Identification Of Axonal Projections Through Deep Brain Stimulation Locations In Mouse
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Introduction
Deep Brain Stimulation (DBS) electrode locations are determined by sub-thalamic nuclei location. However, the primary target of the stimulation is the fiber bundles that pass through these nuclei. Fiber pathways do not appear in histological staining or physiological atases. The Allen Brain Institute has developed a mouse connectivity atlas using viral tracers to map axonal projections. Previous work in our lab has found that differences in lead location vary with behavioral outcomes and stimulation locations in the thalamus have been identified that are correlated to increased arousal, based upon three separate behavioral scores, in mice with traumatic brain injury (TBI).

Objectives
1) Integrate all data and identify fibers bundles passing through stimulation sites
2) Identify common projection targets and the density of projections in these areas

Methods
Behavioral Evaluation: Three behavioral data measures were collected. Horizontal Activity: fidgeting movements, collected by the home cage Accuscan system representing the number of infrared beams broken in the horizontal plane. Total Distance: ambulation, representing non-repeating infrared beam breaks in the horizontal plane. Counts: whole body activity, collected by DSI transmitter and representing changes in field strength between transmitter and receiver as the mouse moves.

Regions of Interest: DBS lead location was determined from post-mortem histology. The volume of tissue activation (VTA) was predicted at this location (Butson et al. 2007). The mouse brain was voxelized and a probabilistic stimulation atlas (PSA) was constructed from each VTA (Butson et al. 2011). Behavioral scores were mapped onto the PSA and averaged at each voxel. The voxels that had an average score above 90% improvement for each of the three behavioral outcomes were used as the ROI.

Results
Connectivity Data: The axonal projection data from the Allen Institute was created with fluorescent viral tracers and two-photon microscopy. Each experiment consisted of a single injection location and 3D reconstruction of the fluorescent axonal projections after approximately 21 days. The experiments used in this analysis were filtered by injection locations only in the thalamus. This gave us a databank of 55 projection experiments. Translation and rotation matrices were applied to transform the projection data into Bregma coordinate space.

Projection Conglomeration: Each projection line in every experiment was first filtered by a normalized intensity value of 0.1. Any projection below this threshold was discarded. With regions of interest (ROIs) identified from the behavioral evaluation. A Euclidean Distance filter was implemented to find all projections within a specified distance of the ROIs.

Conclusion
With this approach we are able to analyze a large projection dataset from the Allen Institute and filter it down to meaningful projections that can be integrated into our own dataset. These projections give us more insight into the possible structures in the cortex that are indirectly targeted by stimulation in the thalamus through these axonal projections. These projections might also provide insight into a potential source of variability in arousal response to stimulation and important DBS targeting locations.

Acknowledgements
Funding provided by the Department of Bioengineering and the Scientific Computing & Imaging (SCI) Institute, University of Utah.