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Quantifying neuronal morphology in a seasonally reproducing rodent, Richardson's ground squirrel (*Urocitellus richardsonii*).

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Introduction

- Seasonal neuroplasticity, neuroanatomical changes in the brain that occur according to season, occur across a range of vertebrate species.
- In songbirds, seasonal neuroplasticity is well studied and the neuroanatomical changes vary across different brain regions.
- Small mammals also undergo seasonal changes in brain anatomy, but are poorly understood.
- Previous studies of hippocampal anatomy in voles and ground squirrels revealed that seasonal neuroplasticity interacts with sex differences such that males differ from females in the breeding season, but not the non-breeding season.
- The extent to which these seasonal variations are the result of changes in neuronal morphology is unknown.
- Our goal is to test for seasonal and sex differences in neuronal morphology in a seasonally reproducing wild rodent with marked sex differences in behaviour: Richardson's ground squirrel.
- We predict that the hippocampal pyramidal neurons will be the largest and most complex in non-breeding season males. This sex difference will disappear in the breeding season, as breeding males will have smaller and less complex pyramidal neurons, which will be comparable to females.

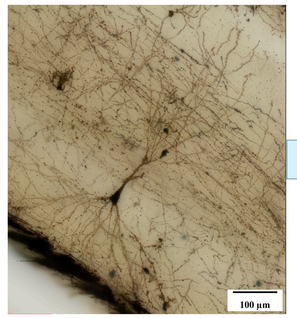
Methods

- Richardson's ground squirrels were live trapped in the field.
- Trapping and processing animals was aided by our field vehicle and mobile lab trailer.

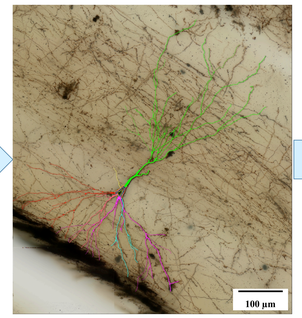
- The brains were extracted in the field and placed immediately into Golgi fixative.
- Following 2 weeks in Golgi solution, the brains were sectioned at 200 μm on a vibratome.

Virtual Microscopy

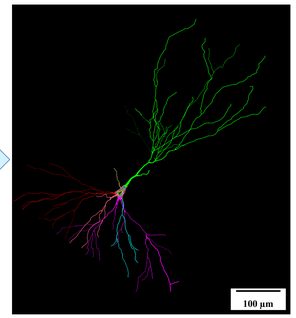
- We first created virtual slides using an Olympus VS-120 slide scanner.
- Using a 40x oil immersion lens (NA = 1.3), z-stacks were scanned at a distance of 0.6μm apart throughout the section.
- The resulting virtual slide was uploaded and neurons traced using Neurolucida 360.
- Hippocampal pyramidal neurons were chosen based on simple criteria, no truncation, full effective staining, not obscured by other neurons and no pixelation in the image scans.
- Neurons were traced in both CA1 and CA3 regions of the hippocampus.



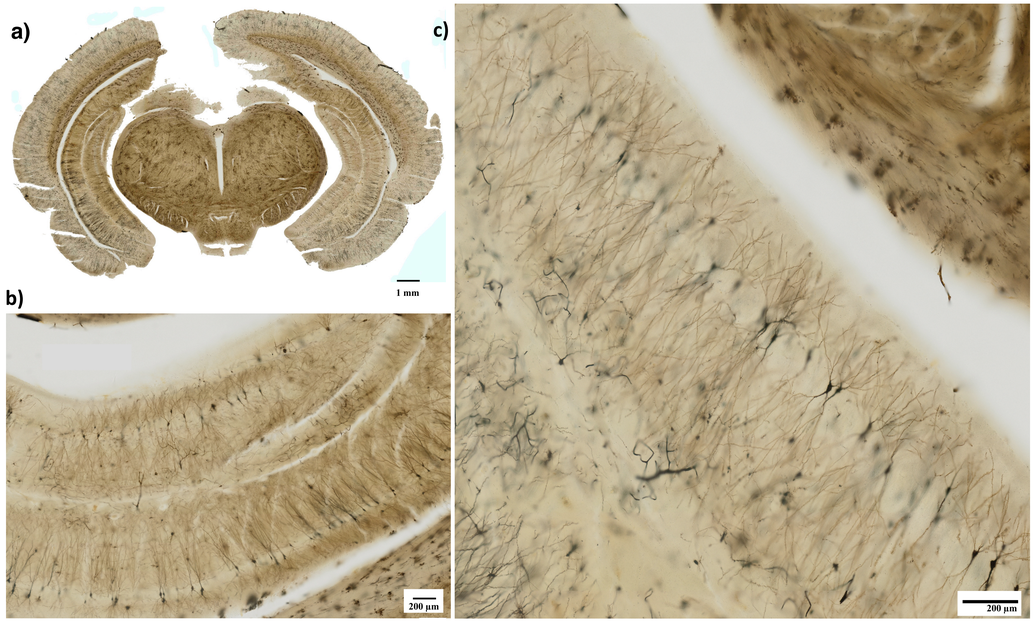
Virtual slide image



Neuron traced on virtual slide

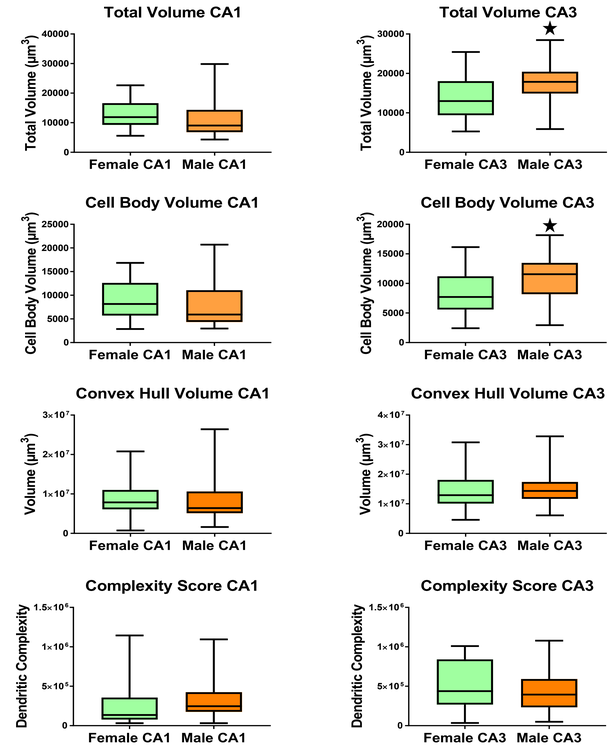


Reconstructed CA3 neuron



Shown above are examples of virtual slides of our Golgi stained tissue: **a)** an overview scan of a caudal section through a Golgi stained Richardson's ground squirrel brain. **b)** and **c)** magnified views of the hippocampus in the same specimen.

Results



- CA3 total cell volume and cell body volume were significantly larger in non-breeding males than females. No significant differences in CA3 Convex hull volume or complexity score.
- No significant sex differences in CA1 neurons.

Discussion

- Although we found a significant sex difference in the size of CA3 neurons, our analyses are thus far constrained to non-breeding.
- Currently, we are doing similar analyses of breeding season males and females to determine if there are seasonal effects.
- Quantification of dendritic spines will also be completed using virtual slides at higher magnification (100x).

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