INTRODUCTION
The locus coeruleus (LC) is a brainstem nucleus known best for being the primary site of noradrenaline (NA) production and has wide projections throughout the cortex. NA acts via 3 classes of receptors (α1, α2, β) and this signaling is critical for optimization of cognitive performance. NA fiber density has not been investigated with density of all 3 NA receptor types, and no protocol exists for fluorescence immunostaining analysis of NA receptors in primates. Here we developed a protocol for staining the NA system along with other cell types known to express noradrenergic receptors.

METHODS

Immunocytochemistry Procedure

Tissue preparation: Tissue from a cohort of adult and aged rhesus macaques was used for this study. Following perfusion, brains were fixed in a solution of 4% PFA and 30% sucrose at 4°C. All brains were cut into 30µm sections in the coronal plane.

Immunostaining Procedures: On the day of staining, sections were first washed in Tris Buffer Solution (TBS). Tissue then underwent an antigen retrieval procedure. Before immunostaining procedures, tissue was washed in TBS, 3% Normal Donkey Serum, and 0.3% TritonX-100. Brain sections were incubated in primary antibodies (see Table 1) overnight. The next day, the tissue was washed in TBS, incubated in secondary antibodies for 2 hours, washed again in TBS then mounted with 80% glycerol in TBS.

Control Immunostaining Experiments: Each antibody was tested at multiple concentrations to find the optimal combination of staining. Control experiments were performed to prove specificity of primary and secondary antibodies. Primary antibodies were preadsorbed with a peptide corresponding to their immunogen sequence and then incubated with the tissue as described above (Figure 1). Anti-adrenergic receptor antibodies were incubated with all other primaries and secondaries, and this was compared to preparations with all antibodies but only the secondary for the receptor (Figure 2). In preparations where primary antibodies were omitted, secondary antibodies did not show any detectable staining.

Visualization: Tissue was imaged at 10, 20, and 40x using a Zeiss LSM 880 confocal microscope. Zen Blue was used to process images.

RESULTS

Figure 1: Characterization of the immunostaining specificity of anti-α1, anti-α2a, and anti-β1 receptor antibodies

Figure 2: Test of primary antibody layer interactions in triple immunofluorescence staining

Table 1: Antibodies and markers used

Table 2: Immunostaining procedure

Table 3: Quantitative analysis

Table 4: Immunostaining solutions

Figure 3: Visualization of the adrenergic receptors alongside adrenergic fibers, microglia, astrocytes, and blood vessels in the hippocampus

CONCLUSIONS
We have developed a staining protocol for the visualization of noradrenergic fibers and receptors in the rhesus macaque hippocampus. Next, we developed a triple immunofluorescence staining with astrocytes, microglia, and blood vessels for visualization of noradrenergic receptors on different cell types. Staining of the α1 receptor was visible on large cell bodies as well as fibers. α2a was predominantly found on fibers, but was present on some cell bodies. α2a also colocalized with microglia as well as vasculature. β1 staining was also evident on both cell bodies and fibers, and colocalized with vasculature. Notably, cells immunopositive for the β1 receptor are smaller than those marked by the α receptor antibodies.

A future direction of this project is to analyze age-related alterations in the density of noradrenergic receptors and fibers.