A NEW RODENT BEHAVIORAL PARADIGM FOR STUDYING CLOSED-LOOP CURSOR CONTROL

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By

Ahsan Ayyaz

February, 2022

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9 February, 2022

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

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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.

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ÖZET

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Biyomedikal Mühendisliği ve Biyoenformatik, Yüksek Lisans

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Şubat, 2022

Bir kumanda kolu kullanarak PC ekranındaki bir imlecin kontrolü, insan ve insan olmayan primatlarda motor beceri öğreniminde yer alan nöral mekanizmaları incelemek için standart bir paradigmadır. Bu paradigma, beyin-makine arayüzü kontrol araştırmalarının başlamasından once, imleç hareketlerinin ödülle ilişkilendirilmesi için insan olmayan primatlarda yaygın olarak kullanılmıştır. Ancak, bu görev, daha basit bir biçimde bile, henüz kemirgenler için mevcut değildir. Bu çalışmada, sıçanların sabit hedeflere ulaşmak için levyeleri kullanarak PC monitöründeki bir imleci yönlendirmesini sağlayan yeni bir davranış paradigması sunmaktayız. İmlecin kontrolü, sıçanların sınırlı bilişsel ve görsel yeteneklerine bağlı olarak tek boyutlu bir alanda gerçekleştirilmektedir. Davranışsal deney düzeneği esas olarak şeffaf, pleksiglas duvarlı bir kafes ve bir kafesin dışında yer alan bir PC monitörü ve bu monitordeki imleç ve iki zıt hedeften oluşmaktadır. Sıçanların dikkati, burada sunulan eğitim prosedürleri aracılığıyla kafesin içinden imleç ve hedeflere kaydırılmaktadır. İmleç kontrol paradigmasında, imleci rastgele seçilen hedefe doğru hareket ettirmek için iki koldan birine bastırmak gereklidir ve imleç, seçilen hedefe doğru hareket etmediği durumlarda hareketlerini düzeltmek için levyeyi bırakma ve bastırma hareketi gereklidir. Deneylerimizde dört sıçandan üçü, imleç hareketini ve imlecin hedefe yakınlığını algılayabilmiş ve günde 2,5 ila 3 saat süren 52±12 günlük eğitimden sonra başarı kriterlerine (ardışık 40 denemede %75 doğruluk oranı) ulaşabilmiştir. Fareler, sağlanan görsel geri bildirime dayalı olarak bir imlecin yörüngesindeki hatayı tespit edebildi ve sonuçlar farelerin yörünge hatasını düzeltmek için görsel motor yeteneğine sahip olduğunu gösterdi. Bu paradigma için bir kavram kanıtı gösterdik, gelecekteki çalışmalar bu paradigma için farelerin öğrenme eğrisine odaklanacaktır. Bu çalışmada sunulan paradigma, görsel geribildirime dayalı imleç kontrolünde yer alan sinir devrelerindeki bilgi işleme ilkelerini araştırmak için kullanılabilir. Bu paradigmayı kullanarak gerçekleştirilen çalışmalar yoluyla elde edilen sinirbilimsel bilgi, beyindeki farklı motor kontrolle ilgili yapılar arasında dağılmış haldeki sinirsel bilgileri izleyerek çalışan yeni beyin-makine arayüzü şifre çözücülerinin geliştirilmesinde kullanılabilecektir. Çalışmada ayrıca yeni nesil yüksek yumuşaklığa sahip dopamin ölçümü ve elektrofizyolojik kayıtlarda kullanılabilecek mikroelektrot dizilerinin üretim yöntemleri tanıtılmaktadır.

Anahtar sözcükler: Davranış, Motor yetenek öğrenme, Görsel geribildirim, Gidiş yolu kontrolü, Yüksek yumuşaklıkta mikroelektrot.

A NEW RODENT BEHAVIORAL PARADIGM FOR STUDYING CLOSED-LOOP CURSOR CONTROL

ABSTRACT

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MSc in Biomedical Engineering and Bioinformatics

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February, 2022

Control of cursor on a PC monitor using a joystick is a standard paradigm to study the neural mechanisms involved in motor skill learning in human and non-human primates. This paradigm is also commonly used in non-human primates for the association of cursor movements with reward before initiation of brain-machine interface control tasks. However, this task, even in a simpler form, has not been available for rodents yet. In this work, we present a novel behavioral paradigm that enables rats to direct a cursor on a PC monitor by operating levers for reaching stationary targets. The control of the cursor is performed in a one-dimensional space depending on the limited cognitive and visual capabilities of rats. The behavioral setup mainly consists of a cage with transparent, plexiglass walls and a PC monitor outside the cage that is used to demonstrate a cursor and two opposite targets to be achieved using the cursor. The attention of the rats was shifted from inside the cage to the cursor and targets through shaping procedures presented here. In the cursor control paradigm, pressing one of two levers is used to move the cursor towards the randomly selected target and a lever release and press sequence was required for correcting the movements of the cursor when it does not move towards the selected target even though the correct lever is pressed. Three out of four rats were able to perceive cursor motion and its proximity from the trial-specific target and achieved inclusion criteria (i.e., 75% accuracy in 40 consecutive trials) for the final step after 52± 12 days of training, with a training session duration of 2.5 to 3 hours per day. The rats were able to detect the error in the trajectory of a cursor based on the visual feedback provided and the results showed that the rats have the visuomotor ability to correct the error in the trajectory. We have demonstrated a proof of concept for this paradigm, future studies will focus on the learning curve of mice for this paradigm. The paradigm presented here can be used to investigate the information processing principles in the neural circuits involved in cursor control based on visual feedback. The neuroscientific knowledge acquired through such studies can be used in the development of novel brain-machine interface decoders which operate by monitoring the neural information distributed across different motor-related structures in the brain. The present study also introduces the methods we developed for the fabrication of a new generation, ultra-flexible microelectrode arrays that can be used for dopamine measurement and electrophysiological recordings.

Keywords: Behavior, Motor skill learning, Visual feedback, Trajectory control, Ultraflexible microelectrode.

CHAPTER 1

1. INTRODUCTION

We interact with our environment through actions, some actions are innate i.e., they are genetically hardwired and do not require pretraining to be executed in response to a stimulus, while others are learned behaviors, such as reaching, grasping [1]. Innate behaviors have defined and hardwired neuronal mechanisms that underly their execution, whereas learned behaviors require neural connections with the ability to generate new and adaptive motor sequences [1]. Identifying the undelaying mechanisms and functions of neural circuits involved in these skilled behaviors is vital as it can guide us in understanding the causes underpinning the neurophysiological diseases related to motor functions. This information can also help in creating better prosthetic solutions for people with motor impairments. Sophisticated technics and models have been employed for this purpose in human and non-human primates, but to understand the neural substrate of the skilled behaviors thoroughly on a larger scale, rodent behavioral paradigms with compatibility for invasive research are desired [2].

Recording and analysis of neural activity while non-human primates perform operant conditioned motor behaviors by interacting with manipulandum such as joystick has contributed immensely over the years in understanding how neural circuits in different brain regions represent these complex skilled movement patterns and cognitive-motor functions [3], [4]. Monkeys provide an unequivocal advantage for this research due to the resemblance of their cortical structures to the human brain that entails advanced cognitive and dexterous motor abilities. Despite extensive research in this area, comprehensive mapping and characterization of the neural circuitry involved in motor skill learning, adaptation, and selection is lacking [1]. This can be partially attributed to the cost and ethical issues involved in housing and handling monkeys, and these constraints make research on non-human primates unfeasible for many labs. The other

constraint has been the lack of robust and compatible paradigms for other species that can mimic the functionality of primates-based motor skill learning.

Rodents on the other hand can provide a versatile and wide repertoire of motor skill learning behavioral paradigms, which was not fully exploited until recently [5]. Despite their limited, cognitive and visuomotor abilities as compared to monkeys, rats provide avenues for high throughput research and ease in collecting scientific knowledge in this field at a rapid pace. The basic neural architecture for motor control is common in rodents and primates, this makes rats capable of acquiring precision demanding and complex motor skills [6]. Moreover, manipulation of neural structures by utilizing genetic tools such as optogenetics has provided an immense advantage in exploring mechanisms that govern motor skill learning using rodents [7]. Rodents use their forelimbs for skilled motor behaviors naturally and paradigms can be designed to utilize this ability to perform operant conditioned behavioral tasks that would be compatible with in-vivo techniques such as electrophysiology [8].

Reaching behavior has been widely studied with a variety of species. A type of reaching task i.e., reaching specific targets from a center position has proved to be quite revealing in terms of scientific findings in brain activity during skilled behaviors [9]-[11]. This paradigm provides reproducible information and allows quantitative analysis of kinematics and spatiotemporal information during the tasks [5]. The use of joystick with head-fixed rats has become a popular method for this type of analysis, while has been around for decades for reaching behavior studies involving human and non-human primates [12], [13]. This method provides the advantage of the correlation between x and y-axis motion dynamics and the underlying neural activity in head-fixed subjects [5]. Some studies have utilized visual feedback through display technologies i.e., studying the control of the trajectory of the cursor to various targets from a center position through a joystick, while others have used control of the position of a sipper tube as visual feedback for control through a joystick [14], [15]. Lever push and pull mechanism with a locally placed sipper on the lever have also been employed [16]. Whereas the use of encoders to monitor the rat's reaching and motor control skills has also been done for this purpose [17]. One common attribute of these methods is that the rats or mice used were head fixed. Although this provides the benefit of exploiting some advance and state-of-the-art neural activity monitoring techniques, it does have some pitfalls. Stress analysis has been performed for head-fixed, fully restrained, and freely

moving rats, and data showed that the level of stress hormone was much higher in head-fixed and fully restrained rats than in freely moving rats [18]. This high level of stress increases the habituation time and can affect the rat's performance during the experiments [18]. Another advantage of designing a paradigm for freely moving rats is the increased flexibility and possibilities of variations in the behavioral setup to suit the goal of the research, such as including nose poke to initiation the trial. Sensing the licking of sipper and whisking movement have been used for motor skill studies but these are not skilled motor movements as these stereotypical movements are governed by the central pattern generators in the brain stem [19], [20]. Another missing aspect in these studies is the analysis of error in trajectory. In these studies, rats either control the position of the cursor to make it reach a specified target based on the visual feedback from an LCD or control the sipper position to make it move to be able to reach it and get a reward. But neither of these studies analyze the inclusion of an error in the trajectory and the activity in brain regions that encodes this error or how the brain adjusts its activity to compensate for the error in the trajectory and corrects it. This aspect of skilled motor behavior has been overlooked so far as there is no rodent paradigm available to undergo this study.

In this study, we tried to address these issues by proposing a closed-loop behavioral paradigm for an operant conditioned one-dimensional one-directional center out reaching control of cursor trajectory based on the visual feedback from an LCD using a lever. The paradigm enables the analysis of the following aspects of the motor skill learning task: (1) decision making in selection between bi-directional randomly selected targets; (2) reaction time, in response to variable cursor speeds, for reaching and pressing the correct lever according to the trial specified target direction; (3) trajectory control of the cursor, i.e., perception of the proximity of cursor to the target and compensation/correction in case of a randomly introduced error by exhibiting a sequence of lever press and release. The one-dimensional motion of the cursor was chosen by considering the limited visuomotor abilities of rats, as controlling and more importantly correcting the errors in the trajectory in 2D space would have been an upheaval task. Nevertheless, one-dimensional control of the trajectory of cursor and error correction holds the potential for significant contribution in scientific knowledge in this field. Rats were free to move in the experimental cage, i.e., no head fixation was done, yet the access to the levers was restricted to the forelimb corresponding to the

target direction, and the shaping procedure insured minimum body movements while operating the levers in response to visual feedback during trials.

The scientific outcomes of elucidating the functionality of different brain structures and neural mechanisms underpinning skilled motor behaviors and cognitive-motor functions can have a huge impact on the development of better brain-machine interfaces (BMIs) and neural prosthetic devices to restore lost motor functions due to neurodegenerative diseases and accidental damage of spinal cord. The development of neural decoders that utilize modulation of neural spike activity in correlation with motor movements has allowed humans and non-human primates to control trajectories of robotic arms and cursors on screens. Recent advances in neural interface technologies to detect neural spike activity have enabled glial scare-free integration of the brain and implantable recording electrodes [21], [22]. In this study, we designed multichannel microelectrodes, that can provide glial scare-free integration with the brain tissue due to less mechanical stiffness and can record up to 8 recording channels simultaneously. Photolithographic procedures were used to prepare these electrodes. Characterization and coating of different chemically active nanoparticles can enhance the sensitivity and durability of these electrodes, but those procedures are out of the scope of this thesis.

1.1. Scope of Thesis

The center-out reaching task is a common method to study the neural correlates of reaching movements in human and non-human primates and requires real-time and precise quantification of these behaviors. Although efforts have been made to replicate this task in rodents by proposing different alternatives. Yet none of the studies addresses or incorporates the introduction of an error in the trajectories during the reaching task and does not propose a methodology to unravel the neural mechanisms and brain structures that encode this error. The availability of a robust and compatible paradigm for in-vivo studies using rodents is imperative for this endeavor. In this study, a new rodent behavioral paradigm is proposed to study a closed-loop control of the trajectory of a cursor based on the visual feedback in a one-dimensional space by operating a lever. Error in the trajectory is introduced randomly and the paradigm allows the rats to utilize the visual feedback to correct the error by a temporally significant sequence of lever release and press. A detailed explanation of hardware and software design, methodology, and steps to train the rats by operant conditioning are provided. We

trained three rats for the paradigm, the results and outcomes of the training are presented. The essential principles of operant conditioning and the visual capabilities of rodents are also discussed.

Secondly, the design and fabrication process and implementation of a new neural implant, gold microelectrode arrays, for a seamless, high throughput, and glial-scare free brain muscle interface are presented.

1.2. Outline of Thesis

Chapter 01 introduces the scientific questions we addressed in this study, the literature review discussing the latest techniques and behavioral paradigms currently employed and our proposed contribution in this regard.

Chapter 02 provides a brief but essential overview of the principles of operant conditioning procedures and a literature review underlining the rat's visual capabilities.

Chapter 03 provides a detailed description of the methods, tools, and materials used for designing the behavioral paradigm proposed in this study.

Chapter 04 discusses the results and findings of this study.

Chapter 05 discusses the procedure and materials for the design and development of gold microelectrodes arrays.

Chapter 06 describes the conclusions and potential avenues of scientific research that can be done by utilizing the findings of this study.

CHAPTER 2

2. THEORETICAL PART

2.1. Reward Function: General Ideas and Historical Background

A common perception of a reward can be explained by an object or a situation one receives or faces for performing a task well. As the appreciation or a favorable outcome of that performance strengthens the behavioral act that caused it, this viewpoint of a reward can be conveniently explained by the concept of instrumental conditioning according to which a reward positively reinforces a behavior. In other words, a feeling of happiness or satiation makes one come for more and perform the same action again. Another perception of reward is related to the subjective inclination, proclivity, or predisposition of an individual for something that produced pleasant outcomes in the past, one likes doing something because it made them happy before. This is termed the hedonic function of the reward. Both these notions fell short of furnishing a comprehensive generalization of the reward function as discussed in the following description.

Ivan Pavlov's theory of classical conditioning provided one of the earliest scientifically driven definitions of the reward function. According to his proposed definition, a reward is an object that brings about a behavior change, also termed learning [23]. When a bell ring sound is paired with a sausage, a dog salivates only when that paired bell sound is presented and not to any other nonpaired sound, this indicates a change in behavioral response (salivation) after food conditioning. This definition does not fully conform with the above-mentioned two notions about reward. The dog does not have to perform any task to get the reward, nor its feelings are pertinent. Despite these discrepancies, this definition of reward function is ubiquitous and a key for neurobiological research.

Thorndike postulated “Law of effect” around the same period, which states that the repeatability of behavior increased if it is rewarded. In other words, if the outcome of a behavior is pleasant it will be repeated and if the outcome is unpleasant the frequency of that behavior will decrease [24]. This definition is closer to operant conditioning as a reward is not received automatically but successful execution of a task is required to gain a reward, unlike Pavlovian conditioning where the reward is acquired automatically. This has a resemblance with Pavlovian conditioning in a way that behaviors with rewarded outcomes are promoted, which is positive reinforcement. Skinner extended this definition of operant conditioning by describing that stimulus-response (S-R) association is reinforced by reward, and there is no subjective causality or conscientiousness involved.

Reward objects used for animal learning are mostly food pellets or liquids such as water or sucrose solution. The motivational value of the reward object is determined by their controlled availability and a calibrated amount is delivered during experimentation. The issue with these foodstuffs and liquids is that it is hard to determine what defines a rewarding effect, is it the taste, smell, sight, drinking, chewing, and swallowing, or the nutritional value associated with the reward object. Which of these constitutes the primary rewarding effect and does for different objects this rewarding effect is associated with different events [25]? In some cases, the taste of the object is rewarding for the animal, even though there is no nutritional value to it, e.g., saccharin, increases the behavioral activity although it has no nutrient to offer. Ultimately, the vegetative parameters of a rewarding object are important, as it is required to maintain a healthy level of electrolytes, proteins, and amino acids in the body, that might be the reason that animals avoid consuming such food that is deficient or lacks nutrients such as amino acids [26]. Nevertheless, reward-based operant learning has provided a plethora of scientific knowledge and is a key to neurophysiological research. Basic principles and terminologies of operant conditioning are discussed in the next section.

2.2. Principles of Operant Conditioning

Operant conditioning sometimes also called instrumental conditioning is a type of learning in which the frequency and intensity of behavior are affected and altered by the nature of its outcome. If an action brings pleasant consequences the probability of it getting repeated increases, and if the consequences are unpleasant the chances of

repeating that action will decrease. This is essentially the gist of Thorndike's law of effect. The change in the behavior based on the outcome results in learning.

There are three basic components involved in operant conditioning: (1) a response that generates a certain outcome (e.g., pressing a lever to get a reward), (2) an outcome that either increases or decreases the probability of the preceding response (e.g., a reward or a punishment), (3) a discriminative stimulus indicating the availability of a certain consequence (e.g., illuminating an LED or a buzzer sound to signal that the lever is now available to be pressed to produce food) [27]. These three components are discussed further in the following section.

2.2.1. Operant behavior

A class of responses that results in a certain consequence is called an operant behavior, the consequence regulates the strength or the future probability that the behavioral response or operant will be repeated or not [27]. For example, the likelihood of pressing the lever in the future increases if the rat gets rewarded. Pressing the lever, in this case, is called an operant response and the consequence decides the probability of it being repeated in the future. The operant behavior is a voluntary response, i.e., pressing the lever delivers a reward, this contrasts with the classical conditioning as the response is elicited by the stimulus (e.g., salivation on the sound of a bell after food conditioning). This gives the impression that the rat is free to choose and perform voluntarily but the operant behavior is changed according to the contingency of reward associated with it and it can be argued that the impression of voluntariness of this behavior is a mere illusion.

2.2.2. Operant consequences: reinforcers and punishers

The outcome that either strengthens or weakens the frequency of a behavior is the second component of operant conditioning. An outcome that follows a behavior is a reinforcer if it increases the probability of repeating that behavior and it is a punisher if it weakens the behavior [27].

Following symbols are used in diagrams of operant conditioning procedures. S^R is used to denote a reinforcing stimulus and S^P is used for punishing stimulus. The operant behavior is denoted by R. So, to show an operating conditioning procedure using a

diagram in which delivery of a food pellet reinforces the lever press operant is shown in **Figure 2.1**.

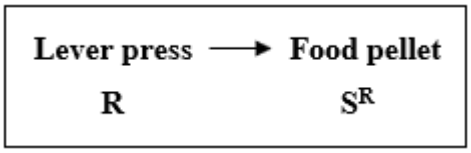

Figure 2.1: Delivery of Food pallet (S^R) reinforces the operant response (R), i.e., a lever press in this case.

For an operating conditioning procedure in which foot shock or punishment weakens the lever press operant is shown as under in **Figure 2.2**.

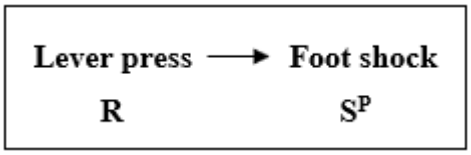

Figure 2.2: A foot shock denoted by (S^P) weakens or curtails an operant response (R).

There is a difference between reinforcer and punisher, and reinforcement and punishment. The reinforcer is a reward that strengthens the behavior, e.g., a food pellet. The punisher is an outcome that weakens the strength of the behavior, e.g., a foot shock. Whereas reinforcement and punishment refer to the procedures by which the outcome strengthens or weakens the behavior.

2.2.3. Operant antecedents: discriminative stimulus

The Operant behavior and the outcome are the two main components of operant conditioning, but in most cases, another discriminative stimulus is employed to indicate that the operant behavior is available, and it will lead to the delivery of a reward. For example, if the lever press operant is only available if there is an LED cue present to receive a reward, this LED cue acts as a discriminative stimulus (S^D) [27]. The behavior is only reinforced when this stimulus is available. This can be described with a diagram in the shown in **Figure 2.3**.

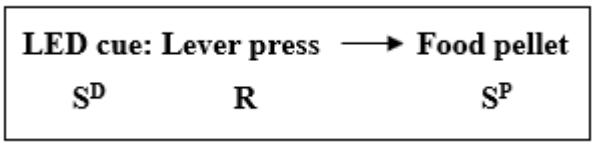

Figure 2.3: A discriminative stimulus indicates (S^D) that the operant behavior (R) is available, and it will generate a reward.

The preceding antecedent stimulus, operant behavior, and the outcome of the behavior are called the three-term contingency. The desired behavior is only strengthened in the presence of a discriminative stimulus and not any other stimulus. It is important to notice that the antecedent stimulus does not act as a conditioned stimulus, the lever is not pressed automatically due to the presence of the stimulus and this behavior is still controlled by the consequence.

2.2.4. Types of contingencies

Contingency means that a stimulus becomes a reward predictor if the frequency of reward presentation is higher when the stimulus is present than when it is absent. If the likelihood of reward is higher in the presence of the stimulus, it induces excitatory conditioning of the stimulus.

Reinforcement and punishment are the two main outcomes of operant conditioning. If a behavior results in a reward it strengthens, which means that there is a contingency to reinforcement. If the behavior results in a punishment, it means that there is a contingency to punishment. Reinforcement and punishment contingencies have further two types: positive and negative [27].

Positive does not mean that the outcome is pleasant, it means that something is added in response to a behavior, it can be something pleasant or unpleasant. Similarly, negative does not mean that outcome is unpleasant, it means something is removed as an outcome of the response. The two subtypes of each contingency are discussed below.

2.2.4.1. Positive reinforcement

If an appetitive stimulus is added as an outcome of the response, it is considered positive reinforcement. Adding something pleasant for the subject increases the future strength of the behavior. For example, delivering food in response to the lever press reinforces the behavior and is positive reinforcement.

2.2.4.2. Negative reinforcement

If an aversive or threatening stimulus is prevented or removed as an outcome of a response and it leads to the strengthening of that response, it is called negative reinforcement. For example, if by pressing a lever foot shock stops, the rat will likely press the lever again when it experiences shock.

2.2.4.3. Positive punishment

If an undesirable or aversive stimulus is added in response to a behavior, this will weaken the strength of that behavior. For example, delivering a shock if the lever is pressed will discourage the rat to press the lever again.

2.2.4.4. Negative punishment

If some desirable stimulus is removed in response to a behavior, it will weaken the strength of the behavior. For example, if food is removed if the trial ends without any reward when a wrong lever is pressed, the probability of repeating this behavior in the future will reduce.

2.2.5. Positive reinforcement: further distinctions

Positive reinforcement is often more effective for behavioral modification than punishments. Various categories of positive reinforcement are discussed below.

2.2.5.1. Contiguity, immediate vs delayed response

Contiguity means that the reward should be delivered in the nearest temporal proximity to the conditioned stimulus (CS) or operant response, an optimum time to deliver the reward is crucial for the strengthening of the operant behavior, the closer the better. If the reward is delivered before the response, it does not lead to learning, it is referred Background conditioning [27].

Dickenson et al designed an experiment to determine the effect of variability of delay (between 2 s to 64 s) on the number of lever presses by the rats, they observed that as the delay was increased the number of lever presses decreased significantly. At a delay of 64s, the lever press activity was almost diminished [28]. Initially, it was thought that it is because the rat forgot what produced the response, but later research showed that rats have very good episodic memory, but the problem is that they could not figure out

which behavior to perform to get the reward. The delay in reinforcement allowed the rat to engage in other behaviors.

2.2.5.2. Primary and secondary reinforcers

Primary reinforcers, also known as unconditioned reinforcers, are objects or events that do not require any training to develop a feeling of liking or persuasion for. These are the events that naturally have a strengthening effect on the behaviors. Food and water are examples of primary reinforcers, and their value is dependent on the deprivation and satiation states. Yet it has been shown in the studies that these do not only include an object that is crucial for survival, and they are not influenced by deprivation of satiation. For example, Butler et al (1954) designed an experiment for color discrimination learning in which the only incentive or rewarding event was to allow monkeys to explore the environment outside the experimental setup [29]. They reported that it was enough to keep monkeys engaged in the task for long hours until they stopped exploring the surrounding environment and that it was very hard to develop resistance to the satiation of this visual exploration [29]. So, this was evidence that visual stimulation is also a primary reinforcer [29].

Secondary reinforcers, also called conditioned reinforcers, are events that have an association with other reinforcers to modify the behavior. Learning is required for developing this association. For example, money is an example of a secondary reinforcer as it can be used to buy primary reinforcers.

2.2.5.3. Extrinsic and intrinsic reinforcement

When we perform a task that gives us happiness and satisfaction it is called intrinsic reinforcement, as the task is pleasurable to perform it has a reinforcing effect by itself. For example, doing exercise improves mood.

When the consequence that is external to behavior has a modification effect on the behavior it is extrinsic reinforcement. For example, writing the thesis because the degree is dependent on it.

2.2.6. Shaping and chaining

Positive reinforcement is used to strengthen a desired behavior, but the problem arises when the desired behavior never occurs. For example, a rat might never touch the lever

while training it to press the lever, there is no way to reinforce it because it does not happen anytime. The shaping procedure is a solution to gradually reinforce smaller steps that lead to the desired behavior, each sequential step is reinforced until the operant behavior is strengthened and a new skill learned.

Training a rat to press a lever can be divided into smaller approximations of the behavior, the first step can be going closer to the lever, the second can be standing with a desired posture near the lever, the third can be placing its paw onto the lever and the final step can be pressing it. When each successive sequence is reinforced and finally, when the rat gets an immediate reward by pressing the lever, it learns the association between pressing the lever and the reward. In this way, a new skill is acquired by reinforcing each sequential step that led to it.

Similarly, if a task consists of several steps, the subject can be trained for one step at a time, and progressing from one step to the other is conditional on learning the previous step completely. This method is called chaining.

2.2.7. Schedules of reinforcement

These are the requirements to be fulfilled through the response to validate a reinforcement. Schedules correspond to the nature of the response that needs to be exhibited to get a reward. For example, does the rat gets food every time it presses the lever, or does it have to press multiple times.

2.2.7.1. Continuous vs intermittent schedules

In a continuous reinforcement schedule, every time a targeted response is shown the subject is rewarded. For example, during the shaping process to train the rat to press the lever, every time it shows an approximation to the targeted behavior it should be rewarded to encourage it to perform the same behavior and to indicate that it was the desired behavior.

In an intermittent reinforcement schedule, a reward is presented after some responses. For example, after pressing a lever 5 times the reward is delivered. Reinforcement can be given after a few responses or after an interval of the last reinforcement when the response is available. The number of responses and intervals can be fixed or variable.

2.2.7.2. Basic intermittent schedules

The four basic types of intermittent schedules are discussed below. A steady-state response is established after the subject has undergone a substantial amount of training, when the schedule is first introduced a more variable state response is observed.

Fixed ratio schedule

Fixed ratio schedule (FR) is the same as continuous reinforcement schedule (CRF). In this schedule, delivery of the reward is dependent on the fixed predetermined number of responses, e.g., in a fixed ratio 2 (FR 2) schedule, to get a reward the rat must press the lever 2 times. The pause between reinforcement and the next schedule is known as postreinforcement pause and it is short in this type of trial. For a "dense schedule", which means the reinforcement will be received after exhibiting a smaller number of responses or easily, postreinforcement pause is smaller, in comparison to a "lean schedule" in which it is harder to get the reinforcement. For example, FR 2 is a dense schedule and FR 100 is a very lean schedule. The response rate in fixed ration schedules is high, which means the rat would quickly exhibit the required number of lever presses to receive the reward.

To increase the schedule ratio from dense to lean schedule also known as "stretching the ratio", smaller steps should be taken, otherwise erratic behaviors or complete collapse in performance can occur. "Ratio strain" refers to the condition when an overly demanding response requirement is imposed on the subject which leads to burnout.

Variable ratio schedule

In variable ratio schedules (VR), reinforcement is delivered at the instance of a randomly selected variable number of responses while keeping the average of the required number of responses the same throughout an experimental session. For example, for a variable ratio schedule 3 (VR 3), the rat gets a reward by pressing the lever between 1 to 5 times and this number varies between the trials while the average number of responses required is kept at 3. The rate of response is high and postreinforcement pause is generally low or none in VR schedules, as the instance of reward is contingent on a variable number of responses, the rat keeps itself engaged in the trial and the intermittent reinforcement involved makes these trials rather addictive for it.

Fixed-interval schedule

In fixed-interval schedules (FI), the rat receives a reward when it gives the first response after a fixed amount of elapsed time. Pressing the lever before the completion of a predictable period is futile as it does not generate any reinforcement. For example, for a fixed interval schedule 60 (FI 60), pressing the lever after an elapsed time of 60 seconds generate a reward, a discriminative stimulus S^D is used to indicate the start of the timer and the availability of reinforcement.

The response rate steadily increases as the elapsed time gets closer to the time limit selected in the FI schedule. The postreinforcement pause is high in this type of trial as a response before the specified time does not provide any incentive so the rat learns to wait.

Variable-interval schedules

In variable-interval schedules (VI), the time for the lever to become available varies randomly and the first response after the time reaches the limit delivers reward. For example, in a variable interval schedule (VI 10), the time for the lever to be available can be between 1 to 20 seconds and it varies from trial to trial with a mean interval of 10 seconds. The response rate is steady in this type of trial, with very low or no postreinforcement pause.

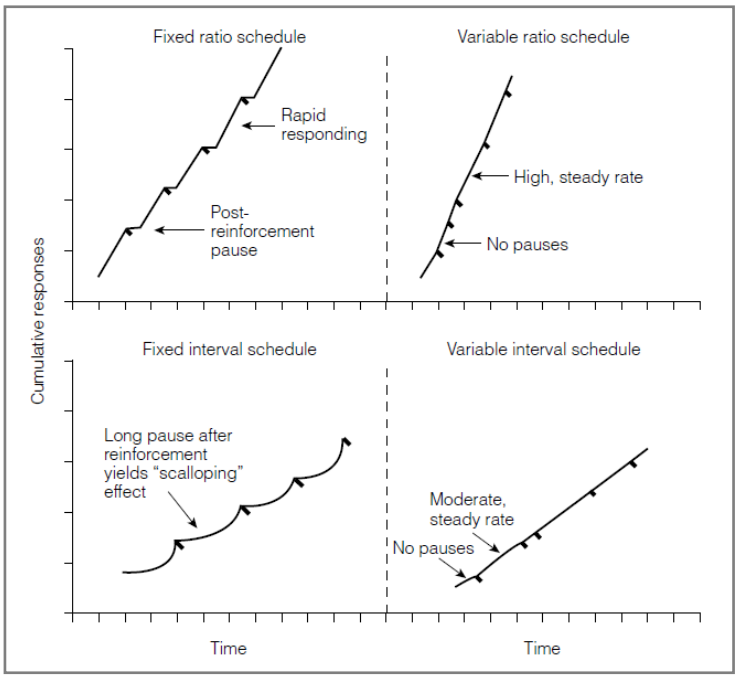

Figure 2.4: Response patterns in intermittent reinforcement schedules [27]-[30].

Comparison of basic intermittent reinforcement schedules

Table 2.1: Comparison of response rates and postreinforcement pauses for intermittent reinforcement schedules [27].

Schedule	FR	VR	FI	VI
Response Rate	High	High	Increasing	Moderate
Postreinforcement Pause	Yes	No	Yes	No

2.2.7.3. Other simple reinforcement schedules

Duration schedules

In a duration schedule, the reward is delivered if a response is consistently exhibited for a certain time. There are two further categories of duration schedules.

In a fixed duration Schedule (FD), a response is consistently required throughout the trial, e.g., in a fixed duration schedule 2 (FD 2), a lever must be kept pressed for a fixed duration of 2 seconds to receive the reward at the end of the period. In a variable duration schedule (VD), a response is consistently required for a variable unpredictable amount of time, this time varies from trial to trial. For example, keeping a lever pressed for a trial dependent variable amount of time to get a reward.

Response-rate schedules

In the case of intermittent reinforcement schedules, the response rate of the behavior varies depending upon the type of schedule, this is merely a secondary effect of different types of intermittent reinforcement schedules. In response-rate schedules, the reward is delivered depending on the rate of response. There are further three types of this schedule.

In differential reinforcement of high rates (DRH), the reward is delivered if the behavior is repeated a specific number of times within a specific prespecified interval. For example, if a lever is pressed 10 times within 1 minute, the reward is delivered at the end of a 1- minute interval.

In different reinforcement of low rates (DRL), the reward is delivered if there is a specific amount of time between two consecutive instances of behavior. For example, if the time between two lever presses is more than 2 seconds limit, the reward is delivered.

If the lever is pressed before 2 seconds finish, the trial will end without any reinforcement.

In differential reinforcement of paced responding (DRP), a specific number of responses are required with a specific rate to validate a reward. For example, a lever should be pressed 5 times with an inter lever press interval between a range of 1 to 2 seconds to get a reward between 5 to 10 seconds after the initiation of the trial.

Noncontingent schedules

In noncontingent schedules, the reward is delivered irrespective of a behavioral response, i.e., behavior and reinforcement are independent of each other. There are two further types of noncontingent schedules.

In a fixed-time schedule (FT), the reward is presented at the end of a fixed period, and the behavioral response is irrelevant to the onset of the reward.

In a variable-time schedule (VT), the reward is delivered at a random time, this random time is selected from within a range of intervals.

Noncontingent schedules can generate behaviors that don't have any context to the trial, these behaviors are termed superstitious behaviors. For example, instead of waiting near to reward delivery dispenser, subjects might circle in the cage or show different repetitive body movements [31]. The performance of a conditioned response also deteriorates by noncontingent rewards [32].

2.2.7.4. Complex reinforcement schedules

In all the schedules discussed above, the only requirement was based on a single response to validate a reward. In complex reinforcement schedules, the behavioral response required is difficult to perform. Three types of complex reinforcement schedules are discussed below.

2.2.7.5. Conjunctive schedules

In a conjunction schedule, more than one schedule is combined in such a way that to receive a reward, requirements of all the schedules are needed to be met. For example, in a FR 2 and FI 1 conjunctive schedule, a lever must be pressed two times and pressed once after 1 second to receive reinforcement.

2.2.7.6. Adjusting schedules

After the rat learns to perform a task well, i.e., above the inclusion criteria, the task difficulty is increased, this is termed adjusting schedule. For example, when the rat's performance in a FR 2 task is satisfactory, it can be shifted to FR 5 schedule.

2.2.7.7. Chained schedules

In a chained schedule, more than one schedule is linked in a sequence such that to get a reward the requirement of an individual schedule is needed to be met in an orderly manner. Each schedule has its discriminative stimulus S^D, which means that the fulfillment of one component of a chain and availability of the next linked schedule is signaled through an S^D. The tasks must be performed in a sequence determined by the schedules of the chain, this is a difference between chained and conjunctive schedules in which sequence is not fixed. A chained schedule can be explained with the following example. Suppose the rat must perform a nose poke two times (FR 2) to start the trial and then keep the lever press for two seconds (FD 2) to get a reward, a discriminator stimulus S^D (LED) lights up when the nose poke is done twice, this acts as an indicator that the nose poke was successful, and the lever is available as described in **Figure 2.5**.

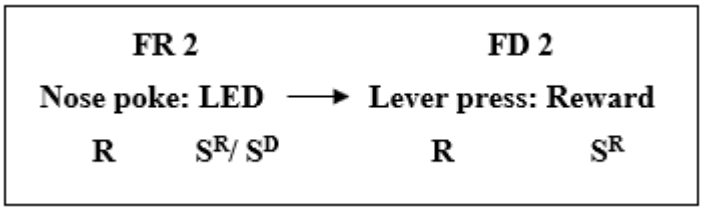

Figure 2.5: A chained schedule comprising of FR 2 and FD 2 schedules to be performed in specified sequence.

2.2.8. Extinction

When a learned operant behavior is followed by a reward, it further reinforces or strengthens the behavior, but if the delivery of reward is ceased after the same behavior it results in extinction. The procedure of extinction consists of cessation of rewards and the process of extinction is the resultant decrease in response. If the response is fully ceased or lost it is extinguished [27].

2.2.8.1. Side effects of extinction

Extinction can lead to some problems, and it is important to recognize the issues instead of taking the impression that the extinction is not working.

Extinction burst

Upon implementing extinction, the response does not fade away immediately, instead an increase in frequency and intensity is seen. For example, if a rat is trained for a FR 2 schedule, when extinction is introduced, it presses the lever several times with greater energy in hope of getting a reward.

Increase in variability

Different variabilities in behavior are induced by the extinction procedure [33]. For example, if the rat does not get the reward by pressing the lever with the hand it used to, it tries to press the lever with both hands or the other hand or might exhibit a different posture to press the lever.

Emotional behavior

When the rat does not get the reward by the response that used to generate reward it displays different emotional reactions such as grooming or showing helplessness and agitation by leaving the reward delivery area.

Aggression

When the rat does not get the reward during the extinction procedure, it displays aggressive behavior. For example, it starts biting the lever.

Resurgence

Rats might start to show a response that was previously learned when the later behavior stopped producing a reward. For example, when the rat does not receive a reward by pressing the left lever it was trained for, it might start pressing the right lever that was used to generate a reward previously.

2.2.8.1.5. Spontaneous recovery

After implementing extinction, the behavior can spontaneously recover after taking a break. For example, if the rat was previously trained to press the left lever to get the

reward, after applying extinction procedure on the left lever press for a day, when the rat is entered into the experimental cage, it starts pressing the left lever again as if it forgot it did not generate a reward in the last session.

2.3. Visual Capabilities of Rodents

Display technologies such as LCDs, touch screens, and projectors have provided ease and flexibility in designing visual feedback-based behavioral models. The introduction of these paradigms can open new avenues for rodent-based research and can help translate findings from this research to other species. The difficulty with these paradigms is that the learning is dependent on the visual capabilities of rodents. As they possess limited visual capabilities as compared to humans and non-human primates, a paradigm design with incompatible settings and without considering the rodent vision can not only hinder learning but also the translation of the findings to other species. In this section, different components related to rodent visual capability are discussed.

2.3.1. Visual acuity

Recognition of separations between dark lines of a grated pattern is called visual acuity. It can also be understood as the sharpness of these gratings, do they look distinct or blurred and continuous without any spacing [34]. Visual acuity is measured in terms of cycles/degree, referred spatial frequency, it is the number of pairs of black and white lines that fit within one degree of the visual field on the retina [34]. The higher the number of pairs of black and white lines in one degree of visual field the higher the visual frequency [34]. Visual acuity is tested by presenting a higher number of cycles per degree [35]. The measure of the maximum visual acuity is the maximum frequency at which a grating stimulus and a solid grey stimulus are indistinguishable [35]. Different species of rats have different visual acuities, pigmented rat strains such as Long Evans have a very low visual acuity and the value is as low as 1.0 cycles/degree [35]-[39]. The Non-pigmented rats such as Wistar and Sprague-Dawley have even inferior visual acuity i.e., 0.5 cycles/second, whereas among different rat strains Fisher-Norway has the highest visual acuity, as they can perceive 1.5 cycles/degree [35], [36]. When compared to humans, rats have very poor visual acuity as humans can distinguish up to 30 cycles/degree, this means that it can be very hard for rats to distinguish and perceive fine details in the grating stimulus [35]. Neural activity in the primary visual

cortex V1 is observed as it can provide evidence perception of changes in the frequency of a grating stimulus in rats. It is reported that in rats as the spatial frequency of the grating stimulus changes from 0.3 cycles/degree, a reduction in the neural activation is observed, this change in the neural activity in response to the change in spatial frequency is observed at up to 1.2 cycles/degree [35], [40], [41]. Most of the neurons in the rat's primary visual cortex V1 show corresponding change in activity with the change in spatial frequency, except 11% of the neurons [40].

2.3.2. Orientation

As discussed earlier, the activity of neurons in rat's V1 provides evidence of orientation perception. The majority of neurons in V1 respond to a change in orientation, around 77% of neurons are responsive or sensitive to a change in orientation, while only 3.5% do not represent any orientation shift [35], [40]-[42]. Neurons in V1 respond to a shift in orientation by decreasing their firing rate when the orientation changes from an optimum value. The shift in orientation in all directions is encoded in this neuronal population, but a significantly larger population represents the change in orientation in the horizontal direction, this population amounts to 35% of the total [35], [40]. This enables much better recognition of the change in orientation in the horizontal direction. The neuronal population representing the orientation shift is much organized in non-human primates, whereas in rodents they spread uniformly in the primary visual cortex [35], [43], [44]. The latest research on this neuronal population however has indicated that they too have an organized distribution [35], [45].

2.3.3. Motion

Rodents have an advanced ability to detect and perceive motion, this capability is enabled by a reliable and enhanced activity of 50% of the V1 neurons in response to a moving stimulus which is not the case for stationary stimuli [35], [40]. To study motion perception a collection of randomly scattered small circular shapes are traversed in a direction. There are two categories of these dots or circular-shaped stimuli, the group of randomly scattered dots that move in a common direction are called signal dots, while the other dots that show motion in random directions are called noise dots [46]. The coherent motion of signal dots gives an impression of motion in a particular direction. To increase the difficulty of these tasks the number of signal dots can be varied, the lesser the number of signal dots the higher the difficulty. The minimum amount of

these signal dots that are required to detect a motion in a direction is called the coherence threshold. Rats have four times higher coherence threshold than humans, and they require at least 25% of the total dots to move in a single direction coherently with 20 degrees to 100 degrees per second to give an impression of motion [47], [48]. As described earlier the perception of motion is better for moving stimulus than a stationary stimulus, this perception can be improved by increasing two factors; (1) the displacement of dots in between consecutive frames, (2) the period of each frame [47], [48]. The optimum range of motion is between 10 to 250 degrees per second, as neuronal activity is reliable and robust in this range, in some cases some neurons respond to up to 700 degrees per second as well which is even higher than in cats [44]. The periodic appearance of a grating stimulus is also perceived as the neurons in V1 are sensitive to these flickers, the frequency range for these flickering grating stimuli is between 0.43 to 6.88 Hz, but a response from some neurons is seen at up to 27 Hz speeds [40]. Some regions in the visual cortex other than V1 also distinguish a global motion from the randomly moving dots [49].

2.3.4. Color

To process visual input, rats like humans possess two types of receptors on their retina, one is specific to processing greyscale input and is called rods, whereas they are dependent on cones for processing colors in their environment. Although these photoreceptors allow rats to navigate and analyze colored objects in their environment, the spectral range of color perception is limited, and they cannot perceive some wavelengths at all. The color processing in cones is enabled by the presence of two types of pigments. These pigments are sensitive to different ranges of wavelengths of light, one pigment is sensitive to blue and ultraviolet light and the maximum sensitivity is at 358nm, whereas the other pigment is sensitive to a longer wavelength of light that corresponds to green color and the maximum sensitivity is at 510nm [50]. Most of the cones are sensitive to green light and only 12% have sensitivity for blue and ultraviolet light [51]. The distribution of cones on rats' retina is not uniform, most of them are found on the bottom of the retina. That is why the perception of colored objects is dependent on the location of the visual field where the image is projected. Rats cannot detect colored objects if they are outside the visual field where cones are not present on the retina. When presented colored stimuli at different locations in their visual field they

could only detect and perceive colors at locations above their visual horizon and not below it [52].

2.3.5. Shape and object recognition

Different shapes display some distinct features, rats, like primates, can process these features to recognize and differentiate between different objects and shapes. Although their visual acuity is limited and much inferior to humans, they can extract distinct decisive features from these shapes which grants them the ability to discern complex shapes regardless of variance in size, shape, color, or orientation [53], [54]. This ability to differentiate between objects, invariant of their low-level features such as size and orientation, suggests that objects have some complex representation. These complex features are used to perform a generalized categorization of an object so that when observed in different contexts and from different viewpoints its category remains invariant. An organization in the visual system is proposed in which the lower-level features, such as brightness, are processed in the earlier layers and the complex features, such as position, that are specific to the object and have less transformational sensitivity are processed in the later stages of the visual system [55].

To analyze the process by which a rat extracts the relevant features of an object for discrimination, an experiment has been used in which bubbles are placed onto the visual objects [56]. After training the rat for discriminating images of two complex objects, bubbles are distributed onto the image such that only the features under the bubbles are visible. The object is identified if features visible under the bubbles provide decisive information about the object. By repeating the trial by changing the position of bubbles, and analyzing the discrimination performance, it can be revealed which complex features specific to the object are important for its identification. Rat creates a template of these features for an object and when the different transformation of the object is shown, it still recognizes the object [57].

CHAPTER 3

3. EXPERIMENTAL PART

3.1. Setup Overview

The main objective of this study was to develop a rodent behavioral paradigm to investigate the closed-loop control of the movement of a cursor by pressing a lever in response to visual feedback from a display. An Arduino Nano and MATLAB-based system were developed to carry out this task. The main apparatus for operant conditioning and motor skill learning is a behavioral cage and an LCD. The cage contains components essential for implementing the experiment that includes: (1) an IR beam; (2) lever assemblies; (3) a water receptacle; (4) cage LEDs; (5) a solenoid valve and water reservoir for reward delivery (**Figure 3.1**). The LCD was used to provide visual feedback. Real-time monitoring of the status and control of all the components was carried out by two-way communication between Arduino and MATLAB. For each trial, the status of all the components was regularly logged in the host computer for the post-experimental analysis.

3.2. Behavioral Apparatus

For operant conditioning and motor skill learning an experimental setup was designed. A custom-made experimental cage was developed to train the rats for stereotypical movements to reach levers while observing the visual cues outside the cage. The experimental setup was comprised of the following components.

3.2.1. Experimental cage

A cage made of transparent plexiglass walls was used for housing the rats during the experiments. The Cage walls were transparent to facilitate visual feedback from an LCD screen and Cage LEDs outside the cage. Levers, water receptacle, and IR sensor were

installed inside the cage at locations easy for the rats to reach and interact with for operant conditioning and motor skill learning tasks.

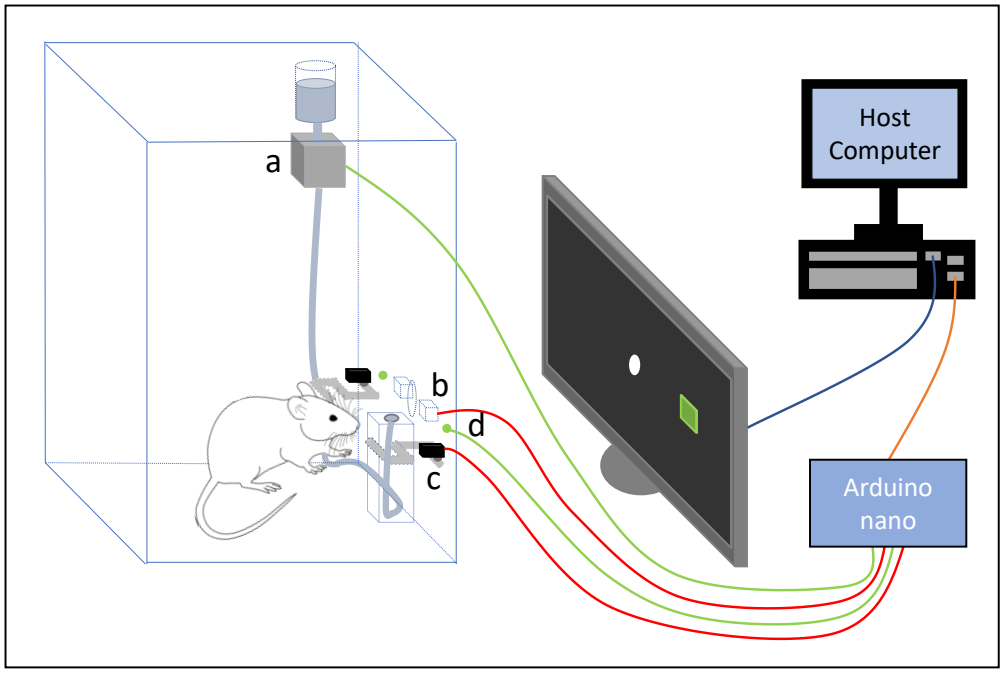

Figure 3.1: Experimental setup, shows rats in a plexiglass cage, **(a)** solenoid valve, **(b)** IR sensor, **(c)** lever **(d)** cage LED. There is an extended LCD in front of the cage and the setup was controlled by an Arduino nano and a MATLAB script on a host computer.

3.2.2. Lever assembly

Two lever assemblies were installed on the front wall of the cage facing the LCD. Lever assemblies were designed using 3D builder which is an open-source 3D model designer application. After designing it was built on the 3D printer. The material of choice was PLA because of its strength and finish. Each assembly was made up of the following parts: cylindrical lever shaft, holder assembly, weight hanger, adjustment screw, and a microswitch. The dimensions of the lever shaft were selected to ensure ease of grasp for the rat using its paw. The shaft pivots inside the holder assembly and at the backside of the shaft, there was a weight hanger, made using a paper clip and attached using dental acrylic, to increase the force required to press the lever during the training process. Microswitch (ZING EAR G5S05) was used to detect the lever presses, it was connected such that in normal conditions when the lever was not pressed the switch was in the normally open state. The microswitch was selected as the force required to press was quite less and the press generated a click sound that served as vital feedback for the rat indicating the lever press was successful. A screw was used to adjust the lever press

travel distance, by changing the length of the screw, penetrating the assembly from the bottom side, the distance the lever needed to travel for a successful press could be adjusted. The lever assembly was covered using a box made up of transparent plexiglass with just a slit available to reach the lever. This was done to induce the same movements by the rats to reach the lever and to restrict their teeth from reaching and biting the lever. The force required to press the lever was also adjustable by adding or removing weight from a weight hanger at the rear end of the lever. Turkish lira coins (1 TL coin weighs 8 g) were hung as weights to induce more force for lever presses as desired during the training.

3.2.3. IR beam

The rats were trained to initiate the trials by interrupting an IR beam. An IR LED and detector pair (HD-DS25CM-3MM) were used to detect the nose poke. A 1.3 cm hole was created on the cage wall facing the LCD, a hole just big enough for the rat to insert its nose and interrupt an IR LED beam. The IR sensor was placed on the outside of the cage wall adjacent to the nose poke hole. The reason behind placing it outside was to avoid unwanted and accidental nose pokes for trial initiation. IR detector module detects an interruption in the IR LED beam and a transition of 5V to 0V occurs at its output.

3.2.4. Cage LEDs

Green color LEDs were mounted above the lever assemblies outside the cage. These LEDs acted as targets and visual cues for target direction in two-lever choice tasks. The brightness of these cage LEDs was adjustable using potentiometers so that it could be decreased as the target selection accuracy improved and to divert the rat's attention towards the targets on the LCD outside the cage.

3.2.5. Reward delivery system

To reinforce the operant behavior the rats were given a reward in the form of water. A water receptacle was placed between the two levers and just below the nose poke area. A pilot-operated solenoid valve (12 VDC CROX 2V025-06) and water reservoir were placed at a height to allow water flow under gravity when the valve was turned on. The magnitude of reward was dependent on the height of the reservoir and the duration of the solenoid valve activation. In our behavioral paradigm, the rat received 30ul of water

as a reward every time it finished the trial successfully. The click sound generated by the valve was the cue for the reward during the trials.

3.2.6. LCD display

An LCD (Philips 221V8A/01) was used as a source of visual feedback. Cursor and targets were drawn while the background was kept dark. The display parameter settings were set to values to achieve the best brightness to contrast ratio. To improve the perceivability of the cursor and the targets the background had to be kept as dark as possible by decreasing the brightness of the screen. The reduced luminescence from the backlight helped to keep the experimental setup dark. High contrast setting was used to make objects more prominent on the black background. In addition, to further reduce luminescence from the backlight and interference in perception, the screen was covered with a pitch-black cloth so that only the cursor and the targets were exposed. The parameter settings of the screen display are given in **Table 3.1**.

Table 3.1: Display parameter settings.

Model	ID 221V8A/01
Aspect ratio	16:9
Brightness	50
Contrast	100
Sharpness	50
Color	6500K

3.3. Behavioral Control Architecture

Arduino nano 3.0 provided the ease and flexibility to interface and control input and output components of the behavioral paradigm. It has an ATmega328 microcontroller onboard which offers 14 digital input/output pins. In our paradigm, to read and control the status of the behavioral components while training, we required 6 of the digital input/output pins (see **Figure 3.2** for the wiring diagram). Digital Inputs: three digital inputs were connected to (1) IR sensor; (2) right lever; (3) left Lever. Digital outputs:

three digital outputs were connected to (1) right cage LED; (2) left cage LED; (3) Solenoid valve.

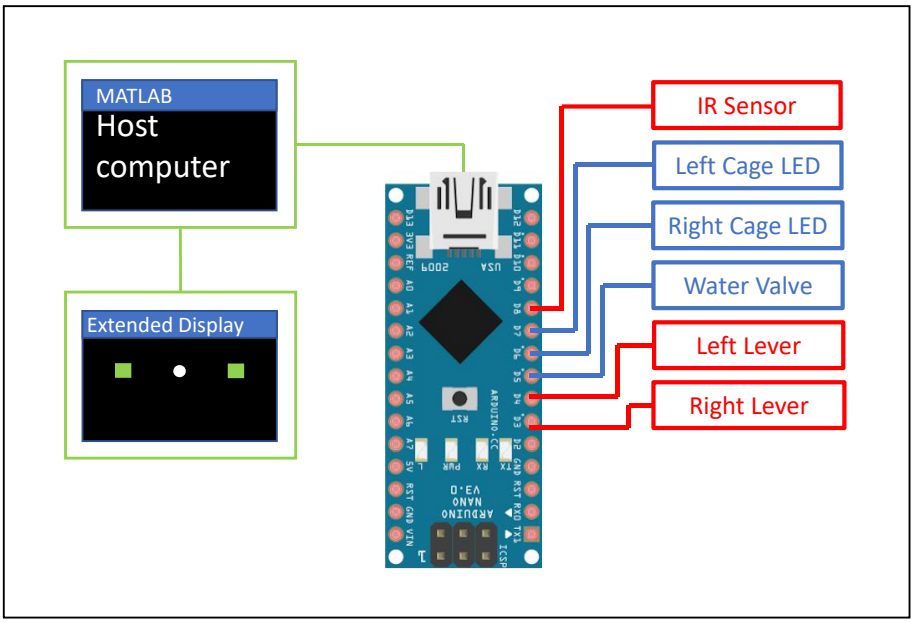

Figure 3.2: Wiring diagram of the Arduino Nano for operant conditioning and motor skill learning task. A MATLAB script monitored input pins on the Arduino and sent TTL signals to control the digital output pins by a two-way communication between the Arduino and the host computer. Visual cues on the extended LCD screen were displayed and controlled, based on the status of the Arduino digital pins in consideration, by the MATLAB script on the host computer.

A MATLAB script was written for two-way communication between Arduino and the host computer in real-time. The MATLAB support package was utilized to establish a connection with Arduino nano via a USB port. The script looped through a set of functions monitoring the status of inputs and sending commands to control the outputs and status of the experiment. When the IR sensor output turned low by a nose poke the trial started. A plot was generated on the extended display in a maximized window with a black background to display a green square-shaped target with grids and a white-colored circular cursor. In the early stage of the habituation, cage LEDs were used to indicate which lever to choose in a two-lever choice task. The status of the lever corresponding to the selected target was continuously monitored. If the rat operated the correct lever interactively by observing the cursor motion and time constraints, the solenoid valve was activated to give a reward, otherwise, the trial was terminated without any reward.

To ensure reliability and easier debugging of the code, data was logged in a log file for all the instances in each execution of the loop for all the trials. To ascertain that the rat perceives the motion of the cursor and behaves accordingly by pressing and releasing the lever, it was important to continuously record the trial parameters. The date and time for all the parameters such as the position of the cursor, status of the lever (pressed or not) were recorded in every iteration of the loop. Nose poke time served as the starting reference point from which the elapsed time of each activity was calculated. The instances of lever press and release during the trials were the deciding factors to understand whether the rat perceives the cursor movement or not and based on that it was either rewarded or punished.

3.4. Rat Operant Conditioning and Motor Skill Learning

The experimental setup for operant conditioning and motor skill learning is illustrated in **Figure 3.3**. Rats were shaped to attend to the cursor motion by sequentially training them to associate lever press and release with the control of motion trajectory of the cursor in one-dimensional space for the center outreaching task. The goal was to make the rat acquire the motor skill to control the cursor trajectory based on the visual feedback from the LCD. In this section, the experimental setup and shaping procedures are discussed.

The experiment essentially consisted of a behavioral cage and an LCD. The behavioral cage was made of transparent plexiglass sheets. The transparent sheets allowed rats to observe cursor and target displayed on the LCD while being enclosed in the cage (**Figure 3.3A**). Two levers were placed on either side of the front wall of the cage facing the LCD. Levers were covered by an enclosure with a slit just large enough to allow grasping of the lever with one paw corresponding to the side of the cage. The enclosure prevented the entry of both forepaws, pressing the lever with the wrong forepaw and biting the lever. A water receptacle was placed in the middle of the two levers. For nose-poke a hole was created just above the receptacle, IR sensor was placed on the outer wall just beside the nose poke area (**Figure 3.3B**). Curved-shaped obstacles were placed on both sides of the cage to make the rats stand in a narrow alley (8cm in width) large enough to accommodate their bodies comfortably. Smooth curve-shaped obstacles, made of thin aluminum sheet, kept the rat standing in between them, in the middle of the two levers. These obstacles also prevented variation in the rat's stance

while operating levers. The setup was designed to make the rat stand in an upright position pointing its head towards the LCD so that the cursor and targets were within the range of its visual field and at the same height as its head. The floor of the cage was flat to make it convenient for the rat to stand. Levers, nose-poke area, and water receptacle were placed strategically to ensure ease of access and to promote stereotypical movements to reach them. A water receptacle was placed on the top of a square block just below the nose-poke area (**Figure 3.3 C, D**), the rate could reach by leaning onto it while standing in the same position. Opening and closing of the solenoid valve made a click sound, delivering 30ul of water. The click sounds from the valve served as a reward cue for the rat during operant conditioning. Cage LEDs were placed above the levers as a visual cue for the trial direction, as the accuracy of the two-lever choice was improved, the brightness of the cage LEDs was reduced to divert the rat's attention to the targets on the LCD. Two LEDs were placed on each side of the back of the cage, these were connected in levers such that when the levers were pressed LED on the corresponding side of the lever turned on. It was done so that the rat's lever press activity during training could be observed and recorded through a camera. Rats fed ad libitum in their home cages and the behavioral cage. To motivate them to perform behavioral tasks they were deprived of water for 21 hours/day and only received water in the experimental cage during training.

The LCD was used to present visual cues in the form of the white-colored circular-shaped cursor and green-colored square-shaped targets. The placement of these objects on the display was adjusted according to the height and visual field of the adult male Wistar rats (**Figure 3.3 C**). The targets were located so that they could be attained by a one-dimensional horizontal motion of the cursor. The experimental environment was kept dark with the help of a black curtain. The background of the LCD was black and the area excluding cursor and targets were covered with a black cloth, reducing interference due to light from the backlight to improve the perceivability of the visual cues. Rat's activity was monitored, in real-time, using two cameras mounted on the front and backside of the cage for a better understanding of the rat's behavior and efficient shaping.

Rat training consisted of three phases: The first phase was applied to make the rat attend to the cursor motion. The second phase was used to associate lever press with the

motion of the cursor and in the third phase, the rat was trained to control and correct the trajectory of the cursor by perceiving its proximity to the target.

At the start of the training, rats were familiarized with the components of the cage. The first step was to teach the rat to access a water receptacle to take water. The manual reward was given every time the rat got closer to the receptacle to familiarize it with the water receptacle. Some rats got intimidated by a sudden click sound from the solenoid valve and feared accessing the receptacle initially. The trick was to fill the receptacle area with multiple manual rewards, as the rats were thirsty enough, they approached the receptacle after some time. To make the rat familiarize with the nose poke area, a manual reward was given each time it took its nose near the nose poke area and gradually trained it to interrupt the IR sensor to get the reward. Rats were operant conditioned to do nose-poke, nose-poke being the operant. After learning to perform the nose-poke, rats were trained to press levers to get the reward, the lever being the operant. The same strategy was adopted to train the rat to press the lever, every time it got closer to the lever a manual reward was given and after some time it learned to access and press the lever inside the enclosure. They were trained to press a lever on one side at a time i.e., training for the right-side lever was done before starting for the left side, rats received a reward each time the lever was pressed. It took one to two days for rats to learn nose-poke, 4 to 5 days to be able to reach levers with correct forepaws. After learning nose-poke and lever press individually, they were trained to perform nose poke then lever press in sequence to get the reward for each side of the cage. When the nose-poke was done, the cage LED above the lever to be pressed and the target on the LCD was turned on immediately and when the lever on that side was pressed the reward was delivered, cage LED and target on the LCD were turned off to indicate the trial has finished. To initiate the next trial rat had to release the lever and repeat the same. In this way, rats learned to do nose poke and lever press in sequence for the side on which the cage LED and target were turned on. When rats learned to use the visual cue from the cage LEDs and LCD to choose a lever, a two-lever choice task was introduced.

In the two-lever choice task, the target was pseudo-randomly selected between left and right. Cage LED and target on the LCD were turned on with the nose poke and reward was delivered when the correct lever was pressed, otherwise in case of the wrong choice trial was ended without reward. Environment turning dark without any reward acted as an indicator of punishment for the rat. Before moving to the random selection of the

target, it was essential to manually shift the target after a few successful trials, when it was observed that rat looked at the target before deciding which lever to press, random target selection was introduced.

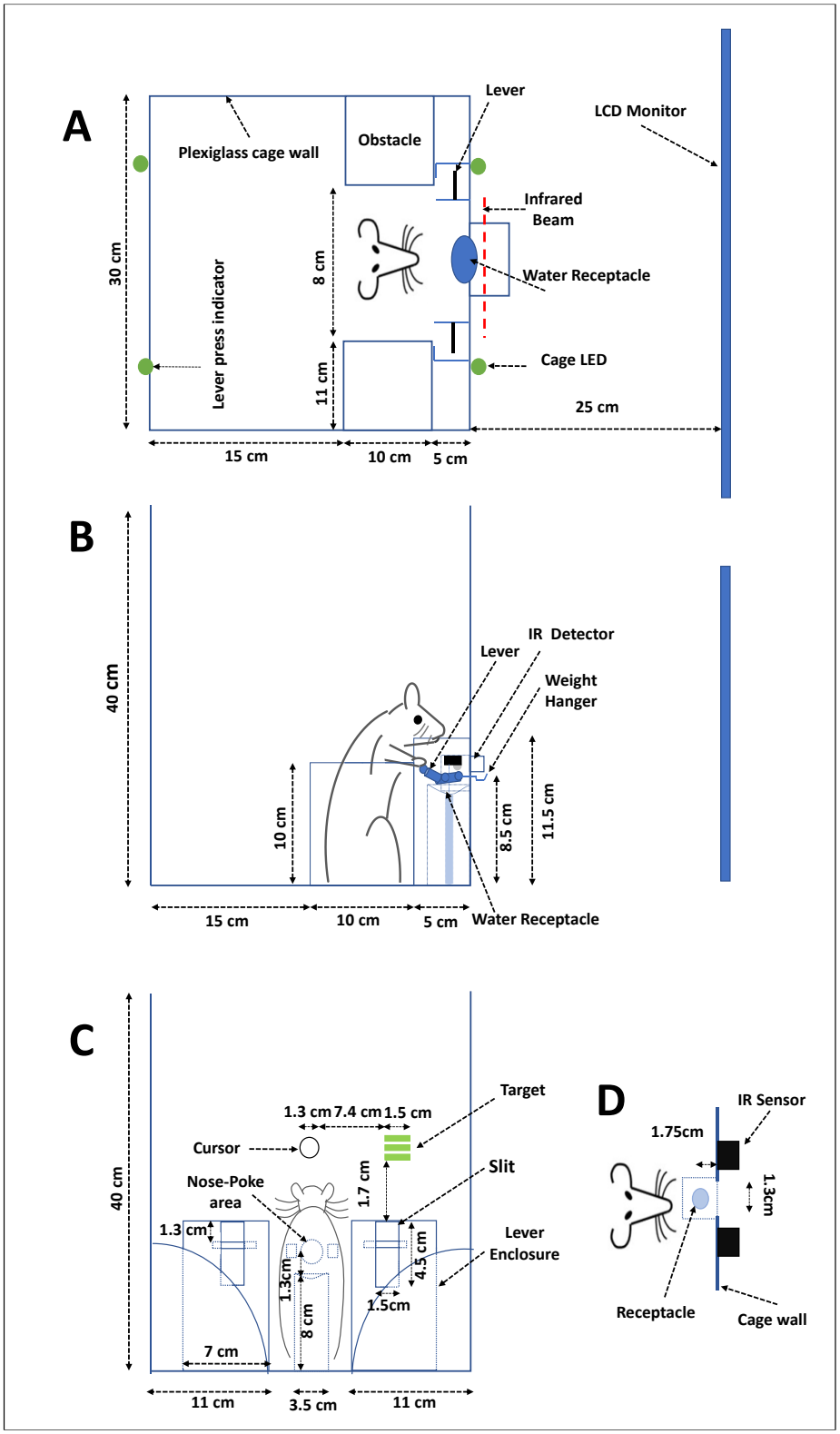

Figure 3.3: Experimental setup. **(A)** Top view. **(B)** Side view. **(C)** Back view. **(D)** Placement of water receptacle, nose-poke area, and IR sensor.

Once rats were trained for the two-lever choice task, they were progressed to the first phase of the paradigm, cage LEDs were not used beyond this point.

The training phases were designed to sequentially and systematically build up the rat's skill to perceive the cursor motion, its proximity to the targets, and behave to control the cursor movement to reach the target based on visual feedback.

3.4.1. Phase I – variable cursor speed

This phase aimed to train the rat to attend to the cursor movement and to ensure that it recognizes when the cursor reaches the target. The trial was initiated by a nose poke, the target was selected randomly, such that no one target was selected more than thrice in a row. Cursor motion speed to reach the target was also randomly selected among the two prespecified speeds i.e., 6.25 cm/s and 3.4 cm/s, that corresponded to 1.2 s and 2.2 s to reach the target respectively. The reason for choosing two different speeds was to elicit a different behavioral response from the rat for each speed to validate that it recognizes the cursor motion. After trial initiation, the cursor and targets were turned on and the cursor automatically started its motion from the center towards the selected target with one of the selected speeds for the trial. The rat had to press the lever between 0.9 s to 1.5 s or between 1.5 s to 2.7 s for 1.2 s and 2.2 s trials to get the reward. The rat was punished, and the trial was finished without any reward in case: (1) Wrong lever was pressed; (2) Rat pressed the correct lever later than the allowed time; (3) Lever was pressed earlier than the start of the allowed interval to press the lever. Action mentioned in point 3 was punished to inhibit an impulsive response from the rat to reach the lever instantaneously just by looking at the targets as the trial starts. Correct lever selection and perception of cursor speeds by distinct lever press behaviors were enforced by reward. At the end of each trial, the cursor and targets were turned off and the environment was turned dark, which acted as an indication that the trial has ended. Rats tried to find an optimum time to press the lever to reduce the effort required to conduct trials. They tried to find a shortcut to maximize the number of rewarded trials with minimum effort by not looking at the screen throughout the trial and finding a point to press the lever during the cursor motion to be

successful for both the speeds. To prevent the rat from finding a loophole or crack in the task the timeout for 1.2 s trials was set to be 1.5s and for 2.2 s trials, the interval to press

the lever started after 1.5 s. In this way, there was no overlap, and the rat could not find an optimum point to press the lever and be successful in both types of trials. A distinct behavior could also be observed visually other than the lever press instances for both types of trials. Rat waited to press the lever in 2.2 s trials, whereas for 1.2 s trials it had to quickly judge the direction and lean towards the lever as there was not enough time to wait. The ideal behavior was to press the lever when the cursor was as close to the target for both the speeds but as rats tried to get the reward as quickly as possible, they tried to press much earlier. For this reason, it was preferable to train the rat for a 2.2 s trial, to make them learn to wait before introducing 1.2 s trials. When the rat reached an accuracy of 75% for 2.2 s trials, 1.2 s trials were introduced at about 20 to 30% of the total trials and gradually increased to 50% as the accuracy improved to above 70% in each increment. It was observed that rats perceived faster cursor motion in 1.2 s trail better than in 2.2 s trials and preferred 1.2 s trials as they received reward earlier. So, the percentage of trials was adjusted to not allow reliance on just one type of trial for reward and intentionally making errors for the other type.

Perception of cursor movement was also verified by turning off the targets. Rats were able to distinguish not only the direction but also the speed of the cursor by selecting the correct levers and displaying a varied behavioral response for both speeds.

3.4.2. Phase II – continuous lever press, linear cursor trajectory

After the rats learned to attend to the cursor motion, the next step was to train them to keep the lever continuously pressed until the cursor reached the target. This was a basic skill to learn for the rats and a precursor to a more difficult step with variable trajectories. By keeping the lever pressed, rats learned an association between continuous lever press and forward one-dimensional movement of the cursor towards the targets. This step involved a two-lever choice task, rats had to choose the lever to continuously press on the side corresponding to the pseudo-randomly selected target direction.

After trial initiation with a nose-poke, the cursor and target was turned on after 40ms. The cursor stayed stationary in the middle of the LCD at the start of the trial. The rat had to choose the correct lever to press continuously for 2.2 s to make the cursor move from the center to the target in a linear one-dimensional motion and get the reward. Although the targets were reached in 2.2 s, the reward was delivered at 2.5 s, which

means that the rat had to keep the lever press for an extra 0.3 s period to get the reward. Rats were trained to keep the lever press until a click sound cue was generated by the solenoid valve. The cursor and target were turned off at the end of the trials. Trials were terminated without a reward if: (1) Rat released lever earlier than 2.5 s after initiating the cursor movement with a continuous lever press; (2) Wrong lever was pressed.

The time to continuously press the lever was incremented in small steps. For instance, in the start the time required to press the lever was kept at 0.2 s, when the rat learned to keep the lever pressed for this amount of time, this period was increased to 0.5 s and so on until it was increased to 2.5s. It was important to keep track of the rat's performance after each increment in the lever press interval. when the rat started to get punished too often i.e., the number of punished trials increased rapidly due to the early release of the lever, it got demotivated and gave up. In such a situation, giving manual rewards and adjusting the lever press period helped to carry on the training by reducing task difficulty. Similarly, the extension period i.e., the period for which the lever must be pressed after reaching the target, was also incremented in gradual steps. At the start, this period was kept at zero seconds, which means the rat had to press for 2.2 s to get the reward. When the rat got used to a 2.2 s period of continuous lever press, this time was increased to 0.1 s then to 0.2 s, and finally to 0.3 s, making the total time to press in the final stage to be 2.5s. The purpose of this extended period of continuous lever press after reaching the target is discussed in the next section. While performing phase II trials, the dominant forelimb of the rat was also identified. In some cases, they tried to press levers on both sides using the same paw, this was an indication of their dominant forelimb. When the rat learned to lean on the curved obstacle to press the lever, it was an indication that the rat was comfortable pressing the lever on that side and this behavior was desirable.

3.4.3. Phase III – variable cursor trajectories

Once the rat learned to press the lever without releasing it until the cursor reached the target, the third and final phase of the training was commenced. The purpose of this phase was to further validate the rat's ability to perceive cursor movement by investigating its behavior when there was a change in the cursor's trajectory. In the previous steps of the training, the cursor always moved towards the targets. In this phase, however, in addition to the trials with linear cursor trajectory, trials with

variation in the cursor trajectory were introduced. These trials with variable cursor trajectory were only introduced for one of the targets specific to the rat's dominant forelimb. For the other target, all the trials were kept linear cursor trajectory trials. Linear cursor trajectory has been discussed in the previous section. In the variable cursor trajectory, after initiating the trial the rat had to press the lever continuously to move the cursor towards the target, instead of reaching the target the cursor started to move towards the center after a certain prespecified amount of time, while the lever was still pressed. To make the cursor move towards the target or to correct the cursor's trajectory, the rat had to release and press the lever within a limited amount of time, signaling that it recognized or identified the change in the cursor's trajectory. Releasing the lever when the cursor was moving towards the center did not change the direction of the cursor's motion, but it served as a signal that the rat recognized the cursor's movement in the wrong direction and responded to correct it. Subsequent lever press when the cursor was still moving towards the center changed its direction towards the target and the rat was rewarded after 0.5 s.

The dominant forelimb of a rat was assessed in phase II through how they reach levers through the slit of the lever enclosure. In this phase, random trajectories were introduced in trials on one side at a time to further validate the dominant side, e.g., 30% of the total trials on the right side were trials with random trajectory, and the rest of 70% were linear trajectory trials. The first variable trajectory introduced consisted of 1.7 s of motion towards the target and then a change in direction towards the center after the 1.7s mark while the lever was still pressed. After nose-poke, the rat pressed the lever to move the cursor towards the target, the cursor changed direction and reached the center of the lever was not released. In the first few sessions, the timeout (time allowed to release the lever to correct the trajectory) for the variable trajectory trials was kept at 5 sec, the trial was terminated after that. The timeout was kept higher in the start to encourage the rat to release and press the lever to correct the cursor trajectory. Later this timeout was reduced to 1.7 s which corresponded to the time it took to reach the center. This timeout was later gradually further reduced to 1.2 s to create a sense of urgency for the rat to act. As the accuracy increased the percentage of trials with random trajectory was increased to 50 % of the trials on the right side. If during random trajectory trials on the right side, the rat could not perform as desired, the target for these trials was changed to the left side. Rat's body had to be stable for it to behave efficiently in these

trials. If it was moving away from the lever to release it or had trouble pressing the lever continuously, the other target side was tried for the random cursor trajectory trials.

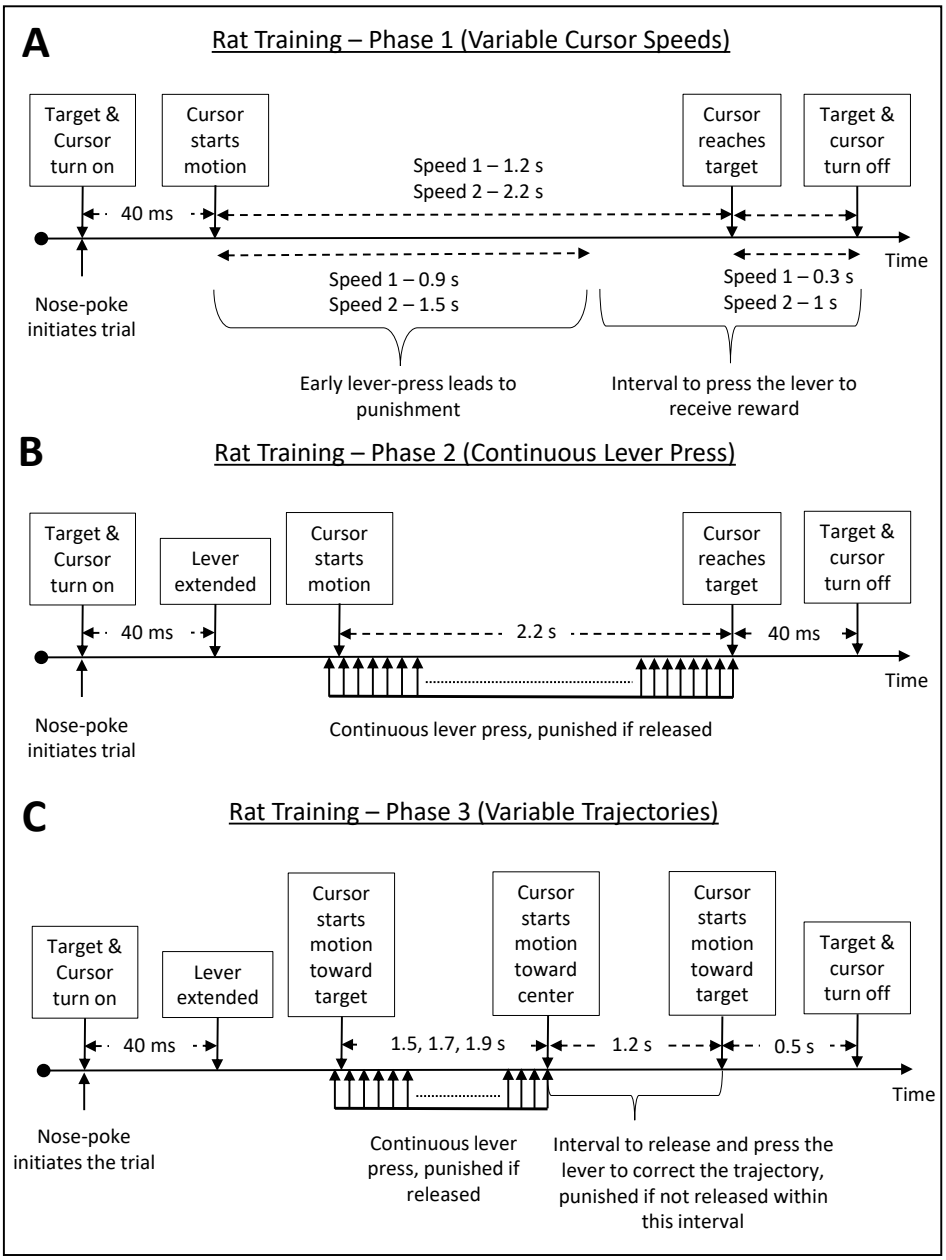

Figure 3.4: Behavioral paradigm for shaping the rat for closed-loop control of a cursor's trajectory in one-dimensional space. **(A)** Paradigm to train the rat to follow cursor motion. **(B)** Paradigm to associate lever press with change in cursor's position. **(C)** Paradigm to train the rat to perceive the cursor's proximity to the target and control and correct the cursor's trajectory.

In linear trajectory (2.2 s) trials rat received a reward at 2.5 s and released the lever after that (typically at around 2.9). So, in the case of the random trajectory (1.7 s), if it released the lever before 2.9 s, the difference in lever release time meant that it perceived the cursor's proximity to the target and its direction of motion and behaved accordingly to correct it.

To further validate this observation, a total of four different trajectories (1.5, 1.7, 1.9, and 2.2 s) were presented on the target side under consideration, while only linear trajectory (2.2 s) trials were presented on the other side.

3.4.4. Problems faced and troubleshooting during the training

All rats were not homogenous when it came to training them. For some rats, it was easier to learn a particular task than for others. Some of the major problems faced during each phase of the training and how they were rectified are discussed in this section.

3.4.4.1. Threatened by the click sound

Some rats found clicking sounds from the solenoid valve threatening during habituation in the experimental setup. The receptacle was filled with water by giving manual rewards and as the rats were thirsty, they gradually started to approach the receptacle. By giving a click sound while the rat was drinking water, it got used to the sound. After checking for 2 to 3 days, if the rat still did not get used to the click sound and refused to come to the receptacle, that rat was suspected to be schizophrenic and was disqualified.

3.4.4.2. Wrong paw for lever press

While training the rat to press levers, if the rat preferred to use the wrong paw to press a lever, automatic reward delivery by pressing the lever was discontinued and the reward was given manually only if the correct paw was used. Increasing the number of manual rewards for correct behavior also significantly helped. For one of the rats, the obstacle had to be removed from the right side to make it learn to use the correct paw, it had learned to use its nose to press the lever and would not use its correct paw. By removing the obstacle, it was trained to first use its paw, it did not matter which paw it used, later after a few trials. The reward was only delivered in case of a lever press with the correct paw. The obstacle was replaced after it learned to press the lever using the correct paw.

3.4.4.3. Multiple lever presses

During training for continuous lever press, the continuous lever press interval was incrementally increased to 2.5 s. For some rats, this phase was challenging as they could not learn to continuously press, even for a smaller interval, and gave up due to extensive punishment. So instead of punishing when they released the lever by finishing the trial,

an alternative approach was used. Rats were allowed to release but the cost to release the lever was increased by adding weights (one or two 1 TL coins were used, 8 g each) so that the force required to press the lever was increased. It was less costly for the rat to keep the lever pressed than to do multiple lever presses to make the cursor move to the targets.

3.4.4.4. Wrong posture

The desired posture to perform continuous lever press and variable trajectory tasks was to press levers while leaning onto the obstacles, keeping the head stationary, and looking towards the LCD. Rats tried to minimize the energy required to perform the task by deciding which lever to press by just looking once at the LCD, then trying to keep the lever pressed while leaning onto the water receptacle to get the reward as early as possible. This was a frequently faced issue with the rats and needed prompt action before this behavior matured. This issue was resolved by putting weights on the levers, starting with a smaller weight (coin), and increasing the number and size according to the requirement. The optimum weight was the one at which the rat had to lean its body onto the obstacle, while still being able to keep the lever pressed. In this way, learning a wrong posture was avoided as it could not keep the lever pressed while leaning towards the receptacle with an optimum amount of force required to press the lever. If the rat was too impulsive and could not learn to stay on the obstacle and was unable to concentrate on the screen by frequent head movements, it was deemed unfit for further training.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1. Results

Four albino Wistar rats, between the ages of 4 to 6 months and weights between 350 to 450g were trained for the behavioral paradigm. All animal procedures presented in this thesis were approved by and conducted in accordance with the regulations of the Istanbul Medipol University Ethics Committee on Animal Maintenance and Experimentation. Three out of four rats were able to reach the final phase of the paradigm. The experimental setup shown in **Figure 3.3** was used for training the rats. The amount of water delivered was initially selected to be 15uL for a single opening and closing of the solenoid valve, but it was later changed to and retained at 30uL per reward delivery as an improvement in the performance was observed with this increment. Three out of four rats reached the inclusion criteria set for Phases I, II, and III as shown in **Figure 3.4**. Rats were shaped to identify the contingency between nose-poke, lever press, and the reward. The click-sound from the solenoid valve, which was the cue for the reward delivery, was threatening for the rats at the start but they started to approach the receptacle and adapted to it within a couple of training sessions. This initial shaping procedure for receptacle identification, learning nose poke to initiate the trials, and lever press on the target side indicated by cage LEDs, in a two-lever choice task took between 9 ± 2 days. To diverts the rat's attention for target selection from the cage LEDs to the cues on the LCD outside the cage, the brightness of the LEDs was gradually reduced to zero as the success rate reached 80%. Rats were quickly able to judge target direction by the cues on the LCD, this took 2 ± 1 additional day to learn.

After operant conditioning the rats to perform two-lever choice tasks based on the visual cues from the LCD outside the cage, Phase I of the behavioral paradigm shown in **Figure 3.4A** was commenced. In this phase, upon the trial initiation by a nose-poke the

cursor on the LCD moved from its center position with a randomly selected speed among the two prespecified speeds (i.e., 1.2s and 2.2s) to reach one of the randomly selected distant targets. The targets were placed at 7.4cm from the center to either side of the center as shown in **Figure 3.3C.** The task was designed to make the rat follow the cursor motion and by judging the speed of the cursor motion press the correct lever within time restrictions (i.e., between 0.9s to 1.5s for 1.2s speed trials and 1.5s to 2.5s for 2.2s speed trials). This was done to make the rat follow the cursor motion and not just rely on the target direction cue by looking at it once at the start of the trial, deciding the trial direction, and pressing the lever as in a simple two-lever choice previously performed based on just the target direction. Learning to press the lever according to the speed of the cursor was imperative for the success in the other two phases of the training. The difference in the distribution of lever press timestamps for both speeds was an indicator that the rat can perceive the difference in the speeds and follows the cursor. If the rat learned to press the lever according to the speed of the cursor i.e., to wait in the 2.2s speed trials and go quickly in the 1.2s trials with an accuracy higher than the 70% for at least 30 consecutive trials, the difference in the behavior for the two types of trials was also clearly visible. This was a difficult task, especially in the case of 2.2s speed trials, for the rats as they are naturally inclined to get the reward as quickly as possible it was tough for the rats to wait as the cursor reached the target after 2.2s. That is why it was important to train the rat for the slower speed first and train them to wait, at least 70% accuracy should be achieved for just the slower speed trials before introducing fast speed trials. The percentage of the 1.2s trials was steadily increased to no more than 40% otherwise rats started to rely on just 1.2s trials and fail 2.2s trials. The interval to press the lever for a successful trial was decreased slowly for 2.2s trials, i.e., the rat was allowed to press the lever after 1s of cursor motion towards the target, to avoid too many punished trials and keep the rat engaged in the task. It was observed that rats perceive the faster speed better than the slower 2.2s speed. Rats tried to find an optimum time to press the lever to compensate and maximize the rewarded number of trials, this was not allowed because of the strict time restrictions forcing the rat to stay focused at the cursor motion and press the levers according to the trial type.

Lever press timestamps, with reference to the trial initiation time, were recorded for each trial. **Figure 4.1A** shows consecutive trials for a session after achieving the 70% inclusion criteria for phase I. The red dots show the trial type, and the blue dots show

the successful and punished trials. For this session of training, 40% of the trials introduced belonged to 2.2s speed. **Figure 4.1B** shows the timestamps for lever presses for each successful trial, red triangles show lever press instances for successful 2.2s speed trials, and blue filled circles for 1.2s trials. The line separating the successful 1.2s and 2.2s speed trials at 1.5s represent the threshold, 1.2s speed trials ended after this and no lever press was allowed after that and the interval to press the lever for successful 2.2s speed trials started after

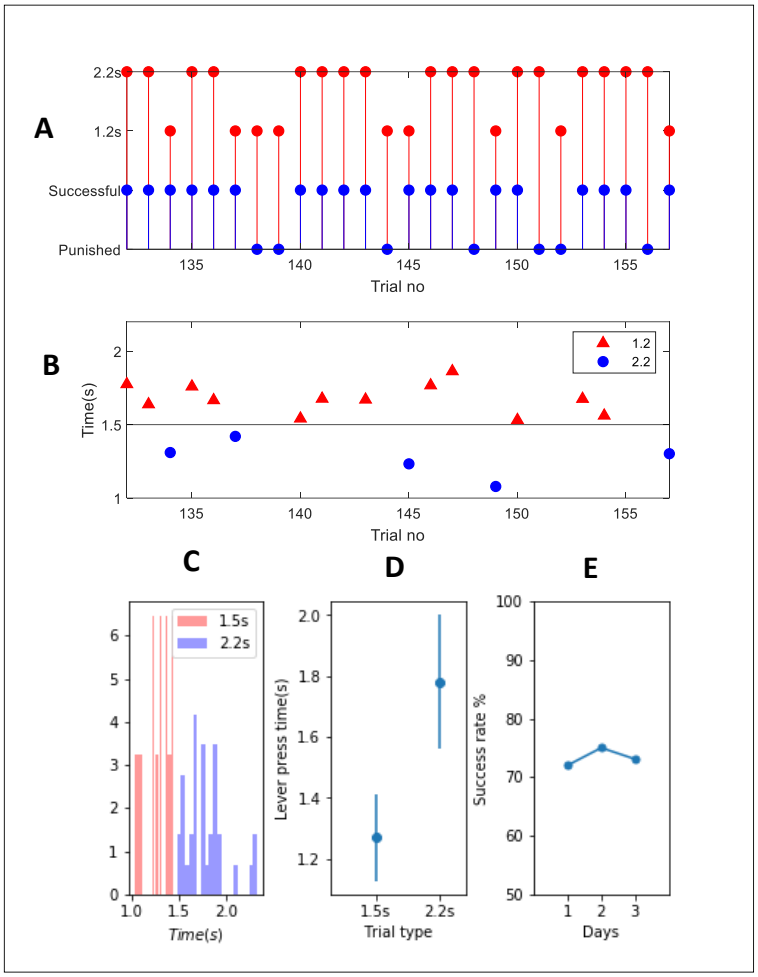

Figure 4.1: Performance of DOPA 96 for trials with variable speed in Phase II. **A)** Illustrates the types and nature of trials (i.e., rewarded or punished) during a section of consecutive trials. **B)** Represents the lever release timestamps to correct the trajectory for the rewarded trials in 1.2s and 2.2s speed trials. **C-E)** Represent the distribution, mean, and variance of lever press timestamps in 1.2s and 2.2s speed trials and success rate for four consecutive days with these settings.

this threshold, lever presses earlier than 1.5s threshold resulted in punishment for 2.2s trials. In this way, the rat could not find an optimum time to press the lever and be successful in both categories of trials. Most of the punished 1.2s speed trials were

caused by the rat being distracted and unable to look towards the LCD in time. Otherwise, the approach behavior towards the lever for the two types of trials was visibly distinct (videos demonstrating the trials with variable speeds are available), which means the rat was able to perceive the cursor motion and responded with different response times for each type of trial. It took 12±3 sessions for the rats to reach the inclusion criteria (70% accuracy for 30 consecutive trials).

Lever press time instances, for each type of trial, for three consecutive days after reaching the inclusion criteria were combined and histograms were drawn to find out the distribution of the two types of trials. As shown in **Figure 4.1C**, the two types of trials have different mean values and if a gaussian mixture model is applied on the distribution, two different peaks would be found denoting the difference in behavior for each type of trial and indicating the ability of the rat to perceive the cursor motion speeds. **Figure 4.1D** illustrates the mean and standard deviation of the two types of trials for data from three consecutive sessions after reaching inclusion criteria. There is no overlap between the error bars representing the standard deviation for the two types of trial and the mean values are different as well, indicating a difference in response time for the two types of trials. **Figure 4.1E** illustrates the success rate for the three consecutive days, this represents a plateau or convergence of the success rate, the performance could not get better any further, so the successful rats were progressed to the next phase of the training.

In the second phase (as shown in **Figure 3.4C**), rats were trained to perform two-lever choice and move the cursor towards the randomly selected trial-specific target by keeping the lever pressed for 2.2s. The purpose of this phase was to train the rat to control the trajectory of the cursor to make it reach a distant target, representing a reaching out task, in a one-dimensional, bidirectional space. This was a critical phase as maximum variation in rat's posture and behavior was seen and measures were taken to keep the behavior consistent for all the rats. Rats were punished if the lever was released before the cursor reached the target. The time required to keep the lever pressed was incremented in steps to gradually increase the difficulty level, once the rat was comfortably able to keep the lever pressed the time required was incremented until increasing it to 2.2s. If the rat tried to keep the lever pressed while leaning towards the water receptacle to drink water as quickly as it could, weight was applied to the levers to make it stay at the lever and look at the LCD. If the rat does not develop an undesired

or wrong behavior it quickly learns to keep the lever pressed while looking at the LCD. The time taken for the rats to achieve inclusion criteria in this phase was 18±4 days. The number of successful trials per day for this phase went above 500 with an accuracy of above 90%, once the rat learned to keep the lever pressed. The success in this phase meant that the rats learned to control the trajectory of the cursor to reach the target by keeping the lever pressed, a prerequisite for the next and final phase of the training.

In the third and final phase of the paradigm (as shown in **Figure 3.4**), an error in the trajectory was introduced randomly i.e., in some trials the continuous lever press for 2.2s made the cursor reach the target (See **Figure 4.2D**) but, in others, while the lever was still pressed, an error in the form of a change in the direction (towards the center or initial position of the cursor) of the trajectory was introduced. The rats were trained to release the lever to indicate the perception of a sudden change in the trajectory and to perform a subsequent single lever press to correct the cursor trajectory and make it move towards the target to receive a reward. To make it easier for the rats, the trials with error in the trajectory were only introduced on one side of the cage i.e., either on left or right and not on both sides, and only 2.2s trials were introduced on the other side. The side chosen for introducing trials with the error was the side corresponding to the dominant forelimb which was identified in Phase II of the paradigm. It was the forelimb with which the rats showed ease and minimum head movement and variations in the posture. The trials shown in **Figure 4.2A-D** are from the rat DOPA 96 with the right forelimb as the dominant forelimb, so the trials with error in trajectory were introduced randomly to the right side only.

To validate that the rats perceive the proximity of the cursor to the target and recognize and correct an error in the trajectory, apart from 2.2s trajectory trials, trials in which the cursor changes its trajectory towards the center after a prespecified interval were introduced randomly. The interval chosen in these trials with error in the trajectory was 1.5s, which means that after traveling towards the target, the cursor automatically changed its direction towards the center, all while the lever was still pressed, and a lever release and a single press was required to correct the trajectory (see **Figure 4.2C**). The term 1.5s trials are used for trials with error in the trajectory from here forward. The metric to substantiate the rat's ability to perceive and correct the error in the trajectory was the timestamp at which it released the lever in 1.5s trials. For 2.2s trials, a latency factor was enforced to make the rat keep the lever pressed for an extended 300ms before

the reward was delivered, which means that the lever release time could not have been earlier than 2.5s in 2.2s trials, which practically was most often higher than 2.8s, as shown in **Figure 4.2B, C**. So, if in the 1.5s trials, the lever release timestamp was earlier than 2.7s and around 2.5s it meant that the rat changed the trajectory in these trials after perceiving the proximity to the target and it was not because it had learned from 2.2s trials to release the lever automatically after waiting for some time. The time allowed for the rat to release the lever in 1.5s trials was gradually reduced to an optimum interval.

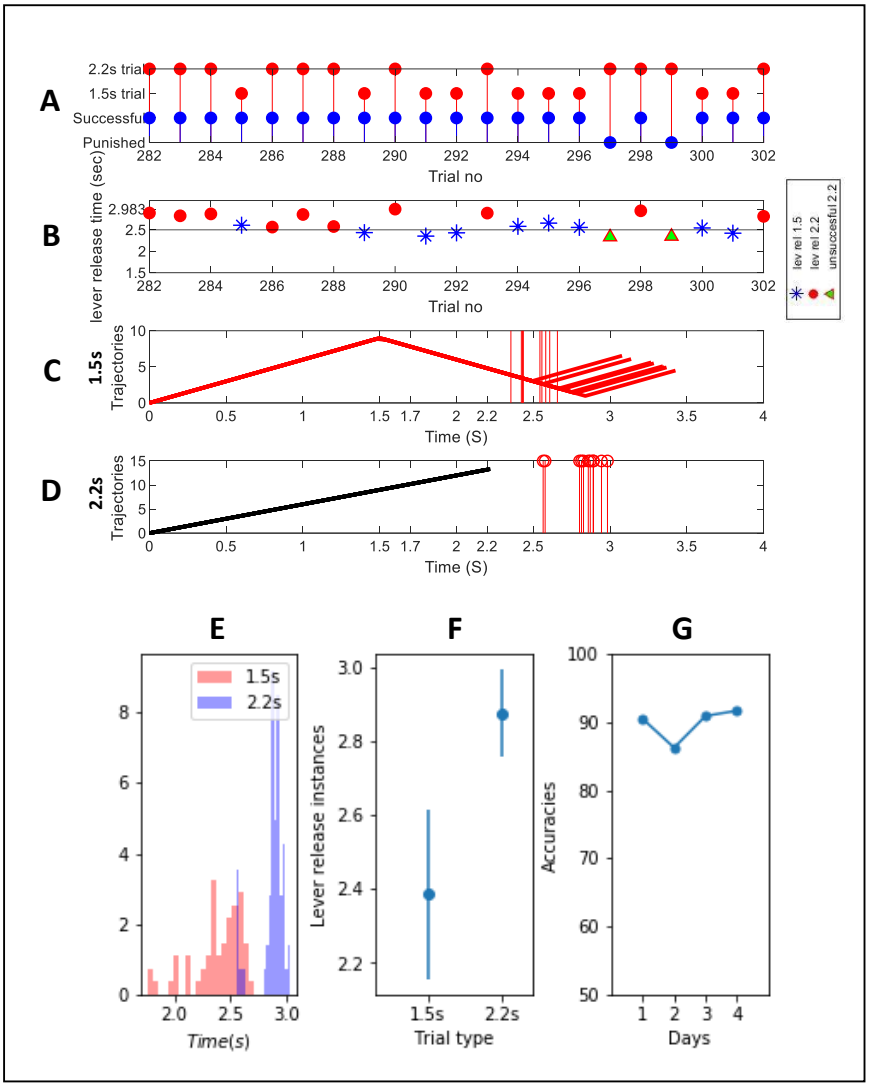

Figure 4.2: Performance of DOPA 96 for trials with various trajectories in Phase III. **A)** Illustrates the types and nature of trials (i.e., rewarded or punished) during a section of consecutive trials. **B)** Represents the lever release timestamps to correct the trajectory (for both rewarded and punished) in 1.5s and 2.2s trials. **C, D)** Illustrate the trajectories and lever release timestamps for 1.5s, and 2.2s trials correspondingly. **E-G)** Represent the distribution, mean, and variance of lever release timestamps in 1.5s and 2.2s trials and success rate for four consecutive days with these settings.

This interval denotes the time which starts at the instance the trajectory changes (at 1.5s from the cursor motion initiation from the center) till the error is corrected. The optimum time allowed to release to lever was set at 1.2s, the trial finished without any reward if the lever was not released before the end of this interval. This interval forced the rat to react before 2.7s (i.e., elapsed time after cursor movement initiation from the center) otherwise it was punished, to keep the recorded timestamps in 1.5s trials as distinct as possible from 2.2s trials. If the rat started to get punished heavily the interval was increased temporarily. It was observed that if the rat follows the cursor, it gains its rhythm again and starts to release the lever within the allowed interval.

The number of training sessions required to reach the inclusion criteria was 22±5. After achieving the inclusion criteria, the lever release timestamps for each category of trial i.e., 1.5s and 2.2, for three consecutive training sessions with the rat DOPA 96 was plotted in a histogram plot, means and standard deviations were also plotted (see **Figure 4.2E, F**). The graphs show no significant overlap between the standard deviations of the two types of trials and the means are at different values. This indicates that the rat perceives the error in the trajectory and corrects it accordingly and it is not because of coincidence or chance or influence from 2.2s trials ($p<0.001$).

In addition to 1.5s error trajectory, 1.7s error trajectory trials were also introduced by keeping the percentage of 2.2s trials 50% and 25% for 1.5s and 1.7s trajectories of the total trials. Although there was some overlapping between lever release instances in 1.5s and 1.7s, there was a trend observed and still, the lever press instances for 2.2s trials were well separated from the other trials. It was observed that in 1.7s trials rats' reaction time was faster than in 1.5s trials (see **Figure 4.3C, D, E**). It can be attributed to the fact that in 1.7s trials cursor get closer to the target than it does in 1.5s trials, which means that the closer the cursor gets to the target the better the perceivability and the faster the reaction time. As the cursor was farther away from the target at the time of change in trajectory, the change in trajectory was recognized a bit later. It indicates that the rat monitors the target and its proximity to reduce the amount of effort during the trials, it develops a strategy to gain maximum reward by just focusing on a narrower region around the target rather than following the cursor to the target. This hypothesis was tested by adding another error trajectory with 1.9s for the change in trajectory as shown in **Figure 4.4E**.

The lever release timestamps for 1.5s trials (see **Figure 4.4C-E**) have the lowest mean and the mean increases in 1.7s and 1.9s trials, the data is not sufficient to support the hypothesis as there is very little difference between the 1.5s, 1.7s, and 1.9s trials.

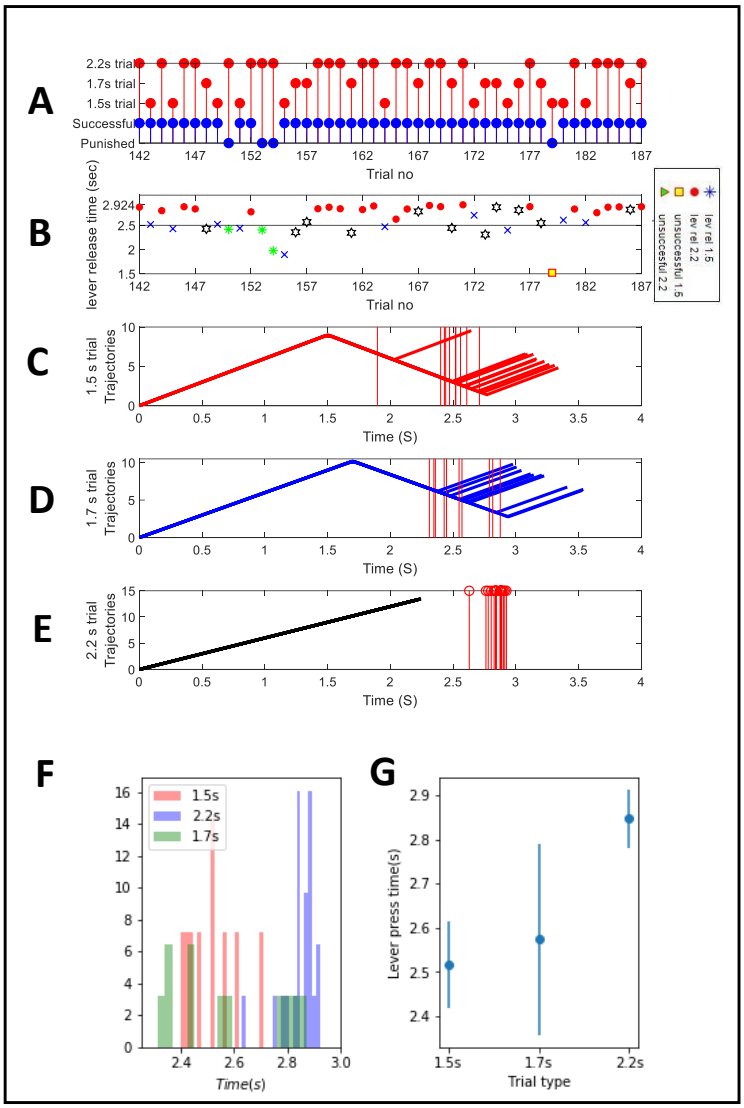

Figure 4.3: Performance of DOPA 96 for trials with various trajectories in Phase III. **A)** Illustrates the types and nature of trials (i.e., rewarded or punished) during a section of consecutive trials. **B)** Represents the lever release timestamps to correct the trajectory (for both rewarded and punished) in 1.5s, 1.7s, and 2.2s trials. **C-E)** Illustrate the trajectories and lever release timestamps correspondingly for 1.5s, 1.7s, and 2.2s trials. **F-G)** Represent the distribution, mean, and variance of lever release timestamps in 1.5s, 1.7s, and 2.2s trials from this section of training.

Nevertheless, the lever release timestamps for error trajectory trials i.e., 1.5s, 1.7s, and 1.9s are distinct from the 2.2s trials, which supports the previous results and explains

that the rats correct the trajectory by deciding to release the lever in case of an error in the trajectory. Rats were able to successfully achieve inclusion criteria until the final phase of the paradigm within 52±13 days. However, a greater number of rats should be trained for the paradigm to assess a more precise learning curve and variations between a group of rats.

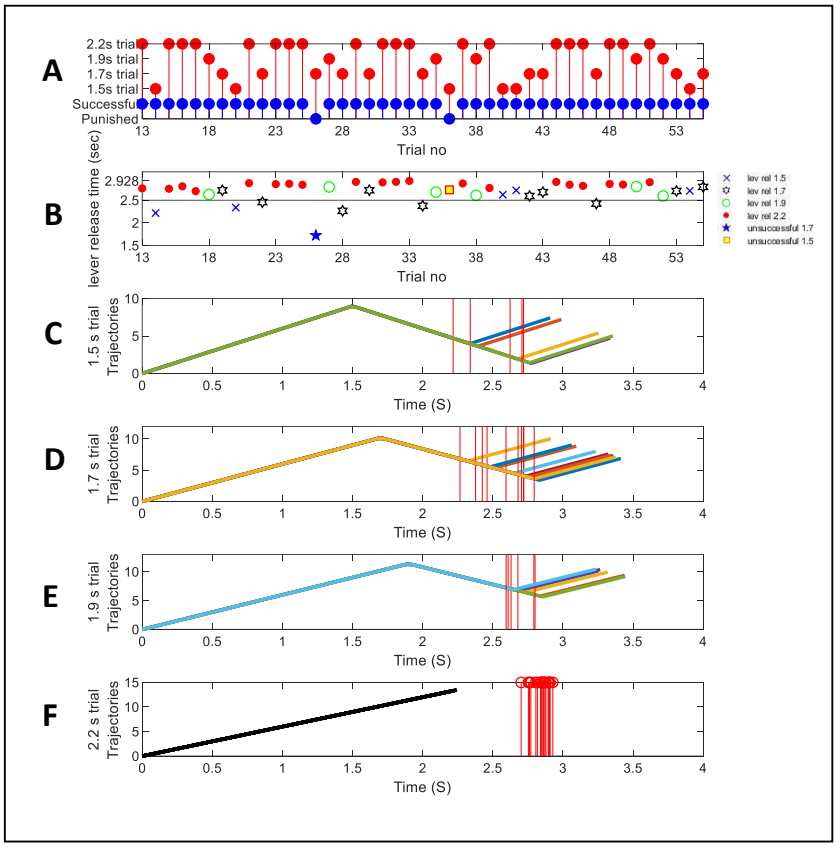

Figure 4.4: Performance of DOPA 96 for trials with various trajectories in Phase III. **A)** Illustrates the types and nature of trials (i.e., rewarded or punished) during a section of consecutive trials. **B)** Represents the lever release timestamps to correct the trajectory (for both rewarded and punished) in 1.5s, 1.7s, and 1.9s trials. **C-F)** Illustrate the trajectories and lever release timestamps for 1.5s, 1.7s, 1.9s, and 2.2s trials correspondingly.

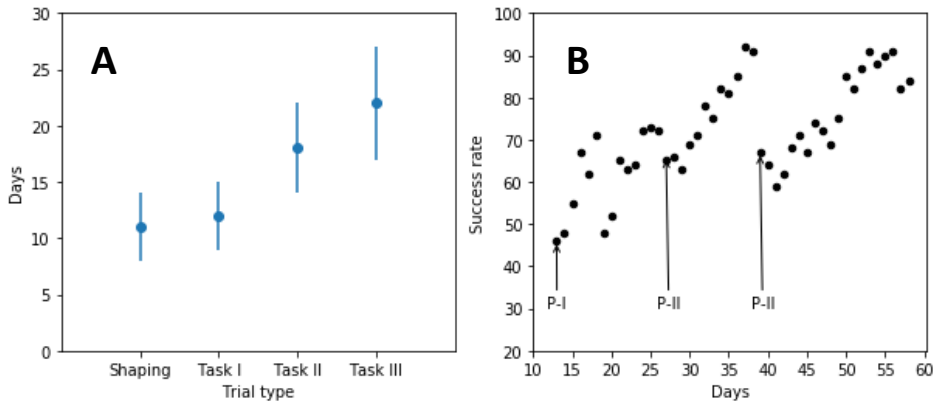

Figure 4.5: A) Represents the number of training sessions required to achieve inclusion criteria for each step of the paradigm. Mean and variance are shown for each step. **B)** Illustrates the success rate in each training session throughout training for DOPA 96, P-I, P-II, and P-II represent the accuracies in the introductory sessions for Phase I, Phase II, and Phase III of the paradigm correspondingly.

4.2. Discussions

In this study, an experimental setup was designed to operantly condition rats to perform center-out reaching tasks by actively controlling the trajectory of a cursor based on visual feedback. An interesting aspect of this setup was the provision to introduce an error in the reaching trajectory in randomly selected trials that have not been addressed yet in any of the rodent models for motor skill learning for center-out reaching tasks.

This study aimed to establish whether rats are visually capable of perceiving the proximity of a moving visual stimulus from a distant target, and more importantly, can they combine motor and visual capabilities to compensate for an error in the direction of the moving visual cue. A practical implementation of the task demanded the fulfillment of two requirements. The first requirement was to develop a behavioral setup to allow the rat to react with learned stereotypical movements in response to a visual cue and the second was the development of a robust visual feedback system to allow the rat to observe the visual cues as effectively as possible. To fulfill the first criteria, a lever mechanism was designed (as shown in **Figure 3.3B, C**) to train the rat to lever press by keeping the body stationary. Levers were placed inside transparent plexiglass enclosures with a slit big enough to enter one paw (see **Figure 3.3C**). This discouraged the rat to use both paws of mouth to press the levers. The lever press mechanism had the provision to apply a dead weight to increase the force required to press the lever. This increase in required force to press the lever restricted excessive head and/or body

movements. Obstacles were placed to keep the rat at the center of the cage and a by-product was the availability of support which aided in keeping the rats still as they preferred to lean on it while keeping the levers pressed. Dimensions of the setup were carefully designed to allow easy and consistent movements to reach levers, receptacle, and nose-poke area while standing on the same spot in the cage (see **Figure 3.3C**). The second requirement was to develop a visual feedback system to train and assist the rats in decision making and to direct them when to respond by pressing or releasing the lever during the trials. A robotic arm with a guide LED for a center-out movement was chosen in the start to act as a moving visual cue. The brightness of the LED was adjustable to fine-tune it and make it just bright enough to ensure the best performance. Two rats were trained with this setup for the behavioral paradigm, and both learned to perceive the direction and speed of the robotic arm movements towards the targets by correctly performing lever press and release. One problem associated with the robotic arm was the noise the servo motor generated while moving, this noise produced induced doubts on the rat's ability to follow and correct the trajectory merely based on the visual feedback as the change in noise level when the robotic arm changed direction could be acting as an auditory cue for the rat to just follow the sound and ignore the visual feedback. Considering this problem posed using servo motor, the paradigm was shifted to presenting visual cues on an LCD. This was much reliable visual feedback as no noise was involved.

In the final implementation of the paradigm, visual cues were displayed on an LCD. A cursor was drawn in the middle of the screen two distant targets depending on the trial type. Different colors were tested for targets and cursor while the background was kept dark, as rats have better capability to distinguish colors near the blue and ultraviolet light wavelengths and for green color, blue color for the cursor was selected initially, while green colored targets were used. Rat's performance for target selection trials was not good with the blue-colored cursor. Different mixtures of blue and green were also used by changing the range of pixel values for these colors between 0 to 255. White color was found to be the best choice for the cursor. Rats are good at distinguishing different objects so to differentiate cursor and targets circular-shaped cursor was used, while a green-colored grid was used for targets [36]. Different sizes of the cursor and the targets were tried, and the dimensions were chosen to be as small as possible to keep the luminance from the cursor at a value that would not create temporary blindness in

the rat by consistently looking at it. The part of the LCD other than the visual cues was covered with a black cloth to keep the interference from the luminance of the backlight at a minimum.

Rats were allowed to freely move in the cage while training, i.e., no head fixation was performed in our paradigm. It is now a commonly used method to restrict movements for better in-vivo recordings as artifacts due to motion and limb movements are negotiated by fixing rat's head, but this method has some pitfalls as it increases the stress hormones in the rats, and it can affect rat's performance [18]. Still, the excessive head movement also has a detrimental effect on the performance, so to overcome this issue a virtual head fixation based on machine learning techniques can be added to this paradigm so that the rat would be punished if it moves to head out of a described area during the trials.

CHAPTER 5

5. DESIGN AND FABRICATION OF MICROELECTRODE ARRAYS

Our goal was to develop a multi-channel microelectrode array that is highly flexible in mechanical properties and suitable for chronic use. The steps followed in the production process of the microelectrode arrays were in accordance with the guidelines described in [21]. The methods we determined for the fabrication of the microelectrode array are described below.

5.1. Photomask Design

Photomask was designed in an AutoCAD environment. As can be seen in **Figure 5.1**, the photomask consists of 4 square-shaped parts. Each portion is designed to fabricate a single layer of the microelectrode array. The upper right (as shown in **Figure 5.1.a**) part is the lowest layer of the microelectrode array consisting of SU8, the lower right (as shown in **Figure 5.1.b**) part is the electrical conduction paths layer made of gold in the microelectrode array, the lower left (as shown in **Figure 5.1.c**) part is the SU8 layer that covers the electrical conduction paths, the left The upper part (as shown in **Figure 5.1.d**) is designed to form the electrode recording area layer made of gold.

The mask is designed to produce 38 microelectrodes at a time. The corresponding part of the photomask was used to create a layer of the microelectrode sequence. The mask was rotated clockwise, it was aligned using the aligner, and the desired layer was created using the relevant photolithographic method. The mask design seen in **Figure 5.1** was carried out in a way that made it possible to produce two types of microelectrode sequences. Fifteen microelectrodes with a length of 3.5 cm in the first type and twenty-three microelectrodes with a length of 2.5 cm in the second type were produced (a total of 38 microelectrode sequences). Having different length

microelectrode sequences will give us an advantage during in vivo experiments. The more practical and less risky will be preferred during implantations.

The photomask is designed to allow the other end of the microelectrode array to be attached to a ZIF (zero insertion force) connector. Figure 10 shows the mask design corresponding to the end of the microelectrode array that will provide the ZIF connector connection. Figure 11B shows the ZIF connector we supplied and Figure 11C shows the ZIF connector connection mechanism. The make and model of the ZIF connector are Hirose FH12A-32S-0.5SH(55). The photomask design has been made to adapt to the pin spacing of the connector. When the microelectrode array is produced, the tip shown in Figure 10 will be inserted into the ZIF connector using a microscope and the connection of the microelectrode array to the connector will be fixed by the mechanism seen in Figure 11C. The ZIF connector will then be connected via a cable or PCB to the Omnetics connector (compatible with the head stage).

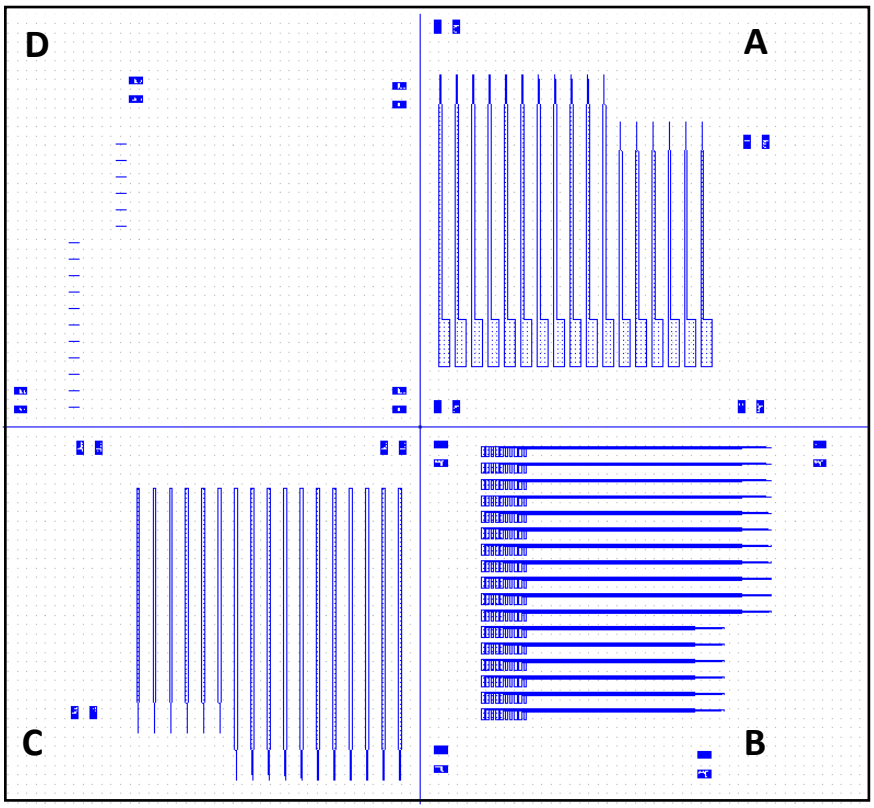

Figure 5.1: Photomask design consisting of 4 parts to produce biocompatible microelectrodes. The numbers of each part are indicated by white numbers.

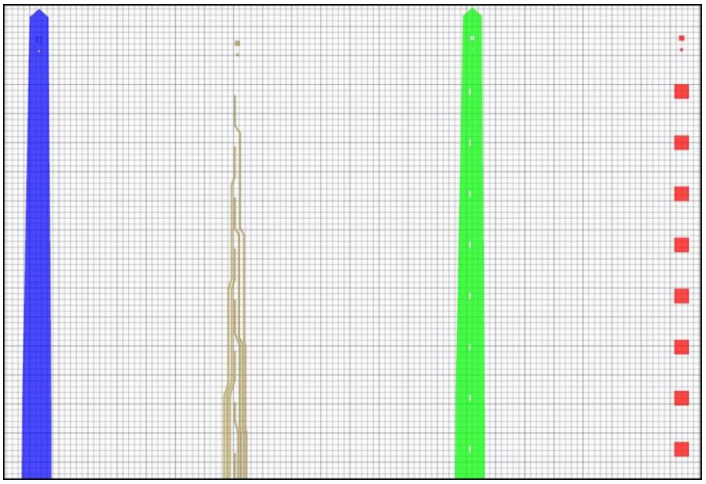

Figure 5.2: 4 different masks prepared to form the 4 layers of the microelectrode array. in this image.

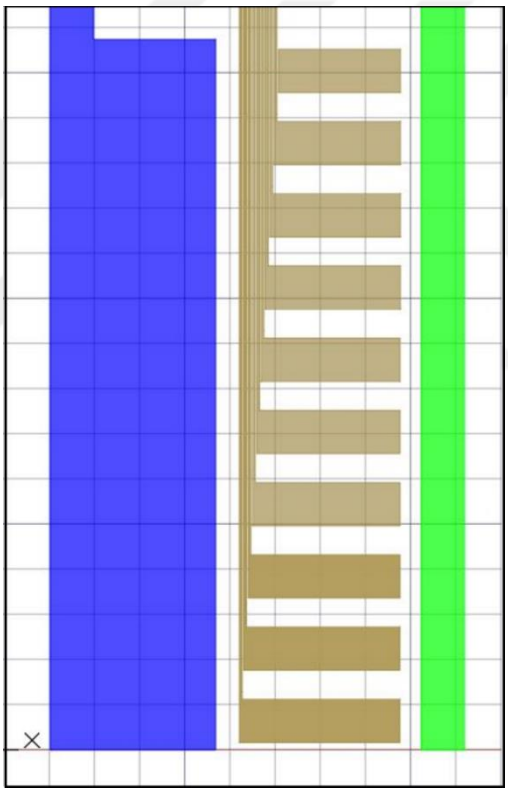

Figure 5.3: Photomask design for connecting the microelectrode array with the ZIF connector. To form the SU8 layer, which is the lowest (first) layer of the blue drawing microelectrode array, the gold conduction paths layer, which is the second layer of the dark yellow drawing microelectrode array, and the SU8 layer, which is the third layer of the green drawing microelectrode array, which will be used to cover the gold conduction pathways. will be used. Thanks to this design, parts of the second layer (gold transmission line layer) that are not under the third layer will be exposed. The ZIF connector will be attached to this part without any soldering.

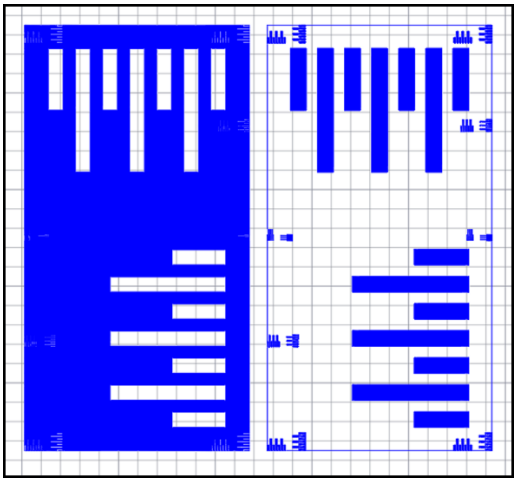

Figure 5.4: Vernier design for photomask. Vernier is used to align the mask during microfabrication. The mask is placed at the 4 corners of each part of the mask shown in **Figure 5.1** to enable alignment.

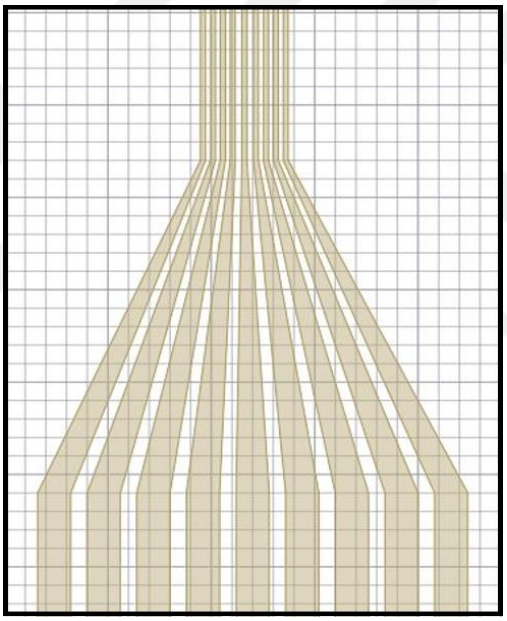

Figure 5.5: Photomask design to create gold conduction pathways. The conduction paths are thinned when approaching the end of the microelectrode array so that the tip of the microelectrode in the target area to be recorded is made as thin and soft as possible. The end of the gold conduction pathways at the tip of the microelectrode array is shown in **Figure 5.2.**

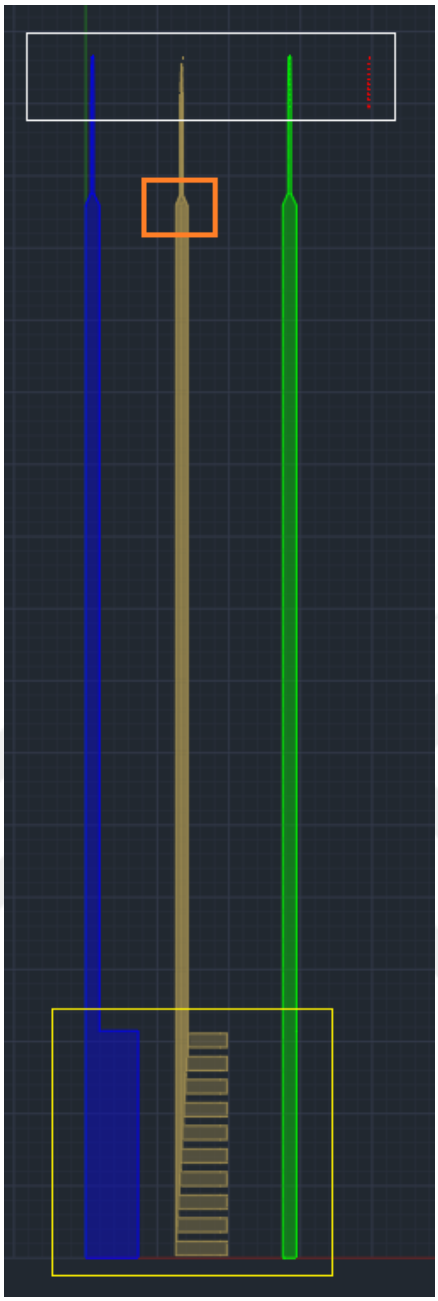

Figure 5.6: Mask design for 4 layers for each microelectrode array. The white box indicates the end of the microelectrode array that penetrates the brain tissue, and the yellow box indicates the part that will be connected to the ZIF connector. A zoomed-in view of the white box is shown in **Figure 5.2**, a zoomed-in yellow box is shown in **Figure 5.3**, and a zoomed-in orange box is shown in Figure 13. The DXF format drawing file of the image can be downloaded from the link https://1drv.ms/u/s!AsMDMspJ-A9ihvUflZExuGlUm_P1eA?e=eR2G9w.

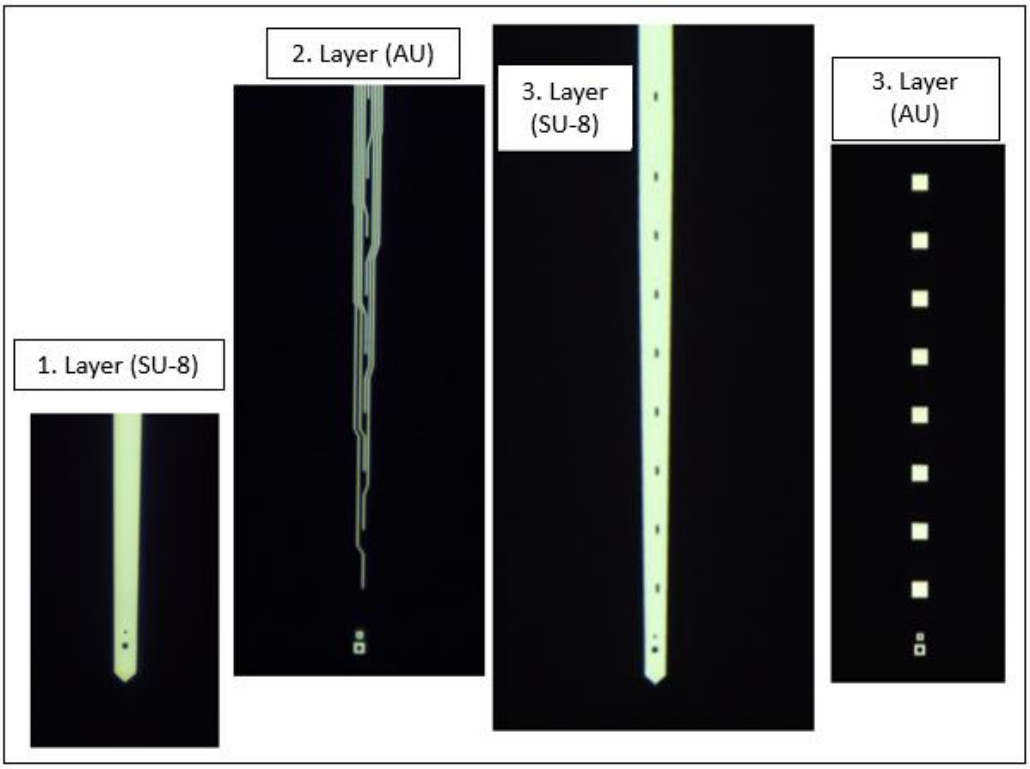

Figure 5.7: A microscope image of each layer's photomask for a microelectrode.

5.2. Photolithography

The materials required were SU-8 2000.5, LOR3A, S1805, SU-8 Developer, CD 26 Developer, and Remover PG. SiO2 layered wafer (substrate) was used in the production of the microelectrode array. It was obtained from Alttaş, Nanografi (Ankara). Substrate properties were selected as follows: Si+SiO2 (wet), Size: 4 inches, Orientation: (100), Boron Doped (p-type), Resistivity: 1 - 10 ohm. cm, single side polished, Thickness: 525 ± 25μm, Sio2 coating thickness: 300nm.

In the first step, 100 nm thick aluminum was coated on the substrate with the thermal evaporation technique. This layer is prepared to remove the microelectrode array by chemical etching after the growth of the microelectrode array and the completion of the augmentation processes, thus separating the microelectrode arrays from the substrate. In other words, the aluminum coating is formed as the sacrificial layer. The aluminum-coated substrate was sliced into 4 equal parts, as ¼ of the photomask was used in each layer. **Figure 5.8** shows an aluminum-coated substrate divided into four.

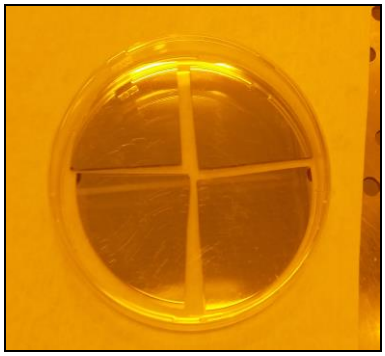

Figure 5.8: Aluminum coated Si+SiO2 substrate.

5.2.1. Layer 1

The materials required were SU-8 2000.5, LOR3A, S1805, SU-8 Developer, CD 26 Developer, and Remover PG. SiO2 layered wafer (substrate) was used in the production of the microelectrode array. It was obtained from Alttaş, Nanografi (Ankara). Substrate properties were selected as follows: Si+SiO2 (wet), Size: 4 inches, Orientation: (100), Boron Doped (p-type), Resistivity: 1 - 10 ohm. cm, single side polished, Thickness: 525 ± 25µm, Sio2 coating thickness: 300nm.

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Figure 5.9: The first layer of the microelectrode array (SU-8). Microscope image (left) and image on the substrate.

5.2.2. Layer 2

After the SU-8 patterns were formed, the second layer containing the gold conductor lines was formed after it was heated to 150 degrees. For this, LOR3A and then S1805 positive photoresists were coated on the surface by spin coating method at 300 nm and 500 nm, respectively. Heating was applied for 5 minutes at 180 degrees for LOR3A and 1 minute at 115 degrees for S1805. After the part of the photomask prepared for the second layer was aligned with the substrate, the photoresists were irradiated with 20mJ/cm2 UV light at 365nm for 2 seconds. Alttaş was placed in CD-26 Developer and gold transmission lines were created. Then, the substrate surface was coated with 3 nm thick chrome and 100nm thick gold by a thermal evaporation method. With Remover PG, lift-off was made for the gold layer and transmission lines were created. The lift-off process and the formed gold transmission lines are shown in Figure 5.10.

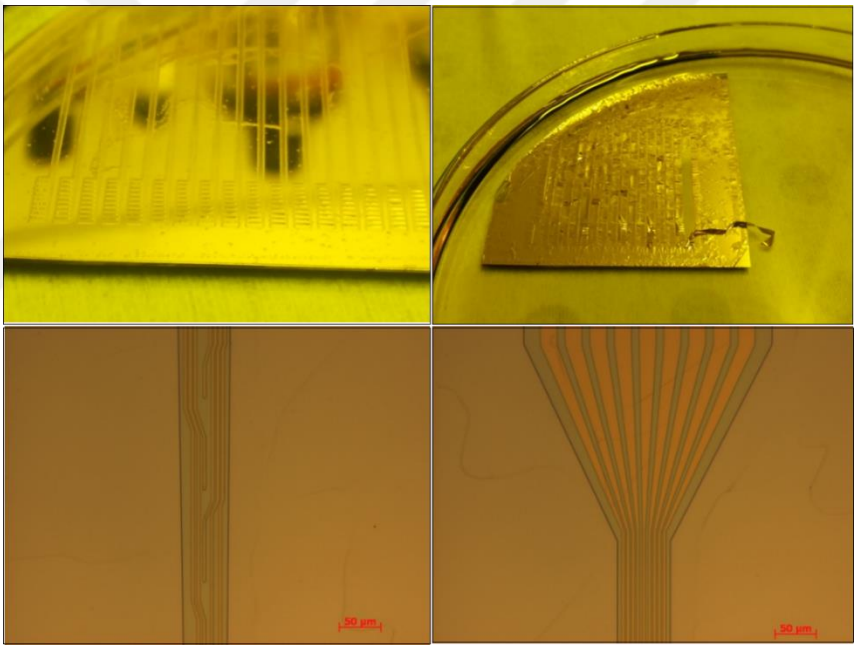

Figure 5.10: Creation of the second layer (gold transmission paths). The gold-plated substrate is in the **upper left**, the gold lift-off process is in the **upper right**, the formed 3 micron-wide gold paths are in the **lower left**, and the 20-micron gold paths leading in the connector are seen in the **lower right**.

5.2.3. Layer 3

After the creation of the golden paths, the third layer was created. In this layer, SU-8 is used as a biocompatible material to provide electrical insulation, as in the bottom layer. To create this layer, the methods used in the formation of the lowest SU-8 layer were applied.

Figure 5.11: Microelectrode array image after forming the third layer (SU-8).

5.2.4. Layer 4

After the creation of the third layer, the process of creating the gold register areas was started. The recording fields form the last layer (fourth layer) of the microelectrode array. In the formation of this layer, the chrome-gold plating and lift-off processes in the formation of the second layer, which can be seen in **Figure 5.10**, were followed. The gold record fields created are shown in **Figure 5.11**.

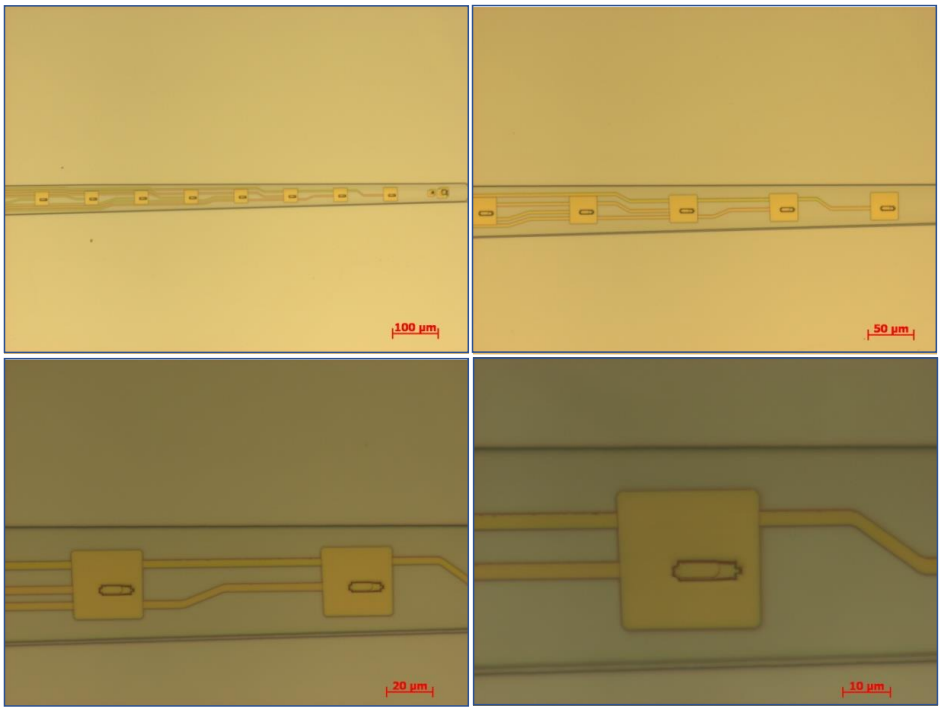

Figure 5.12: A microscope image of the microelectrode array at different zooms after the formation of the fourth layer (the gold recording fields). Recording fields are seen as square-shaped. The third layer (SU-8 layer) provides electrical isolation between the transmission paths (second layer) and the recording areas (fourth layer).

After the fourth layer was formed, the production process of the microelectrode array was completed. The wall thickness of the microelectrode array was measured as 1.85

µm with the surface profiler. We think that this thickness will provide sufficient mechanical softness. The conductivity between the recording areas of the microelectrode array and the connector legs was also measured. As a result of the measurement, the resistance between the recording areas and the connector legs was measured as 316 ohms. Connector feet and recording area used for resistance measurement are shown in **Figure 5.13**.

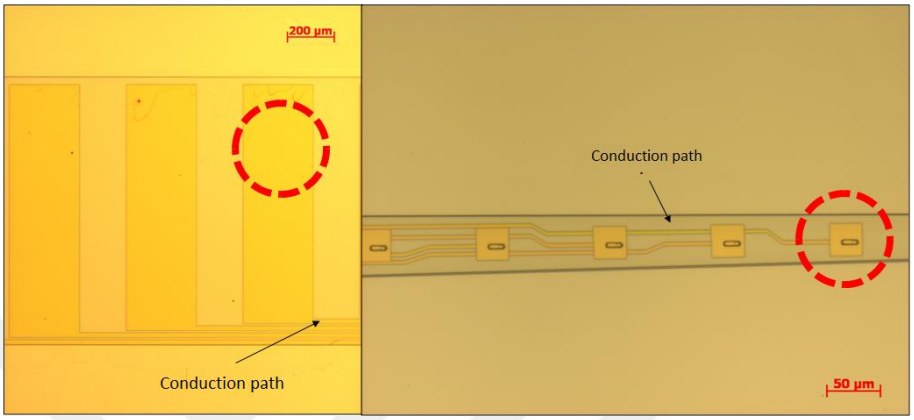

Figure 5.13: Microelectrode array connector legs **(left)** and recording areas **(right)**. The connector legs of a microelectrode array are shown on the left. The recording areas of a microelectrode array are shown on the right. One each of the connector legs and the registration area is indicated by a red dashed circle. Transmission paths are indicated by black arrows. The transmission path provides signal transmission between the recording area and the connector legs.

The microelectrode array needs to be separated from the substrate before connecting to the Hirose ZIF connector from the legs seen in the left photograph in **Figure 5.13**, and the microelectrode array will be connected to our electrophysiological and voltammetric recording systems via this connector.

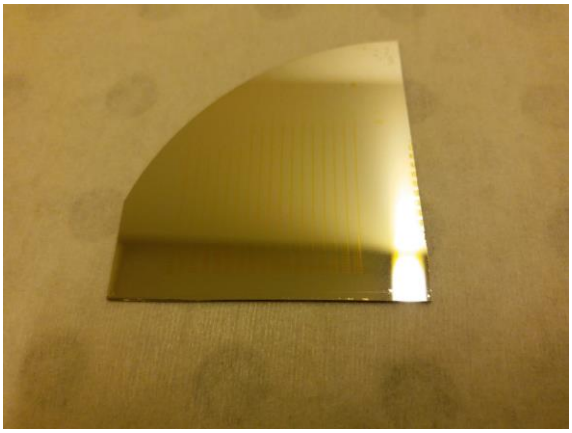

Figure 5.14: The condition of the completed microelectrode arrays (17 in total) on the substrate. With the aluminum etching process, the microelectrode knees will be separated from the substrate.

5.3. Etching

For removing the microelectrode array from the substrate, a chemical etching process of the sacrificial aluminum layer was required. First, tungsten etchant (667498-500ML, Sigma Aldrich), which is stated to be effective for aluminum, was ordered and tested. We chose this etchant as both tungsten and aluminum etchants, thinking that we could also thin the tungsten wire that we will use as a guide to advance the microelectrode array through the brain tissue. However, in our experiments, it was observed that the etching process progressed more slowly than desired. On top of that, hydrochloric acid (37%), iron (III) chloride (40%), and distilled water were mixed in a ratio of 1:1:20 as another etchant that would provide faster etching and not disrupt the structure of the microelectrode array. It was observed that the desired etching speed was achieved (**Figure 5.15** and **Figure 5.16**). It was observed that the microelectrode arrays remaining in the etchant in approximately 4-5 hours were separated from the substrate surface.

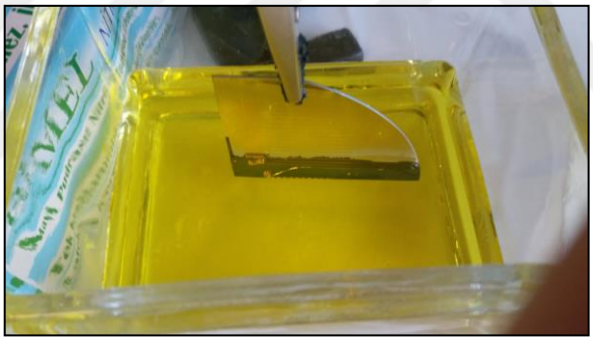

Figure 5.15: Separation of the microelectrode array from the substrate by chemical etching

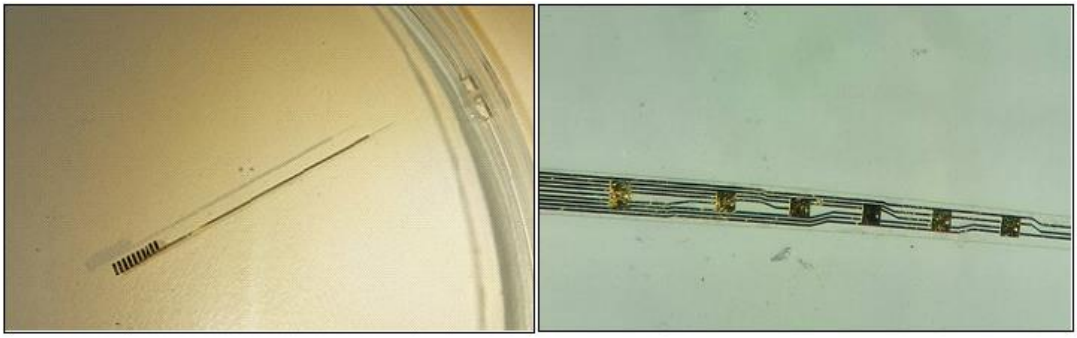

Figure 5.16: An image of the microelectrode array separated from the substrate floating on distilled water (left) and the image of the microelectrode array removed from the substrate on a glass slide.

It was observed that the microelectrode arrays separated from the substrate were hydrophobic and therefore floated in the water. It was observed that it adhered permanently to the surface when it came into contact with glass surfaces, as in the photo on the right in Figure 3. On top of this, the microelectrode array was hydrophilized by the oxygen plasma method to prevent sticking to glass surfaces and to facilitate subsequent processes. Oxygen plasma was applied to a microelectrode array, which was previously separated from the substrate, with the same parameters, and it was observed that it floated in the water, became hydrophilic, and did not suffer any damage on the surface of the microelectrode array.

Oxygen plasma application to the microelectrode array provided hydrophilization. However, it has been observed that aluminum is oxidized when oxygen plasma is applied while the microelectrode array is still on the aluminum-coated substrate, and therefore the aluminum etchant we use (a mixture of hydrochloric acid, iron (III) chloride, distilled water) remains very slow in etching the oxidized aluminum surface. In **Figure 5.17**, it was observed that some microelectrode arrays did not separate from the substrate surface even after 10 days. However, it was observed that these microelectrode arrays were also separated from the surface in the following days.

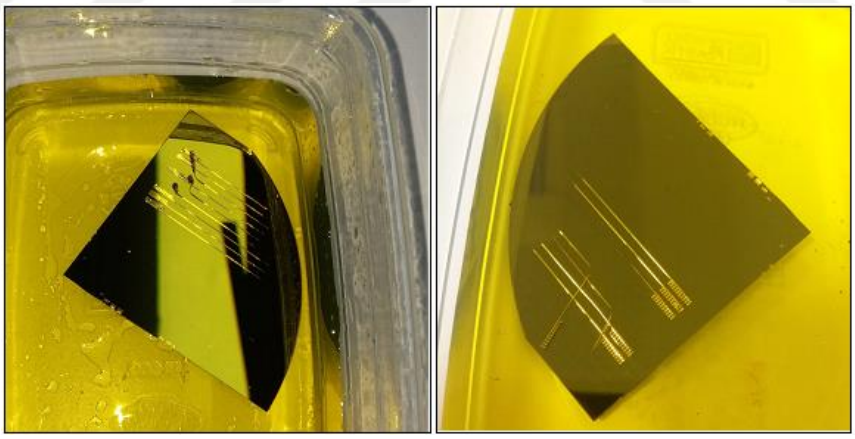

Figure 5.17: After 24 hours (left) and 10 days (right) in the etchant.

After this experiment, it was decided to apply oxygen plasma to the microelectrodes after they were separated from the substrate surface in the next productions. In addition, it was thought that it would be appropriate to carry out further processing by holding the microelectrode arrays separated from the substrate with plastic materials (e.g., plastic tweezers or sticks).

A microelectrode array, smoothly separated from the substrate surface, was connected to the 32-channel connector (Hirose FH12A-32S-0.5SH(55)) and made ready for implantation. For this, a low-cost method has been developed. The template shown in the left photo in **Figure 5.18** was printed on transparent acetate paper from a printer with toner. The lines on the template are formed to correspond to the connecting legs of the Hirose FH12A-32S-0.5SH(55) connector. Thus, the microelectrode array seen on the left in **Figure 5.16**, separated from the substrate, is aligned on the template in the photo on the left in **Figure 5.18**, it shows a microscope image of the aligned microelectrode array in the right photograph.

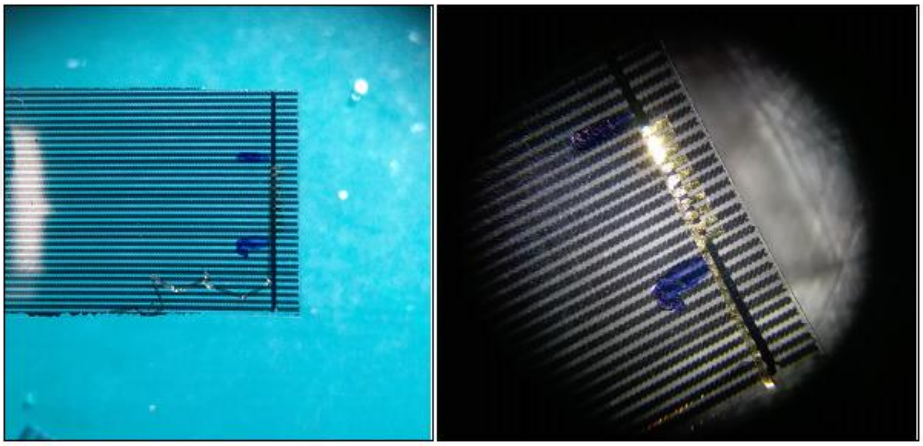

Figure 5.18: Alignment of the microelectrode array with the connector template.

After the microelectrode array was aligned on the template shown in **Figure 5.18**, it was attached to the Hirose connector soldered to the PCB. The left photo in **Figure 5.19** shows the connection between this microelectrode array and the connector. After this process, a glass capillary tube (VitroCom CV1017) with an inner diameter of 100 µm and an outer diameter of 170µm was glued on the template as seen in the right photo in **Figure 5.19**. The length of the glass capillary tube and the width of the template are equal. Then, a 50micron thick tungsten microwire was passed through a glass capillary tube, again as seen in the photo on the right in **Figure 5.19**. This tungsten microwire will be used as a guide to penetrate and advance the microelectrode array, which is made of very soft material, into the brain tissue.

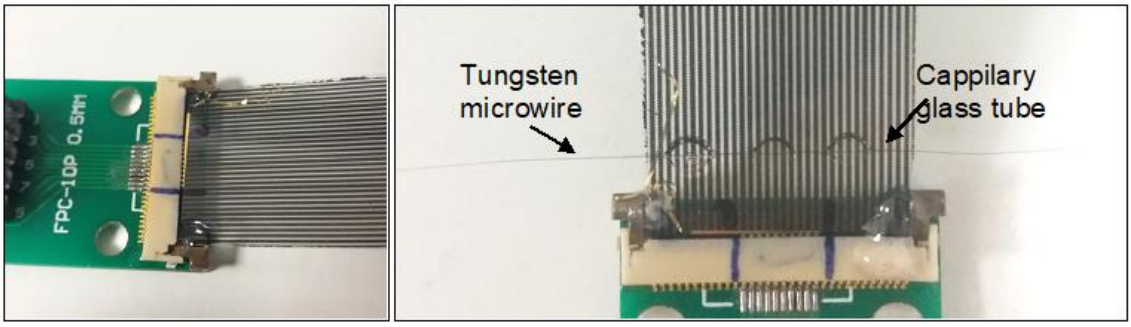

Figure 5.19: Connecting the microelectrode array with the Hirose connector (left) and fixing the glass capillary tube (inner diameter: 100 µm, outer diameter: 170 µm) with a 50 µm thick tungsten microwire inserted on the template with adhesive (right).

Polyethylene glycol (PEG) was used as a biocompatible material to attach the microelectrode array to the tungsten microwire to be used as a guide. Since the thickness of the microelectrode array is only 1.8 µm, it is not possible to hold it with tweezers and align it on the tungsten microwire, the microelectrode may be damaged. The setup we prepared for the process is shown in **Figure 5.20**.

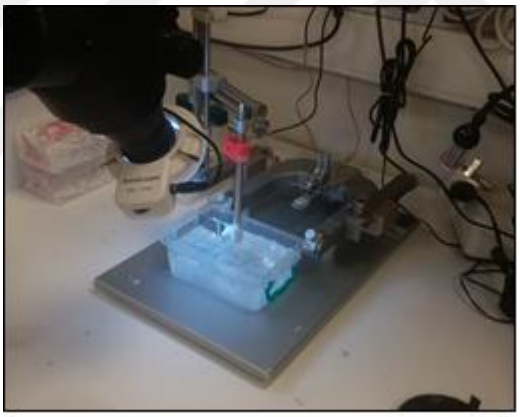

Figure 5.20: Device for attaching the microelectrode array to the tungsten microwire.

As shown in the left photo in **Figure 5.21**, the microelectrode array was brought close to distilled water and the tip was made to float on the water with surface tension. Likewise, the tip of the tungsten microwire is immersed in water. With a very soft and thin Teflon rod, the microelectrode array was brought close to the tungsten microwire and adhered to the microwire by adhesion. The microwire array and microelectrode array were then slowly moved upwards together.

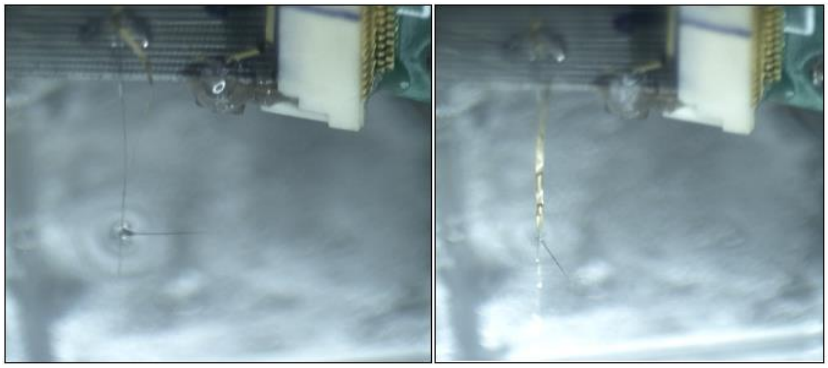

Figure 5.21: Attachment of the microelectrode array to the tungsten microwire.

As seen in the right photo in **Figure 5.21**, as the microwire and microelectrode array move upwards, the surface that adheres to each other increases. Then, as the microwire and microelectrode array came out of the water, 5% w/v PEG solution was applied to the microwire with a syringe. Thus, permanent adhesion of the microelectrode array to the tungsten microwire is ensured. **Figure 5.22** shows the microelectrode array attached to a 50μm thick tungsten microwire via PEG.

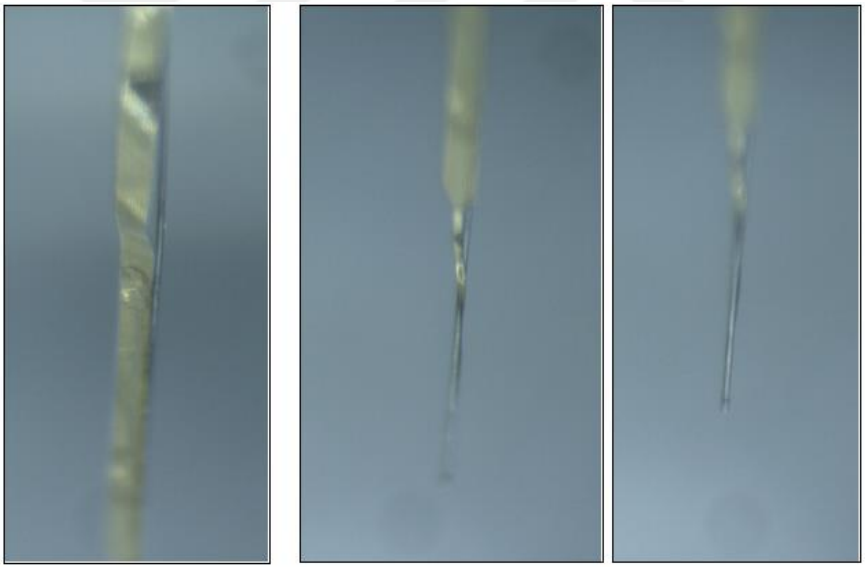

Figure 5.22: Permanent attachment of the microelectrode array to the tungsten microwire with PEG. The middle of the microelectrode array with the conduction lines and its attachment to the tungsten microwire with PEG (left). The attachment of the thin tip of the microelectrode array with the recording areas to the tungsten microwire with PEG (middle and right).

The microelectrode array ready for implantation into brain tissue attached to the tungsten microwire is shown in **Figure 5.23**. The microelectrode array can be connected to electrophysiology and voltammetry systems via the green PCB shown in the figure and the header connector on this PCB. After the tungsten microwire and microelectrode

array are placed in the brain tissue, spontaneous dissolution of PEG in the tissue and separation of the tungsten microwire and microelectrode array from each other will be ensured [22]. After the microelectrode array and tungsten microwire are separated from each other, the tungsten microwire will be pulled up through the glass capillary tube and removed from the brain tissue. Such microelectrode array will remain within the brain tissue. In the next phase, the implantation of the microelectrode array in the brain tissue and in vivo recording processes will be started. The design and fabrication of the microelectrode arrays are finished.

CHAPTER 6

6. CONCLUSIONS AND FUTURE WORK

In this study, we proposed a new rodent behavioral paradigm for closed-loop control of the trajectory of a cursor based on visual feedback. This is an effort to propose an alternative methodology to study the center-out reaching task in rodents with an addition of the introduction of errors in the trajectories and methodology to train the rats to be able to correct the errors that have not been addressed before in behavioral studies pertinent to this task. The findings of this study can contribute significantly towards the development of better BMIs and neuroprosthetic devices. The outcomes of this study are as follows; a fine-tuned behavioral setup is presented for the perception of trajectory error of cursor, rats can perceive the error in the trajectory of a cursor based on the visual feedback provided, rats possess the visuomotor capability to correct the error in trajectory.

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