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A comparison of neuroinflammation to implanted microelectrodes in rat and mouse models

Kelsey A. Potter-Baker [a,b,1], Madhumitha Ravikumar [a,b,1], Alan A. Burke [a], William D. Meador [a], Kyle T. Householder [a,b], Amy C. Buck [a,b], Smrithi Sunil [a,b], Wade G. Stewart [a], Jake P. Anna [a], William H. Tomaszewski [a], Jeffrey R. Capadona [a,b,*]

[a] Department of Biomedical Engineering, Case Western Reserve University, 2071 Martin Luther King Jr. Drive, Wickenden Bldg., Cleveland, OH 44106, USA
[b] Advanced Platform Technology Center, L. Stokes Cleveland VA Medical Center, 10701 East Blvd. Mail Stop 151 AW/APT, Cleveland, OH 44106-1702, USA

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ABSTRACT

Rat models have emerged as a common tool to study neuroinflammation to intracortical microelectrodes. While a number of studies have attempted to understand the factors resulting in neuroinflammation using rat models, a complete understanding of key mechanistic pathways remains elusive. Transgenic mouse models, however, could facilitate a deeper understanding of mechanistic pathways due to an ease of genetic alteration. Therefore, the goal of the present study is to compare neuroinflammation following microelectrode implantation between the rat and the mouse model. Our study suggests that subtle differences in the classic neuroinflammatory markers exist between the animal models at both two and sixteen weeks post implantation. Most notably, neuronal densities surrounding microelectrodes were significantly lower in the rat model at two weeks, while similar densities were observed between the animal models at sixteen weeks. Physiological differences between the species and slight alterations in surgical methods are likely key contributors to the observed differences. Moving forward, we propose that differences in the time course of neuroinflammation between the animal models should be considered when trying to understand and prevent intracortical microelectrode failure.

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1. Introduction

Intracortical microelectrode technology has emerged as a promising tool in both basic neuroscience and functional rehabilitation [1,2]. For example, intracortical microelectrodes have been utilized to functionally map neuronal circuits that may play a role in the progression of neurological diseases [3–6]. Additionally, intracortical microelectrodes have the potential to record neuronal signals, which can be translated into functional outputs such as moving a prosthetic device or computer cursor [7–9]. Unfortunately, long-term clinical use of microelectrode technology is limited primarily due to chronic device instability [10–12]. To this end, multiple studies have been conducted to better understand the failure modes of intracortical microelectrodes. Specifically, recent studies by Prasad *et al.* and Barrese *et al.* have suggested a dominant role of neuroinflammation directly contributing to mechanical, material and biological failure modes following microelectrode implantation [12–14].

Given the correlation between the neuroinflammation and device failure, several groups have begun to implicate the role of individual or small groups of pro-inflammatory molecules in facilitating neurodegeneration, device corrosion or the propagation of the neuroinflammatory response [11,15–21]. For example, Karumbaiah *et al.* and Moshayedi *et al.* each utilized quantitative PCR to identify specific genes (IL-36Ra and TLR-4, respectively) that were up-regulated following microglia responses to increased tissue strain [16,17]. Skousen *et al.* demonstrated the potential correlation between tumor necrosis factor – alpha (TNFα) and neuronal loss at the microelectrode–tissue interface using both computer models and histological assessment of rats implanted with microelectrodes [18]. Furthermore, Potter *et al.* recently provided a correlation between reactive oxygen species accumulation and the up-regulation of TLR-4 and catalase combined with increased neurodegeneration

* Corresponding author. Case Western Reserve University, 2071 Martin Luther King Jr. Drive, Cleveland, OH 44107, USA. Tel.: +1 216 368 5486 (office); fax: +1 216 368 1509.
E-mail addresses: jeffrey.capadona@case.edu, jrc35@case.edu, jcapadona@aptcenter.org (J.R. Capadona).
[1] Co-first authors.

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[19]. While each of these studies suggested a correlation between a specific inflammatory gene or pathway and neuroinflammation, none are capable of determining definitive conclusions regarding the temporal relationship between an individual pathway and microelectrode performance. The inability to draw such definitive conclusions is due to the fact that multiple pathways play a role simultaneously in propagating neuroinflammation following microelectrode implantation [22,23]. In order to draw such conclusions, the field must begin to look at transgenic 'knock-out' or 'knock-in' mouse models.

In contrast to rat models, transgenic mouse models have gained popularity in the biosciences for mechanistic studies aided in the discovery of novel therapeutics. For example, using an Alzheimer's disease (AD) mouse model, Cramer *et al.* recently reported on therapeutics capable of rapidly clearing amyloid plaques [24]. Additionally, the use of a mouse model lacking superoxide dismutase has resulted in the development of therapeutics for amyotrophic lateral sclerosis and AD [25–27].

As the intracortical microelectrode fields moves closer to identifying a specific gene, cell type or neuroinflammatory pathway, transgenic mouse models may provide definitive answers to factors associated with mitigating device failure. In order to investigate a new animal model for the application of intracortical microelectrodes, it is critical establish a comparative baseline between the widely accepted rat model, and the inevitable mouse model. Hence, the goal of the current study was to compare neuroinflammation to implanted intracortical microelectrodes between the rat and the mouse model. In this study, we focused our analysis on the quantification of the most common immunohistological markers used in the field at both initial (2 weeks) and chronic (16 weeks) time points post implantation.

2. Materials and methods

2.1. Animals and surgical implantation

All procedures and animal care practices for both animal models utilized in this study were done in accordance with the Case Western Reserve University Institutional and Animal Use and Care Committee (IACUC). Rats were obtained from Charles Laboratory and age-matched to 7–8 weeks of age (~200–250 g) at the time of surgery. Similarly, C57-BL6 mice were obtained from Jackson Laboratory and age-matched to ~6 weeks of age (~20 g) at the time of surgery.

Surgical procedures followed our previously published protocols for both animal models [21,28,29]. Briefly, rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) administered by intraperitoneal (IP) injection and maintained on anesthesia using isofluorane (1–2%) for the duration of the surgery. Similarly, mice were anesthetized with isofluorane (3–5%) and maintained at 1–2% throughout the surgery. Following administration of anesthesia, the surgical area was shaved and the animal was mounted onto a stereotaxic frame. A subcutaneous (SQ) injection of Marcaine (~100 μl; 0.25%) was then administered below the incision site as a local anesthetic. To ensure a sterile surgical field, the shaved surgical site was scrubbed with alternating passes of betadine and 70% isopropanol. It is also important to note, that all surgical procedures for mice were performed in a class II sterile hood using microisolator techniques. To prevent retinal drying, ophthalmic ointment was used throughout the surgery. Additionally, prior to surgery all animals received the antibiotic cefazolin (16 mg/kg) and analgesic meloxicam (1 mg/kg (rats), 4 mg/kg (mice)) by SQ injection to prevent infection and manage pain, respectively.

An incision was created down midline until lambda and bregma were visible, and the surrounding tissue was retracted to expose the skull. The skull was penetrated using a biopsy punch (P/N #536, PSS Select) to create 3-mm hole, exposing the brain tissue (3 mm lateral to the midline and 4 mm caudal to bregma (rats); 1 mm lateral to the midline and 2 mm caudal to bregma (mice). For rats, the dura was then reflected manually using a 45° dura pick. Dura reflection was not necessary for mice. A non-functional single shank 'Michigan'-type electrode (2 mm × 123 μm × 15 μm) was then slowly inserted into cortical tissue by hand in both animal models, while taking precaution to avoid any visible vasculature. Microelectrodes were implanted approximately 2 mm into the cortex and implant orientation was maintained across all surgeries. All implanted electrodes used in this study underwent ethylene oxide (EtO) gas sterilization [28]. Prior to implantation, all electrodes were degassed for a minimum of 12 h. Following electrode placement, kwik-sil (World Precision Instruments) was applied over the exposed cortical tissue to prevent cortical drying. Inserted electrodes were then securely anchored to the skull using ultra-violet (UV) curing dental acrylic (Fusio/Flow-it ALC, Pentron Dental). Finally, the incision site was closed using 5-0 monofilament polypropylene suture and topical triple antibiotic ointment was applied over the sutured area to prevent drying and infection.

2.2. Tissue extraction and processing

The neuroinflammatory response to intracortical microelectrodes between the rat and the mouse model was compared at pre-determined time points of either two or sixteen weeks [21,28]. Prior to perfusion, animals were heavily anesthetized with an IP injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Each animal was then transcardially perfused with 1× phosphate buffered saline (PBS) until the exudate was clear (approximately 500 mL per rat and 20 mL per mouse). Tissue was subsequently fixed with 4% paraformaldehyde (PFA). Brain tissue was carefully extracted and placed in fresh 4% PFA for 48–72 hours at 4 °C until cryoprotected.

Rat brains were then cryoprotected using a stepwise gradient of sucrose (10%–20%–30%) in 1× PBS. Mouse brains were cryoprotected in 30% sucrose in PBS for 48 hours. Following cryoprotection, both rat and mouse brains were frozen in optimal cutting temperature (OCT) medium (Tissue Tek). Brain tissue was then sliced into either 20 μm (rats) or 16 μm (mice) thick axial sections and directly mounted onto glass slides (SuperFrost Plus). All mounted sections were stored at −80 °C until immunohistochemistry was performed.

2.3. Immunohistochemistry

Two critical time points (two and sixteen weeks) important in the assessment of neurodegenerative events were analyzed and compared between the two models [21,30]. At each pre-determined end-points of two and sixteen weeks, the neuroinflammatory response was assessed between the animal models using common immunohistochemical markers used in the field [31–33].

2.3.1. Antibodies

The following primary antibodies were used to evaluate rat cortical tissue in this study: mouse anti-glial fibrillary acidic protein (GFAP) (1:500, #A-21282, Life Technologies), mouse anti-CD68 (1:100, #MAB1435, Chemicon), mouse anti-neuronal nuclei (NeuN) (1:250, #MAB377, Chemicon) and rabbit anti-immunoglobulin G (IgG) (1:100, #618601, AbD Serotec).

The following primary antibodies were used to evaluate mouse cortical tissue in this study: rat mAb to CD68 [FA-11] (1:500, ab53444, Abcam), GFAP polyclonal rabbit antiserum (1:500, RA22101, Neuromics), rabbit anti-Ms IgG (1:1000, STAR26B AbD Serotec), and mouse anti-NeuN clone A60 (1:250, MAB377, Millipore).

2.3.2. Immunofluorescent antigen tissue labeling

Immunohistochemistry was performed using previously established methodology [28,29]. Briefly, tissue sections were removed from −80 °C and were equilibrated to room temperature (RT) for approximately 15 min. Tissue was then washed three times with 1× PBS to remove remaining OCT and permeated with 1× PBS with 0.1% Triton-X 100 (Sigma) (1× PBS-T) for 15 minutes. Tissue sections were then incubated in blocking buffer (4% v/v serum, 0.3% v/v Triton-X 100, 0.1% w/v sodium azide) for 1 hour at RT. To stain rat tissue sections, goat serum was used in the blocking buffer. Similarly, to stain mouse tissue sections, chicken serum was used in the blocking buffer. Next, primary antibodies (diluted in blocking buffer) were added to tissue sections and incubated for 18–22 hour at 4 °C.

Unbound primary antibodies were then removed using six subsequent washes with 1× PBS-T. Next, species-specific secondary antibodies (anti-rabbit, anti-goat, anti-mouse or anti-rat Alexa-Fluor 488 and anti-rabbit, anti-goat or anti-mouse Alexa-Fluor 594, Molecular Probes) were diluted 1:1000 in blocking buffer and incubated with tissue sections for 2 hours at RT. Total cell population was also labeled in all tissue samples using 4′,6-diamidino-2-phenylindole (DAPI, Life Technologies). Following secondary incubation, unbound secondary antibody was removed using six subsequent washes with 1× PBS-T. Next, detergent (Triton-X) was removed with an additional three washes with 1× PBS. Additionally, tissue autofluorescence was removed using a 10 min treatment with 0.5 mM copper sulfate buffer (50 mM ammonium acetate, pH 5.0) [29]. Finally, slides were thoroughly rinsed with ddH_2O and coverslipped using Fluoromount-G (Southern Biotech). All slides were air dried at RT at then stored in the dark at 4 °C.

2.3.3. Chromogenic labeling of neuronal nuclei

For mouse tissue, neuronal nuclei densities were assessed using a Diaminobenzidine (DAB) histochemistry kit (SuperPicture Polymer DAB kit, Life Technologies) using methods previously described [28]. Briefly, tissue sections were permeated for staining as described in Section 2.3.2. Sections were then blocked in blocking buffer (containing goat serum) for 1 hour at RT. Following blocking, primary antibody (diluted in blocking buffer) was added to tissue sections and incubated for 1 hour at RT. Unbound primary antibody was then removed with three washes of 1× PBS. Next, 100 μL of horseradish peroxidase (HRP) polymer conjugate was added to each tissue section for 10 minutes at RT. Unbound HRP was then removed with three washes of 1× PBS. Next, 100 μL of DAB chromogen was added to each tissue section for 5 minutes at RT. Finally, slides were thoroughly