FY19 PRARP Overarching Challenge: quality of life, devices benefiting those living with TBI and AD/ADRD.

FY1 PRARP Focus Area: quality of life; nonpharmacological inventions and devices.

Background – Alzheimer's Disease (AD) and traumatic brain injury (TBI). Dementia, broadly defined, incurs devastating individual human cost with an increasingly substantial societal burden. AD represents the clinically most diagnosed form of dementia, seconded by vascular dementia (Masters et al, 2015). With regard to TBI and AD, Schaffert et al (2018) found in their epidemiological study that a history of TBI with loss of consciousness represented a meaningful risk factor for the onset of AD – importantly, as confirmed by autopsy. As reviewed in Van Den Heuvel et al (2007), these epidemiological observations comport with the observed rapid (hours to days) accumulation of excessive amyloid precursor proteins (APP), then Aβ in soluble and plaque forms, within and around traumatically injured axons after moderate to severe TBI. Roberts et al (1994) is an early example of an autopsy-based study demonstrating this rapid onset after severe TBI in 30% (13/40) patients, days to about a week after injury, with more Aβ accumulation in older patients. Marklund et al (2014) also observed rapid production of APP and Aβ using cerebral microdialysis in 10/10 severe head injury patients up to 14 days after injury.

Motivation – *Ultrasound can address AD histopathology and behavior, in vivo* . We note that there exists recently evolving literature demonstrating that transcranially delivered ultrasound can temporarily and safely activate central neural circuits, shown in rodents, sheep, primates, and humans (reviewed in Bobola et al, 2018; Blackmore et al, 2019). Motivated by this literature and by Iaccarino et al, our preliminary data demonstrates that transcranial, pulsed, and focused ultrasound, delivered with a PRF of 40 Hz can activate 5XFAD (C57BL6) mouse brain acutely at 40 Hz and activate microglia to co-localize with Aβ plaque relative to sham after one hour of ultrasound plus an additional hour of sedation. Moreover, daily exposure for one week of each hemisphere of 5XFAD (C57BL6) mouse brains to the same ultrasound protocol shows a quantitative reduction in Aβ plaque in each hemisphere relative to sham application.

Hypothesis. Optimized transcranial near-diagnostic ultrasound can improve AD symptoms after TBI, *in vivo*, through activation of microglia and upregulation of eNOS that reduce Aβ and tau burdens

Aim #1: Optimize, acutely, tnDU activation of microglia and upregulation of eNOS, in AD brains.
Proposed study: Expose one brain hemisphere per mouse of male and female wild type C57BL6**,** 5XFAD (C57BL6) and APPPS1 AD mice to tnDU that generates 40 Hz brain activity, using the other hemisphere as a control. Optimize within the tnDU field the percentage of activated microglia that overlap with Aβ, acutely, and eNOS upregulation, after 24 hours, guided by histological analysis that highlights candidate biological impacts of ultrasound on AD brain. *Anticipated outcomes:* Identification of two optimal tnDU protocols, one maximizing co-location of activated microglia and Aβ, the other, upregulation of eNOS, all within the ultrasound field and relative to the contralateral hemisphere.

Aim #2: Test the ability of tnDU protocols from Aim #1 to improve AD symptoms after TBI.
Proposed study: Apply or sham apply optimal tnDU protocols from Aim #1 to each brain hemisphere of separate cohorts of male and female C57BL6 and female 3xTG AD mice, at different ages and after induction of contusion-based TBI in one brain hemisphere. Assay AD mouse behavior before and after TBI as well as before and after tnDU therapy, itself delivered for one week starting after TBI induction. Euthanize subsets of mice at several time points during this study and perform histological analysis.
Anticipated outcomes: Identification of two tnDU protocols that improve learning and memory deficits after TBI, one that correlates with activation of microglia co-localized with Aβ, the other, with increased markers for cerebral vasodilation, each in turn correlated with reductions in Aβ and tau burdens, with all correlations varying inversely with mouse age at time of TBI.

Innovation. We propose refinement and testing of an entirely new paradigm, ultrasound, to treat AD after TBI, and possibly other dementias, through activation of microglia and/or induction of cerebral vasodilation.

Anticipated impact. Here we seek to determine whether or not ultrasound applied transcranially to the brains of mouse models of AD after TBI can improve memory-related behavior, through induction of a combination of activated microglia into a phagocytotic rather than pro-inflammatory state, and cerebral vasodilation (where vasodilation would likely produce additional benefits) that reduce Aβ burden. We anticipate that the useful ultrasound protocols we identify will approach the near diagnostic intensity levels of ultrasound shown safe in mouse, rat, sheep, rabbit, non-human primate and human brains. Therefore, successful completion of the proposed work could lead relatively rapidly to preclinical human trials for safety then efficacy of our approach applied to AD and, possibly, to other dementias, after TBI.