



Report & Data analysis

UG – Research Experiments

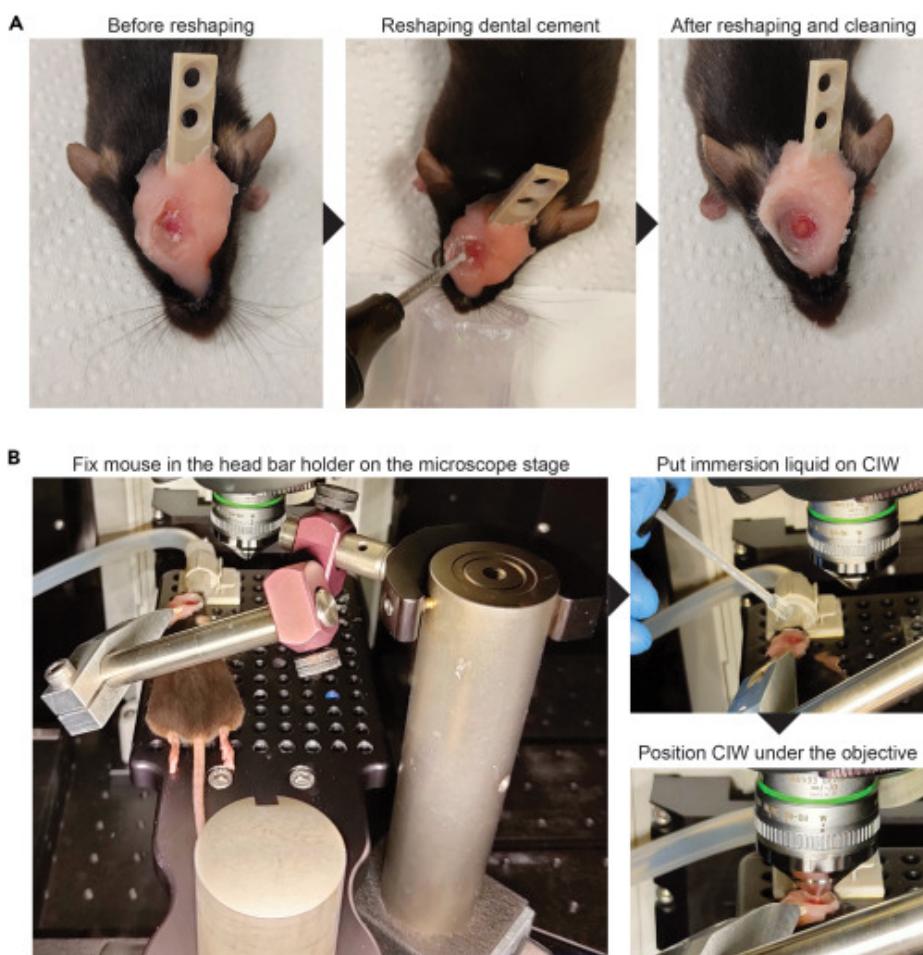
Module 2 - Neurobiological methods

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In Module 2 we studied Basic Neurobiology, Optogenetics, Mouse Handling and Training, Mouse Blood Perfusions and Surgeries. We also did fun Backyard Brain experiments recording action potentials from cockroaches legs. We also did Calcium imaging of mouse brain to record firings in Neuron clusters. In this report I would talk and discuss all that we learned in the module 2 both the neurology methods the top down approach (tissue to behavior) and bottoms up approach (behavior to tissue) and its techniques discussed.

Optogenetics

Optogenetics techniques are used to control the firing of neurons using lights of different wavelengths. These techniques use genetically modified organisms (GMO) expressing channelrhodopsin which allows influx for efflux of ions in the presence of a specific wavelength of a light in the neurons eventually leading to there activation and generation of action potential. This technique is used to study the effects of particular group of neurons on the animal behavior.



In the lab we used ChR2 channelrhodopsin genetically modified (GM) mouse which are allowed in 2 setups : 1) Movement restricted mouse - In this setup the mouse has surgically implanted screw attached to a firm stand and a LED bulb of the specific wavelength is shined and a control LED on top of the setup. Here we studied the change in the licking pattern in the mouse due to different odors and training them to learn reward and threat. 2) Freely moving Mouse - in this setup the mouse is allowed to freely move in a setup keeping rest of the setup same.

Mouse is trained to detect different odor by providing them water as reward and studied their learning behavior after days. And can we replicate similar behavior by artificially activating those network of neurons using channelrhodopsins.

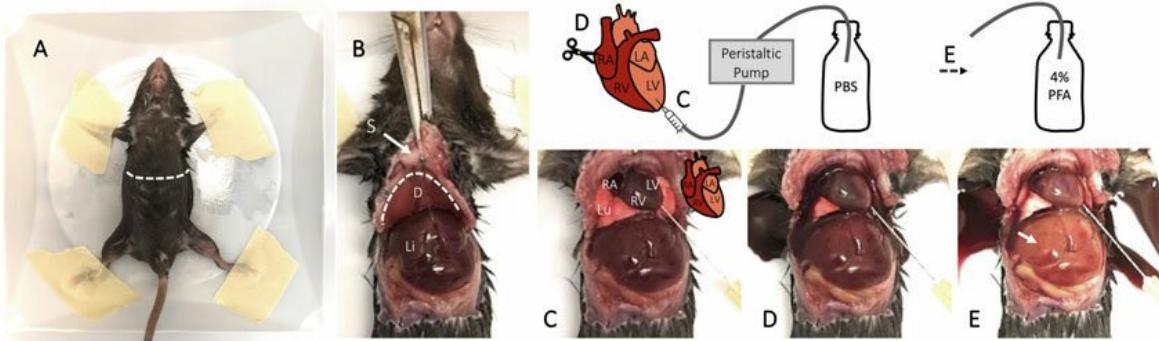
Mouse Handelling

In this module we also learnt to handle mouse as a model organism and how to vaccinate them there feeding behavior and anesthetize them for surgeries using Ketamine and saw few surgical operations over them. How to check for responses after anesthesia and saw how to operate using microscope.

Blood Perfusion and Brain Extraction

Blood perfusion is a crucial step in neuroscience research, used to remove blood and fix tissues for histological analysis later on. It basically freezes the tissue intact to perform imaging techniques later on.

It involves first anesthetize the mouse using 10% Ketamine, 0.1–0.2 mL per 25 g mouse. Then we made a midline incision over the thoracic cavity cut open the diaphragm and expose the heart. A needle is placed in the left ventricle and the is flushed with **PBS** (phosphate-buffered saline) preventing clot formation and allowing for better fixation. The liver should turn pale, indicating successful blood removal.

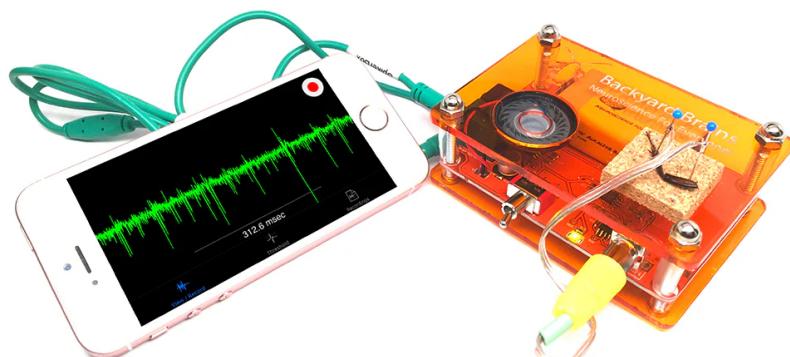


Then the body is flushed with 4% Paraformaldehyde **PFA** a perfuse fixative solution (~10–20 mL) to preserve brain and tissue structure. Body stiffens up. This fixes the tissue (freezes it in whatever state) so that could be used later for imaging.

We then decapitated the mouse for the brain extraction then slowly cut the skin through the midline incision and then carefully cut open the skull without damaging the brain. And slowly pickup the brain using a spatula and cutting all the connections of the olfactory bulb and any other tissue holding it carefully. Extract the brain and preserve it in formalin.

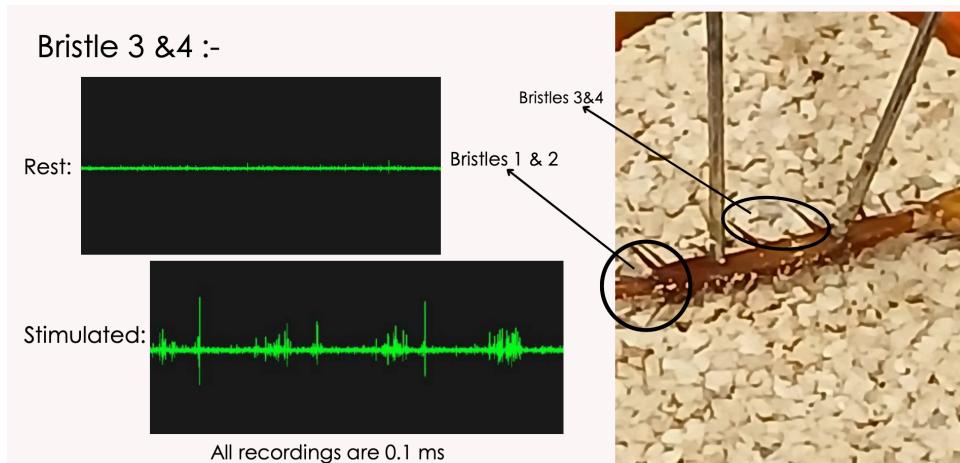
Backyard Brains

These were a fun set of experiments giving us a way to visualize the action potentials in the cockroaches legs. Cockroaches have a alive leg even when detached from the body these legs can still fire action potentials when simulated by touch on the hairs on the legs. 2 pins were attached to the legs one for reference and the other to get the potential difference with is them amplified by the apparatus and then is converted to sounds and waves for us to visualize the intensity of the signals.



We also tried putting ice on the leg and got very intense signals which indicates multi modal input signals in the cockroach and then we recorded the recordings from different hairs of the cockroach legs once without touching and once continuously touching to compare.

We also sent motor signals to the cockroach legs using the electrodes and saw dancing leg like behavior where the leg moved with the beats.



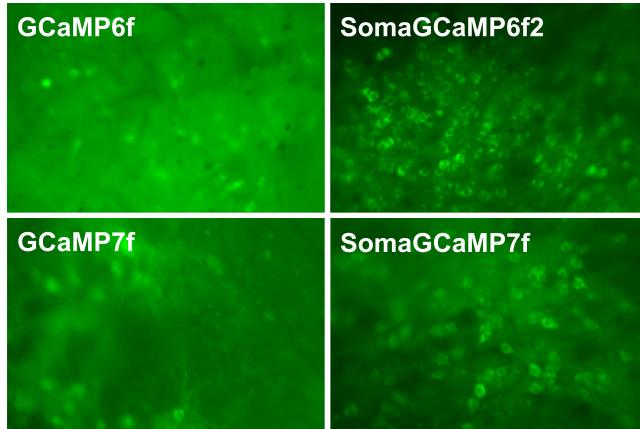
Effects of Nicotine - Backyard Brains

Here we studied the effects of nicotine on the motor signaling and sensory signaling in the cockroach legs using the same Backyard Brains apparatus by artificially injecting nicotine. We did inject the cockroaches with nicotine to see a bit enhanced sensory neurons activity. We saw very little increase in activity while activated but no significant increase in the basal levels of the sensory activity without any activation.

Calcium Imaging

Calcium imaging is a technique used in neuroscience to visualize and measure neural activity in living cells and tissues. It relies on genetically modified fluorescent indicators that bind to calcium ions (Ca^{2+}) and emit light when excited by a specific wavelength. Since calcium influx is closely linked to neuronal action potentials and

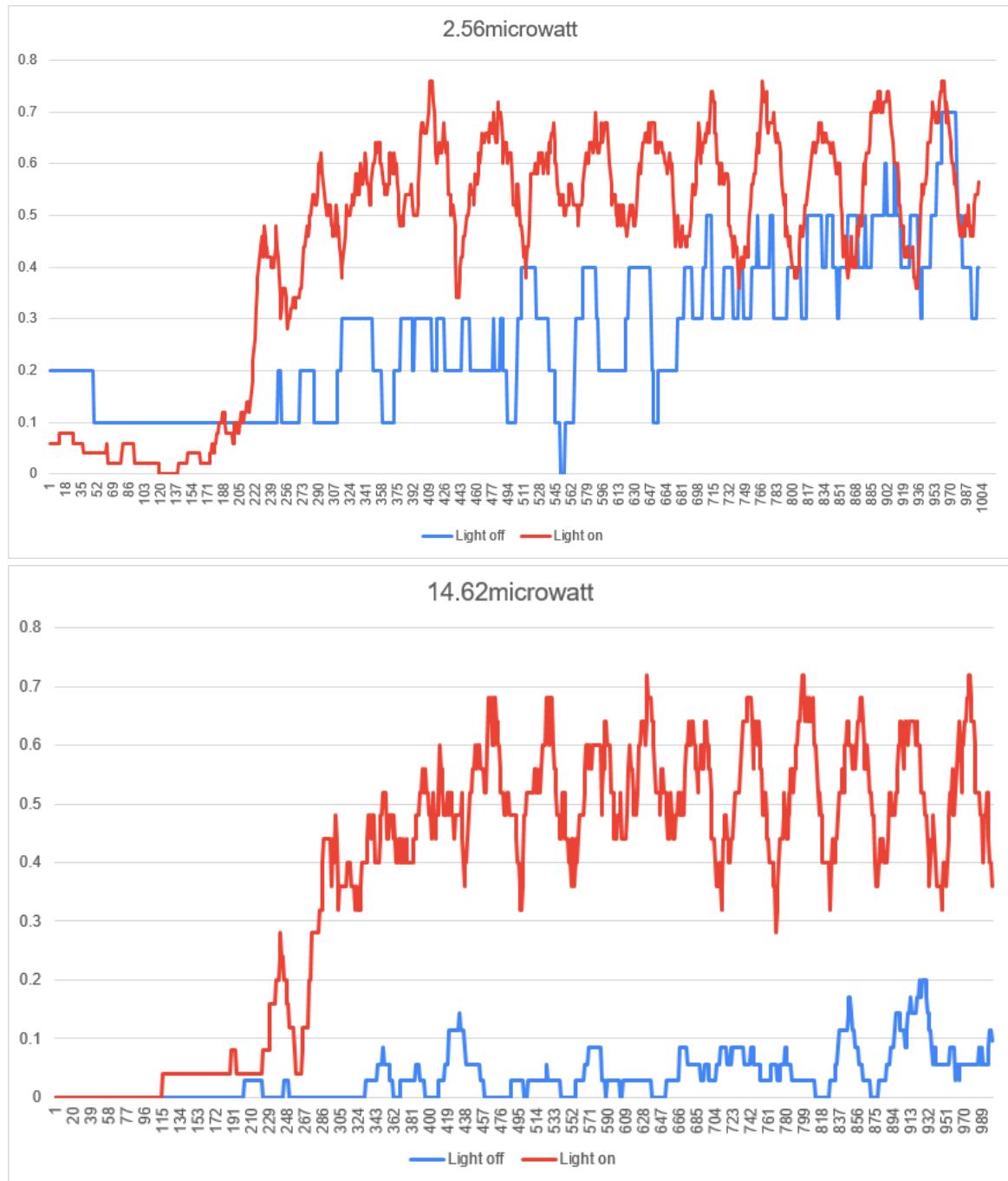
synaptic transmission, this method provides a real-time view of neuronal activity at both sparse single cells and network of neurons. Genetically encoded calcium indicators (GECIs) like GCaMP have revolutionized the field, allowing long-term imaging in live animals.



The process involves loading calcium-sensitive dyes or expressing GECIs in neurons, a lens is surgically implanted in the mouse and a small camera is moved which mounted on top with a led and captures images at high FPS. Compared to traditional electrophysiology, calcium imaging provides detailed recordings of large neuronal populations but has lower time related resolution.

Data Analysis

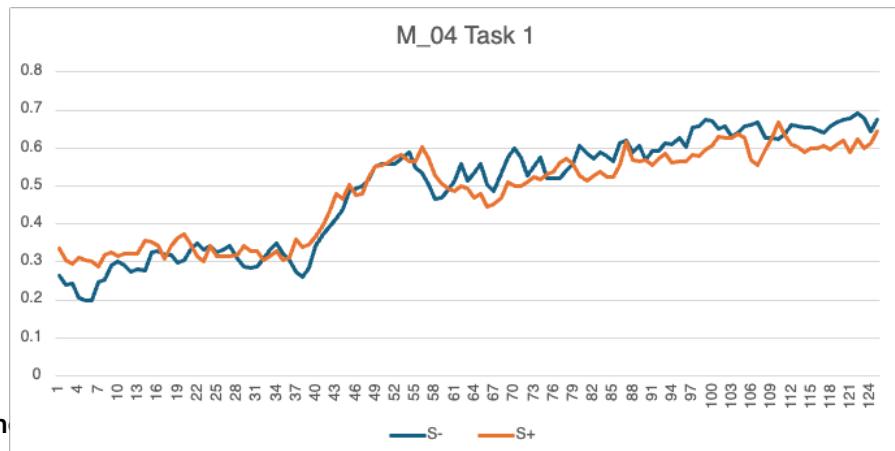
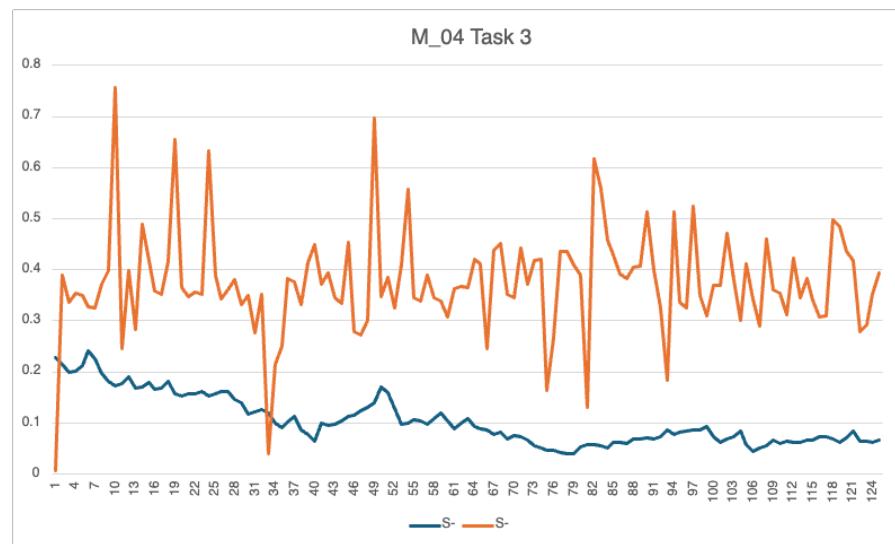
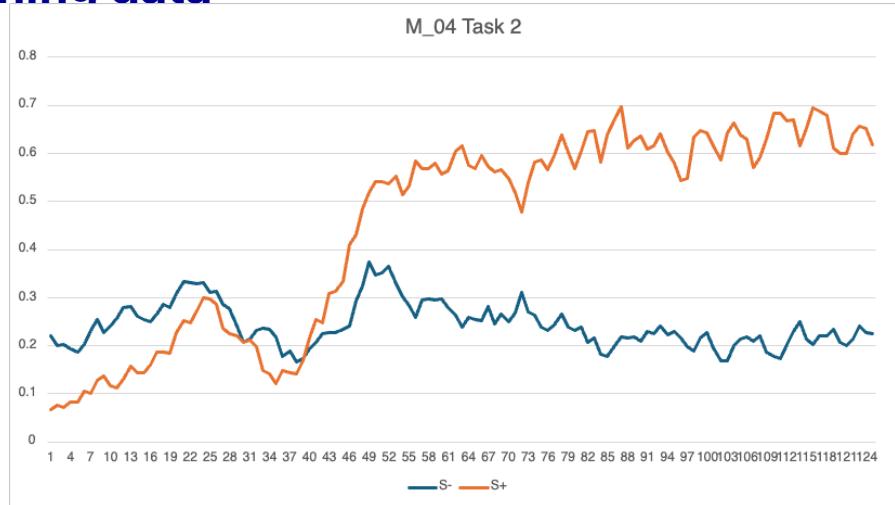
1. Optogenetics - data

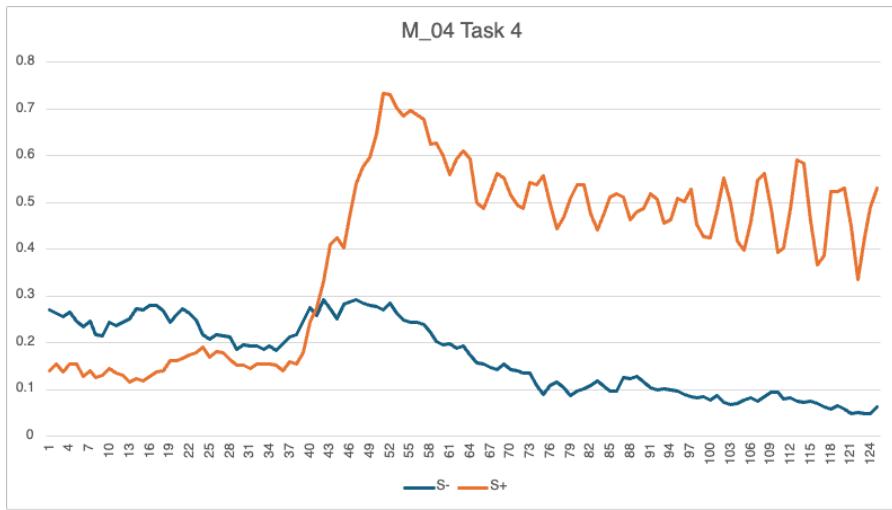


This graph shows the learning of the organism with time as the training progresses. The organism learns to differentiate between odors while optogenetically simulated. The training involved conditioning the mice to associate specific odors with a reward (water here). The data illustrates the percentage of correct responses for positive (S+) and negative (S-) odor

trials. The graph shows a trend of improvement in task performance over time under 14.62 microwatt but not in 2.56 microwatt, indicating successful learning under 14.62 microwatt. The results support the hypothesis that optogenetic modulation can influence sensory perception and learning in odor-based tasks. These graphs shows successful learning in 14.62 microwatt but not in 2.56 microwatt.

2. Training data

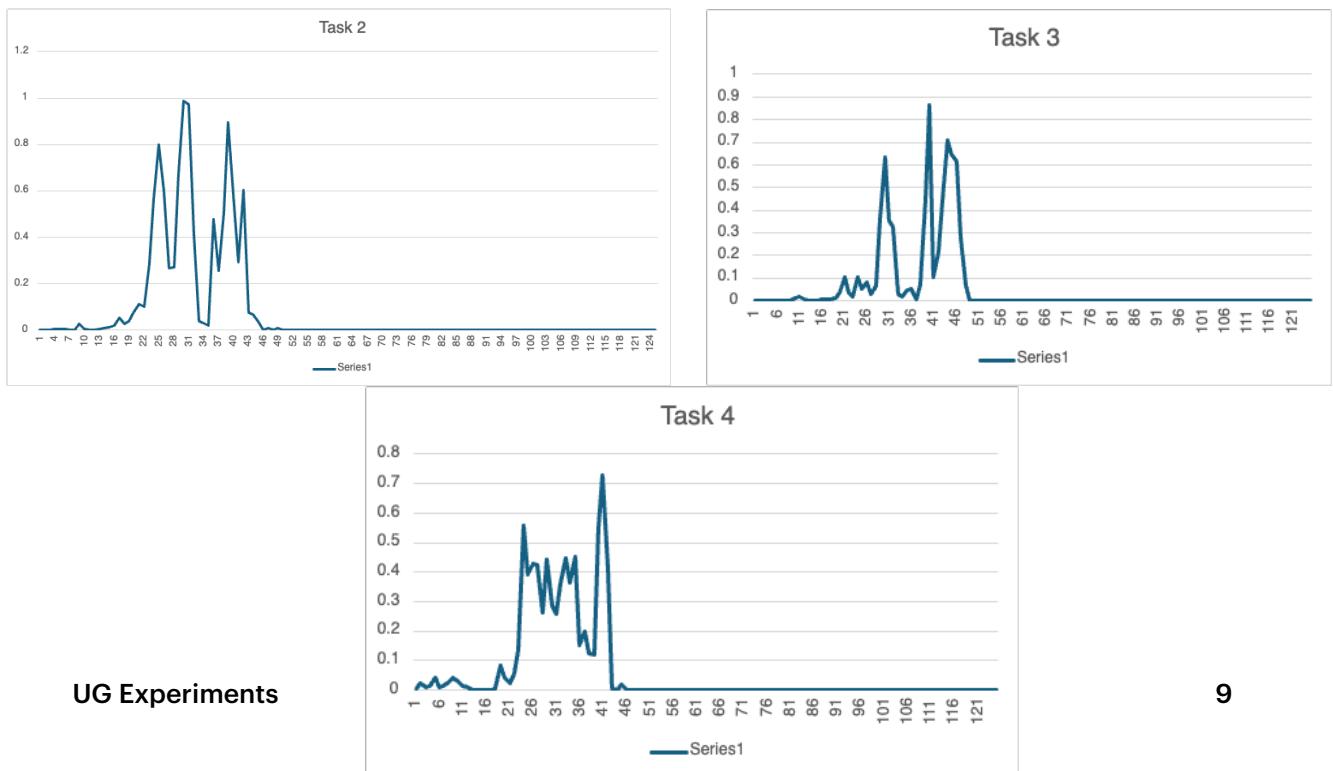




The graphs show the performance across four tasks (M_04 Task 1 to Task 4), with correct responses to positive stimuli (S+) and negative stimuli (S-) plotted over time. In Task 1, both S+ and S- responses show an increasing trend, indicating gradual learning. Task 2 shows a sharp improvement in S+ responses, with S- responses remaining consistently low, suggesting better differentiation between rewarded and non-rewarded odors. Task 3 exhibits high variability in S+ responses, may be due to inconsistent behavior or external influences during training. Task 4 demonstrates a improvement in S+ accuracy followed by stable pattern, while S- responses decline, showing strong learning. These results indicate that the mice successfully learned to differentiate odors over time.

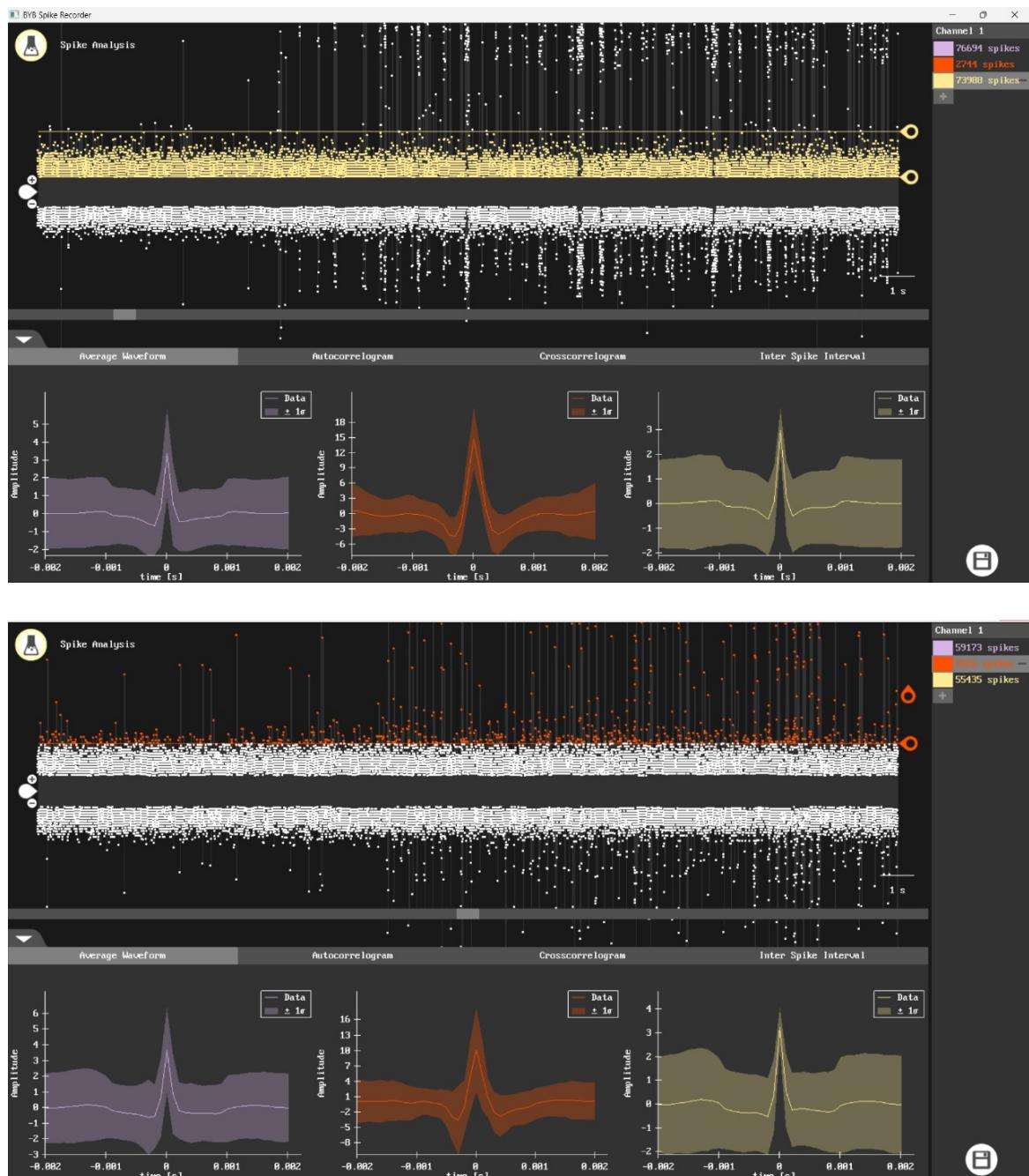
3. Discrimination time

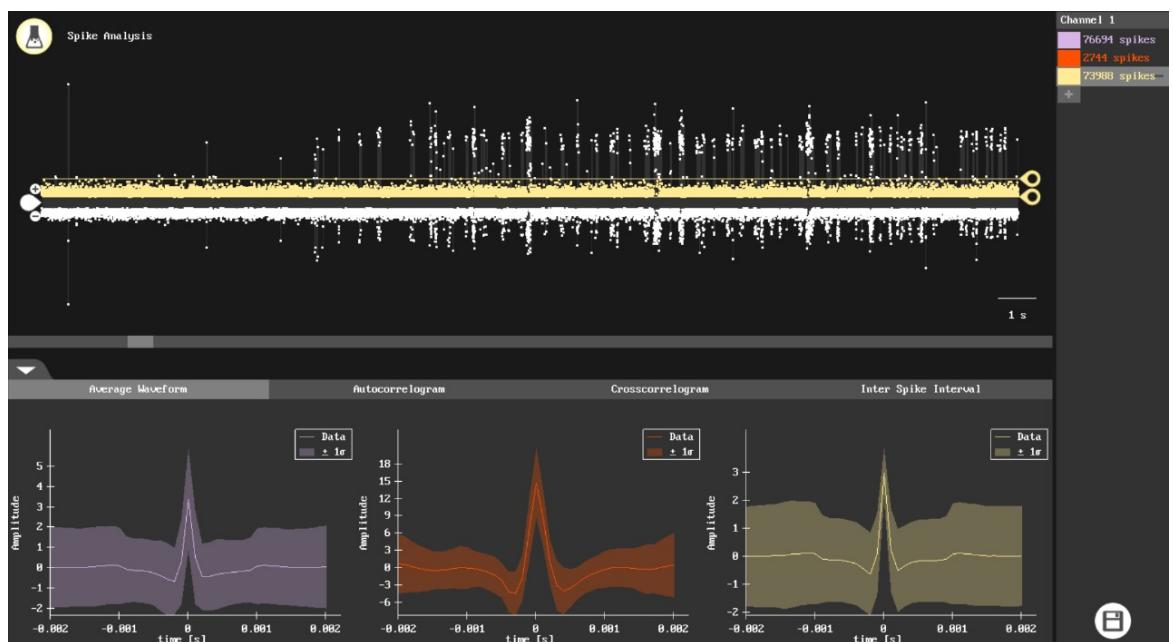
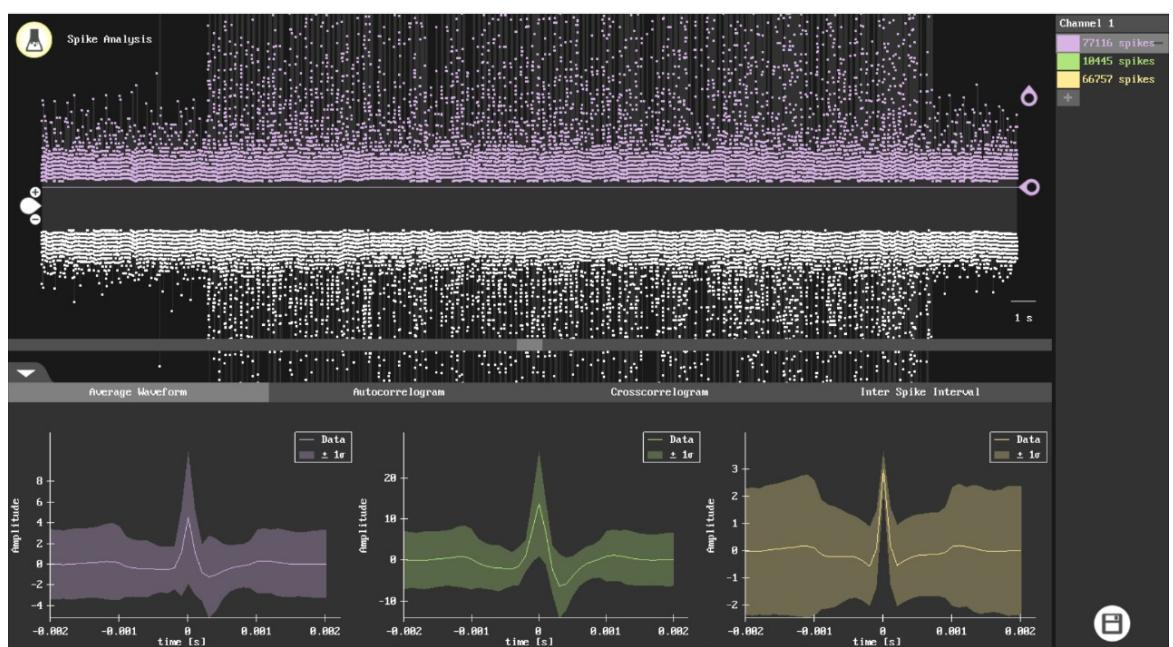
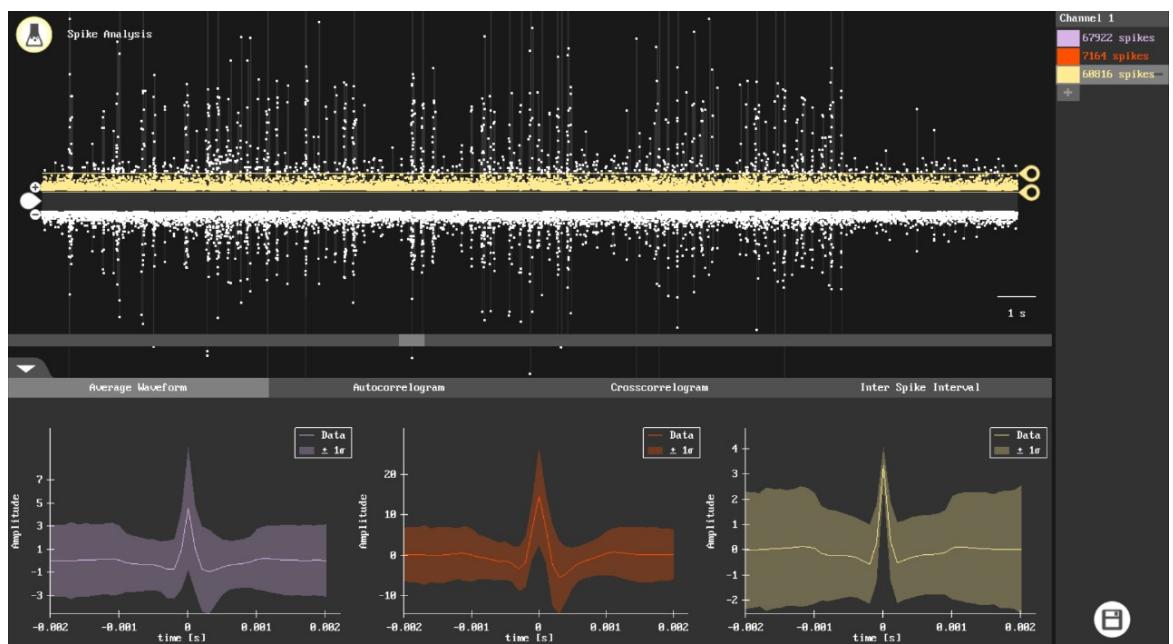
Discrimination time for task 2 is around 0.92 sec and for task 3 is around the bin 0.96 sec for task 4 it is also 0.96 with significance 0.01 after 2 sided t-test.



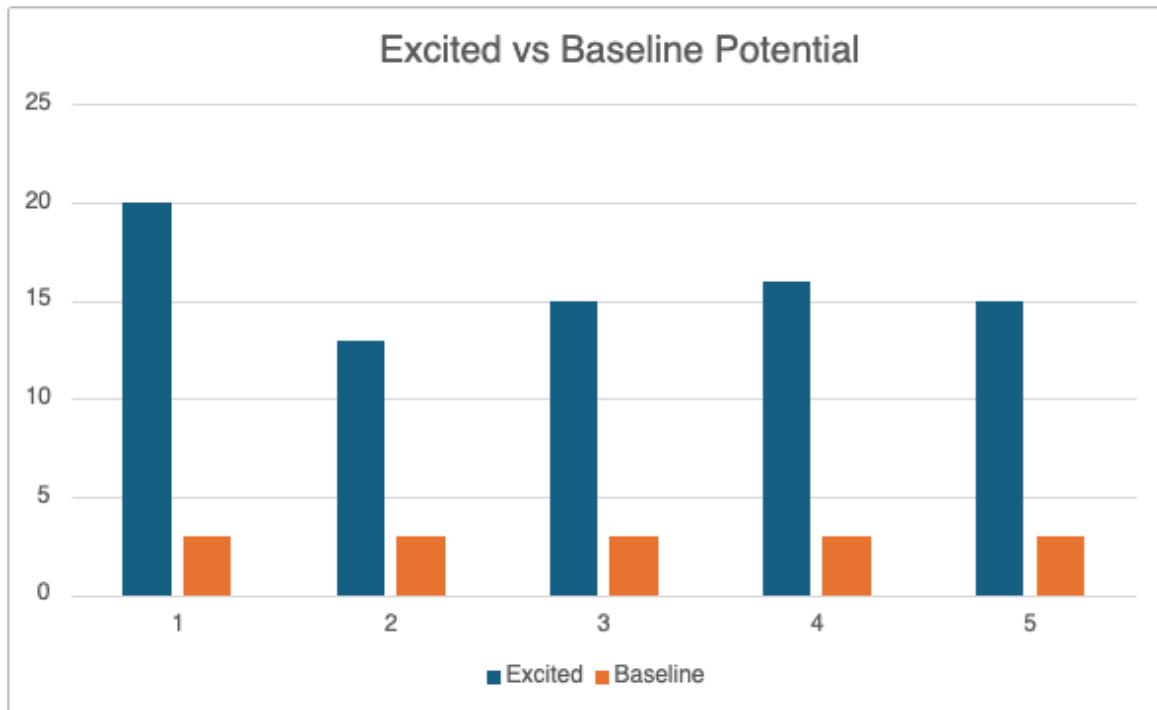
These graph shows the p-value of the bins in task 2, 3 and 4.

4. Cockroach action potential data





The below left most graph represents the baseline potential and the bottom middle graph represents the excited potential of the cockroach. (When the leg bristles were disturbed) These are 2 mins readings of 5 different bristles. We see a clear difference between the amplitude of the excited and baseline potential in all 5 trials while increasing the distance from the electrodes.



I could not see any distance dependent relationship on the leg in the bristles, first it decreased and then increased as in above graph. We can also clearly notice the shape of the action potential in all the 5 trials in the bottom middle graph.

Conclusion

The learning of the animal was successful it showed clear differentiation in licking pattern in all the tasks and at even after similar times within the experiment. The animal could clearly differentiate between the 2 odors after the training significantly. We could clearly notice the action potential and the significant increase in the potential when bristles were excited.