

**A VIDEO TRACKING SYSTEM FOR DETECTING AND RESTRAINING  
MOVEMENTS OF FREELY MOVING RODENTS**

A THESIS SUBMITTED TO  
THE GRADUATE SCHOOL OF  
ENGINEERING AND NATURAL SCIENCES  
OF ISTANBUL MEDIPOL UNIVERSITY  
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF  
MASTER OF SCIENCE  
IN  
BIOMEDICAL ENGINEERING AND BIOINFORMATICS

By

Bihter Ozhan

February, 2023

A VIDEO TRACKING SYSTEM FOR DETECTING AND RESTRAINING  
MOVEMENTS OF FREELY MOVING RODENTS

By Bihter Ozhan

3 February 2023

We certify that we have read this dissertation and that in our opinion it is fully adequate,  
in scope and in quality, as a dissertation for the degree of Master of Science.

---

Assist. Prof. Mehmet Kocatürk (Advisor)

---

Assist. Prof. Özge Şensoy

---

Assoc. Prof. Dr. Pınar Öz

Approved by the Graduate School of Engineering and Natural Sciences:

---

Prof. Dr. Yasemin Yüksel Durmaz

Director of the Graduate School of Engineering and Natural Sciences

I hereby declare that all information in this document has been obtained and presented in accordance with the academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Signature :

Name, Surname: Bihter Ozhan

## **ACKNOWLEDGEMENT**

I would like to thank to my supervisor Dr. Mehmet Kocaturk for his guidance and support. I could not have completed this long and difficult process without my family and friends and special gratitude for their motivation, support, and encouragement to make this work possible. And I would like to thank my husband, Talha Zaidi, who always and under all circumstances supported me.

Bihter Ozhan

February, 2023



# CONTENTS

	<u>Page</u>
<b>ACKNOWLEDGEMENT</b> .....	<b>iv</b>
<b>CONTENTS</b> .....	<b>v</b>
<b>LIST OF FIGURES</b> .....	<b>vi</b>
<b>ÖZET</b> .....	<b>vii</b>
<b>ABSTRACT</b> .....	<b>ix</b>
<b>1. INTRODUCTION</b> .....	<b>1</b>
1.1. Scope of Thesis .....	3
1.2. Outline of Thesis .....	3
<b>2. THEORETICAL PART</b> .....	<b>4</b>
2.1. Reward Function: General Ideas and Historical Background .....	4
2.2. Schedule of Reinforcement.....	6
2.3. Extinction and Its Side Effects.....	7
2.4. Rodent's Visual Capabilities .....	8
2.4.1. Visual acuity .....	8
2.4.2. Orientation .....	8
2.4.3. Motion.....	8
2.4.4. Color .....	9
2.4.5. Shape and object recognition.....	9
2.5. Head Fixation of Rodents .....	9
2.5.1. Types of head fixation .....	10
2.5.1.1. Actual head fixation.....	10
2.5.1.2. Virtual head fixation .....	10
<b>3. EXPERIMENTAL PART</b> .....	<b>12</b>
3.1. Setup Overview .....	12
3.2. Behavioral Cage Environment .....	12
3.2.1. Experimental cage.....	12
3.2.2. IR beam.....	14
3.2.3. Lever assembly .....	14
3.2.4. Reward delivery system.....	14
3.2.5. Cage LEDs.....	14
3.3. LCD Display .....	14
3.4. Top View Camera System .....	15
3.5. Operation to Place the LED .....	15
3.6. Background Control Architecture.....	16
3.7. Experimental Process.....	17
3.8. Experimental Troubleshooting .....	19
3.9. Methods .....	19
<b>4. RESULTS AND DISCUSSION</b> .....	<b>26</b>
4.1. Results .....	26
4.2. Discussions.....	29
<b>5. CONCLUSIONS AND FUTURE WORK</b> .....	<b>31</b>
<b>BIBLIOGRAPHY</b> .....	<b>32</b>
<b>CURRICULUM VITAE</b> .....	<b>34</b>

## LIST OF FIGURES

<b>Figure 3.1:</b> Experimental setup, <b>a)</b> Top view, <b>b)</b> Side view, <b>c)</b> Back view, <b>d)</b> Placement of water receptacle, nose-poke area, and IR sensor.....	13
<b>Figure 3.2:</b> Circuit created with a 3-volt battery, switch and 10-ohm resistor and was mounted on the head of the rat.....	15
<b>Figure 3.3:</b> Image of the screw placed on the rat head after the operation. Thanks to the dowel mounted on the LED circuit, they pass into each other and reached a fixed position.....	16
<b>Figure 3.4:</b> The flow chart of the top camera code which is sending output to paradigm code.....	23
<b>Figure 4.1:</b> The rat's position and three camera angles when rat comes to the middle of the cage for a nose poke or for a reward.....	27
<b>Figure 4.2:</b> The rat's position and three camera angles when rat comes to press the lever to perform a paradigm.....	27
<b>Figure 4.3:</b> The resolution and frame rate relationship.....	28



# SERBESTÇE HAREKET EDEN KEMİRGENLERİN HAREKETLERİNİ TESPİT ETMEK VE KISITLAMAK İÇİN VIDEO TAKİP SİSTEMİ

## ÖZET

Bihter Özhan

Biyomedikal Mühendisliği ve Biyoenformatik, Yüksek Lisans

Tez Danışmanı: Dr. Öğr. Üye. Mehmet Kocatürk

Şubat, 2023

Kafa sabitleme, kemirgenleri sınırlandırıp bir ekrandan görsel uyarın almalarını sağlamak için yararlı bir yöntemdir. Bu teknik, görsel uyarınların deneye uyanırken iletilmesini garanti etse de, davranışsal çalışmalarda fiziksel kısıtlamanın yarattığı stres çoğunlukla göz ardı edilemez. Ek olarak, teknik, konuyu ele almak için uzmanlık ve incelenecek davranışsal görev sırasında deneyin yeterince sessiz kalmasını sağlamak için birkaç gün hatta haftalarca aşamalı eğitim gerektirir. Bu çalışmada, serbestçe hareket eden farelerin hareketlerini kısıtlamaya izin veren ve olumlu ve olumsuz pekiştirmeler kullanarak bir PC monitöründen görsel uyarınlar almalarını sağlayan bir yöntem sunuyoruz. Sistem temel olarak deney ortamının üzerine yerleştirilmiş bir video kamera, kameradan alınan video akışını işlemek için bir PC ve PC'den komutlar almak ve serbestçe hareket eden özneye zamanında takviye ipuçları vermek için mikrodenetleyici tabanlı bir cihaz kullanır. Kameradan gelen video akışını elde etmek, işlemek ve görselleştirmek için MATLAB kullanılarak bir program geliştirilmiştir. Deneyci, programı kullanarak görselleştirilmiş kamera görüntüsünde sanal bir dikdörtgen alan oluşturur ve program, deneyin deneysel ortamda belirtilen alanda olup olmadığını belirtmek için mikrodenetleyicinin ikili çıktısını sürekli olarak sürer. Deneyin ortamdaki konumunu ve hareketlerini algılamak için deneyin başına küçük bir madeni para piliyle çalışan kırmızı bir LED takıldı ve elde edilen her video karesi üzerinden LED'in anlık konumu çıkarıldı. Mevcut sistemin uygulanabilirliğini doğrulamak için, onu, erkek Wistar farelerinin bir PC monitöründe bir imlecin kontrolünü öğrendiği bir görsel-motor görevine entegre ettik. Sistem denekleri başarılı bir şekilde dar bir koridorda tuttu ve koridordan çıktıklarında negatif pekiştirme vererek PC monitörüne doğru yönlendirdi. Sonuç olarak, denek etkili bir şekilde koridorda kalacak ve görsel uyarınları deneme yanılma yoluyla takip edecek şekilde şekillendirildi. Burada sunulan davranışsal paradigma için çevreden düşük gecikmeli görüntülerin alınmasında, öznenin konumunun saptanmasında ve pekiştirme ipuçlarının zamanında iletilmesinde önemli bir parametre olan işlenmiş video akışının kare hızı, 160x120 görüntü çözünürlüğü için  $25.87 \pm 9.50$  Hz idi. Çalışmamız, burada sunulan video izleme sisteminin, farelerin hareketlerini, davranışsal bir görevin belirli bir döneminde yasaklanmış bir hareketi gerçekleştirdikleri gibi, olumsuz pekiştirme sağlayarak kısıtlayabildiğini gösteriyor. Mevcut sistemde konum göstergesi olarak kullanılan LED'in mevcut kafa aşaması bileşenlerine kolayca monte edilebilmesi nedeniyle, mevcut sistemin, beyin aktivitesini izlemek için farelerin varsayılan olarak kafatasına implantlar aldığı çalışmalara da entegre edilebileceğine inanıyoruz.



Anahtar sözcükler: Hayvan davranışı, Pekiştirme, Video işleme, Nesne algılama.

# **A VIDEO TRACKING SYSTEM FOR DETECTING AND RESTRAINING MOVEMENTS OF FREELY MOVING RODENTS**

## **ABSTRACT**

Bihter Ozhan

MSc in Biomedical Engineering and Bioinformatics

Advisor: Assist. Prof. Dr. Mehmet Kocatürk

February, 2023

Head fixation is a useful method to keep the rodents restrained and make them receive visual stimuli from a screen. Although this technique guarantees delivering the visual stimuli to the subject while it is waking, the stress caused by physical restraining is mostly not negligible in behavioral studies. In addition, the technique requires expertise for handling the subject and several days or even weeks of progressive training to make the subject stay sufficiently quiet during the behavioral task to be studied. In the present work, we introduce a method which allows restraining the movements of freely moving rats and makes them receive visual stimuli from a PC monitor using positive and negative reinforcements. The system basically utilizes a video camera placed on top of the experimental environment, a PC to process the video stream acquired from the camera and a microcontroller-based device to receive commands from the PC and deliver timely reinforcement cues to the freely moving subject. A program was developed using MATLAB to acquire the video stream from camera, and process and visualize it. Using the program, the experimenter creates a virtual rectangular area on the visualized camera view and the program continuously drives the binary output of the microcontroller to indicate whether the subject is in the specified area in the experimental environment. In order to detect the position and movements of the subject in the environment, a red LED powered by a small coin battery was attached to the head of the subject and the instantaneous position of the LED was extracted through each acquired video frame. In order to validate the practicality of the present system, we integrated it into a visuomotor task where male Wistar rats learned the control of a cursor on a PC monitor. The system successfully kept the subjects in a narrow corridor and oriented them toward the PC monitor by delivering negative reinforcement whenever they left the corridor. Consequently, the subject was effectively shaped to stay in the corridor and follow the visual stimuli through trial-and-error. The frame rate of the processed video stream, an important parameter in acquiring low-latency images from the environment, detecting the position of the subject and delivering timely reinforcement cues, was  $25.87 \pm 9.50$  Hz for 160x120 image resolution, which was appropriate for the behavioral task presented here. Our work indicates that the video tracking system presented here was capable of restraining the movements of rats via introducing negative reinforcement just as they perform a prohibited movement during a specified period of a behavioral task. We believe the present system can also be integrated into the studies where the rats by default receive implants into their skull for monitoring the brain activity since the LED used as the

indicator of position in the present system can be easily mounted onto the existing head stage components of the brain activity monitoring systems.



Keywords: Animal behavior, Reinforcement, Video processing, Object detection.

## **CHAPTER 1**

### **1. INTRODUCTION**

Motor learning is defined as the process by which an individual acquires motor skills and acquires as a function of practice or experience with a relatively permanent change in performance. The motor movement ability allows the movement of living things using structures in the central nervous system. These structures are motor cortex, basal ganglia, brain stem, cerebellum, and spinal cord [1]. Motor skills are associated with the structures, it defines the ability to move the muscles. Motor skill learning reorganizes the motor cortex; therefore, it allows to examine the activity occurring in the brain [2]. Rats are used to understand the neural mechanisms that are involved in motor skill learning in general.

Neurons in the human brain make connections based on the intensity, repetition, level and state of internal and external inputs, stimuli (impulses). This interneuron connection can be either weakened and discarded depending on the needs of the body and the expectations of the brain, whether it is meaningful or not, or if it is too strong, it can establish new connections by being scraped into the brain. Such activities are linked by the connections of brain networks and provide important information for understanding brain networks [19].

Knowing the mechanisms of neural circuits formed by behaviors acquired with motor learning skills will be pioneering information for neurophysiological diseases and motor disorders. It may guide the knowledge of the causes of neurophysiological diseases and the development of treatment methods. It may also contribute to neurological prosthesis studies. In this study, we present a paradigm that will be beneficial in the investigation of neurological diseases.

The study of visuomotor skills in rodents has long been a crucial area of research in neuroscience, with important implications for understanding both normal brain function and the neural mechanisms underlying various neurological and behavioral disorders. In this thesis, the topic is a new rodent visuomotor skill learning paradigm that enables control of a cursor on a PC monitor. The ability to control a cursor on a computer monitor is a fundamental skill in today's technologically advanced society.

However, traditional methods for studying visuomotor skills in rodents have often been limited by the need for restrictive physical restraint, which can impede the animals' ability to move and learn. To address this issue, this thesis proposes a new paradigm for studying visuomotor skills in rodents, utilizing a movement restraining system based on online video tracking. In this method, animals are free to move within a designated area of their cage, while a top camera system tracks the animal's head movements and uses this information to control the paradigm. This allows for a more natural and unrestricted learning environment for the animals, while still providing the researcher with precise control over the task demands. The study utilizes virtual head fixation, a method in which the animal is free to move within a designated area determined by a video camera system placed on top of the experimental environment. The system is a kind of virtual head fixation method since it targets to restrain the head movements of rodents by operant conditioning. The general questions this research aims to address include:

- How does the use of a virtual head fixation system impact the ability of rodents to learn and perform visuomotor tasks?
- To what degree can rodents learn to control a cursor on a PC monitor using this new paradigm?
- How does this new paradigm compare to traditional methods of rodent visuomotor skill learning?

To address these questions, this thesis will present the results of a series of experiments in which rodents are trained to perform a variety of visuomotor tasks using the new paradigm, and the results will be compared to those obtained using traditional methods.

Overall, this thesis aims to contribute to the field of rodent neuroscience by developing and testing a novel approach for studying visuomotor skills in rodents.

## **1.1. Scope of Thesis**

In the development of brain-machine interfaces, it is of great importance to examine the neural mechanisms involved in motor skill learning. To study these neural mechanisms in human and non-human primates, we present a lever-controlled paradigm to control the cursor on a computer monitor. In this paradigm, the aim of the experiment is to provide the target that appears on the monitor with the task of getting the cursor out of the center, and it requires correcting the error occurred in the experiment with visual feedback. Although many studies have been done, it has not addressed an orbital error during the decentralization mission in rodents and does not propose a method for the neural mechanisms encoding this error, so the existence of a suitable paradigm for in-vivo studies using rodents is imperative. In addition, although the subject was allowed to move freely in the experimental environment, in the paradigm we developed, head movement restraining was applied to prevent complications in operant conditioning caused by the rat's limb movements. Thus, when the rat goes out of the designated area, it is punished and since there is no physical movement restraining of head-fixation, it does not have any effect that will stress the rat during the experiment. This thesis discusses the experimental paradigm and the detailed explanation of the training process.

## **1.2. Outline of Thesis**

Chapter 01 brief introduction about general questions which addressed in the study. Chapter 02 provides a literature review discussing about the study. Chapter 03 provides a detailed description of experimental environment, materials and working steps in this study. Chapter 04 discusses the results of this study. Chapter 05 discusses the conclusions about the scientific research that can be done by using the findings of this study.

## **CHAPTER 2**

### **2. THEORETICAL PART**

#### **2.1. Reward Function: General Ideas and Historical Background**

The perception of reward can be explained with the happiness or favorable outcome that can strengthen the repeatability of the ones act. This viewpoint of reward also refers as instrumental conditioning reward positively reinforces the behavior. Another perception of reward is subjective inclination of an individual for the task that gives happiness or pleasant outcomes in the past. This is referred as hedonic function of the reward. Both notations generalize the reward function, discusses as following.

The theory of classical conditioning proposed by Ivan Pavlov, described the reward as an object that provides a behavioral change or learning [3]. This can be explained with the example of a dog that salivates only when a doorbell ring paired with the sausage is presented to dog. This shows the behavioral conditioning for food. As in this case, dog does not have to do any task to achieve the reward, so this reward does not fully conform the above mentioned two reward notions. Despite all of this, the discussing theory of reward is a key for neurobiological research. The operant conditioning for behavioral research states that the repeatability of behaviors can be achieved if they are rewarded properly. Positive reward can provide high frequency of specific actions and vice versa. This is also known as ‘law of effect’ postulated by Thorndike [5]. This concept has a little difference with what presented by Pavlov, in which rewards are presented without performing any action. For animals’ behavioral experiments, different reward objects are utilized which mostly includes food pallets, liquids, water, sucrose etc. The controlled quantities of these reward objects defined their motivational value. Determining the rewarding effect of these rewards is quite difficult task. The primary rewarding effect can be taste, smell, chewing, sighting, swallowing or nutrition valve. This rewarding effect is also associated with different events for different objects [4]. Nevertheless, the reward

based operant conditioning provided a strong scientific platform for neurophysiological research.

Some of the basic components in operant conditioning, includes the response, outcome of reward and stimulus of reward. The response is actually the voluntary act of operant, which it performs in the pursuit of reward. Pressing the lever to get the reward object is the response or operant behavior and represented as R. The outcome of reward tells the frequency of the operant behavior. If the outcome increases the probability of repeating the behavior, then it is reinforcer and if it weakens the behavior then it is punisher. Both reinforcer and punisher are represented as  $S \rightarrow R$  and  $SP$ , respectively. The stimulus is employed to indicate that the operant behavior is available which will lead to the reward. LED cue in lever press example acted as the discriminative stimulus  $S \rightarrow D$  [6].

Sometimes the likelihood of reward is higher in the presence of stimulus and less in the absence of it, which means stimulus acts as a reward predictor. This case is called the Contingency. If a behavior acts as reinforcer, then it's a contingency to reinforcement and if it acts as a punisher then it's the contingency to punishment. Both are further distributed into positive and negative [6]. A positive for reinforcer will be the stimuli that can strengthen the reinforcement and positive for punisher will be the stimuli that can strengthen the punishment. Delivering food and delivering shock in response to the lever press are two examples for positive reinforcer and positive punisher, respectively. Similarly, Negative reinforcer and punisher are opposite to the positive, which weakens the main strategy. Removing the shock with lever press, and removing the food if trial ends without reward are the examples of negative reinforcer and negative punisher, respectively. Positive reinforcements are usually more effective than punishments, for behavioral experiments.

There is another classification of reinforcers, termed as primary and secondary reinforcer. Primary reinforcers are those which do not require any training to develop a behavioral emotion and naturally have a strengthening effect on behaviors. Food and water are the examples of primary reinforcers. On the other hand, secondary reinforcers are those that have associations with other reinforcers to modulate the behavior. Learning is required in this case to develop these associations.

To achieve the desired behavioral tasks, positive reinforcers are used. But the problem arises when the animal never performs the desired tasks to get the reward. In such cases

a shaping procedure is utilized to encourage the animal to perform the desired task. This shaping procedure includes the sequential steps which gradually strengthen the operant behavior. In case of lever press example, smaller sequential steps include the going near to the lever, stand in right position, stand in right posture, looking towards target and press the lever. Each sequential step is reinforced to achieve the desired behavior, and this is known as shaping procedure. The subject can be trained for each step, one at a time and eventually it will learn the desired behavior. This progression from one step to another is also known as chaining.

## **2.2. Schedule of Reinforcement**

Schedule of rewards is usually corresponding to the responses which are responsible to achieve the reward does rat gets food or water, every time it presses the lever, or it must press the lever multiple times for that. This gives rise to the classification of schedules in continuous and intermittent. In continuous schedules, subject can get the reward every time when it performs the targeted response. In shaping process rat gets the water whenever it comes in the approximation. In intermittent schedule, reward is presented after some responses in lever press example, reward is presented whenever lever is pressed 3 times.

The intermittent schedules are further discussed in four categories. If the reward delivery depends upon the fixed predetermined number of responses, then it's a fixed ratio schedule in fixed ratio 3 (FR3) rat must press the lever 3 times to get the reward. If the reward is given at the instance of randomly selected variable number of responses while keeping the average of required responses same, throughout the experiment, then it's a variable ratio schedule. In variable ratio 3 (VR3) to get the reward, rat must press the lever between 1-5 times, it varies in trials but overall, the average should be 3. In some other cases, subjects get the reward by performing the response after a fixed interval of time, pressing the lever after 60 seconds, this is known as fixed interval scheduling. On the other hand, if the time for the lever to become available varies randomly and the first response after the time reaches the limit, gives the reward, then it's a variable interval scheduling.

Reward scheduling sometimes also classified based on the response's duration. In some cases, a continuous response is required for a fixed amount of time, this is fixed duration schedule. E.g., press the lever for 2 seconds to get the reward. If the duration of response

is not fixed and linked to some trial parameter then it's a variable duration schedule, e.g., press the lever until cursor reaches to destination.

Response rate of the behavior also sometimes varies with the schedule type. In such cases reward is delivered based on response rate, If the same type of response repeated specific number of times within specified duration to get the reward, then it's a differential reinforcement of high rates. Lever should be presses 3 times within 30 second then reward is delivered. If user specifies some time interval between two consecutive instances of behaviors, then its differential reinforcement of low rates. There should be a gap of 3 second between two consecutive lever press.

In complex reinforcement schedules, reward schedule is not limited to a single response, which makes it a challenging problem. In these cases, if all the schedules are arranged in such a way that to receive the reward all schedules' requirements must be met then it's a conjunctive schedule. lever must be pressed two times and then one time after the delay of second, to achieve the reward. If the sequence of schedules must also be followed in conjunctive schedules, then it becomes chained schedule nose poke to initiate the trial, press lever two times then wait for the actuator to reach the destination and turns the LED on and then press the right button.

### **2.3. Extinction of Its Side Effects**

Sometimes delivery of reward for the specific behavior response is ceased to analyze the subject response. This process is known as extinction. It can result in decreased number of behavioral responses from the subject. It is noticed that the effect of learned behavior does not fade away so easily in extinction process, in fact it sometimes increases the frequency of same learned behavior, if rat learned to get reward after two lever presses, then in extinction if reward is removed, the rat will press the lever with more high frequencies in the hope of reward. Similarly, rat can also show some variability in behavior, previously if it was pressing the lever with one hand then after extinction it can press the lever with left hand or both hands. It will try to change its behavior in the hope of reward. It can also show the aggression by biting the lever and can display emotional responses in the form of grooming or agitating by leaving the reward delivery area. It might also possible that rat started to show previously learned behavior when the later behavior stopped producing the reward. It is also observed that to recover the effect of extinctions, a break in trials can also be helpful. If rat learned to get reward after pressing

lever two times, then after implementing extinction for one day, the next day rat will again try the same old behavior of pressing the lever two times, as it forgot that yesterday this behavior couldn't generate responses.

## **2.4. Rodent's Visual Capabilities**

The behavioral learning of the rodents depends upon how they perceive the environment in which they are trained. As it is clear evident that the visual capabilities of rodents are not same as compared to the humans or non-human primates, so the experimental environment must use the tools and settings through which they can understand the environment in a better way for behavioral learning. To understand this different component of rodent's visual capabilities are discussed as follows:

### **2.4.1. Visual acuity**

Recognition of dark lines of grating pattern is Visual acuity. It is measured in terms of spatial frequency i.e., number of pairs of black and white lines that can fit in one degree of visual field on retina. [7]. Different rat species have different visual acuity pigmented rat strains (Long Evans) have very low visual acuity of 1.0 cycle/degree [11], [12] non-pigmented rats (Wistar, Sprague-Dawley) have even more less visual acuity of 0.5 cycles/degree, [11], [12] whereas Fisher-Norway rats have highest visual acuity of 4.5 cycles/degree [11], [12]. These visual acuities are very less as compared to humans who have 30 cycles/degree, which shows that how hard it is for them to perceive the fine details of environment. The neural activity in rat's primary visual cortex also shows changes with the change in spatial frequency [13].

### **2.4.2. Orientation**

It is observed that the change in orientation of objects caused the high response from around 77% of the neurons in rat's visual cortex V1, while only 3.5% does not respond to any orientation shift [11], [13], [14]. The response of neurons against the optimum orientation shift of objects is in the form of decrease in their firing rates. The most significant response is observed against the horizontal orientation shift [11], [13].

### **2.4.3. Motion**

The advanced ability of detecting and perceiving the motion is observed in rodents. They are more responsive towards moving stimuli as compared to the stationary stimuli [11],

[13]. This has been observed by studying the single and same movement of randomly scattered dots. The task difficulty is increased if the number of scattered dots decreased. The minimum number of these dots required to detect the motion is coherence threshold. Rats have four times higher coherence threshold than humans. [15], [16]. The perception of these moving dots can also be increased by improving the displacement of these dots and increasing the frame period.

#### **2.4.4. Colors**

Just like humans, rats possess two types of receptors on their retina, in which one of them process greyscale input, called rods. These are dependent on cons for processing colors in environments. The spectral range of these photoreceptor in rats are limited and does not allow them to perceive some wavelengths. There are some pigments in cons which helps them for color processing. One of the pigments is sensitive to blue and ultraviolet light whereas other is sensitive to longer wavelength of light corresponds to green color. It is also observed that most of the cones are sensitive to green light and only 12% have sensitivity for blue and ultraviolet [8]. The distribution of cons on rat's retina is not uniform and most of them lies on the bottom side of retina, because of this the perception of colored objects depends upon the location of the visual field, where the image is projected.

#### **2.4.5. Shapes and object recognition**

Rats have the abilities tom extract different features of the objects which are helpful for them to distinguish between different objects. Though the ability of rats to recognize the objects are much inferior to humans and non-human primates, buts still they could differentiate different size, shapes, color and orientation of objects. [9], [10]. The complex features are used to generalize the object categories, which in some cases remain invariants for different complex objects.

### **2.5. Head Fixation of Rodents**

Head fixation is a commonly used technique in behavioral neuroscience research with rodents, particularly in visual motor skill learning studies. This technique involves immobilizing the head of the rodent in a fixed position using a headrest or a specially designed head holder. The purpose of head fixation is to prevent the rodent from moving

its head during the task, allowing for more precise measurement of visual and motor responses.

One of the main advantages of using head fixation is that it allows for greater control over the visual stimulus presented to the rodent. By keeping the head in a fixed position, the researcher can ensure that the visual stimulus is presented to the same location on the retina during each trial. This is important for studying visual perception and visual motor learning, as it ensures that any changes in behavior are a result of the task rather than variations in the visual stimulus.

Another advantage of head fixation is that it allows for the use of more complex visual stimuli, such as moving or dynamic images. By keeping the head in a fixed position, the researcher can present the stimulus to a specific location on the retina, making it possible to study visual processing and motor responses in a more precise way.

However, head fixation also has some disadvantages. One of the main disadvantages is that it can cause stress and discomfort to the rodent, which can affect the results of the study. Additionally, head fixation can also limit the rodent's natural behavior, which can make it more difficult to study certain aspects of behavior.

Despite these disadvantages, head fixation is widely used in behavioral neuroscience research, particularly in studies of visual perception and visual motor learning. This method is often used in combination with other techniques, such as eye tracking or electrophysiology, to provide a more comprehensive understanding of the neural mechanisms underlying visual processing and motor learning [18].

### **2.5.1. Types of head fixation**

There are two main types of head fixation: virtual and actual. Their details are discussed as follow.

#### **2.5.1.1. Actual head fixation**

Actual head fixation involves physically immobilization of the animal's head, typically using a headrest or a surgical implant. This method is more invasive and can cause more stress to the animal, but it provides a higher degree of control over the animal's gaze. This method involves attaching a small metal plate to the rodent's skull using dental cement. The plate is then secured to a platform, preventing the rodent from moving its head. This method is easy to setup but provides more discomfort to the animal [17].

### **2.5.1.2. Virtual head fixation (restriction of head movement via reinforcement learning)**

Virtual head fixation is achieved by using a computer-controlled tracking system that follows the animal's head movements and visual stimuli. This allows the animal to move its head freely within a defined range, but still maintains control over the animal's gaze. If the animal tries to move out of the defined boundaries, then the system punishes it in the form of ending the trial without giving the reward. This method causes less stress to the animal as compared to the actual head fixation and animal can perform more trials.

In general, virtual head fixation is the better option because it is less invasive and causes less stress to the animal. However, actual head fixation may be necessary in certain situations, such as when extremely precise control over the animal's gaze is required or when the animal's head movements are too large to be effectively controlled by a virtual tracking system.

Optogenetics and head-mounted microscopes are two additional methods that can be used for virtual head fixation of rats in a cage environment.

Optogenetics is a technique that uses light to control the activity of specific neurons in the brain. In optogenetic experiments, the rat's head is often immobilized using a head post or head mount, while a fiber optic cable is used to deliver light to the targeted brain region. This allows the experimenter to precisely control the timing and location of neural activity in the brain, while the rat is free to move around the cage [21].

Head-mounted microscopes are another tool used in neuroscience research to monitor the activity of neurons in the brain. These microscopes are lightweight and can be attached to the rat's head, allowing for the recording of neural activity in real-time as the rat moves around the cage. In order to achieve virtual head fixation, the microscope must be equipped with a tracking system that monitors the position of the rat's head in real-time and adjusts the field of view of the microscope accordingly [20].

In both cases, the goal of virtual head fixation is to immobilize the rat's head while allowing its body to move freely, in order to study the neural activity associated with specific behaviors or stimuli. The choice of method depends on the specific experimental design and the research question being addressed.

## **CHAPTER 3**

### **3. EXPERIMENTAL PART**

#### **3.1. Setup Overview**

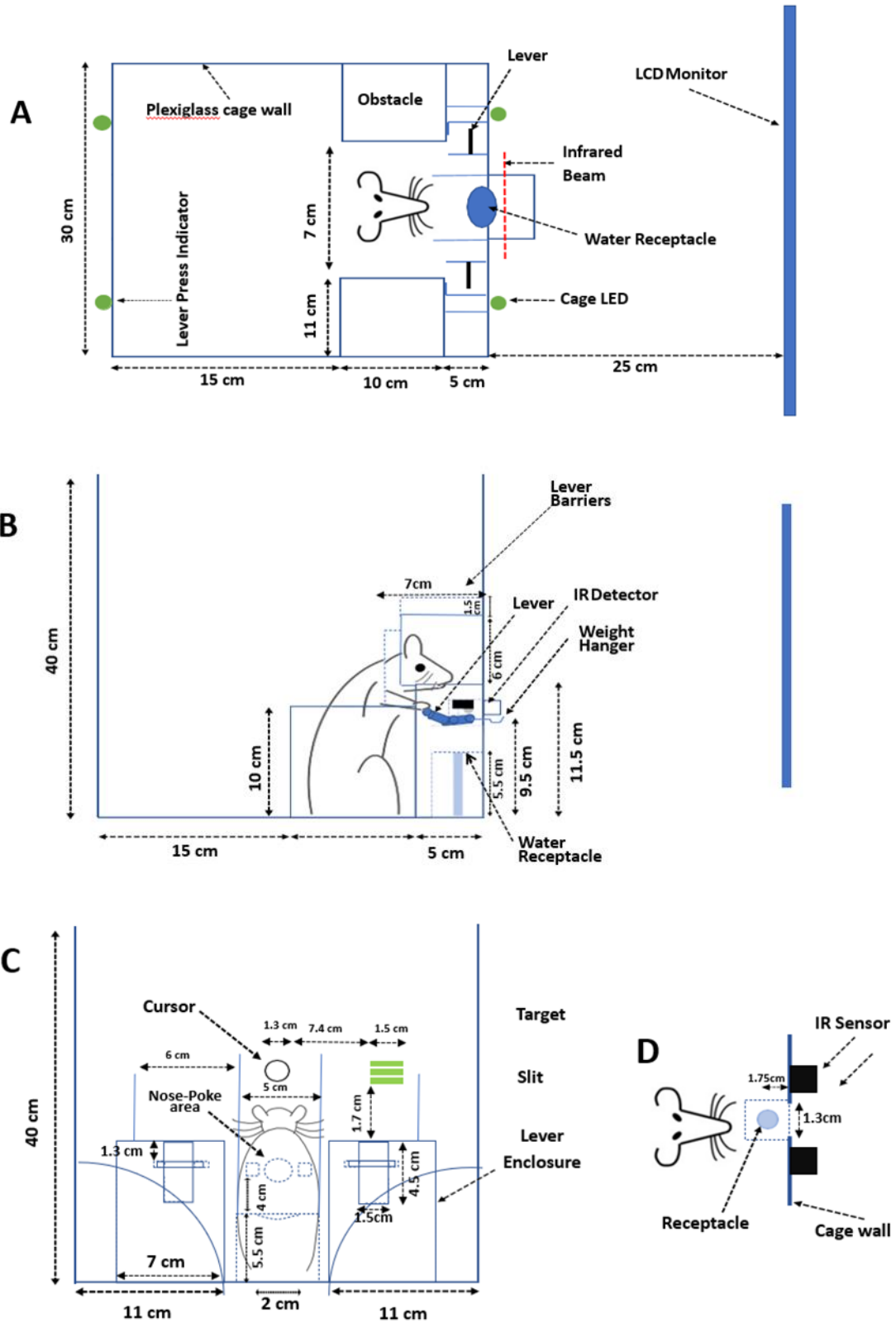
In the study, main aim was to create a behavioral paradigm with the visual feedback from a display in the closed-loop control. Two different system were gathered to perform the task. The system which is controlling the main paradigm and the camera system which is controlling the rodent to make stable in the determined location worked together to create a better version task. An Arduino Nano which was controlling the connection system and MATLAB-based system which was controlling the coding part were developed separately for both main paradigm and camera system. Behavioral cage, which was the environment for performing the task, LCD which was giving visual feedback to rodent to perform a paradigm and LED system which was inserted to head of rodent to control the experimental area by controlling the top camera were the main apparatus in the study.

#### **3.2. Behavioral Cage Environment**

The experimental setup was designed in the laboratory by custom hand made to train the rats.

##### **3.2.1. Experimental cage**

Transparent plexiglass walls were used to create an experimental cage environment to maintain the visual feedback between inside the cage and outside the cage. With the camera system positioned on the cage, top control was provided. The top of the cage was left open for the camera system to work properly.



**Figure 3.1:** Experimental setup, **a)** Top view, **b)** Side view, **c)** Back view, **d)** Placement of water receptacle, nose-poke area, and IR sensor.

### **3.2.2. IR beam**

The rat must do nose poke to start the trial so, first a 1.3 cm hole was opened in front of the cage wall which is towards to LED screen. Then, IR LED and detector pair were placed opposite sides of the created hole from the outside of the plexiglass cage wall to prevent accidental nose poke.

### **3.2.3. Lever assembly**

Two lever assemblies were designed in the 3D builder program which is a open source and then printed in the 3D printer using PLA as a material for levers. Two lever assemblies were located on the front wall and the dimension of the lever shaft was determined according to the rat to give easiness to press. Microswitch was chosen to detect the lever presses because of its less force required feature. The lever assembly was covered with the form which printed from 3D printer, and it has an only slit part to give pressing area for rat to create same movements and prevent biting the lever.

### **3.2.4. Reward delivery system**

Water which was located between the lever assemblies and below the nose poke area was given as reward to make strong the operant behavior. 12 VDC CROX 2V025-06 solenoid valve and water reservoir work together to deliver the water with the help of the gravity so placed at a high position near cage wall. The amount of incoming water could be adjusted by changing the opening time of the valve, so that each rat's motivation status could be considered separately.

### **3.2.5. Cage LEDs**

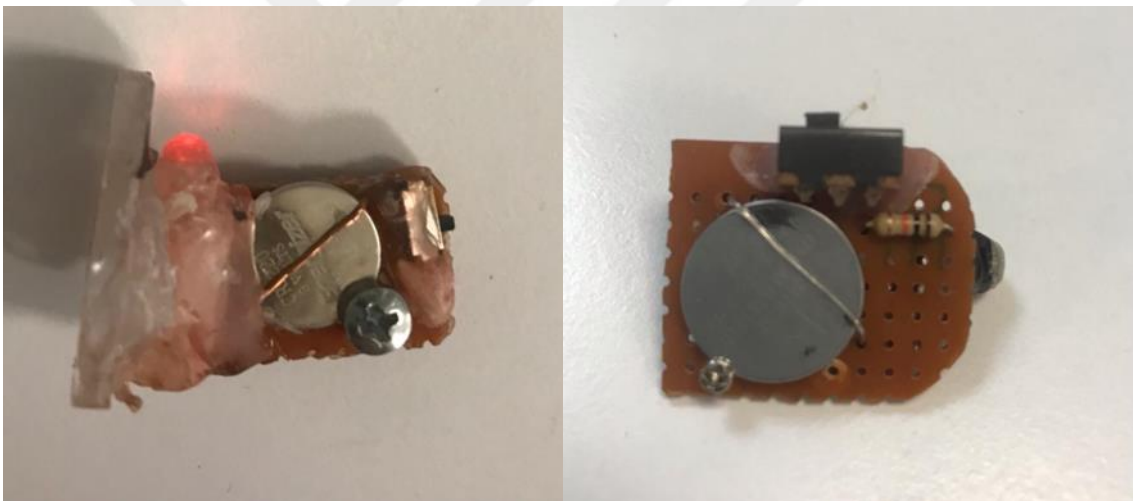
There are four cage LEDs in total, two of which are on the front cage wall and two on the rear cage wall. The front LEDs were placed externally in front of the lever sides for convenience in the two-lever choice phase before LCD was used in the task. Two rear LEDs (right and left) were placed on the cage which are connected to the right and left lever press, respectively. These LEDs indicated that which lever is pressed by the rat inside the cage, while performing the behavioral experiment. The camera is also placed behind the cage, which recorded these LEDs indication and rat behavioral experiment.

### 3.3. LCD Display

Philips 221V8A/01 LCD display was in front of the cage for visual feedback source in the experiment. The screen parameters were adjusted to keep the background as dark as possible, and the cursor and targets were drawn. To block the brightness from the backlight of the LCD screen, it was covered with a black band film, revealing only the cursor and targets.

### 3.4. Top View Camera System

A virtual head fixation was applied to prevent artifacts caused by the rat's limb movements. For this purpose, an image-taking camera was placed at the top of the cage. On the other hand, a small LED circuit was mounted on the head of the rat by performing an operation.



**Figure 3.2:** Circuit created with a 3-volt battery, switch and 10-ohm resistor and was mounted on the head of the rat.

A small circuit was prepared for mounting on the rat head. First, the circuit, which was placed flat without inclination, was tested on the rat over time, and the optimum tilt angle was obtained. Since the rats hit the circuit in their heads against the cage wall during the experiment, 45 degrees inclination was given as a standard value and the rat was placed on the head. This not only contributed to the movement of the rat, but also prevented the sound of hitting the cage wall during the experiment, allowing the rat to focus on the experiment.

### 3.5. Operation of Place the LED

LED fixation on head of the rat was performed after injection of a mixture of ketamine and xylazine anesthesia and anesthesia continuity was supported with isoflurane. Rat was fixed in stereotaxic instrument after anesthesia and the incision was created on the rat's head. Tissue residues were cleaned with the hydrogen peroxide. Then, 4 screws with a diameter of 1.6 mm, two in the front and two in the back, were inserted into the skull of the rat and chamfers also were created on the skull. These processes were carried out to ensure that the led on the rat head is reliably and permanently attached to the skull. The screw with a length of 20 mm was positioned in the rat skull on the center of bregma. The diameter of the threaded part of the screw was 4 mm(M4). Dental acrylic was prepared, and open skull was covered with it and waited until it becomes hard. The rat was observed on the following day to become sure about operation success and when anesthesia effect is over, free water and food were given.



**Figure 3.3:** Image of the screw placed on the rat head after the operation. Thanks to the dowel mounted on the LED circuit, they pass into each other and reach a fixed position.

### **3.6. Background Control Architecture**

The Arduino nano was used to control input and output components in the paradigm. A MATLAB script was written to enable real-time two-way communication between the Arduino and the host computer. The MATLAB support package was employed to establish a connection through a USB port with the Arduino nano. Two different Arduino and MATLAB systems were created separately for both the main paradigm and the upper camera system. The Arduino of the main paradigm was continuing and ending the experiment with the information it received from the camera system. To ensure the reliability and ease of debugging the code, data was logged in a log file for all instances during each execution of the loop for all trials. To ensure that the rats were responding to the cursor's motion and behaving accordingly by pressing and releasing the lever, it was important to continuously record the trial parameters. This included recording the position of the cursor, status of the lever (pressed or not), and the date and time for each iteration of the loop.

### **3.7. Experimental Process**

The rats were rewarded by performing the task of pressing and releasing the lever according to the right or left targets which was appearing randomly on the LCD screen. The aim of the study was to control the cursor movement with visual feedback from the LCD screen and thus to provide motor skill acquisition. The LCD screen and the experimental cage were positioned according to the rat's viewing distance, and when the rat is in an upright position, the cursor and targets on the LCD screen are within the field of view.

First, the rat was allowed to spend a certain amount of time in the cage every day to adapt to the cage environment. As the rat approached the water receptacle, it was taught the location of the water receptacle by giving a reward manually. Same thing was carried out for levers. Except during the experiment, the rats were not given water, so the reward mechanism was provided with water. The location of each component had to be taught, so after the position of the water receptacle and the levers were taught, the nose poke area was also gradually taught. Each time the rat approached the nose poke area, it was guided by giving a reward with manual reward. After learning the components separately, first the nose poking and then the lever press in sequence were trained for rat right and left

side. The cage LEDs were turned on, after the rats had completed their learning period of the cage LEDs. The targets on the LCD screen were turned on at the same time as the cage LEDs. Thus, the interest of the rat was tried to be directed to the LCD LEDs. Two lever choice step was performed when rats were able to understand the visual feedback from the target LEDs and LCD targets. In two lever choice steps, right or left target was chosen randomly when rat performed nose poke and cage LED and target which is on LCD screen become on. After rat pressed the correct lever according to the randomly chosen target, reward was given otherwise in wrong lever chosen trial was ended. When the rat succeeds in the two-lever choice phase, the cage LEDs are turned off and continue the experiment with visual feedback only from the LCD screen. After this process, the training phase begins and the paradigm which is based on understanding the cursor movement and the proximity of the cursor to the target is used.

In the final version of the paradigm, while one of the right or left targets is selected randomly, the cursor also becomes on. We can start the cursor at certain distances to the targets at certain intervals. After the random selection, if the left target was selected, it tries to reach the cursor by pressing continuously the left lever without any limitations. In this part, it doesn't matter whether the rat gets visual feedback or not. The aim is to ensure that the two-lever choice selection is not forgotten by the rat, and the distinction between right and left is not forgotten. Rat needs to understand that when the left target is selected, he will constantly press the lever, and on the other hand, to realize the paradigm with the visual feedback he receives from the screen when the right target is selected.

For the right side, we can start the cursor from the distances determined to the target according to our adjustments in the code. Right target and cursor become on after nose poke. The cursor appears at a predetermined distance from the target. According to the last level reached, the cursor appears at seventy percent from the target. Then, the rat must press the lever when it is inside the area determined according to the image taken from the top camera system. If the rat does not press the lever inside this area, it cannot start the experiment. The important point here is that we understand which region the rat's head is in, and at the same time we make sure that it sees the monitor screen. If the rat pressed the lever in the area determined in the top camera system, it could start the experiment. There are two identified situations at this stage. In the first case, the rat will constantly press the lever and when the cursor reaches the target, it will receive its reward. In the second case, the rat advances the cursor by continuously pressing the lever, but the cursor

moves back after a certain stage. At this stage, the rat makes a release-pressing movement on the lever with the feedback it receives from the monitor. Another important point is that it does not leave the area determined in the top camera system until the award comes. If it does, the experiment is terminated. The visual feedback from the monitor is very important, as the rat is unaware of what situation will come. It is also possible to set when the cursor will make the comeback movement from the code. If the rat is constantly pressing or constantly releasing, the rat can be trained by changing the probability of these two situations in the code. We keep the probabilities of these two situations as close to each other as possible so that the rat is unaware of what condition will come. During the experiment, rat activity was monitored and recorded in real time with the help of the cameras which were placed in the front and back of the cage.

### **3.8. Experimental Troubleshooting**

Unexpected situations were observed in rats during the training phase. Especially bad posture, wrong learning by leaning on the obstacles in the cage, pressing the lever whenever one can reach instead of doing the tasks in order during the training phase, and making head-shaking movements, especially by not standing still, created undesirable situations in the experiment.

To prevent these situations, different solutions were tried to be produced. Barriers were placed between the tape and the levers to prevent the rat from extending its paw to the lever even when receiving a reward. On the other hand, the biggest problem was the strange head movements that the rat made during the experiment. The rat was not focused on the experiment, did not make the paradigm in an orderly, and most importantly, it was not sure whether it saw the target. For this reason, a virtual head fixation was made to keep the rat in a certain area. After rat does a nose poke and starting the experiment, the experiment was terminated by giving a punishment when it went out of the determined trajectory when it was supposed to press the lever on the randomly coming target side.

### **3.9. Methods**

The study aimed to control cursor movement on an LCD screen using visual feedback and to investigate motor skill acquisition in rats. To prevent artifacts caused by the rat's limb movements, a virtual head fixation was applied. An image-taking camera was placed

at the top of the cage, and a small LED circuit was mounted on the rat's head. The circuit was initially placed flat without inclination and tested on the rat over time to determine the optimum tilt angle. As the rats were found to hit the circuit against the cage wall during the experiment, a 45-degree inclination was applied as a standard value to prevent this from happening. This not only contributed to the rat's movement, but also prevented the sound of hitting the cage wall during the experiment, allowing the rat to focus on the task at hand. The rats were rewarded for correctly pressing and releasing levers according to the randomly appearing right or left targets on the LCD screen. The experimental cage and LCD screen were positioned to ensure that the cursor and targets were within the rat's field of view when in an upright position.

Generally, male Wistar rats weighing around 450 grams were used in the experiment. The rats were first acclimated to the cage environment by spending a certain amount of time in the cage each day. They spent approximately 2-3 hours a day in the cage environment. They were tried to adapt to the cage environment for 2-3 days. The location of the water receptacle and levers were taught to the rats by providing manual rewards as they approached them. After the rats learned the location of the water receptacle and levers, they were gradually taught the location of the nose poke area and were rewarded with manual rewards each time they approached it. Once the rats learned the location of each component separately, they were trained to perform nose poking and lever pressing in sequence for both their right and left sides. It took approximately 10 days, with the initial shaping, learning the location of the components in the cage, and learning the two-lever choice step. After the rats completed their learning period of the cage LEDs, the cage LEDs were turned on, and the targets on the LCD screen were also turned on at the same time in order to direct the rat's interest to the LCD LEDs. The rats were then tested in a two-lever choice step, where a random right or left target was chosen, and the rat had to perform a nose poke and press the correct lever corresponding to the target to receive a reward. It took approximately 2-3 days for the rats to learn the stages of receiving visual feedback from the monitor instead of the cage leads. In the final step of the paradigm, the learning of the rats varied. In this part, virtual head fixation is now included in the paradigm and the difficulty level of the paradigm has also increased. Depending on their motivation, the amount of reward from the tap was used differently to motivate the rats. More than 80% success was achieved. Although the mean values for the learning time of the rats were given, they differed for each rat.

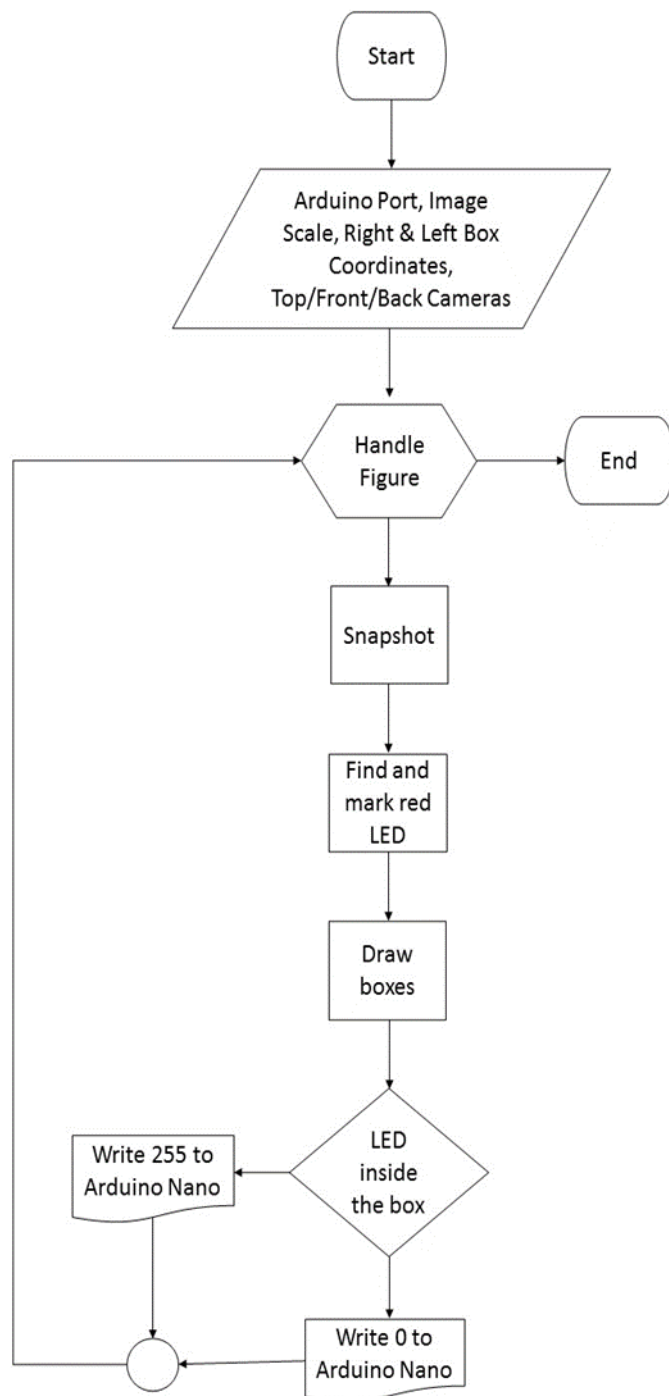
Camera code was written in MATLAB and is used for a virtual head fixation technique in a laboratory setting.

The code is using a webcam to capture images and track a red LED. It then sends a signal to an Arduino board via a serial port. It starts by setting some settings, including the Arduino port, image scale, and box coordinates for the left and right boxes. Then it connects to the Arduino board through the specified port and connects to three webcams - one in the front, one in the top, and one in the back. It also creates a figure window to display the images captured by the three webcams. It captures the image from the top webcam and runs a function called "find\_red\_led()" on it, which is used to locate the red LED. It then marks the LED location on the image using a function called "mark\_led\_on\_image()", and draws the boxes on the image using a function called "draw\_boxes()". It then checks if the LED is inside one of the boxes and sends a signal to the arduino board accordingly. The code also has a loop that runs indefinitely, updating the image and LED location until the figure window is closed. It also has some performance and buffer related variable which are also used to check the performance of the code.

The code starts by setting several variables that are used throughout the rest of the code. The Arduino port variable specifies which serial port the Arduino board is connected to, the image scale variable is used to adjust the size of the images displayed on the screen, and the `box_coordinates_left` and `box_coordinates_right` variables contain the coordinates of the two boxes that are drawn on the image. Next, it connects to the Arduino board by creating an Arduino device object and specifying the port and baud rate. Then it connects to the three webcams by creating `cam_front`, `cam_top` and `cam_back` objects and setting their resolutions. Then it creates a figure window and sets it as a fullscreen window with no menu or toolbar. After that, it creates three subplots in this figure window to display the images captured by the three webcams. It then enters a while loop, which runs indefinitely until the figure window is closed. Inside the loop, it captures an image from the top webcam using the `snapshot()` function, runs the `find_red_led()` function on it to locate the red LED, marks the LED location on the image using the `mark_led_on_image()` function, and draws the boxes on the image using the `draw_boxes()` function. Then it checks whether the LED is inside one of the boxes using the `is_led_inside_one_of_boxes()` function. If the LED is inside one of the boxes, it sends a signal to the arduino board by writing the value 255 to the arduino device using the

write() function. If the LED is not inside one of the boxes, it sends a signal of 0 to the arduino board. It also includes some performance related variables such as prev, cntr, performance\_buffer\_size, performance. These variables are used to keep track of the performance of the code and the buffer size used to store the performance data. Overall, the code is using the webcams to capture images and track the location of a red LED and using that information to send signals to the arduino board. The webcams are also used to display the images on the screen with the boxes and LED location marked on the them.

1. The code starts by setting several variables including the arduino port, image scale, and box coordinates for the left and right boxes.
2. It connects to the arduino board through the specified port and connects to three webcams - one in the front, one in the top, and one in the back.
3. It also creates a figure window to display the images captured by the three webcams.
4. It enters into a while loop, which runs indefinitely until the figure window is closed.
5. Inside the loop, it captures an image from the top webcam using the snapshot() function, runs the find\_red\_led() function on it to locate the red LED.
6. It then marks the LED location on the image using the mark\_led\_on\_image() function, and draws the boxes on the image using the draw\_boxes() function.
7. Then it checks whether the LED is inside one of the boxes using the is\_led\_inside\_one\_of\_boxes() function.
8. If the LED is inside one of the boxes, it sends a signal to the arduino board by writing the value 255 to the arduino device using the write() function.
9. If the LED is not inside one of the boxes, it sends a signal of 0 to the arduino board.
10. After that, it updates the images and the LED location and repeat the process.
11. The loop continues until the figure window is closed.



**Figure 3.4:** The flow chart of the top camera code which is sending output to paradigm code.

As the resolution of an image increases, the number of pixels in the image also increases, which means more data needs to be processed. This can lead to a decrease in the frame rate because the camera or processing system may not be able to keep up with the increased amount of data. This is because in order to produce high-resolution images, the camera needs to capture more data, which takes more time, and therefore it can capture fewer frames per second. It's worth mentioning that this relationship is not a linear one, and it may also depend on other factors such as the camera's sensor, lens, lighting conditions, and the processing power of the system. Additionally, some cameras are designed to produce high-resolution images at a high frame rate, but they tend to be more expensive. In general, when designing a system that captures images or videos, the trade-off between resolution and frame rate is considered and a balance is sought between the two. The relationship between resolution and frame rate is that as the resolution of an image increases, the frame rate tends to decrease, and as the resolution decreases, the frame rate tends to increase. Resolution refers to the number of pixels in an image and is typically measured in width and height, such as 1920x1080. The higher the resolution, the more pixels there are in an image, resulting in more detail and a higher-quality image. However, this also means that more data needs to be processed, which can increase the computational resources required and decrease the frame rate. Frame rate, on the other hand, refers to the number of images captured per second and is typically measured in frames per second (FPS). A higher frame rate means that more images are captured in each period of time, resulting in a smoother video and better motion capture. However, capturing more images per second also requires more computational resources and can decrease the resolution of the images. Therefore, when designing a system that captures images or videos, there is usually a trade-off between resolution and frame rate. If high resolution is desired, a lower frame rate may be accepted, and if high frame rate is desired, a lower resolution may be accepted. In general, when it comes to video applications such as surveillance, video conferencing, or streaming, high frame rate is more important as it gives smooth video and better motion capture. However, in some applications such as photography or scientific imaging, high resolution is more important as it gives high-quality and detailed images.

The resolution refers to the number of pixels in the captured image, and it is set to "160x120" for the top webcam, "640x480" for the back webcam, and "320x240" for the front webcam. A higher resolution means that the image will have more pixels and

therefore more detail, but it also means that more data needs to be processed, which can increase the computational resources required. This can lead to a decrease in the frame rate, which is the number of images captured per second. A lower resolution means that the image will have fewer pixels and therefore less detail, but it also means that less data needs to be processed, which can decrease the computational resources required. This can lead to an increase in the frame rate. In this code, the front webcam has the lowest resolution, and the back webcam has the highest resolution. This suggests that the back webcam may be capturing images at a lower frame rate than the front webcam. The top webcam which is A4 TECH PK-635G model and supporting 30 FPS maximum has the intermediate resolution, so it may be capturing images at a frame rate that is between the front and back webcams. It is also possible that the frame rate is determined by the webcam's hardware and the code is not changing it.

## **CHAPTER 4**

### **4. RESULTS AND DISCUSSION**

#### **4.1. Results**

A total of 6 rats were used in the experiment. All the rats learned to stay within the limits set in the camera system. Rats 210 and 207 did not perform experiments with a specific repetition motion before the top camera system was used. After they made a nose poke movement and started the experiment, they went to press the lever, then left it unfinished and spent time in the cage, and then they came again. They were not looking at the monitor and were not getting visual feedback also. Experimental success also increased as the rats were forced to stay within a certain area after the camera system was used. Since the experiment was terminated when the rats did not stay in the designated area, they did not leave that area during the experiment.

When the left side was selected, the rat received a reward only when it moved the cursor to the target by continuously pressing the lever. When the right side was selected, he performed the paradigm by taking visual feedback from the monitor. No requirements were set for the left side. Even if the boundaries were drawn with the top camera system, it was not active, and the experiment was not stopped even if the rat left the determined area. The reason the left side had such a function was for the rat to continue without losing its right and left balance. Even though there was no punishment for the left region, it was seen during the experiment that the rat still did not come out of that region. He turned the movement he made during the paradigm into a stereotypical movement and established the right-left balance.

Since the rats could not see the red light, the LED was chosen especially in red color. In this way, even the slightest effect that could affect the experiment was tried to be eliminated. In addition, the light is prevented from scattering by covering the led with

tape. In this way, the LED detection quality of the top camera system has also been increased.



**Figure 4.1:** The rat's position and three camera angles when rat comes to the middle of the cage for a nose poke or for a reward.

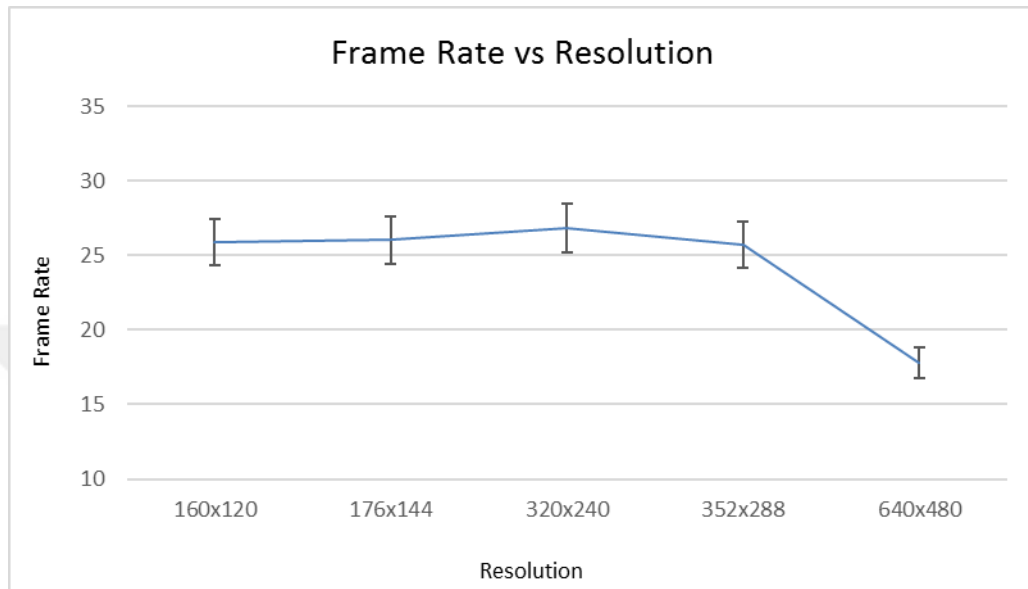
**Figure 4.1** and **Figure 4.2** show images of the rat taken from three camera angles during the experiment. When the rat makes a nose poke, it looks like in **Figure 4.1**. Then, when the cursor and target become on, rat should press the lever in the determined region as in **Figure 4.2** and stay in the determined region until the end of the experiment. When rat successfully completes the experiment, should get his reward from the tap again as shown in **Figure 4.1**.



**Figure 4.2:** The rat's position and three camera angles when rat comes to press the lever to perform a paradigm.

First, the experiment was conducted with rats without activating the top camera system and the experiment was video recorded. Then, the top camera system was activated, and

the experiment was video recorded in the same way after the rat learned. The percentages of success in the two experiments were compared. When the top camera system was not active, the rat could not show stereotypical movement and achieved a maximum success rate of 50 percent. Rat success was measured as at least 85 percent when learn with the top camera system was completed.



Resolution	160x120	176x144	320x240	352x288	640x480
Frame Rate (fps)	25.87 ± 9.50	26.03 ± 9.03	26.84 ± 9.02	25.71 ± 8.65	17.79 ± 3.65

**Figure 4.3:** The resolution and frame rate relationship.

In the code provided, there does not appear to be any explicit delay functions that would pause the execution of the code for a set amount of time. However, there are a few factors that could contribute to delays in the code. First, the `snapshot()` function captures an image from the webcam, which takes some amount of time depending on the webcam's hardware and the resolution of the image. This delay would occur every time the code enters the while loop and captures a new image. Second, the `find_red_led()` function, `mark_led_on_image()` function and `draw_boxes()` function might take some amount of time to process the image, which would add to the overall delay. Third, the `write()` function is used to send data to the arduino board through the serial port. The time it takes for the data to be sent could add a small delay. Finally, the `is_led_inside_one_of_boxes()` function is used to check if the LED is inside one of the boxes, which could also take some time to process. All of these factors, when combined, could result in a delay between

the time the image is captured and the time it is displayed on the screen. It is also worth noting that the frame rate of the webcam might impact the delay of the code. If the frame rate is low, the delay between the images captured will be high, and if the frame rate is high, the delay between the images captured will be low.

On the other side, the higher the resolution is kept without sacrificing frame rate, the more useful the system becomes for wide applications. Therefore, the frame rate of the processed video stream, an important parameter in acquiring low-latency images from the environment, detecting the position of the subject, and delivering timely reinforcement cues was used as  $25.87 \pm 9.50$  Hz for 160x120 image resolution. When the resolution value increases, the system processes more data due to the increase in the number of pixels. This can also cause freezes in the continuous image taken, causing the animal's movements to be missed by the camera in the experiment, resulting in a delay in punishment and as a result the system is not working efficiently.

## **4.2. Discussions**

The most important points in the study were the error of the rats in the task of reaching out from the center. While the cursor was taken straight in some trials during the experiment, it was returned with an error in some trials and feedback was expected from the rat. It was studied whether the rats could detect visual proximity and, more importantly, whether they could combine the error in orbit with their motor abilities using visual feedback. Another important point was the virtual head fixation applied to the rat. Strange head movements were observed in some rats during the experiment. This both affected the experiment, and we could not be sure if the rat was getting visual feedback. The rat was moving freely during the experiment before virtual head fixation. He could not perform the experiment in a stereotypical way since he could do the experiment at any distance from which his arm reached the lever. Thanks to the camera system placed on the cage, the rat was kept fixed within a determined area. He was only able to press the lever while in this area. Thus, he was able to perform his experiment in a stereotypical way and to look at the monitor from a certain angle.

For the head fixation of the rat, firstly, real head fixation was tried instead of virtual head fixation. In real head fixation, a 3D-printed apparatus was placed on the rat head. A mechanism was prepared in the middle of the cage where the apparatus on the rat head would be placed and fixed. Without experimentation, only head fixation was tried to be

used in the rat. Standing still stressed the rat. After months of trials, no success was achieved. The rat was very stressed, fluttering and sometimes even shouting. A movable tape was also prepared to reduce stress. Water was given freely from the tape to rat to get used to the situation. Even this situation did not reduce the degree of stress. He was startled by the water coming from the tape and making fluttering movements. For this reason, it was decided that the virtual head fixation would be a better option. Even though the rat was kept in a certain area with the upper camera system, it did not create any stress factor as it could move freely. At the same time, we knew from which angle he was looking at the monitor because he was standing in a certain area.

The upper camera system was able to detect the position of the rat with the feedback it received from the LED placed on the rat's head. In the first studies, the LED placed on the rat head was not inclined and was placed straight. As time passed, it was observed that the rats hit the led on the cage wall during the experiment. Therefore, instead of placing it straight, it was placed at a 45-degree angle towards the body of the rats.

In summary, this thesis has introduced a new head movement restriction system which is a promising approach for studying visuomotor skills in rodents. It has the potential to be adapted to study other types of learning and brain function, with important implications for both basic science and the development of new therapies for neurological and behavioral disorders. We believe the present system can be used not only in visuomotor tasks but also any behavioral experiment where the movements of the rodents are needed to be tracked and the subjects are reinforced based on a determined location in the experimental environment.

## **CHAPTER 5**

### **5. CONCLUSIONS AND FUTURE WORK**

The results of this thesis demonstrate that the use of head movement restraining via video processing, in other words virtual head fixation, in rodent visuomotor skill learning can lead to faster and more accurate learning, as well as better generalization and retention of learned skills, when compared to traditional methods of rodent visuomotor skill learning. Additionally, this thesis provides evidence that the neural mechanisms underlying the animals' performance are like those observed in traditional methods. The use of virtual head fixation has several advantages over traditional methods, such as allowing the animals to move freely in their cage and making the learning more naturalistic. These advantages suggest that the use of virtual head fixation could be useful in a wide range of visuomotor learning studies and could also be adapted to study other types of learning and brain function.

As for future work, one next step could be to investigate the effects of virtual head fixation in other rodent species and other types of visuomotor tasks. Additionally, it could be interesting to study the effects of different types of feedback, such as haptic or auditory feedback, on the learning process. Furthermore, this approach could be used to investigate the neural mechanisms of visuomotor learning in more detail, for example by using optogenetics or chemo genetics to manipulate specific neural populations. Another possibility is to investigate how this paradigm could be used to study the neural basis of visuomotor deficits in rodent models of neurological and behavioral disorders, such as Parkinson's disease or schizophrenia. This could lead to a better understanding of the neural mechanisms underlying these disorders and could inform the development of new therapies.

## BIBLIOGRAPHY

- [1] J.A. Kleim, E. Lussnig, E.R. Schwarz, T.A. Comery, W.T. Greenough, Synaptogenesis and FOS expression in the motor cortex of the adult rat after motor skill learning, *J. Neurosci.* 16 (1996) 4529–4535. <https://doi.org/10.1523/jneurosci.16-14-04529.1996>.
- [2] J.A. Kleim, S. Barbay, R.J. Nudo, Functional reorganization of the rat motor cortex following motor skill learning, *J. Neurophysiol.* 80 (1998) 3321–3325. <https://doi.org/10.1152/jn.1998.80.6.3321>.
- [3] F. Fearing, I.P. Pavlov, G. V. Anrep, Conditioned Reflexes. An Investigation of the Physiological Activity of the Cerebral Cortex, *J. Am. Inst. Crim. Law Criminol.* 20 (1929) 153. <https://doi.org/10.2307/1134737>.
- [4] R.A. Wise, Brain reward circuitry: Insights from unsensed incentives, *Neuron.* 36 (2002) 229–240. [https://doi.org/10.1016/S0896-6273\(02\)00965-0](https://doi.org/10.1016/S0896-6273(02)00965-0).
- [5] E. L. Thorndike, Animal intelligence: Experimental studies, New York, *The Macmillan Company*, 1911.
- [6] L. Powell, Russell; Symbaluk, Diane; Honey, Introduction to Learning and Behavior, 2009.
- [7] M. Landy, "Perception Lecture Notes: Spatial Frequency Channels," 22 Mar 2004.[Online].Available:<https://www.cns.nyu.edu/~david/courses/perception/lecturenotes/channels/channels.html>. [Accessed 25 Jan 2022].
- [8] Á. Szél, P. Röhlich, A.R. Caffé, T. Van Veen, Distribution of cone photoreceptors in the mammalian retina, *Microsc. Res. Tech.* 35 (1996) 445–462.[https://doi.org/10.1002/\(SICI\)1097-0029\(19961215\)35:6<445::AID-JEMT4>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-0029(19961215)35:6<445::AID-JEMT4>3.0.CO;2-H).
- [9] D. Zoccolan, Invariant visual object recognition and shape processing in rats, *Behav. Brain Res.* 285 (2015) 10–33. <https://doi.org/10.1016/j.bbr.2014.12.053>.
- [10] D. Zoccolan, N. Oertelt, J.J. DiCarlo, D.D. Cox, A rodent model for the study of invariant visual object recognition, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 8748–8753. <https://doi.org/10.1073/pnas.0811583106>.
- [11] M.B. Broschard, J. Kim, B.C. Love, J.H. Freeman, Category learning in rodents using touchscreen-based tasks, *Genes, Brain Behav.* 20 (2021) 1–21. <https://doi.org/10.1111/gbb.12665>.
- [12] G.T. Prusky, K.T. Harker, R.M. Douglas, I.Q. Whishaw, Variation in visual acuity within pigmented, and between pigmented and albino rat strains, *Behav. Brain Res.* 136 (2002) 339–348. [https://doi.org/10.1016/S0166-4328\(02\)00126-2](https://doi.org/10.1016/S0166-4328(02)00126-2).
- [13] S. V. Girman, Y. Sauvé, R.D. Lund, Receptive field properties of single neurons in rat primary visual cortex, *J. Neurophysiol.* 82 (1999) 301–311. <https://doi.org/10.1152/jn.1999.82.1.301>.
- [14] G. Matteucci, R.B. Marotti, M. Riggi, F.B. Rosselli, D. Zoccolan, Nonlinear processing of shape information in rat lateral extrastriate cortex, *J. Neurosci.* 39 (2019) 1649–1670. <https://doi.org/10.1523/JNEUROSCI.1938-18.2018>.

- [15] S.K. Petrino, R.E. Clark, P. Reinagel, Evidence That Primary Visual Cortex Is Required for Image, Orientation, and Motion Discrimination by Rats, *PLoS One*. 8 (2013) 1–10. <https://doi.org/10.1371/journal.pone.0056543>.
- [16] R.M. Douglas, A. Neve, J.P. Quittenbaum, N.M. Alam, G.T. Prusky, Perception of visual motion coherence by rats and mice, *Vision Res.* 46 (2006) 2842–2847. <https://doi.org/10.1016/j.visres.2006.02.025>.
- [17] P.P. Belsey, M.A. Nicholas, E.A. Yttri, Open-source joystick manipulandum for decision-making, reaching, and motor control studies in mice, *eNeuro*. 7 (2020) 1–9. <https://doi.org/10.1523/ENEURO.0523-19.2020>.
- [18] R. Kimura, A. Saiki, Y. Fujiwara-Tsukamoto, F. Ohkubo, K. Kitamura, M. Matsuzaki, Y. Sakai, Y. Isomura, Reinforcing operandum: Rapid and reliable learning of skilled forelimb movements by head-fixed rodents, *J. Neurophysiol.* 108 (2012) 1781–1792.
- [19] A.I. Selverston, Are central pattern generators understandable?, *Behav. Brain Sci.* 3 (1980) 535–540. <https://doi.org/10.1017/S0140525X00006580>.
- [20] S.L. Resendez, J.H. Jennings, R.L. Ung, V.M.K. Namboodiri, Z.C. Zhou, J.M. Otis, H. Nomura, J.A. Mchenry, O. Kosyk, G.D. Stuber, Visualization of cortical, subcortical and deep brain neural circuit dynamics during naturalistic mammalian behavior with head-mounted microscopes and chronically implanted lenses, *Nat. Protoc.* 11 (2016) 566–597. <https://doi.org/10.1038/nprot.2016.021>.
- [21] Solari, N., Sviatkó, K., Laszlovszky, T., Hegedüs, P., & Hangya, B. Open Source Tools for Temporally Controlled Rodent Behavior Suitable for Electrophysiology and Optogenetic Manipulations, *Frontiers in Systems Neuroscience*, (2018) 12. doi:10.3389/fnsys.2018.00018.

## **CURRICULUM VITAE**

Name Surname : Bihter Özhan

Place and Date of Birth :

E-Mail :

### **EDUCATION:**

B.Sc. : 2020, Istanbul Medipol University, Biomedical Engineering.



# A VIDEO TRACKING SYSTEM FOR DETECTING AND RESTRAINING MOVEMENTS OF FREELY MOVING RODENTS

## ORIGINALITY REPORT

4%

SIMILARITY INDEX

3%

INTERNET SOURCES

2%

PUBLICATIONS

2%

STUDENT PAPERS

## PRIMARY SOURCES

1	<a href="http://acikerisim.medipol.edu.tr">acikerisim.medipol.edu.tr</a> Internet Source	2%
2	<a href="http://www.biorxiv.org">www.biorxiv.org</a> Internet Source	<1%
3	<a href="http://www.cogpsy.uni-osnabrueck.de">www.cogpsy.uni-osnabrueck.de</a> Internet Source	<1%
4	<a href="http://epdf.tips">epdf.tips</a> Internet Source	<1%
5	Submitted to University of Cumbria Student Paper	<1%
6	Shilpa J. Buch. "Neuroimmune Pharmacology as an Emerging Curriculum for Pre-Medical Students", Journal of Neuroimmune Pharmacology, 2010 Publication	<1%
7	<a href="http://www.nature.com">www.nature.com</a> Internet Source	<1%
8	<a href="http://d-nb.info">d-nb.info</a> Internet Source	<1%