PROJECT SUMMARY (See instructions):

Ideally one would want to study the brain by examining the cellular properties of individual neurons interconnected within circuits *in vivo*. Current state of the art methods for single-neuron studies in living tissue are time-consuming, labor intensive, expensive, and more importantly, can capture information from only a few cells pooled together. Here, we propose a new method that uses femtosecond laser ablation to dissect and isolate many single neurons from living tissue and has the potential to give simultaneous information about a cell's transcriptome and potentially proteome. The method will be used to investigate encoding of emotional valence in the basolateral amygdala of mice through next generation RNA-sequencing of neuronal ensembles. We hypothesize that our approach will overcome current technological limitations and enable transcript information to be obtained from groups of individual neurons activated during a behavior. To process the large amounts of single neurons expected from dissection, we also propose a novel, inexpensive, and relatively unbiased RNA library preparation protocol for RNA-sequencing.

Femtosecond laser capture microdissection (fs-LCM) is suitable for capturing neurons from three-dimensional tissue slices thanks to its high resolution, which allows dissection of small, individual neurons without damaging the soma. Additionally, the method can isolate many single neurons from the same tissue without pooling cells together, enabling the study of interconnected neuron circuits at the single-neuron level for the first time. The novel *Arc-CreER:Ai14* mouse strain developed at the University of Texas at Austin will be used to obtain BLA sections with neurons labeled during fear or reward learning. *Arc-CreER:Ai14* mice are engineered to express the fluorescent marker tdTomato in neurons activated by a particular behavioral manipulation. A custom built dissection microscope will identify labeled neurons through two-photon autofluorescence imaging overlapped on the large field of view with fluorescence images that will be collected simultaneously. A femtosecond laser will then precisely ablate connective tissue surrounding the targeted neuron without damaging it. Once freed from the tissue, the neuron will be collected by a high-precision micropipette system and deposited directly into a microcentrifuge tube containing RNA fragmentation buffer for library preparation. By lysing the cell into fragmentation buffer, our proposed library preparation method omits expensive and time-consuming steps that are unnecessary for single neurons.

We expect to discover mechanisms underlying the recruitment of valence-encoding neurons in fear and reward responses in mice. The femtosecond laser microdissection method will provide a novel and scalable approach to gathering full transcriptome data from many single cells without sacrificing their integrity or information regarding their location within the tissue ensemble. We expect the approach to push forward the development of additional single-cell analysis methods. Our novel RNA library preparation method is expected to make single-cell transcriptome analysis more accessible by reducing protocol complexity and costs while maintaining accuracy.

RELEVANCE (See instructions):

Our proposed project forms a new collaboration between teams from diverse colleges (Engineering, Pharmacy, Medicine, and Natural Sciences) at the University of Texas at Austin. The group is highly interdisciplinary, with expertise in laser dissection (Ben-Yakar), two-photon microscopy (Ben-Yakar), neuroscience (Messing, Mayfield), and next-generation sequencing and analysis (Mayfield, Hunicke-Smith). Our project will develop a new technique to isolate multiple single cells within behaviorally activated ensembles for complete cellular analysis, which is not possible with current methods. Using our technique we hope to uncover new information about the cellular phenotypes of neurons that regulate fear versus reward behaviors. The UT BRAIN initiative will enable us to obtain preliminary results that will lead to applications for larger NIH grants to expand and scale our methodology for more extensive studies of neuronal ensembles that regulate specific behaviors. With full automation, this new laser dissection method has the potential to map the full transcriptome, genome, and proteome of individual neurons across the whole brain.