İstanbul Medipol University

School of Engineering and Natural Sciences

Graduation Project Report 2

PROJECT II FINAL REPORT - 2022-2023

PROJECT TITLE
Deep Learning for Odor Sensing
PROJECT ADVISOR
Prof. Dr. Mehmet Kemal ÖZDEMİR
TEAM MEMBERS
Tayseer M. Hussein Buse Nur FİDAN Senanur DEMİRCİ

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Project Team Members:	Tayseer M. Hussein - Buse Nur FİDAN - Senanur DEMİRCİ
Sponsor Company (if any):	TÜBİTAK

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PROJECT PLAN Duration in Weeks	28 Weeks	28 Weeks
STARTING DATE	26.09.2022	26.09.2022

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Project Title: Deep Learning for Odor Sensing
Project Advisor: Prof. Dr. Mehmet Kemal ÖZDEMİR
Team Members: Buse Nur FİDAN, Senanur DEMİRCİ, Tayseer M. HUSSEIN
Project Group Title: Odor Sensing

ABSTRACT

Chemical identification by scent has been the focus of research lately, as it has a wide range of applications, from airport security to mine sweeping, from the diagnosis of certain disorders to the detection of narcotic chemicals. [1,2,3]. Unlike light and sound, the odor detection process does not involve a simple method. This is due to the rapid spread of odor molecules in the environment. It can be said that odor consists of chemical molecules, but it cannot be said that every chemical molecule has an odor. Odor sensing is the ability to detect and identify odors, which is important for a variety of applications in different fields. The olfactory system is responsible for detecting and identifying odors, and it has been the focus of extensive research in fields such as genetics, cellular biology, biochemistry, and neurophysiology. Odor sensing has massive practical applications, including the protection of airports agents, bomb threats, drug smuggling, and the precautional diagnosis of diseases through smell, etc. It is also used in the food and agriculture industry to ensure product quality and safety, as well as in the Cosmetic industry to develop fragrances and beauty products. Odor-sensing technology has the potential to revolutionize various industries and improve the lives of people around the world, by utilizing odor-sensing technology just like previously utilized senses like sight, where cameras and screens were developed, hearing, where microphones and speakers were developed and touch, where digitizers and haptic devices where developed.

However, when we look at the studies on odor classification, it is seen that there is a system called electronic nose. An odor detection system has been developed using chemical sensors in the electronic nose. However, this system has some disadvantages. The operation of the electronic nose is limited, because when odor diversity increases, it can cause the same results for different odors. In addition, as the number of odors to be classified increases, the sensors in the electronic nose must be reprocessed.

The Odor sensing project, on the other hand, aims to obtain odor data by placing an electrode in the olfactory bulb of the mouse rather than an electronic sensor, and to classify these data with an accuracy rate of over 80% using various artificial intelligence algorithms. As the name of the study implies, a mouse is used in an odor-sensing setup, along with an electronic system to capture, amplify and filter the olfactory bulb signal and a deep learning model to detect odors from the captured olfactory bulb signal. The basic goals of the project are accomplished through a number of processes covered in this study. The four major goals of this research are to develop a data collection system, collect data from the olfactory bulb, process the data using deep learning, and then identify the chemical responsible for the odor.

Artificial intelligence is a sub-branch of computer science. Artificial intelligence can simply mean the intelligence found in machines. The aim here is that machines can imitate skills such as decision-making, learning, thinking and classification, similar to living things with natural intelligence. Artificial intelligence consists of synthesized mathematical equations to perform these operations. The reason for using artificial intelligence in the project is to enable machines to distinguish odor in a way that the human brain can. In order to achieve this, the classification feature of artificial intelligence is used. As the name of the project implies, using deep learning, odor detection is also performed in this project. A dataset is needed for the deep learning model to train on in order to conduct the classification using deep learning. In order to create a dataset, signals from a mice or another lab animal's olfactory bulb must first be collected. Single-channel electrodes were employed to make that happen. One can observe the process, the signal's type and value, and its value can be used to determine what should be tested and expected by taking a signal from a single electrode. In the beginning, the electrode holder was designed with the help of a 3D printer and the electrodes were placed on the holder with the help of silver paint. After testing and measuring the electrodes, new electrodes were generated. According to the results that are taken in this experiment, electrodes should have around 120k ohm impedance. (Circuit results). After all preparations were completed, the experiments were started. At the end of the experiments, the electrodes were attached to the animal's head together with the designed electrode holder. But the animal easily took it off its head and threw it away. For this reason, a change was made in the design, but due to the lack of resin for the 3D printer, the printing process could not be done just in time. There were some problems that are discussed throughout the discussion part which are related to the lab noise, surgical issues, and animal based. Since, while recording the data, there were some problems encountered, the dataset required to train the deep learning model was not created. Therefore, an EEG dataset was found and utilized as the B plan and the best accuracy of the classification was 96%.

There are several technologies that are used for odor sensing, including mass spectrometry, gas chromatography, and e-noses, which are devices that use chemical sensors to detect odors and can classify them based on their chemical signature.

Keywords: Odor, Olfactory Bulb, Signal Processing, Multi-Channel Electrode, Deep Learning, Odor Classification.

1. OBJECTIVE OF THE PROJECT:

The main objective of this project is to use deep learning techniques to improve the accuracy and efficiency of discrimination of odors. Specifically, the objectives of the project are:

1. Developing a data collection system to gather data from the olfactory bulb, which is the part of the brain that is responsible for detecting and identifying odors.

2. Developing electrical hardware that amplifies. filters the low amplitude signals, and high noise signals collected from the olfactory bulb region.

3. Using deep learning algorithms to process the data and identify the chemical compounds that produce odors.

4. Evaluating the effectiveness of the deep learning-based odor sensing system through experiments and comparing its performance to traditional methods.

5. Exploring the potential applications of the deep learning-based odor sensing system in various industries, such as the detection of chemicals, the precautional diagnosis of diseases through smell, and more. Helping in improving the technological developments and Research on taste and smell.

6. Helping in developing research infrastructure for smell and odor-focused devices in different sectors. To achieve the same level of advancements in current other senses-based technologies.

Overall, the goal of this project is to demonstrate the prospect of using deep learning to improve the accuracy and efficiency of odor discrimination and to identify the potential applications of this technology, such as assisting individuals with anosmia.

2. LITERATURE REVIEW:

The olfactory and brain systems function in mysterious ways. The characteristics of neural signals and cell activities allow for the observation of diverse responses to various scents. After the odor molecules reach the nose, they pass through mucus and reach the receptors. There are two basic views about the way these receptors, which enable them to detect an odor, work. The first of these is the shape method. According to this method, a puzzle-like relationship exists between the shape of the odor-sensing receptors and the shape of the odor molecules. Another view is the vibration model. According to this model, the vibration of odor molecules is different from each other. Olfactory receptors can detect odor thanks to this difference. A side goal of the Deep Learning for Odor Sensor project is to test if the vibration theory is in agreement with the data collected from the mice.

There are odor sensors and e-noses that can only distinguish very strong chemical odors. However, there are many disadvantages to using them for odor detection. First of all, these sensors are both costly and can only distinguish strong chemicals. There are numerous studies that use machine learning to detect and categorize odors. These studies generally used e-noses or sensors to obtain odor data.

Additionally, publications about odor discrimination utilizing the mouse olfactory bulb are also performed. Since mice are highly developed creatures in sense of smell and taste, they can be trained in a laboratory environment. It also provides the opportunity to classify more odors. As it will be explained in the originality section, taking data from the mouse and classifying this data with artificial intelligence is a special and unique study that distinguishes the project from the literature. Basically, we let the mouse's olfactory bulb do the sensing while deep learning replaces the mouse brain and makes the decisions for the odors.

We can divide the studies in literature into two groups. First, there are numerous machine-deep learning techniques and research in this area, however, due to the same or related techniques, some of them were described in detail and others were mentioned in a few phrases. Studies are given according to the year of the publication.

Omatu, Araki, Fujinaka, and Yano used MOG and QCM sensors to acquire odor data [4]. These sensors are commercially available sensors and can only detect certain chemicals. They did three experiments in total. First, they conducted a total of three experiments. 500 samples were gathered for the test after several iterations using four different kinds of tea. A total of 5 coffees were classified in the second experiment. For each coffee type, there were 35 samples, and the dataset was reset 100 times. 1500 samples were left over at the end for classification. For the classification of the odor data, a 3 layered neural network was adopted based on the error backpropagation method. The accuracy rate for the first experiment is 96.2% and for the second experiment is 88.8% on average. The purpose of this publication is actually to measure the distinguishability of odors when odors are mixed. They used ethanol, water, metal salicylate, and triethyl-aminate as odorants and grouped them into two groups. When the smells are combined, the classification's accuracy declines.

A deep learning method was created by B. Grodniyomchai and his colleagues utilizing e-nose to classify odors [5]. Their Deep Neural Networks (DNN) structure consists of 7 input neurons, 4 output neurons, and 5 hidden layers. 5 hidden layers consist of 128,64,32,16 and 8 neurons, respectively. They used 4 different odors. Odorless, beer, whiskey, and wine. MLP, Decision Tree, NB, and DNN Confusion Matrix were developed. Each one of the odor identifications is entirely accurate. The results for MLP, Decision tree, NB, and DNN are 99.01%, 97.90%, 95.43%, and 99.26%, respectively, after 10 trials. DNN performs better than others in this area.

It has been demonstrated by Chomtip P. and Piyorot K. that altering the size of hidden neurons results in a difference in performance [6]. The Naive Bayes approach was used by Francesca Arcelli F. and other authors with The Data Class Code and the God Class Code both having

95.4% accuracy. Convolutional Neural Network (CNN) and Support Vector Machine (SVM) was used by PeiFeng Q and his colleagues to transform sensor data into a grayscale image, reaching 95.7% and 92.9% accuracy, respectively [7]. Chomtip P. and Natt S. used the rule-base model and neural networks to determine the type of beer (with 25x28x10 structure) accuracy of 87.5% was attained [8].

By considering atoms as nodes and bonds as edges, Benjamin Sanchez-Lengeling, Jennifer N. Wei, and their colleagues trained a graph neural network to identify the scents, or to predict the fate of an odor [9]. In this method, GNNs convert atoms and bonds into vectors with predefined lengths that are then processed by a fully connected neural network. A GNN was trained to anticipate the connection between a molecule's structure and its scent using a special and extensive dataset of expertly labeled single-molecule scents.

According to N. Bodyak, operant conditioning was used to train mice for the purpose of odor detection through discrete trials like go/no-go sessions [10]. The outcomes of this study are completely different from a classification using deep learning, but they train mice to provide superior results, either in terms of the ratio of odors they present for the greatest degree of accuracy or in terms of how they show the odors to the mice.

In a study, Juan Guo, Yu Cheng, Dehan Luo, Kin-Yeung Wong, Kevin Huke, and Xin Li used the CNN, LSTM, BP, and PCA RF algorithms to classify the data they had gathered via e-nose and then compared the outcomes. They suggest a paradigm for predicting deep odor descriptor ratings. They separated the data from the E-nose into equal frames first. They then provided the ConvLSTM network with each frame as input. 16 input channels, 14 hidden channels, a kernel size of 16, 4, and a size stride of 1 are all included in the model's single hidden layer [11].

As for the second group they used mice for data collection. Because of technology limitations, Fuqiang Xu and his associates concluded that it was still impossible to see and compare whole patterns with different odorants in the same species [12]. They used functional MRI with high resolution to show responses of olfactory bulbs. Software called ODORMAPBUILDER was created specifically to adapt to anatomical MRI and fMRI data. Analysis of the spatial correlation between odor maps was done to judge how similar they were.

Kyung-Jin You and his friends used micro-wire electrodes placed in the mitral/tufted cell layers of MOB to record the neuronal responses to several scents [13]. They did five trials using four different rats. For olfactory analysis, the maximum likelihood estimate (ML) based decoding method was applied. The average decoding accuracy for these four rats was approximately 100.0%, 96.0%, 84.0%, and 100.0%.

Kensaku Mori, Yuji K. Takahashi, Kei Igarashi, and Shin Nagayama mentioned Molecular-feature clusters and mitral & middle-tufted cells in their study on where the olfactory receptors are located. In glomerular maps, odorant receptors (OR) are spatially represented [14]. Optical imaging or fMRI techniques are utilized to map odorant-mediated glomerular activity. MRR (molecular receptive range) is calculated in relation to stimuli. This judgment is made using the dorsal area (zone 1). Each cluster glomerulus, each of which is situated in a different region of the dorsal surface, responds in some way to molecules with a certain functional group. Since olfactory information can be processed, the functional physical distinction between mitral and middle tufted cells makes a difference in how the odor maps are deciphered. The dorsal surface cluster A has been studied by the authors.

Liujing Zhuang and his associates created an in vitro capturing and assessing process13 for the long-term and repeatable recognition of olfactory stimuli. They placed probing micro-wire array electrodes in the olfactory bulb of awake rats in order to record odor-evoked electrical activities [15]. Micro-wire array electrodes that were handmade, 16-channel, and chronically implanted were utilized to collect data. Each of the eight microwires has two rows and is connected to a PCB by a tiny 20-pin plastic connector. In order to implant electrodes, the rats underwent surgery while under anesthesia. The rats were prepared for odorant administration after their procedure. Following the odorant response, the experimenters waited between 5 and 15 minutes after each odor was administered for 5 seconds. Rat's neuronal olfactory bulb impulses may be monitored by connecting the implanted electrode connector to the data acquisition device's head stage cable preamplifier. The experimenters used MATLAB to do some signal-processing operations on the raw data before analyzing it. The correctness of the results for various odorant concentrations expressed as mol/L was mostly reviewed in the results section.

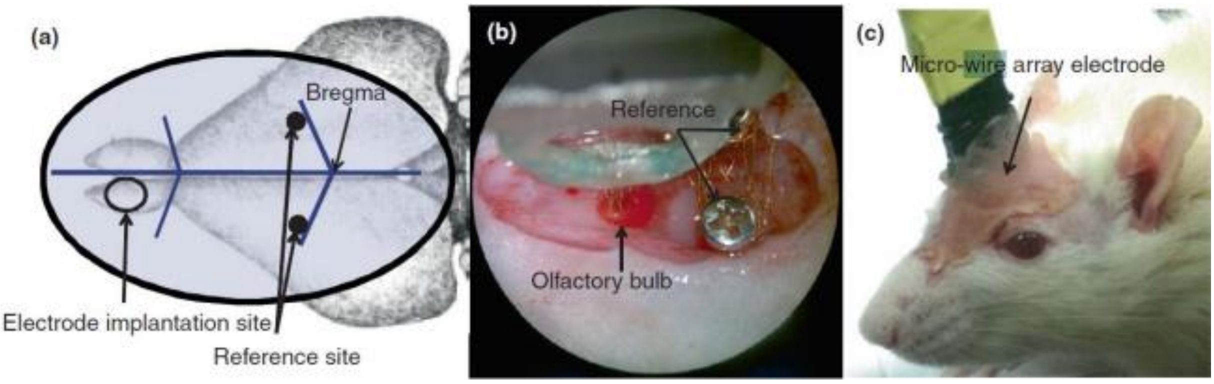


Figure 1. *(a) The electrode location. (b) 16-channel electrode array integration with Olfactory Bulb (c) Electrode array was implanted in the OB of the rat.[12]*

Anesthetized rats and a 16-channel microwire electrode array are used in Jun ZHOU, Qi DONG, Liu-jing ZHUANG, Rong LI, and Ping WANG's study [16]. Anisole, carvone, citral, and isoamyl acetate are the 4 pure compounds that are used for odor generation. It employs principal component analysis. They thought that the first breathing cycle had been finished via odor discrimination. 16-channel microwire electrode arrays were first used after electrode preparation. An electrode was placed during the procedure. Then, in the odorant distribution part, each 2ml of odor is sent in 0.5s and 60s of fresh air. Then, utilizing an OmniPlex system, electrophysiological recording, spike sorting, and band-pass filtered digitization were carried out. They also included some preparation time for breathing in the fresh air and smells. The MATLAB environment was used for all data analysis.

The researchers Kaiqi Su, Liujing Zhuang, Duanxi Cao, Tiantian Guo, Bin Zhang, Jie Zhou, Ning Hu, and Ping Wang found that the mice's behavior was significantly constrained by the circuits and cables used to receive its data [17]. In order to record wirelessly, they employed a device known as the wearable wireless neural recording system (WONRS). To demonstrate the system's use, continuous brain recordings from an awake mice's olfactory bulb have been made over extraordinarily large distances from the monitor station. In order to test the stability of the developed biosensing system for continuous recording, WONRS was used to identify and evaluate the responses of M/T cells from the same recording channel over a period of days. WONRS is composed mostly of two components. a user interfaces with an HTTP2 server and user applications, as well as a hardware interface with printed circuit boards for the head stage and backpack (PCB). This allowed for the evaluation of the system's data collection capabilities.

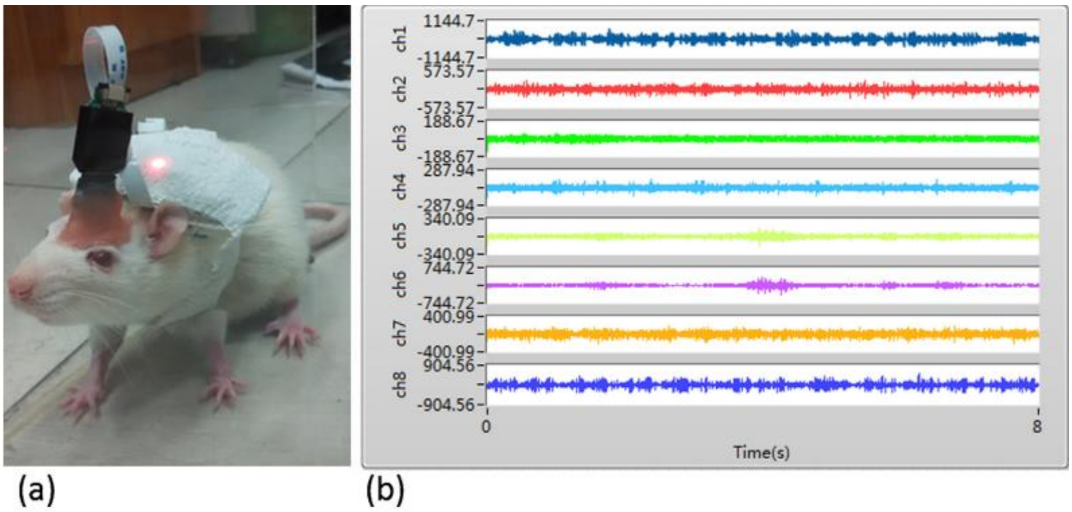


Figure 2. *(a) The WONRS prototype is being used with a well-behaved adult rat at the moment. (b) All 8 channels of the WONRS recorded waveforms are displayed online in real-time strip charts [17].*

3. ORIGINALITY:

Smell detection is a field that has been interesting since ancient times and has appeared in various studies and in different forms. Odor, which does not contain a sharp difference in itself such as light or sound, is based on chemical molecules and this is one of the challenges to tackle when dealing with gas molecules. As described in the literature, researchers use a sensor called e-nose to detect the odor. Although electronic noses provide odor detection, they have serious disadvantages. Electronic noses detect very strong chemical odors. This situation limits the range of odors to be detected. However, collecting data from the olfactory bulb eliminates this limitation by the various responses of numerous neurons. In contrast to other odor-detecting techniques such as using an electronic nose, mice is used as experimental animals in this investigation. The compounds are first taken in by the mice's nasal cavity after the odor has reached it.

The mice's olfactory bulb receives neurological information from the cells in its nasal cavity. The classification of odors is typically carried out by the mouse brain using information from the olfactory bulb. However, in this work, the mouse's olfactory bulb signals were collected using electrodes, transported to the computer environment, and then analyzed. Mice were used in this experiment because they can classify a very large spectrum of odors more quickly and affordably than electronic nose applications. There is no study in the literature that classifies the data obtained from the olfactory bulb of mice with artificial intelligence. The project differ from the studies in literature by aiming to classify the data from the olfactory bulb of mice in artificial intelligence.

4. SCOPE OF THE PROJECT AND EXPERIMENTS/METHODS:

The progressive aim of the project is to design a multi-channel electrode system that collects the signals from the olfactory bulb for a selected amount of odor and transfers it to a data center, and to develop a deep learning algorithm that predicts which odor molecule corresponds to the olfactory bulb signal coming to the artificial intelligence data center.

A modular approach has been applied in the execution of the project. The general flow chart of the project is shown in Figure 3. The project is basically divided into three parts. It should be known that if the whole project is considered as a system and each part as a subsystem, each subsystem is expected to reach the success criteria determined by itself. For this, simultaneous studies are carried out for different subsystems. Modular systems is integrated when each section is successfully completed. At this point, it is expected that the whole system meet the overall success rate as a result of the combination of individually functioning modules.

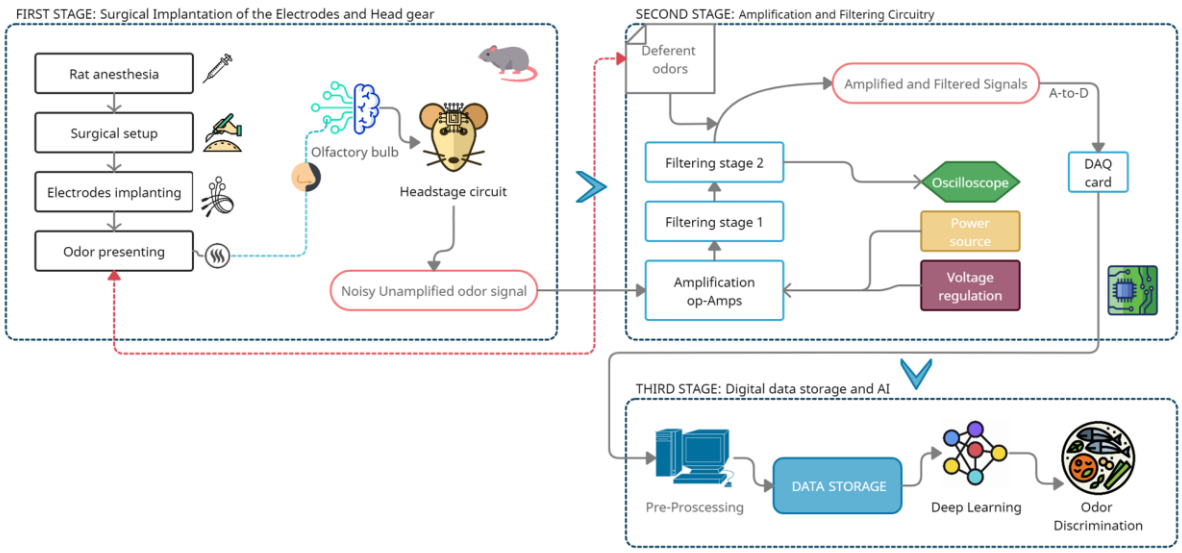


Figure 3. *Overall Stages of Project*

As stated in Figure 3, the first stage of the project is to receive electrophysiological signals from the living organism. Then, in the second stage, these received signals are filtered and amplified. In the third stage, the signals whose spikes are detected are made suitable for classification by applying signal processing algorithms. And lastly, the odors are separated by various artificial intelligence algorithms according to the pattern created by the odors.

THE FIRST STAGE: Surgical Implantation of the Electrodes and Hand Gear

- **Determining the Olfactory Bulb Location**

The first stage of the project is the acquisition of electrophysiological olfactory signals from the olfactory bulb of the mouse. In order to carry out these animal experiments, a certificate and ethics committee approval are required, and necessary documents are provided. At this point, it was determined that the behavior of the olfactory cells was in the Mitral/Tufted layer of the olfactory bulb [13]. Then, the exact location of this layer was learned from Mouse Atlas and the coordinates were found as X=3.920mm, Y=0.6mm, and Z=1mm.

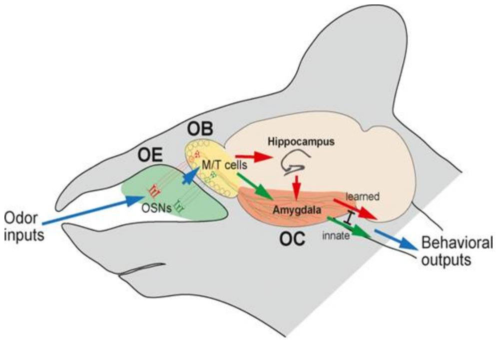


Figure 4. *Mouse Olfactory Circuits*

- **Electrode System Design and Production**

Electrode structures are used in studies to observe the electrical changes caused by olfactory signals in neurons, like other signals formed in the brain. Because of their compatibility and recording quality in living cells, tungsten wires are preferred here.

The electrode structure created with tungsten wires provides the opportunity to take in vivo recordings from living organisms. During this process, the action potential formed in the nerve cells is received by the conductive tungsten wires. When creating this structure, two different wires, coated and uncoated, are used. While the uncoated recording electrode is placed at the target point where interaction is expected, the coated reference electrode is placed in the area where there is no neural activity. In this way, the electrical difference between neurons is recorded.

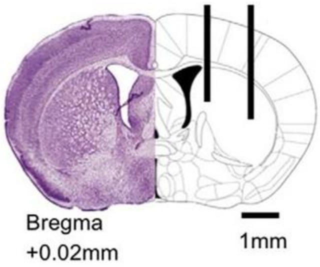


Figure 5. *Section of Mouse Brain Showing Electrode Placement* [15]

An original method has been applied in the preparation of the electrodes. First of all, it is aimed to use a multi-channel electrode structure in case of success with a single channel in the continuation of the project. However, since successful recording could not be obtained with the electrodes designed to be single channel, the design and production of multi-channel electrodes was not carried out within the scope of the project. Instead, single-channel electrode designs with different structures were developed and applied.

The following steps were followed in the formation of the mentioned single-channel electrode structures.

1. The electrodes were designed in accordance with the skull structure of the mouse.

2. The electrode holder structure specified in Figure 12 and the header are fixed to each other to provide the connection to transmit the electrical change.

3. After the implementation of the 1st and 2nd steps, structures as in Figure 13-14 were obtained. In order to immobilize these structures to the stereotactic table where the mouse is placed, the structure in Figure x was designed and printed from a 3D printer.
4. After the production of the electrode holder structures, when the tungsten wires are placed, and the design is completed; record electrodes are placed in one of the cavities inside the holder and reference electrodes are placed in the other.
5. The coat of the part of the recording electrode that remains in the holder is peeled off by heating. The part to be applied to the head of the mouse is left coated.
6. The length of the part of the two electrodes outside the structure is Z = 1 mm by measuring with a caliper and cutting.
7. Afterwards, the structure is fixed by applying conductive silver paint to the structure.
8. Finally, the recording electrode is made ready for impedance measurement.

- **Implementation**

Certain steps were followed during the preparation of the mouse and the surgical environment and the placement of the electrode into the olfactory bulb for each electrophysiological recording.

Preparation Process of Mouse and Surgical Environment

1. Ambient cleaning and temperature adjustment.
2. Making the settings of the stereotactic table where the surgery performed.
3. Adjusting the microscope to be used in the process.
4. Adjusting the dosage for Ketalar injection / isoflurane which are the anesthesia methods to be used.
5. Making sure that the drill, which is used to hold the electrode to the mouse's head after the procedure, is working.
6. Preparing the appropriate amount of cement to be used to fix the electrode.
7. Shaving the area to be operated on.

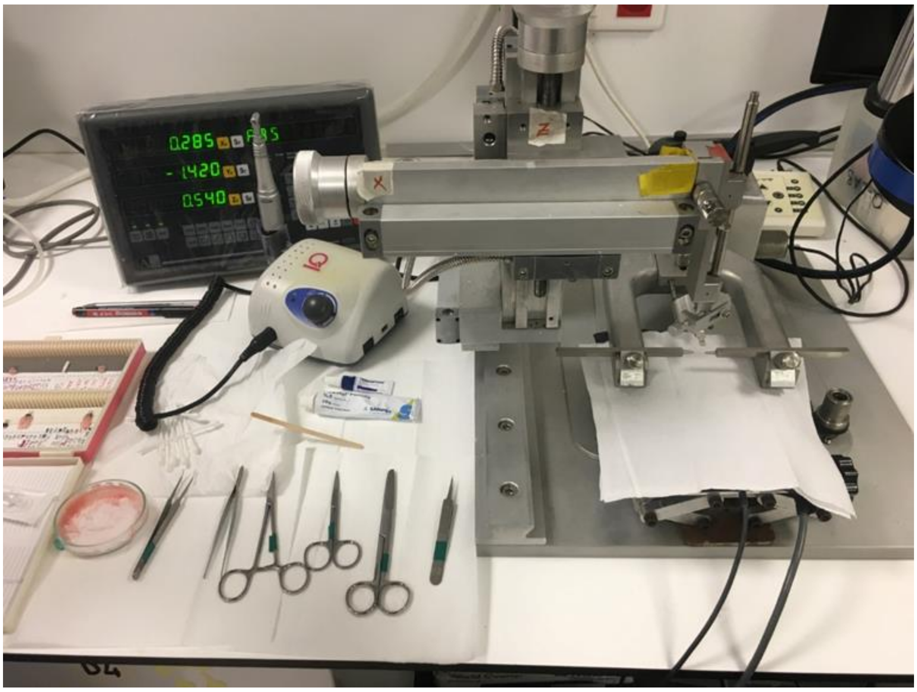


Figure 6. *Prepared Operation Setup*

Implementation of Electrode to Olfactory Bulb

1. The shaved area is cut.
2. Then the skull was cut, and the area was cleaned with H_2O_2.
3. Bregma point is determined and marked.
4. Coordinates are set to 0 in bregma.
5. Lambda point is found and marked according to the Z value.
6. The correct point is marked by providing X and Y coordinate values.
7. The electrode is placed at this point.
8. The procedure was completed by placing electrodes with dental cement.

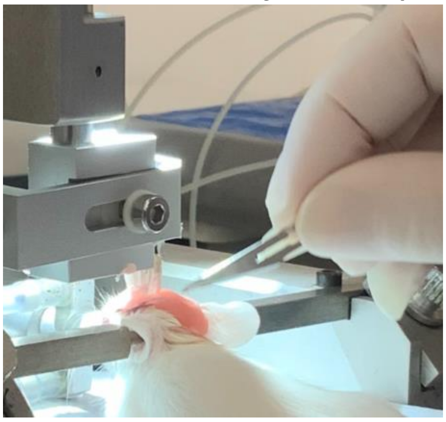


Figure 7. *Experimental Animal in the Surgical Environment After Anesthesia*

Transmission of the Odor Signal to the Digital Domain

1. After the electrode is placed and fixed with dental cement, it is connected to an experimental circuit prepared to filter and amplify the signal.
2. The analog signal, which is the output of the circuit, is digitized with the NI USB-6001 USB DAQ data acquisition card.
3. The digital signal was imported into MATLAB for monitoring and processing.
4. The digital signals obtained for different odors were recorded.

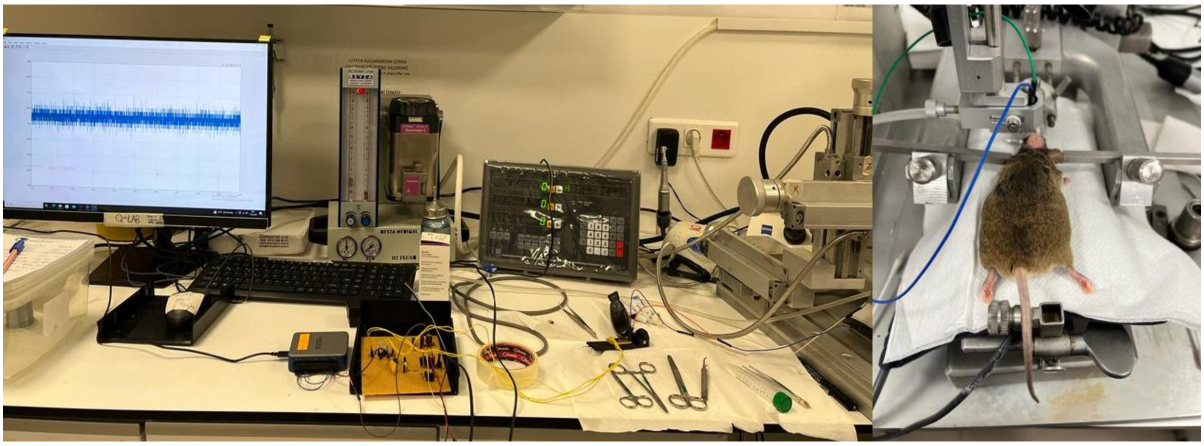


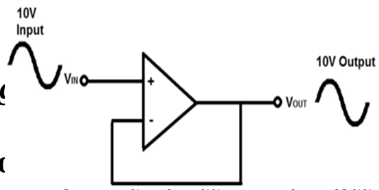
Figure 8. *Electrical and Digital System Setup*

THE SECOND STAGE:

- **Amplification and Filtering Circuitry:**

This part shows the detailed stages of the circuit used to get a usable signal to be used as a dataset. The circuit is powered with a power supply in conjunction with a power regulator IC like the 7905 to maintain a stable voltage on the rails at all times. A better power source would be a 9V battery with power regulation since it has less noise and is portable. Making the circuit fully self-dependent in one enclosure.

Stage-1: Head stage:
Since the experimental procedure requires working with a live mouse, in order to have a flexible system, a head stage circuit is designed and implanted on the head of the mouse. This stage serves as the initial input receiver of the electrodes. In this stage, an Op-amp is used in the voltage-following configuration, where the inverting input is connected directly to the output. This serves the purpose of buffering by drawing a small amount of current since it has a high input impedance and a low output impedance. They are also called impedance-matching amplifiers. Along with the impedance of the electrode, they act like a voltage-dividing circuit. As long as the impedance of the buffer is higher than the input impedance, the voltage across the buffer is maintained. Thus, any good op-amp can be used for this stage and is used in the next stage as well.



Fi *natic*

Stage-2:(Updated) Instrumentati
A typical instrumentation amplifier consists of a buffer and a differential op-amp configuration, where it takes 2 inputs and outputs the difference between them. This helps to get rid of unwanted noises with a gain factor that is determined by the resistor's configuration. Ideally, an amplifier with input deference should give an output of the deference multiplied by the gain. But in practice, op-amps deal with common offsets that influence the output. Thus, such op-amps have a Common Mode Rejection Ratio (CMRR) that corresponds to the ratio of ignored common offset values between the input's terminals. Although an Instrumentation Amplifier can be designed using discrete components and ready-to-use IC can be used like the AD620. where it shows a lot better performance and much lower noise. The AD620 uses only 1 resistor RG to set the Gain up to 10000. Pins 1 and 8 are used for that.

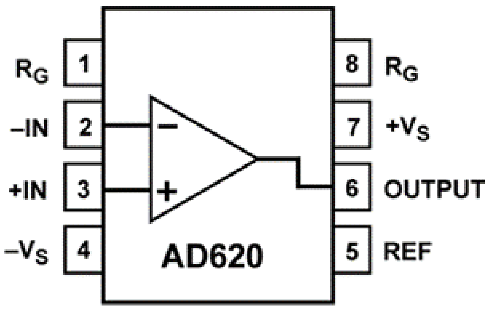


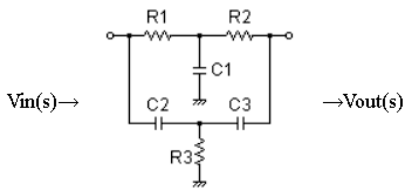
Figure 10. *AD620 Schematic*

Looking at the datasheet, we can see that the gain of this IC is G = 1 + (49.4kΩ/RG). So, if we were to set the RG resistor to be 8kΩ, the gain would be G = 1 + (49.4kΩ/250Ω) = 200. This gain value can easily be changed using only one resistor or a potentiometer for fine adjustments.

For testing purposes, the gain is set to have 2000 V/V in this stage. Further amplification can be added using another amplifier if needed. But this stage is sufficient to provide most of the gain in the prototyping period.

Stage-3:(updated) 50-60 Hz Twin – T Notch Filter:

A big source of interference and noise is caused by the power line interference and coupling which is usually around 50-60Hz. Using a twin tee Notch filter at this stage eliminates interference and gives a better result overall.



Center rejection frequency

$$\begin{cases} f_0 = \dfrac{1}{2\pi}\sqrt{\dfrac{\dfrac{1}{C3}+\dfrac{1}{C2}}{C1R1R2}} \\ f_0 = \dfrac{1}{2\pi}\sqrt{\dfrac{1}{C2C3R3(R1+R2)}} \end{cases}$$

Figure 11. *Twin – T Notch Filter*

Stage-4:(updated) Active Bandpass Filter:

A bandpass filter is the opposite of a notch filter, where a band of frequencies is passed and the frequencies out of that bandwidth are attenuated. This is determined by the values of the resistors and capacitors used. From studies, we know that the range of frequencies in an olfactory lay around 0.5 and 1kHz. Including action potentials in the olfactory sensory neurons OSNs, Local field potentials LFPs, and Electroencephalography EEG in the olfactory cortex. Although these ranges may vary depending on the animal's age, type, and specific neural activity, this range is a good starting point since also the stop bands of the Bandpass are not ideal, so having a wider range can help with filtering the desired responses. Thus, we aim to have a range of around 8KHz. Previously we aimed to use a 4th order active bandpass filter but after further research, a better approach is to use a single stage for band filter for a couple of reasons. Firstly, to lower the power consumption of the overall circuit. Which also results in lower power lines interference and lower components, making the circuit much smaller while achieving the same effect. Thus, a band pass active filter is used.

Additional circuit as Plan B for extra amplification:

An additional amplification unit can be used by an inverting amplifier with a 50k ohm potentiometer. In this way, the overall gain of the amplifier circuit can be changed to 2000, 3500, and 4500 according to the needs.

Additional circuit as Plan B for 50-60 Hz noise removal:

In case the instrumentation amplifier common mode did not remove the noise of 50-60 Hz or twin tee notch filters used in this stage do not behave as expected, a much better solution is to use part of the circuit called the right-leg driving circuit. This part of the circuit is usually used in ECG applications where a ground terminal is connected to the right leg of the patient which is the furthest to the heart. This type of circuit is useful since the common mode noise between the two inputs is feedback into the body again through an inverting op-amp. So, this acts like sound-canceling headphones where the inverted noise is feedback and is canceled due to the common mode configuration of the instrumentation amplifier. This part of the circuit can be added to the circuit and adapted for the electrode placements.

Safety features for the experiment mice and handling the circuit:

The circuit itself is power efficient. since the usage of A620 Inst-Amp and the usage of 9V batteries is regulated down to 5.5V. This ensures that the circuit do not increase temperature thus lowering the resistance and having a much higher current draw overall. However, for precautionary measurements, a high-value current limiting resistor is used in case a short between the V+ and any node of the circuit occurs. meaning that if a 250k ohm resistor is used. and a short between the power input of 5.5V produces a current of I = 5.5/250k = 22 μA on the body. which is considered an exceedingly small value and is considered safe level.

In addition, heat shrink is used on exposed connections to ensure that they are insulated from the elements. An isolated case holds all components tightly. Moreover, switches are used for the battery holder to make sure that the circuit is not running when it is not in use and to draw down the battery.

THE THIRD STAGE: Digital Data Storage and Classification with AI

The size of the data is considered to determine the most efficient algorithm to be performed for the solution. However, when looking at the data collected in the literature, the spike detection algorithm is applied to the odor signals. As a result, the data is observed and unobserved odors within a certain time period. Considering this, it has been seen in the literature that the CNN model gives more accurate results for classification. However, considering the data size, the algorithms that are planned to be used can be divided into 3 groups.

1-1D Convolutional Network: 1D CNNs are made up of 1D CNN layer and one regularization layer. This algorithm appears to provide recognition tasks with a small amount of data.

2- Convolutional Neural Network: Convolutional neural networks, a branch of deep learning, are usually applied for visual analysis. If the data size is large, it is the algorithm to be used.

3-LSTM: Typically, EEG signals have strong characteristics. It is referred to as higher dimensional or dimensional. Methods for deep learning are very dimensional. It makes signal processing easier. On the other hand, LSTM captures EEG signals. It is one of the widely used categorization deep learning techniques.

5. PROJECT TARGETS AND SUCCESS CRITERIA:

Targets:

The ultimate goal of the project is to be able to classify odors with artificial intelligence. In order to achieve this, sub-goals have been determined.

1. Developing an electrode structure for taking electrophysiological recordings.
2. Placing the electrode at the correct point in the olfactory bulb.
3. Recording and digitizing the electrical activity in the brain when an odor is given.
4. Developing a controlled environment for data to be collected for different scents.
5. Processing digitized signals in MATLAB for use in AI.
6. Creating a dataset with the processed olfactory signals taken.
7. Classification of odors with >80% accuracy using this dataset

Success Criteria

In order to evaluate the progress of the project and the work carried out, different targets and success rates have been determined for each stage mentioned in the Experimental Methods section of the report. Target and success criteria have been determined for Project 1 and Project 2 separately.

Project 1

At this stage, four objectives were determined:

1. Developing the electrode structure to be used in data collection. Contribution to overall success is %5.
2. Performing data acquisition studies from the mouse with this electrode. Contribution to overall success is 10%.
3. By building a multi-stage amplifier circuit, getting at least 1000 times voltage gain. Contribution to overall success is 10%.
4. Carrying out classification studies with different algorithm models and datasets. Contribution to overall success is 20%.

Project 2

In the project, which is divided into 3 main sections, each section consists of a different number of work packages. In Project2, these work packages have been increased.

- In WP1, all members are required to review the literature again. The reason for this work package is to observe how similar problems are solved in the literature in order to eliminate the problems encountered in Project 1. WP1 was carried out with a given 5% success rate.

- WP2 is "Electrode Construction and Measurements". Here, the electrodes produced at the end of Project1 were measured and it was seen that the electrode was an open circuit. For this reason, the electrodes were reconstructed with different methods and measured with an impedance meter. The required impedance value was provided, and the work package met the 10% success rate.

- WP3 is the work package "Development of Data Cleaning Algorithms," which aimed to collect data with properly functioning electrodes and process the collected data. This work package was being carried out using continuous time signals that were distinct from odor. However, its performance on odor data remained unknown at that time since the data had not yet been made available. As it was not possible to collect accurate odor data, these algorithms were tested using various time-dependent datasets instead. Consequently, succeeded as %100 in this data.

- WP4, which is “Design and Simulations of Electrical Circuits on Tools”, has completed the success rate of 10%, as it can provide the signal with the desired gain and SNR values as predicted from simulation.

- WP5 is the implementation of amplifier and filter circuits designed with electrical elements. When the whole system is created, the results match the simulation results in WP4. Thus, the success rate of 15% is met.

- System designs were made for WP6, which is “Electrode and Environmental Setup Modeling”. There have been disruptions in the supply of some of the materials required for the controlled environment. Due to the fact that his contribution to the project was not very high, the implementation phase of this step was postponed.

- WP7 and one of the key points of the project which is “Electrophysiological Recording”, is delayed as a different problem comes to light in each trial. Many attempts were made, but no record could be obtained. For this reason, this work package could not meet the 20% success rate completely.

- WP8 is another crucial block of the project, which is “Developing AI Algorithms for Different Dataset”. This work package continues to be developed by changing the size, time-dependent behavior, and content of the data, as well as the models used. Since there is no odor data, the developed models have not yet been integrated and tested with odor data. However, the developed models were tested with many different EEG datasets and reached the targeted accuracy rate. With the tried data, the algorithms met the success rate with an accuracy of over 80%.

- WP9 is the last work package and is the integration of the outputs of all work packages with each other to obtain a fully worked system. Currently designed electrode, electrical amplifier circuit and digitization of data are interconnected and work in harmony. However, since the collected data could not be transferred to artificial intelligence, a connection with the second half of the project could not be established. For this reason, this work package works with a 50% success rate.

To conclude, most of the work packages in the project were completed and reached the determined success rates. However, due to the interdependence of the work packages, the disruption in one affects the success rates of the others. The levels of achievement of success rates are summarized in Table5.

6. RISKS AND B PLANS:

Table 1. *Riska and B – Plans*

WP #	RISKS	B-PLANS
2	Produced electrodes did not meet the required impedance value	Electrodes were made again in different materials and shapes
3	Developed algorithms do not work properly in odor data	Confident with algorithms provided in the literature
5	Circuit does not have the desired gain level and SNR	Reduce the circuit for less power and higher SNR
7	Unable to obtain data / Obtaining with high noise	Attempted to re-register with new methods Different signals from time-varying odor were used
8	Deep learning algorithm result is less than 80%	The parameters of the optimum model were updated, and new models were developed
9	Individually working systems do not provide the desired success rate when they come together	Troubleshooting problems in integration and making blocks compatible with each other

7. WORK TIME PLAN OF THE PROJECT:

In the project, a weekly work plan was determined for each work package that continues throughout the term. Here, first, it was necessary to conduct a literature review of all team members in all weeks until the integration process to eliminate the problems and difficulties encountered due to the developments in each work package. Then, for the second work package, electrode structures were created by Buse Nur Fidan before the experiment and their measurements were completed. At the same time, the development of the third work package, the filtering and gaining algorithms, was carried out.

During this period, the circuit design process was in progress and simulations were carried out for gaining and filtering the analog signal. In this process, materials were ordered for the realization of the circuit. While recording attempts were being made, it was waited for the circuit to be built and the trials to be completed. However, the realization process was delayed due to the latency in the transportation of the materials. Electrical circuit design and development was led by Tayseer Hussein.

At the same time, it was aimed to develop the electrode model and the experimental environment as a result of the experiences gained from the recording trials. In this process, 3D model formation was studied. At the same time, AI algorithms continue to be developed to be ready when odor data is reached using different datasets and models. These two tasks are carried out by Senanur Demirci.

Apart from this, taking electrophysiological recording, which is one of the most important steps to observe whether all work packages are working at the determined success rates and to complete the project, is another work package that needs to be worked on throughout the entire term. However, due to reasons such as the camera resolution that developed throughout the process, the inadequacy of the electrode structure, and the electrode not being fixed on the mouse's head, this package was worked on in the week 4 and 8 and no success was achieved. In this package, medical faculty students Nazende Yağmur Uysal and Rufeyda Yağcı are the main attendants.

The final work package depends on the achievement of all the above-mentioned work packages within the specified period of time. If Table4 can be followed properly, it is expected that the integration of all systems i carried out by the whole team in the last month.

8. DEMO PLAN:

This project aims to distinguish odors by using artificial intelligence. Due to the project hardware and software integration, the hardware part of the project which works with living animals in combination with the acquired live data is demonstrated with a demo video. Including the surgical setup and the main parts of the project.

9. FINANCIAL EVALUATION:

This project is financially supported by TÜBİTAK 2209-A Research Project Support Program for Undergraduate Students. At the same time, some expenses for the project were provided from the Istanbul Medipol University fund. Detailed explanations are given in Tables 10 and 11.

10. RESULTS:

10.1 Result for Implementation

In the project, it is aimed to use the multi-array electrode structure. For this reason, an 8-channel electrode holder structure was designed and printed from a 3D printer, as in Figure 12. The upper part of this structure is wide enough to fit any 6 headers on the market. Considering parameters such as taking up less space on the mouse's head and the proximity of the electrodes to each other, it has been gradually thinned as one goes down.

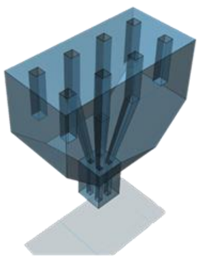


Figure 12. *8-Channel Electrode Structure*

The part that remains inside the electrode structure of the coated tungsten wire is heated and separated from its coating in order to provide transmission. In this way, the connection of the system with the next circuit is accomplished. Next, the wires are passed through the thin hollows in the holder and fixed using silver paint to ensure conductivity.

However, before starting the recording trials with these multiple structures, it was thought that the use of a single-channel electrode structure would allow the operation of the process to be followed more accurately at the beginning. For this reason, first of all, the single-channel electrode structure seen in Figure 13 was designed and produced by going through the mentioned steps.

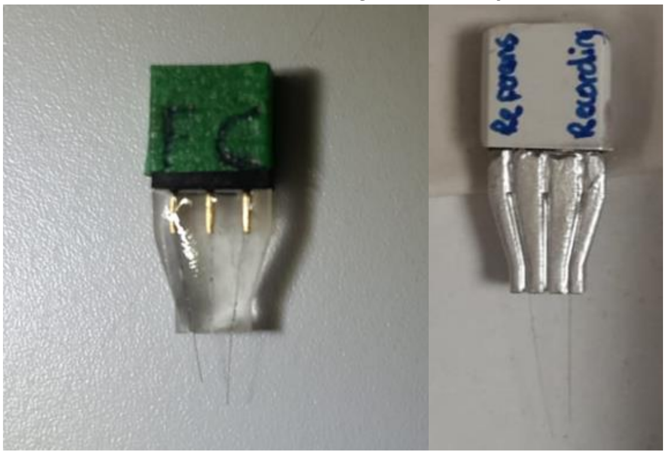
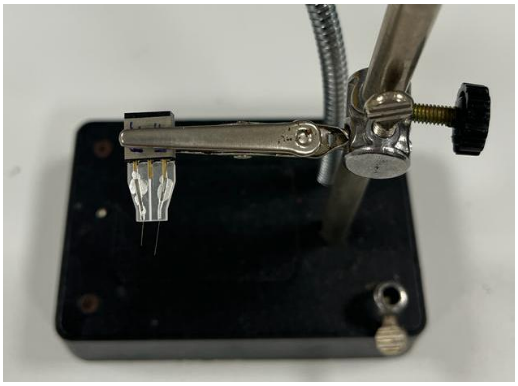


Figure 13. *Created Single-Channel Electrodes*

Another important and final step after the electrodes is formed is to measure the impedance of the electrodes. Although this value can be calculated theoretically, the impedance of the wires may vary due to micro scratches during the processes and due to the added paint. For this reason, the measurements of the electrodes, which are completed with an impedance meter, is made.

The electrodes shown in Figure 13 were measured, but the desired impedance value could not be achieved. As seen in Figure 15a, the impedance value due to the short circuit of the system was measured as 0 ohms.

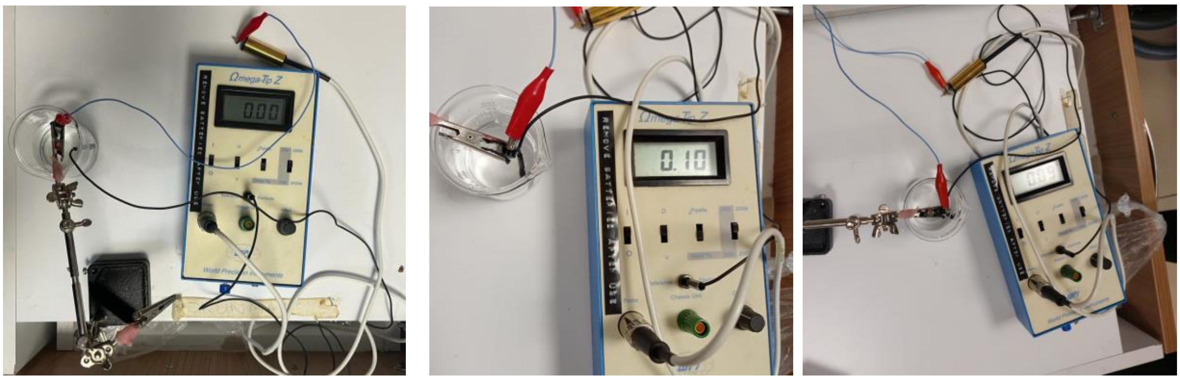
Then, two different development methods were established, and electrode trial was performed again. Here again, conductive silver paint was used as the material. Here, the two electrodes in Figure 14b were developed using a micropipette and an injector. Then, when the impedance measurements were made again, impedance was seen around 100 and 90 kohms. These values were found suitable for use in data acquisition. Measurement results are shown in Figure 15b and Figure 15c, respectively.



(a)

(b)

Figure 14. *(a) Electrode Development Set-up and Production of the First Working Electrode (b) Electrodes with the Correct Impedance Value*

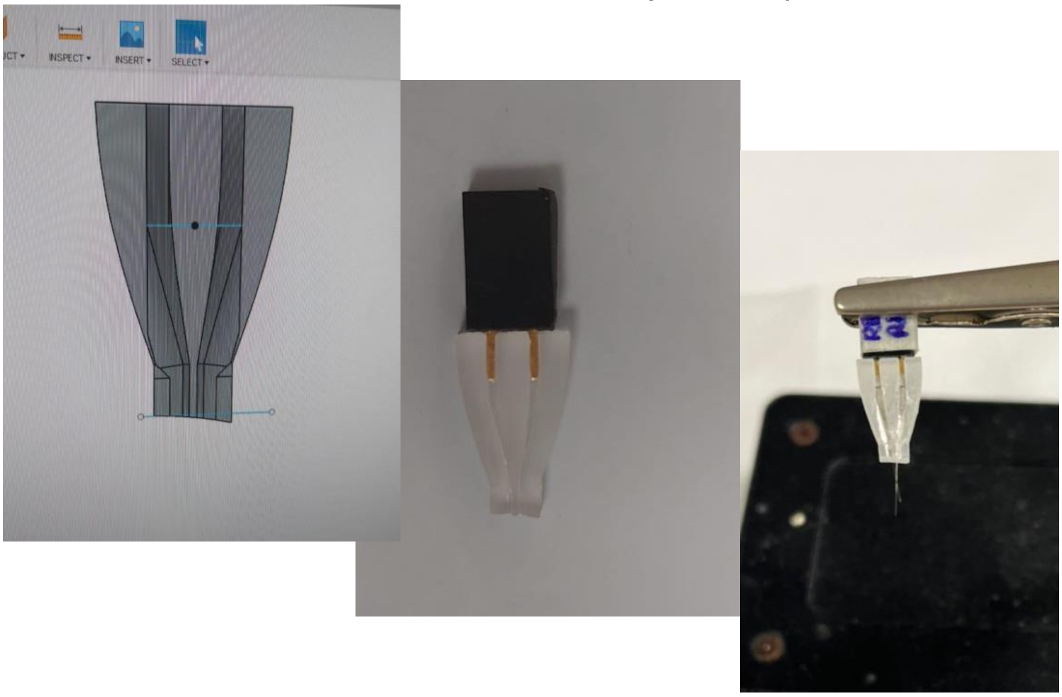


(a) *(b)* *(c)*

Figure 15. *(a) The measurement results of the first electrode produced with conductive silver paint in Figure 13 (b) The measurement result of the electrode developed by pouring silver with the help of an injector (c) The measurement result of the electrode developed using a micro pipette*

The electrodes were suitable for recording in terms of impedance value. However, since the part of the designed holder structure that comes to the mouse's head is flat, it was difficult to fix it. After the operations, while the animal was in the recovery process, the electrodes came out of the animal's head. Figures 28 a and b in part 10.4. Result for Electrophysiological Recording show a mouse removing the electrode from its head after surgery and the protruding electrode.

In order to better adapt the electrode to the mouse's head, the tip of the electrode was designed in a curved structure instead of being straight. Printed from a 3D printer with biocompatible resin.



(a) (b) (c)

Figure 17. *(a) Curved Electrode on Design Tool b) 3D Printed Curved Electrode with Biocompatible Resin (c) Electrode Structure Created with Wires*

10.2. Circuit Design Simulation Results

The previously designed circuit was redesigned and optimized to have fewer components and achieve the same effect as the original design.

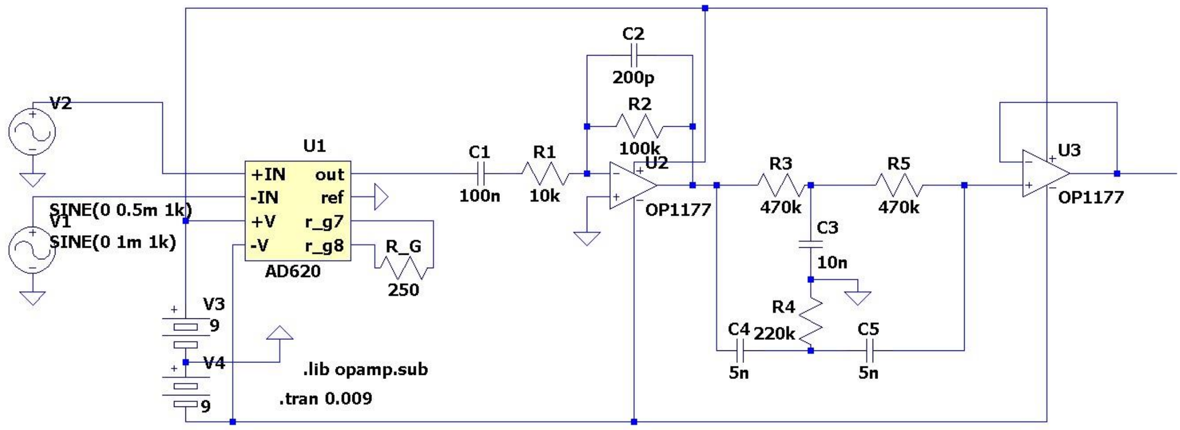


Figure 17. *Main Parts of the Circuit*

as seen a 250-ohm resistor is used for the AD620 to set the gain to 2000. According to the data sheet, this amplification rate is feasible within the frequency range needed for the project. Where the frequencies in the range of 1 – 10kHz are not much attenuated due to the high gain margin. Moreover, the bandpass filter is set to have a band of 1 to 1kHz, but this can be modified easily later for the specific range seen through tests. The time domain results are shown below.

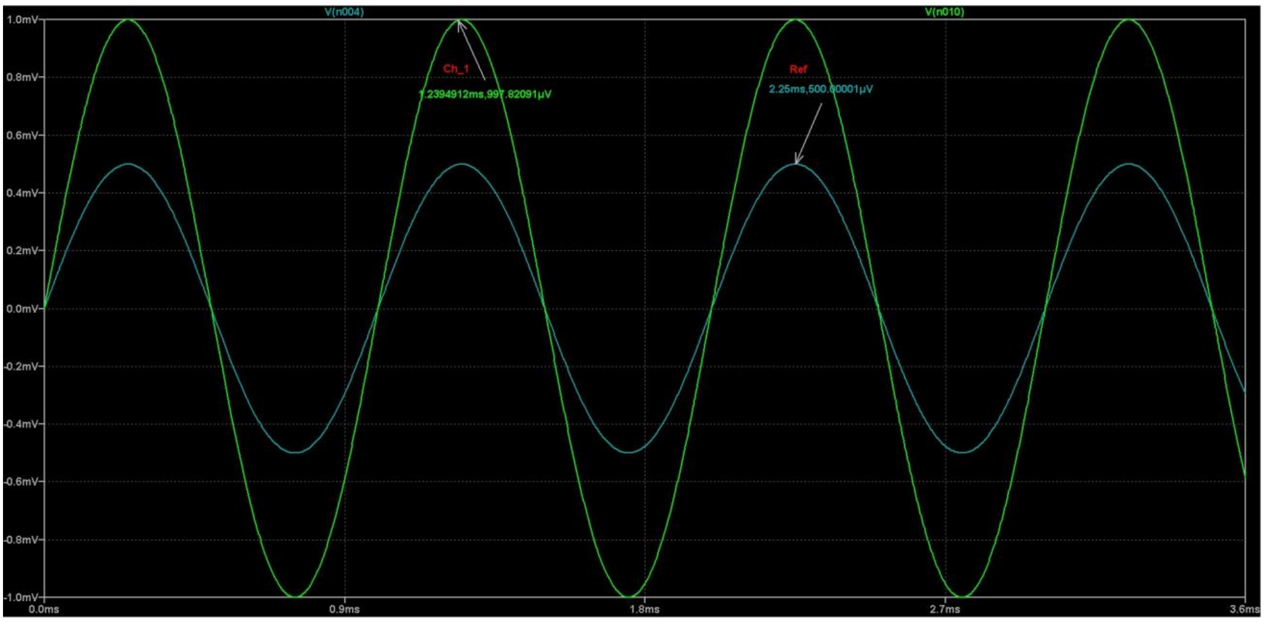


Figure 18. *Transient analysis of input signals Ch_1 and Ref*

As seen in Figure 18 an input signal at channel 1 is given a value of 1mV and a 0.5mV signal is given for the reference input. Making the deference output of the head stage 0.5mV. which to be amplified.

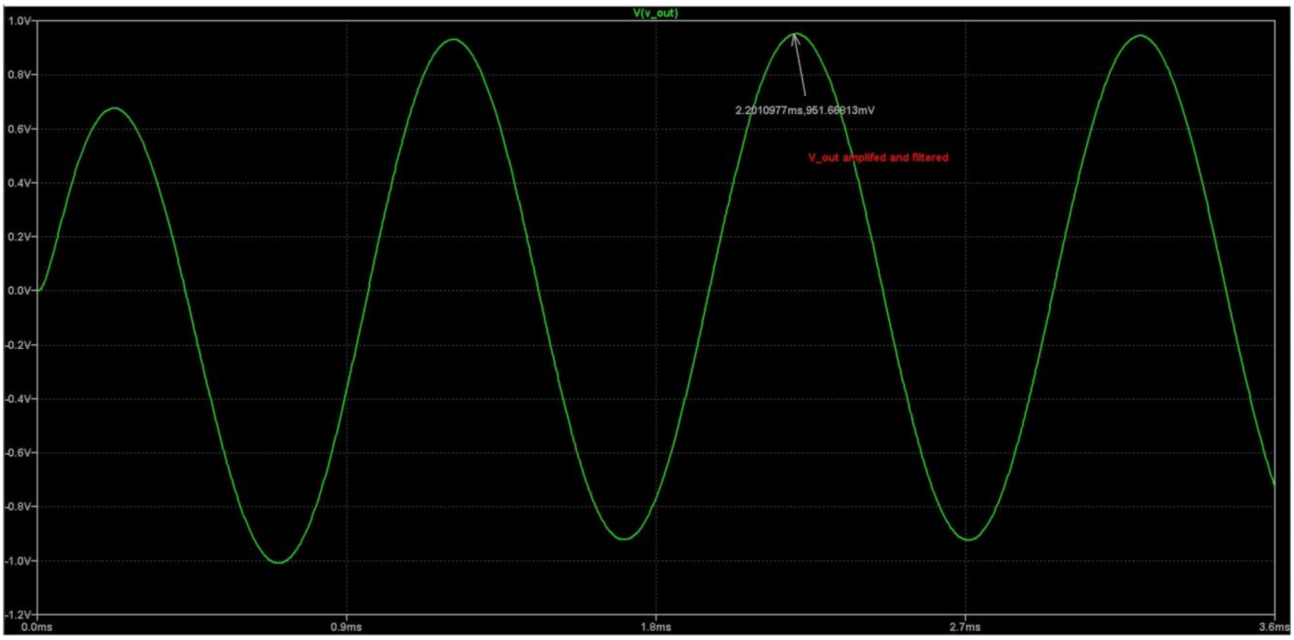


Figure 19. *Filtered and amplified output time domain signal*

Looking at the output amplified signal in the above figure, we can see after the signal is stable, the peak value is around 1 V. taking into consideration that the difference input was 0.5mV in Figure 15, we have a gain value of 1/0.5mV = 2000 V/V. Which is sufficient for the initial tests and is within the needed range.

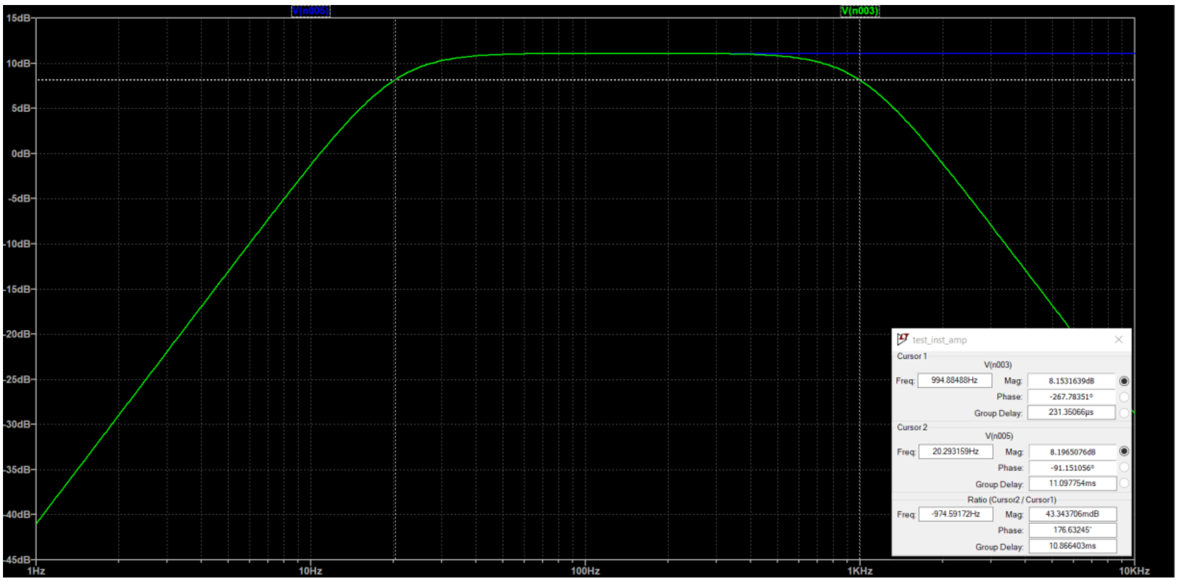


Figure 20. *Band-Pass Filter Response*

This gives us the desired range of frequencies needed for the dataset. Yet this bandpass filter can be adjusted according to the further tests that is done.

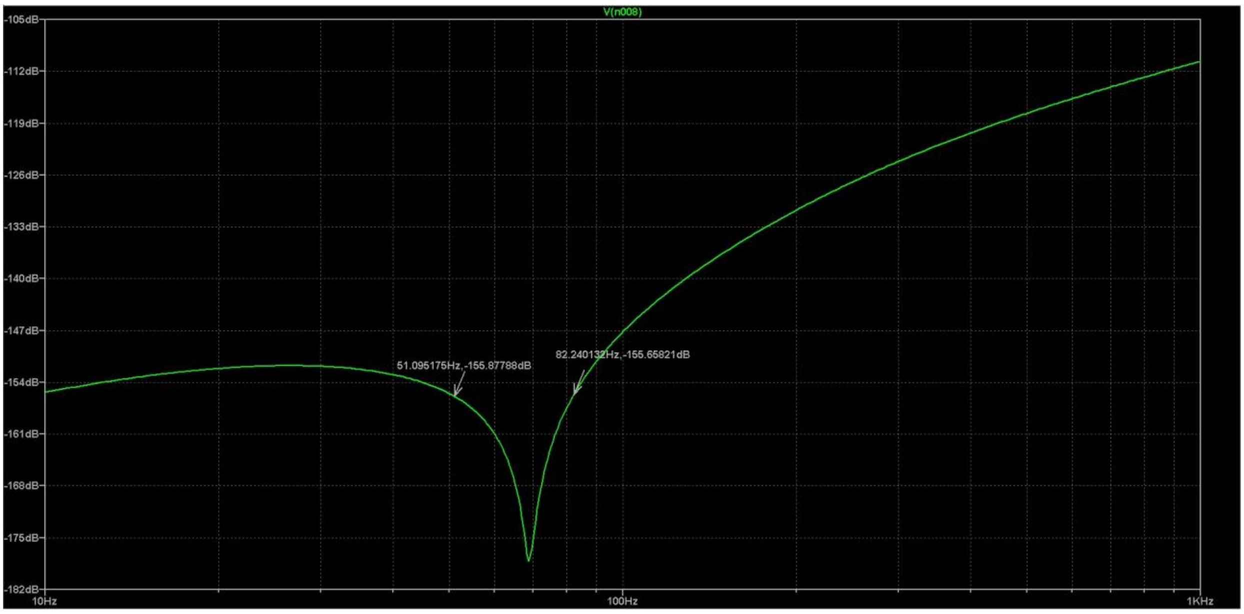


Figure 21. *Notch filter response*

For the twin tee Notch filter, a range between 50Hz and 80Hz is used to reduce the effect of 50-60Hz power lines interference and noise. This range is designed to make sure that tolerances and inconsistencies in the resistors and capacitors do not affect the notch filter, thus a wider range is used.

After Bulding the circuit, the tests show the predicted Gain and SNR As follows:

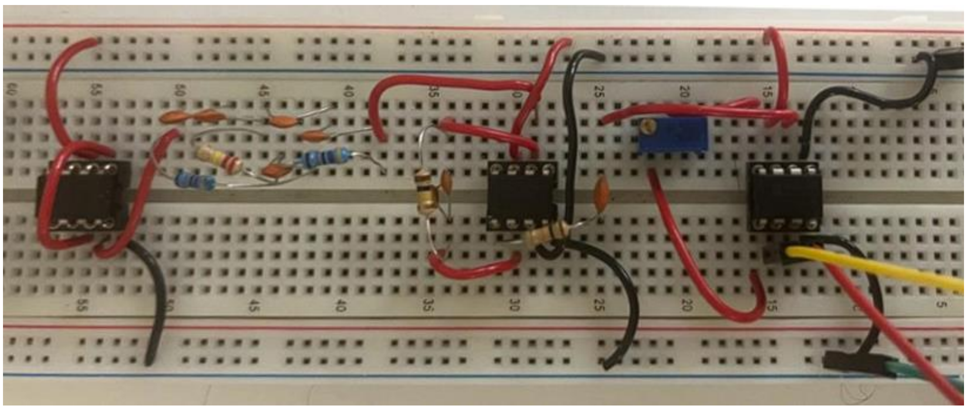


Figure 21. *Circuit implementation*

The circuit was tested by inputting a 2mVpp, which is the smallest voltage that the function generator can generate and observing the output in blue. The yellow input shows a value of 140mV, but it is due to the oscilloscope lower limit of voltage detection. The output sine wave has a peak-to-peak value of 5.36V. meaning the gain is 2680 V/V. This gain value varies depending on the input frequency but within the range intended to be used for the spike detection, the gain range is 1500 – 4398 V/V.

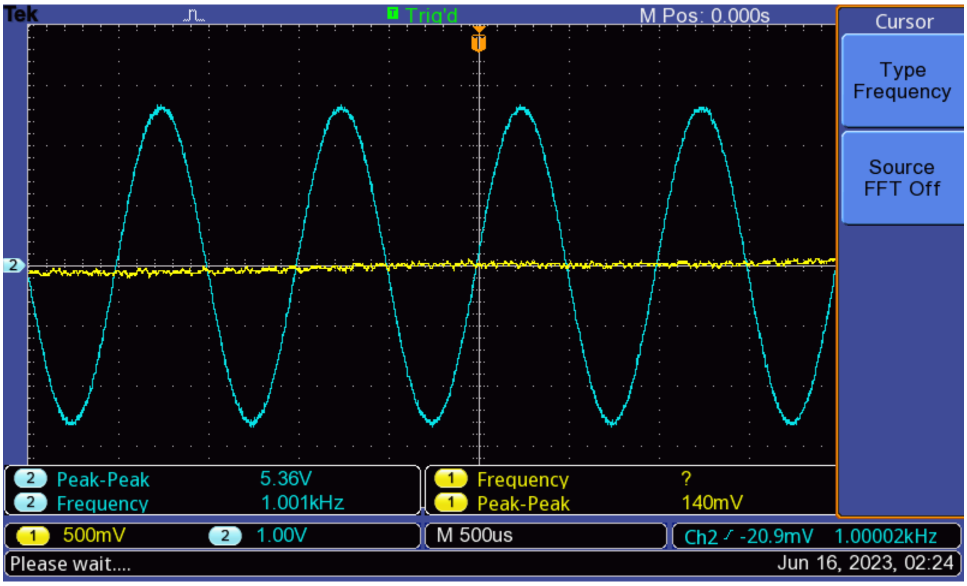


Figure 22. *Transient Analysis of the circuit*

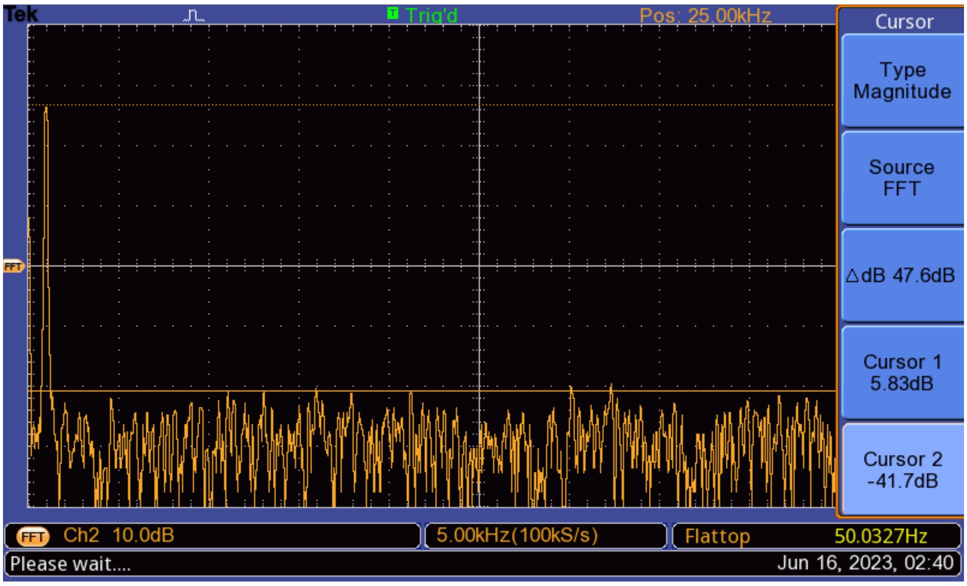


Figure 23. FFT of the circuit

When inputting a 1KHz signal and observing the output in frequency domain as seen above from Figure-23, courses were used to find the noise and signal levels, where in this range the signal gain is 5.85 dB and the noise level to be –41.7 dB. Thus, the signal to noise value is 5.83 - (-41.7) = 47.53 dB. Also sweeping the input to test the band-pass filter shows that the passband is within the simulated range and does its job to attenuate high frequency components of the input. The same goes for the notice filter for the 50-60 Hz noise where a –10 dB value is at the range of the mains voltage frequency and gradually increases at the passbands.

10.3. Result for Deep Learning Implementation

The aim of the project is to classify the odor data taken from the mouse using a deep learning algorithm. For the preliminary work, convolutional deep learning (CNN) models were built as an example for the main purpose of this project. For this purpose, two CNN models were created and tested. Fashion-MNIST[16] was used to test the models. This dataset is imported from Keras.

```
model = Sequential()#defining a model
model.add(Conv2D(16, (3, 3), activation='relu', input_shape=(IMG_ROWS, IMG_COLS, 1)))#adding convolutional layer
model.add(MaxPooling2D((2, 2)))#maxpooling the output of the previous layer with 2x2
model.add(Conv2D(32, (3, 3), activation='relu'))
model.add(MaxPooling2D((2, 2)))
model.add(Dropout(0.25))#inorder prevent over fitting we are using drop out boxes
model.add(Conv2D(64, (3, 3), activation='relu'))#repeating the above process 3 times
model.add(MaxPooling2D((2, 2)))
model.add(Dropout(0.25))
model.add(Flatten())
model.add(Dense(128, activation='relu'))#adding dense layers which helps to get us kernel which deals with seprating classes
model.add(Dropout(0.25))
model.add(Dense(NUM_CLASSES, activation='softmax'))
```

Figure 22.

Figure 22 shows the first CNN model. When the Fashion-MNIST dataset was tested with this model, it was seen that the test accuracy value was 0.89. In order to improve the observed test accuracy, another model was tested.

```
fashion_model.add(Conv2D(32, kernel_size=(3, 3),
                 activation='relu',
                 kernel_initializer='he_normal',
                 input_shape=(IMG_ROWS, IMG_COLS, 1)))
fashion_model.add(MaxPooling2D((2, 2)))
# Add dropouts to the model
fashion_model.add(Dropout(0.25))
fashion_model.add(Conv2D(64,
                 kernel_size=(3, 3),
                 activation='relu'))
fashion_model.add(MaxPooling2D(pool_size=(2, 2)))
# Add dropouts to the model
fashion_model.add(Dropout(0.25))
fashion_model.add(Conv2D(128, (3, 3), activation='relu'))
# Add dropouts to the model
fashion_model.add(Dropout(0.4))
fashion_model.add(Flatten())
fashion_model.add(Dense(128, activation='relu'))
# Add dropouts to the model
fashion_model.add(Dropout(0.3))
fashion_model.add(Dense(NUM_CLASSES, activation='softmax'))
```

Figure 23.

Figure 23 shows another CNN model. When the same dataset was tested with this model, it was seen that the test accuracy value was 0.92. The classification algorithms to be used in the project vary according to the data type. As stated in the relevant sections, data acquisition from mice could not be performed for various reasons. This situation was stated as a risk and plan B was prepared. Feeling emotions data set was used as the ready data set. This data set is readily available on Kaggle. According to research by Jordan J. Bird and colleagues, Muse recorded microvoltage from the TP9, AF7, AF8, and TP10 electrodes using an EEG headband [17]. 2 subjects, 1 male and 1 female, were used. They had the subjects watch 12 minutes of 6 movies and recorded the responses. Then they classified these outputs as positive, negative and neutral. Based on this study, data with 708 positive, 708 negative and 716 neutral labels in the emotion dataset were classified. First, the data were classified using the CNN algorithm. The accuracy value of the classification made to the CNN algorithm was less than expected. This is because spike detection is not applied to the eeg data, which is the signal. The data is only preprocessed signal data free of noise. Looking at the literature studies, it has been seen that the LSTM algorithm is also used in eeg signal classifications. Figure.24 shows the LSTM model used for classification. Model classified the data with 96.5% accuracy. In order to try different algorithms, a model was prepared and tested by using convolution and LSTM together. Figure.25 shows the model. This model classified the data with an accuracy rate of 96.7%. Finally, the DNN algorithm was used. Figure.26 shows the model used. This model classified the data with an accuracy rate of 97.5%. The algorithm and classification accuracy values used can be seen in Table.2.

```
model_2 = Sequential()
model_2.add(LSTM(200, return_sequences=True, stateful=False,
          recurrent_dropout=0.5, dropout = 0.5, input_shape=((1,2548))))

model_2.add(Flatten())


model_2.add(Dense(100))
model_2.add(BatchNormalization(axis=-1))
model_2.add(Activation('relu'))
model_2.add(Dropout(0.5))


model_2.add(Dense(3, activation='softmax'))

model_2.compile(loss='categorical_crossentropy',
              optimizer='rmsprop',
              metrics=['accuracy'])
```

Figure.24 - LSTM Model

```
model.add(Conv1D(40, kernel_size=12, strides=4,
          input_shape=((1,2548)),padding='same'))
model.add(Activation('relu'))
model.add(Dropout(0.5))
model.add(BatchNormalization())
model.add(LSTM(30, return_sequences=True, stateful=False))
model.add(Dropout(0.5))
model.add(BatchNormalization())
model.add(LSTM(20, return_sequences=True, stateful=False))
model.add(Dropout(0.5))
model.add(BatchNormalization())
model.add(Flatten())
model.add(Dense(3, activation='sigmoid'))
```

Figure.25 - CNN + LSTM Model

```
input_dnn = Input(shape=(2548, ))

x_dnn = Dense(2548, activation='relu')(input_dnn)
x_dnn = BatchNormalization()(x_dnn)
x_dnn = Dropout(0.25)(x_dnn)

x_dnn = Dense(3822, activation='relu')(x_dnn)
x_dnn = BatchNormalization()(x_dnn)
x_dnn = Dropout(0.27)(x_dnn)

x_dnn = Dense(5096, activation='relu')(x_dnn)
x_dnn = BatchNormalization()(x_dnn)
x_dnn = Dropout(0.3)(x_dnn)

x_dnn = Dense(3822, activation='relu')(x_dnn)
x_dnn = BatchNormalization()(x_dnn)
x_dnn = Dropout(0.27)(x_dnn)

x_dnn = Dense(2548, activation='relu')(x_dnn)
x_dnn = BatchNormalization()(x_dnn)
x_dnn = Dropout(0.25)(x_dnn)

y_dnn = Dense(3, activation='softmax')(x_dnn)
```

Figure.26 - DNN Model

Table.2 - Accuracy Results

Algorithm	Accuracy Result
LSTM	96.562%
CNN + LSTM	96.719%
DNN	97.500%

10.4. Result for Electrophysiological Recording

One of the most important steps that connects all work packages and main parts in the project and provides the relationship between them is to take an electrophysiological recording. For this work package, it is important to conduct trials until successful registration is achieved. The recording attempt was made twice.

In the first recording attempt, one of the electrodes that were deemed to work properly as a result of the measurement results was selected and used. During the experiment, the experimental animal was first anesthetized with isoflurane. Then, the head was shaved and the area to be operated on was cleaned and prepared. The olfactory bulb region, where the reference and record electrodes enter, was punctured and marked with a single-needle holder developed to mark the point where the data is taken. These two marked points were drilled, and a suitable environment was prepared for the electrodes to enter. Afterwards, it was aimed to place the electrodes in a way that coincides with these two points. However, this process could not be carried out as expected due to the camera angle. Electrodes and microscope camera angle are shown in Figure 24.

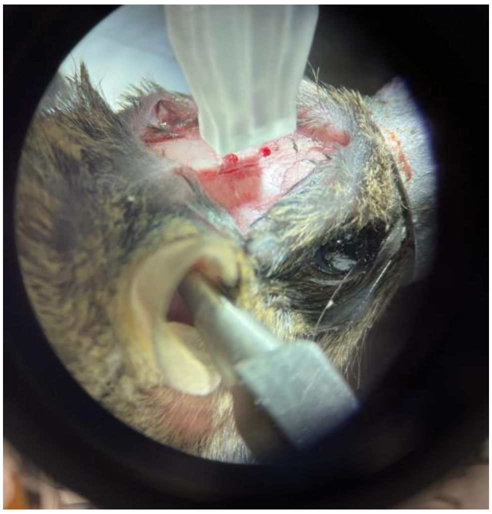


Figure 24. *Electrodes and Microscope Camera Angle*

After the electrodes were placed at the points thought to be correct. The electrode structure then needs to be fixed so that it is ready for recording in a stable state throughout the experiment and afterwards as the mouse continues to live. For this reason, the prepared dendrite mixture was applied to the area to fix the electrode to the skull of the mouse. Prior to this, grid-shaped cavities were created in the skull of the mouse with the help of a drill so that the dendrite could adhere better to the skull.

After the mixture dries the electrode structure was combined with the appropriately designed header. Header is responsible for providing the connection between the headstage circuit and the electrodes. The headstage circuit is the first step of the circuit board where the bandpass filter and amplifier circuits are. The situation of the mouse with electrodes and connections on the stereotactic table after the circuit connection is given in Figure 25.

Figure 25. *The situation of the mouse with electrodes and connections on the stereotactic table after surgery*

An analog to digital converter was used to observe the analog scent signal received from these circuits in MATLAB. Here, the Natural Instruments (NI) DAQmx card and graphical user interface (GUI) were used for this task. The digitally converted signals were sent to MATLAB for viewing and recording.

After all connections were made, the digitalized version of the received signal was observed. An abrupt change in signal was expected by placing the raspberry essence on the nose of the anesthetized mouse. However, this change could not be observed clearly. The signals generated during this process were recorded. One of them is given in Figure 26. In addition, the smell of orange was tried to get results, but the expected change in the signal could not be observed.

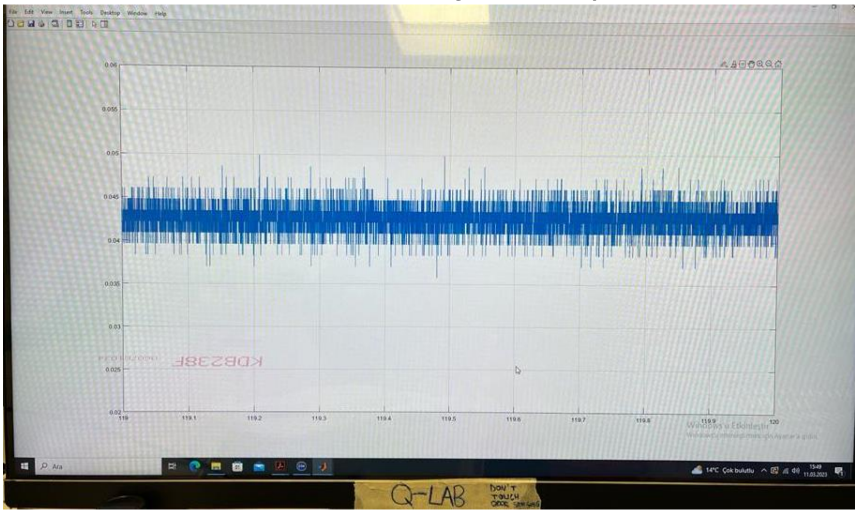
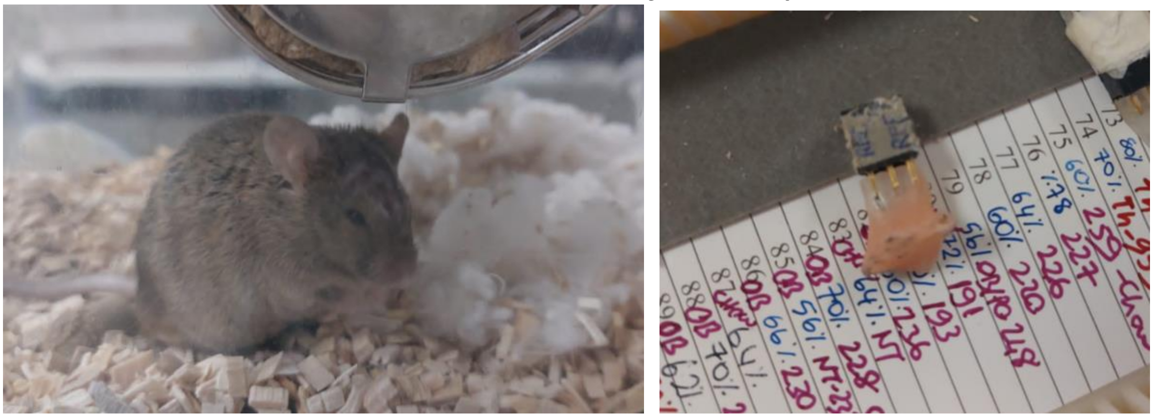


Figure 26. *Digital Received Signal on MATLAB*

The animal, whose experimental process was completed, was taken to its cage for recovery. In the next period, the vital signs of the animal were followed up. The state of the mouse under anesthesia in the living area after surgery is given in Figure 27.

Figure 27. *Mouse in Cage After Surgery*

After the operation was performed, it was observed that the mouse removed the electrode placed on its head during the healing process. The ejected electrode, together with the dental cement, could not adhere to the skull of the mouse. This situation is shown in Figure 28.



(a) (b)

Figure 28. *(a) Mouse with Electrode Opening on Its Head (b) Ejected Electrode*

It was thought that the reason for this situation was that the flat structure of the electrode was not compatible with the skull of the mouse. For this reason, the electrode holder is designed in a curved structure to be compatible with the mouse as seen in Figure 17. With this structure, electrodes were produced again, and the operation process was repeated. Since dental acrylic could not be found in this operation, the electrode was fixed to the mouse's head with resin and then sutured.

Figure 29. *Mouse in Cage After the Last Surgery*

11. **DISCUSSION**:

The project aims to create a reliable setup for odor data acquisition using electrodes and an amplification and filtering circuit in conjunction with a DAC to have a dataset that then can be digitally processed and used for deep learning algorithms to classify odors.

The project has different work packs connected to each other and are important for the project's success. In Figure-3, the overall flow of the project is shown in 3 main stages. The first stage consists of the surgical setup, where the mouse is placed in the operation setup shown in Figure-6. Anesthesia injections are given to the mouse according to its weight, size, and age of the mouse. Then the surgery starts with cutting the tissues of the mouse’s head to reach the Olfactory bulb shown in Figure-1(b). Then, the tungsten-coated single-channel electrodes shown in Figure-13 are implanted into the electrode implantation site and the reference electrode is further up near the bregma as shown in Figure-1(a) in the exact location shown in the methods section. Once each microwire is secure in the correct places, the mouse is stitched, and a medical implant cement is used to make sure that the electrodes do not move after the mouse recovers from anesthesia. This is shown in Figure-1(a). The electrodes' impedance is calculated shortly since the tungsten spray was delayed in shipping.

The second main stage of the project involves the amplification and filtering of the signals coming from the implanted electrodes. As a first step, a voltage buffer TLC2274 configured op-amp is used to have a high impedance along with the electrode. to maintain the voltage on the circuit terminals and limit the attenuation from the electrodes. Next, the AD620 Instrumentation amplifier is used due to its common mode characteristics. The data sheet of the inst-opAmp shows that the CMRR gain is about 2000V/V which is sufficient for this stage. According to the datasheet, the effect of 50-60Hz power line interference is removed due to the CMRR. but in any case, a twin tee notch filter stages is added shown in Figure-18. according to the potential known ranges of frequencies and voltage amplitudes. We know that the range of voltage is 0.1-5mV and frequencies in an olfactory lay around 0.5 and 1kHz. Including action potentials in the olfactory sensory neurons OSNs, Local field potentials LFPs, and Electroencephalography EEG in the olfactory cortex. Although these ranges may vary depending on the mouse’s age, type, and specific neural activity, this range is a good starting point since also the stop bands of the Bandpass are not ideal, so having a wider range can help with filtering the desired responses. Thus, we aim to have a lower cut of around 20 Hz and a higher cut of 1kHz. These ranges are subject to change according to the first prototype’s results and may vary depending on the observed ranges. Figure-17 shows the band-Pass and the result of the simulation. Figure-14 shows the overall main parts of the circuit. The overall gain of the circuit is around 2000v/v and can be lowered or increased using a 50k ohm potentiometer at the amplification stage. The modifications and updates done to this stage aimed to have a lower number of components and a lower signal to noise to ratio. Due to delays in the materials needed for the first prototype, this circuit is more robust and has a lower risk of failure than the previously designed circuit.

In the third main stage which is the deep learning part, challenges were mostly caused due to the lack of finding datasets which are published on the internet because there was not any dataset which was created with the signals from the olfactory bulb of an animal.

While the experiments are being carried out, the deep learning algorithm must be ready simultaneously. First, a dataset that was created with the signals from the olfactory bulb of an animal was investigated. However, no ready-to-use dataset matching this condition was found. While the search for the dataset continued, the CNN model was created and tested on the

Fashion MNIST dataset. The Fashion MNIST dataset is a dataset that can be imported from Keras. This 28x28 dataset includes 60000 train sets and 10000 test sets. This dataset was tested using 2 different models. The first model consists of 3 convolutional layers and a kernel size of 3. Each convolutional layer is followed by the ReLU activation function as a last-layer output layer with 10 nodes since the dataset has 10 classes that are classified. This model's test accuracy value is 0.89. The other model's accuracy value is 0.92. It has been observed that the second model gives a better result. The structures of the models are given in Figure.17 and Figure.18. These algorithms were also tested with emotion EEG datasets. The emotion dataset is a dataset that has been processed but not spiked detection applied. Therefore, when the CNN models mentioned were tested, the accuracy rate was low. However, it has been observed that LSTM and DNN algorithms give better results in classifying the data set. Table.1 shows the accuracy rate.

12. CONCLUSION:

To conclude the whole project, the aim of the project is to observe the effects of the smell on the brain, to take these changes with the multi-channel electrode structure and to classify them with the artificial intelligence algorithm developed after passing through some filtering and amplification circuits set up and extracting the useful parts. For the project to successfully achieve its goal of odor classification, each intermediate target is expected to meet the determined success criteria. In this case, the final goal is achieved with a cumulatively increasing success rate. Intermediate targets for Project 1 and their contribution to overall success are given in the Project Targets and Success Criteria section as four stages. As a result of the progress, the results of project 1 were evaluated with these success rates.

In the first stage, it was aimed to develop the electrode structure. Here, an 8-channel electrode holder structure was designed since it is primarily aimed to use multi-channel electrodes. Afterwards, since it was decided to use a single-channel one before that, experiments were made to make electrodes using only two channels of this designed structure. Here, it is aimed to obtain the most accurate result by using more than one method. In the result part, the single-channel electrodes in Figure.13 were developed. In order to observe whether these electrode trials meet the determined success criteria, impedance measurements should be made. Studies on this subject are still ongoing. For this reason, it can be said that this stage provides the required success rate of 50%.

In the second stage electrophysiological recording and digitization experiments are expected from mice with the electrode structure developed. Since the electrodes were not ready yet, experiments were carried out using previously prepared electrode structures for the project. Since the mice died as a result of the anesthesia method used in this process, a successful recording operation could not be performed. However, subjects such as using experimental animals, following the operation processes, determining the location of the olfactory bulb were reinforced. Since the success criterion determined at this point is based on the studies on this subject, 60% has been met.

The third stage is on building and testing the filtering and amplification circuits. At this stage, the input signal to be used in this circuit system cannot be an electrophysiological signal since they are not taken yet. Instead, it has been tested and observed by creating a system in the simulation environment with a low voltage input signal with similar properties. The most important characteristic of the developed system is that it provides high voltage gain. A gain of at least 1000 times is targeted for this stage. In the studies, a system whose gain can be changed with a potentiometer was obtained and gains of 1000, 1500 and 2500 were obtained in the simulation environment. According to these results, it is seen that the success criterion for this stage is 100% met.

In the fourth stage, studies with different datasets and deep learning algorithms are expected to obtain results with high accuracy rates. The purpose of this step is to gain familiarity with creating a model based on the dataset and to quickly develop and implement the model when odor data is collected. Here, different from the odor data, classification studies were carried out using MNIST and emotion EEG data and various accuracy rates were obtained. Targeted studies were carried out and the determined success criterion was achieved at a high rate. As stated in the result section, emotion EEG data is classified by LSTM, LSTM with convolution layer and DNN algorithms. Classification percentages are given in Table.2 and it is seen that the algorithm with the highest classification value is DNN which is 97.5%.

Consequently, the project encompasses four primary stages and nine distinct work packages. Each work package was assigned success criteria based on its contribution to the overall project objectives. Most of the work packages have successfully met the designated success criteria. However, the Electrophysiological Recording, a critical step within WP7, has achieved only a 60% success rate. Given the interdependence among the work packages and WP7, several of them could not fulfill the specified criteria entirely. Nevertheless, upon evaluating the project as a whole, it becomes evident that it has fulfilled 90% of the success requirements.

13. ASSESSMENT OF ENGINEERING COURSES:

A lot of courses conducted at the university from freshman to senior helped in the research and implementation of this project. Starting with the basics of circuit designs in Circuits 1 and 2, where operational amplifiers were explained and taught, and Signals and Systems and Digital Signal Processing courses where signal processing in both CT and DT domains was taught extensively. This course helps a lot in digitally processing data and evaluating data to be used for datasets. As the name of the project suggests, it includes deep learning classification. For this purpose, Machine Learning and Deep Learning courses have also been effective.

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15. PROJECT ACTIVITIES AND WORK PLAN

Table 3. *The Work-Activity Plan for Project*

Work and Activity Project	Responsible Group Member	Timeline													
		1. week	2. week	3. week	4. week	5. week	6. week	7. week	8. week	9. week	10. week	11. week	12. week	13. week	14. week
1. Literature Review	All Members														
2. Electrode Construction and Measurements	Buse Nur FİDAN														
3. Development of Data Cleaning Algorithms	Buse Nur FİDAN														
4. Design and Simulations of Electrical Circuits on Tools	Tayseer M. HUSSEİN														

5. Building and Control Circuits	Tayseer M. HUSSEİN														
6. Electrode and Enviromental Setup Modeling	Senanur DEMİRCİ														
7. Electrophysiological Recording	Nazende Yağmur UYSAL Rufeyda YAĞCI														
8. Developing AI Algorithms for Different Dataset	Senanur DEMİRCİ														
9. Implementation of All Blocks	All Members														

16. LIST OF WORK PACKAGES

Table 4. *Detailed Definition of Work and Activity for Project*

WP No	Detailed Definition of Work and Activity
1	Re-examination of the literature for the solution of the problems encountered while carrying out the work packages of Project1 and Project2
2	Creating new electrodes by changing the materials used and the method of construction and measuring them with an impedance meter
3	Working on algorithms such as adaptive noise filtering to reduce the noise level of the data and thus increase the SNR value
4	Creating and simulating amplifier and filter design with LTSpice and Cadence
5	Implementation of circuits with integrated and components and testing the system in a laboratory environment and comparing the results with simulation
6	Developing controlled experiment set-up and electrode structure 3D models for more accurate recording
7	Data acquisition from the olfactory bulb with the electrode structure developed
8	Obtaining the AI model with the optimum result by changing the Data Set and the model
9	Integration of each individual work package and attaining a fully working system

Table 5. *Work package targets, their assessment, and the contribution of each work package to the overall project success.*

Work Package	Target	Measurable Outcome	Contribution to Overall Success	Completion Percentage
1	Literature Review		1%	100%
2	Electrode Construction and Measurements	Electrode Impedance	10%	100%

		Around 120k Ohm		
3	Development of Data Cleaning Algorithms	High SNR / Noise-Free Odor Signals	10%	100%
4	Design and Simulations of Electrical Circuits on Tools	High Amplitude and SNR Value Data	10%	100%
5	Building and Control Circuits	Results Matching Simulation Results	15%	100%
6	Electrode and Environmental Setup Modeling	Proper Working Electrode and Suitable Experimental Environment	2%	50%
7	Electrophysiological Recording	Acquired Odor Signals	20%	60%
8	Developing AI Algorithms for Different Dataset	>80% Success and More than 5 Odors Classification	30%	100%
9	Implementation of All Blocks	Fully working system	2%	50%
			Total:100%	Total: 90%

Table 6. *Work package targets, their assessment, and the contribution of each work package to the overall project success.*

WORK PACKAGE DISTRIBUTION									
Project Member	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9

SD	25%	10%	10%	10%	10%	70%	5%	80%	25%
BF	25%	80%	80%	10%	10%	10%	5%	10%	25%
TH	25%	0%	10%	80%	80%	10%	5%	10%	25%
NU & RY	25%	10%	0%	0%	0%	10%	85%	0%	25%
Total	100%	100%	100 %	100 %	100 %	100 %	100 %	100 %	100%

SD: Senanur Demirci
BF: Buse Nur Fidan
TH: Tayseer M. Hussein
NU & RY: Nazende Yağmur Uysal & Rufeyda Yağcı

17. BUDGET

Table 7. *Proposed Budget in TL*

	ITEMS				
	PEOPLE	MACHINE-INSTRUMENT	MATERIALS	SERVICE	TRAVEL
IMU FUND	-	3D Printer Equipments	-	Certificate to Use Experimental Animals	-
SPONSOR COMPANY FUND	-	-	Electrical Components	-	
TOTAL		3200 TL	1150 TL		

Table 8. *Actual Budget in TL (what you spent indeed)*

	ITEMS				
	PEOPLE	MACHINE-INSTRUMENT *	MATERIALS*	SERVICE	TRAVEL
IMU FUND	-			Certificate to Use	

				Experimental Animals	
SPONSOR COMPANY FUND	-		Silver Paint Steel Springs Biocompatible Resin		
TOTAL			267,62 TL 280,0 TL 4.723,56 TL		

*Provide proforma invoice for machines and materials to be purchased.
*Provide technical specifications for machines and services to be purchased.
*Make a contract for services if necessary

18. CURRICULUM VITAE

19. SUPPORT LETTERS (if any)

Deep Learning for Odor Sensing project is supported by TÜBİTAK 2209-A Research Project Support Program for Undergraduate Students.

2209-A Üniversite Öğrencileri Araştırma Projeleri Destekleme Programı 2022/1

Başvuru Numarası	Durumu	Başvuru Belgeleri		
1919B012212364	Desteklenmesine Karar Verildi	Belgeleri Göster	Onaylı Başvuru/İzleme Formları	Aktif İzleme Süreci Aşamaları