial cells is the ability for NG2 glia to form synapses with neurons and receive synaptic input. NG2 glia express both GABAergic receptors and glutamatergic receptors, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA). Activation of AMPA receptors by glutamatergic signaling modulates the proliferation and differentiation of NG2 glia.[2] The presence of these channels allow NG2 glia to sense and modulate neuronal activity. The selective ablation of NG2 glia results in reduced glutamatergic transmission and decreased neuronal viability in pyramidal neurons.[2] This perturbation in glutamatergic signaling due to a loss of NG2 glia can trigger depressive-like behavior. NG2 glia also express dopaminergic and serotonergic receptors but their functional significance is currently unknown. While altered glutamate signaling and NG2 glia abnormalities are observed in neurodegenerative diseases such as multiple sclerosis (MS) and Alzheimer's disease (AD), the functional state of myelin and oligodendrocytes most directly dictate neuronal viability.[2]

Oligodendrocytes, while expressed in higher relative density in white matter, are also present in the cortex and play several important roles. This cell type is responsible for the extension of myelin membrane around axon fibers, assisting in neurotransmission and synaptic signaling via the propagation of action potentials. They are critical for neuronal survival through the secretion of essential neurotrophic factors including brain-derived neurotrophic

factor (BDNF), glial-derived neurotrophic factor (GDNF), and insulin-like growth factor 1 (IGF-1) as well as the supply of glucose from the extracellular space for ATP production.[3] Likewise, the expression of glutamate transporters (GLUT1 and GLAST) in myelin are important in maintaining glutamate homeostasis.[1] However, the constant production and maintenance of myelin around axons exerts a strong metabolic dependence on oligodendrocytes, making them particularly vulnerable to incidences of ischemia and hypoxia which are defining features of stroke. Oligodendrocytes, as well as their precursors, store limited amounts of the antioxidant glutathione making them susceptible to oxidative stresses involving increased ROS production. Damage to oligodendrocytes is also mediated by glutamate oversignaling via NMDA receptors in myelin, resulting in excitotoxicity due to the rapid increase in intracellular calcium.[2] Compromising oligodendrocyte viability during device insertion could leave neurons demyelinated and without trophic support. In incidences of demyelination, NG2 glia will differentiate into myelinating oligodendrocytes. However, if NG2 glia are preferentially differentiated into astrocytes after injury and are participating in the formation of a glia scar, then they will be unable to supplement the loss of oligodendrocyte cells and neurons will remain demyelinated until proinflammatory forces overwhelm the cell, leading to apoptosis and a reduction in neurochemical signaling.

Lastly, pericytes reside along the vascular membrane of the blood-brain barrier surrounding endothelial cells. Identified by their expression of PDGF-β receptors as well as the NG2 proteoglycan, pericytes are responsible for regulating blood flow, maintenance of the BBB, as well as removal of toxic byproducts produced from cellular metabolism. Neurotransmitters dictate blood flow by altering the contractility and dilation of the vasculature through pericytes, activating downstream messengers. Glutamate, GABA, dopamine, and adenosine are vasodilatory while norepinephrine is vaso-constrictory.[4] Pericytes help clear tissue debris, foreign pathogens, and amyloid-β toxins present in AD.[4] They are also involved in endothelial cell-mediated recruitment and adhesion of peripheral leukocytes, most common around areas lacking pericytes.[4] Their viability is dependent on PDGF-β secretion from endothelial cells and the inability for pericytes to perform normal physiological functions could compromise the integrity of the blood-brain barrier as well as clearance of toxic waste. In incidences of stroke or spinal cord injury, pericytes are known to detach from vascular membranes and migrate toward the site of injury where the BBB is compromised, possibly in an attempt to initiate angiogenesis and neovascularization. Similar expression patterns have been observed around chronically implanted intracortical sensors.[1] Pericyte morphology changes within 72 h postinsertion with signs of angiogenesis occurring near the device shank (Figure 2C). Also, pericytes are capable of phagocytosis owing to their ability to mediate waste clearance from the brain. Observed by the autofluorescence emitted from the oxidative breakdown of waste products, pericytes display phagocytotic behavior around inserted probes (Figure 2D). Different from microglia and astrocytes, pericytes appear to be important for remodeling tissue after injury by reestablishing the BBB, the first step toward closing off the parenchyma from peripheral inflammatory cells and pathogens and returning to a normal neurochemical environment.

NOVEL APPROACHES TO ATTENUATE INFLAMMATORY TISSUE RESPONSE TO NEUROCHEMICAL SENSORS

Intervention strategies that maintain the integrity of both the probe and host tissue have been investigated to sustain long-term stability and signal sensitivity of neural interfaces and neurochemical sensors. Development of biocompatible coatings, bioactive surfaces, drug-releasing polymers, microfluidic drug-releasing devices, or even smaller, softer, more flexible substrates attenuates the initial inflammatory events initiated upon probe insertion in an attempt to reduce glial scarring and neuronal loss. The simplest device modification involves developing neurochemical sensors that are less hydrophobic via functionalization of hydrophilic or zwitterionic polymer coatings to prevent biofouling, which is the absorption of proinflammatory proteins responsible for the recruitment of activated glial cells onto the surface of the probe. These coatings prevent hydrophobic domains of plasma proteins from adhering to the surface, while deterring hydrophilic protein attachment through the formation of a water layer at the device–tissue interface.

Functionalizing the device with bioactive compounds such as cell surface proteins, proinflammatory receptor antagonists, or ROS scavengers have the potential to modulate cellular behavior. For example, the cell adhesion molecule L1 has shown promise as a biomimetic surface coating to attenuate the microglial ensheathment of implanted probes.[5] Two-photon in vivo imaging has revealed significant reduction in microglia surface coverage of L1-coated probes compared to controls as well as reduced tissue radius (from 130 to 103.5 μm) of microglial activation (Figure 3A,B). L1 has the dual function of promoting neuronal survival while deterring the recruitment and attachment of microglial cells. Preventing the formation of a physical glial barrier, reducing the amount of activated glia pathology, and improving the survivability of neuronal networks has the potential to improve neurochemical signaling at the device-tissue interface. Other cell surface proteins, extracellular matrix proteins, and enzymes are currently being explored to reduce inflammation, promote neuronal survival, and minimize negative alterations on neural activity.

Additionally, pharmacological interventions through the secretion or administration of anti-inflammatory and antioxidative substances can reduce harmful cellular reactions. Previously, retrodialysis release of dexamethasone, an anti-inflammatory corticosteroid, has shown to mitigate the microglia reaction to inserted probes. When compared to artificial cerebrospinal fluid (aCSF), dexamethasone retrodialysis from probes resulted in distinct differences in microglial activation and morphology up to 6 h after implantation observed through live in vivo imaging (Figure 3C,D), minimizing the radius of activation from 177.1 μm with the aCSF control to 93.0 μm with dexamethasone treatment.[6] However, long-term side-effects and variability of anti-inflammatory corticosteroid use on brain tissue adjacent to inserted probes have yet to be determined.

Neural activity and neurotransmitter dynamics can be greatly influenced by the activity of supporting glial cells and the neurovasculature. Growing evidence in the literature points to fact that these non-neuronal cell types play a major role on the activity of neurons, the release of neurotransmitters, and the clearance of the secreted neurotransmitters. A better

systems level understanding is likely to help identify novel probe designs and new intervention strategies as well as the time course of those interventions to improve the information content of sensor readout and the longevity of these neurochemical sensors.

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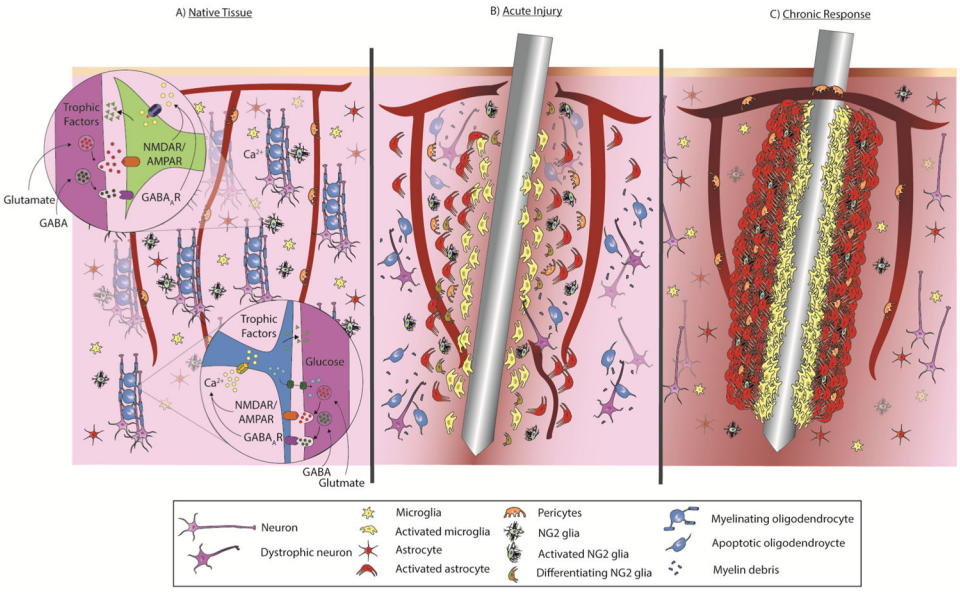

Figure 1.

Visualization of foreign body response to implantable devices in the brain. (A) Cellular components in healthy brain tissue. Axons signal both oligodendrocytes and NG2 glia for neurotrophic support and neuronal modulation via glutamatergic and GABAergic neurotransmitter release. (B) Acute injury after device insertion. Disrupted blood vessels leak inflammatory factors into the parenchyma. Microglia, astrocytes, and NG2 glia become activated and migrate toward the surface of the device. NG2 glia differentiate into scar-forming astrocytes around the implant. Pericytes detach from surrounding vasculature and migrate toward the site of injury to repair broken vasculature. Oligodendrocytes and neurons suffer injury due to oxidative stress and exposure to proinflammatory factors. Axon-growth inhibitory myelin debris is deposited around the lesion site as a result of insertion trauma. (C) Chronic immune response to implanted devices. Glial cells form a chemical and mechanical barrier around the device, preventing the transmission of ions, charged solutes, and neurochemical signals. Angiogenesis (formation of new blood vessels) is mediated by pericytes around the device. Axons remain demyelinated due to oligodendrocyte cell death and reduced NG2 differentiation.

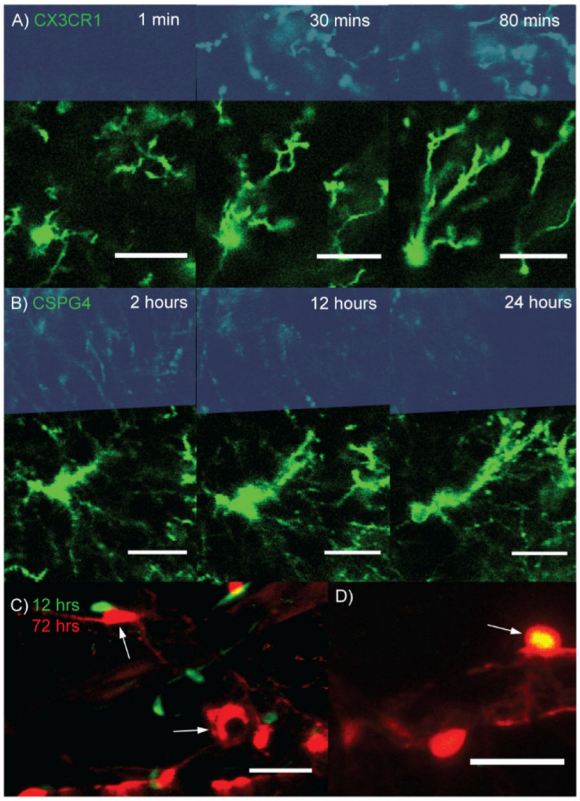

Figure 2.

Acute tissue reaction of oligodendrocyte precursors and pericytes to inserted electrodes. (A) Microglia cells expressing GFP under the CX3cr1 promoter extend their processes immediately after electrode insertion and establish contact with the probe surface (shaded blue) within the first 30 min. (B) Oligodendrocyte precursors expressing GFP under the *Cspg4* promoter extend processes around 12 h postinsertion until 24–48 h postinsertion before cell body migration begins to occur. (C) NG2-expressing pericytes show changes in cell morphology and signs of formation of new blood vessels (white arrows) 72 h following electrode insertion. (D) NG2-expressing pericytes displaying lipofuscin autofluorescence after oxidative breakdown of waste products following electrode insertion. Scale bars = 25 μm.

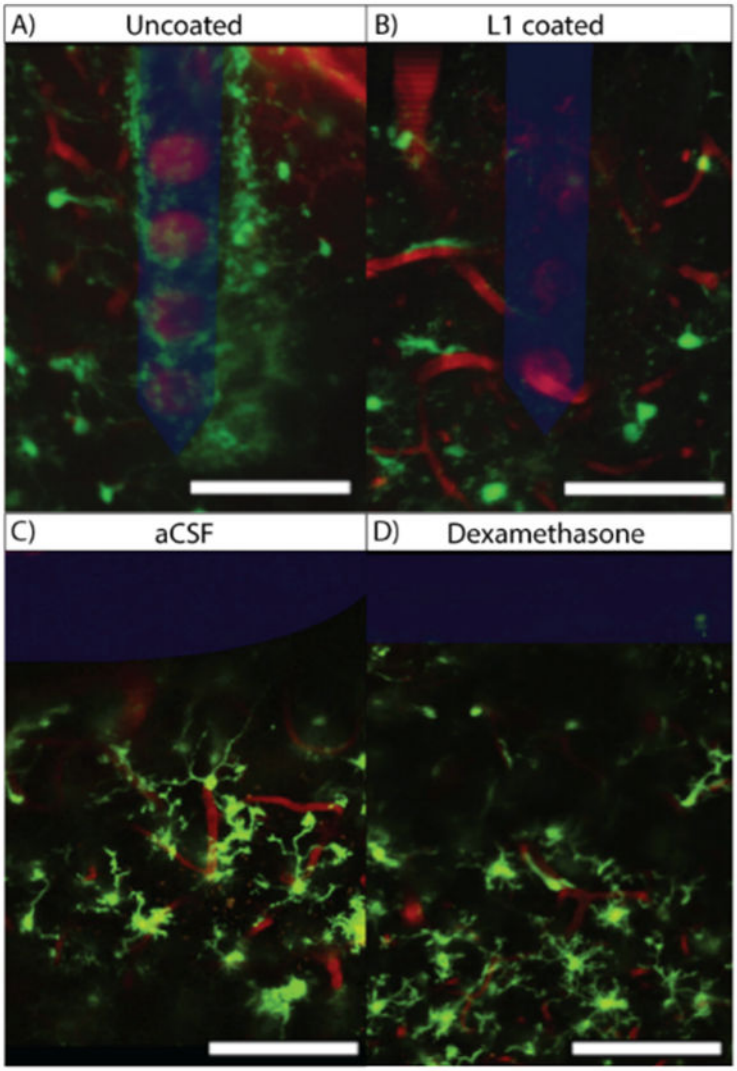

Figure 3.

Surface functionalization or release of anti-inflammatory drug attenuates microglia response to implanted devices. Microglia cells (green) in *CX3cr1* mice encapsulate uncoated electrodes (A) while remaining in ramified or nontransitional states around L1-coated electrodes (B). Adapted from ref 5. Copyright 2017, with permission from Elsevier. Likewise, microglia cells show activated morphology around implants that secrete artificial cerebral spinal fluid (C) compared to electrodes that secrete anti-inflammatory dexamethasone (D). Blood vessels are labeled with SR101 (red) and the electrode surface is outlined in blue. Adapted from ref 6. Copyright 2017, with permission from Elsevier. Scale bars = 100 μm.