NOTE: The study outlined below falls within the scope of an NIH R01 proposal, with the highlighted experiments to be supported by this seed grant. We hope to collect proof-of-principle preliminary data from these experiments to support a future NIH R01 application.

The striatum, a key player in decision-making, learning, and reward processing, is implicated in various neurological and psychiatric disorders. The tail striatum resides at the caudal end and receives sensory information from the thalamus and cortex. Recent studies have highlighted the tail striatum's unique function in sensory-cued, outcome-driven decision-making. These behaviors, established through associative learning, involve neuronal plasticity-driven changes in sensory perception. Since neurons are actively modulated by the local network, including glial cells, the research focus has been primarily on neuronal circuits. Despite these, the role of tail striatal glial cells in sensory perception during associative learning has not been extensively studied and remains largely unexplored.

Our previous research revealed essential roles of the tail striatum in two distinct auditory-cued behaviors: auditory frequency discrimination and fear conditioning. Acquisition of both behaviors involves associative learning where auditory perception is adapted by altered representations of auditory cues in tail striatal spiny projection neurons (SPNs). Importantly, inhibition of neuronal plasticity in the tail striatum impaired learning in both behaviors, highlighting its crucial role. These findings prompted us to investigate the underlying mechanisms driving the formation of neuronal plasticity in this region. In collaboration with the Wu Lab (co-PI), a bioinformatics group, we performed single-cell RNA sequencing (scRNAseq) to profile the transcriptome of the tail striatum. Comparing the transcriptomes of naïve and trained mice, we identified a cluster of genes upregulated specifically in SPNs of trained mice. Intriguingly, many of these genes are linked to glial function, particularly astrocyte function.

Astrocytes are known for their diverse mechanisms in modulating synaptic transmission and plasticity. To investigate whether they contribute to tail striatal function in auditory-cued behaviors, we employed an *in vivo* Ca^{2+} imaging method to analyze their activity during associative learning. A preliminary result showed that cue-evoked Ca^{2+} responses in tail striatal astrocytes was significantly potentiated after fear conditioning, mirroring the increases in cue-evoked neuronal response and behavioral response (freezing). This finding aligns with the possibility that astrocytes modulate tail striatal neuronal plasticity during learning. Furthermore, scRNAseq analysis provides us with potential pathways mediating interactions between astrocytes and neurons in the tail striatum during learning.

Building on our pilot studies and established research, we hypothesize that tail striatal astrocytes actively contribute to neuronal plasticity, supporting changes in auditory perception during associative learning. To test this hypothesis, we propose a series of experiments utilizing established methods from the Xiong laboratory (PI), including mouse behavioral paradigms, *in vivo* Ca^{2+} imaging, chemogenetic manipulations, and viral and histological techniques. Additionally, the expertise of the Wu laboratory (Co-PI) in bioinformatics and biostatistics will be crucial for analyzing and interpreting scRNAseq data.

Aim 1 To examine the activity of tail striatal astrocytes during associative learning. We will first utilize transgenic mice and confocal imaging to determine whether learning induces alterations in the morphology and cell density of tail striatal astrocytes. Next, using *in vivo* Ca^{2+} imaging in freely behaving mice, we will monitor the dynamics of Ca^{2+} signaling in tail striatal astrocytes throughout the learning period. This aim will establish a potential correlation between trail striatal astrocyte activity and associative learning.

Aim 2 To determine the regulatory role of tail striatal astrocytes in auditory perception during associative learning. We will first selectively eliminate astrocytes in the tail striatum and assess their necessity for successful associative learning. We will then investigate whether astrocytic Ca^{2+} signaling plays a role in learning by employing viral vectors to specifically suppress Gq signaling in tail striatal astrocytes. Lastly, we will explore the link between astrocytes and learning by examining whether the above manipulations lead to changes in auditory cue representations in tail striatal SPNs. Establishing such a connection would solidify the hypothesis that tail striatal astrocytes regulate auditory perception during associative learning.

Aim 3 To determine signaling pathways underlying tail striatal astrocyte-neuron interactions during associative learning. We will further analyze our scRNAseq results to identify a focused set of candidate genes likely involved in astrocytic modulation of SPNs. Based on the prioritized candidates, we will then generate shRNA viral vectors to specifically knockdown their expression in tail striatal astrocytes. Finally, we will combine the gene knockdown approach with *in vivo* Ca^{2+} imaging to determine how manipulating specific signaling molecules affects both auditory cue representations in tail striatal SPNs and overall learning performance. By observing changes in these measures, we can directly evaluate the roles of the identified signaling molecules in astrocyte-neuron communication during learning.