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Effects of caspase-1 knockout on chronic neural recording quality and longevity: Insight into cellular and molecular mechanisms of the reactive tissue response

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Abstract

Chronic implantation of microelectrodes into the cortex has been shown to lead to inflammatory gliosis and neuronal loss in the microenvironment immediately surrounding the probe, a hypothesized cause of neural recording failure. Caspase-1 (aka Interleukin 1 β converting enzyme) is known to play a key role in both inflammation and programmed cell death, particularly in stroke and neurodegenerative diseases. Caspase-1 knockout (KO) mice are resistant to apoptosis and these mice have preserved neurologic function by reducing ischemia-induced brain injury in stroke models. Local ischemic injury can occur following neural probe insertion and thus in this study we investigated the hypothesis that caspase-1 KO mice would have less ischemic injury surrounding the neural probe. In this study, caspase-1 KO mice were implanted with chronic single shank 3mm Michigan probes into V1m cortex. Electrophysiology recording showed significantly improved single-unit recording performance (yield and signal to noise ratio) of caspase-1 KO mice compared to wild type C57B6 (WT) mice over the course of up to 6 months for the majority of the depth. The higher yield is supported by the improved neuronal survival in the caspase-1 KO mice. Impedance fluctuates over time but appears to be steadier in the caspase-1 KO especially at longer time points, suggesting milder glia scarring. These findings show that caspase-1 is a promising target for pharmacologic interventions.

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Keywords

Caspase-1; Interleukin-1 β ; Interleukin-1 β converting enzyme; intracortical electrodes; microelectrodes; neural interface; reactive tissue response; chronic electrophysiological recording; mechanical tissue strain; pericyte; oligodendrocytes; Blood-Brain Barrier; BBB; inflammation; impedance; Foreign Body Response

2. Introduction

Penetrating cortical neural microelectrodes are a critical frontend component of brain-computer interfaces [1, 2]. The ability to monitor direct neuronal output with high stability and sensitivity would yield powerful neuroscience tools for understanding behavior, memory, plasticity, connectivity, and neural circuitry. While long-term neural implants for spike recordings have demonstrated feasibility [3–5], large variability of implant performance and poor longitudinal reliability has been a major challenge limiting the adoption of this technology [6–8]. This *variability* and *unreliability* is understood to be the result of complex multimodal failure mechanisms [9]. These include, but are not limited to: material failure such as corrosion, insulation failure, material degradation, electrical lead breakage, electrode delamination and biological responses including biofouling, neural degeneration, and inflammatory gliosis [10]. The present study is focused on dissecting the molecular pathways behind the biological responses that are related to chronic neural recording performance.

The *variability* in intracortical hemorrhaging resulting from microelectrode insertion was first demonstrated under *in vivo* two-photon imaging [11]. It was shown that penetrating a single large intracortical blood vessel resulted in significantly larger BBB bleeding areas compared with penetrating through many small capillaries. This study also revealed the unpredictability of disrupting or avoiding these large intracortical BBB vessels if only the surface vasculatures are avoided during insertion. More recently, it has been shown that implanting ultrasmall electrodes closer to major penetrating blood vessels leads to increased astrocytic GFAP activity [10, 12].

The disruption of BBB leads to the deposition of plasma proteins foreign to the CNS including albumin, globulins, fibrin/fibrinogen, thrombin, plasmin, complement, red blood cells (hemosiderin), increased acidosis, and reactive oxygen species [13–24], each of which has been shown to induce inflammation in the CNS [25–35]. For example, albumin has been shown to bind to Transforming Growth Factor- β Receptors (TGF β R) in astrocytes [25], leading to upregulation of Myosin Light Chain Kinase (MLCK) immunoreactivity [36]. MLCK phosphorylates myosin light chain (MLC), thereby inducing contractions and weakening endothelial cell-cell adhesion [37, 38]. Further, albumin has been shown to activate astrocytes and microglia through the mitogen-activated protein kinase pathway (MAPK) resulting in increased levels of interleukin (IL)-1 β and nitric oxide as well as CX3CL1 in astrocytes [39].

Disruption of the BBB and insertion of probes have also been shown to immediately activate nearby microglia [40]. These cells persistently produce high levels of pro-inflammatory

cytokines (interleukin-1 and TNF α) and chemokines (such as MCP-1) for the duration of the implantation, which could lead to neuronal degeneration and demyelination [41–48]. In addition, microglia-initiated inflammation cascades result in the progression of the glial sheath that forms an ionic and growth barrier between electrodes and neurons, which may reduce the recording quality [49, 50]. Activated microglia also induce dysfunction of the BBB by releasing IL-1 β which upregulates MMP-9, a matrix metalloproteinase known to degrade the gap junction of BBB endothelial cells [51]. Persistent BBB breach at the location of indwelling brain implants has been observed, and can have a negative effect on the function of chronic neural implants through recruitment of pro-inflammatory myeloid cells and increased presence of neurotoxic factors. Among these factors, MMP-9 is found to be more highly expressed in the tissue nearby the electrodes compared with non-implant control tissue [52]. One recent study examined the cytokines and soluble factors present around the implanted microelectrode arrays using laser capturing microdissection and gene expression analysis and found elevated levels of several pro-inflammatory and neurotoxic cytokines as well as tumor necrosis factor α (TNF α). Among these, upregulation of IL-1 β mRNA is the most significant across all types of electrode designs tested [53].

IL-1 β is a key pro-inflammatory cytokine and plays a critical role in inflammation and programmed cell death [54]. The synthesis of IL-1 β precursors (pro-IL-1 β) is induced by Toll-like receptors or RIG-like receptors, but pro-IL-1 β must be cleaved and activated by caspase-1. Caspase-1 in turn, must be activated by inflammasomes, which are mediated by complex cytoplasmic pattern recognition receptors signaling in response to cell injury. Caspase-1 is the only known enzyme that cleaves *in vivo* pro-IL-1 β into mature IL-1 β . Furthermore, caspase-1 activation is an early event detected in neuronal cell death associated with ischemia as well as in chronic neurodegeneration [55, 56]. A previous study has revealed loss of perfusion *in vivo* in multiple adjacent capillaries around an implanted electrode, which creates an ischemic microenvironment around the probe [40]. Using murine models, it has been demonstrated that inhibition of caspase-1 activity, either through knockout of the gene or by overexpression of a dominant negative construct, can slow neurodegeneration caused by a diverse set of circumstances including Huntington's Disease, Amyotrophic Lateral Sclerosis, traumatic brain injury, and ischemic stroke [57–64].

Since caspase-1 plays critical and relevant roles in neuronal cell death and activation of inflammatory pathways, we hypothesize that it mediates these processes after electrode implantation. The ability to prolong neuronal survival in the presence of neuronal stress is a key function of caspase-1 inhibition, and this may be leveraged to decrease the kill zone and inflammation around brain implants, thereby improving neural recording. To understand the effect of caspase-1 inhibition on electrophysiological performance, microelectrode arrays were implanted into primary monocular visual cortex of age-matched caspase-1 KO and WT mice. Neural activity was evoked using visual cortex stimulation paradigms, and recording performance was quantified over months. Here we show that caspase-1 plays an important role in the biological failure mode of chronically implanted electrodes and that the inhibition of caspase-1 improves chronic recording performance.

3. Method

This study characterizes and compares the chronic neural recording characteristics of intracortical neural electrodes in C57B6 WT mice and C57B6-caspase-1 KO mice along multiple layers of the cortex and hippocampus. A visually evoked recording model is used (Fig. 1) [65–67], and electrical recording characteristics were evaluated across the metrics of single-unit yield, single-unit SNR, single-unit amplitude, impedance, and noise floor as previously established [68]. The molecular, cellular, and vascular response around the recording sites of implants was examined with postmortem immunohistochemistry at the end of recording periods.

3.1. Surgical Implantation

Single shank Michigan electrodes (A16-3mm-100-703-CM15) were implanted unilaterally into the left primary monocular visual cortex, (V1m) of 9 wk old caspase-1 KO (n=9) and WT (n=9) female mice. Each mouse was anesthetized under 1.5% isoflurane and mounted onto a stereotaxic frame (Kopf Instruments, Tujunga, CA). The top surface of the skull was exposed and a drill sized craniotomy was made centered at 1 mm anterior to Lambda and 1.5 mm lateral to midline using a high speed dental drill and a .007 drill bit. Saline was applied continuously onto the skull to dissipate heat from the high-speed drill. Extra care was taken to prevent damage to the dura by reducing the drill speed and gently manually feeling the resistance of the skull when the dural blood vessels were visible through the opaque thin skull. A total of three bone screws were installed bilaterally over the primary motor cortex as well as over the contralateral visual cortex. The reference wire was connected to the bone screw over the contralateral visual cortex, while the ground wire was connected to both bone screws over the motor cortex. Arrays were inserted at ~2mm/s using a stereotaxic manipulator until the top edge of the last recording site was at the edge of the brain surface. Insertion of the array was visualized by a tilted surgical scope. The silicon device and the craniotomy were sealed carefully with silicone (Kwik-sil) and a headcap was created using UV-cured dental cement (Pentron Clinical, Orange CA). Body temperature was maintained throughout the procedure using a warm water pad (HTP 1500, Adroit Medical Systems, Loudon TN). 0.3mg/kg buprenorphine was administered twice daily for three days as a post-operative analgesic. All animal care and procedures were performed under the approval of the University of Pittsburgh Institutional Animal Care and Use Committee and in accordance with regulations specified by the Division of Laboratory Animal Resources. (For WT: N=9 for 1–8 days, N=8 for 14–35 days, N=6 for 42–133 days, and N=4 for 140–185 days. For caspase-1 KO: N=9 for 14–35 days, N=7 for 42–133 days, N=5 for 140–175 days, and N=4 for 182 days.)

3.2. Neurophysiological Recording

Neural electrophysiology was recorded as previously described [68] and extensively described in a companion study (Kozai, TDY; Du, Z; Smith, MA; Chase, SM; Bodily, LM; Caparosa, EM; Friedlander, RM; Cui XT). Spontaneous recording was conducted in a dark room. Visual stimuli was presented using the MATLAB-based Psychophysics toolbox [65, 67, 69] on a 24" LCD (V243H, Acer. Xizhi, New Taipei City, Taiwan) monitor placed 20 cm from the eye contralateral to the implant, spanning a visual field of 60° wide by 60°

high. Solid black and white bar gratings were presented drifting in a perpendicular direction and synchronized with the recording system (RX7, Tucker-Davis Technologies, Alachua FL) at 24,414 Hz. Each 1 second grating presentation (rotated in 45° increments) was separated by a 1 second dark screen period and the entire set was repeated 8 times per recording. The raw data stream was filtered from 0.3–5 kHz to produce spike data streams. The spike data stream was further pre-processed using published methods [70, 71]. Possible spikes were detected using a fixed negative threshold value of 3.5 SD. Offline spike sorting was carried out using a custom MATLAB script modified from previously published methods [68, 72]. Average signal-to-noise ratio (averaging the amplitudes of single-units for each channel) and average amplitude of noise (2 SD) were used to quantify electrode recording performance. Only channels exhibiting sortable single-unit spikes with SNR >2 were analyzed. Candidate units with SNR between 2 and 3 were manually confirmed or excluded by examining the combination of waveform shape, auto-correlogram, peak threshold crossing offset, and peri-stimulus time histogram (PSTH) with 50 ms bins. Candidate units with SNR below 2 were discarded, and candidate units with SNR greater than 3 were manually confirmed by examining the waveform shape.

Current source density (CSD) to the evoked visual stimulus was used to identify Layer IV [73, 74]. Briefly, CSD was performed by computing the average evoked (stimulus-locked) LFP at each site, smoothing these signals across sites, and then calculating the second spatial derivative [73, 75]. The CSDs across 64 stimulus trials were averaged and the location of the minimum value of the CSD in the first 100 ms was determined to be Layer IV. Polarity inversion of LFP was also examined to supplement the CSD analysis [76]. Recording data along the probe shank across mice was aligned to a common Layer IV.

3.3. Impedance Spectroscopy

Electrochemical impedance was measured immediately after each neural recording session. While under anesthesia, the implanted array was connected to an Autolab potentiostat using a 16 channel multiplexer. Impedance was measured for each channel using a 10 mV RMS sine wave from 10 Hz to 32 kHz, employing a 15 multisine paradigm to shorten the time required for measurement. In this work, the 1 kHz impedance is reported unless indicated otherwise.

3.4. Immunohistochemistry

Mice were sacrificed and perfused according to University of Pittsburgh IACUC approved methods. Each mouse was deeply anesthetized using a 90 mg/kg ketamine, 9 mg/kg xylazine cocktail. Once the proper plane of anesthesia was observed, mice were transcardially perfused using a warm phosphate buffered saline (PBS) flush at ~70 mm Hg followed by ice cold 4% paraformaldehyde at 70–90 mmHg. Mice were decapitated, and heads were post-fixed in a 4% paraformaldehyde bath at 4°C for 4–6 hrs. The skulls containing the brain were then removed and soaked in a 15% sucrose bath at 4°C overnight followed by a 30% sucrose bath for 36–48 hours. Following sucrose protection, the bottom and sides of the skull was dissected and the brain was gently removed from the electrode array and headcap. Brains were then blocked and carefully frozen in a 2:1 20% sucrose in PBS:optimal cutting

temperature compound (Tissue-Tek, Miles Inc., Elkhart, IN) blocking media blend with dry ice. Tissue was horizontally sectioned using a 25 μ m slice thickness.

Tissue sections were rehydrated with 5 min washes with 1x PBS, repeated twice. The tissues were then incubated in a humidified chamber with pH 6.0, 0.01M sodium citrate buffer for 30 min at 60°C. Then, a peroxidase block was performed in PBS with 10% v/v methanol and 3% v/v hydrogen peroxide for 20 min on a table shaker. Next, tissue sections were incubated in a blocking solution (5% goat serum, 0.1% triton X-100) for 30 min at room temperature. Lastly, the tissue samples were blocked with AffiniPure Fab Fragment (Alexa-647 115-607-003 Jackson ImmunoResearch Laboratories, Inc.) for 2 hours then vigorously rinsed 8 times (8×4 min) with 1x PBS.

Following blocking, sections were incubated in a primary antibody solution consisting of 5% goat serum, 0.1% triton X-100, and antibodies against neuronal nuclei (1:250 NeuN MAB377 Millipore), microglia (1:500 Iba-1 NC9288364 Fisher), Neurofilament (1:250 NF200 MAB5256 Millipore), GFAP (1:500 Z033401 DAKO), PDGFR β (1:250 3169S Cell Signaling Technology), APC/CC1 (1:200 OP80-100UG EMD Millipore), activated Caspase-3 (1:250 Asp175 9661S Cell Signaling), and/or tomato-plant lectin (1:200 B-1175 Vector Labs) 18 hours at 4°C. Sections were then washed with PBS (3×5 min) and incubated in a secondary solution consisting of 5% goat serum, 0.1% triton X-100, and antibodies (1:500 goat anti-mouse Alexa 488, Invitrogen, and 1:500 goat anti-rabbit Alexa 568, Invitrogen, Carlsbad CA) for two hours at room temperature. Sections were then rinsed with PBS for 5 minutes, exposed to 1:1000 Hoechst 33342 (Invitrogen) for 10 minutes, and washed in PBS (3×5 min) before being coverslipped with Fluoromount-G (Southern Biotech, Birmingham AL). Sections were promptly imaged using confocal microscopy (FluoView 1000, Olympus, Inc., Tokyo, Japan) at 20X magnification.

3.5. Statistics

Variances were calculated and compared between WT and caspase-1 KO. Large differences in variance and standard deviation (as indicated by error bars) were observed between the two groups. Therefore, comparison between two groups was performed using Welch's T-test (unequal variance). For all tests, $\alpha < 0.05$ indicated a significant result.

3.6 In Vivo Two-Photon Imaging

Based on the observation that IL-1 β is upregulated in the presence of increased mechanical strains [77], we examined the effect of implant induced strain on surrounding neurons. Acute 3 mm long four-shank Michigan silicon electrodes (A4×4-3mm-100-177-A15, Neuronexus Technologies, Ann Arbor, MI) and a carbon fiber Microthread electrode were used. In order to better visualize the probe, fluorescent molecules were loaded into a conducting polymer polyethylenedioxythiophene (PEDOT) coating electrodeposited on the recording sites as previously described [40, 78]. 4–8 week old WT mice obtained from Jackson Laboratories (Bar Harbor, ME) and weighing 20–25 g were prepared for cortical implants using previously established methods [40]. The mice were anesthetized with a mixture of 90 mg/kg ketamine and 9 mg/kg xylazine administered intraperitoneally (IP) with regular updates of 17.5 mg/kg every 30 min or as needed. Care was taken to prevent

vascular damage during drilling and removal of the bone. All experimental protocols were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee.

0.8 mM Oregon Green 488 BAPTA-1AM (OGB) was dissolved in DMSO with 20% Pluronic Acid and mixed in artificial cerebral spinal fluid containing 50 μ M Sulfarhodamine101 as previously described [79, 80]. Briefly, this was filled into a custom pulled patch pipette with a tip diameter of 1–3 μ m. The pipette was inserted ~300 μ m deep and pressure-ejected at 5–10psi for sixty 1s pulses. The electrode was aligned with a stereotaxic manipulator over the cortex away from major vasculature at a 30°–35° angle as previously described [40]. Probes were inserted using a z-axis automated microdrive (MO-81, Narishige, Japan) at 50 μ m/s for 500 μ m such that the tip of the electrode was approximately 200–250 μ m (~Layer 2/3) below the surface of the brain. Little or no bleeding was observed during insertion. A two-photon laser scanning microscope was used for *in vivo* imaging. The microscope consisted of a scan head (Prairie Technologies, Madison, WI) and a Ti: sapphire laser (Mai Tai DS; Spectra-Physics, Menlo Park, CA) providing 100 fs pulses at 80 MHz tuned at a wavelength of 920 nm for this study. Fluorescence was detected using non-descanned photomultiplier tubes (Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, Japan) in whole-field detection mode. Images were acquired using Prairie View software.

4. Results

4.1. Recording Depth Analysis Using Current Source Density

Current source density (CSD) was used to identify Layer IV in the visual cortex (Fig. 1a). Immediately following an 'ON' stimulus, a strong electrical current sink (neural source) can be observed using CSD as large populations of Layer IV input neurons cell bodies depolarize, which is then followed by an electric source. In addition, LFP polarity inversion can typically be observed around the border of Layer IV to help identify its depth [81]. Delayed sources in Layer II/III and Layer V also help contrast the location of Layer IV.

For the purposes of this analysis, the depth of Layer IV on the day of the surgery is defined as 0 μ m. Examining the changes in the depth of Layer IV over time shows that while there are some subtle fluctuations early on in both WT and caspase-1 KO groups, the depth is mostly stable (Fig. 1b). After 100+ days there is a mean decrease in the depth position of Layer IV as well as an increase in variation. Further examination of the magnitude of depth change over time shows that most of the depth fluctuation occurs during the first week, stabilizing by 14 days (Fig. 1c). In particular, the caspase-1 KO group appears to show greater Layer IV position drift compared with the WT group. Therefore, the average depth of Layer IV between day 14 and day 100 was used for depth related analysis of the electrode's chronic performance.

4.2. Depth Independent Electrode Performance Analysis

First, electrode performance comparison between WT and caspase-1 KO was made by averaging performance metrics of all channels along the probe shank regardless of the depth. In such depth independent analysis, single-unit yield (percentage of electrode sites able to detect a single-unit) for WT mice began acutely at $59.0 \pm 2.4\%$ and declined to $11.1 \pm 2.8\%$ on

day 77 where it stabilized for the remainder of the 7 month recording duration (Fig. 1d). On the other hand, caspase-1 KO mice started with a yield of $60.4 \pm 8.5\%$ and declined to $38.4 \pm 5.0\%$ on day 77. The electrodes in the caspase-1 KO animal had a significantly greater yield ($p < 0.05$) than the WT between days 14 and 133. Mean single-unit SNR was initially 2.13 ± 0.08 for WT and 3.05 ± 0.11 for caspase-1 KO (channels with no detectable single-units were considered to have SNR=0), then declined to 0.32 ± 0.04 (WT) and 1.14 ± 0.04 (KO) on day 77 where it stabilized (Fig. 1e). In general, significantly greater SNR ($p < 0.05$) was detected in the caspase-1 KO mice between day 2 and day 140. Of the electrodes sites that were recording single-units (channels with no detectable single-units were ignored), the average SNR was significantly greater ($p < 0.05$) for the caspase-1 KO mice over the first three days, but significantly greater for the WT after day 98 (Fig. 1f). However, by this point, the yield from the WT had decreased to an average of one site per array.

The average amplitude of the single-units started at $54.93 \pm 3.62 \mu\text{V}$ (WT) and $75.95 \pm 6.05 \mu\text{V}$ (caspase-1 KO) on the day of surgery and increased to $92.2 \pm 6.3 \mu\text{V}$ (WT) and $92.2 \pm 6.4 \mu\text{V}$ (caspase-1 KO) on day 6 (Fig. 1e). On day 14, it had declined back to $73.2 \pm 4.1 \mu\text{V}$ (WT) and $73.5 \pm 3.6 \mu\text{V}$ (caspase-1 KO), then more slowly declined to $55.8 \pm 8.32 \mu\text{V}$ (WT) and $61.1 \pm 3.1 \mu\text{V}$ on day 77. Interestingly, the single-unit amplitude was greater for the WT between days 105 and 154. Noise floor was at $13.4 \pm 0.4 \mu\text{V}$ (WT) and $16.0 \pm 0.9 \mu\text{V}$ (caspase-1 KO) on the day of the surgery, and increased to $19.1 \pm 0.4 \mu\text{V}$ (WT) and $19.5 \pm 0.3 \mu\text{V}$ (caspase-1 KO) by day 14 where it generally remained stable (Fig. 1g). The noise floor was significantly greater ($p < 0.05$) in the caspase-1 KO mice over the first week. Impedance increased from $675 \pm 18 \text{ k}\Omega$ (WT) and $696 \pm 18 \text{ k}\Omega$ (caspase-1 KO) to $1,280 \pm 102 \text{ k}\Omega$ (WT) and $1,185 \pm 74 \text{ k}\Omega$ (caspase-1 KO) over the first 14 days (Fig. 1i). Note that the sharp decline at day 140 is due to the removal (sacrifice) of the highest impedance mice (2 each of WT and caspase-1 KO) that were no longer recording any single-units.

4.3. Depth Dependent Electrode Performance

Probe implant depths were aligned across animals at their average Layer IV depth between day 14 and day 100 (Fig. 1). Layer IV was determined with CSD following the visual stimulus. Electrode performance metrics at different cortical Layer/depth over time were plotted in heatmaps in Figure 2–3. Interpolation smoothing was used to enhance visualization of signal change over time. Cheverons were used to denote discrete sampling time points, while circles were used to denote discrete sampling depths. Chronic single-unit yield showed very strong layer dependence (Fig. 2a,b). Both WT and caspase-1 KO show strong acute/early chronic recording yield in Layer IV and deep in CA1, and poor yield in Layer I. Implants in caspase-1 KO mice also demonstrate robust yield from Layer II/III, V, VI, and a broader region of CA1. Chronically, caspase-1 KO mice show more robust yield in the cortex, especially around Layer IV, V. On the other hand, WT mice show lower yield in the cortex, predominantly limited to Layer IV and some in Layer II/III. In CA1, most of the recording yield was lost around day 40 in both WT and caspase-1 KO mice. Overall, caspase-1 KOs demonstrated much greater yield across the cortex than WTs.

Single-unit SNR followed a similar trend to the single-unit yield (Fig. 2c,d). Both groups again showed little signal in Layer I. During the acute/early chronic phase, SNR was most

strongly detected around Layer IV, Layer V, and deeper CA1 in WT mice. In caspase-1 KO mice the SNR was greatest in Layer IV, and followed by Layer V, Layer VI, and CA1. Chronically, SNR in WT mice was predominantly in Layer IV and Layer II/III, while caspase-1 KO recorded strong signals in Layer II through VI. Additionally, greater SNR was detected in the caspase-1 KO mice than their WT counterpart. In CA1, caspase-1 KO recorded signal for longer compared with WT.

Noise floor showed interesting properties (Fig. 3a,b). In the cortex the noise floor was greater in caspase-1 KO mice early on, but greater in WT mice at later time points. This transition occurred around week 8 and 12. In CA1, the noise floor was greater in WT in the acute/early chronic phase, but declined more quickly than the caspase-1 KO counterpart. The impedance followed a similar profile for the WT (Fig. 3c). Impedance was generally lower in the cortex early on, but increased between weeks 8 and 12. However, in caspase-1 KO cortex the impedance was generally very steady with high impedance around Layer I and mildly in Layer II/III (Fig. 3d). The increased impedance in these layers was generally consistent. Interestingly, jumps in impedances can be detected in the shallow layers of the white matter early on. Later, these increases can be seen in Layer V as well as Layer VI. These jumps in impedance become more limited after 12 weeks. In deep CA1, impedance increased and then decreased more rapidly in WT mice compared with caspase-1 KO animals.

4.4. Histology

In general, histology showed stabilized interfaces from the 4+ month implanted mice. Despite this more than 50% of the WT mice showed some neurons co-labeled with caspase-3 near the probe track (an apoptotic marker) (Fig. 4a), while only three neurons co-labeled with caspase-3 were found in two caspase-1 KO mice (Fig. 4c). It should be noted that in both WT and caspase-1 KO mice, non-neuronal sub-cellular features were frequently labeled with caspase-3 (Fig. 4b,d).

Just as recording performance along the electrode depth is not uniform (Fig. 2–3), the histology along the electrode depth varies (Fig. 4e,f). Tissue sections of 25 μ m thickness were carefully collected while tracking the total depth of the sections from the surface of the brain. The biological layers of the sections are identified by cross-referencing tissue depth, neuron size, neuron density, and the morphology. For example, Layer I only has a few scattered interneurons, which make the identification of the border between Layer I and Layer II easy to identify. This information is then compared to the electrical performance metrics (SNR, Impedance) by aligning the tissue to the evoked CSD data (Fig. 4g–l). When showing the histological features, recording metrics are displayed whenever possible to help correlate the biological observation to physiological outcome.

In WT, detection of substantial IgG leakage (see paragraph below for the definition of chronic IgG leakage) from the vasculature corresponded with sizeable glial scar, loss of neuronal density or elevated caspase-3 activity and could be detected along with loss of electrophysiological activity (Fig 5a,c, 6a). However, caspase-1 KO mice with similar levels of IgG leakage maintained healthy electrode interface, good neural population around the electrode, and strong electrophysiological recordings (Fig. 5d–f, 6d–e). Even when a large

tissue infarction was observed near the implant, the electrode in the caspase-1 KO mice was able to detect single-units (Fig. 6f).

Upon close examination of the histology, asymmetrical glial response and BBB leakage could be commonly detected around the perimeter of an implant track cross section. Likewise, intensity and distribution of markers are found to vary significantly along the implant shank. Some IgG labels are found in the cells and others are found in extracellular space. IgG has been used as a marker of BBB leakage in recent publications [82]. Previous studies have demonstrated that IgG that enters the CNS is sequestered by astroglia and microglia where they have been shown to remain for at least 9 months [83–85]. We similarly showed that throughout the tissue, IgG around the implant is mostly sequestered by glial cells at the surface of the implant. Sequestered IgG is not considered a sign of chronic BBB leakage as the IgG release from the blood vessel may have occurred at the initial implantation time or from blood borne glial cells that crossed the BBB. We therefore define IgG presence in the extracellular space as an indicator of chronic BBB leakage. IgG leakage in the extracellular matrix of 4+ month implanted tissue was detected in Layer I/Layer II in approximately half of both WT and caspase-1 KO mice, while below Layer II/III only two WT and two caspase-1 KO mice showed chronic IgG leakage (Fig. 5a–e). Furthermore, IgG leakage in the extracellular space of one tissue section did not equate to chronic IgG leakage along the entire length of the implant (Fig. 5a). In one example, at a greater depth in the tissue, IgG was entirely sequestered within the glial scar (Fig. 5b). Such asymmetry and heterogeneity of the histological features invalidates the use of averaged histological features to predict electrophysiology performance (Fig. 4).

Lastly, to further understand the role of BBB on the reactive tissue response and electrophysiology, pericytes and oligodendrocytes were labeled with antibodies of platelet derived growth factor receptor β (PDGFR β) and Adenomatous polyposis coli (APC/CC1), respectively (Fig. 7). Over gross qualitative evaluation, APC labelled oligodendrocytes appeared more uniformly distributed with closer proximity to the probe than the corresponding neuronal population. Remarkably, pericytes were not only detected on nearby BBB, but also at the probe/tissue interface for both WT and caspase-1 KO tissue.

5. Discussion

Sixteen channel linear silicon electrode arrays were implanted into mouse left visual cortex, and neural activity was evoked by showing whole field drifting gratings on a computer screen. Single-units were compared across arrays as well as across depth. Overall, chronic electrical performance showed significant differences between caspase-1 KO and the control group (Fig. 1). For this study we elected to use the visual cortex model due to the simplicity and the ability to drive activity without introducing substantial electromagnetic or electrostatic radiation (i.e, motion artifact) as well as the surgical accessibility of the cortical region. The visual cortex lacks the curvature and dense surface vascularization of barrel cortex, and the comparative lack of columnar structures in V1 should conceptually lead to more uniform and homogenous neural activity across all array sites with stimulation [86].

5.1 Chronic Electrophysiology

In Figure 1, data were averaged across recording sites to provide an overall comparison between WT and caspase-1 KO. Since neural recording yield depends dramatically on the cortical layer in which the electrode recording site resides, we further demonstrate the recording metrics in a layer dependent manner in Figure 2–3 to provide a more precise picture of the changes over time. It is important to point out that while averaging across depth is acceptable when comparing the same type of electrodes in the same brain region, it can greatly bias the results when comparing different probe designs where electrode sites are located at different depth.

5.1.1 Acute to Early Chronic (0–7 days)—In the acute to early chronic phase, recording yield and SNR is generally robust in both WT and caspase-1 KO mice. Yield was not significantly different during this period. However, SNR significantly decreases ($p < 0.05$) in the WT mice from day 3 compared with caspase-1 KO. During this phase the average SNR of electrodes actively recording single-units is significantly greater ($p < 0.05$) in caspase-1 KO than WT.

When examining cortical layers, greater SNR was recorded in caspase-1 KO mice excluding Layer I (Layer I predominantly contains only axons, dendritic tufts and very few scattered inhibitory neurons). In contrast, WT mice record the strongest SNR from Layer IV and Layer V. Interestingly, the noise floor is significantly greater ($p < 0.05$) in the caspase-1 KO mice compared with WT mice during this period, though generally lower than other time points. In contrast, impedance slowly increases in both conditions, indicating very little impact from the caspase-1. Increase in noise is normally a consequence of increase in impedance due to microgliosis or increase in background neural activity. The lack of impedance difference between WT and caspase-1 KO indicates that more distant low amplitude units were detected in the caspase-1 KO mice. This could be a result of higher neuronal viability around the electrodes in the caspase-1 KO mice.

Unlike the cortex, the noise floor increases sharply in CA1 in both WT and caspase-1 KO, with greater increase in WT. Impedance also peaks around day 5 in both WT and caspase-1 KO, with greater increase in WT mice. It has been shown that the tip of the electrode is the focal point of mechanically induced tissue strain of probes fixed to the skull [87]. The sharp increase here may be due to a more severe tissue response resulting from the strain concentration at the tip [87]. In addition, the hippocampus is known for its role in hippocampal cells' sensitivity to activity-dependent changes [88–91]. Tissue in the hippocampus region, which is important for learning and houses endogenous adult progenitors, may be more sensitive to injury [92]. Increases in noise and impedance were greater in WT, suggesting reduced acute inflammation in the caspase-1 KO mice. Additional research is necessary to understand the role of caspase-1 on electrode induced acute injury and strain, and their impact on electrophysiological performance.

It should be noted that the variance of single-unit yield, SNR, and single-unit amplitude are greater for the caspase-1 KO than the WT in the acute period as indicated by the error bars. In addition, the position of Layer IV changes more dramatically in caspase-1 KO than WT during this period. Interestingly, increases in IL-1 β have also been shown to lead to the acute

release of high-mobility group box 1 (HMGB1) through ERK, a member of the mitogen activate protein kinase (MAPK) family, and chromosome region maintenance 1 signaling following an injury [93]. HMGB1 has been shown to enhance tissue repair and promote cell survival [94–96]. This suggests that some early inflammation can be beneficial. The beneficial acute inflammation may explain why the recording variance is initially high in the acute to early phase in the caspase-1 KO compared with the WT by stabilizing variability from the insertion. WT mice are able to mediate acute inflammation through the IL-1 β pathway to contain the variability of the initial injury. Nevertheless, recording performance variance in the caspase-1 KOs decreased and stabilized over the first week while it increases in the WT over the first week. This suggests that caspase-1 may be an important signaling pathway for acute pro-recovery signaling, but may negatively impact SNR and performance over the chronic period.

5.1.2 Early Chronic (1–4 weeks)—During this period, the average SNR substantially decreases. The strongest signals are limited to the region around Layer II–V, but mostly localized to Layer IV by the end of 4 weeks in both WT and caspase-1 KO. On the other hand, most of the yield can be seen in Layer II–IV and CA1 in WT mice with CA1 showing the best yield. In caspase-1 KO mice, high yield with minimum change occurs, except in CA1 where the yield began to decrease towards the later part of the phase. Nevertheless, implants in caspase-1 KO mice perform significantly better ($p < 0.05$) over single-unit yield and SNR as compared with WT.

Impedances in Layer I and II/III are greater in caspase-1 KO mice, which may be influenced by meningeal cells [97], though it is unclear why caspase-1 KO mice would be more impacted than WT. Jumps in impedances are also observed in the callosum and alveus layers of the hippocampus. These layers immediately below the cortex contains bundles of myelinated axons that facilitate interhemispheric communication or project towards the fimbria [98, 99]. Glial cells in this region may be more sensitive to the implanted electrode, though additional research is necessary. As expected, the noise floor increases at this depth during this period.

5.1.3 Early chronic stabilization (4–7 wks)—During this period, the electrical properties in the cortex of the implant generally stabilize, though continue to subtly decline. In the lower regions of the hippocampus, the recording yield and SNR drop off dramatically. Similarly noise floor and impedance drop off dramatically. Between Layer V and the white matter fiber track region, sharp increases and decreases in impedance could be a result of glial cells crawling along the shanks of the devices and temporarily covering the recording sites as they move. In WT mice, the average impedance decreases below the pre-implant impedance values. This may be due to the oxidation of the iridium recording sites or breakdown in the insulation, though additional studies will be necessary to determine the specific mode of material failure. Interestingly, such impedance decrease was not observed in caspase-1 KO mice. One hypothesis is that the milder inflammatory environment of the caspase-1 KO mice produces less reactive oxygen and nitrogen species that degrade the implant materials. Further research exploring the relationship between abiotic and biotic factors will be needed to test this hypothesis.

5.1.4 Early chronic to chronic transition (8–12 wks)—Impedances of recording electrodes drastically increased 10 weeks post-implant in WT mice, while small decreases were seen in caspase-1 KO mice. The noise floor followed a similar trend of increasing in WT mice and decreasing in caspase-1 KO mice. This was followed by a drop in yield and SNR between 9 and 11 weeks post-implant. This increase in impedance could be a result of glial scar consolidation or meningeal fibroblasts. The observed smaller change in caspase-1 KO suggests a milder glial scarring due to lack of IL-1 β release. Overall, these findings suggest that there is a transition to a chronic phase that impacts electrical performance metrics of chronically implanted cortical electrode arrays in mice. Further research should focus on understanding the biological and mechanical contributions to this stabilizing change.

5.1.5 Chronic (12+ weeks)—Around this period, most of the performance metrics stabilize in mice. Implants in caspase-1 KO mice continue to demonstrate significantly greater ($p<0.05$) yield and SNR. After approximately 14 weeks, only 2–3 recording sites in two of the four WT mice were still detecting single-units, whereas 33 recording sites in all four KO mice were still detecting single-units. Impedance and noise levels remained similar throughout this period.

5.1.6 Electrophysiology Performance Summary—These results show that caspase-1 knockout dramatically improves recording performance over the chronic period. While recent in vitro studies suggest the role of caspase-8 as a parallel activator of pro-IL-1 β when the receptor dectin-1 is activated by certain fungal or mycobacterial antigens, it is expected that these antigen are not present on properly sterilized microelectrodes [147]. Furthermore, the relevant contribution of caspase-8 activation of IL-1 β remains to be addressed [148]. Nevertheless, caspase-1 knockout substantially improved the longitudinal single-unit yield and SNR, strongly supporting the important role of caspase-1 in mediating neuronal death and inflammation associated with chronic electrode implantation.

5.2 Histology

Caspase-3 labeling showed that cell death around implanted electrodes continues 4–6 months after implantation (Fig. 4). The fact that fewer caspase-3 positive neurons were detected in caspase-1 KO mice is consistent with the higher recording yield. It is worth pointing out that caspase-3 activity was not limited to the neuronal population (Fig. 4b,d). Non-neuronal caspase-3 was detected in both WT and caspase-1 KO mice, especially inside the glial scar. These caspase-3 positive cells in the glia may be cells that are engulfed, digested, or being pulled apart by other glial cells. Other caspase-3 labeling was observed as scattered sub-cellular structures; these may include processes, neurites, axons, or myelin of nearby cells located at a different depth. Identifying these cell types and their role in the chronic tissue response may provide additional insight towards intervention strategies. In general, the glial response demonstrates reduced inflammation and glial scarring in the caspase-1 KO mice as compared with WT.

Recording performance and histology along an electrode array's depth is not uniform (Fig. 2–6) due to the inherent layered structure and heterogeneity of the brain tissue. In fact, even

in the same tissue section, the histology shows that the tissue reaction is not uniform, with substantial asymmetry around the implant (e.g. Fig 5d, Fig6c,e,f). Therefore, radial averaging around an electrode (as has been done in most of the neural tissue interface literature) can distort histological metrics of planar electrodes that face laterally in one direction, which may further confound any attempts aimed at correlating tissue reaction to recording metrics. Therefore, this study raises new questions in quantitative correlations of tissue histology and electrophysiological performance (Fig. 4–6). Because electrophysiology was extensively quantified, we use qualitative histology to identify unique features and outliers, instead of homogeneously evaluating histology at random depths to relate to electrophysiological outcomes. Figure 4e,f shows an example of tissue histology with good recordings from shallower layers while no recordings from deeper layers. Figure 4g–l also show histological slices that have close neuronal cell body proximity ($\ll 100\ \mu\text{m}$) but poor recordings (Fig. 4). Figure 5a,d,e show robust glial encapsulation, however the electrode in Fig 5e is still capable of recording single-units from all nearby electrodes. Figure 5f shows limited glial encapsulation, but good recording capabilities. On the other hand, Figure 5b,d show milder encapsulation with close neuronal proximity, and good impedances, but demonstrated poor chronic recording capabilities. Figure 5–6 also show that IgG leakage is not always directly tied to electrophysiological outcomes.

In most of the 4–6 month implanted tissue, IgG accumulation was only found at the surface of the electrode and sequestered by glial cells. Previous studies have demonstrated that IgG that enters the parenchyma of the CNS is sequestered by astroglia and microglia where they have been shown to remain for at least 9 months [83–85]. In addition, IgG can also be carried into the CNS by blood borne macrophages [100]. Therefore the presence of IgG in the tissue is not necessarily indicative of chronic IgG leakage, as the IgG detected may be from the initial surgical implantation. In addition, pharmaceutically increasing BBB leakage throughout the CNS did not, in and of itself, result in the activation of astrocytes or microglia as seen following CNS injury [83]. Combined these paint a more complex relationship between the BBB and electrophysiological performance than previously hypothesized.

In some WT and caspase-1 KO mice, IgG leakage, as defined by the presence of IgG labeling in extracellular space, was only detected in the shallow layers of the cortex. Chronic IgG leakage in these mice may be dominated by vascular leakage from the bone or dura mater rather than from the brain parenchyma.

In two WT and two caspase-1 KO mice IgG leakage was detected at a chronic time point below Layer II/III. Of these four mice, two WT and one caspase-1 KO mice had no single-unit detectable at the time sacrifice (Fig. 5a,c 6a,d), while the last caspase-1 KO mouse with similar IgG leakage recorded single-units from 50% of the recording sites (Fig 5d,e. 6e, 7e). This suggests that IgG leakage is not sufficient for the loss of electrophysiological recordings at least when the caspase-1 signaling cascade is disrupted, though additional research is necessary to understand the dynamic relationships.

Lastly, to further understand the role of BBB on the reactive tissue response and electrophysiology, pericytes and oligodendrocytes were examined with PDGFR β and APC,

respectively (Fig. 7). Pericytes are tightly coupled to the capillary neurovascular endothelial cells and sustained PDGF- β signaling secreted by the endothelium is thought to be required for pericyte survival in the adult CNS [101–105]. These cells play a critical role in reducing BBB permeability, regulating blood flow, and clearing neurotoxic cellular byproducts [101, 104, 106–108]. This has particularly resounding implications as loss of perfusion (BBB occlusion) has been previously demonstrated in the microenvironment surrounding implanted microelectrodes [40]. Dysfunction or deficiency in pericytes is associated with BBB breakdown which has been shown to cause CNS degeneration as discussed in the introduction [101, 104, 109]. Remarkably, pericytes were not only detected on nearby BBB, but also at the probe/tissue interface for all WT and caspase-1 KO tissue. In the adult CNS of mice, PDGFR β is expressed in pericytes [110], and blood-borne bone marrow progenitor cells have been shown to contribute to CNS pericytes following ischemic injury [111–113]. Pericytes have many additional roles associated with the BBB such as secreting essential extracellular matrix proteins (e.g. laminin) [108, 114]. Further, pericytes have been shown to possess macrophage-like phagocytotic properties to clear toxic cellular byproducts [104, 108]. In addition, CNS pericytes have been suggested to regulate T-cell entry into the brain [115]. There are many ways pericytes may contribute to the reactive tissue response which need to be investigated. While the mechanisms governing the interaction between the electrode and pericytes have yet to be explored, these findings show that pericytes are reoccurring elements of the chronic electrode-tissue interface.

Over gross qualitative evaluation, APC labeled oligodendrocytes appeared more uniformly distributed with closer proximity to the probe than the corresponding neuronal population (Fig. 6). APC/CC1 has been primarily used to identify mature oligodendrocyte cell bodies through cross reactivity of an antigen other than APC [116, 117]. APC is a tumor suppressing protein whose *Apc* mRNA in the CNS is largely localized in neurons [116]. Following CNS injury, APC has been shown to be transiently expressed in oligodendrocyte lineage cells during oligodendrocyte regeneration [118]. Therefore, it is not surprising to find APC expression around the implanted electrode. Cross-referencing the corresponding adjacent tissue section in Figure 6 and 7 show that PDGFR β + / APC+ (yellow) cells show positional expression patterns similar to caspase-3 labeling (Fig. 6&7). While additional research is necessary to determine these relationships, this study points to new research avenues for understanding cellular and molecular contributions involved in the chronic implantable brain-machine interface.

5.3 Biochemical Pathways

Mechanical strain caused by mechanical mismatch between the probe and the brain has been investigated as a source of persistent chronic tissue reaction around an implant [87, 119–122]. *In vivo* imaging has shown that the tissue strain surrounding the probe remains hours after insertion [40], though it is unclear how much of that is due to release in friction tension between the tissue and the implant or due to edema, inflammation swelling, or impaired blood flow induced change in intracranial pressure [123]. Culture studies have shown that shear strain on astrocytes and neurons results in loss of neurites as well as cell death [124]. Stretch-induced injury of brain tissue has been shown to activate calcium-dependent ERK

through increased extracellular ATP and P2 purinergic receptor signaling in astrocytes [125]. The ERK in turn has been shown to mediate the increase in IL-1 β [39].

It is possible that flexible implants may reduce the expression level of IL-1 β below the threshold for additional injury. In Figure 8, we show that reducing the feature size of the implant may reduce the mechanical strain experienced by the cells from the accommodation of the probe volume after insertion. In addition, reducing probe volume typically results in reduced surface area, which may play a role in reducing biofouling and improve diffusion and cleanup of soluble signaling molecules. Experimental evidence has shown improved recording quality and histology when probe volume and surface area is drastically reduced [68, 126, 127]. However, large variability in recording performance and histology is still observed across implants with the same design (surface chemistry, surface area, volume, cross-sectional area, and stiffness) [6, 7, 12]. Even within same implant, and same tissue section, asymmetric tissue reaction can be observed (Fig. 4–7). Figures 4–6 show that neuronal shape (circular or ellipsoid) around the electrode can greatly vary across mice and implant depth. Combined with the demonstration that some of the devices can record single-unit signals over very long periods of time suggests that mechanical mismatch is not the primary source of variability and unreliability. A follow-up study aims to characterize the non-biological failure modes of planar microelectrodes.

It has been demonstrated that continuous expression of IL-1 β leads to continuous BBB leakage [128]. Surprisingly, continuous IL-1 β expression, infiltration of leukocytes, and BBB leakage *in vivo* are not sufficient alone in causing neurotoxicity or neurodegeneration [128–131]. Increases in IL-1 β and caspase-1 activity likely lower the threshold towards neurotoxicity and magnify the neurodegeneration response as demonstrated when ischemia and excitotoxicity injuries were combined with increased IL-1 β [132, 133]. In a similar way, neuroprotective molecules such as L1CAM may raise the threshold for neurodegeneration, leading to better neural interfaces [134–136]. This may be particularly important in multishank arrays such as the Utah “bed-of-needles” Array that are more difficult to position to avoid key vascular features during insertion. Injury of key BBB features on the surface or superficial layers may result in ischemic damage and large tissue infarction in the tissue below where the recording sites are typically located [11, 137–139].

These results emphasize the complexity of the biological pathways that govern the reactive tissue response and longitudinal electrophysiological recordings from penetrating electrode arrays. While there is a relationship between IL-1 β and the BBB, BBB injury is not limited to chronic BBB leakage [11], but can include vascular occlusion, edema, and ischemia/hypoxia [40]. Knocking out caspase-1 clearly improves recording performance but does not eliminate the degradation of some electrical performance metrics (SNR). Eliminating a pro-apoptotic factor can minimize secondary injury and propagation of cell death to nearby cells, however, it may not eliminate metabolic depletion (e.g. oxygen) from vascular disruption [11]. Additional research may be needed to focus on replenishing the tissue (e.g., O₂, metabolites), removing toxins (e.g. pericytes), and accelerating tissue repair (e.g. HMGB1).

Conclusion

We have shown here that genetic models provide a platform for directly exploring molecular and cellular pathways impacting chronic electrophysiology performance and can aid in identifying specific targets for pharmaceutical or technological intervention. Knocking out caspase-1 can significantly improve recording quality of chronically implanted cortical electrodes. A number of promising pharmacologic interventions for the treatment of neurodegeneration have been demonstrated to inhibit activation of caspase-1 [140–146]. These drugs may be applied with either systemic administration or local release to improve the neural recording yield and longevity.

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Figure 1.

Chronic electrode performance comparison between caspase-1 KO (blue dashed) and WT (black solid) mice. * indicates $p < 0.05$. **a)** Current Source Density following the ‘ON’ stimulus identifies Layer IV. (Sink=Red; Source=Blue). **b)** Average depth position of Layer IV compared with day 0. **c)** Magnitude of average depth change shows that Layer IV fluctuates greatly during the first two weeks, but stabilizes thereafter. **d)** Single-unit yield over days. **e)** Single-unit SNR (mean amplitude over $2 \times \text{STD}$ noise). **f)** Average SNR of recording sites able to detect a single-unit. **g)** Voltage amplitude of single-units. **h)** Voltage amplitude of the noise floor. **i)** Average 1 kHz impedance.

Figure 2.

Single-unit recording performance as a function of depth and time. Black circles indicate discrete sampling depths of recording sites and black chevrons indicate discrete sampling time points. **a)** WT single-unit yield. **b)** Caspase-1 KO single-unit yield. **c)** WT single-unit SNR. **d)** Caspase-1 KO single-unit SNR. Note: Interpolation was used to help visualize the change in yield over depth and time. (Similar to line scatterplots and CSDs). Zeroes were ignored in the SNR heatmaps to better illustrate the signal quality across layers. Probability of recording a significant SNR can be examined via the yield plots (a-b).

Figure 3. Chronic electrode property as a function of depth and time. Black circles indicate discrete sampling depths of recording sites and black chevrons indicate discrete sampling time points. **a)** WT noise floor voltage. **b)** Caspase-1 KO noise floor voltage. **c)** WT 1kHz Impedance. **d)** Caspase-1 KO 1kHz Impedance.

Figure 4.

Example histology of neuronal health in WT (a, b, e, g-i) and caspase-1 KO (c, d, f, j-l) mice. **a, c**) Activated caspase-3 positive neurons (co-labeled in yellow/orange). **b, d**) Caspase-3 positive non-neurons (red). Note: **a, d**) There are no neuronal nuclei in the callosum or alveus axon tracks normally. The lack of neurons is not due to neuronal degeneration. **e, f**) Sections along multiple depth of same mice. **e**) WT 203 days post-implant, Layer I/II (left), Layer II/III (middle), Layer VI (right). **f**) KO 189 days post-implant, Layer II/III (left), Layer III/IV (middle), Layer V (right). **g-l**) Chronic time points show poor correlation between neuronal proximity and recording performance. **g**) Tissue from day 35 around Layer IV where single-units were detected on all nearby channels. **h**) Tissue from day 182 around Layer III-IV. Single-units were only detected from recording sites much deeper than this tissue depth. **i**) Tissue from day 133 around Layer IV which did not recording single-units on any channel of the electrode for the last 2 months prior to sacrifice. **j**) Tissue from day 35 around Layer III-IV where single-units were recorded. **k**) Tissue from day 133 around Layer II/III where single-units at this depth did not record any units for 2 months prior to sacrifice. **l**) Tissue from day 189 around Layer V-VI. Single-units were not recorded below Layer IV for the last 2 weeks prior to sacrifice. Recording sites always face left. The recording performance (SNR) and impedance are shown at the bottom of corresponding images. '[]'s show the 1kHz impedance range of the 3 nearest recording sites. Scale bar = 100 μ m.

Figure 5.

Example histology of Chronic BBB injury and IgG leakage in extracellular space 4–6 months post implant. Recording sites always face left. **a)** IgG leakage surrounding the implant around Layer II/III. **b)** same as (a), except in CA1. However, most of the IgG is sequestered by the glial cells and leakage into the extracellular space is very limited. Note Caspase3 activity within the IgG+ glial scar. **c)** Layer III tissue shows asymmetric leakage surrounding the implant and only at the vasculature near the top left corner of the probe footprint. IgG and Caspase-3 show localization. IgG distant from the leakage is sequestered by glial processes and accumulation in the extracellular space is limited. **d)** IgG leakage in Layer II/III. Here leakage is more easily distinguished as IgG+ and IgG- extracellular space is easier to identify. **e)** Same as (d), except in Layer V–VI. **f)** IgG leakage in Layer II/III IgG leakage. Note: Tissue infarction can be seen in the top left corner of the image. Across adjacent tissue sections, a major vessel can be seen projecting from the edge of implant toward the tissue infarction. However, single-units were still detected from electrode at this depth. '[]'s show the 1 kHz impedance range of the 3 nearest recording sites. Scale bar = 100 μ m.

Figure 6.

Example multi-labelled histology of WT (a–c) and caspase-1 KO (d–f) 4–6 months post-implant. Vertical columns show tissue from adjacent tissue section of the same animal. Rows show different staining combinations. Recording sites always face left. The recording performance and impedance are shown at the bottom of image. '[]'s show the 1kHz impedance range of the 4 nearest recording sites. **a)** Around Layer IV–V where single-units were not detected. **b)** Around Layer III–IV where single-units were not detected. **c)** Around Layer VI where single-units were not detected. **d)** Layer V–VI where single-units were not detected. **e)** Layer V–VI where robust single-units were detected. **f)** Layer IV–V where robust single-units were detected.

Figure 7.

Example histology of WT (a–c) and caspase-1 KO (d–f) 4–6 months post-implant. PDGFR β + (green) and PDGFR β +/APC+ (yellow) cells can be seen near the implant or along the implant surface. Recording sites always face left. **f**) shows IgG trapped in non-perfused capillaries that project from the surface of the implant. PDGFR β + pericyte cells can be seen along the capillaries and probe/tissue interface surface.

Figure 8. 2 photon imaging of tissue strain *in vivo* from a 4 shank Michigan Electrode Array (a) and a carbon fiber Microthread electrode (b). Neurons are green (Oregon Green Bapta-1 AM) while recording sites and astrocytes are red (PEDOT/PSS/Rhodamine and Sulfarhodamine101, respectively). Cyan outline highlights Microthread electrode. **c–d**) (a) and (b) green channel with neuron shapes highlighted with blue. Neurons in (a) and (c) are much more compressed and oval/elliptical than neurons in (b) and (d), indicating increased mechanical strain from the embedded probe volume.