

Developing Whole-Hive Pavlovian Conditioning to Promote Attraction of *Apis mellifera* to the Synthetic Scent of the Endangered Orchid *Prosthechea cochleata* for Conservation

Background

- Habitat fragmentation creates boundaries between populations causing:
 - Less visitation of plants by native pollinators and thus decreased genetic diversity and population size
- Honeybees are efficient, but non-native and generalist pollinators:
 - Honeybees evaluate the quality of food sources as a hive, optimizing foraging by visiting sources with a high nectar and pollen yield
 - Did not co-evolve to forage from or pollinate native plants

Native pollinators are often the best suited for pollinating native plants but if their populations decline, it is important to investigate methods to encourage honeybees to aid in pollinating endangered plant species like *Prosthechea cochleata*



Prosthechea cochleata. Conservatory of Flowers. <https://conservatoryofflowers.org/bloom/prosthechea-cochleata/>

Methods

Setup:

- EAG records electrical activity in antennas to determine strength of signals sent to the brain after contact with stimuli



Antenna creating single path for electrical current to flow through in EAG setup:

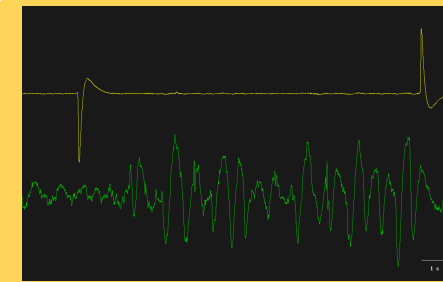
- Allows EAG to record changes in olfactory neurons of the antenna upon introduction of VOCs

Stimuli:

- Introduce only filter paper (negative control), pseudocumene, nonanal, mesitylene, limonene, decanal, benzaldehyde, and α -pinene

Analysis of Data:

- Peaks indicate deviation from the resting state
- Photoresistor helps align data



Raw EAG data from mesitylene

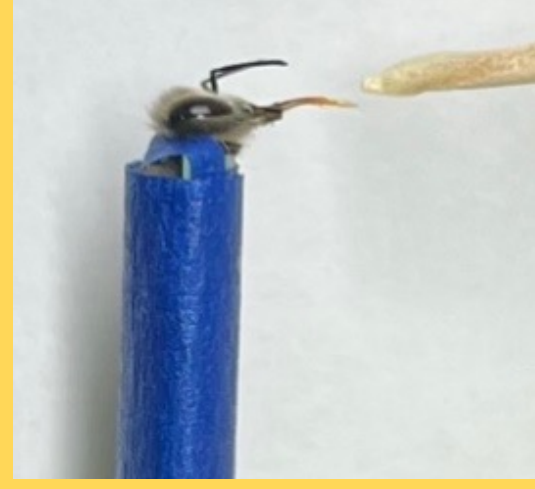
- Yellow is photoresistor data
- Green is signal from antenna
- Data shows olfactory detection of VOC when sample is present

Stimulus



- Waft synthetic scent/VOC or hexane (control group) over bee for 15 seconds

Reward



- Reward is a 50% (by weight) sucrose and water solution
- Introduce sucrose reward let sit for 10 minutes
- Repeat previous steps 5 times

PER exhibition

- Waft synthetic scent of VOC over bee for 15 seconds and observe PER exhibition

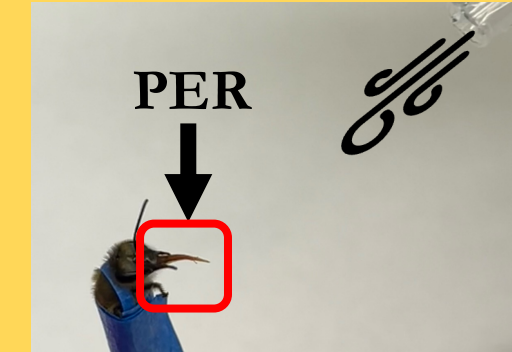
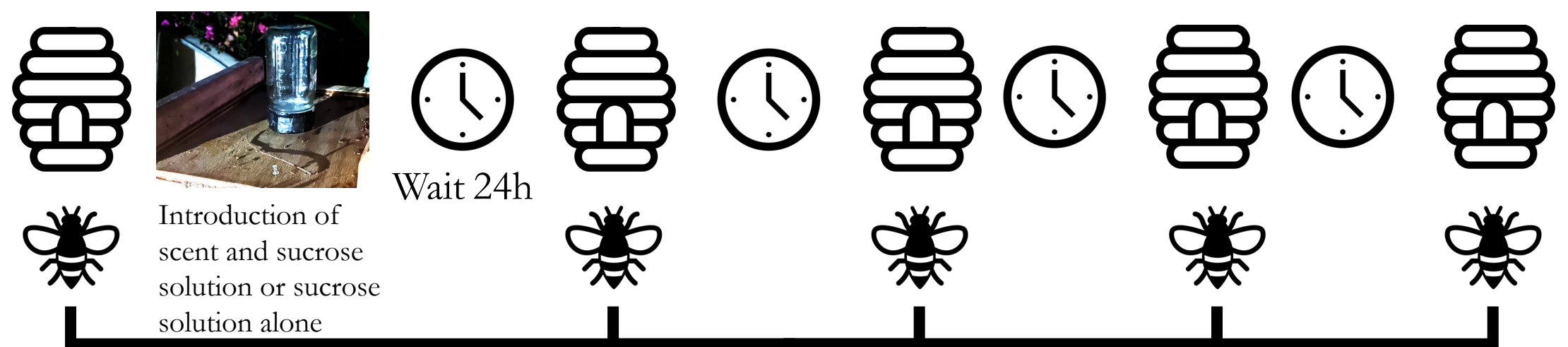


Fig 10 & 11: Synthetic scent presented to a bee conditioned with the scent (left) and to a bee conditioned with hexane (right) indicates successful conditioning



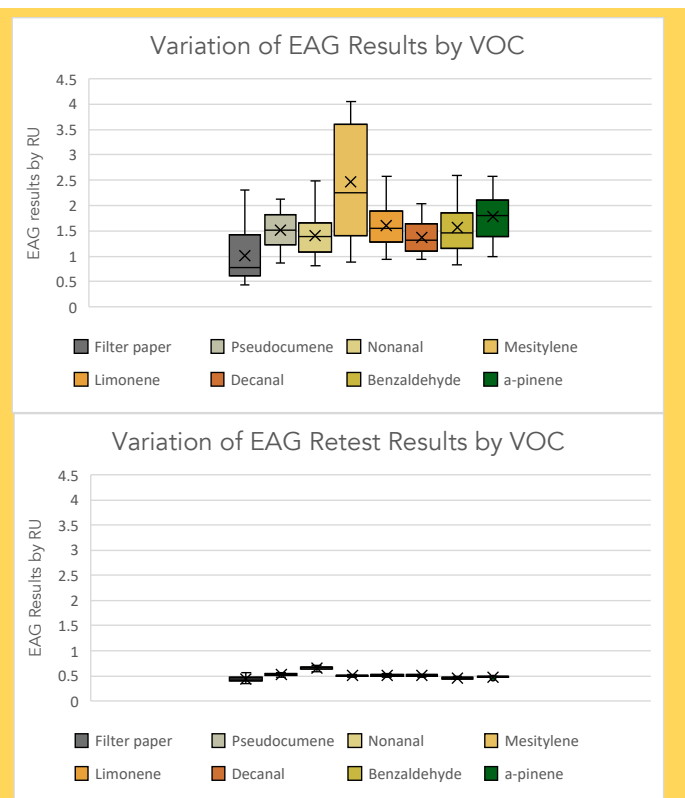
Repetition, Concentration, and Persistence:

- Repeat Pavlovian conditioning in 6 groups conditioned 1-6 times to observe the impacts of repetition
- Feed bees with a mixture of synthetic scent and sucrose reward at various concentrations
 - Observe PER after 5 rounds of conditioning
 - Observe sustained PER after multiple unrewarding experiences and a singular round of conditioning



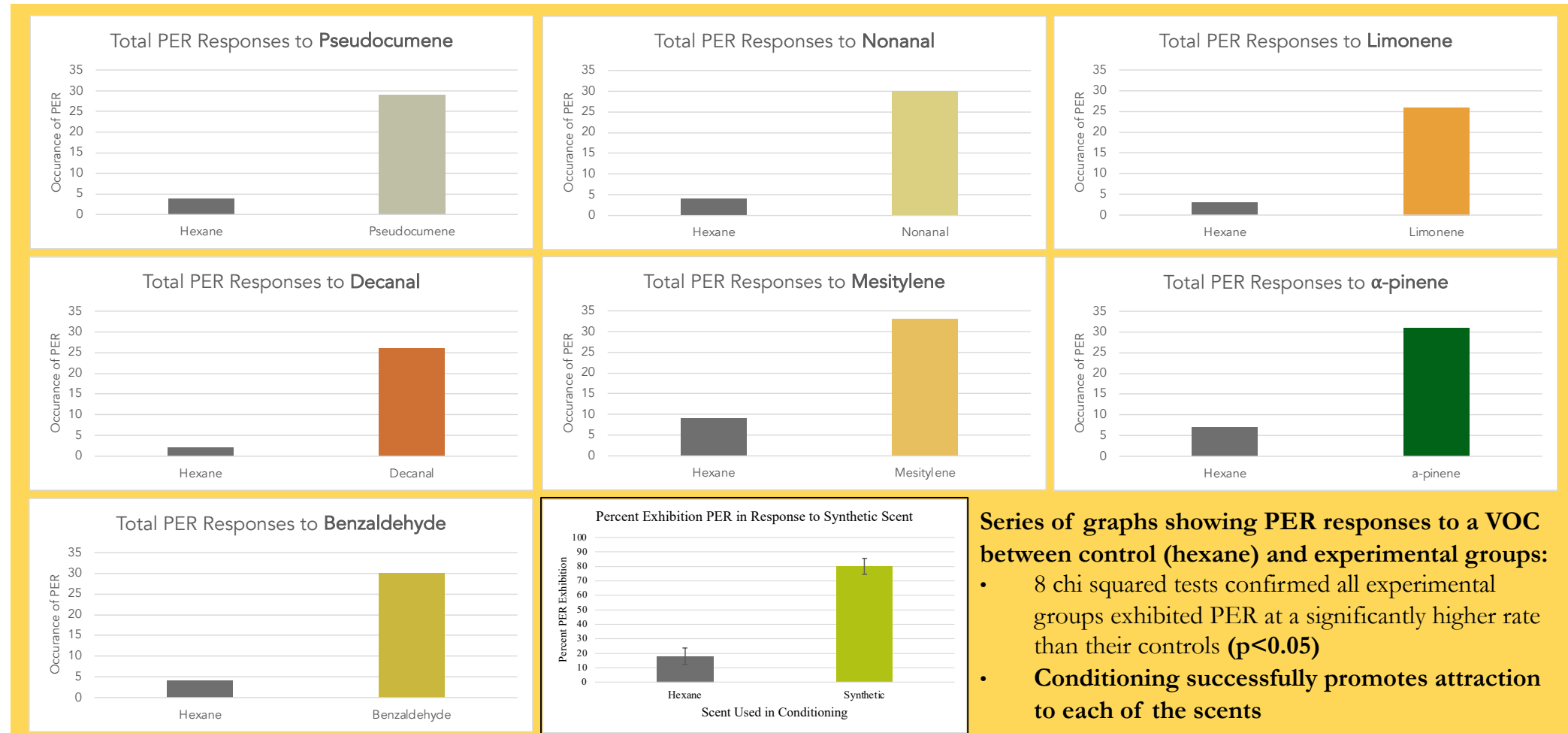
Expose all groups to synthetic scent 6 times and observe PER after unrewarding experiences

Results



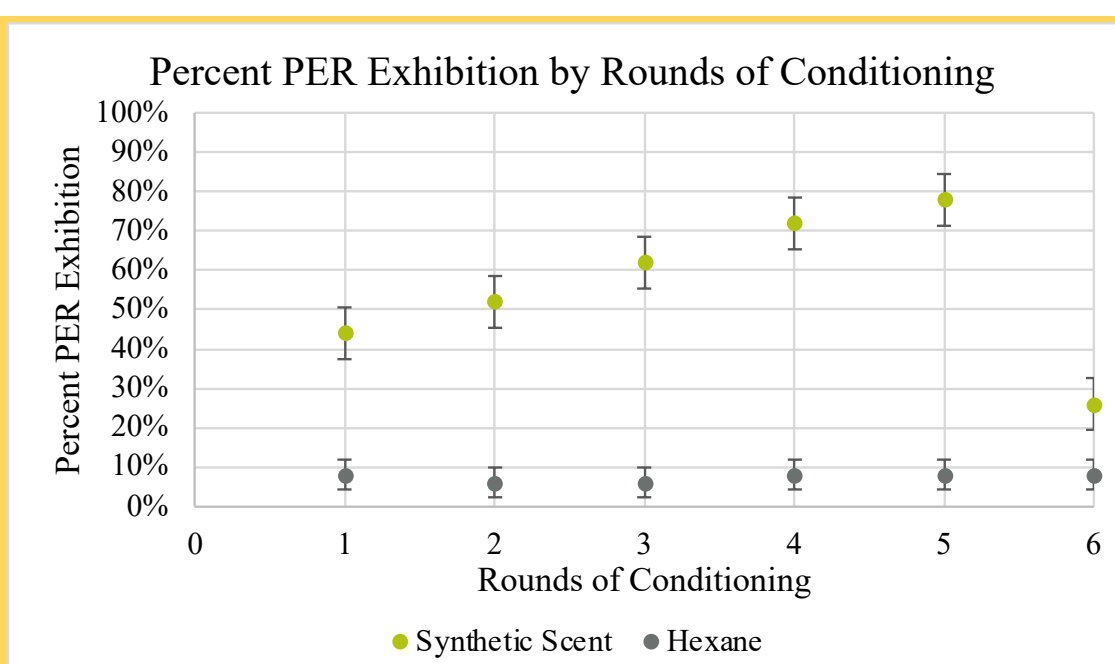
Box plots comparing EAG results original and revised EAG setup where revised shows changes consistent with a more reliable setup

- No measurement tool available in EAG software, so relative units (RU) used to quantify results with average control response=1 RU
- Revised EAG setup resulted in more reliable data with an **R&R value of 16.78%**
- Tukey-Kramer tests found 5 VOCs yielded higher results than filter paper ($p < 0.05$)
 - α -pinene and benzaldehyde results were not significantly different from filter paper ($p > 0.10$)



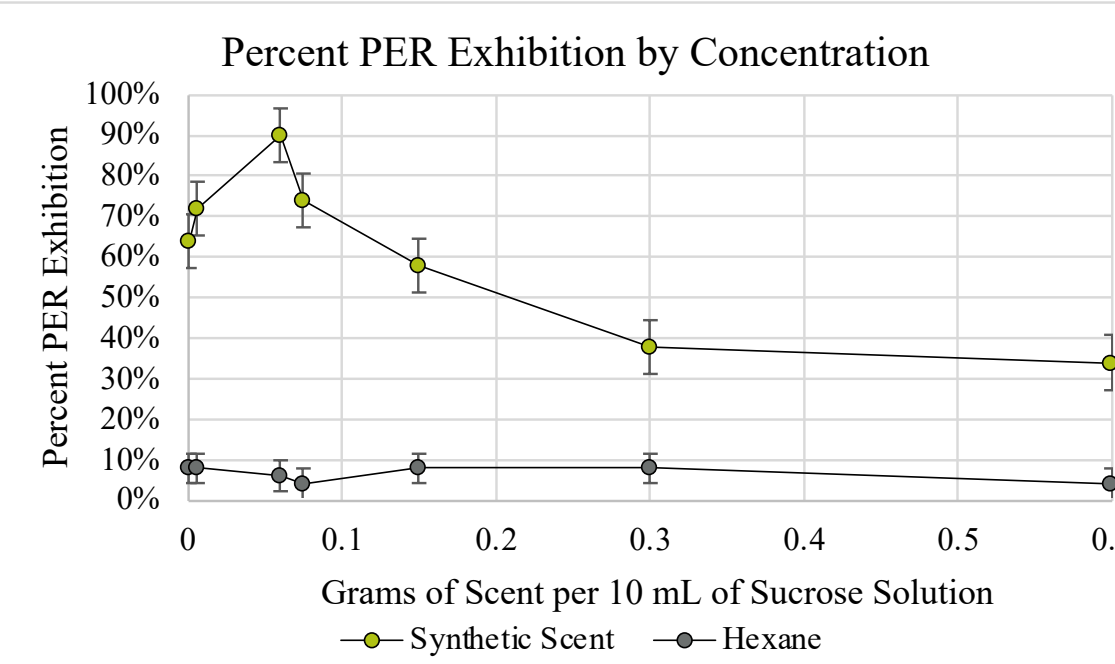
Series of graphs showing PER responses to a VOC between control (hexane) and experimental groups:

- 8 chi squared tests confirmed all experimental groups exhibited PER at a significantly higher rate than their controls ($p < 0.05$)
- Conditioning successfully promotes attraction to each of the scents



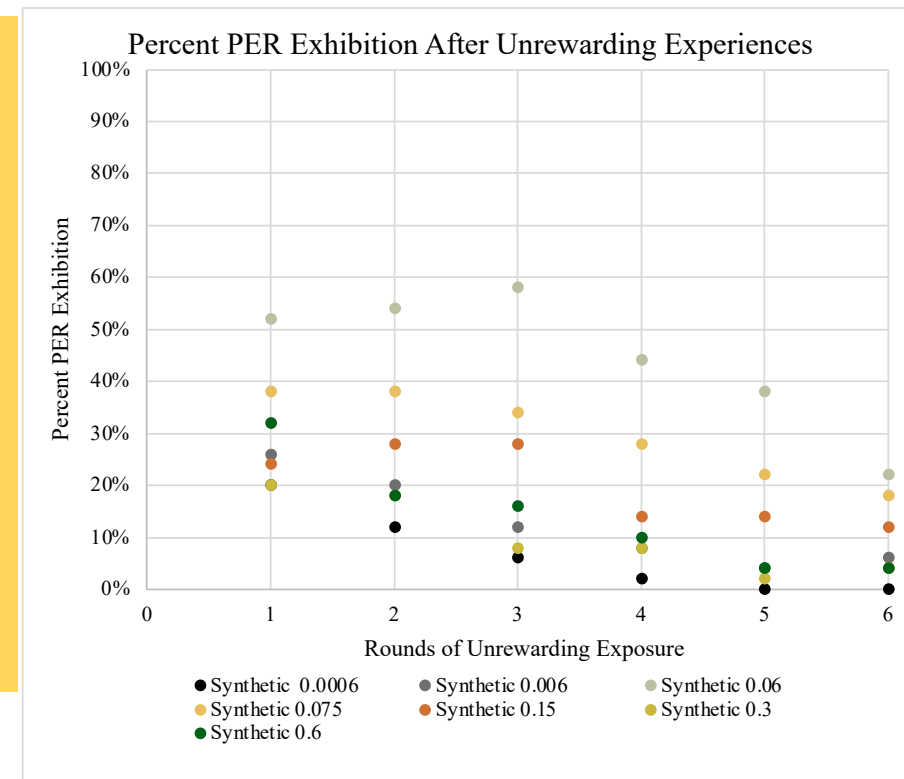
Percent of bees that exhibited PER in response to the synthetic scent after conditioning 1-6 times with hexane or synthetic scent

- Logistic regression confirmed experimental group for 5 rounds of conditioning to be significantly higher than the experimental groups for 1,2,3, and 6 rounds of conditioning ($p < 0.05$), but not 4 rounds



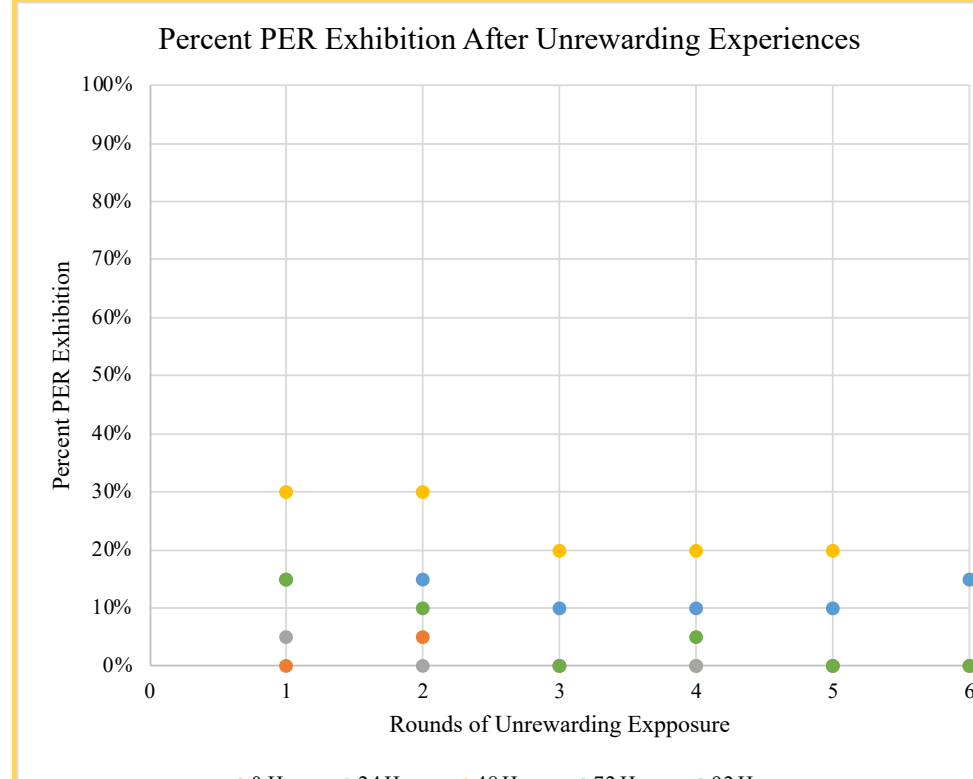
Percent of bees that exhibited PER in response to synthetic scent after feeding five times with various concentrations of hexane or scent in a sucrose solution

- Logistic regression confirmed 0.06g/10mL yields significantly higher PER exhibition than all other groups tested ($p < 0.05$)



Percent of bees that exhibited PER in response to repeated unrewarding experiences with synthetic scent after one feeding with 0.0006, 0.006, 0.06, 0.075, 0.15, 0.3, 0.6 g of hexane (left) or the synthetic scent (right) per 10mL of sucrose and water solution

- Logistic regression confirmed 0.06g/10 mL elicits highest sustained PER exhibition of tested groups throughout each unrewarding exposure ($p < 0.05$)



Percent of bees that exhibited PER in response to repeated unrewarding experiences with synthetic scent after 0, 24, 48, 72, or 98 hours of whole-hive feeding with sucrose solution (left) or sucrose solution and synthetic scent (right)

- 48 hours elicits highest sustained PER exhibition of tested groups when averaged across unrewarding exposure ($p < 0.05$), but is not statistically higher than 72 rounds at later unrewarding experiences