Disruption of Endogenous Damage Receptor Signaling to Improve Performance of Intracortical Microelectrodes

John K. Hermann, Madhumitha Ravikumar, Jessica K. Nguyen, Shruti Sudhakar, Priya Srivastava, Jeffrey R. Capadona
Case Western Reserve University

Statement of Purpose: Intracortical microelectrodes have the potential to restore function and sensation to severely paralyzed individuals[1]. Unfortunately intracortical microelectrodes are unable to function reliably long term. Failure is thought to be associated with the tissue response to the implanted microelectrode, especially neuronal loss, blood-brain barrier disruption, and microglial activation[2-5]. Activation of microglia in response to necrotic cells and infiltrating serum causes the release of pro-inflammatory cytokines and reactive oxygen species, which are harmful to the blood-brain barrier and neurons. Toll-like receptors 2 (TLR2) and 4 (TLR4) on the surface of microglia recognize necrotic cells and infiltrating serum[6, 7]. Co-receptor cluster of differentiation 14 (CD14) coordinates the ligand binding of TLR2 and TLR4, as well as other Toll-like receptors. Therefore, the current study investigated if TLR2, TLR4, and CD14 inhibition will attenuate intracortical microelectrode mediated neurodegeneration and improve intracortical microelectrode performance.

Methods: TLR2, TLR4, and CD14 knockout mice (*Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, and *Cd14*$^{-/-}$, respectively) were implanted in the cortex with microelectrodes for 2 or 16 weeks. Horizontal sections of cortical tissue were stained for immunohistochemical markers evaluating microglial activation, astrocytic encapsulation, blood-brain barrier disruption, and neuronal dieback. We are currently implanting transgenic mice lacking the CD14 co-receptor with functional microelectrodes to record neural activity for up to 16 weeks. The quality and stability of neural signals will be compared to the performance of identical devices implanted in control wildtype animals. Histological evaluation will track both neuroinflammation and blood-brain barrier stability over time. Electrophysiological evaluation will correlate neuroinflammation to device performance over time.

Results:

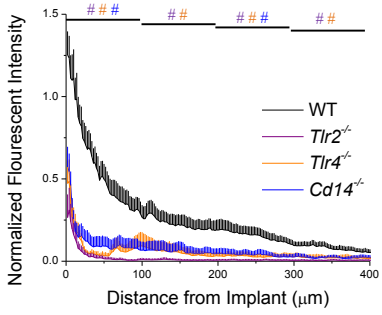

Figure 1. Microglial activation reduced in *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, and *CD14*$^{-/-}$ mice relative to wildtype mice at two weeks post-implant. # = ($p<.05$ vs wildtype).

All three knockout conditions demonstrated significant ($p<.05$) reductions in microglial activation at the two week time point (Fig. 1). *Cd14*$^{-/-}$ mice also demonstrated significantly reduced astrocytic encapsulation at the two week time point. *Tlr2*$^{-/-}$ mice demonstrated significantly reduced blood-brain barrier permeability at both the 2 and 16 week time points. *Cd14*$^{-/-}$ mice demonstrated enhanced neuronal survival around the implanted electrode at the 2 and 16 week time points (Fig. 2).

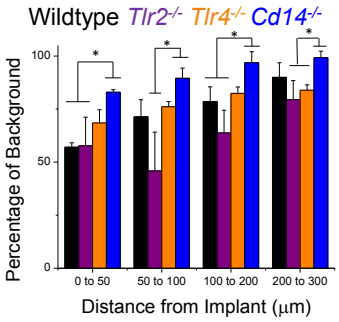

Figure 2. NeuN positive cells as a function of distance from the microelectrode surface at 16 weeks post-implantation * = ($p<0.05$).

Conclusions: Removal of endogenous damage receptor signaling pathways effectively reduced inflammation in response to an implanted intracortical mictroelectrodes. However the downstream consequences of each specific pathway are not uniform. For example, *Tlr2*$^{-/-}$ mice, missing a receptor involved in the recognition of necrotic cell debris, had a more stable blood-brain barrier and reduced microglial activation, but did not demonstrate reduced neuronal dieback around the implant site. *Tlr4*$^{-/-}$ mice, missing a receptor involved in the recognition of serum proteins, did not exhibit any significant improvements beyond microglial activation. *Cd14*$^{-/-}$ mice, missing a co-factor to both TLR2 and TLR4 signaling pathways, demonstrated reduced astrocytic scarring and neuronal dieback relative to wildtype mice. Thus, *Cd14*$^{-/-}$ mice were chosen for ongoing functional recording studies. The reduction in neuronal dieback in *Cd14*$^{-/-}$ mice may translate to higher quality signals and improved stability in the long term. In addition, the reduced astrocytic scarring may also translate to reduced recording impedance.

References:

1.Hochberg, L.R., Nature, 2012. **485**(7398): p. 372-5.
2.Saxena, T., Biomat. , 2013. **34**(20): p. 4703-4713.
3. Rennaker, R.L., J Neural Eng, 2007. **4**(2): p. L1-5.
4. McConnell, G.C., J Neural Eng, 2009. **6**(5): p. 056003.
5. Tresco, P.A. and B.D. Winslow, Crit. Rev. in Biomed. Eng., 2011. **39**(1): p. 29-44.
6. Sims, G.P., Annu. Rev. Immunol., 2010. **28**: p. 367-388.
7. Beg, A.A., Trends in Immunol., 2002. **23**(11): p. 509-512.

Funding: Funding from PECASE, Case Western Start Up Funds, the Dept. of Veterans Affairs issued Career Development Award-2 for J. Capadona (Grant No. B6344W) as well as Dept. of Veterans Affairs Merit Review (Capadona, Grant No. B7122R), NIH NINDS (R01 NS 082404), and the APT Center are gratefully acknowledged.