POLITECNICO DI TORINO

Master of Science in Biomedical Engineering

Master's Thesis

USING TAPERED FIBERS FOR RECORDING AND MANIPULATING NEURAL ACTIVITY IN BEHAVING MICE

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Abstract

Self-initiated movements are a very hot topic among the neuroscientists. In this regard, a hypothesis suggests dopamine neurons play an important role since basal ganglia disorders also show problems related to movement initiation, like Parkinson's disease. Different approaches try highlighting the structures involved, but optogenetics is, to date, the best compromise.

This work uses for the first time tapered optical fibers to record the activity of dopamine neurons in head-fixed transgenic mice. According to several scientific articles, this novel approach is minimally invasive if compared with the flat-faced optical fiber. Moreover, they have one or more windows that permit to direct the light towards new brain structures. The movement artifact due to this novel approach is then investigated. This isn't negligible, but it doesn't compromise the findings. Finally, the tapered optical fibers are used to manipulate the neural activity in behaving mice.

The results show tapered optical fibers can withdraw a fluorescence signal. Moreover, calcium signals recorded on two diverse locations into the mammalian brain are similar, suggesting dopamine neurons involved during voluntary movements. About the stimulation through the tapered optical fibers, it appears they induce an early reaction, confirmed by a statistical analysis.

Classification tools could be used to improve the reliability of these results eliminating grooming movements. Moreover, the experiment could be repeated using a mirror system to direct the light through windows of tapered optical fibers.

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Introduction

Voluntary movements involve the initiation of movement but also the inhibition of unwanted movements when at rest.
In primates, Basal Ganglia (BG) are involved in action selection. They represent a collection of nuclei interconnected through several pathways, that work together to produce the desired movement.
This study focuses the attention on the Nigrostriatal Pathway, also called Dopaminergic since it involves dopamine to modulate the activity of several structures in the brain. In particular, this work tries understanding if dopamine neurons are related to voluntary movements.
Nowadays, much information, about how BG work, comes from the analysis of the clinical phenomenology of some its human disorders [1].
Some diseases involve a loss of dopaminergic neurons and problems related to the movement. It isn't still clear how these two effects are related together. Several laboratories are trying to understand which are the structures involved in the voluntary movements and how they are linked together.
Focusing only on clinical evidence it is unrealistic since the BG disorders involve several symptoms, not only related to the movement.

This work tries to verify an interesting hypothesis that states that the dopamine neurons are involved in self-initiated movements.
This study focuses the attention on two locations into the mammalian brain: the

cell bodies, where the dopamine neurons are, and the terminals, where the dopamine signal is transmitted.

The thesis is divided into five chapters: the first one explore the voluntary movement, indicating on the principal structures involved. In this regard, some disorders, like Parkinson's disease, that show a loss of dopamine neurons and problem to self-initiate an action are studied. Subsequently, the principal method to investigate the action potential into a mammalian brain is proposed. The classical approach, electrophysiology, is compared to optogenetics. It is decided to explore several techniques. In this regard, the tapered optical fibers are described, comparing with the flat-faced optical fibers. The third part of this work explores the photometric approach to record simultaneously the neural activity *in vivo* in two different spots. This was done injecting a fluorescence protein, GCaMP6f, into two DAT-cre mice and placing in the terminal the novel fiber and in the cell body the cut optical fibers. This part also investigated the movement artifacts due to the tapered optical fiber. The analysis was possible through two transgenic mice and a fluorescent protein, GFP. The fourth chapter explores the use of the optical fibers to manipulate the neural activity in head-fixed transgenic mice. Manipulation involved alternate stimulation days through tapered optical fibers or flat-faced fibers. In the concluding two chapter, the results are presented and then discussed.

This research work permitted to analyze for the first time the use of the tapered fibers in a photometric approach.
The results of these experiments show that the tapered fibers have a great potential to record and manipulate the neural signal activity in behaving mice.

Chapter 1

Basal Ganglia: pathways and disorders

This chapter explores voluntary movements, focusing the attention on the action initiation and the inhibition of unwanted motions when at rest.
The first section describes basal ganglia, the part of the brain involved for this purpose.
The second part focuses the attention on two significant pathways, the direct and indirect. This section proposes two competitive models that try to explain how they work.
The first one assigns the action initiation to the direct pathway whereas the movement inhibition to that on indirect. The second model proposes a coordinated activation of both paths during a voluntary action.
The latter part concentrates on the symptomatology due to basal ganglia's disorders. The Parkinson's disease is investigated, focusing on its typical signs related to the movements.

1.1 Basal Ganglia

The basal ganglia are part of the prosencephalon. They are usually involved in transferring the cerebral cortex activity to direct action, like control of movements or cognitive actions or reward-based selection of results.
The basal ganglia receive input from all four lobes of the cerebral cortex, but provide efferent projections only to the frontal cortex, via the thalamus.
The basal ganglia (Fig. 1.1) consist of five interconnected nuclei: [2]

- the caudate nucleus
- putamen
- globus pallidus
- subthalamic nucleus
- substantia nigra.

The striatum is the largest nucleus and the main input structure of the basal ganglia. It consists of the caudate nucleus and putamen, called dorsal striatum, and the nucleus accumbens, named ventral striatum. Caudate nucleus and putamen are the input nuclei for the basal ganglia.
The globus pallidus is divided into the internal and external part.
The internal globus pallidus in an output of the basal ganglia with the substantia nigra pars reticulata.
In particular, the substantia nigra is divided into two parts, the previously cited the substantia nigra pars reticulata, and the substantia nigra pars compacta.
The second one assumes an important role in the nigrostriatal pathway, also called the dopaminergic pathway since involves the dopamine. Finally, the subthalamic nucleus lies below the thalamus. It directly communicates with the globus pallidus and the substantia nigra pars reticulata.

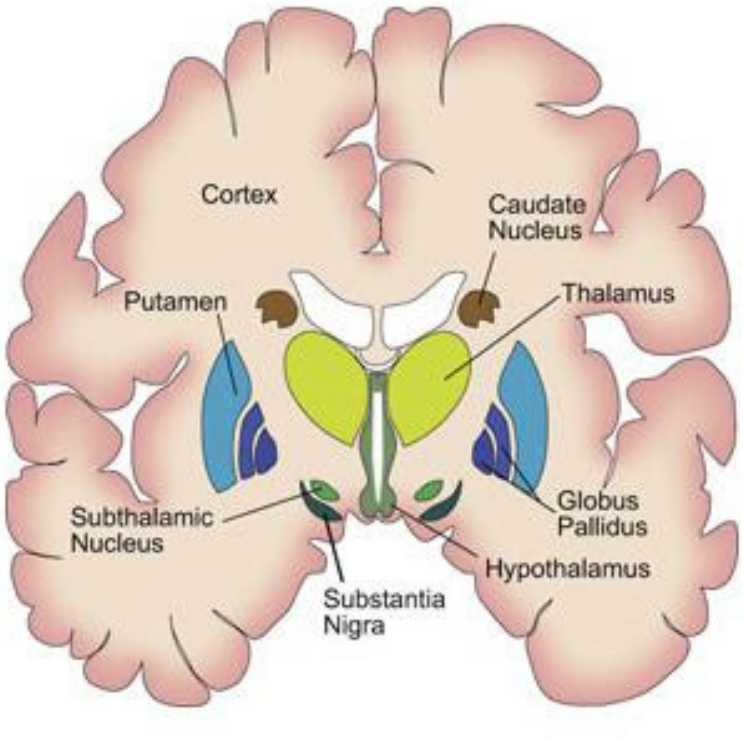

Figure 1.1: Basal ganglia representation. The cortex, the thalamus and the hypothalamus are also included since related to the movements.
Source:`https://2.bp.blogspot.com/_kaQ5P19FVgk/Sg7a0dM4FfI/AAAAAAAAC90/8_NQlwUxk_4/s400/BasalGanglia2.JPG`

All these structures, including the thalamus, work together to regulate the movements.

The globus pallidus and the substantia nigra pars reticulata provide the output of the basal ganglia through inhibitory projection, also called GABAergic projection since this involves GABA neurons.

The execution of the desired movement is related to the projection of the thalamic nuclei to the motor cortex. Depending on the power of the connection, it is possible the planning and execution of movement behavior.

Most of the hypotheses, about how the basal ganglia work, come from the clinical observation of some diseases related to itself. Using the phenomenology of this

disorder to comprehend how the basal ganglia work leads to intrinsic advantages and disadvantages [1].
Sometimes, this simplifies the analysis since the clinical observation is intuitive. On the other hand, the principal disadvantage is that disorders to the basal ganglia involve various symptoms not only related to the movement.

According to Parkinson's disease, there is a loss of dopaminergic neurons in the substantia nigra pars compacta and the symptomatology of this disease suggests this part of the brain is necessary for spontaneous movement.
This issue will be investigated more in details in the following sections.

1.2 Direct pathway and Indirect Pathway

The output of the basal ganglia converges to the thalamus which is typically under inhibition. The restriction of the thalamus leads to the excitation of the cortex. The greater the inhibition, the more probable the movement is.
The cortex has a direct linking with the muscle involved in the movement selection. There are two major paths that work to induce the desired action, the direct and indirect pathway (fig. 1.2).
The direct pathway provides a connection between the output neurons of the basal ganglia and both globus pallidus internal segment and the substantia nigra pars.
In this case, the cortex transmits excitatory messages to the corpus striatum releasing glutamate.
These excitatory nerve cells create synapsis with the inhibitory neurons located in the striatum. Successively, the striatum becomes more active and its inhibitory neurons can inhibit the globus pallidus internal through GABAergic release.

Since the internal part of the globus pallidus is restrained by the corpus striatum neurons, its activity is turned down and it can't inhibit the thalamus so much.

In this way, the thalamus is able to get a bit more excited and project excitatory messages to the motor cortex.
Successively, the motor cortex becomes more active, and it sends excitatory messages to the muscles to develop the desired movement.

According to Gerfen and Surmeier [3], it appears dopamine receptors, located in substantia nigra pars compacta, involve into the two striatal projection systems. The neurons of the direct pathway bind D1 dopamine receptors, instead, D2 receptors are involved in the indirect pathway.
According to their model, when dopaminergic neurons bind these receptors, this increases the activity of the direct pathway and vice versa for the indirect way.

Indirect striatal projection involves the activity of the globus pallidus external and subthalamic nucleus. The first one receives GABAergic input from the striatum. These inhibitory neurons are sent to the subthalamic nucleus. In turn, this one provides excitatory projections to the substantia nigra pars and globus pallidus internal. They are both the output neurons of the basal ganglia.

The fig. 1.3 provides an illustration of two mice's brain. In the first, the direct pathway was promoted. To do this, a mouse expressing Drd1a promoter was used. The fluorescent image highlights the principal structures involved in this striatal projection. On the right, by contrast, a mouse promoted Drd2 gene was used. It is interesting to note the difference between the two. Focusing the attention on the globus pallidus external, it is possible to detect the silent activity in the direct pathway, in contrast with the indirect striatal projection.

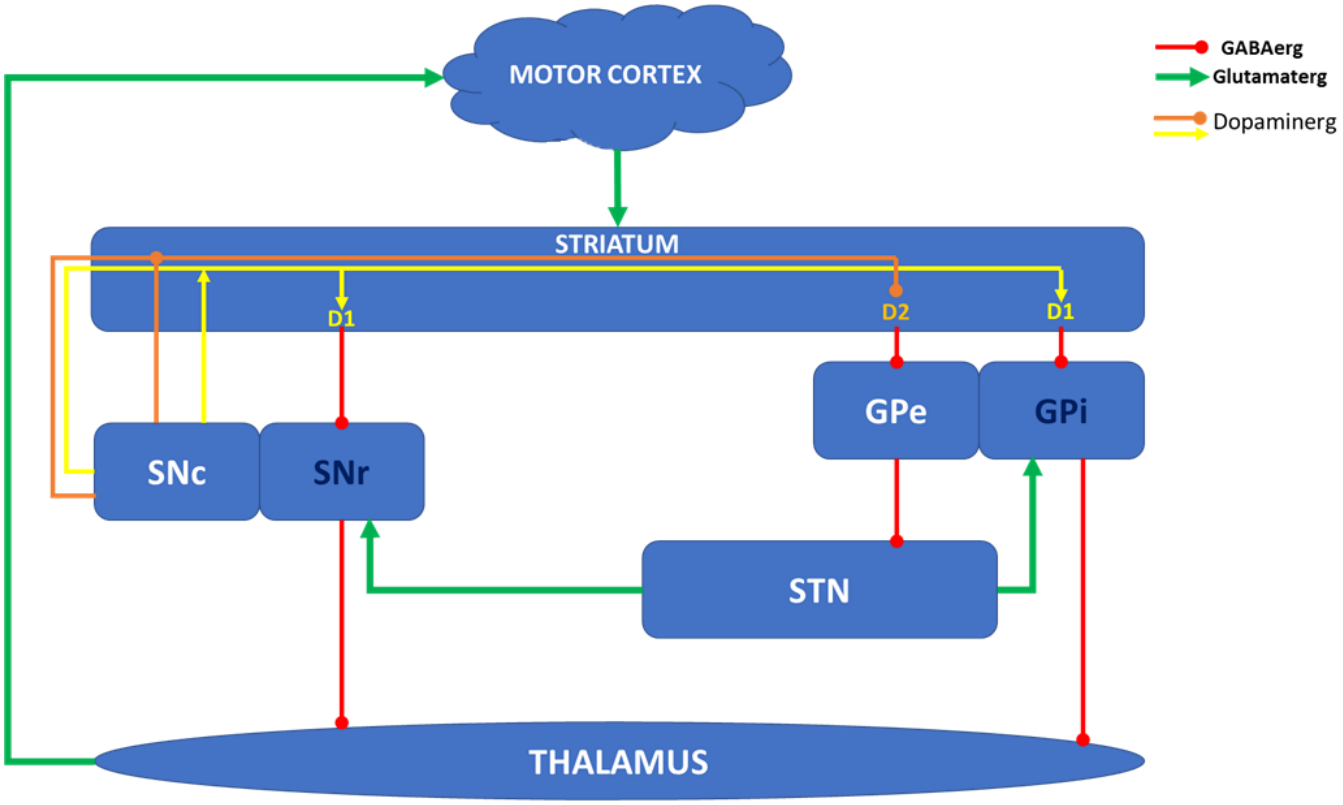

Figure 1.2: The cortex provides excitatory inputs to the Striatum, that provides inhibitory input to globulus pallidus internal (Gpi) and substantia nigra pars reticulata (SNr). These two are able to disinhibit the thalamus, through GABA neurons. The disinhibition of the thalamus leads to the activation of the motor cortex, that permits the movement. In this pathway, the substantia nigra pars compacta (SNc) plays an important role. In fact, this gets stronger the direct pathway through D1 receptors that modulate the dopamine release. The indirect pathway leads to a minor inhibition to the thalamus since the striatum interacts firstly with globus pallidus external (Gpe). The Gpe release GABA neurons to the subthalamic nucleus (STN) that sends excitatory inputs to Gpi and SNr. The major excitation of these two leads to a minor disinhibition of the thalamus. In this way, the thalamus can't stimulate properly the cortex. Also, in this case, the SNc plays an important role since it releases dopamine to the striatum through the D2 receptor that is able to get stronger the stimulation of the Gpe.

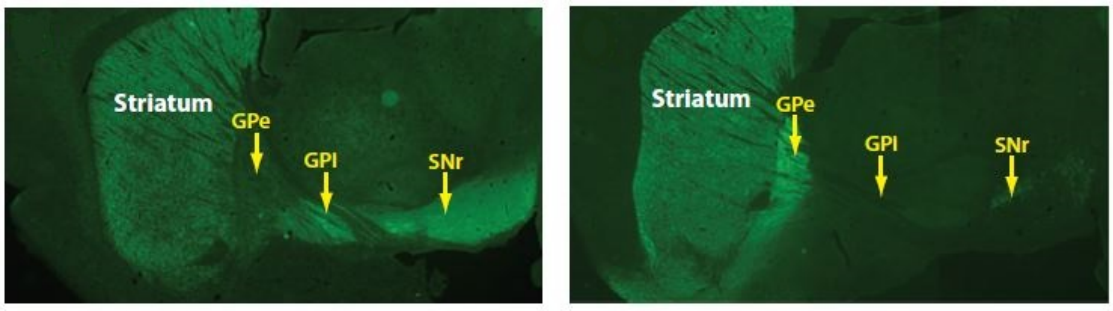

Figure 1.3: On the left, fluorescent imaging of a mouse brain under regulation of the Drd1a promoter shows direct pathway. On the right, fluorescent imaging of a Drd2-eGFP mouse shows the indirect pathway. [3]

1.3 Parkinson's disease

Parkinson's disease is a progressive and neurodegenerative disorder. It worsens over time because there is a loss, a degeneration of neurons in the brain.
In Parkinson's disease, there is a loss of dopamine neurons. It appears these are crucial for allowing to make normal movements.
It is possible to see in patients with this disorder a loss of the neuromelanin in substantia nigra pars compacta. In turn, there is a disappearance of dopaminergic neurons. This equally affects the presence of dopamine in the striatum.

There are several signs typically involved in patients with this disorder, but this work focuses the attention only with those related to the movements.

1. Shakiness as tremors in the hand or the finger.
2. Stiffness, it is felt when the person bends part of their body like the arm or leg or wrist. In this case the movement becomes extremely slow, It doesn't feel very fluid anymore and instead, it feels really rigid.
3. Slowed-down movement, they can execute them, but it just takes a longer time to do them

4. Problem with balance, feeling really unstable and unsteady when standing or walking around.

The first three are typically used to decide whether or not someone has Parkinson's disease. In any case, not all the patient with this disorder will have all of these problems.

The critical issue remains the impossibility of dealing with the cause of this disease. The mechanism, that leads to a loss of dopaminergic neurons in the substantia nigra and in the striatum, is still unknown.
For the majority of them, it's unknown what triggered the disease, so these cases are called idiopathic since the origin is unknown. In many cases, there is no family history of the disorder, about 15% of people do have a family history. For these individuals, the Parkinson's disease appears to be provoked by a mutation in one of the genes.
Depending on which gene is the culprit, a mutation can either cause Parkinson's disease or it is able to increase a person's chance of developing this disorder. So genetics is the culprit once in a while, but most of the time it is impossible to know the causes of this condition.
Despite this, there are some risk factors associated with developing Parkinson's disease[4]:

- having a certain gene mutation;
- exposure to certain pesticides and some cleaning chemicals;
- older ages, after 60 years old;
- history of concussions;
- gender, men are a bit more likely than women;
- regularly breathing in heavy metal particles like copper or manganese or lead.

It is possible to study Parkinson's disease in an animal model using MPTP [5].

This is a synthetic substance able to cause in the animal the same motor symptomatology typically seen in patients with Parkinson's.
The symptoms are variegated and not necessarily related exclusively to the motor field; moreover, they change from person to person.
Mahlong DeLong [5] proposed a model to investigate the movement problems related to basal ganglia disorders. An alteration of the output provided by internal globus pallidus and substantia nigra pars compacta leads to basal ganglia disorders. This means there isn't the balance between the direct and indirect pathway, anymore.
If the indirect path prevails, the effect of completely inhibiting the movements leading to bradykinesia would be obtained. Otherwise, if there is a prevalence of the direct pathway, hyperkinesia expects.

Cui, Jin et al. [6] confirm this model investigating on freely mice. Each mouse selectively promoted the direct and indirect pathway, and GCaMP3 protein was expressed to examine the fluorescent signal.
According to their results, when the movement disorders appear, this is due to a not appropriate coordination between the direct and indirect pathway. Moreover, they explained the animal rest as the shutdown of both pathways.
This appears to be in contrast with the prevalent model, which relates the movement to the direct pathway and the rest to the indirect pathway.
The classical model explains the bradykinesia only related to that one indirect; that is, the direct pathway is completely turned off and the movement is controlled by the inhibition of the indirect pathway. On the other hand, the hyperkinesia is explained as the inefficiency of that one indirect, completely turned off.
This other model [6], instead, relates the movements impairment to an inefficiency coordination of both pathways. Bradykinesia and hyperkinesia are both controlled by the direct and indirect pathway, but unable to produce the desired effects.

Chapter 2

Optogenetic tools

This chapter explores the optogenetic tools, proposing new approaches and introducing tapered optical fibers.
The first part analyzes alternative methods to investigate neural activity. Several authors have proposed alternative approaches because, to date, there is no optimal technique. Each device is described, highlighting advantages and limitations.
From the analysis, optical fibers with a ferrule are the best compromise between costs and results.
The subsequent part focuses on two types of optical fibers, one flat and one tapered.
In particular, the second type represents the novel approach studied during this work.

2.1 State of art

The principal purpose of all the authors that will be presented is to study the neural activity in freely moving mice.
Warden, Cardin and Deisseroth [7] offer a review of the principal methods to examine the mammalian brain.

Electrophysiology is one of the most important techniques to investigate and record neural activity. Even if this is an excellent method there are some limitation, mainly if the neuroscientists want to investigate the neural activity in freely mice.
Optogenetics represents a possible approach. There are several works about optogenetic technologies.
This method represents a science that combines optical tools to genetic manipulation, to control or manipulate the neural activity.
The figure 2.1 is taken from Warden et al. work, [7]. The part above shows the classical method, electrophysiology, compared with the novel approach, optogenetics.
The principal advantage of using optogenetics is the selectivity given by the genetic modification. In recent years, this multifaceted science has been applied to various regions of the mammalian brain, as this work [8] demonstrate. Chen et al. described what an appropriate practice should include during the optogenetic analysis.

1. A light-activated protein, capable of interact with neural activity highlighting distinct cellular process, depending on the purpose and the macromolecule used.

2. A viral vector, that permits to direct the protein towards the target, genetically modifying the neural response. Particularly, this allows the neuron to react to light.

3. The light, able to excite or inhibit the modified neurons, depending on the protein used.

4. A readout system to investigate and analyze the collected data.

Their paper [8] summarizes the principal works in several regions of the mammalian brain as the cerebral cortex, thalamus, hippocampus, ventral tegmental

area, nucleus accumbens, striatum and others.

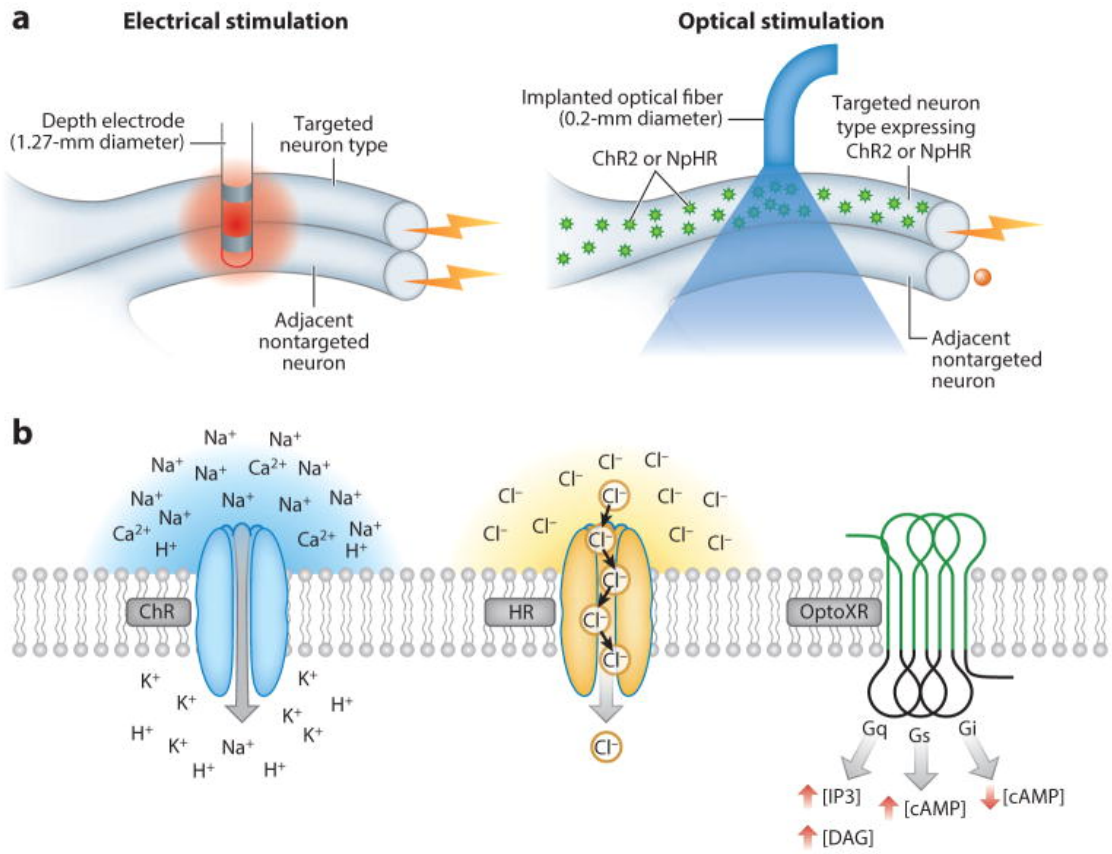

Figure 2.1: The upper part of the figure illustrates the difference between the use of electrophysiology and optogenetics. The optical approach offers a better selectivity because it also works genetically. In part b of the figure, three different optical tools are presented. ChR is the most important family capable of inducing an action potential. The HR-family inhibits the neural activity. OptoXR-family groups several engineering tools that can modulate or record the neural activity. [7]

The key goal of the last few years is to investigate neuronal activity in the brains of allowed mice to move. Over time, several approaches are proposed. A brief overview is presented [7].

The first application dates back to 2007 using a cannula. This cannula was implanted by means craniotomy. Through the same hole, a virus was injected. This

virus was a ChR2 expressing. The cannula was, additionally, used to insert the optical fiber during the experiment. With this approach it was possible, for the first time, to stimulate a targeted area. Moreover, it offered to manipulate genetically and optically at the same time. Typically, it is preferred an implantation for a protracted period, to reduce the cost and the impact on the rodent.
The subsequent work, in fact, proposed the implantation of a flat-faced optical fiber.
The fiber was composed by a metal ferrule placed above the glass part. The ferrule was used during the experiment to connect the optical fiber to the external readout system.
Once inserted, it was impossible to repeat the insertion since the optical fiber was glued to the scalp. This decreased the possibility to produce damage on the neural tissues of interest, but at the same time reduced the malleability of the experiment. Several approaches tried overcoming this problem.
A work has proposed a multi-fiber system to simulate multiple sites at the same time. This was problematic to implant and also very expensive to carry out.
As previously explained, the light carries out a fundamental role in the optical science. Different light sources have been used by neuroscientists.
The first approach was the laser setup because the light emitted could attain an adequate intensity, despite possible losses during coupling. On the other hand, this system is expensive, compared with LED apparatus, and not well performed for all the wavelengths.
Nowadays, the majority of labs uses LED light to illuminate regions of the brain through the optical fiber. The laser systems have many advantages; most of them listed in [7].
However, there are some disadvantages:

- it is difficult to obtain high light power;
- it has some problems with the multicolor experiments.

- it could create artifact due to connection between LED and the electrical wires.

Other two remarkable works are important to explore.
One concerns the multi-site recording and stimulation. In this regards, there are many innovative approaches only at the level of research. Some models remain valid only in theory because extremely complicated to perform or expensive for *in vivo* testing,
Anikeeva et al. [9] have proposed an optetrode useful for recording and stimulation of neural activity in transgenic mice. The figure 2.2 illustrates the constitutive parts of the device.
The optical fiber is inserted in the center of the device, inside the perforated screw which constitutes the support for the entire device. Sixteen microwires are contained in four tetrodes and they are all connected to a breadboard. The readout system is constituted by the ferrule of the optical fiber and the breadboard.
They were able to assemble a compact device with minimal invasiveness. The output diameter is about 200 μm.
To stimulate and record the neural activity an optical fiber was used, capable of light delivering.
Four tetrodes, instead, were able to investigate, virtually, the neural activity of different neurons at the same time. They were connected to a breadboard. A readout system could be able to obtain the action potential from each microwire.
The novel approach is represented by the use of a perforated screw with a dual purpose: a support for the entire device and a way for the insertion of the fiber.

The last design proposed in this review is still not well performed, but it represents a possible future approach.
Since the principal purpose remains the neural activity investigation on freely moving mice, several wireless devices are proposed.

They are based on LED light source and, theoretically, they permit the examination of more rodents at the same time.
Typically, the proposed dimensions are compact with obvious advantages, but at the same time a limitation about the heat dissipation. Another drawback is the low power output. Noways, only the blue light seems to be well performed. Kim et al. [10] proposed a device which integrated many functions including the possibility of wireless control.
The figure 2.3 illustrates on the left all the structures which component the microdevice. On the right, instead, two freely moving mice with the wireless device are photographed.
This optogenetic injectable apparatus includes a light source, a temperature sensor, a needle for the injection, microelectrodes for multi-recording and an inorganic photodetector.

All the presented devices have the same purpose, that is, the examination of the neural activity in behaving mice. Some of these devices are very expensive to manufacture or require a different readout system than usual, already used in the laboratories. Novel approaches have been proposed and the optical fiber is, to date, one of the most used tools to record and stimulate neural activity in mammalian brains.
The photometric apparatus is not expensive if compared with other systems and it offers a reliable measurement. As already explained, this approach has limitations. For this reason, the novel technique, the tapered optical fiber, will be investigated. This presents great advantages, as it will be explained well later; moreover, it doesn't need a new recording and stimulation apparatus different from that one already used in laboratories.

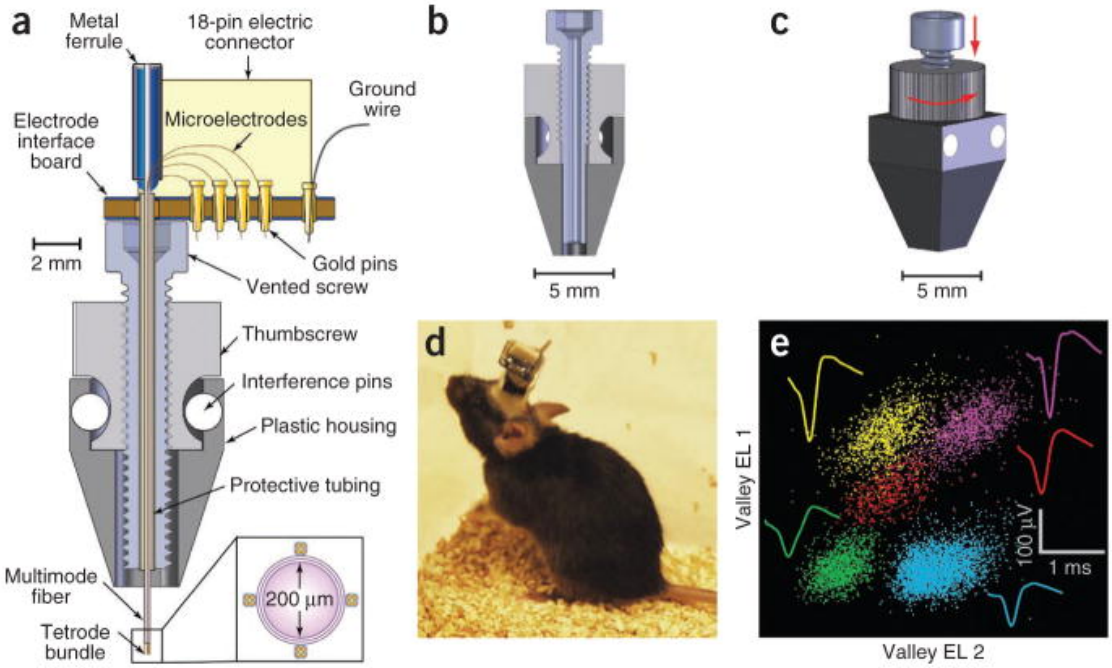

Figure 2.2: On the left, a schematic illustration of the device is presented. The dimension of the craniotomy is limited, preventing the neural damage or excessive invasiveness. The figure b and c present a vertical cross-section and a 3D view. They are both useful to see the screw mechanism able to change the region investigated. In part d, a freely moving mouse is photographed. The part e illustrates different clustered action potential obtained by the optetrode. [9]

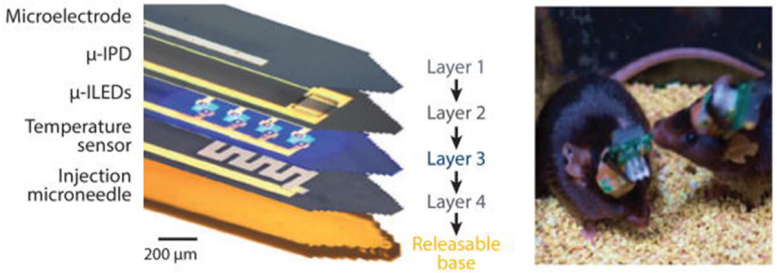

Figure 2.3: On the left all the structures which component the micro-device is presented. On the right, instead, two freely moving mice with the wireless device are photographed.[7]

2.2 Optical fibers

The optical fiber is a very thin plastic or glass strand [23]. The optical fiber is composed of a glass fiber with a cylindrical structure, made of three layers.
The core is in the center, which has a higher refractive index. Outside of core, the cladding layer has a lower refractive index than the core. The external layer is the buffer plastic coating that doesn't affect the fiber optics performance. It is there for mechanical protection.
The light is coupled into the fiber's core, traveling along the length of the fiber. The core and the cladding are both based on fused silica, with almost no impurities. This transparency is significant because it permits the light to travel along the fiber also for as hundreds of kilometers with a minimum loss.
The light doesn't leak out of the fiber because of the total internal reflection.
Snell's law guides how light travels at the interface of the core and cladding. The core has the refractive index equal to 1.5, while the cladding has a lower refractive index equal to 1.4. When the light impacts at the interface, there is an angle between the core and the cladding. Some power is reflected back, instead, some power enters into the cladding refracted. When the incident angle is increased until the critical angle, all light is reflected into the core. This is the phenomenon called total internal reflection.
When the input angle is less than the critical angle, the light will leak out of the fiber; otherwise, if the angle is greater than the critical angle the light is bounced back and forward at the interface of the core and the cladding.
A simple communication system of the light, typically, has at least three key components. The light source, which typically is a LED. A driving circuit can turn on or off the ligh. The original electronic signal is accurately translated into a light signal. The fiber allows the transfer of the light also in long distances. The photodetector, instantly detecting the light, generates electronic current. This light

signal could the translate back to an electronic signal.
The optical fiber is a tool that allows studying the neural activity in a mouse's brain, under certain condition. It is required to have transgenic mice, where the neurons could be excitable by the light.
The virus and the light, in a transgenic mouse, allow to record the population neural activity or exciting the neurons to have the desired behavior.
There are several types of fibers, but this work will focus the attention on the flat fiber, the traditional approach, and the tapered fiber, the new tool.
In this chapter, it will be explored what already exists in literature and the new possible approach during recording and manipulation of neural activity.
The figure 2.4 shows the cut fiber. As illustrated, it is possible to cut the fiber of the desired size and to couple it to a laser or LED system for the light propagation[11].

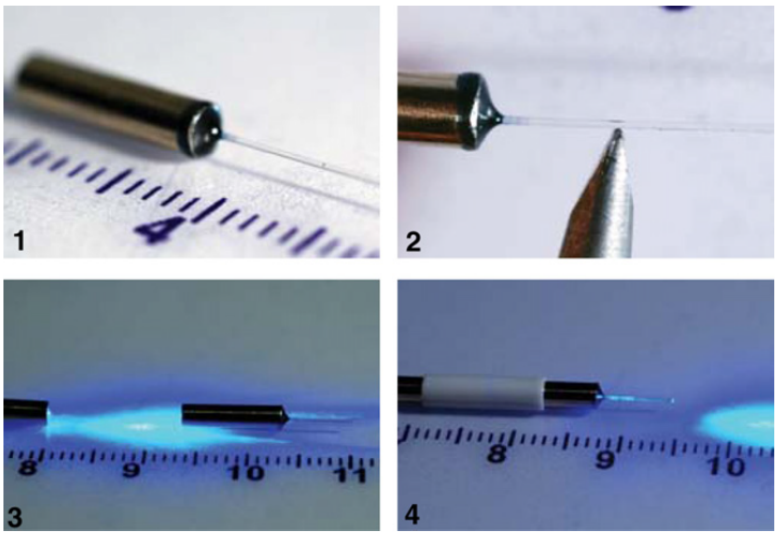

Figure 2.4: The part 1 and 2 show the cut fiber with a metal ferrule, the normal dimension and the possibility to cut it of the desired length. The underlying part (3-4) shows light propagation. It is possible to couple the metal ferrule with a light source to have the light delivery. [11]

The core and the cladding, in this case, were made of glass, instead, the ferrule, the part above the core, was made of stainless. The flat fiber is customized, it is

possible to cut it of the desired length. There are several steps normally followed to cut the fiber. Firstly, it is important to measure the transmittance with the light meter of the uncut fiber. To do this, it is necessary to adjust the power out of the laser coupled fiber to $\sim$ 1mW. Then coupling each assembly to the laser, it is possible to measure the output power at the tip of the fiber. In this step it is really important to avoid any possibility of interference with the ambient light, this one could affect the measurement. After cutting the fiber, it is possible to note an improvement of the transmittance. Usually, it increases by 5%.

2.2.1 Tapered fiber optics

In the last few years, optogenetics has taken an important role in the study of the brain since this method offers a better precision and selectivity. As previously explained, the classical approach, the cleaved optical fiber, has two intrinsic limitations: [12]:

- sometimes, the diameter of the fiber optics is too large and this could create damage on the region of interest.
- after the installation, it is not possible to redirect the light in different regions of the brain.

About the first one, the researchers usually prefer to put the fiber optics above the region of interest. In this way it is possible to avoid damages, but, at the same time, it is not possible to give the right illumination to the spot desired.
It is important to redirect the light because of the heterogeneous architecture of the brain, so to investigate other regions, the only alternative is to do another surgery, localizing the new fiber in the new spot. This, of course, implies an increase of the experiment time with a subsequent growth of the costs.
All these solutions cannot solve the problem but, only, they try to get around the problem.

There are several innovative approaches. Some of them use a complex fabrication process, others are particularly invasive. Most of them are not tested *in vivo* because they remain difficult to implement and the testing of their effectiveness is only theoretical.

This work used the tapered optical fiber, a very simple and innovative tool that provides high selectiveness and minimal invasiveness. Its easy availability leads to this choice; moreover, they offer a good reproducibility of the experiment with a low-cost impact. In fact, it is possible to use the tapered optical fiber with the same optical apparatus already existing in the research laboratories.

Using the tapered optical fiber, Ferruccio et al. (2017, [13]) tried to control the activity of the dorsal striatum in freely mice. Firstly, they dealt with the comparison between the cut optical fiber and the tapered fiber. In figure 2.5, it is possible to have an intuitive overview of the principal advantages while using the tapered optical fibers.

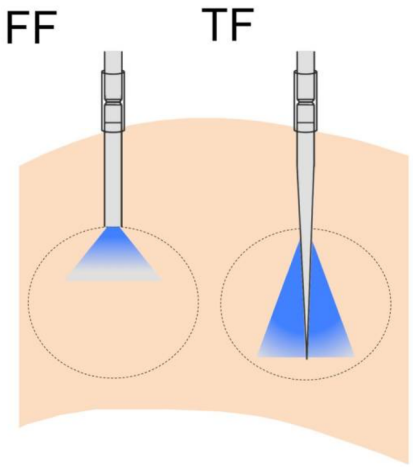

Figure 2.5: The picture shows two different types of optical fiber, the flat fiber (FF) and the tapered fiber (TF). The picture helps is to have a general overview of the principal advantages of the tapered fiber. In particular, it is evident the different size of the diameter at the tip. [13]

The flat optical fibers offer a different light distribution to the surrounding tissues. The other inconvenient is the larger size of the diameter at the tip. This could create damage to the brain tissues. For this reason, it is preferred to insert the cut optical fiber above the region of interest.

In contrast to this fiber, the tapered fibers can release a uniform, but, at the same time, a selective illumination, limiting potential damage during the insertion.

The light is transmitted along the fiber thanks to the total internal reflection. Along the fiber, the angle of ray propagation rises proportionally to the taper angle. Avoiding the critical angle, it is possible to have selectiveness in the brain, increasing the input angle of the light. Another important feature is the fiber numerical aperture; in fact, it is possible to choose the light output according to a proper fiber numerical aperture and a taper angle.

The taper angle influences the length of the emitted light along the fiber.Interesting, the light emitted changes according to the taper angle 2.6. The authors [13] compared two different fiber numerical aperture. This analysis is not exposed here since only the tapered fiber with fiber numerical aperture equal to 0.39 were used during the experiment.

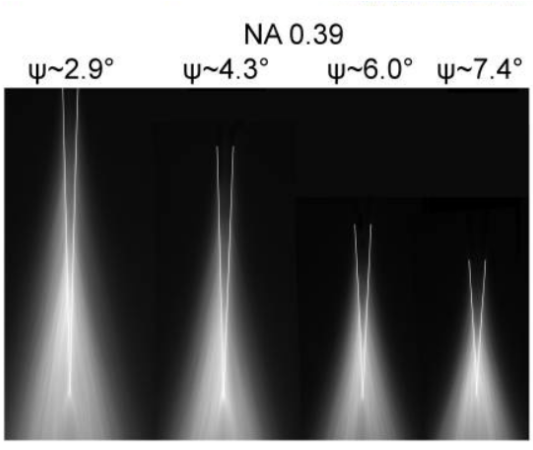

Figure 2.6: This picture shows that the dimension of the taper angle is inversely proportional to the output light.[13]

The tapered optical fibers have another important property. In fact, they have the possibility to emit light from several points by a single instrument. This is achieved by means windows extracted along the length of the optical fibers.In literature, there are several papers about this.

In particular, there are two studies, Pisanello Ferruccio et al. (2014, [14]) and Pisanello Marco et al (2015, [12]), that investigate to different models, analyzing which is the best compromise with respect to the needs.

In the first study, the authors analyzed three different types of tapered fibers. Their differences were the location and the number of the windows. The authors led a comparative analysis in two different means, that are an ideal environment and the brain.

They found that it is possible to stimulate different parts of the brain, simultaneously and with different wavelengths. Moreover, coupling the tapered fiber to a microelectrode array, the researchers can stimulate and record in real time. The validation *in vivo* confirmed what they already found in the ideal test. The tapered optical fiber with two windows offered a better behavior in both cases, in term of selectiveness and illumination. The fabrication process allows to choose the pattern for the windows; in this case, they chose the square shape.

It is really interesting to notice how changes the light varying the input angle in a fluorescent solution (figure 2.7).

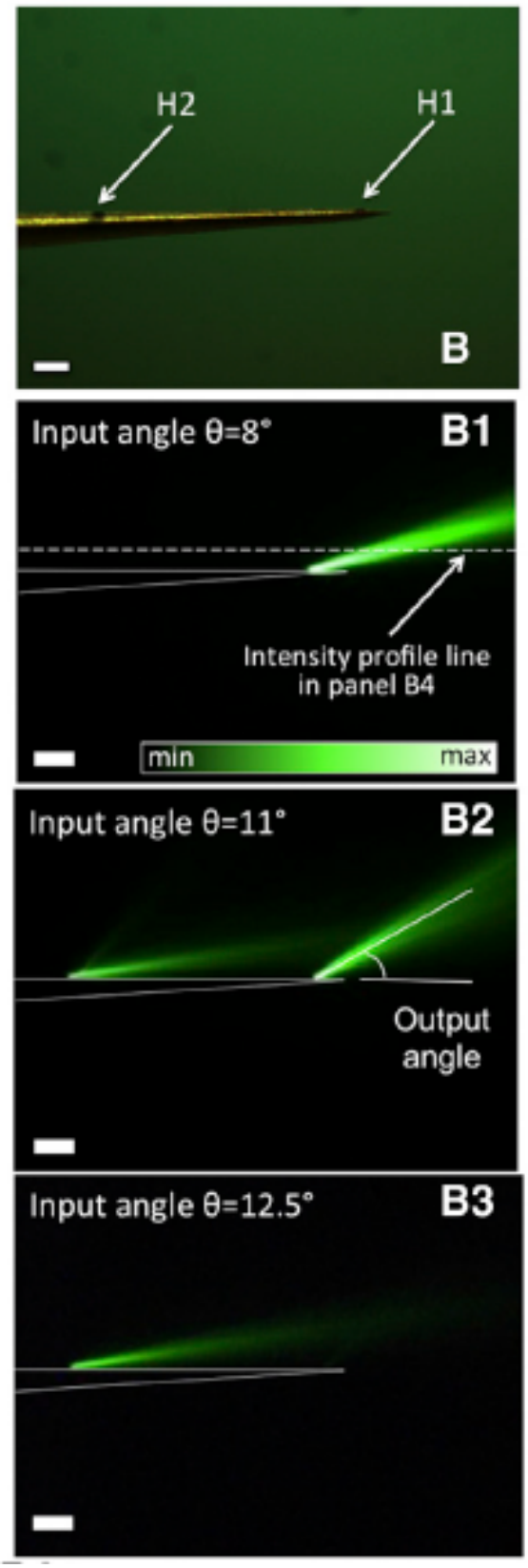

Figure 2.7: In figure B it is shown how the fiber looks like, and the localization of the two windows are indicated. In the following three pictures, it is possible to see how the region of interest changes varying the input angle.[14]

The second study [12], instead, analyzed three different devices, nanopatterned with gold coating. The first two had only a window but in two different spots. The third device had two windows at the same spots of the previous fibers.
They explained the physical principles of their devices, verifying, through a mathematical model, the possibility to obtain a modal multiplexer. In fact,it is possible to give, as input, to the fiber different wavelengths, that means two or more colors. These are put into a single tapered fiber, carrying different information per second.
There is another remarkable study [15] about the testing of tapered fibers with different numerical aperture. They evaluated divices with increasing numerical aperture, starting from 0.22 until 0.66.
An important founding was the relation between the emitting length, that is how long is the ray emitted from the fiber, and the intensity of the light. Hight power is expected when there is a lower emitting length.
They found that if the numerical aperture increases, the ratio between the First Emission Diameter and the core size increases, too; so higher levels of First Emission Diameter permits to have larger diameter of the core and a major numerical aperture, without interfering with thee taper angle.

2.2.2 Comparison

This section helps to summarize the comparison between two types of fibers explained in the previous part: the flat-faced optical fiber and the tapered optical fiber.
Sometimes, during construction, researchers want to amend their experiment, for example stimulating o recording the neural activity in distinct parts of the brain, respect to where the fiber is inserted. This is possible only with the tapered optical fibers. These, thanks to their windows, can emit light, simultaneously, in separate parts of the brain. This aspect of the tapered fiber has been already analyzed more

in details previously.

The cut fiber has a diameter of the tip bigger than whose of the tapered fiber. This could be a problem when the purpose is to stimulate or record some delicate parts of the brain, being sure to not create any damage.

To go beyond this problem, the classic approach typically involves the insertion of the fiber in the region above the one of interest. The intuitive limitation is properly the practical impossibility to record directly the region desired with related crosstalk problems.

The tapered fibers, otherwise, since they have an extremely thin tip, can also be inserted within the region of interest, with a lower probability of causing damage. Therefore, the tapers have a less invasiveness if compared with the flat fibers.

The cut fiber creates a dissimilar illumination. The tapers improve this aspect giving both selectiveness and heterogeneity. The selectiveness is achieved with their shape and their windows, changing the light input angle with a system of mirrors. This lead to a rise in the cost of the experiment using the tapered optical fiber.

The cut fiber creates a heterogeneous illumination. The tapers improve this aspect giving both selectiveness and heterogeneity. The selectiveness is achieved with their shape and their windows, changing the light input angle with a system of mirrors. This leads to a rise in the cost of the experiment utilizing the tapered optical fibers. Finally, it has to be considered that the flat cleaved fibers are customizable, cutting it of the desired length. This is impossible with the other fiber since it is merely possible to purchase different fibers with already determined lengths.

Chapter 3

Neural activity recording

This chapter focuses on recording the activity of a population of neurons. This method is widely used in research laboratories.
This work used the classical photometric apparatus with an innovative tool, explained in the previous chapter, the tapered optical fibers.
The new fibers were inserted into the terminal spot, instead, the flat-faced optical fiber into the cell body location.
The principal aim of this work was to understand if the novel approach can replace that classic one to highlight the dopamine pathway.
It is suggested that this path is involved in voluntary movement, as explained in the first chapter, but it is not yet completely clear.
The first part will explain the materials and methods utilized. After describing the surgical approach and the photometric setup used, it will be explained how teach the task to the mice. In this part, the task will be explained in detail.
The third part will analyze all the results obtained. The interesting thing is that, as it will be explained later, the results lead to other two experiments.
The first experiment concludes this chapter in the fourth section. The movement artifacts due to the fibers have been studied to realize how much the results are interfering.

3.1 Materials and Methods

The purpose of this part of the work is to explore the tapered fibers using the photometric approach. These were compared with the cut fibers, to understand if it is possible to replace them in photometry. To perform this, two transgenic mice were used. The transgenic mice are widely used in neuroscience research to understand how movement, cognition and other behavioral function are related to the different pathways present in the brain.
DAT-Cre mice were use. These animals, under certain conditions, can highlight the dopamine neurons.
The virus used was AAV1-Syn1-FLEX-GCaMP6f. The GCaMP6f protein is a fluorescent calcium indicator.
AAV1 represents the type of virus. Syn1 is the promoter. FLEX is a gene that can be only activated by the presence of dopamine neurons. This is the reason why DAT-Cre mice are used.

3.1.1 Surgery

The followed procedure was according with NIH Guide for the Care and Use of Laboratory Animals and the Animal welfare Act. After anesthetizing the mouse with isoflurane, it was placed into a stereotactic apparatus. The head was fixed with ear-bars and leveled using Bregma and Lambda reference points. The craniotomy was performed in two sites:

1. in dorsal striatum and substantia nigra pars compacta left for a mouse;
2. in dorsal striatum and substantia nigra pars compacta right for the second mouse.

In each of these mice, the virus was injected in SNc using a glass pipette. The injection was controlled by a pump at 50 nl/min for 2 ml.
For all of the mice, the tapered fibers was inserted in the terminal spot, while the

cut fiber in cell body spot. A headpost was fixed in the scalp with Metabond, an adhesive cement, and the identification number of each animal was written on the cement.
More details about the mouse and the surgery are presented in the table.

Virus Injection As anticipated previously, GCaMP6f is protein used to highlight the action potential. There are several possible approaches, but this macromolecule is the faster between the GCaMP-family. GCaMP6s is, instead, the brightest calcium indicator protein. [16]
Different from GFP (green fluorescence protein), GCaMP6f is able to emit a fluorescence signal when changing the concentration of calcium inside the cells.
This fluorescent protein becomes brighter when the calcium binds with it and its shape changes.
On the other side, GFP is a protein that becomes fluorescent when excited with blue light. In this case, it isn't related to the calcium concentration.
Measuring broadly the presence of calcium inside the neurons, it is possible to record the neural activity.
When action potential comes down the axon, the calcium enters the cell and binds to the GCaMP protein. The cell gets brighter. When the action potential goes down from the cell body, it is possible to see a lot of calcium at the terminal.
Sometimes it is possible to note a calcium buffering at the cell body. There are several hypotheses try to explain this phenomenon. A possibility could be the cell body is bigger.
The GCaMP brightness depends on how much light is delivered through the optical fiber; how much protein is injected through the viral vector; according to how much calcium is in the region of interest and calcium is proportional to the amount of neural activity produced by cells.

3.1.2 Behavior and Recording

According to the Harvard Medical School guidelines, in the first four days after the rodent surgery, the activity, incision and pain are monitored. In both mice, the activity and the incision were normal and there was an absence of pain, before starting the experiments. After these days, the mice were water deprived to motivate them during the task.
Every day, the weight before launching the experiment and after was calculated, to be sure to stay above the 80% of the normal weight.
The head-fixed mouse was placed on a platform, that was connected to the ground.
The accelerometer was put in contact with the mouse's back.
The juice was poured into a syringe and was checked it came out of the nozzle.
The PlexBright LD-1 Single Channel LED Driver was turned on.
After cleaning the ferrules of both optical fibers, these were connected to an optical path cable with a ceramic mating sleeve.
The Spike2 software and the MATLAB code, that manages Arduino, were run.

The experiment was interrupted when the mouse didn't feel confident anymore or after, at least, six hundred trials were completed.
Before putting the mouse in its cage without water, it was weighed again and offered a little bit of water.

Each day after recording, the light was turned off. This was done to avoid beaching and to preserve the fluorescent signal. These days were called behavioral days.
Putting the mice in same record conditions, their optical fibers weren't connected to the photometric apparatus and only the MATLAB code was run.

Optical setup

The figure 3.1 presents how a photometric apparatus looks like.
Several instruments are used to direct the light through the mammalian brain and then to investigate the fluorescence signals.
A typical photometric apparatus, used for voluntarily moving animals, contains a luminous source and system able to direct the light in numerous tools. In particular, a PlexBright Optical Path cable connected the optical fiber with the Doric fluorescence mini cube with three ports.
This cable includes two different connectors. The LC ferrule tip was designed to couple it with the optical cannula, installed into the rodent brain, via a ceramic coupling sleeve. The FC connector, on the other hand, can promote a connection between the mouse and the photometric system. Each port of the Doric Fluorescence mini cube includes a distinct purpose.
The LED blue light, 465 nm, coming from a power source driver, is direct toward the excitation input.
This light is then steered, by means a beam splitter, to the sample gate. The S port can connect the fluorescence mini cube to a 200 μm core diameter optical fiber.
Finally, the fluorescence emission port, set-up to 525 nm, is connected with the Doric Photodetector.
All connections are mediated by the Doric Lenses Fiber-optic Patch Cords that integrated an attenuating filter.
The fluorescence light can be collected with one photoreceiver module that addresses the signal information straight to the CED Power1401-3A.
This multi-channel processor can receive as input the fluorescence signal and can represent on a monitor how the signal looks like. The cue, a blue LED, and the camera are controlled by Arduino. The power source used in this experiment is a LED blue light. Two tools can generate the light and direct it, the Table-top LED Modules and the LED Driver LD-1 Single Channel, respectively. These two are

manufactured by Plexon.

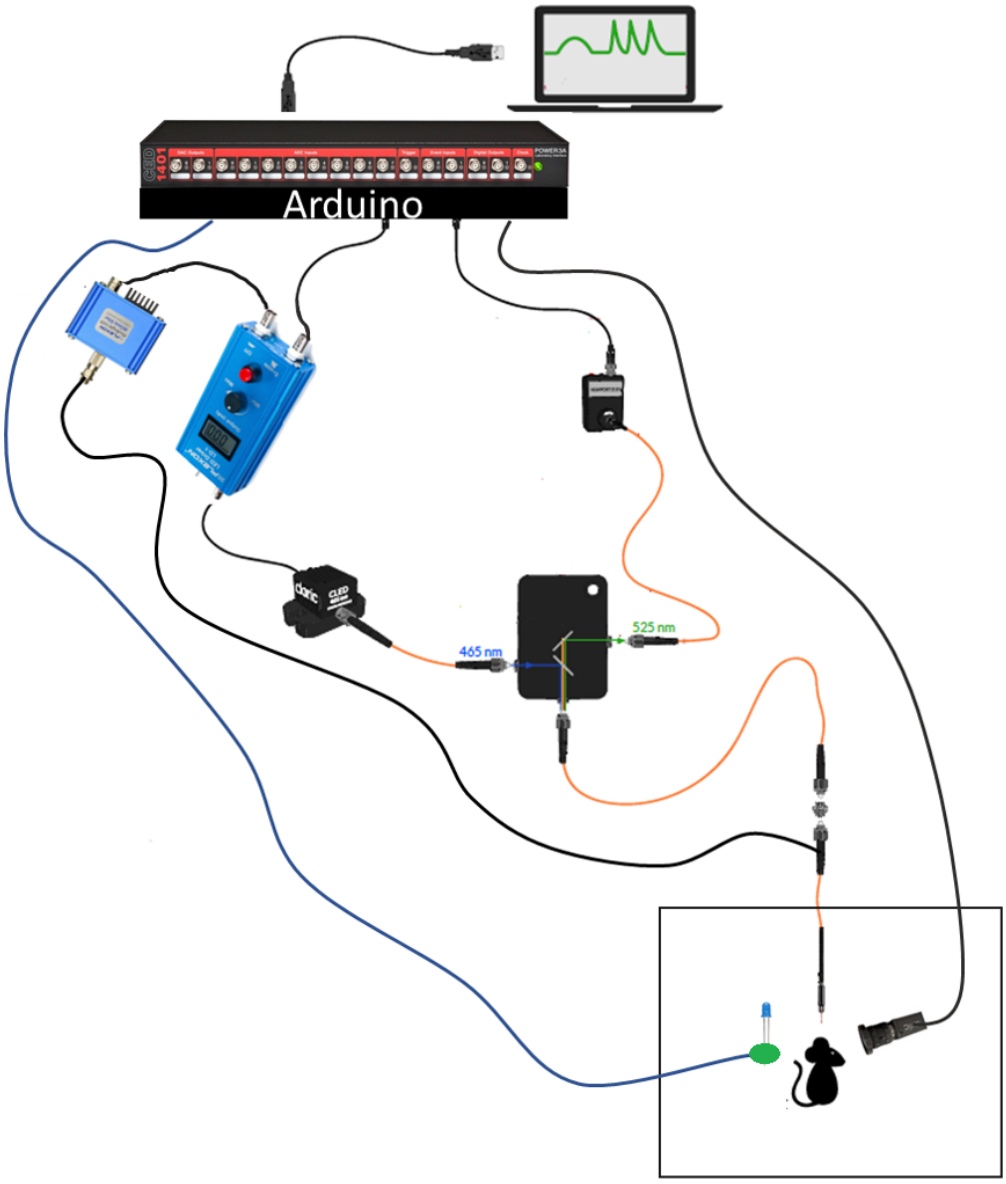

Figure 3.1: Fiber photometric apparatus.
All the pictures are taken from three website:
`https://campdeninstruments.com/listing/optogenetics`,
`http://doriclenses.com/life-sciences/323-fiber-photometry-systems`,
`http://ced.co.uk/products/pow3in`

Task structure

There are several works that correlate the dopaminergic system with voluntary actions.

The mouse was trained to get a reward at a specific time. The task involves the timing and the decision of movement from the mouse because the dopaminergic system is hypothesized to be involved in moving.

The mouse was head-fixed to be sure it makes the same motion every time; moreover, different parts of the brain are responsible for several movements.

The timing task permits to put the mouse in the same case every single trial. This condition increases the probability of the same neurons are activated at that time. It is crucial to not permit any action before the movement of interest.

Moreover, since this work studies self-initiated movements, it is significant to select only the voluntary action.

The rodent receives a light cue and has to wait for a relatively prolonged time before self-initiating a movement to trigger reward. Since the mouse can't move, it has to rely on some internal processes to generate movement.

In this case, the self-initiated motions are defined as generated through an internal process triggered by a light stimulus.

Thus, two things ensure the self-initiated movement is done on every test, the timing and the fact that the mouse was head-fixed.

The hypothesis proposed by Hamilos [17] is that the animal will move when the balance is directed towards the direct pathway.

It is expected to note earlier movement when the balance flavors the direct pathway. For this reason, the timing task is a good compromise to test the hypothesis.

Direct and indirect spiny projection neurons are observed during this timing task.

The trial period is divided into four intervals as illustrated in the figure 3.2 part A.

After an arbitrary delay of 400-1500ms, head-fixed mice receive a cue to start timing.

The first is called Post-Cue interval. During this period the mouse demonstrates an impulsive response. Since the principal aim of this work remains the voluntary movement this period will not be considered in data analysis.
The second interval is a period of time that doesn't allow any reward if the mouse licks. In addition, the trial will abort.
At 3.33 s, until 7 s, there is the reward window. This period is divided into two sub-periods. The operant window and the post target. If the mouse withholds licks during the No-Lick Interval, it can get an apple juice reward in two ways: operant or Pavlovian.
Pavlovian conditioning is the first learning procedure adopted so that mice pair the light stimulus with a juice reward.
Operant conditioning is more complicated since it happens at the of the No-Lick interval. Only the first lick is rewarded.
At the last moment, there is ITI window that finishes after 17 s from the beginning of the cue.
The second part of the figure 3.2 (B, C, D), shows, through the histograms, the mouse preference to lick during the operant window than the post-target.
During the days, it was noticed that the mouse preferred the operant conditioning even if it was more likely the probability to not get any reward.
The timing task and the difference between the operant and Pavlovian timing task are well explained in Hamilos work, [17].

The tables 3.2 and 3.4 present all the days of recording in a schematic way.
Focusing on the column called "DLS", it is possible to observe an increase in the current supplied through the tapered optical fiber.
On the fist day, the experiment was performed under conditions known from previous works.
This set level light was unable to record the fluorescence signal coming from the

terminal spot.

For this reason, an appropriate level light was set every day, trying to avoid photo-bleaching.

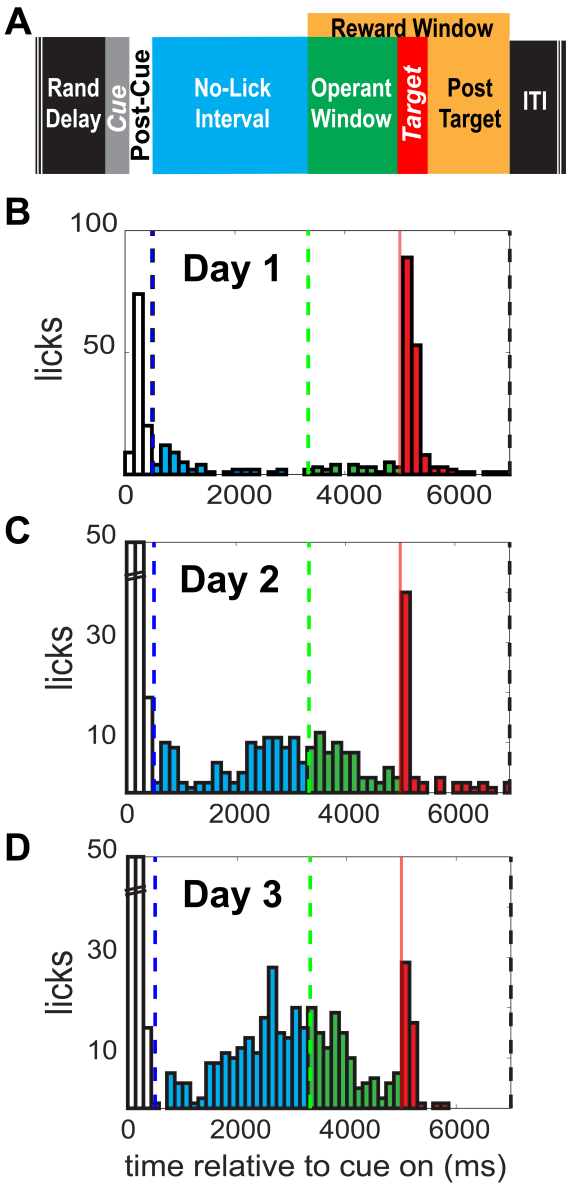

Figure 3.2: Part A shows time task division. The pictures B, C and D present histograms of three days. It is possible to note the spontaneous adaptation of the mouse behavior. The first day it adopted a Pavlovian mode, instead, after three days there is an operant conduct.[17]

Data acquisition

The first day after the surgery both optical fiber were bleached. After three days of water deprivation, the signal was recorded from both spots, cell body and terminal.

The picture 3.3 show how the signal looks like from the CED signal. Signals are referred to the terminal spot (DLS) and to cell body spot (SNc). It is possible to observe in both cases the bleaching phenomenon.

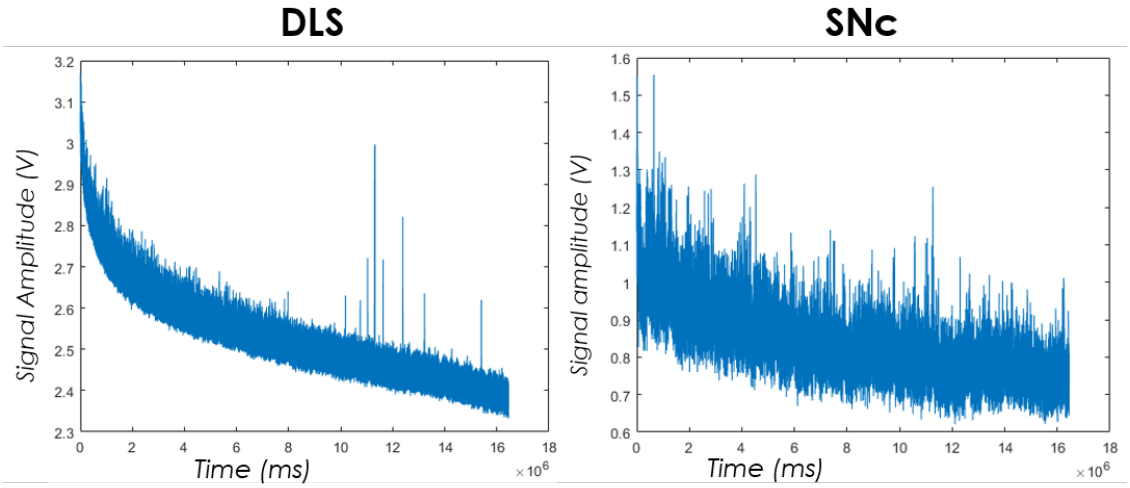

Figure 3.3: The figure present two signals withdrawn from cell body and the terminal as obtained by Spike2. The bleaching phenomenon is evident in both cases.

Without compromising the shape, a Gaussian filter is applied to smooth the signal.

Over the experiment, the bleaching phenomenon remains a problem.

There are many possibilities to safely analyze photometry signals.

This work utilized the $\triangle$F/F correction. This method was chosen to not produce artifacts in the cleaned up data.

The baseline firing of DA neurons could be informative of the timing of the self-initiated movement on a given trial, and its correction could cause problems.
For this reason, it was chosen to examine a moving window with ten trials. The average fluorescence intensity of this interval was called F0(t). F(t) is, instead, the fluorescence intensity at a given time. Then the $\triangle$F/F signal was calculated as:

$$\frac{F(t) - F0(t)}{F0(t)} \tag{3.1}$$

3.2 Results

It was hypothesized that the signal related to the movement is transmitted from the cell body to the terminal.
Moreover, a characteristic of the dopamine neurons is they present a ramp.
With this in mind, the calcium signal is examined. On one side, this work tries to verify that biological hypothesis; from the other, a technological problem is explored. Data examined, in fact, clarify if tapered optical fibers represent a satisfactory compromise for the neural activity recording.
The plots presented in this section try suggesting an interpretation to the fluorescence signal recorded at the terminal with the tapered optical fiber and at the cell body, withdrawn with flat-faced optical fiber.

Before showing the data obtained from this approach, a briefly introduction will explain, in general, what the figures represent.
The following analysis will present a combination of two plots representing the lick-triggered averages (LTA) of calcium signals.
On the x-axis there is the time relative to the first lick; on the ordinate, the amplitude of $\triangle$F/F signal, calculated from the 3.1.
On the right, the fluorescence signal during the no-reward window, taken from a

given point into the brain, is shown. This one is divided into two temporal intervals only to facilitate the analysis.
On the left, the reward interval is illustrated and also in this case the signal is divided into two times.
The zero coordinate is calculated by averaging first licks of each trial in the respective range.

The illustration 3.4 represents the fluorescence signal recorded at terminal spot with the tapered fiber. The plot on the right, the calcium signal of the first licks occurred in the time interval rewarded, is on the same scale with that one on the left.
Focusing on the negative part of the graphs, a ramp is shown after a period of latency. It appears they are dopamine neurons since this is a their typical characteristic.
From this plots, it appears the mouse prepare its movement before to do it, that it, this predicts when the mouse wants to move. This suggests confirming the hypothesis that sees dopamine neurons involved in self-initiated movements.

The figure 3.5 presents, instead, the fluorescence signal withdrawn with the flat-faced optical fiber from the cell body spot.
This is the classical photometric approach. Most of the works, in this field, use this type of optical fiber to highlight the neural activity. It was chosen to not use the tapered optical fiber also in this spot to have a positive control about what is already known from previous works.
To explain this graph the same approach seen before is applied.The calcium signals are represented on the same scale.
A big ramp after a short latency period is shown. This ramp star before to have the movement, the fist action is, in fact, synchronized to the zero coordinate.

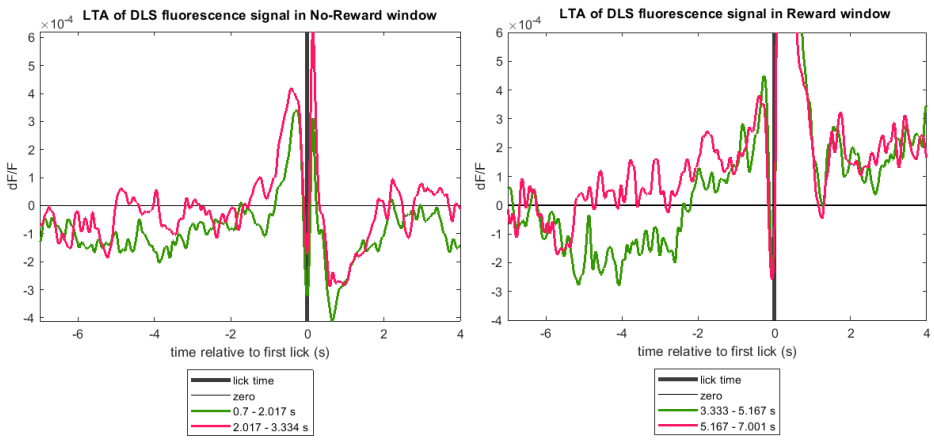

Figure 3.4: Mouse H15. The figure is divided into two parts. The difference between plots on the left and the right is the timing interval, one no-rewarded and the other rewarded, respectively. These signals were withdrawn with the tapered optical fiber from the terminal spot. In both graphs, it is possible to note a ramp after a latency period. This is a typical dopamine neurons behavior. Effectively, dopaminergic cells are transmitted from the substantia nigra par reticulata to the dorsal striatum. These graphs suggest the transmission occurs when the mouse decides to move.

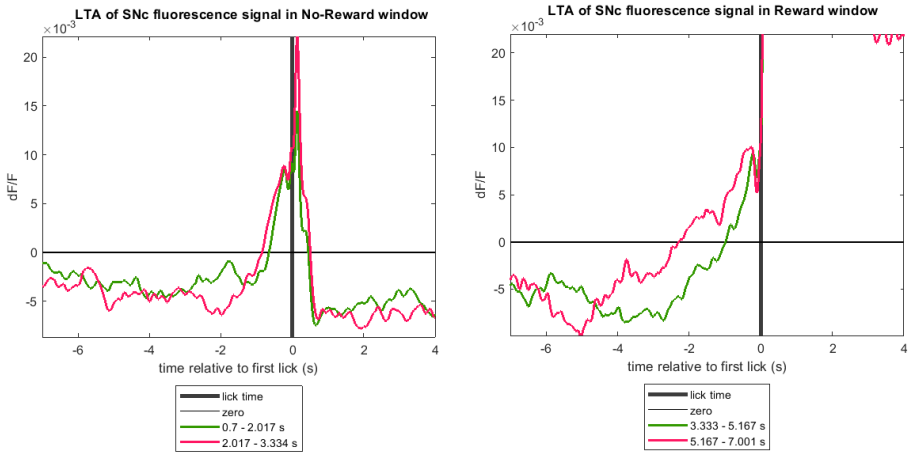

Figure 3.5: Mouse H15. Fluorescence signals withdrawn from the cell body spot. It is possible to note in both graphs a ramp after a latency period, typical dopamine neurons behavior. This ramp start before movement occurs, suggesting the mouse was preparing to move.

It is possible to compare the two figures (fig. 3.4 and fig. 3.5) just described. They present some similitudes. Dopamine neurons, usually, show a ramp in the proximity to the movement. In this case, their typical behavior is confirmed.
Moreover, it appears the ramp start before movement occurs, suggesting dopamine neurons involved in self-initiated actions. It could be, the mouse was preparing the movement.

This work presents the same plots on different scale, too (fig. 3.6 and fig. 3.7). This permits to analyze other characteristic of the fluorescence signals withdrawn from two difference spots.
Exploring all the graph taken from different locations and in distinct timing intervals, when the animal is close to boundary to get rewarded, this bin is persistently higher.
The prominent neural activity recorded, close to 3.33 s, suggests more dopamine neurons activated. On one side this could be due to the dopamine role into the mammalian brain. It is involved in several mechanism, not only related to the movement. This is in part demonstrated by the diseases that show a degeneration of dopamine pathway.
On the other hand, The mouse shows a predilection to lick as soon as possible, even if this could compromise its reward. Close to 3.33 s, in fact, increases the probability to not get any reward.

However, there is a different shape if the not scaled signal withdrawn from two locations are compared.
The right part of the figure 3.7 presents a calcium buffering. There several possible approach that could explain this phenomenon.
Thinking on the function of dopamine neurons, the presence of the calcium buffering could be related to the fact that these neurons are not merely involved in

movements.

On the other hand, it is also real that the cell body spot is bigger and this needs more time to stop the calcium release.

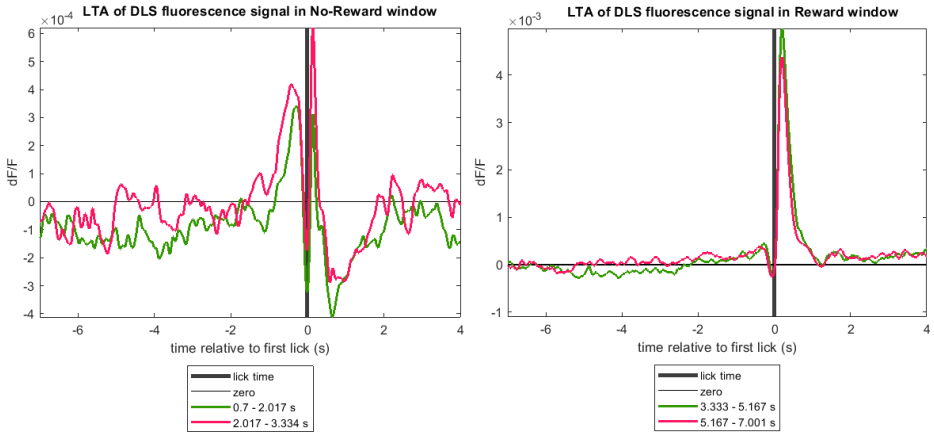

Figure 3.6: Mouse H15. LTA of the signal taken from DLS spot into two different scales, during two timing interval. The bin close to 3.33 s is higher in both graphs. After the movement, the signal goes straight down.

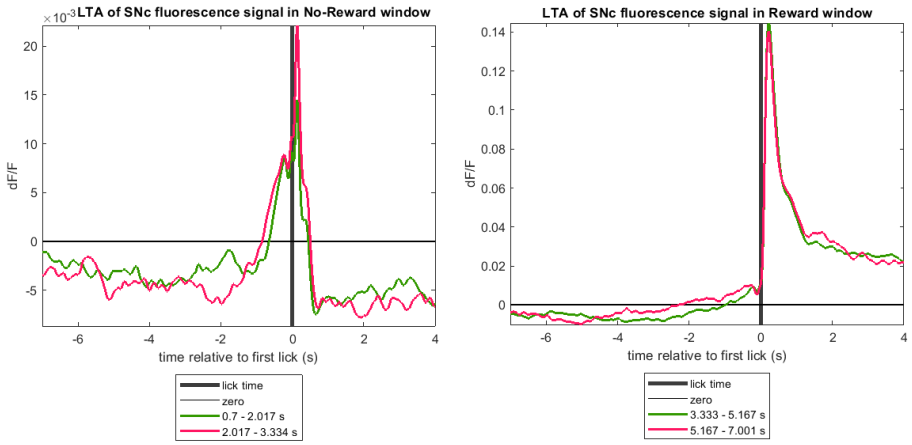

Figure 3.7: Mouse H15. LTA of signal taken from SNc spot into two different scales. Both plots present a prominent neural activity in the proximity to 3.33 s. There are several possible explanations to this phenomenon. Focusing on the right part of the figure, there is a calcium buffering.

Comparing the signal to noise ratio in each case, both optical fiber demonstrates optimal performance. This is evident, mainly, in the plot illustrating the time interval with reward.

All these graphs confirm, also, an important technological achievement.
The tapered optical fibers can be used as well as the flat fibers in the photometric approach.
This is a great advantage knowing the properties of this approach, already explained in chapter 2.

The analysis is completed with the results of the second rodent. All the plots confirm what already explained for the mouse H15.
Different from the previous analysis, this case doesn't present a calcium buffering. The signal goes straight after the voluntary movement down in both dorsal striatum and substantia nigra pars reticulata spots.

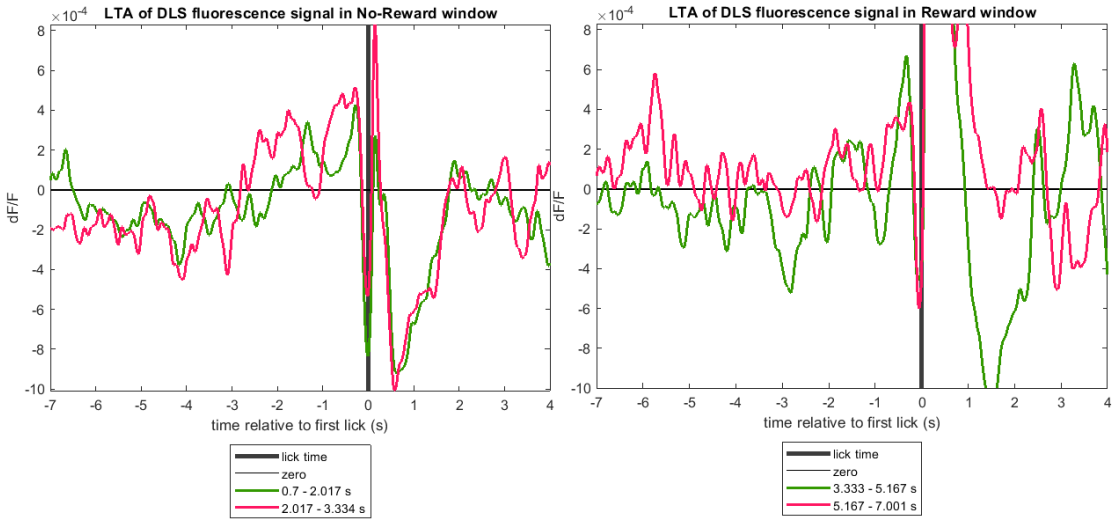

Figure 3.8: Mouse H14. Representation on the same scale of the LTA of signal taken from DLS spot. On the left the first lick that didn't get rewarded are presented and vice versa on the left.

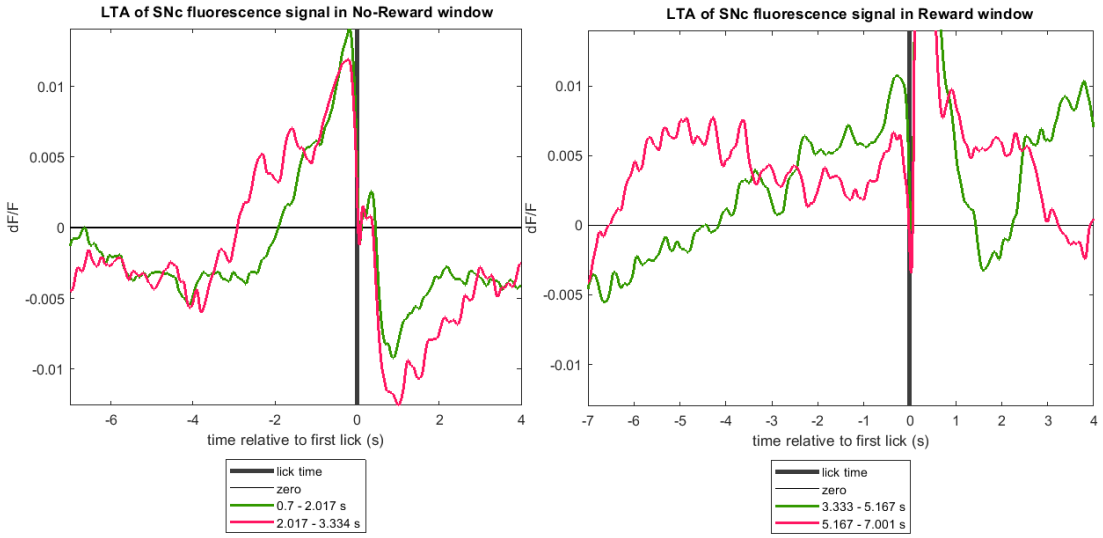

Figure 3.9: Mouse H14. Representation on the same scale of the LTA of signal taken from SNc spot. On the left the first lick that didn't get rewarded are presented and vice versa on the left.

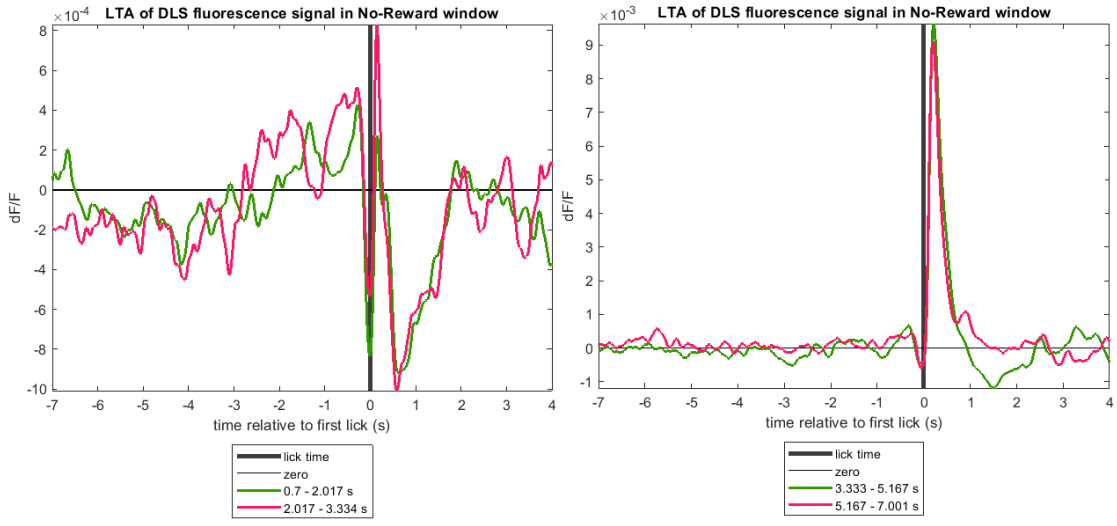

Figure 3.10: Mouse H14. LTA of the signal taken from DLS spot into two different scales. On the left the first lick that didn't get rewarded are presented and vice versa on the left.

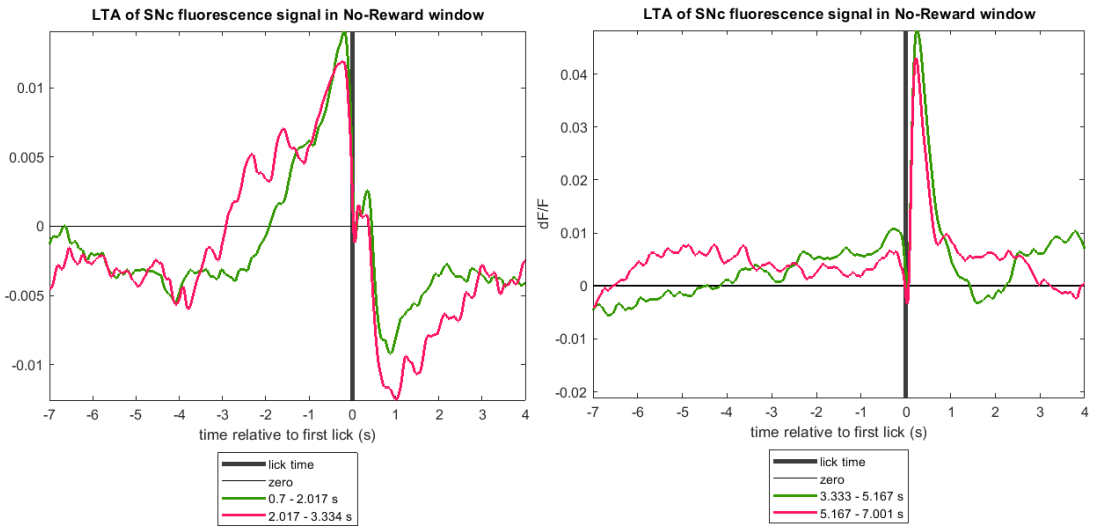

Figure 3.11: Mouse H14. LTA of the signal taken from SNc spot into two different scales. On the left the first lick that didn't get rewarded are presented and vice versa on the left.

3.3 Movement artifact

The purpose of this experiment was to analyze how much the tapered optical fibers are affected by the movement artifacts. In particular, it was explored if the calcium signal recorded by the photometric approach could be influenced by this problem.

Two transgenic DAT-cre mice were labeled with GFP proteins.

The GFP is a green fluorescent protein that exhibits bright green fluorescence when exposed to light in the blue or in the ultraviolet.

A possible approach is to use the tdTomato fluorescent protein in the same mice used to record the neural activity, as already done in the Assad Lab. In this case, they noticed a big crosstalk-effect between GCaMP6f ad tdTomato proteins. For this reason, it was thought to explore another technique injecting the AAV-SYn-Flex-GFP virus into the two diverse DAT-cre mice.

3.3.1 Material and Methods

All the steps followed during the surgery were provided by the Institutional Animal Care and Use Committee of Harvard Medical School.
Different from the photometric approach, the GFP protein was injected since the focus of the experiment wasn't the neural activity.
In this case, the movement artifacts due to the tapered optical fiber were explored. To do this, these fibers were put on both terminal spot, in two different animals.

In the tables 3.6 and 3.8, there is a schematic presentation of all the details about the surgery and the animals.

Recording

The timing task, explained previously, was taught to two mice.
It was expected to observe a signal as flat as possible since the GFP protein isn't an indicator of the neural activity.
This signal, detected by the GFP, should reflect the relative movement due to the fiber into the brain when the mouse was moving.
To maximize this effect, the modal current has been set to the maximum possible, avoiding the saturation of the signal.
The column DLS of each table 3.5 and 3.7 indicates this approach.
Higher is the level of the light, more cell will be detected and higher will be the movement artifact.
The details about the recording, for each mouse, are shown in the tables 3.5 and 3.7.

3.3.2 Results

All the collected data are combined together. Different from GCaMP case, it was uniquely significant to verify the presence of the motion artifacts. For this reason, there isn't the equivalent amount of collected data.
The results are presented in a figure with two plots. On the left there is the GFP signal, coming from the left terminal, in the no-reward interval. On the left, the fluorescence signal during the reward window is presented. The zero coordinate is synchronized with the average of all the first lick in the respective time interval.
The figure 3.12 shows the case of the tapered optical fiber put in the left terminal.

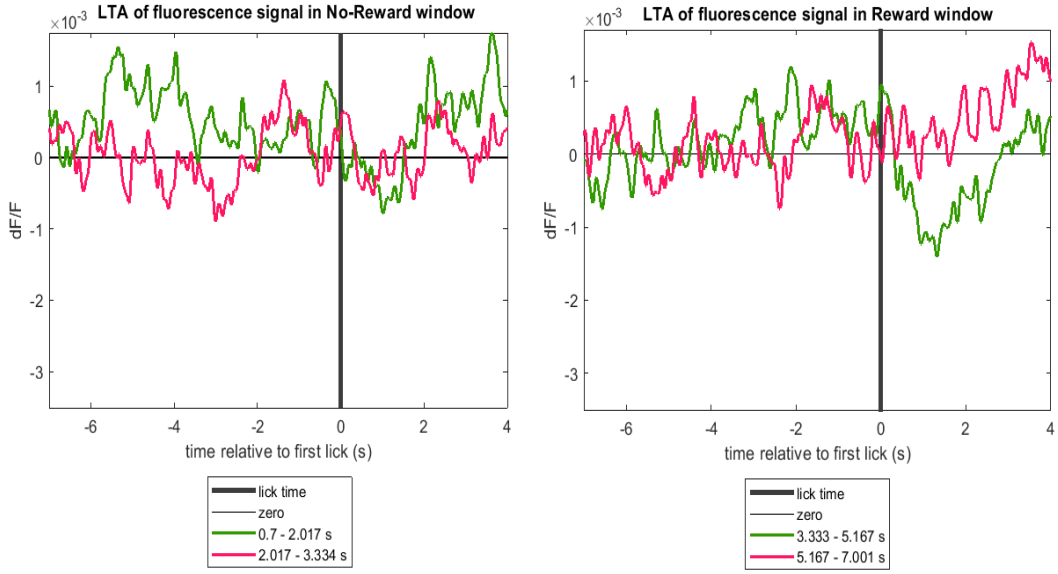

Figure 3.12: The figure shows the combined data divided in different temporal intervals. On the right, it is shown the signal if the mouse licked in the unrewarded window. On the left, it is shown the signal when the mouse licked in the rewarded window.

The movement artifact is not negligible. The plots show a lower SNR compared with the GCaMP signal. Nevertheless, there some significant findings, analyzing the graphs. There are two remarkable regions in the plots with the calcium signal.

On one side the ramp before the voluntary movement and the significant spike when the movement occurs.
The motion artifacts due to the tapered optical fiber don't compromise this analysis since they are circumscribed in determined regions and limited in amplitude.
Definitely, this analysis was important to quantify, in a qualitative way, the interference of artifacts to understand if this problem influenced the findings.

For completeness, this analysis was done on the right side, too. It is possible to draw the same conclusions. The figure 3.13 presents the signal recorded from the second mouse.

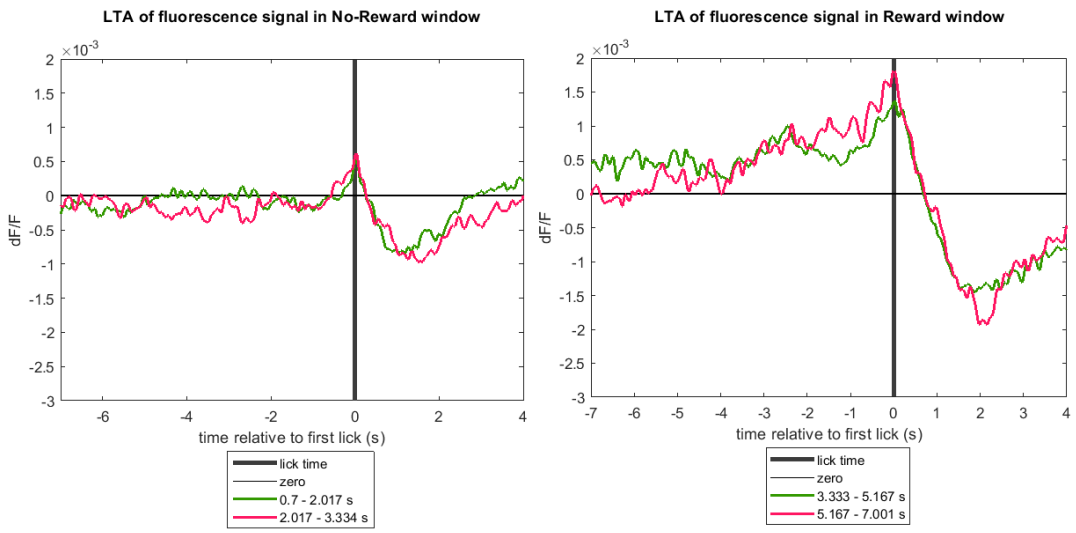

Figure 3.13: The figure shows the combined data divided in different temporal intervals. On the right, it is shown the signal if the mouse licked in the unrewarded window. On the left, it is shown the signal when the mouse licked in the rewarded window.

Table 3.1: The table summarized more details about the first GCaMP mouse, the fibers used and the days processed.

H14	
Name	Flo
Mouse	DAT-cre
Gender	Female
Virus injected	AAV1-Syn1-FLEX-gCaMP6f
Virus injection spot	Cell body (Ventral location = 4.2 mm)
Rate of injection	50 nl/min
Total virus injected	2.5 ml
Tapered fiber location	Terminal left (Ventral location = 3.82 mm)
NA taper fibers	0.39
Emitting length taper fibers	1.5 mm
Tapered fiber length	15 mm
Flat fiber location	Cell body right (Ventral location = 4 mm)
Flat fiber transmittance	58%
Flat fiber length	2.5 mm
Experiment duration	18 days
Started	7/9/18
Finished	7/27/18
Recording days	9/9 processed
Behavior days	9

Table 3.2: The table summarized details about the neural activity recording of the first GCaMP mouse.

H14				
Rig	Date	Day	SNc (mA)	DLS (mA)
1	7/10/18	3	2	3
1	7/12/18	5	2	15
1	7/14/18	7	2	15
1	7/16/18	8	2	15
1	7/18/18	10	2	25
1	7/20/18	12	2	27
1	7/23/18	14	2	15
1	7/25/18	16	2	15
1	7/27/18	18	2	15

Table 3.3: The table summarized more details about the second GCaMP mouse, the fibers used and the days processed.

H15	
Name	Eve
Mouse	DAT-cre
Gender	Female
Virus injected	AAV1-Syn1-FLEX-gCaMP6f
Virus injection spot	Cell body (Ventral location = 4.2 mm)
Rate of injection	50 nl/min
Total virus injected	2.5 ml
Tapered fiber location	Terminal right (Ventral location = 3.863 mm)
NA taper fibers	0.39
Emitting length taper fibers	1.5 mm
Tapered fiber length	15 mm
Flat fiber location	Cell body right (Ventral location = 4 mm)
Flat fiber transmittance	58%
Flat fiber length	25 mm
Experiment duration	18 days
Started	7/9/18
Finished	7/27/18
Recording days	9/9 processed
Behavior days	9

Table 3.4: The table summarized details about the neural activity recording of the second GCaMP mouse.

H15				
Rig	Date	Day	SNc (mA)	DLS (mA)
1	7/10/18	3	2	3
1	7/12/18	5	2	20
1	7/14/18	7	2	20
1	7/16/18	8	2	15
1	7/18/18	10	2	15
1	7/20/18	12	2	20
1	7/23/18	14	2	20
1	7/25/18	16	2	20
1	7/27/18	18	2	20

Table 3.5: The table summarized more details about the first GFP mouse, the fibers used and the days processed.

H18	
Name	Lea
Mouse	DAT-cre
Gender	Female
Virus injected	AAV-FLEX-GFP
Virus injection spot	Cell body left (Ventral location = 4.2 mm)
Rate of injection	2.5 ml at 2 times of magnification
Tapered fiber location	Terminal left (Ventral location = 3.80 mm)
NA taper fibers	0.39
Emitting length taper fibers	1.5 mm
Tapered fiber length	15 mm
Experiment duration	10 days
Started	8/17/18
Finished	9/21/18
Recording days	6/6 processed
Behavior days	4

Table 3.6: The table summarized details about the neural activity recording of the first GFP mouse.

H18			
Rig	Date	Day	DLS left (mA)
1	8/20/18	3	3
1	8/23/18	5	3
3	8/25/18	7	6
3	9/17/18	8	14
3	9/19/18	9	19
3	9/21/18	10	27

Table 3.7: The table summarized more details about the second GFP mouse, the fibers used and the days processed.

H19	
Name	Gin
Mouse	DAT-cre
Gender	Female
Virus injected	AAV-FLEX-GFP
Virus injection spot	Cell body right (Ventral location = 4.2 mm)
Rate of injection	2.5 ml at 2 times of magnification
Tapered fiber location	Terminal right (Ventral location = 3.811 mm)
NA taper fibers	0.39
Emitting length taper fibers	1.5 mm
Tapered fiber length	15 mm
Experiment duration	9 days
Started	8/20/18
Finished	9/21/18
Recording days	6/6 processed
Behavior days	3

Table 3.8: The table summarized details about the neural activity recording of the second GFP mouse.

H19			
Rig	Date	Day	DLS rigth (mA)
1	8/20/18	2	3
1	8/23/18	5	3
3	8/25/18	6	6
3	9/17/18	7	24
3	9/19/18	8	27
3	9/21/18	9	27

Chapter 4

Neural activity modulation

The similar approach seen used to record the neural activity is, in addition, used in modulation. The same instruments are used but changing the configuration. This chapter will explore the neuro-modulation in the mammalian brain.
The first section will analyze the methods and the materials used. The surgical approach will broadly follow the steps already explained in the previous chapter, but there are some peculiarities.
The significant part concerns the stimulation's type. There are three types: the first one will represent a sham stimulation, the other two will be a bilateral modulation. This aspect will be explained more in detail later.
The second section will describe the outcomes obtained, comparing each result in the three distinct stimulations.

4.1 Materials and Methods

The principal purpose of this part of the work was the modulation of the neural activity through an engineered protein ChR2-EYFP, injected via viral vector.
It is possible to stimulate the brain of a transgenic mouse by means the blue light.
It was used the same instruments already explained in the previous chapter.

4.1.1 Surgery

Most of the steps followed are similar to the case of photometry. All the steps followed the guideline from Harvard Medical School Animal Care and Use Committee. In this case, each mouse had four fibers. The tapered optical fibers were placed in both terminal, instead, the flat-faced fibers were inserted in the cell bodies spots. Before of the fiber insertion, the virus was injected. Different from the previous experiment, a viral vector carrying ChR2-enhanced yellow fluorescent protein was injected in both cell bodies spots, through stereotaxis. This ChR2-EYFP fusion protein combines the properties of both genes. This gene fused is excited with blue laser light.
After the five canonical days of post-surgery monitoring, the mice were water deprived.
More details about the mice, the fiber locations and the surgery are presented in the tables 4.1 and 4.3.

Optical setup

Several instruments are connected together to direct the light through the mammalian brain.
There are some remarkable differences respect to the classic photometric apparatus 3.1. The purpose of this part is the modulation of the neural activity, different from photometry, where the calcium signal was recorded.

For this reason, the Led Driver is not connected to the photodetector.
At the beginning, the light delivered was set to a power equal to 50 mW.
The stimulation protocol was controlled by Arduino. The protocol used was according Soares [18] work.
The stimulus lasted 7000 ms with duty cycle equal to 20 ms at 10 Hz of frequency.
The stimulated trials were the 30% of trials at time 0ms with respect to the cue.

4.1.2 Modulation

After the five days of post-surgery monitoring, the mice were water deprived.
The first ten days were used to teach the task to the mice. The task was the same the photometric approach.
Different from photometry, this part is useful because the only feedback during the experiment is the mouse's behavior.
To teach the task, the head-fixed mouse was placed in the rig in front of the nozzle, and it was in the condition explained in Sham section.
It was decided to excite bilaterally. This because the principal purpose of this work is to see if the tapered fibers could be used in the terminal spots during optogenetics stimulation.
In particular, after teaching the task, the mouse was submitted to the tapered fibers modulation. The following day it was decided to not stimulate, the so-called sham days, and then the flat-faced fibers were stimulated.
It was decided to improve the procedure, modulating in two separate days two distinct spots, while the third days was with the light turned off.
All the types of stimulation are explained in details in the following paragraphs.

Sham Stimulation

The sham days are so-called since there wasn't a real stimulation.
Before to start with the experiment, it was crucial to verify that the PlexBright LD-1 Single Channel LED Driver was disconnected from the detector and turned off. This routine procedure ensured the light didn't come out.
Two different MATLAB codes were run. Firstly the optogenetics code that was used to program Arduino. Even if in this case the light was turned off all the same input were sent to Arduino.
The panel with a LED permitted to verify the correctness of the modulation outside the rig.
Subsequently, the mouse's behavior code was run. Launching this code, the experiment started.
The first days the codes were stopped not before three hundred completed trials. Successively, it was preferred to stop everything when the mouse had became uncomfortable.
Moreover, during the first week, the hybrid conditioning was preferred. As already noticed in photometry, this approach is useful for the mouse in order to become familiar with the task.

Real Stimulation

The real simulation occurred through two bilateral modulation types:

- the tapered optical fibers;
- the flat-faced optical fibers.

They were done in two separate days, preventing the stimulation on the exact spot in two consecutive days. Before starting, mouse preparation gained importance. Typically, there were several simple steps useful to make it comfortable to get good

results.

At the beginning, it was meaningful to monitor the mouse's weight. This should be above the 80% of the measured weight before starting with water deprivation.

The mouse was located on a platform where the head could be fixed. This was the most critical step in the whole experiment preparation because the mouse could feel uncomfortable and jeopardize everything.

Successively, the ferrule of both bilateral optical fibers was cleaned with a 70% ethanol solution.

Unlike the fictional days, the PlexBright LD-1 Single Channel LED Driver had to be turned on.

After running the of optogenetics code, already explained in the sham case, it was a recommended practice to verify the light emitted with the frequency specified by the protocol.

In the end, the mouse was placed in the rig, connecting the fibers and the mouse's behavior code was executed.

The experiment was interrupted when it no longer felt confident, or not before six hundred completed trials.

Before putting the mouse in its cage without water, it was weighed again and given a little bit of water.

4.2 Results

The following sections will present, in a graphic way, the results divided according to the type of modulation.
For simplicity, days without a real stimulation are called Sham. Taper is used to name the days when the tapered optical fibers were stimulated and Cut those in which the stimulation of the cut fiber occurred.
For each section are presented five plots:

- Raser plot;
- the cumulative distribution function (CDF);
- the CDF in an interval between 0.7 and 7 s;
- the permuted difference;
- the permuted variance ratio.

The raster plot has on the x-axis the first lick time, on the y-axis the trial number. All the trials of all the same type of stimulation are put together.
All the first lick are indicated with a circle that could be of two distinctive colors. The blue indicates the first lick occurred during no stimulation and the orange vice versa.

The cumulative distribution function has on the abscissa the first lick time with respect to the cue and on the ordinate the probability of occurrence. This plot indicates the likelihood that mouse licked under stimulation before or at the same time respect to the unstimulated trials.
All the trials of the whole experiment are put together.

The third graph illustrates the cumulative distribution function only in the interval from 0.7 s until 7 s. In this plot, the instinctive reaction to the cue and the ITI are not considered since it isn't still clear how to interpreter the ITI interval.

The latter two plots are permutation test. All the trials are mix together e random combination of a thousand trials is selected. This is done to remove the dependence on time.
The first one calculates the difference between the area under the curve of the CDF of the unstimulated trials and the area under the curve of the CDF of the stimulated trials.
This measurement was done several times as permutation test, do not correlate the time and the stimulation.
On the x-axis the permuted difference between the area under the curve of no-stimulated trials and the stimulated tasks is presented, on the ordinate its frequency.
For each plot, the p-value was calculated and compared with the actual value to realize if it is possible or not to reject the null hypothesis.
According to this plot, it is possible to understand if the early lick is exclusively related to a coincidence or it is a really voluntary preference of the mouse. This plot, in fact, is able to highlight if the bilateral modulation of the optical fiber could transform the mouse's behavior.

The last plot is a permutation test, too. In this case, the x component of the accelerometer was considered. This was done to understand if the mouse during stimulation moved more.
To do this, the ratio between the no-stimulated trials and that one stimulated was calculated and then the variance of this ratio.
A set of a thousand trials are taken and put together to not associate the time to the type f stimulation. This was repeated for several times.
Also, in this case, the p-value was compared with the actual value to reject or not the null hypothesis.

In this case, it was investigated if the hypothetic more movement during stimulation is hardly due to chance or not.

In general, when the light was turned off, there shouldn't be a preference of behavior; whereas when the light was turned on, depending on the stimulated region, there were two distinct behaviors.

4.2.1 Taper days

This section presents the five graphs explained before for each mouse.
Raster plot 4.1 shows, on the x-axis, the first movement after the cue.
The interval starts from 0.7 s, after the reaction window, to be sure that the analysis considers only the voluntary actions.
The first days the power light was set to 50 mW, but as it is possible to see from the figure, it was insufficient to modulate the neural activity.
Therefore, it was chosen to heighten the power source to the maximum, i.e. 101 mW, based on previous results.
It is possible to see a pattern starting around the 800th trial. Initially, the first lick concentration is higher on the upper left corner; instead, the last days it is more significant on the lower right corner, into the reward boundary.
In the end, the mouse exhibits an adaptive behavior. This could be due either to a major virus expression or a learning effect.

The cumulative distribution function represents a statistical analysis to appreciate what is the first lick under stimulation occurred before that one without stimulation.
There are two CDF plots. The first 4.2 indicates all the task interval, instead the second one 4.3 is cut at 7 s, when there is the reward boundary.

In both plots, it is possible to note that first lick under stimulation occurred before that the others.

This work completes the analysis with two permutation tests. Both plots were explained in the previous section.
Through the first plot 4.4 it is possible to understand if the null hypothesis could be rejected or not.
The null hypothesis states there is no difference between the first lick with or without stimulation. The only observed difference can be attributed solely to chance.
To eliminate the correlation between lick and time, a thousand permutations are calculated.
The p-value equal to 0.046 makes it possible to reject the null hypothesis since the significance value is equal to 0.05. The alternative hypothesis that established there is a preference not due to the chance is therefore real.

The second permutation 4.5 test concerns the accelerometer signal. The alternative hypothesis states that during the modulation the mouse moves more than without stimulation. On the other hand, the null hypothesis establishes this is purely due to change. Also, in this case, the p-value equals to 0.01 permits to reject the null hypothesis.

Through the significant test, it is possible to state the results are statistically significant.

For completeness, the same plots about the second mouse are reported. In this case, the results in part confirm what previously explained.
The permutation test calculated by subtracting the area under the CDF curve without and with stimulation, respectively, has a p-value equal to 0.193.
Since the significance level is 0.05, it is impossible to reject the null hypothesis.

In most cases, plots show there wasn't an optimized level of power light. It isn't discernible if the light was too much or not enough.

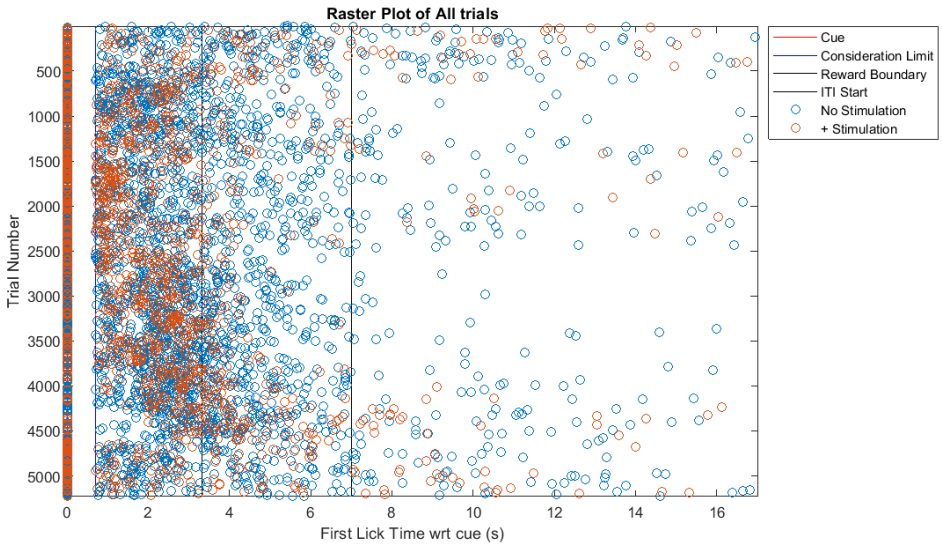

Figure 4.1: H16 taper Raster Plot

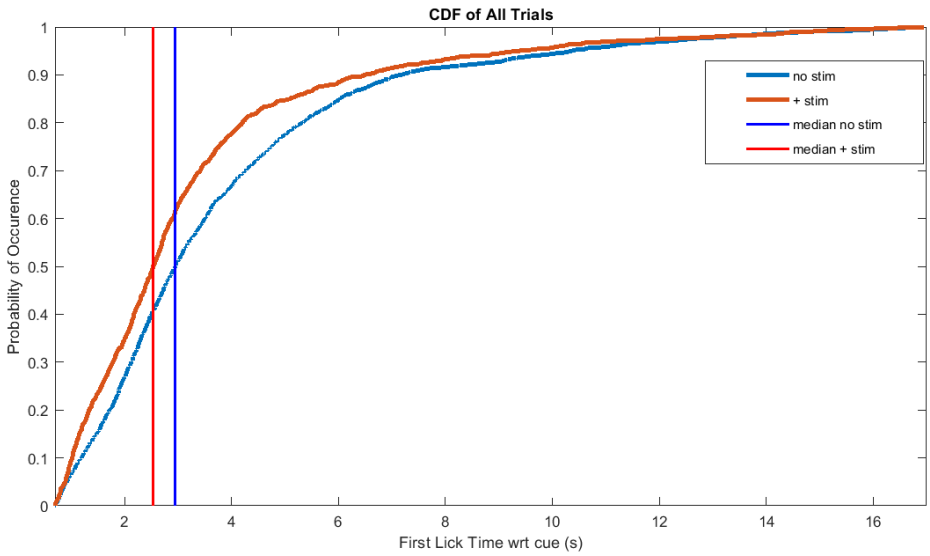

Figure 4.2: H16 taper CDF

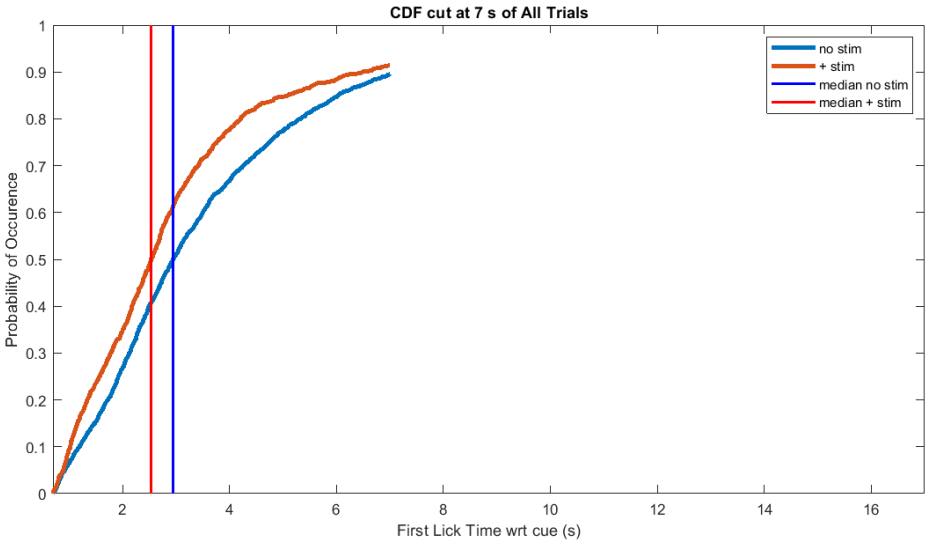

Figure 4.3: H16 taper CDF cut

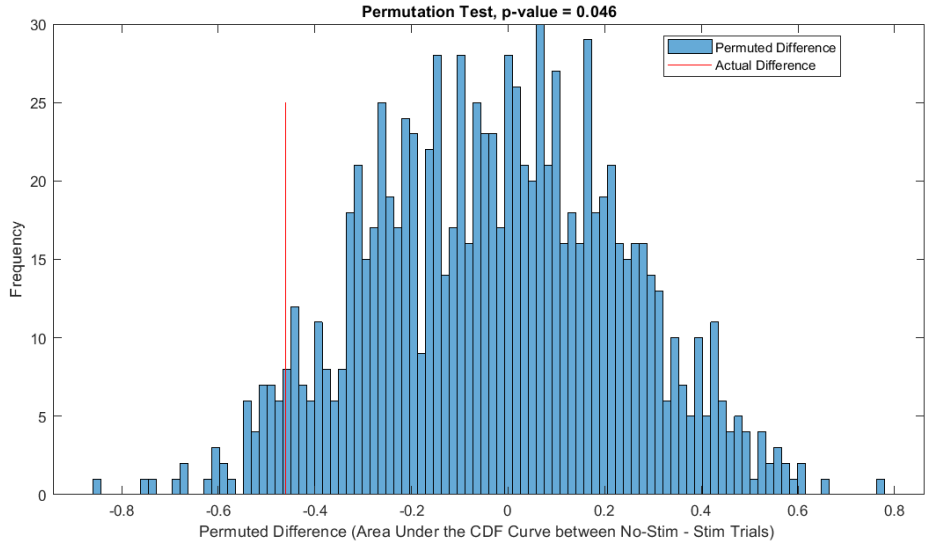

Figure 4.4: H16 taper Permuted Difference

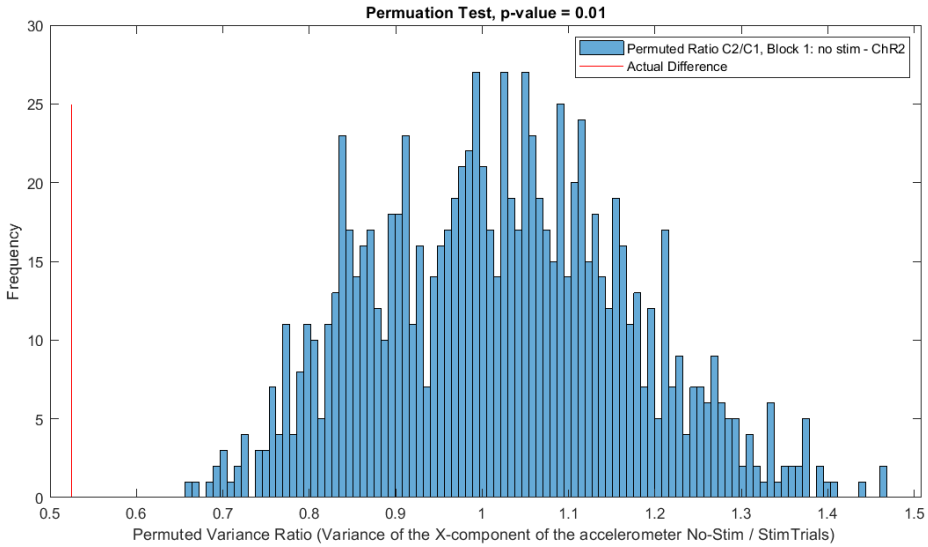

Figure 4.5: H16 taper Permuted Variance Ratio

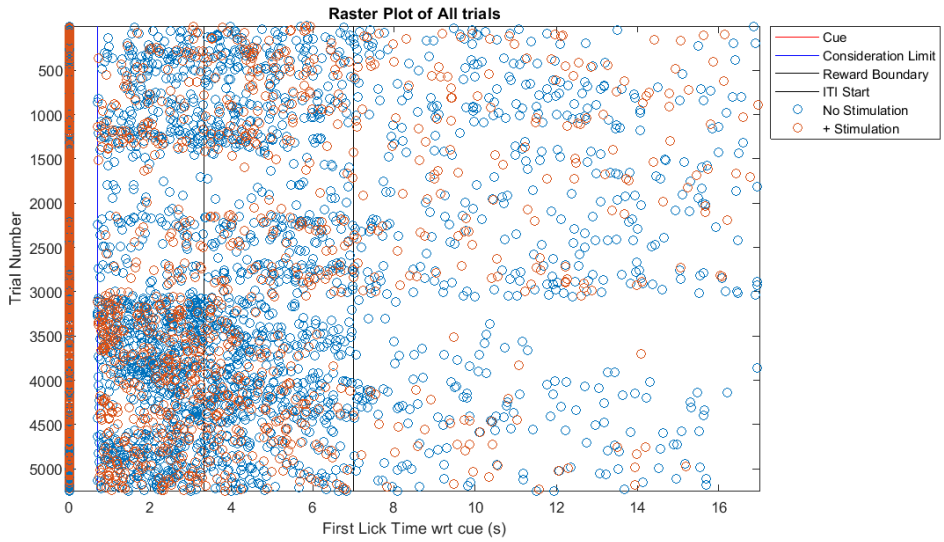

Figure 4.6: H17 taper Raster Plot

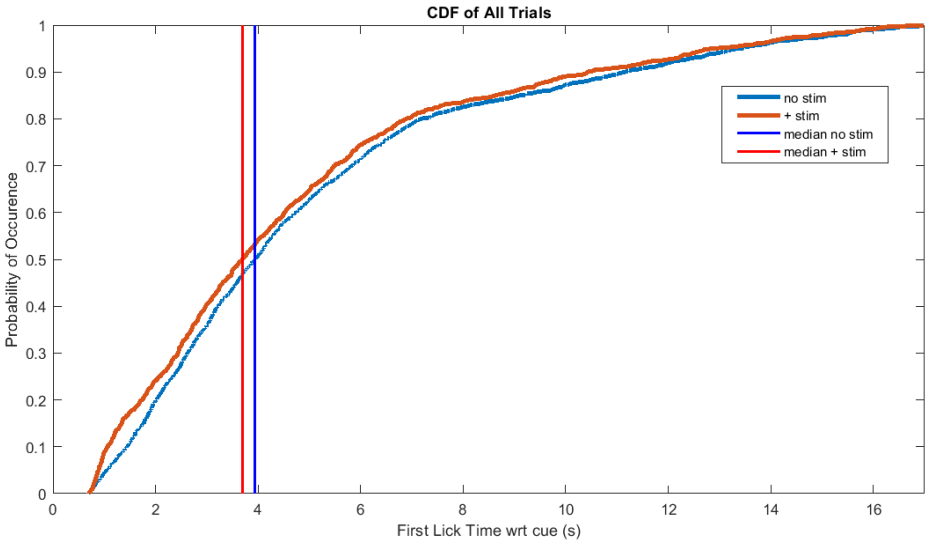

Figure 4.7: H17 taper CDF

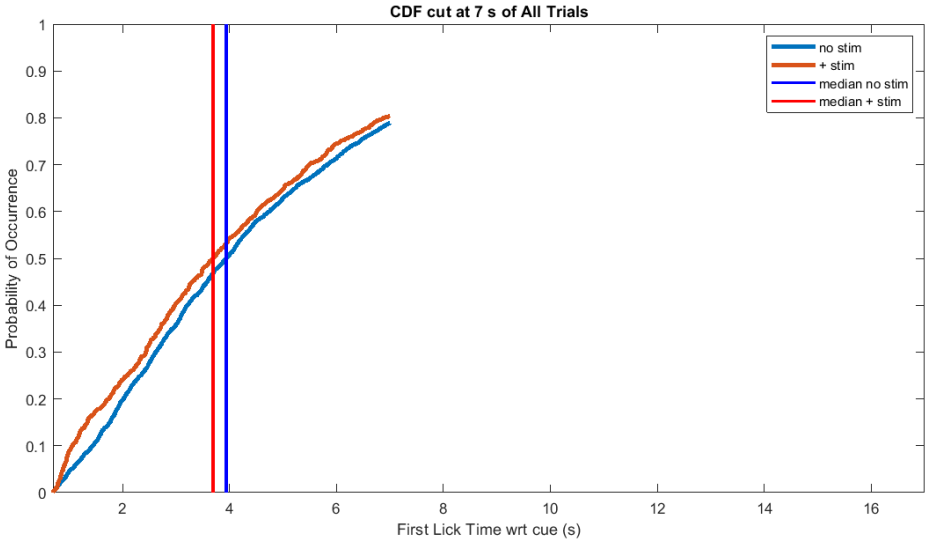

Figure 4.8: H17 taper CDF cut

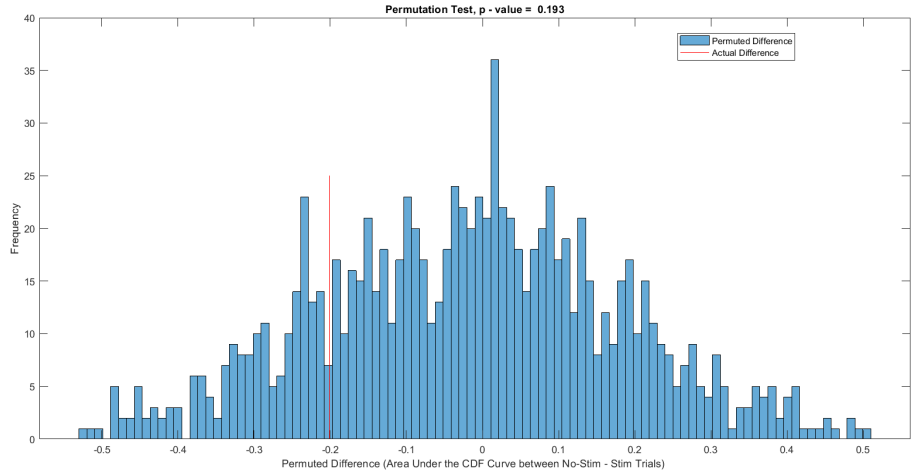

Figure 4.9: H17 taper Permuted Difference

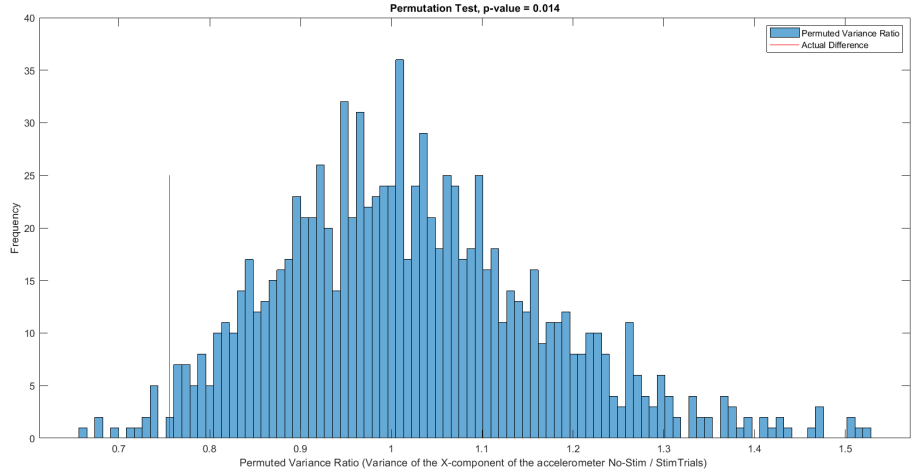

Figure 4.10: H17 taper Permuted Variance Ratio

4.2.2 Sham days

The sham days are used as control. This days shouldn't have any preference behavior, since the light was turned off. Effectively, analyzing all the plots for each animal there isn't any statistical significance.

The p-value calculated for all the permutation test is above the significance level.

Figure 4.11: H16 Sham Raster Plot

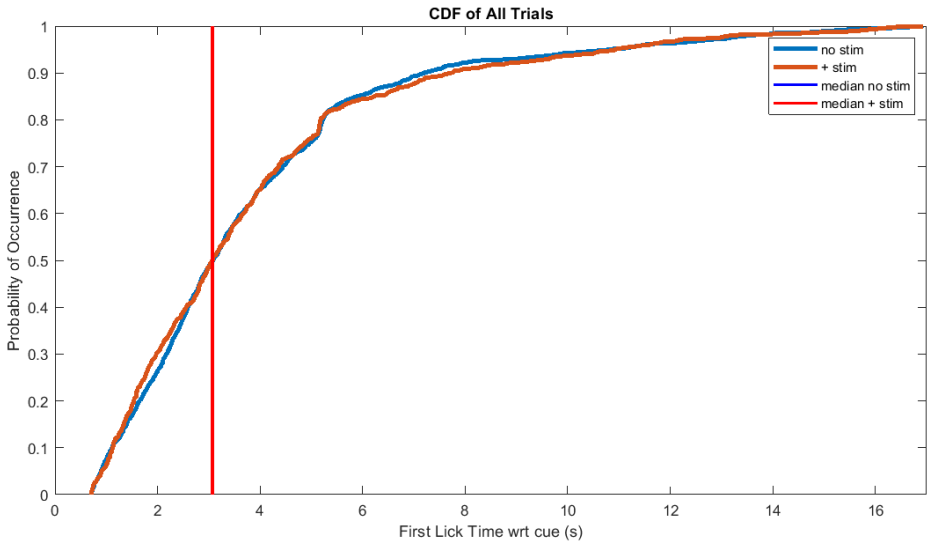

Figure 4.12: H16 Sham CDF

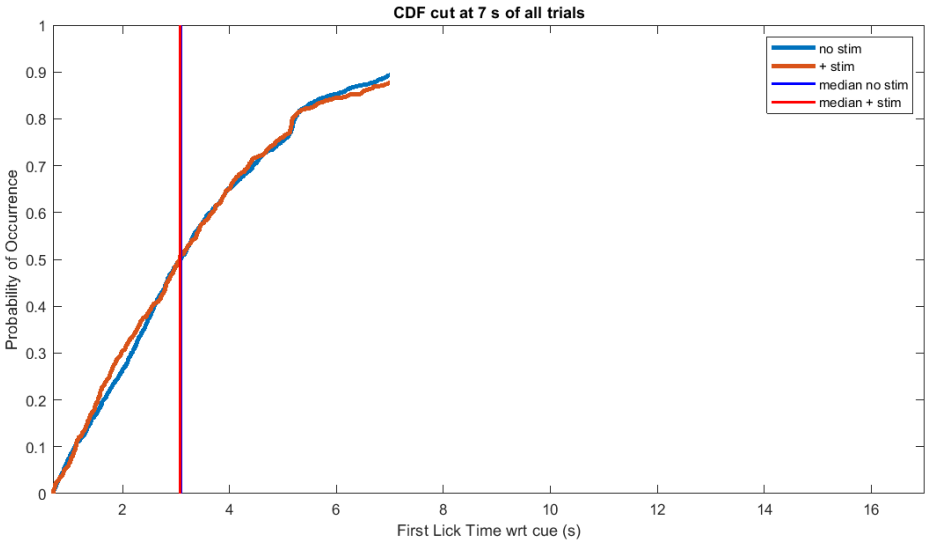

Figure 4.13: H16 Sham CDF cut

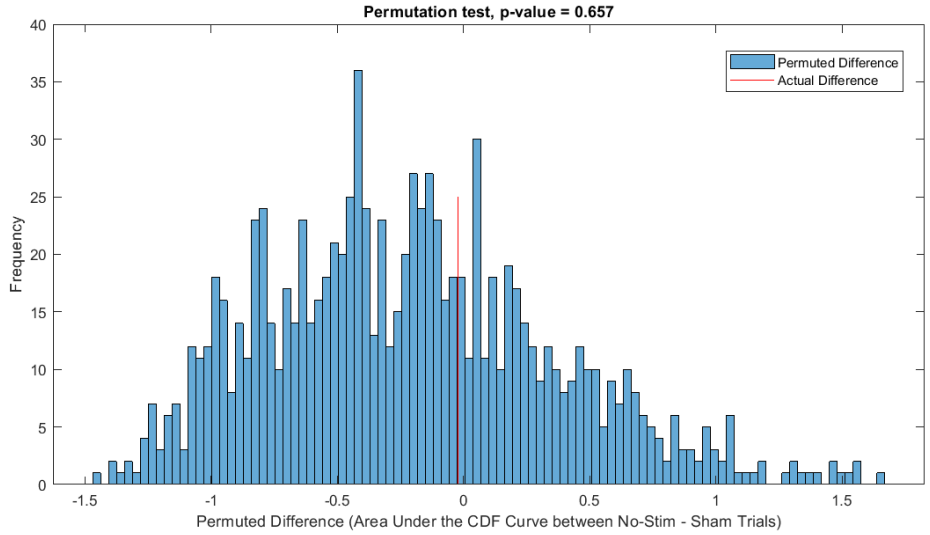

Figure 4.14: H16 Sham Permuted Difference

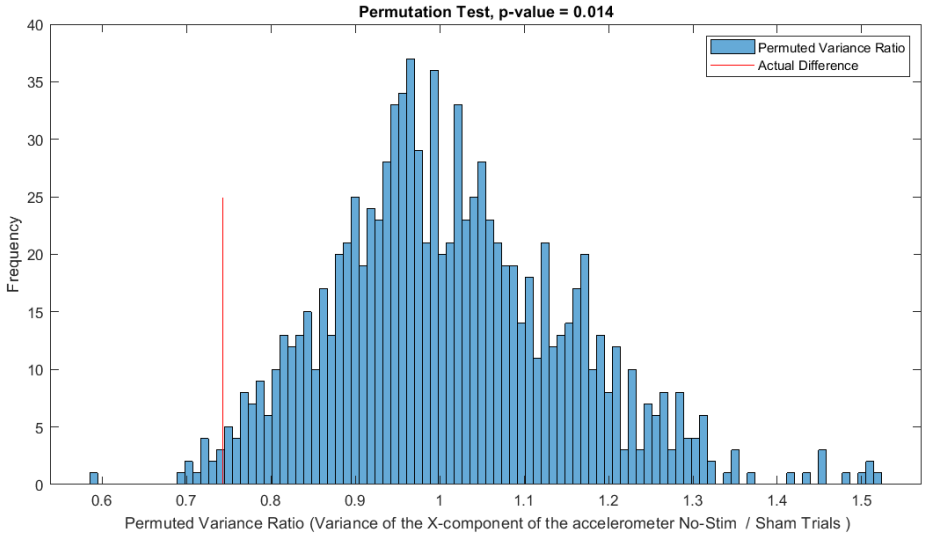

Figure 4.15: H16 Sham Permuted Variance Ratio

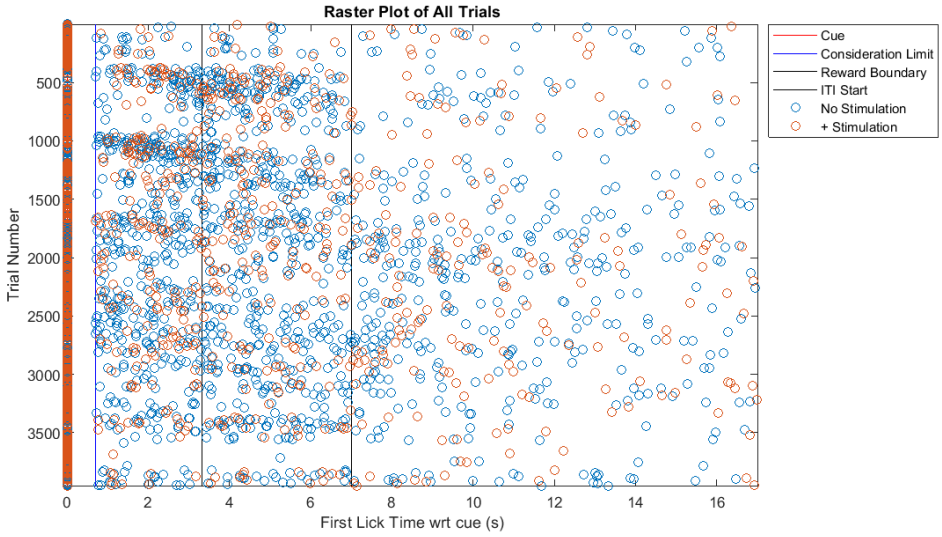

Figure 4.16: H17 Sham Raster Plot

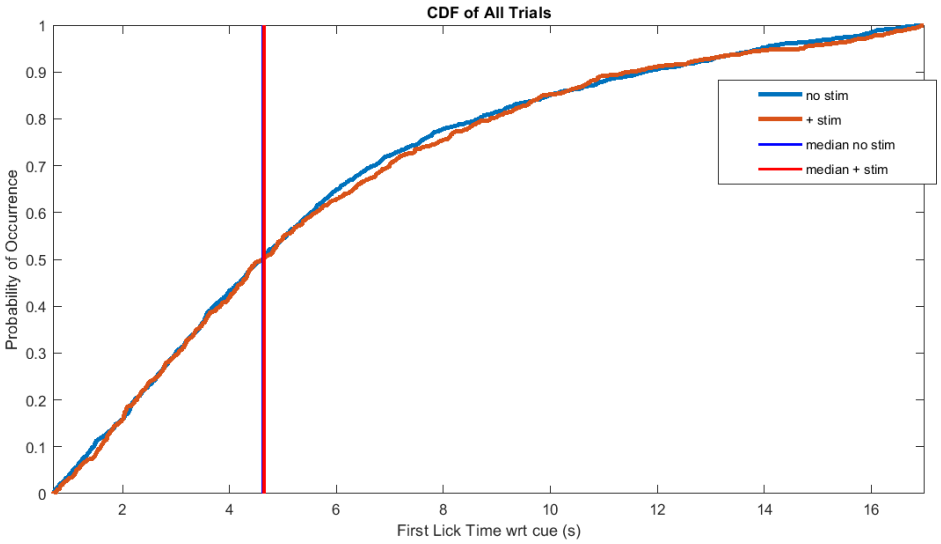

Figure 4.17: H17 Sham CDF

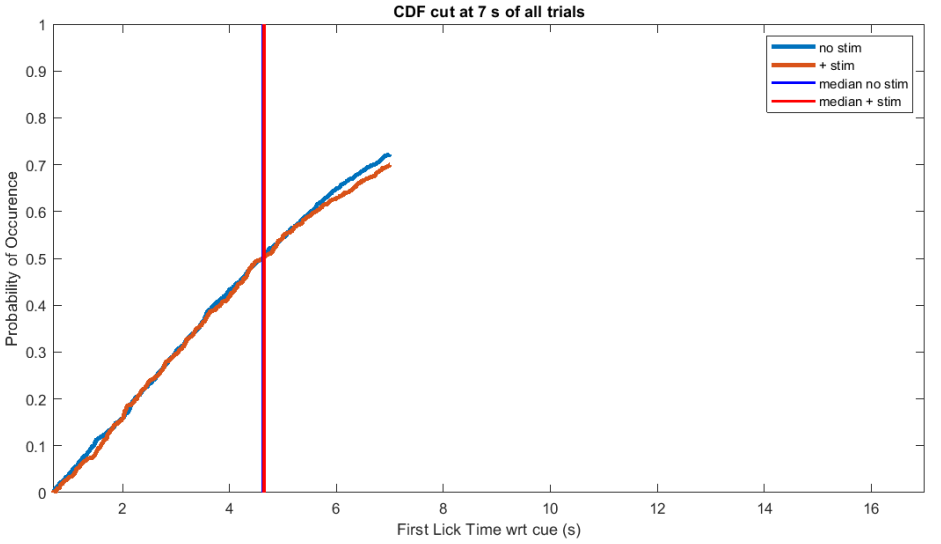

Figure 4.18: H17 Sham CDF cut

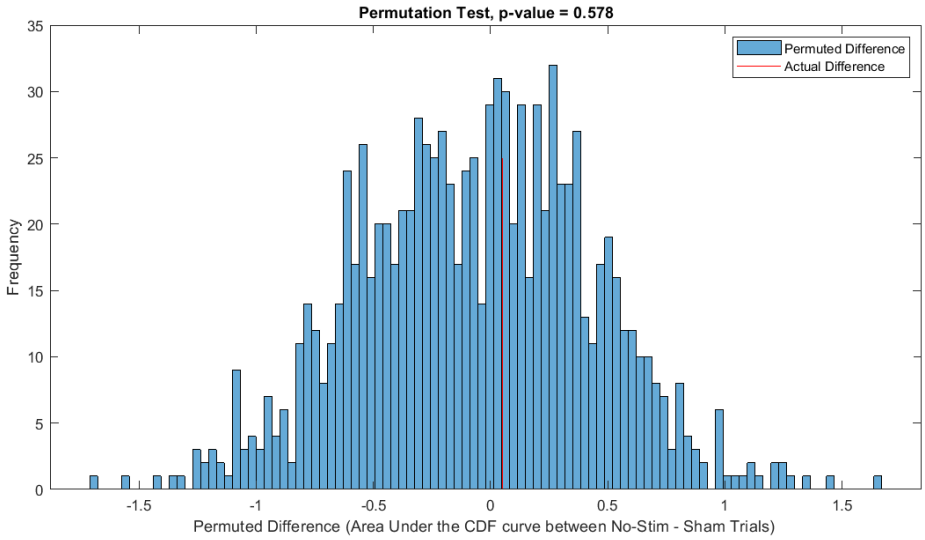

Figure 4.19: H17 Sham Permuted Difference

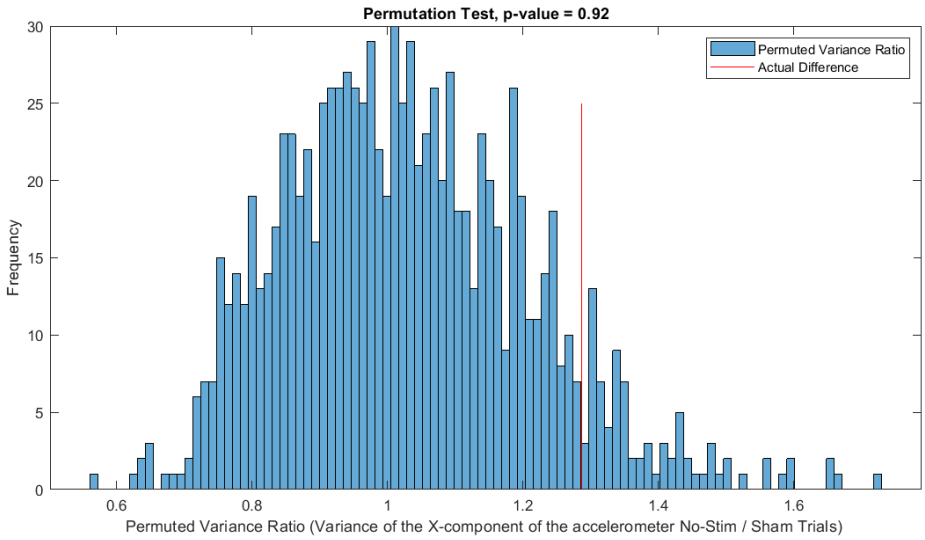

Figure 4.20: H17 Sham Permuted Variance Ratio

4.2.3 Cut days

When the stimulation concerned only the flat-faced optical fiber, those are named cut days.
This was used as a positive control since this is the classical approach to modulate the neural activity.
After the first day, the level light was heightened.

The Raster Plot 4.21 shows a concentration of first licks at the beginning of each day with a trend to not lick anymore at the end. In particular, after several days, it looks like the mouse prefers to not lick anymore, as suggesting a behavior. A patient with Parkinson's disease usually shows these involuntary movements when there is a withdrawal of drug treatment.

Furthermore, the cumulative distribution function is plotted (fig. 4.22 and 4.23). Different from when the tapered optical fibers were stimulated, there isn't an early reaction during the stimulation.
The mouse seems in general overstimulated, suggesting the light level was too much during the stimulation.

The permutation test 4.24, that calculates the difference between the area under CDF curve in case of stimulation and no stimulation, doesn't recommend to reject the null hypothesis.
The early reaction under modulation can be merely attributed to chance.

The dyskinesia observed during the experiment is also confirmed by the second permutation test 4.25.
In this case, the variance of the x component of the accelerometer was investigated.

The p-value equal to 0.01 is lower than the significance level, suggesting the rejection of the null hypothesis.

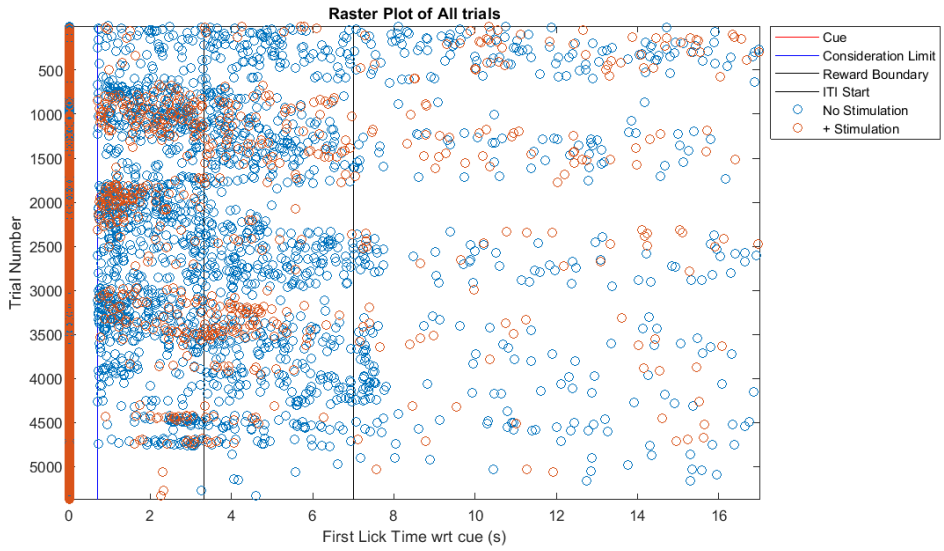

Figure 4.21: H16 cut Raster Plot

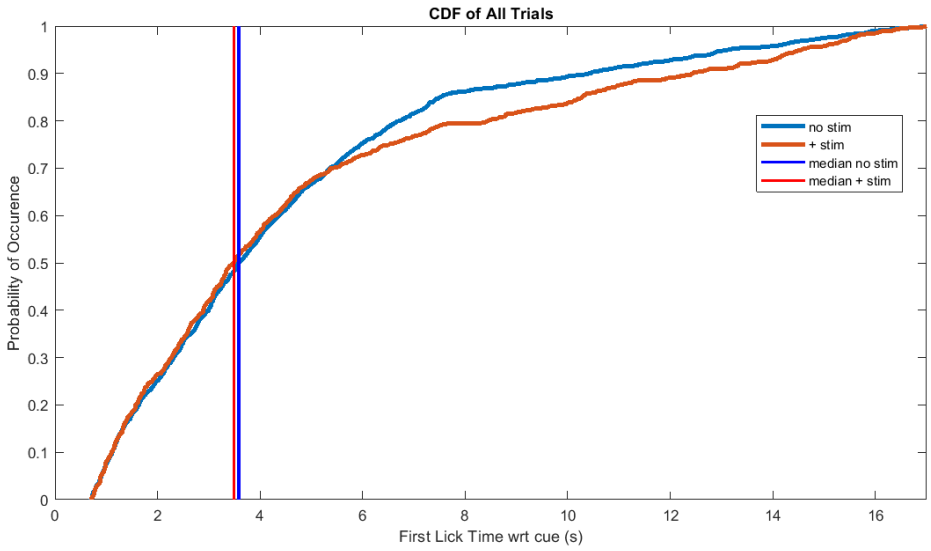

Figure 4.22: H16 cut CDF

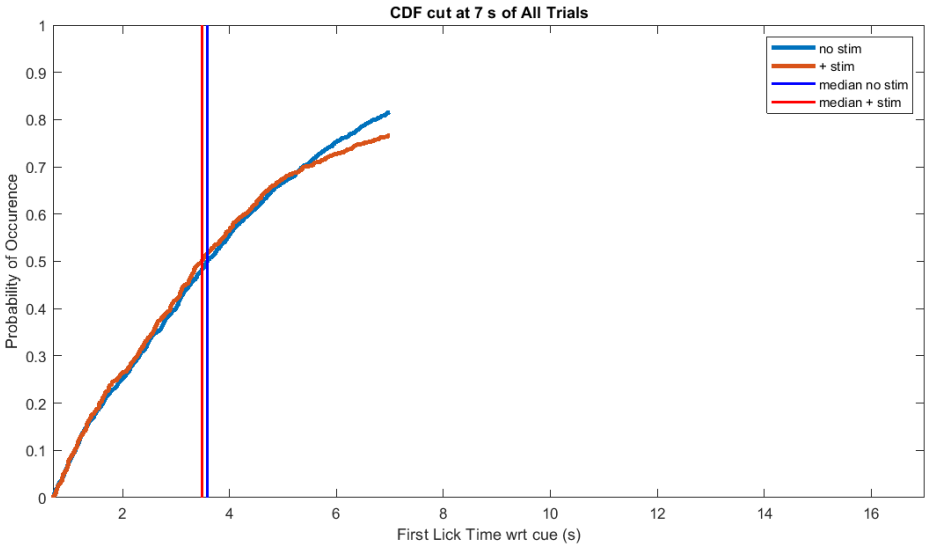

Figure 4.23: H16 cut CDF cut

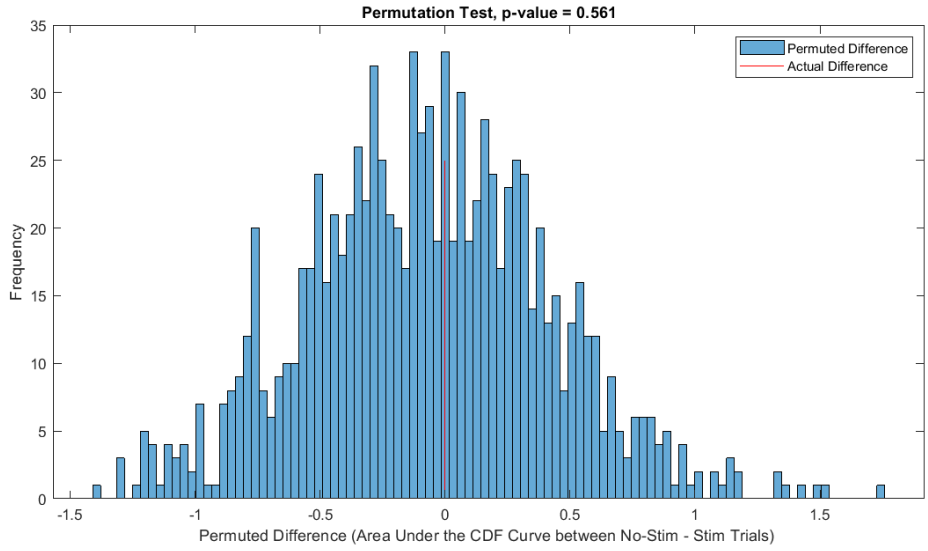

Figure 4.24: H16 cut Permuted Difference

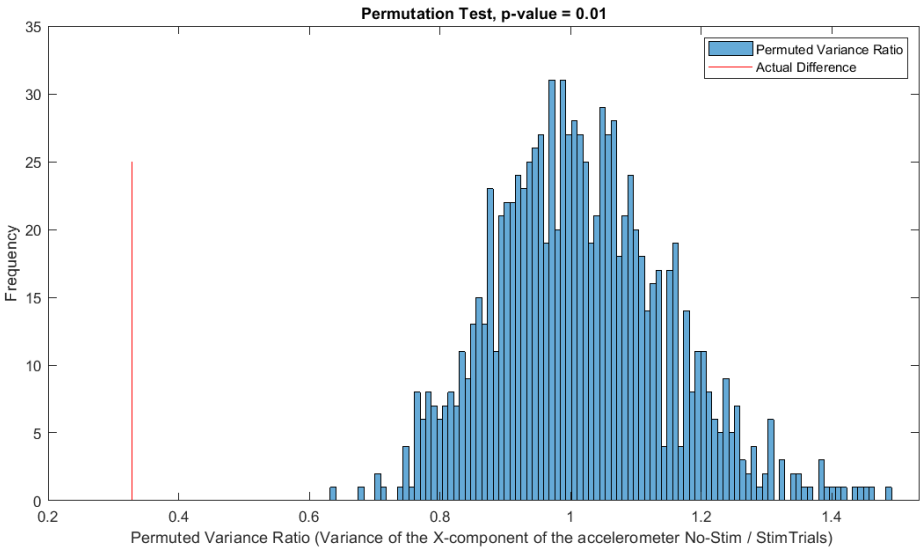

Figure 4.25: H16 cut Permuted Variance Ratio

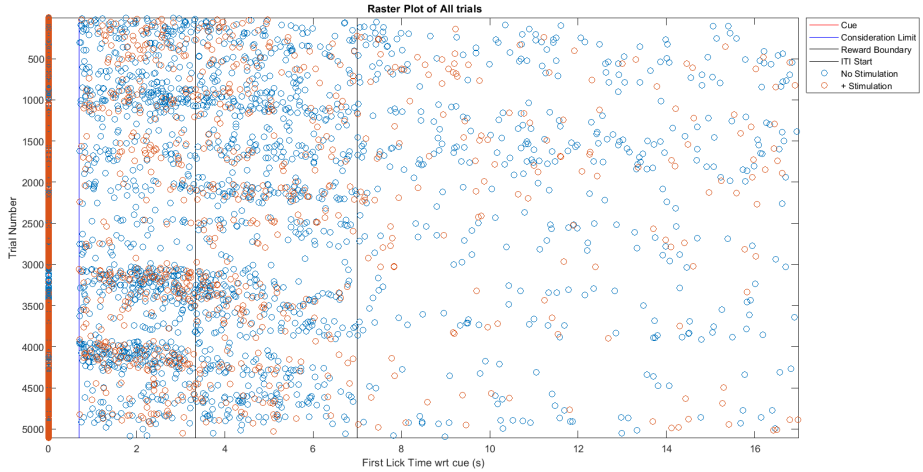

Figure 4.26: H17 cut Raster Plot

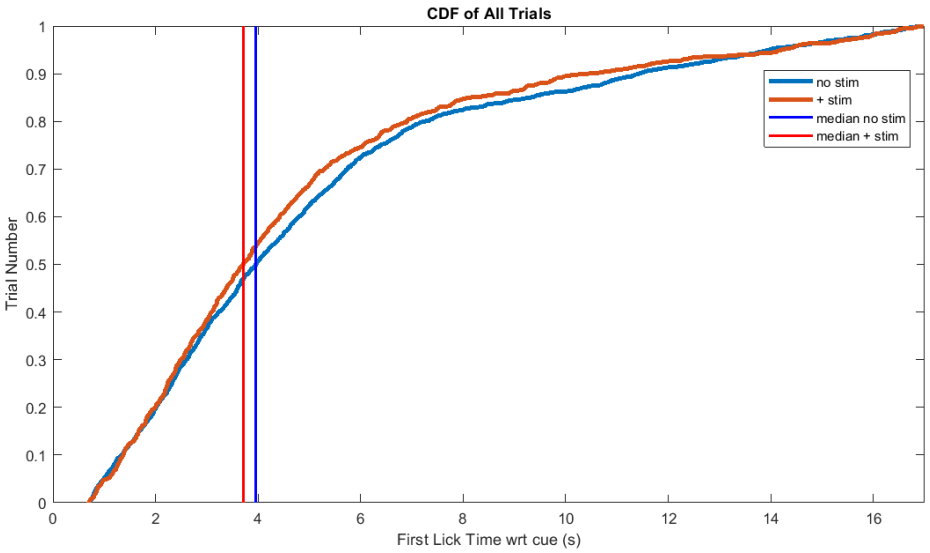

Figure 4.27: H17 cut CDF

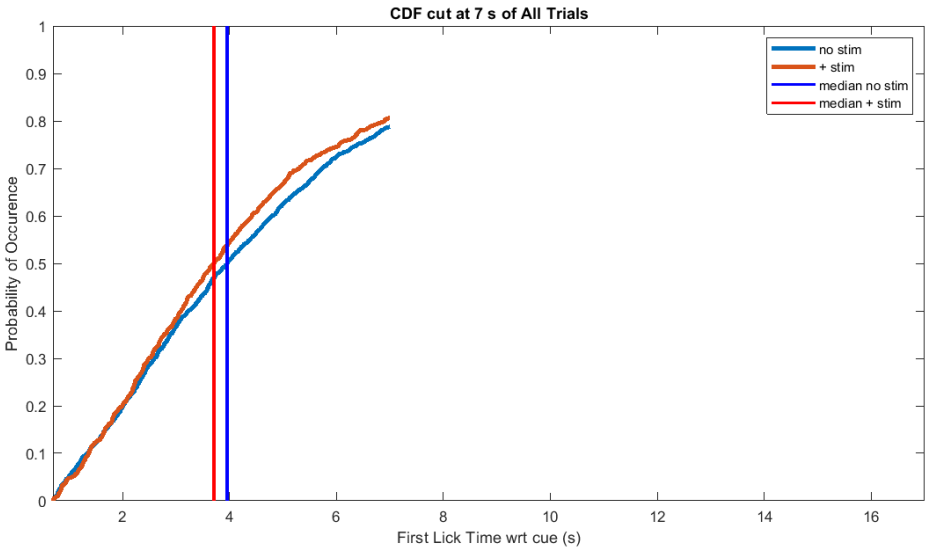

Figure 4.28: H17 cut CDF cut

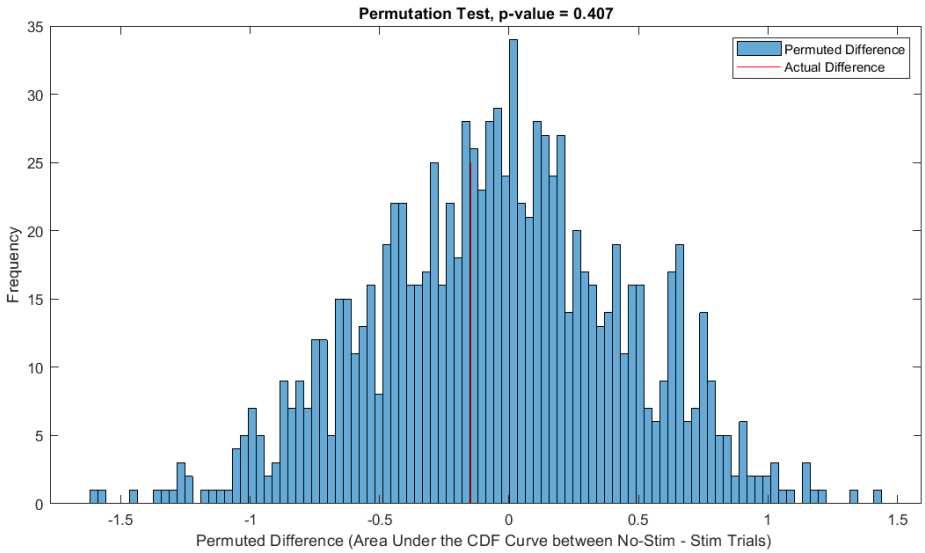

Figure 4.29: H17 cut Permuted Difference

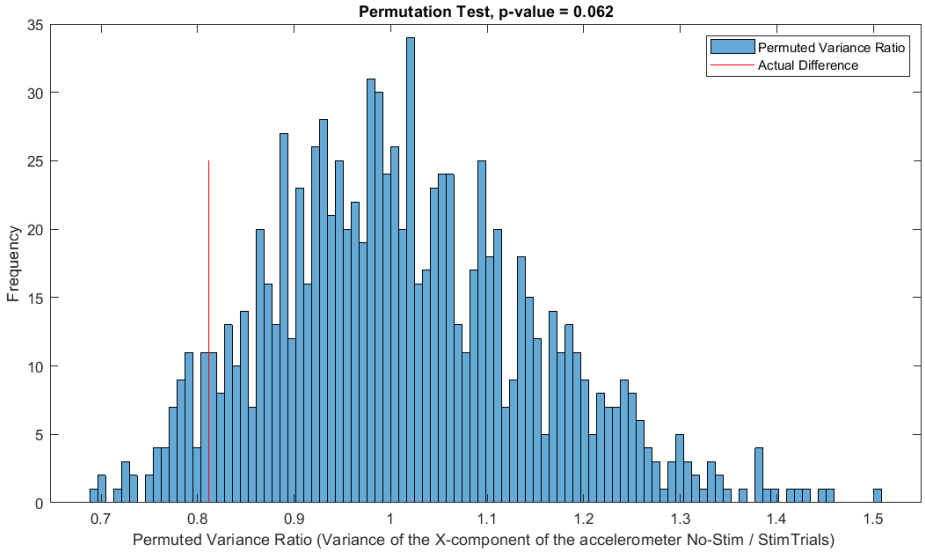

Figure 4.30: H17 cut Permuted Variance Ratio

Table 4.1: The table summarized more details about the first mouse, the fibers used and the days processed.

	H16
Name	Liz
Mouse	DAT-cre
Gender	Female
Virus injected	AAV5-CAG-FLEX-ChR2-eYFP
Virus injection	Cell bodies (Z = 4.2 mm)
Rate of injection	200 µl at 2 times magnification
Number of injections	2
1st taper fiber	Terminal left (Z = 3.8 mm)
2nd taper fiber	Terminal right (Z = 3.8 mm)
NA taper fibers	0.39
1st cut fiber	Cell body left
2nd cut fiber	Cell body right
1st cut fiber transmittance	55%
2nd cut fiber transmittance	55%
Experiment duration	30 days
Started	7/16/18
Finished	8/25/18
Sham days	7/14 processed
Taper days	7/7 processed
Cut days	7/7 processed

Table 4.2: The table summarized details about the neural activity manipulation of the first mouse.

H16					
Rig	Date	Day	Mode	Stimulation	# Trials
1	7/16/18	1	Hybrid 500	Sham	190
1	7/17/18	2	Hybrid 500	Sham	279
3	7/18/18	3	Hybrid 500	Sham	314
4	7/20/18	5	Operant 0	Sham	367
3	7/24/18	8	Operant 0	Sham	846
3	7/27/18	11	Operant 0	Sham	599
3	8/1/18	12	Operant 0	Taper	584
1	8/2/18	13	Operant 0	Sham	568
4	8/3/18	14	Operant 0	Cut	668
4	8/6/18	15	Operant 0	Sham	234
4	8/6/18	15	Operant 0	Taper	590
4	8/7/18	16	Operant 0	Cut	876
3	8/8/18	17	Operant 0	Sham	665
3	8/9/18	18	Operant 0	Taper	920
3	8/10/18	19	Operant 0	Cut	1181
3	8/13/18	20	Operant 0	Taper	1008
3	8/14/18	21	Operant 0	Cut	883
3	8/15/18	22	Operant 0	Sham	745
3	8/17/18	23	Operant 0	Cut	442
3	8/18/18	24	Operant 0	Taper	906
4	8/20/18	25	Operant 0	Taper	706
4	8/21/18	26	Operant 0	Cut	264
3	8/22/18	27	Operant 0	Sham	600
4	8/23/18	28	Operant 0	Taper	512
4	8/24/18	29	Operant 0	Cut	694
4	8/25/18	30	Operant 0	Sham	567

Table 4.3: The table summarized more details about the second mouse, the fibers used and the days processed.

	H17
Name	Meg
Mouse	DAT-cre
Gender	Female
Virus injected	AAV5-CAG-FLEX-ChR2-eYFP
Virus injection	Cell bodies (Z = 4.2 mm)
Rate of injection	200 µl at 2 times magnification
Number of injections	2
1^{st} taper fiber	Terminal left (Z = 3.8 mm)
2^{nd} taper fiber	Terminal right (Z = 3.8 mm)
NA taper fibers	0.39
1^{st} cut fiber	Cell body left
2^{nd} cut fiber	Cell body right
1^{st} cut fiber transmittance	56%
2^{nd} cut fiber transmittance	58%
Experiment duration	30 days
Started	7/18/18
Finished	8/26/18
Sham days	7/14 processed
Taper days	7/7 processed
Cut days	7/7 processed

Table 4.4: The table summarized details about the neural activity manipulation of the first mouse.

H17					
Rig	Date	Day	Mode	Stimulation	# Trials
1	7/18/18	1	Hybrid 500	Sham	369
3	7/20/18	3	Hybrid 500	Sham	368
3	7/21/18	4	Hybrid 500	Sham	401
3	7/23/18	5	Hybrid 500	Sham	700
3	7/25/18	7	Operant 0	Sham	896
4	7/25/18	8	Operant 0	Sham	1179
4	8/1/18	10	Operant 0	Taper	655
4	8/2/18	11	Operant 0	Sham	648
3	8/3/18	12	Operant 0	Cut	319
3	8/6/18	13	Operant 0	Taper	225
3	8/7/18	14	Operant 0	Cut	854
4	8/8/18	15	Operant 0	Sham	699
4	8/9/18	16	Operant 0	Taper	936
4	8/10/18	17	Operant 0	Cut	1161
4	8/13/18	18	Operant 0	Taper	969
4	8/14/18	19	Operant 0	Cut	436
4	8/15/18	20	Operant 0	Sham	448
4	8/17/18	21	Operant 0	Cut	594
4	8/18/18	22	Operant 0	Taper	927
4	8/20/18	23	Operant 0	Taper	672
4	8/21/18	24	Operant 0	Cut	886
4	8/22/18	25	Operant 0	Sham	586
4	8/23/18	26	Operant 0	Taper	870
4	8/24/18	27	Operant 0	Cut	862
4	8/25/18	28	Operant 0	Sham	661

Chapter 5

Discussion

This project investigated the role of the tapered optical fibers during the recording and the modulation of the neural activity in behaving mice.
It is still unclear the role of the dopamine during the voluntary movement.
The first chapter studied the basal ganglia where the action selection occurs, focusing on two fundamental pathways, the direct and the indirect.
Two patterns are proposed to comprehend how they work during the movement.
The classical model states the direct pathway related to the movement, instead, that one indirect related to the rest.
The alternative model, instead, proposes a concurrent activation of both pathways during the action selection. During the movement, the direct and the indirect pathway are both activated, but the balance is shifted toward that one direct and vice versa during the rest.
This topic is really interesting since several diseases involve the loss of dopamine in substantia nigra pars compacta.
Understanding the role of the dopamine in the course of the movement could provide a novel approach during the treatment of Parkinson's disease, for instance.

The main approaches used to investigate neural activity during the movement

are then proposed.
Unlike electrophysiology, optogenetics provides high selectivity during stimulation. For this reason, this method is widely used.
The debate on the most suitable model to investigate the action potential in behaving mice is still open. This project proposed the most remarkable according to Warden, Cardin and Deisseroth.
This analysis converges to the flat-faced optical fiber. They represent an optogenetic tool widely used in research laboratories since they provide minimal impact on the behaving mice.
It is then proposed a novel approach that involves the tapered optical fibers. These utilize the same photometric apparatus already available in the laboratories, so they don't have a big effect on the costs.
These fibers present several advantages thanks to their conical shape on the tip. They are, in fact, minimally invasive and highly selective, and moreover, they are able to direct the light through two o more windows placed along the fiber.
This is a considerable benefit since it is possible to modify the experiment in progress.

Knowing these great advantages proposed by Pisanello, De Vittorio and others, it was decided to utilize this new tool to record the neural activity in behaving mice.
A GCaMP6f protein was used to highlight the neural activity of a neurons population in two transgenic DAT-cre mice. This fluorescent protein, through a viral vector, was injected and it was able to bind with Calcium during the action potential propagation.
The mice were trained to learn a timing task. This was done while the mouse was head-fixed since different parts of the brain responsible for distinctive movements. The mouse, in this way, was put in the same case every single trial. Furthermore,

activated neurons are more likely to be the same because for each test the rodent should do the same movement.
Analyzing the signal after the first recording day, it was seen the modal current delivered through the tapered optical fiber was insufficient for this purpose. For this reason, the level light was increased to a value that was a good compromise for each mouse between bleaching and recorded signal.

After the experiment was completed, all data were combined together and the activity of the neurons population was investigated. The $\triangle$F/F was plotted, triggered according to the average of the all first licks during a determinate time interval.
It was possible to note that dopamine neurons rapidly rises and then plummets after the movement. Moreover, the signal withdrawn at the terminal with the tapered optical fiber goes down before the signal recorded at the substantia nigra pars compacta.
It is interesting to observe the behavior of these neurons before the movement occurs. Every plot shows a ramp after a short latency period triggered with the cue. This could suggest the mouse is thinking to lick to get a reward.

The movement artifacts due to the tapered optical fibers were investigated in two different transgenic DAT-cre mice.
In this case, the GFP protein through a virus was injected. This only made dopaminergic neurons fluorescent, without any correlation with their calcium signal.
Also, in this case, the mouse was trained to learn a timing task and the $\triangle$F/F signal was plotted.
The graphs show movement artifacts are not negligible. On the other hand, it is possible to see they have amplitude limited, so the results found with GCaMP mice

are still consistent.

The final part of this work explored the modulation of the neural activity through the flat-faced and the tapered optical fibers in behaving mice.
The first days were called sham because even if the mouse was prepared as a real stimulation, the light was turned off. During this time interval, the mouse was learning the timing task.
After these days, the protocol included two days of stimulation and a fictitious one. As far as the first two are concerned, the tapered optical fibers or the flat-faced ones were separately stimulated.
From GCaMp mice, it emerged that the level light played a fundamental role when tapered optical fibers are used.
For this reason after two bilateral modulation days, the power of the light was increased to around 100 mW.
The results were analyzed according to which type of fibers were used during stimulation and a section dedicated to the sham days is also presented.
During the days with stimulation through the tapered optical fibers, the first licks occurred before under modulation than without. This was confirmed by a statistical analysis.
In this regard, a permutation test calculated the difference between the areas under the CDF curve without stimulation and with stimulation. The p-value equal to 0.046 was lower than the significance level. For this reason, the results are statistically significant.
A second statistical analysis was performed analyzing the x component of the accelerometer. It was calculated the variance of this signal in two cases, under stimulation and without. It was found that the mouse moved more under modulation. The p-value equal to 0.01 provides a statistical significance to these results.
During the stimulation through cut optical fibers, it was seen an over-stimulation

confirmed by the raster plot and the statistical analysis. The mouse showed typical involuntary and impaired movements equally seen in a Parkinsonian patient after years with L-dopa treatments.
To conclude, the sham days don't present a remarkable difference. As expected, since the light was turned off there wasn't a real stimulation.

At the end of all the experiments, the mice were sacrificed and perfused. This was done to study through a fluorescence microscope the exact location of the fibers and the expression of the virus.
The images confirmed that the fibers were placed in the right spots and the virus, injected at the cell body, propagated also into the striatum.

The results obtained during recording and modulation confirm tapered optical fibers are a good compromise to investigate the neural activity in behaving mice. They are more expensive than the flat-faced fibers but they offer several advantages, as minimal invasiveness and possibility to modify the experiment in progress.

Future Improvements

These results suggest possible new studies, deepening biological or technological aspects.
It is interesting to understand if it is possible to modulate the neural activity of the mouse through the flat-faced optical fibers with an appropriate light level.
For this reason, it could be useful to repeat the same modulation experiment modifying the light power.
However, the over-stimulation could provide a new model to study dyskinesia.
Another problem concerns the grooming. The mouse during the task should only

lick. In reality, analyzing its behavior through a screen, sometimes the mouse performs other types of movements.

There isn't a tool that permits to automatically eliminate all the grooming detected as first licks. Moreover, the task lasts several hours and it is impossible to watch the screen for all the experiment time. This work eliminated only the evident grooming without a deep analysis.

Comparing the first licks with the grooming the second ones show, usually, a higher frequency. It could be useful to develop new classification tools capable of automatically eliminate the movements not related to the licks.

Finally, since with the tapered optical fibers is possible to direct the light through their windows in several regions, it could be useful to repeat the experiment connecting the photometric apparatus to a mirror system.

Chapter 6

Conclusion

In summary, the tapered optical fibers were analyzed to answer to biological and technological questions.

On one side, it is still unclear the role of dopaminergic neurons during the movement. There are proposed two models, this work adopted the concurrent activation model of both direct and indirect pathways. It was tried to verify the hypothesis that dopamine neurons are involved during voluntary movements with the propagation of the action potential by the cell body along the axon towards the terminal. Two DAT-cre mice and a GCamP6f protein were used to study the calcium signal. On the other side, this analysis involved also the tapered optical fibers to understand if they can replace the flat-faced optical fibers.

The results show a very similar signal withdrawn from two different locations with two diverse fibers, suggesting that this is the same signal propagated when the voluntary movement occurs.

During the experiment, it was important to adjust the modal current delivered through tapered optical fibers for each mouse since the known level already used with the cut fibers was insufficient.

The movement artifact due to the novel tool was investigated in two different mice where the GFP protein was injected. This isn't negligible, but it doesn't appear to

affect the previous results.

To understand if it is possible to modulate the neural activity in behaving mice, two DAT-cre mice and a ChR2-eYFP protein were used.

Each rodent had four fibers, two tapered fibers located at the terminal and two flat-faced fibers placed at the cell body.

The results show an early reaction of the mice under stimulation of the tapered fibers. On the other side, the light level during the stimulation of the cut fibers wasn't optimal, so the mouse showed dyskinesia. It could be useful to repeat the experiment by optimizing the power used.

In both experiments, the results are able to highlight the great potentiality of the tapered optical fibers. They are minimally invasive and it is possible to modify the experiment in progress through their windows.

However, future improvements involve new classification tools to automatically eliminate the grooming movements and use of a mirror system to direct the light in different regions of the mammalian brain.

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