



INSECTS AND CANCER

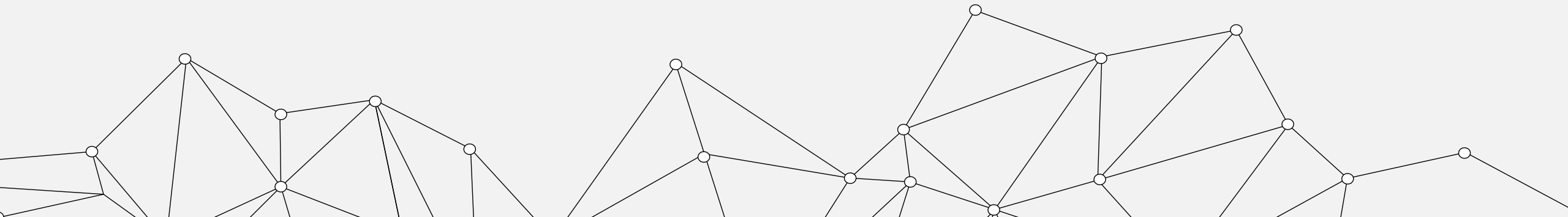
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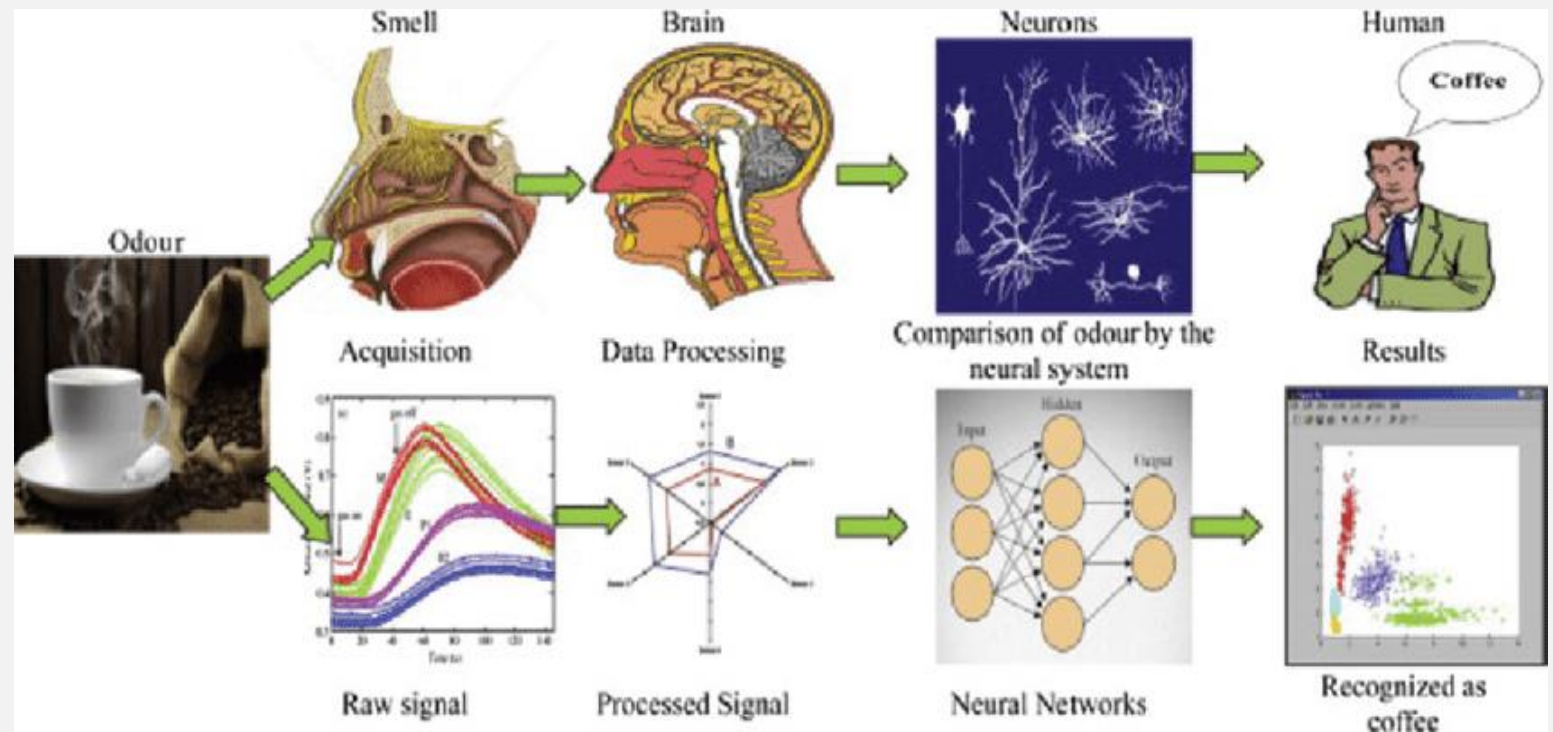
INTRODUCTION

- Cancer cells possess specific features such as a deregulated cellular energetic metabolism, the ability to self-sustain themselves with proliferating signals or by exploiting tumor-promoting inflammation factors.
- The metabolism of each type of cancer produces volatile organic compounds (VOCs), these can act as biomarkers for cancer diagnosis through gas chromatography or artificial olfactory systems.



WHAT ARE ARTIFICIAL OLFACTORY SYSTEMS?

- Artificial olfactory systems, often referred to as "artificial noses," are sensor technologies designed to mimic the human sense of smell. These systems utilize various sensor units based on different recognition principles, such as bioelectric effects, to detect and analyze chemical compositions, particularly volatile organic compounds (VOCs).
- However, the results of gas chromatography analyses are extremely variable and most of the E-nose systems need to be optimized and are still at the prototype stage.

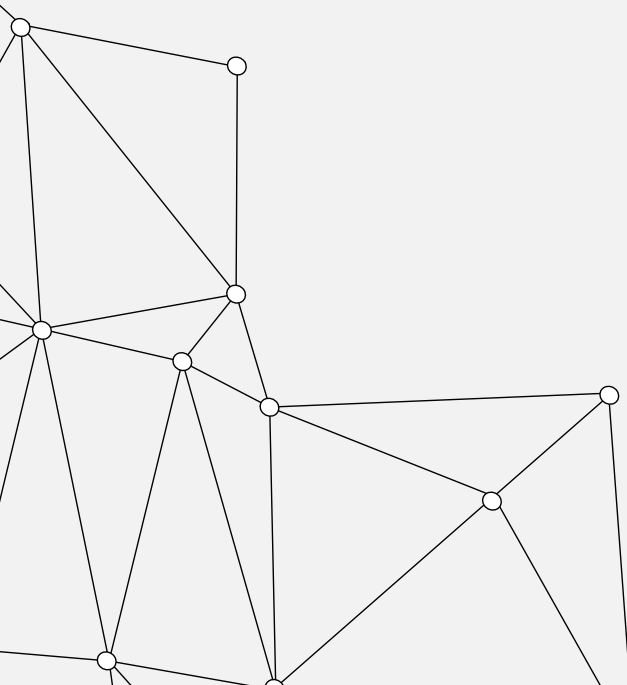


ADDRESSING THE PROBLEM

- Cancer is among the world's leading causes of death.

- The current methods used for detection are invasive, expensive, and time consuming.

- This poses a critical challenge for public health in order to develop a noninvasive, inexpensive, and efficient tool for early cancer detection.



CAN DOGS BE HELPFUL?

- Dogs' noses are well suited for medical diagnosis, and are being used for the detection of cancer-specific VOCs.
- However, training dogs is both expensive and time consuming.
- The conditioning phase, in particular, takes several months and hundreds of trials are needed before the dog is operative.



CAN DOGS BE HELPFUL?

- For instance, in a study in 2019:
(90.3%) of correct identification was achieved using two dogs, 5 months of training, and 1531 conditioning trials to perform 31 memory tests.



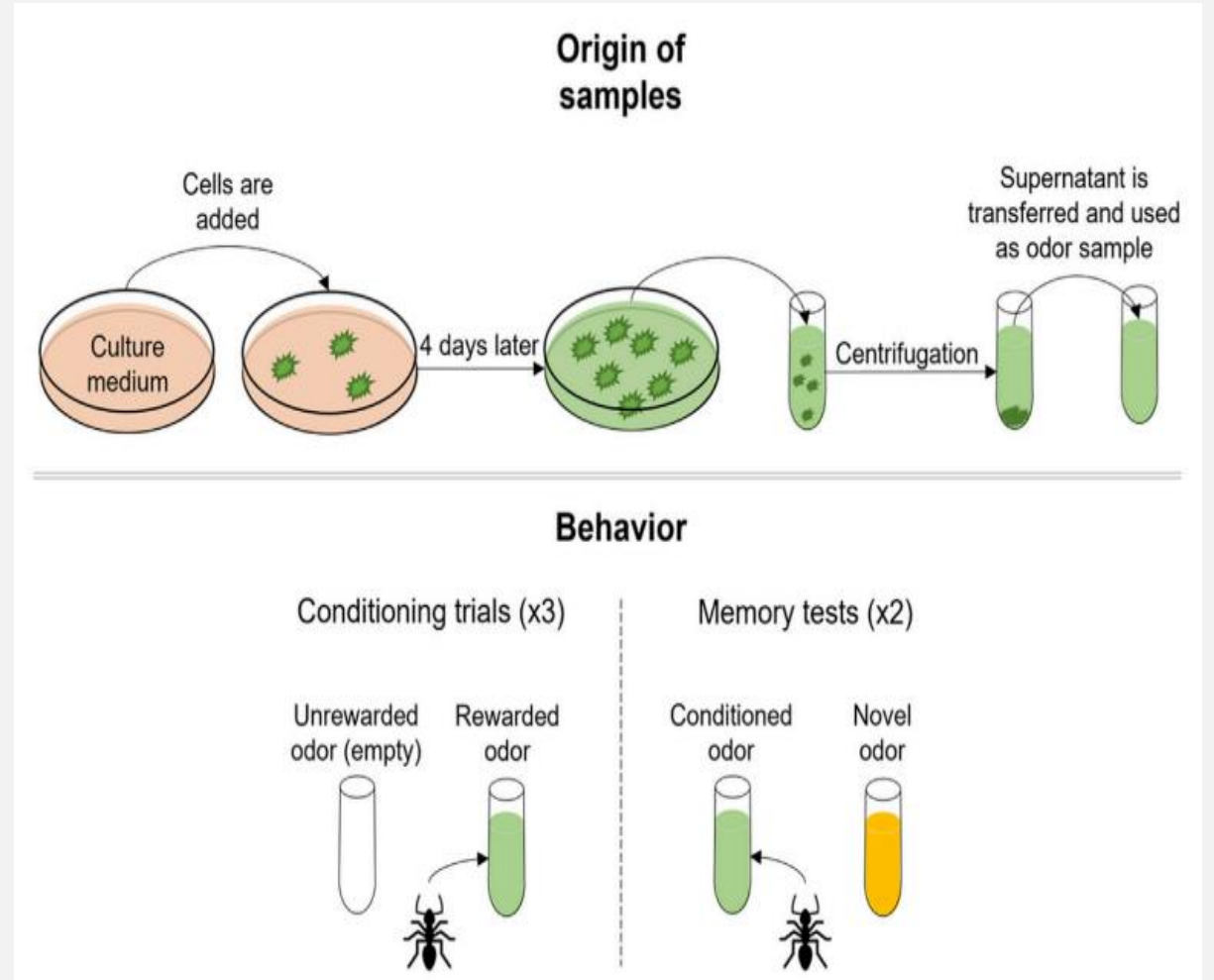
WHAT ABOUT INSECTS?

- Compared to dogs, insects can be easily reared in controlled conditions, they are inexpensive, they have a very well-developed olfactory system, , and hundreds of individuals can be conditioned with very few trials.
- For instance, a study on fruit flies in (2014) reported that: The odors from different cancer cell lines evoked specific olfactory receptor activity patterns in the antenna, suggesting that such insects could be used as cancer biodetectors.



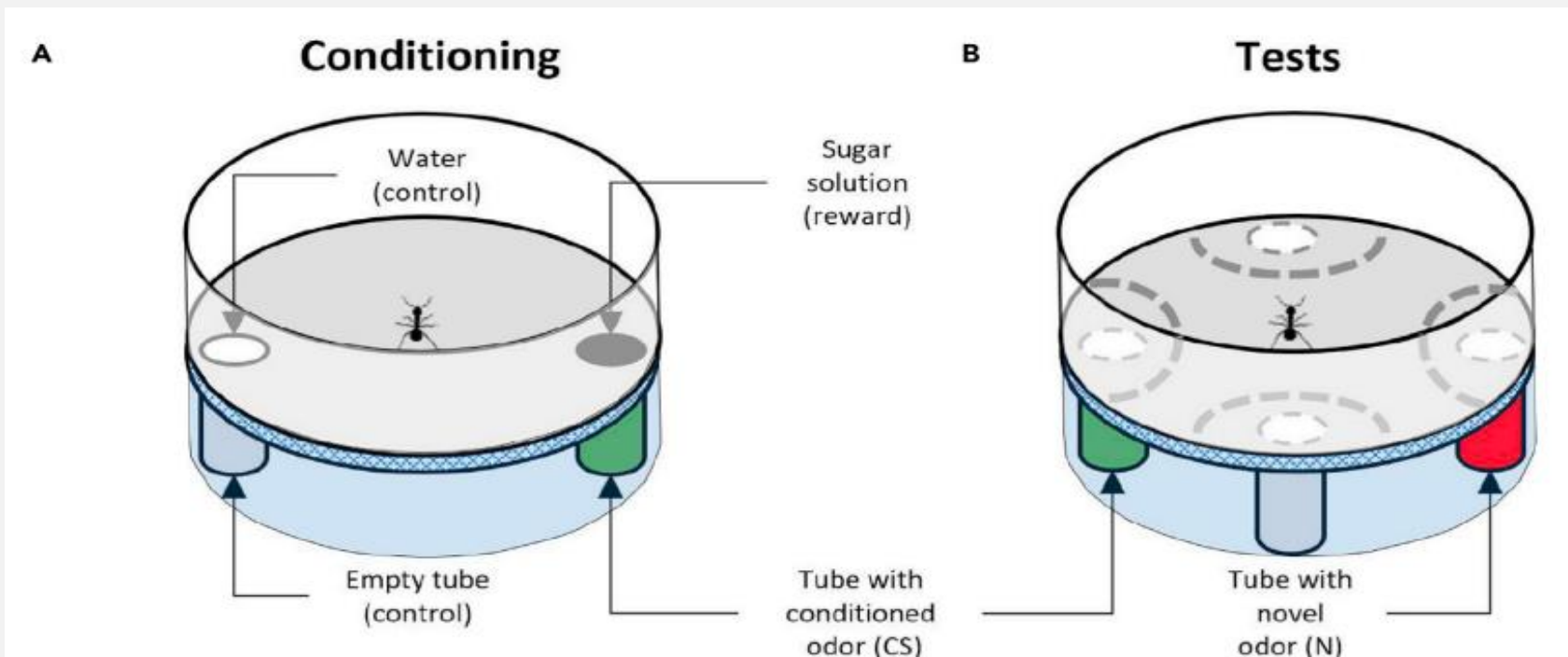
WHAT ABOUT INSECTS?

- Additionally, a recent study in (2022) reported the use of the ant *Formica fusca*, where they demonstrated that individual worker ants of this species quickly learn to associate an olfactory stimulus with a food reward and retain this information for an extensive period of time (several days).



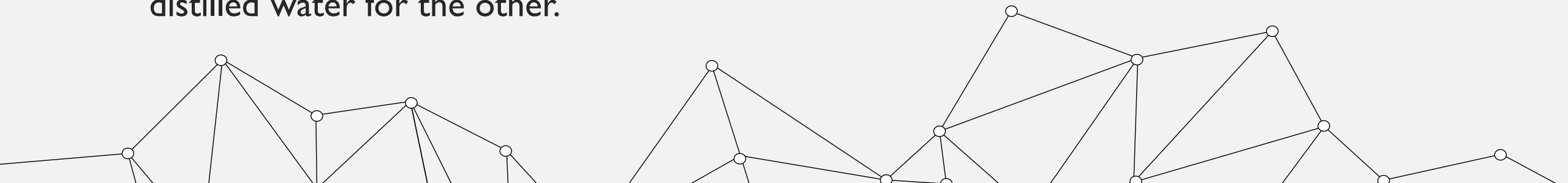
METHODOLOGY

- In the study, individual ants were trained to associate the odor of a cell sample with food reward, and later had to discriminate this learned sample against a new one.
- Tested ants were foragers (ants that leave the nest to search for food), and were individually marked with a dot on the abdomen or thorax using oil-based paint (Mitsubishi Pencil).



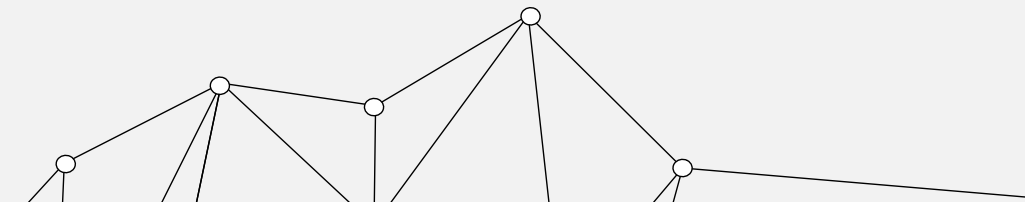
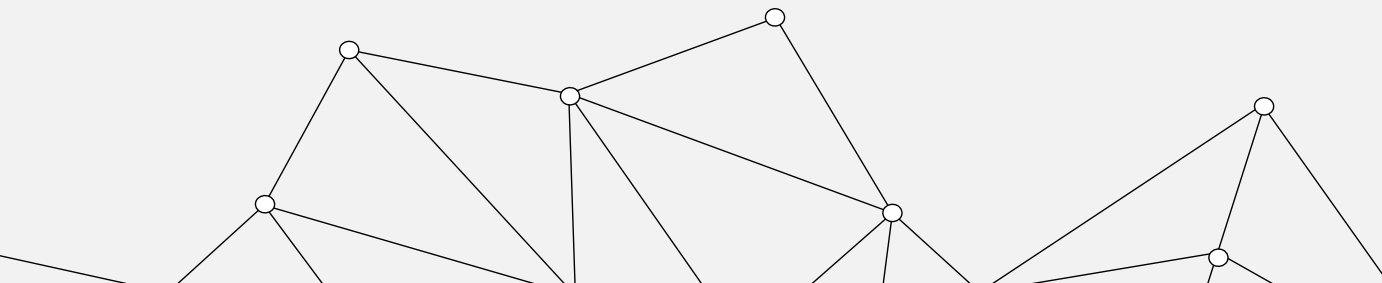
METHODOLOGY

- Ants were individually placed in a circular arena, with clean filter paper at the bottom. The arena had two holes at the ground level (10 cm between the holes). Two glass vials were each placed below one of the holes with the opening toward the arena. One vial was filled with 4 mL of the cell supernatant, whereas the other was empty.
- As cells were cultivated at 37°C, they were kept at this temperature throughout the experiment by inserting the vials in a water bath placed just below the arena. To avoid any effect of heat on ants' learning and memory performances, polystyrene foil (insulating material) was placed between the water bath and the arena.
- The portion of filter paper above the vials was pierced with an entomological pin to allow natural diffusion of the odor. Small plastic discs were placed above each hole and received a 1 mL drop of sugar solution (30% w/w) for the conditioning odor and of distilled water for the other.



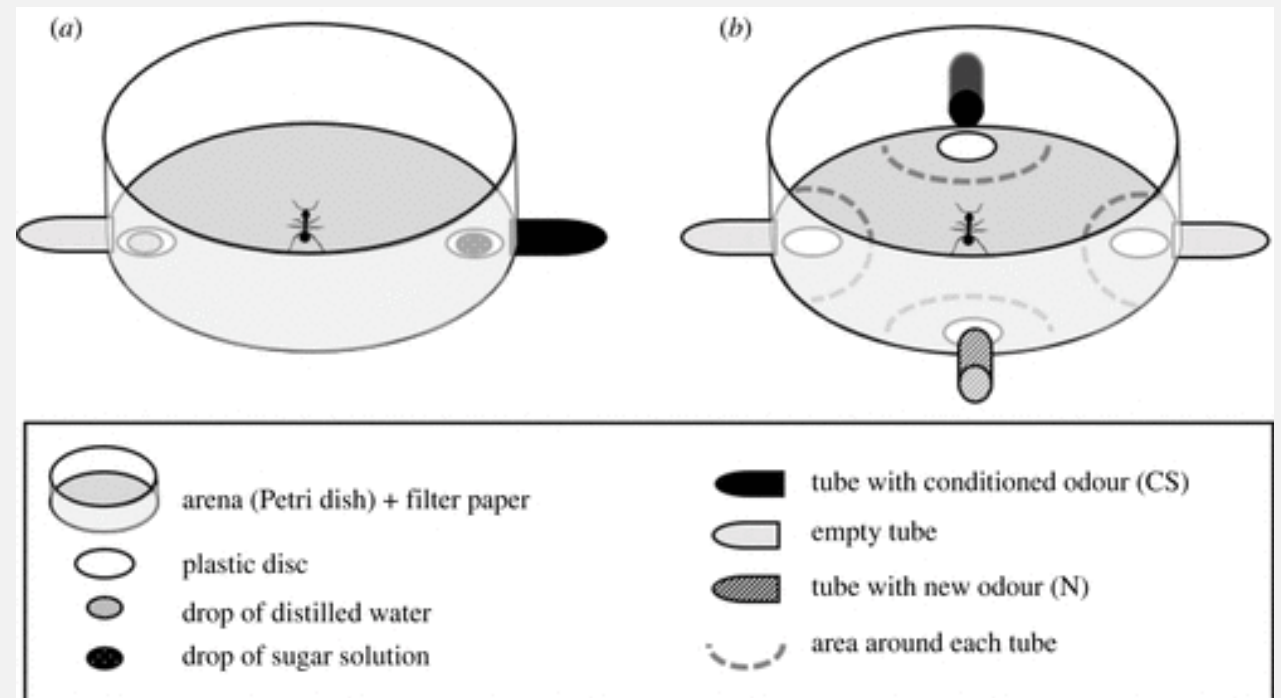
METHODOLOGY

- The ant was allowed to drink the drop of sugar solution and was then returned to the colony, where it could perform trophallaxis (mouth to mouth exchange of liquid food) with her nestmates.
- Without trophallaxis, the crop of tested ants would be full in only a few conditioning trials and the ants would not be motivated to find more food.
- The ant was allowed to drink the drop of sugar solution and was then returned to the colony. Tested ants were left for about 3 min in the colony (inter-trial interval), during which they terminated trophallaxis and came back spontaneously to the foraging arena, where they were picked up for the next training trial.



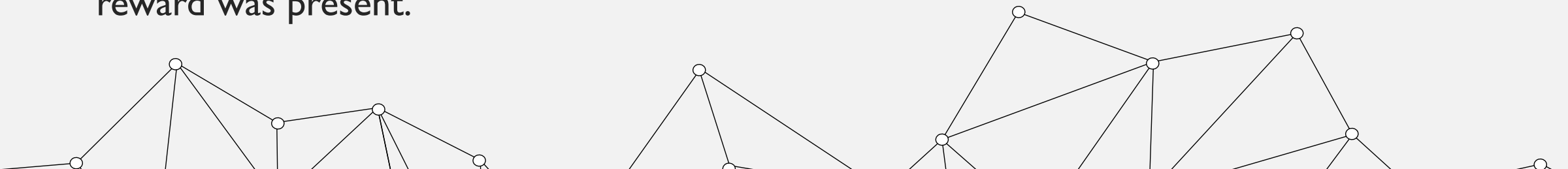
METHODOLOGY

- The filter paper at the bottom of the arena and the plastic discs were replaced with clean ones to remove any possible chemical cues left by the ant at the previous trial.
- The orientation of the arena and the position of the experimenter were also modified between trials to limit the possible use of visual or other spatial cues.
- Then, to test whether ants have learned that the CS is a predictor for reward, a memory tests were performed in which the reward was absent.



RESULTS

- Ants can detect cells through olfaction.
- Individual ants ($n = 36$) were subjected to three training trials in a circular arena, during which the odor of a human cancer cell sample (IGROV-I, ovarian cancer) cultured in medium (DMEM) was associated with a reward of sugar solution.
- The time the ants needed to find the reward decreased over the trials, indicating that they had learned to detect the presence of cells based on their emitted volatiles.
- This was confirmed by ants performing two consecutive memory tests in which no reward was present.



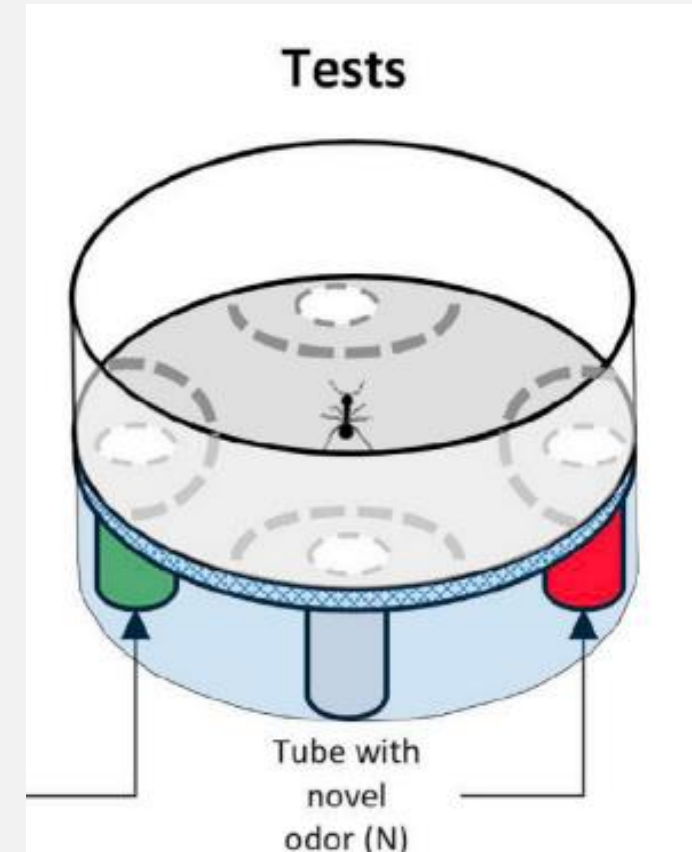
RESULTS

- In a similar circular arena, the time spent by the ants investigating two different odors was measured:

The odor of the cells (IGROV-I, ovarian cancer) (conditioned stimulus) and the odor of the culture medium alone (DMEM) (novel odor).

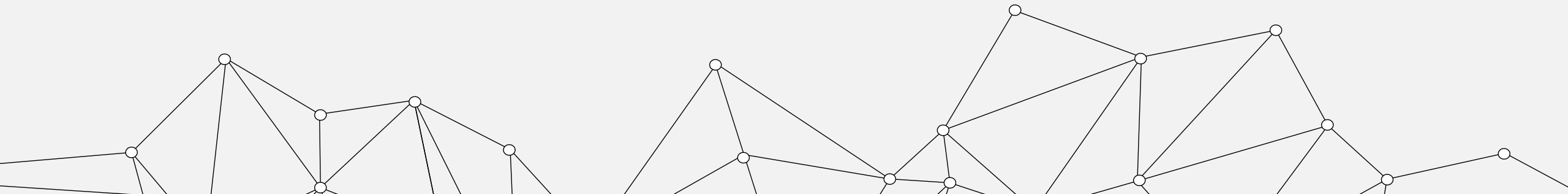
Two empty tubes were also present as controls.

During these memory tests, ants spent significantly more time near the conditioned odor (cancer cells) than near the culture medium alone, demonstrating that ants can recognize the presence of cells in a sample.



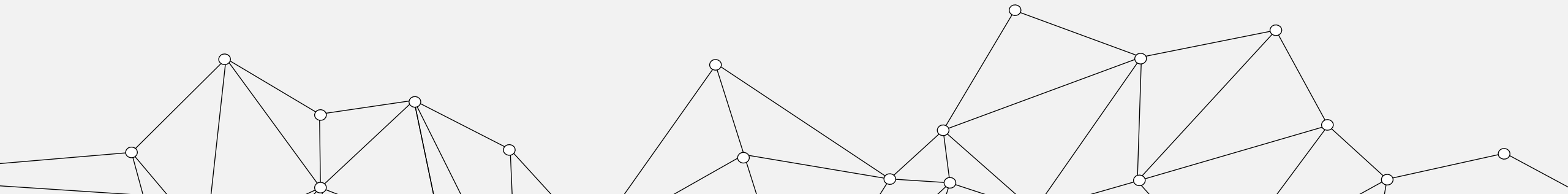
DISCRIMINATION BETWEEN CANCEROUS AND HEALTHY CELLS

- Next they investigated whether ants could discriminate cancer cells from healthy ones by using two breast cell lines: an epithelium cancer cell line derived from adenocarcinoma breast cancer, MCF-7, and a healthy breast cell line.
- Ants spent significantly more time near the conditioned odor, demonstrating that they can discriminate a cancerous cell line from a healthy one, and exhibit this ability after a simple, 3-trial olfactory learning protocol.



DISCRIMINATION OF TWO CANCEROUS LINES

- Finally, they tested if ants can differentiate between two different cancerous cell lines: the MCF-7 cell line and the MDA-MD-231 breast cancer cell line.
- Ants were either conditioned to the MCF-7 ($n = 25$) odor, or the MDA-MD-231 odor ($n = 24$). And were tested in an arena with the conditioned odor and the novel odor (as above).
- Ants of each group spent significantly more time near the conditioned odor than the novel one, showing that they can indeed discriminate between two different cancer cell lines.



DISCUSSION

- Using a simple conditioning protocol, *F. fusca* ants can detect the VOCs emitted by cancer cells. A conditioning protocol based on only three training trials was sufficient for ants to associate cell derived VOCs with a reward.
- Ants were able to:
 - i) Perceive the presence of cells in a medium.
 - ii) Differentiate cancerous VOCs from non-cancerous ones.
 - iii) Differentiate between two cancerous samples based on VOCs.



DISCUSSION

- *F. fusca* ants learn fast and retain a learned association after only three trials, using ecologically relevant odors (floral, food, or cuticular ant hydrocarbons). Such remarkable learning abilities were recently described in the same species (2019), and in other ant species such as *Lasius niger* (2020), *Camponotus spp.* (2009), , and *Linepithema humile* (2020).

Lasius niger



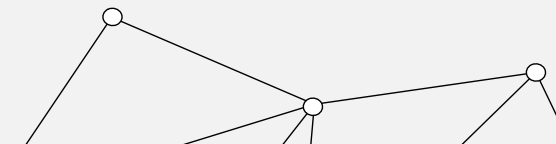
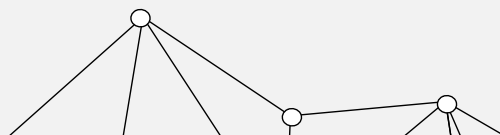
Camponotus spp.



Linepithema humile



Formica fusca



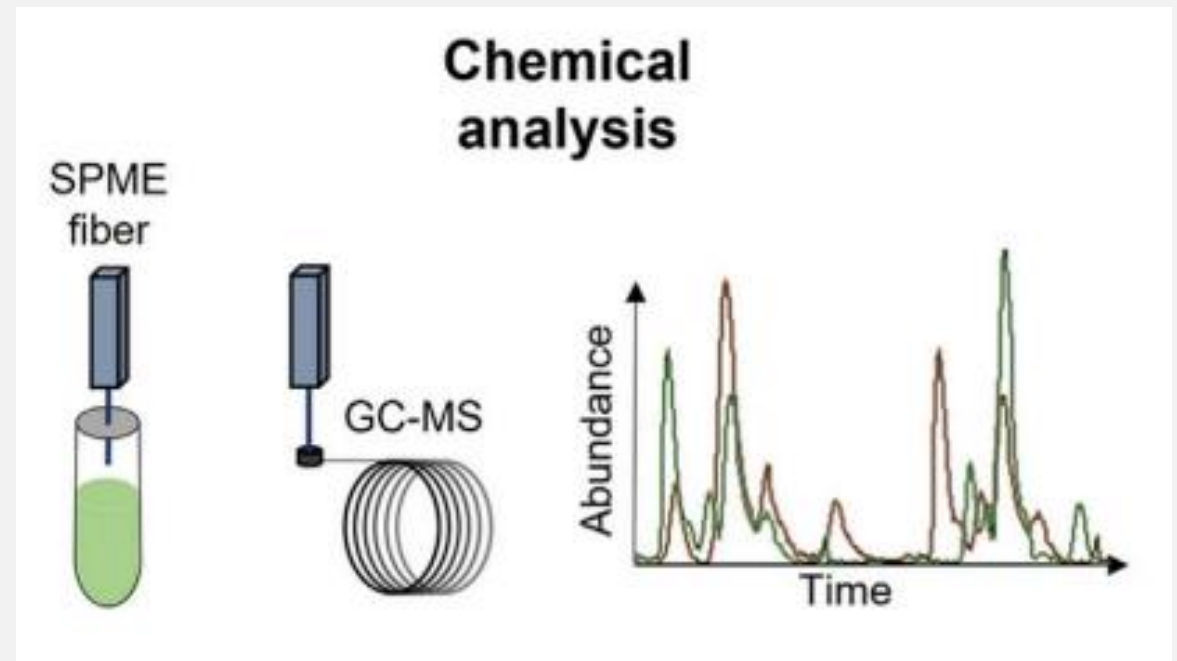
EXPLANATION

- Cancer is a complex disease, it is likely that each type of cancer (lung, breast, prostate, etc.) produces its own pattern of biomarkers, and not a single common molecule.
- For example, in the MCF-7 cell line, styrene and dodecane were more present whereas benzaldehyde was less abundant compared to the control. MCF-7 also produced more phenol than the other breast cell lines.
- In (2015), These VOCs were also found in higher abundance in the breath of patients with breast and ovarian cancers. While decanal was less abundant, and indeed it was found that this compound is consumed by the cancer cells from the medium.



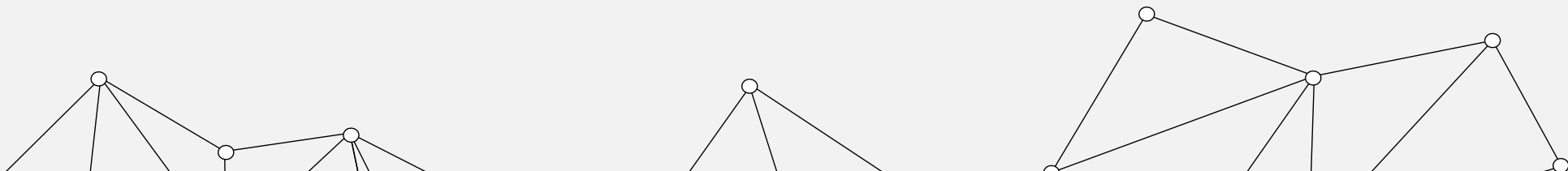
BENEFIT IN GAS CHROMATOGRAPHY

- the acquisition of data may differ, as no standard procedures were established yet.
- using MCF-7 cell line, and SPME/GC-MS analysis, in one study (Silva et al., 2017), the SPME fiber was left in the headspace of the cell line for 45 min at 37 C, and then injected into a GC for 10 min at 250 C, whereas in a similar study (Liu et al., 2019), the fiber was left in the headspace for 30 min at the same temperature of 37 C, but then injected into a GC at a higher temperature (270C) for a shorter time (5 min).
- SPME/GC-MS tools are extremely powerful for the identification of the VOCs composition of samples, but these methods are still lacking a proper standardization to be broadly used and reliable in real screening situations.



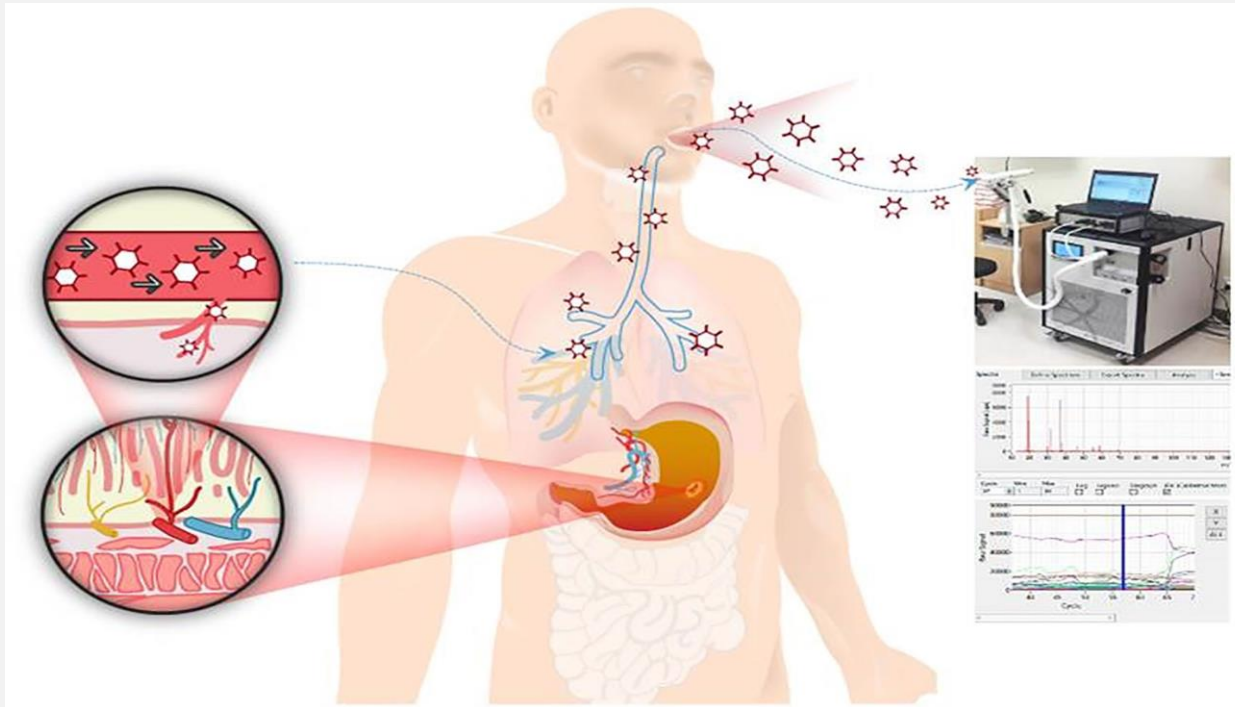
CONCLUSION

- Ants are available in great numbers, and collectively choose the right odor with a very high probability ($p < 0.01$ in all experiments).
- Ants are thus equivalent to dogs — the most studied bio-detectors — in terms of detection abilities. In some respects, ants surpass dogs because they need an extremely shorter training time (30 min compared to 6–12 months for a dog) and a reduced cost of training and maintenance.
- Individual *F. fusca* ants can also be used more than once. A previous study showed that: With a single conditioning trial, ants could be tested up to nine times before response extinction.



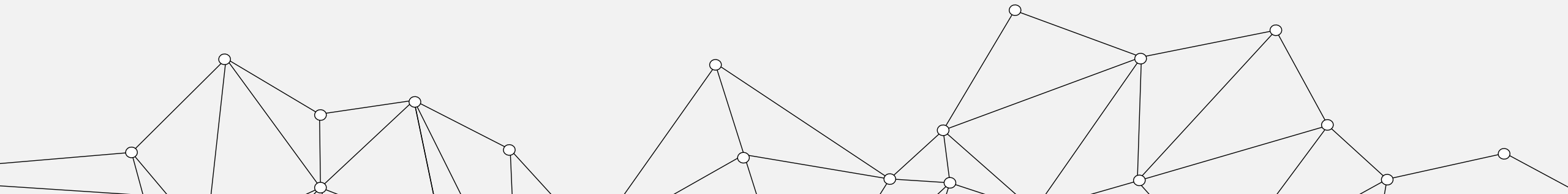
CONCLUSION

- This approach could potentially be adapted to a range of other complex odor detection tasks, including the detection of: Narcotics, explosives, spoiled food, or other diseases (malaria, infections, diabetes for instance).
- Research will now aim to widen the range of cancer-related odors that can be detected by ants, moving to the detection of body-emitted odors.



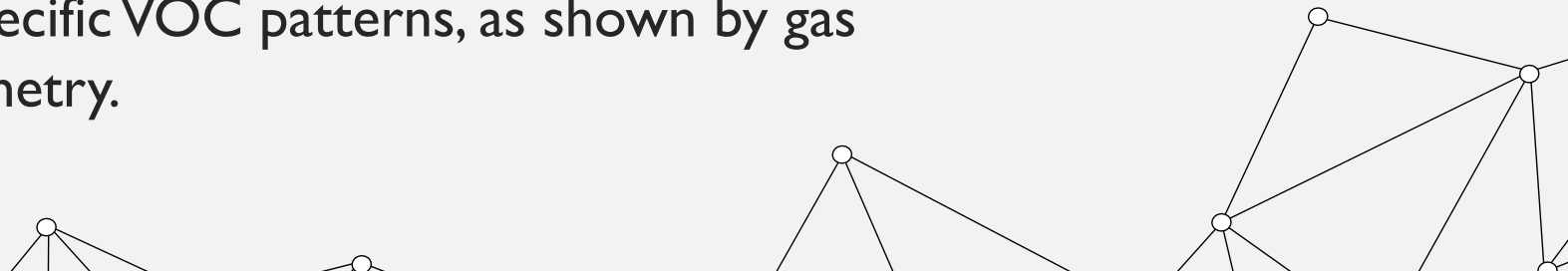
LIMITATIONS OF THE STUDY

- This study was performed on a single population of *F. fusca* ants. Although there are no reasons to believe that this population could be the only one to have developed refined olfactory abilities allowing the detection of human cancer volatiles, it would be of interest to test our protocol with individuals from other populations of *F. fusca*, as well as with other ant species.
- We cannot exclude that some of the detected VOCs may still be contaminants. Ideally, the validity of the VOCs as biomarkers could be tested in behavioral experiments in which ants are trained with the full cell odor and tested only with a solution containing the potential biomarkers.



SUMMARY

- Cancer cells are characterized by an altered metabolism, producing unique patterns of volatile organic compounds (VOCs) that can be used as cancer biomarkers.
- Dogs can detect VOCs via olfactory associative learning, but training dogs is costly and time-consuming. While insects, such as ants, have a refined sense of smell and can be rapidly trained.
- Individual ants need only a few training trials to learn, memorize, and reliably detect the odor of human cancer cells.
- These performances rely on specific VOC patterns, as shown by gas chromatography/mass spectrometry.





THANK YOU FOR LISTENING