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Classification of Hippocampal Interneuron Types Using Optical Clearing and Immunostaining

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Although hippocampal neuronal activity is required for spatial navigation and learning, little is understood about how the pyramidal neuronal network is controlled during these behavioral states. The wide anatomical and molecular diversity of hippocampal interneurons suggests that these neurons play a critical role in shaping network activity and plasticity. To better understand the role of interneurons during ongoing behavior, we recorded the calcium activity of identified interneuron types from head-fixed animals performing a spatial navigation task in virtual reality. By using cre-driver lines to drive calcium indicator expression, we can study the activity patterns of broad classes of interneurons; however, more precise tools are required to further subdivide these categories so that function can be mapped onto individual interneuronal cell-types. To do this, we performed post-hoc brain clearing and antibody staining using the AbSca*l*e method. AbSca*l*e uses urea, sugar alcohols, and various ionic detergents to wash out lipids and clear intact tissue. We are able to preserve genetically encoded calcium indicator fluorescence while using immunofluorescent labeling to stain for markers of different subtypes of interneuron. These technical advances will allow us to assign functional activity to specific subtypes of interneurons in order to elucidate how diverse interneuron types interact to regulate hippocampal activity during movement and learning.