Contributions from A17 Amacrine cells to the Oscillatory Potentials in the Full Field Electroretinogram (ERG) of Mice

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INTRODUCTION

Rationale

Retinal diseases can develop at various stages of life and impede vision and quality of life. As such, ophthalmologists and researchers need to understand the retina's underlying mechanisms and physiology to effectively address the related pathology.

The Electroretinogram (ERG)

Electroretinography is a widely used technique to evaluate retinal function in a non-invasive manner in the clinic and laboratory (Smith et al. 2015). The retinal field potential generated by the sum of cellular responses to a flash of light is depicted in the electroretinogram (Smith et al. 2017).

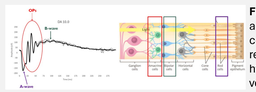

Figure 1. Representative electroretinogram showing a- and b-waves, oscillatory potentials, and which cells they correspond to in the retina. Diagram of retina by https://www.istockphoto.com/illustrations/retina-vector

Amacrine cells

A17 Amacrine cells in the inner plexiform layer of the retina play a key role in scotopic conditions (Jearth et al. 2016) and in the rod pathway by creating an inhibitory circuit, channeling their synaptic output back onto rod bipolar cells (Dong and Hare 2003). The precise role of GABAergic A17 feedback in scotopic processing is unclear as rod bipolar cells receive input from multiple classes of amacrine cells (Dong and Hare 2003). The synapses between rod bipolar cells and GABAergic A17 amacrine cells are believed to be the main mechanism underlying OP generation (Liao et al. 2023).

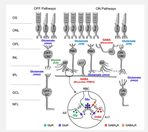

Figure 2. Diagram by Liao et al. 2023 depicting key synapses and neurotransmitters in the retina

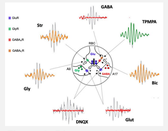

Figure 3. Diagram by Liao et al. 2023 highlighting the site of action of chemical agents.

Overall Goal

This study aims to determine the effect of the pharmacological ablation of A17 amacrine cells on oscillatory potentials in the mouse.

MATERIALS AND METHODS

Experimental Design

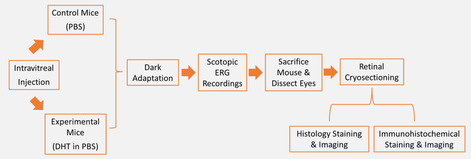

Figure 4. Overview of experimental design.

Intravitreal Injections

Figure 5. Isoflurane is administered continuously.

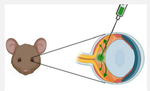

Figure 6. Representation of an intravitreal injection. Photo by Garanto 2022.

Electroretinography

Figure 7. Celeris ERG system. https://www.diagnosysllc.com/preclinical/preclinical-products/.

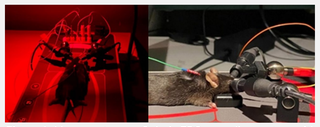

Figure 8. Mouse set up on Celeris ERG recording system with active, reference, and ground electrodes visible.

RESULTS

Mouse 1 Right Eye ERGs at DIM and STRONG flashes

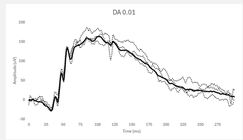

Figure 9. ERG recording depicting the response of the right eye of a normative C56BL/6 mouse to a light intensity of 0.01 cd*s/m^2 with amplitude in microvolts on the y-axis and implicit time in milliseconds on the x-axis.

Name	μV	ms
a	23.95± 8.87	33.14± 1.50
b	183.53± 69.78	110.44± 9.35

Table 1. Mean amplitude (μV) and implicit time (ms) of the a- and b-waves on the ERG recording of the response of the right eye of a normative C56BL/6 mouse in response to a light intensity of 0.01 cd*s/m^2 .

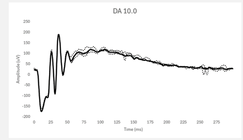

Figure 10. ERG recording depicting the response of the right eye of a normative C56BL/6 mouse to a light intensity of 10.0 cd*s/m^2 with amplitude in microvolts on the y-axis and implicit time in milliseconds on the x-axis.

Name	μV	ms
a	163.59± 50.60	11.03± 0.83
b	306.01± 116.54	88.28± 11.51

Table 2. Mean amplitude (μV) and implicit time (ms) of the a- and b-waves on the ERG recording of the response of the right eye of 10 normative C56BL/6 mice in response to a light intensity of 10.0 cd*s/m^2.

Mouse 1 Right Eye Isolated Oscillatory Potentials at DIM and STRONG flashes

Figure 11. Isolated oscillatory potentials from the ERG recording of the right eye of a normative C56BL/6 mouse in response to a light intensity of 0.01 cd*s/m^2 with amplitude in microvolts on the y-axis and implicit time in milliseconds on the x-axis.

Name	μV	ms
OP1	7.05± 2.66	35.91± 3.16
OP2	15.34± 6.47	45.75± 3.37
OP3	18.35± 7.08	54.75± 3.52
OP4	10.43± 5.64	65.72± 4.31
OP5	3.74± 3.10	79.40± 4.71

Table 3. Mean amplitude (μV) and implicit time (ms) for oscillatory potentials 1-5 on the ERG recording of the response of the right eye of a normative C56BL/6 mouse in response to a light intensity of 0.01 cd*s/m^2 .

Figure 12. Isolated oscillatory potentials from the ERG recording of the right eye of a normative C56BL/6 mouse in response to a light intensity of 10.0 cd*s/m^2 with amplitude in microvolts on the y-axis and implicit time in milliseconds on the x-axis.

Name	μV	ms
OP1	44.99± 14.45	22.56± 3.57
OP2	92.94± 32.51	32.88± 3.48
OP3	78.34± 36.20	44± 4.20
OP4	36.28± 19.19	55.97± 4.59
OP5	13.27± 5.94	69± 5.14

Table 4. Mean amplitude (μV) and implicit time (ms) for oscillatory potentials 1-5 on the ERG recording of the response of the right eye of a normative C56BL/6 mouse in response to a light intensity of 10.0 cd*s/m^2 .

Postmortem Studies

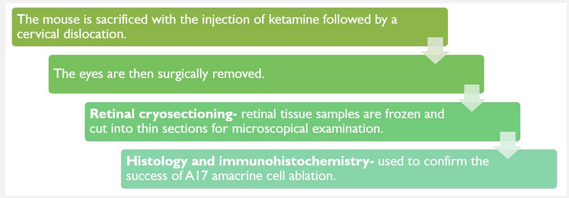

Figure 13. Flowchart depicting the steps involved in postmortem studies following the completion of ERG recordings.

Detection of DHT autofluorescence used as an indicator of drug access to retinal cells: anticipated results

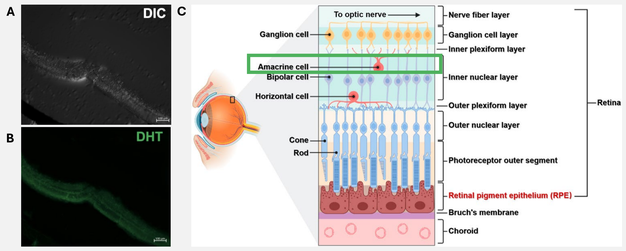

Figure 14. (A) Differential interference contrast (DIC) image taken of 5,7-dihydroxytryptamine hydrobromide (5-7 DHT) injected eye. (B) Image of 5,7-DHT injected eye shows the presence of autofluorescence in the retinal layers, confirming that the A17 amacrine cells have been successfully ablated. Images by Becca Henderson and the Côté lab. (C) Autofluorescence detection in retinal layers will confirm the successful ablation of A17 amacrine cells, image by Yang et al. 2021.

Timeline

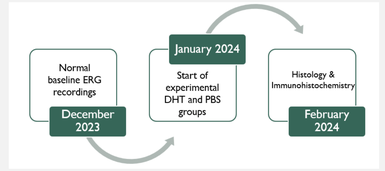

Figure 15. Flowchart depicting the timeline for the study. Normal baseline ERG recordings have been completed as of December 2023, experimental DHT and PBS injections started in January and will continue into February along with the histology and immunochemistry analyses.

CONCLUSION AND CLINICAL RELEVANCE

- ERGs provide important information on retinal function in the laboratory and clinic.
- Provide an improved understanding of the cells involved in OP generation.
- May help ophthalmologists and researchers to determine if A17 amacrine cell inhibitory circuits are involved in human pathological states.

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