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Beesearch: smillin' urine and discriminatin'sex

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Introduction

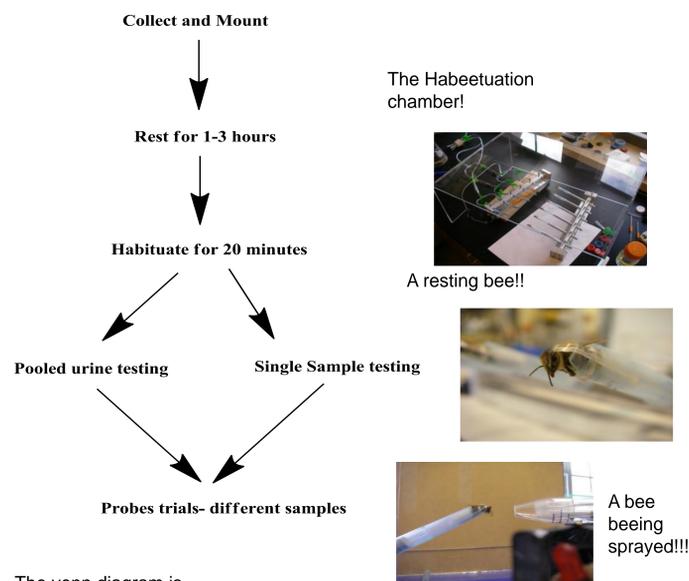
- Identifying a single substance in a mixture of substances is important in today's world: cancer biomarker tests, drug screening, and blood analysis all look to find some sort of chemical needle in a haystack.
- Animals, mainly dogs, have shown an ability to identify cancerous urine and stool samples.
- For a dog to identify between samples it must receive olfactory training which can take years.
- Honeybees, on the other hand, only take about an hour to train and have flexed their olfactory muscles in discriminating between nest mates, flowers, and mock urine with great accuracy.
- Because bees are much easier to train and have similar olfactory capabilities as dogs, it may be useful to use bees to identify clinical samples.
- To test this, the bees were charged with discriminating sex using male and female mouse urine samples.

Question:

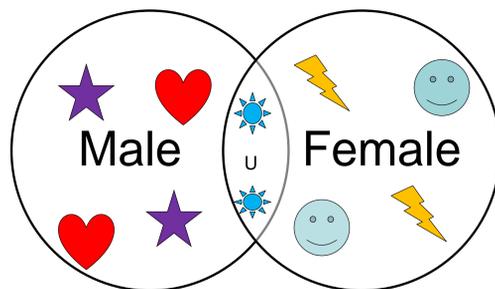
- Can we use bees to identify male and female mice urine?
- Can the bees take it a step further and correctly classify a novel sample of the same sex after training?

Methods

- Bees were collected from a bee club hive at Puget Sound then harnessed and left to rest for 1-3 hours.
- After resting, the bees were habituated to urea for at least 20 minutes before testing.
- Testing odors during training consisted of male and female mouse urine.
- The bees were tested using two different methods: pooled urine testing and single urine sample testing.
- The pooled trials used a mixture of three different urine samples of the same sex as the odors. The single sample trials used one male and one female sample.
- Each training session tested the bees in 10 counterbalanced trials. The bees were exposed to the testing odor for 5 seconds each trial. One odor was paired with a sugar reward (S+), while the other odor was unrewarded (S-).
- After each session the bees were probed with urine samples from different mice than were used throughout that session.

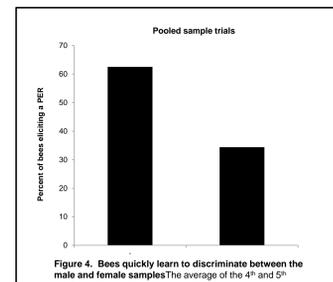
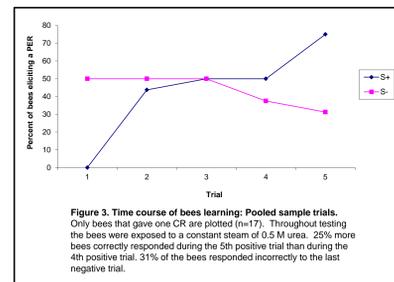
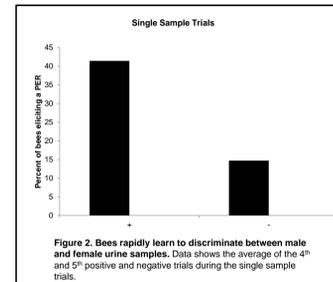
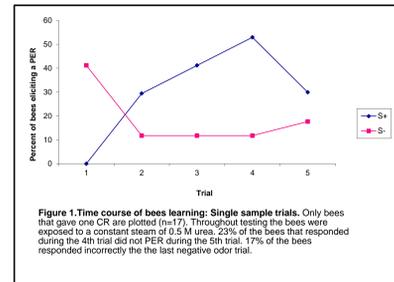


The venn diagram is meant to show that male and female urine will have characteristics in common, but also have distinct features that will help the bees to classify them correctly. In this case urea and a blue sun are aspects they share while the smile faces, lightning bolts, hearts, and stars are all distinct features of that urine.

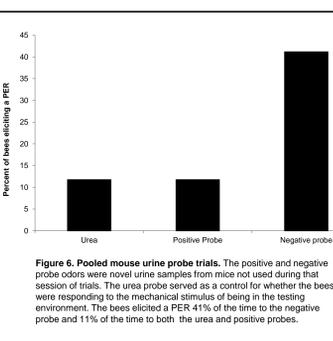
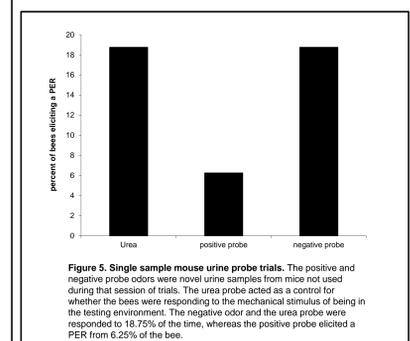


My question: Can bees discriminate between male and female mice urine and, if so, can they apply that to a novel odor of the same sex?

Hypothesis: The bees will be able to discriminate amongst the pooled male and female odors the same as with single samples, but that during probe trials the pooled urine tested bees will discriminate more accurately.

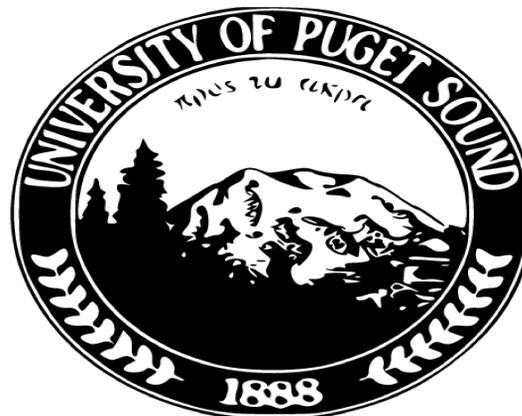


The probe trials act as a test for the bees to determine whether they can apply what they learned throughout the 10 counterbalanced trials and apply to a novel sample from mice of the same sex.



How did the Bees do?

- The results show that the bees can discriminate between urine samples from male and female mice. Both the results from the pooled urine and single urine sample trials show a higher percentage of bees eliciting a PER to the positive odor at the 4th and 5th trials.
- The bees could not accurately discriminate the positive odor of a novel mouse sample, and in fact elicited a PER more to the negative odor in both the pooled sample and single samples probe trials

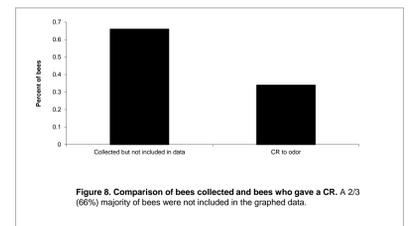
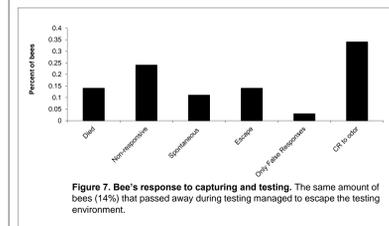


Conclusions

- Pooled urine testing seems to yield better results based on the large dip in the data (figure 1) collected during the single urine sample trials.
- If the bees were ever to be used as a screening technique for diseases, they would constantly be presented with novel samples from persons whom they have never smelled. The probe trials were meant to mimic this scenario. Because the bees could not apply their knowledge to novel stimuli (at least under the training conditions I used), it seems their chance at clinical fame is a bust, however PER could still be used as long as the odorant came in a less complex form.
- The single urine sample testing positive response dip may be due to satiation. Perhaps the responsive bees just could not fit any more sugar into their honey stomachs and so did not elicit a response.
- Research and discovery can be extremely frustrating, but still well worth the time and effort.

Future Work

- Throughout research this summer there were many hurdles, most of which I feel should be improved in future work:
 - The proper air flow for the background air, habituation chamber, and testing odor should be tweaked and maintained in order to assure the bees are not responding to the mechanical stimulus, but are still receiving an adequate amount of odor.
 - I used 5 microliters of urine each session, but perhaps a higher volume could help the bees to recognize the odor.
 - Creating a closed habituation chamber.
 - Avoid being stung by bees.
 - Minimize wasted bee collection:



- The large number of bees being collected but whose data cannot be used is wasteful. Bees that die or are non-responsive could be due to a variety of factors: hive health, weather, mounting technique could all be a few.
- In the end it would be more useful to use PER research to help understand more about how bees can help themselves instead of making them smell foreign odors that are not of importance to them.
- In this vein, an experiment set up to test how the bees are affected by pesticides using PER testing could contribute to the on going debate on bee health and Colony Collapse Disorder.



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References

- Alexander Titus (photos at left and flow chart idea)