Optimizing a CO_2 Activation Assay to Quantify Host-Seeking Behavior in *Anopheles gambiae*

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Introduction

- Mosquito host-seeking behavior is essential for malaria transmission
- Carbon dioxide (CO_2) is a key activator that triggers and enhances host-seeking flight
- Changes in CO_2 sensitivity could alter biting behavior and transmission risk
- Measuring CO_2 responsiveness requires a controlled, quantitative behavioral assay

→ A validated, standardized platform to reliably measure CO_2-evoked activation is currently lacking in our system.

Research Questions

- Can we develop and validate a reliable CO_2 activation assay to accurately quantify host-seeking responsiveness in *Anopheles gambiae?*

Does variation in CO_2 responsiveness provide a measurable behavioral pathway through which biological states (e.g. infection or circadian timing) alter host seeking behavior?

Assay Overview

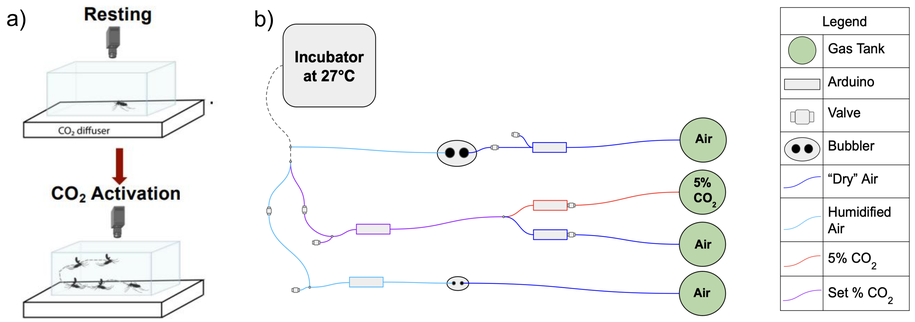

Figure 1. CO_2 Activation Assay Design and Gas Delivery System
a) Schematic of CO_2 activation assay used to quantify sensitivity to controlled CO_2 stimulation. Individual *Anopheles gambiae* females tracked within an arena to calculate speed, displacement, and activation threshold. Following acclimation, mosquitoes are exposed to humidified air (control) or defined CO_2 concentrations. High-throughput and generates individual-level behavioral data. Courtesy of Hannah Sproch and Dr. Photini Sinnis.
b) Gas delivery and valve system adapted from the McMeniman lab (previously used in *Aedes*) and optimized for *Anopheles*. Red indicates CO_2 , purple indicates calibrated CO_2 mixtures, and light blue indicates humidified air to prevent desiccation. Valves open briefly prior to stimulation to relieve pressure and ensure rapid stable switching between control and CO_2 .

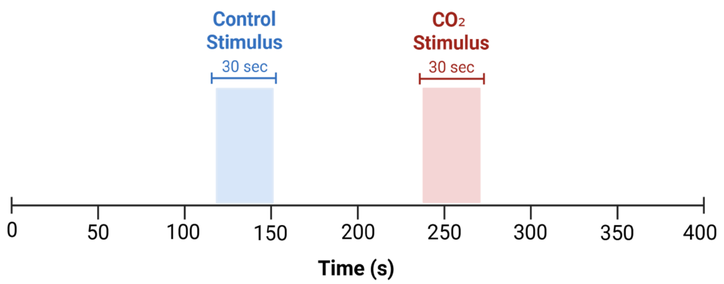

Figure 2. Experimental Timeline
Mosquitoes were acclimated in a 27°C incubator for 10 minutes prior to imaging. Baseline locomotion was recorded, followed by a 30-second humidified air control stimulus at 121 seconds and a 30-second CO_2 stimulus at 241 seconds. Total recording duration was 360 seconds. This design enables within-individual comparison of baseline, control, and CO_2-evoked activation. Courtesy of Hannah Sproch.

Assay Validation

Does handling stress affect CO_2 responsiveness?

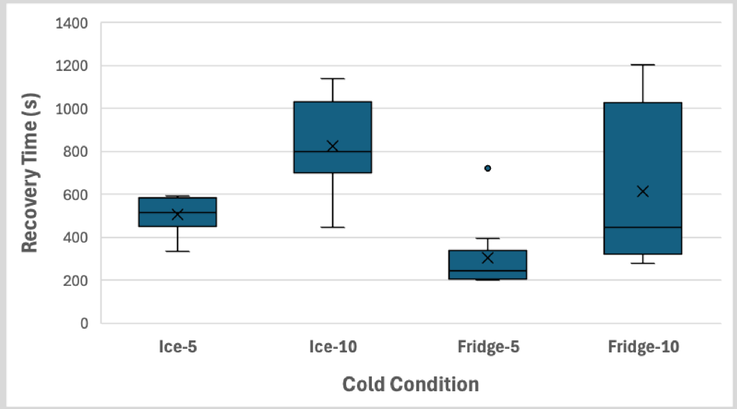

Figure 3. Recovery Time Following Cold Knockdown
Recovery time of female *Anopheles gambiae* after cold knockdown under four conditions (5 or 10 min on ice; 5 or 10 min in fridge). Fridge 5 min resulted in the fastest and most consistent recovery. Courtesy of Hannah Sproch.

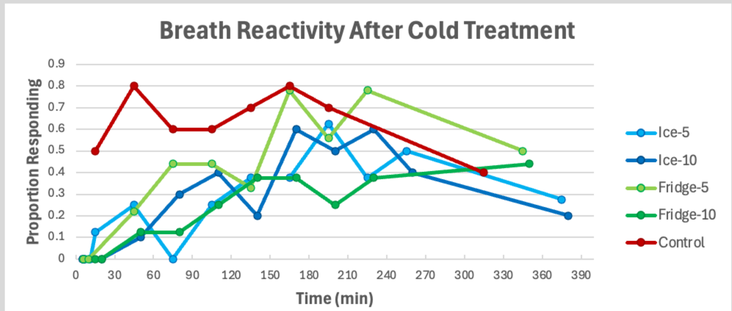

Figure 4. CO_2 Reactivity After Cold Treatment
Proportion of mosquitoes responding to CO_2 over time following different cold knockdown methods. Ice treatments reduced and delayed CO_2 responsiveness, whereas short fridge exposure preserved activation. Courtesy of Hannah Sproch.

Figure 5. Cold Knockdown and Assay Preparation Workflow
Representative images of mosquito chilling, recovery, and transfer to assay chambers prior to behavioral testing.

Optimized protocol: ~2.5 minute fridge knockdown
Short fridge exposure preserved rapid recovery and CO_2 responsiveness

Biological Sensitivity

Time-of-Day Modulates CO_2 Activation

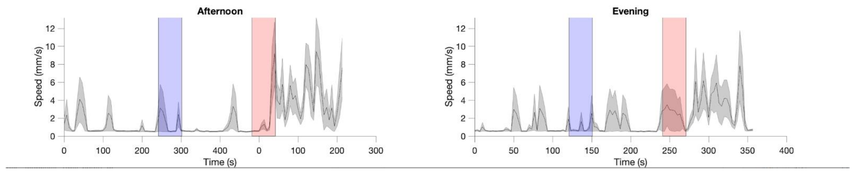

Figure 6. Time-of-Day Modulates CO_2-Evoked Activation
Cumulative comparison of locomotor speed in afternoon versus evening trials. Evening mosquitoes exhibit stronger CO_2-evoked activation relative to baseline and air control, indicating circadian modulation of host-seeking sensitivity.

CO_2 Concentration Sensitivity

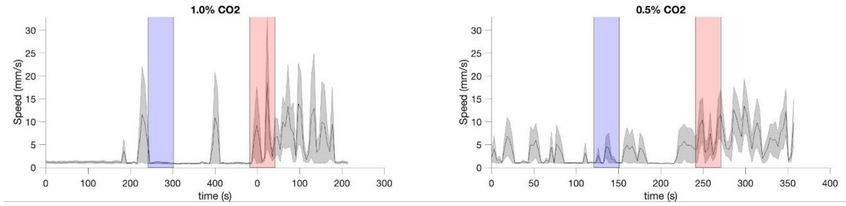

Figure 7. CO_2 Concentration Sensitivity
Comparison of mosquito activation in response to 1.0% and 0.5% CO_2. Both concentrations elicit significant activation above baseline and air control, demonstrating that the assay detects graded, concentration-dependent responsiveness.

Conclusion and Future Directions

Conclusions
- Established a reliable, quantitative CO_2 activation assay
- Optimized handling to prevent behavioral confounds
- Assay detects biologically meaningful modulation
- Platform is ready for infection-based studies

Future Directions
- Test Plasmodium-infected mosquitoes
- Define CO_2 activation thresholds
- Apply in high-containment conditions

Acknowledgments

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