BLOOD DRAWS TO ASSESS BIOMARKERS OF INFLAMMATION IN PERIPHERAL BLOOD MONOCYTES IN RESPONSE TO CHRONIC IMPLANTS FOR DEEP BRAIN STIMULATION (DBS)

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INTRODUCTION

- Chronic implants cause an inflammatory response that can negatively impact the function of the implant. However, current methods to assess inflammation are terminal involving histology.
- Implant stimulation could exacerbate inflammation that in turn, can impact the efficacy of DBS therapy requiring frequent tuning by an expert.
- Glial fibrillary acidic protein (GFAP), expressed in reactive astrocytes, is a widely used biomarker to assess brain tissue response to inflammatory stressors.
- Peripheral blood monocytes (PBMCs) are recruited to the brain to clear inflammation debris and can re-enter the blood stream.
- We propose here a non-invasive method for assessing the GFAP response in brain tissue using blood-draw that examines the peripheral blood monocytes

METHODS

- We used the following 4 experimental cohorts (n=3 rats in each cohort implanted with 4 teflon-insulated tungsten electrode arrays) to assess GFAP levels in PBMC extracts:

  Cohort 1: surgery, craniotomy, no implant
  Cohort 2: surgery, implant, no electrical stimulation
  Cohort 3: surgery, implant, electrical stimulation, **Stim1** - 9 nC/phase and 25 μC/cm^2 with a GSA of 35,000 μm^2 (below Shannon limit)
  Cohort 4: surgery, implant, electrical stimulation, **Stim2** - 36 nC/phase and 1834 μC/cm^2 with a GSA of 1900 μm^2 (above Shannon limit)

- For each rat ~1 ml of blood was drawn 2 weeks before and at various times after surgery, followed by a terminal bleed 10 weeks after surgery
- Cohorts in experimental conditions 3 & 4 were stimulated every day for 1 hr for the first 4 weeks
- Stimulation conditions were biphasic, charge-balanced at 100 Hz with amplitude of 180 μA and 50 μs/phase (condition 3) or 360 μA and 100 μs/phase (condition 4)
- Blood samples were analyzed by a) fluorescence-activated cell sorting (FACS) to measure the number of GFAP containing cells and b) single-sided ELISA (ELISA) to determine GFAP levels in extracts of peripheral blood monocytes at ZelosDX, Tucson, AZ
- Electrical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were performed periodically

RESULTS

- In cohort 3, there was a 3- to 4-fold increase in GFAP expression at 24 hours and 2 weeks post-implantation compared to baseline.
- The levels of GFAP were found to decrease progressively at 4-, 6-, and 10-weeks post-implantation in cohorts 2 and 4.
- Results of GFAP in PBMC extracts in all the cohorts are consistent with terminal GFAP assessments in brain tissue using immunohistochemistry techniques.
- FACS analysis of the percentage of monocytes in the blood showed a similar GFAP expression profile over time of implantation, but did not show any significant differences between cohorts 3 & 4
- EIS and CV measurements showed consistent electrode function during experiments

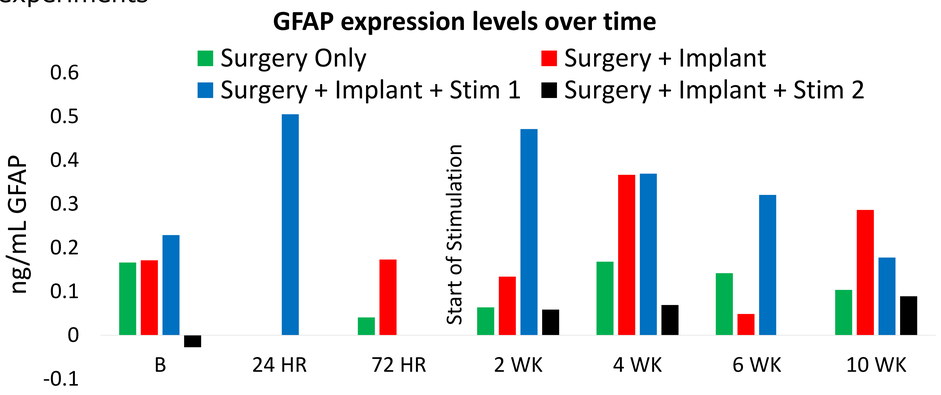


Figure 1: Mean GFAP expression levels in PBMC extracts over time in different cohorts.

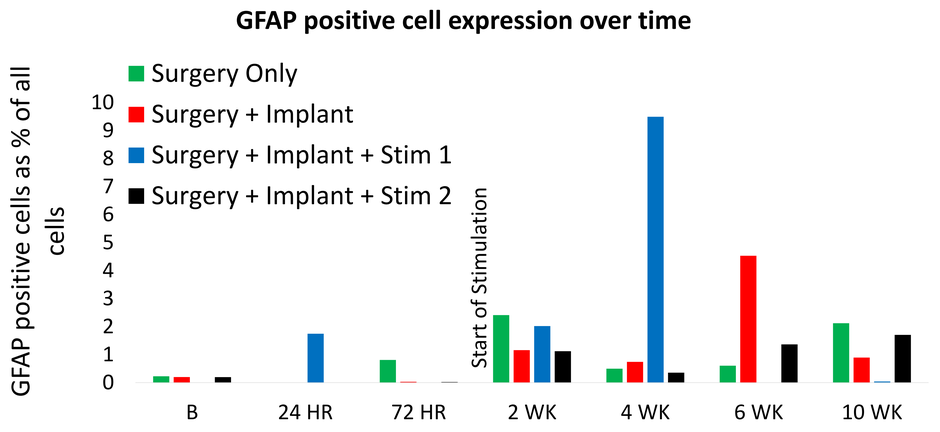
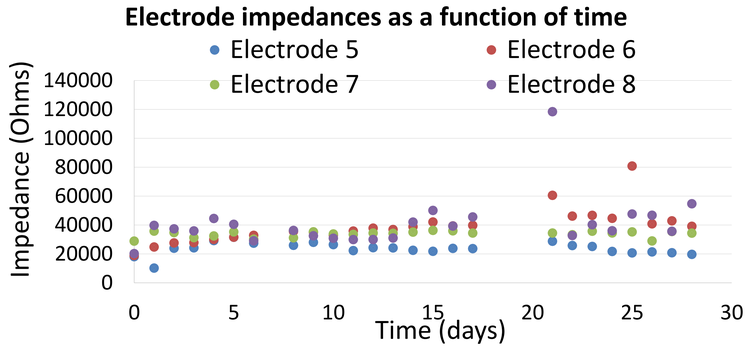


Figure 2: Percentage of GFAP positive cells (determined using FACS) over time in different cohorts.



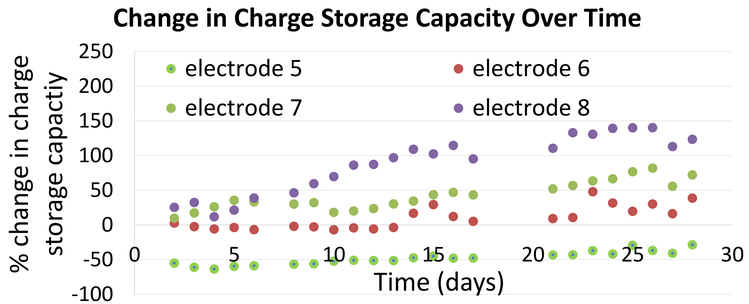


Figure 3: Typical impedances at 1 kHz (top) and charge storage capacity (bottom) in an array over the duration of a chronic experiment show no significant changes.

- Electrical impedances at 1 kHz and charge storage capacities did not change significantly during the experiment indicating the integrity of the neural interface.
- The proposed novel assay involving PBMCs allows for multiple GFAP assessments at 2-week intervals in longitudinal rodent experiments for the first time.
- This will allow us, for the first time, to track changes in *expression of GFAP in rodent brain tissue* due to any interventions introduced during the experiment.

CONCLUSION

In conclusion, we have shown that the novel blood-based assay is capable of detecting trace amounts of GFAP in response to microelectrode implantation and DBS activation in rodent peripheral blood monocytes in chronic experiments

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