Exploring Retinal Projection to the Medial Amygdala: Laterality, Sex, and Cell Types

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Abstract

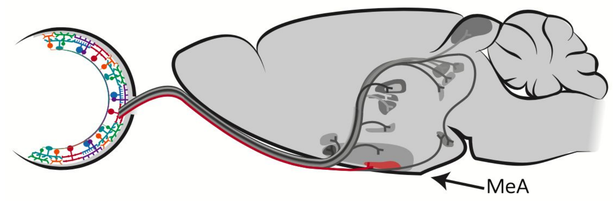
How do Retinal Ganglion Cells project to the medial amygdala when we consider laterality, sex, and subtypes?

In the mouse brain, there are 59 retinorecipient regions, including the medial amygdala (MeA). The MeA is a sensory integrating region where social information is processed, especially those related to sexual selection, aggression, and pup retrieval. The neurons that link visual input to the brain are known as retinal ganglion cells (RGCs). There are ~47 mouse RGC subtypes, each with their own light stimulus sensitivities and activity patterns. As the MeA is notorious as the sexually dimorphic social behavioral hub, it is worthwhile beginning to explore sex differences or laterality differences in this retinorecipient area. Using anterograde, intravitreal injections to label RGCs, we measured the retinal terminal densities at the MeA from cable length, fill volume, tortuosity, and branch points, differences between the left MeA and the right MeA in male and female mice were explored. To identify which RGC subtypes are expressed in the MeA, we used functional and morphological analysis after retroviral tracing. These findings determined a broad overrepresentation of direction-selective and orientation-selective subtypes. Determining the functional specificity of RGCs projecting to the MeA may provide insight on the role of visual input in these medial amygdala-related behaviors.

Introduction

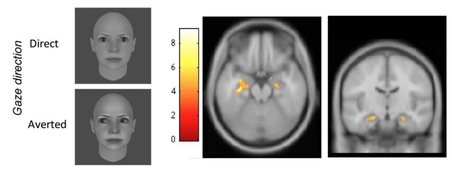
Medial Amygdala

- In the mouse brain, there are about 59 retinorecipient regions, including the medial amygdala (MeA).
- The MeA is a region responsible for processing social cues involved in aggression, mating, recognition, and more.
- Retinal ganglion cell subtypes projecting to the MeA and their impacts on MeA associated behavior is not greatly understood.



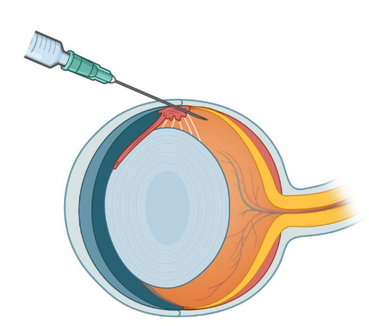
Retinal Contributions to Medial Amygdala

- There are approximately 47 different subtypes of RGCs in the mouse retina, each with their own unique light stimulus preferences and firing patterns
- In human subjects, the amygdala response to eye contact does not require an intact primary visual cortex
- In the subject, there was increased right amygdala activation in an fMRI paradigm in reaction to direct gaze, as opposed to an averted gaze

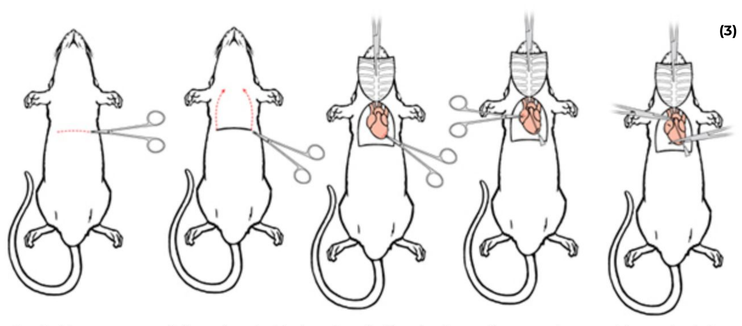


Methods

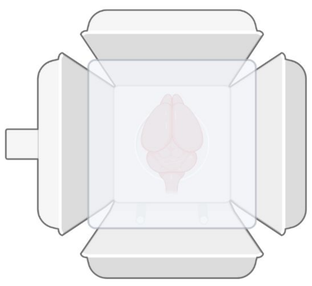
Determining Laterality and Sex Differences (Anterograde Injection)



Day 1 - Unresponsive mouse's sclera stereotaxically injected with CTB-647 anterograde circuit tracer (far-red)



Day 7 - Mouse transcardially perfused with phosphate-buffered saline until tremors in extremities noticed, then decapitated for brain extraction. Brain soaked in 4% paraformaldehyde for 24 hours



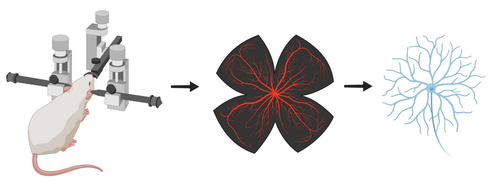
Day 8 - Brain soaked in 30% sucrose for 24 hours as a cryoprotectant. Brain embedded in optimal cutting temperature (OCT) compound before mounting onto cryostat.

Brain sliced in cryostat at 60 µm thickness, aiming at entire medial amygdala

Brain slices imaged under Nikon A1R GaAsP confocal microscope

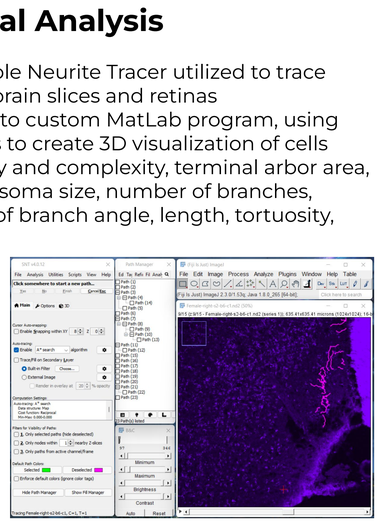
Determining RGC Subtypes (Retrograde Injection)

- Brain exposed using craniotomy
- MeA stereotaxically injected with g-deleted rabies, MeA projecting circuit tracer
- Eye dissected and retina extracted
- Retina imaged under Nikon Ti2 Widefield epi-fluorescent microscope



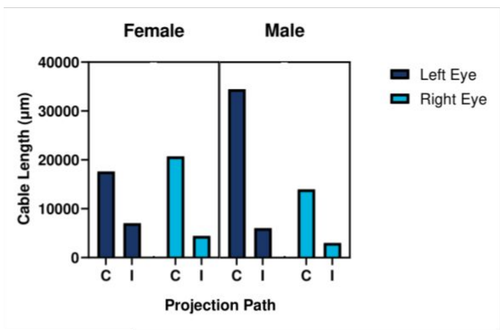
Morphological Analysis

- Fiji/ImageJ Simple Neurite Tracer utilized to trace circuits in both brain slices and retinas
- Traces exported to custom MatLab program, using surface matrices to create 3D visualization of cells
  - Arbor density and complexity, terminal arbor area, total length, soma size, number of branches, distribution of branch angle, length, tortuosity, and z-range

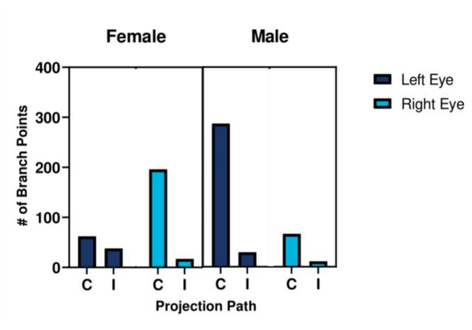


Results

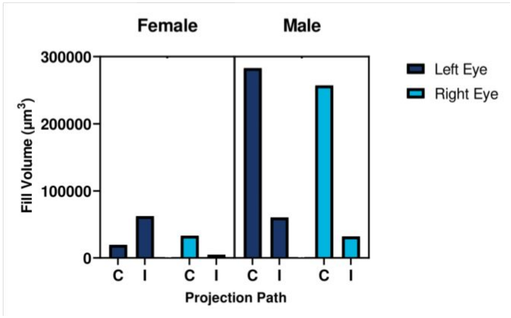
A Cable Length



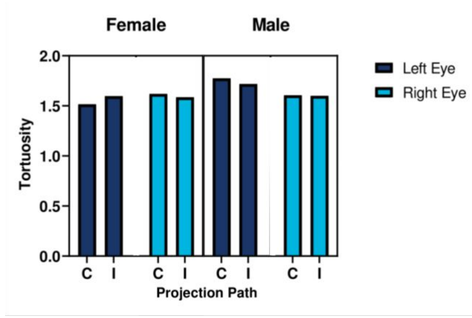
B Branch Points



C Fill Volume

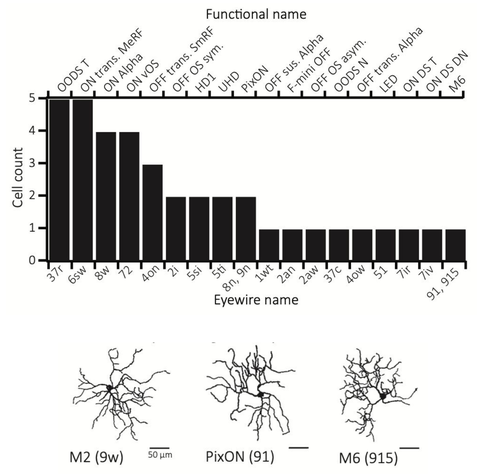


D Tortuosity



A - D. Tables of retinal ganglion cell circuits projecting to the MeA traced (n=225). Cable length, # of branch points, fill volume, and tortuosity described based on contralateral (c) or ipsilateral (i) projection direction **(lower x-axis)**, eye, and sex.

E RGC Count and Example Patterning



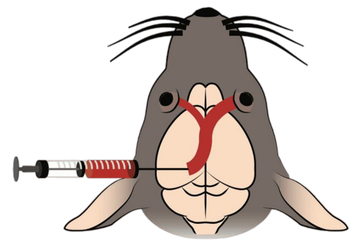
E. Retinal ganglion cell subtypes which project to MeA identified. Cell types are described using both Eyewire nomenclature **(lower x-axis)** and common names used in retinal literature **(upper x-axis)**. Y-axis is the frequency which they were identified.

Conclusions

- Anterograde tracing revealed laterality differences in male mice where contralateral cable lengths were greater compared to ipsilateral projections.
  - Also revealed that contralateral projections in left and right male eyes differed (10,000 µm) significantly more than other eyes, specifically female (~3,000 µm).
- Female right eyes had more branch points while male left eyes had greater branch points
- Laterality differences also exist where male mice fill volumes were greater than female mice.

Future Directions:

- Tracing more circuits to greater justify conclusions
- Diving deeper into RGC subtyping to determine functional specificity of RGCs projecting to the MeA
  - Provide insight on the role of visual input in medial amygdala-related behaviors
- Examining how these axons projecting to the MeA respond to ethologically relevant stimuli to find repercussions on MeA-related behavior



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