Master's Thesis

Master's degree in Neuroengineering and Rehabilitation

Neuroimaging research on olfactory rehabilitation after COVID-19

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RESUM

Els estudis i les evidències dels avantatges que aporten les teràpies de rehabilitació en relació a la neuroplasticitat son cada vegada més abundants. En aquest projecte, amb col·laboració amb el laboratori LAIMBIO (Laboratori d'Anàlisi d'Imatge Mèdica i Biometria) de la Universitat Rey Juan Carlos, s'ha fet un estudi per investigar la rehabilitació olfactiva de pacients amb anòsmia després d'haver passat COVID-19.

El TFM s'ha desenvolupat dins del projecte finançat per la URJC "Nova teràpia de rehabilitació olfactòria per al tractament de persones amb seqüeles a l'olfacte per afectació pel COVID-19".

El problema principal és que la malaltia del COVID-19 és molt recent i encara es desconeixen molts dels seus efectes a nivell neuronal. En aquest projecte s'utilitzen tècniques de neuroimatge per veure l'efecte a nivell cerebral. Es comparen imatges del cervell dels pacients abans de fer teràpia de rehabilitació olfactiva, i després de fer-la. En aquest estudi s'ha treballat amb imatges de ressonància magnètica funcional (fMRI) obtingudes a l'Hospital Universitari Quironsalud Madrid. S'han inclòs quatre subjectes a l'estudi i s'han aconseguit identificar les zones cerebrals amb activació abans de fer la teràpia olfactiva i després de fer-la.

Per últim, s'han obtingut resultats de les imatges a nivell de preprocessat, anàlisi estadístic (de primer i segon nivell) i anàlisis ROI (*region of interest*). Aquests resultats han estat interpretats i s'han extret les respectives conclusions.

RESUMEN

Los estudios y las evidencias de las ventajas que aportan las terapias de rehabilitación en relación con la neuroplasticidad son cada vez más abundantes. En este proyecto, en colaboración con el laboratorio LAIMBIO (Laboratorio de Análisis de Imagen Médica y Biometría) de la Universidad Rey Juan Carlos, se ha realizado un estudio para investigar la rehabilitación olfativa de pacientes con anosmia después de haber pasado COVID-19.

El TFM se ha desarrollado dentro del proyecto financiado por la URJC "Nueva terapia de rehabilitación olfatoria para el tratamiento de personas con secuelas en el olfato por afectación por el COVID-19".

El principal problema es que la enfermedad del COVID-19 es muy reciente y todavía se desconocen muchos de sus efectos a nivel neuronal. En este proyecto se utilizan técnicas de neuroimagen para ver el efecto a nivel cerebral. Se comparan imágenes del cerebro de los pacientes antes de realizar terapia de rehabilitación olfativa, y después de realizarla. En este estudio se ha trabajado con imágenes de resonancia magnética funcional (fMRI) obtenidas en el Hospital Universitario Quironsalud Madrid. Se han incluido cuatro sujetos en el estudio y se ha conseguido identificar las zonas cerebrales con activación antes de hacer la terápia olfativa y después de hacerla.

Por último, se han obtenido resultados de las imágenes a nivel de preprocesado, análisis estadístico (de primer y segundo nivel) y análisis ROI (*region of interest*). Estos resultados han sido interpretados y se han extraído las respectivas conclusiones.

ABSTRACT

Studies and evidence of the benefits of rehabilitation therapies in relation to neuroplasticity are increasingly abundant. In this project, in collaboration with the LAIMBIO laboratory (Laboratory of Medical Image Analysis and Biometry) of the Rey Juan Carlos University, a study has been carried out to investigate the olfactory rehabilitation of patients with anosmia after COVID-19.

The project has been developed within the project funded by the URJC "New olfactory rehabilitation therapy for the treatment of people with olfactory sequelae affected by COVID-19".

The main problem is that COVID-19 disease is very recent and many of its effects at the neuronal level are still unknown. In this project, neuroimaging techniques are used to see the effect at the brain level. Images of the brain of patients before and after olfactory rehabilitation therapy are compared. This study has worked with functional magnetic resonance imaging (fMRI) obtained at the University Hospital Quironsalud Madrid. Four subjects were included in the study, and it was possible to identify the brain areas with activation before and after olfactory therapy.

Finally, results were obtained from the images at the level of preprocessing, statistical analysis (first and second level) and ROI (region of interest) analysis. These results have been interpreted and respective conclusions have been drawn.

ACKNOWLEDGMENTS

I would like to start thanking *Universidad Rey Juan Carlos* (URJC), concretely to *Laboratorio de Análisis de Imagen Médica y Biometría* (LAIMBIO), for the opportunity to offer me to work in this project and for hosting me these months to do my internship and thus, be able to finish my master's studies. Special thanks to Angel Torrado Carvajal, tutor of my TFM in URJC, who has guided and advised me in different stages of the lifetime of this project. I would also like to thank Susana Borromeo López, co-tutor of the project at the URJC. In addition, I would like to thank my colleagues at LAIMBIO laboratory with whom I have shared hours of work, and for helping each other with any doubts that may have arisen during the work done.

Secondly, I would like to thank to *Universitat Politècnica de Catalunya* (UPC) and its teachers who have accompanied me in the course of the master's degree in Neuroengineering and Rehabilitation. Moreover, a special gratitude to Miguel Ángel Mañanas, tutor of my TFM at the UPC.

Thirdly, thanks to Pablo Garcia, who was working in a similar area to my project and I could share some results with his professional opinion.

Last but not least, I also want to thank my family, my partner and my friends for their support throughout the master's degree. Thanks to their daily encouragement and to the trust they have in me, they have made me feel immensely proud of the amount of work performed in this project.

GLOSSARY

TFM: *Trabajo final de master*

SARS: Severe acute respiratory syndrome

MERS: Middle East respiratory syndrome

WHO: World health organization

NCS: Nervous central system

TMS: Transcranial magnetic stimulation

PCR: Polymerase chain reaction

LFTs: Lateral flow tests

UPSIT: University of Pennsylvania smell identification test

VAS: Visual analog scale

CT: Computed tomography

MRI: Magnetic resonance imaging

fMRI: Functional magnetic resonance imaging

PET: Positron emission tomography

EEG: Electroencephalography

MEG: Magnetoencephalography

DWI: Diffusion weight imaging

DTI: Diffusion tensor imaging

BOLD: Blood oxygenation level dependent

HRF: Hemodynamic response function

GLM: General linear model

OD: Olfactory dysfunction

Matlab: Matrix laboratory

SPM: Statistic parametric mapping

ROI: Region of interest

UPC: *Universitat Politècnica de Catalunya*

URJC: *Universidad Rey Juan Carlos*

LAIMBIO: *Laboratorio de Análisis de Imagen Médica y Biometría*

BIDS: Brain imaging data structure

DICOM: Digital imaging and communication on medicine

TR: Repetition time

TA: Acquisition time

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1. PREFACE

In the last two years, COVID-19 disease has played a major role in people's lives, completely changing the course of their lives. It led to lockdown and isolating citizens from the world for many months, and although it is now returning to the new normality, COVID-19 is still present in our society. COVID-19 has been milder in some cases and in others it has been more severe, even causing death. Moreover, in some cases COVID-19 has caused long term or even permanent sequelae such as the cases of patients with anosmia after suffering from COVID-19, on which this project is based. [10]

1.1 Project origin

Recovering from COVID-19 can be frustrating for patients as they must be confined for two weeks approximately. Moreover, the recovery is still an enigma because some patients have a medical profile that differs from others. For instance, some of them loose smell sense while others do not. However, in many cases it has been proved that smell recovery can be solved by applying a certain therapy to the subject. And here is where this project was born, in order to understand better the neuroplasticity of smell recovery. In addition, URJC has had previous projects on studying smell impairments, therefore, this TFM is a continuation of this line of work. Finally, MAPFRE company agreed to fund this project, so this is one more reason to do research in this area.

1.2 Motivation

There are several reasons why so much interest and motivation were in the neuroimaging research on olfactory rehabilitation after suffering COVID-19.

The first motivating reason is that this pandemic is a very recent occurrence that is still present in our lives and research on its effects is very motivating. In addition, the symptom of loss of smell is very curious and not yet known in depth, which makes it even more interesting to study. Moreover, if this study is able to help further patients with these characteristics (with anosmia after suffering COVID-19), it will be so gratifying. Preliminary data from ISCIII's 'ENE-COVID-19' [31] show that 43 percent of people with antibodies have reported a sudden loss of smell. On the other hand, when compared with other viruses which cause respiratory infection, it is

estimated that 20 percent of the subjects affected by COVID-19 continue to present olfactory alterations as a sequel.

Another reason is to learn more about neuroimaging techniques and discover the utilities of several software created for this purpose, like SPM or CONN software. Furthermore, the neuroimaging techniques can be challenging as they are still under development. And everything that is challenging, is encouraging. It is so satisfying working with a really innovative technology and learning more about its tools.

Lastly, the URJC research team has previous experience in the use of olfactory rehabilitation for the recovery of olfaction in other neurosensory anosmia, including those of viral origin. In previous projects, the efficacy of such olfactory rehabilitation treatment in patients with different types of anosmia assessed by fMRI was observed. Therefore, using the same technique of olfactory rehabilitation treatment in this project is thought to achieve good results.

1.3 Prerequisites

On one hand, there are some necessary tools that have been used to carry out the project which are listed below. These tools are explained better in section 5 where software and hardware components are detailed.

- Computer with its corresponding accessories (screen, mouse, keyboard)
- Magnetic Resonance scanner, model: Signa Premier 3T (General Electric Healthcare)
- Horos software
- Matlab software
- SPM12 software
- CONN software
- MRIcron software
- Xjview software

On the other hand, and the most important thing for the correct development of the research, a previous knowledge is needed: a good command of the use of the SPM and CONN program, which also means that the user has a good level in processing neuroimaging functional data. Moreover, the user has to know how to use the Matlab and Horos program and how to transfer DICOM files into NIfTI files (in this case, with MRIcron program). Also, an understanding of the viewer program Xjview is necessary for the development of the study.

2. INTRODUCTION

This work explains how the process has been from beginning to the end of a neuroimaging research based on medical images of the brain of four subjects with anosmia after suffering COVID-19. To do this, steps have been followed that were stipulated at the beginning of the work in order to carry out all the objectives stablished. The following of the different work sections shows how it has evolved.

The first part is based on an information search phase where all the theoretical concepts to be applied in a practical way throughout the project are found. Moreover, a state of art is developed. In this way, a good academic basis to carry out this work will be included.

Secondly, there is a more detailed explanation of this procedure related to the use of neuroimaging techniques in order to find rehabilitation changes in anosmia patients' brain after suffering COVID-19. Furthermore, results and discussion of these findings will be presented.

And finally, at the end of the project are the annexes where all the scripts used in Matlab software are placed.

2.1 Hypothesis and Objectives

As it has been mentioned, this project's main objective is to identify which brain areas are activated and/or which connections are in patients' brain who have suffered COVID-19 with anosmia, before and after olfactory rehabilitation therapy. The initial hypothesis is that differences between the pre-therapy acquisitions and the post-therapy acquisitions will be found. It is believed that the olfactory area in the brain will be impaired in pre-therapy images, and it will have more activation on post-therapy images or maybe in post-therapy images other brain areas are more activated because they have learnt the functions of the impaired area due to neuroplasticity. Moreover, the results in pre-therapy images of the different subjects will not differ a lot between them, and the same between the post-therapy images of the four subjects.

Therefore, we can sum up the main objectives in the following list:

- Identify brain areas activated or inactivated before the rehabilitation therapy.

- Identify brain areas activated or inactivated after the rehabilitation therapy.
- Identify brain connections in fMRI images before the rehabilitation therapy.
- Identify brain connections in fMRI images after the rehabilitation therapy.
- Establish comparations between subjects and extract conclusions.
- Establish comparations between pre and post therapy acquisitions and extract conclusions.

Seeing as the technological world advances and that new neuroimaging techniques are developed; this work can be classified as innovative and pioneering (see section 3.4 for references of similar studies). By complying with the prerequisites for this project, it is possible to carry out these objectives through a correct work methodology by working with SPM12 and CONN softwares (as these programs were previously used in the LAIMBIO team in previous projects).

2.2 Scope of Work

The final scope of this project is to evaluate the COVID-19 condition and sustained anosmia, and how an olfactory rehabilitation treatment can help to improve this olfactory loss. It will be carried out a scientific research on olfactory rehabilitation in patients who suffered from COVID-19, using neuroimaging techniques. To accomplish that, at the end of this project this study needs to satisfy the stipulated requirements (commented in section 4). Furthermore, this work will be finished when the results of the statistical analysis are obtained with each corresponding discussion.

3. STATE OF THE ART

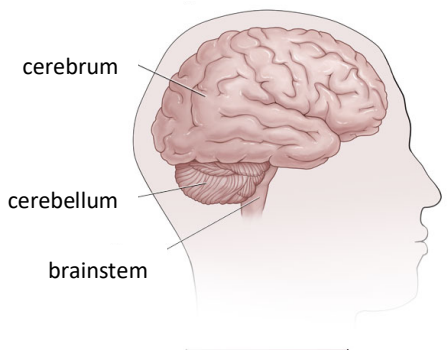
It is important to take into account the background of the project by investigating the state of the art. In order to do so, first the anatomy and physiology of the human brain and olfactory system will be explained, as these structures are involved in the study. Second, research on the pathologies suffered by the patients of this study will be generated. Therefore, a review of COVID-19 disease and a review of anosmia disease will be made. Last but not least, a report of the most current neuroimaging techniques will be carried out being focused on magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI). Afterwards, projects based on previous work in olfaction using fMRI technique will be examined and summarized.

3.1 Anatomy and physiology

In this section, the anatomy and physiology of the human brain and the olfactory system are going to be presented in order to be more conscious of the scope of the anosmia disease which the patients of this study are suffering after COVID-19. Moreover, it will help to understand better the relationship between the brain and the smell sense, and how a cerebral impairment can affect the olfactory function. Furthermore, having the fundamentals of human anatomy will allow to make better comparisons of the results at the end of this project.

3.1.1 Human brain

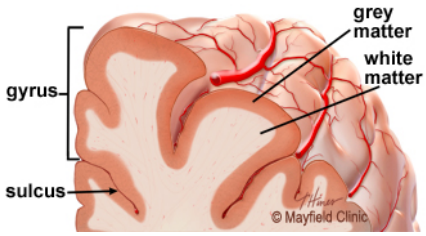
The brain is a complex organ, protected inside the skull, which receives information through the five senses: touch, hearing, sight, smell and taste. It processes the received information in order to have a meaning for the human. Then, it can store this information in human's memory. Moreover, the brain controls any function of the body. It can control voluntary movements, speech, intelligence, memory, emotions, creativity, etc. The basic anatomical subdivisions of the brain are the cerebrum, cerebellum and brainstem. [2][3]



[**Fig 1.** Basic anatomical divisions of the brain.]

First, the cerebrum is going to be explained. The surface of the cerebrum is called the cortex which is a sheet of neurons that represents half the weight of the brain. Its surface has a folded appearance where the folds are called gyrus and the groove between folds is called sulcus. This appearance allows to increase the area of the cortex in order to fit more neurons inside. Moreover, in the cortex there are 16 billion neurons distributed in layers. These neurons have the property of receiving, processing and transmiting information by emitting electrical impulses to hundreds of other neurons. The transmission of information between neurons is produced in a tiny gap between them called a synapse. Furthermore, apart from the nerve cells (neurons), the brain is also made up of glia cells which provide neurons with structural support, nutrition and protection.

In addition, the cortex has a grey-brown color because of the nerve cell bodies, that is why it is called gray matter. These neurons are connected to other brain areas by long nerve fibers placed under the cortex called axons. The axons are usually recovered with myelin, what gives them a pale coloration and that is why it is called white matter.



[**Fig 2.** Cortex of the brain.]

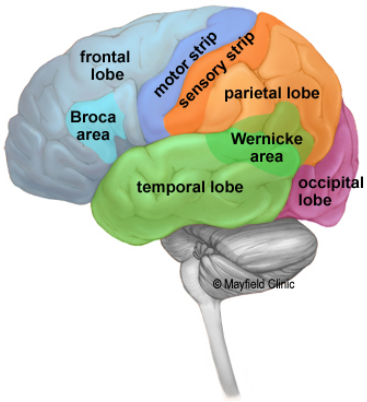
After having explained the cortex area, let's focus on the cerebrum. The cerebrum is formed by two brain hemispheres and the diencephalon. On one hand, the brain is divided on two hemispheres (the left hemisphere and the right hemisphere) where each hemisphere controls the opposite side of the body. These two hemispheres can transmit messages from one side to the other thanks to corpus callosum. Corpus callosum consist on millions of nerve fibers connected and it is placed at the center of the cerebrum.

Furthermore, each hemisphere of the cerebrum can be divided it in four parts called lobes: [4][5]

- Frontal lobe: it is the biggest section of the brain placed in the front of the head which is related with personality characteristics and with the movement. It is in charge of deciding the appropriate motor behavior in each case. There is also placed the Broca's

Area which is associated with speech ability. Lastly, the smell recognition usually involves parts of the frontal lobe too.

- Parietal lobe: it is placed on the middle of the brain. The parietal lobe allows to interpret language and words, and also interprets signals from vision, hearing, motor, sensory and memory. Moreover, it allows to identify objects and to understand the spatial and visual perception like where is the person's body placed in relation with the objects from his surroundings.
- Occipital lobe: it is placed on the back of the brain and it helps the person to interpret visual information like light or color.
- Temporal lobe: it is placed on the sides of the brain. They are important for interpreting voice and sounds in order to understand spoken language because Wernicke's Area can be found in this lobe. The temporal lobe also allows short-term memory, hearing, sequencing and organization of concepts. It is also involved in some degree of smell recognition. Lastly, it is the place where important memory structures (hippocampus) and the unconscious emotional system (limbic system) are located.

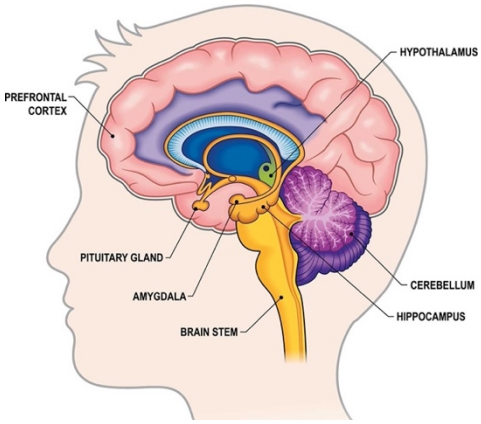


[**Fig 3.** The four brain lobes.]

On the other hand, the diencephalon of the cerebrum is placed under the hemispheres and it contains the thalamus, the hypothalamus and other structures. The diencephalon regulates the hormonal secretion of the pituitary gland through the hypothalamus and also connects the telencephalon with the brainstem through fibers. Moreover, the thalamus is the structure that processes the sensory and motor information, and influence the level of attention and alertness. [1]

Furthermore, the diencephalon is part of the limbic system which is formed mainly by the thalamus, hypothalamus, hippocampus, and amygdala. The limbic system is involved in

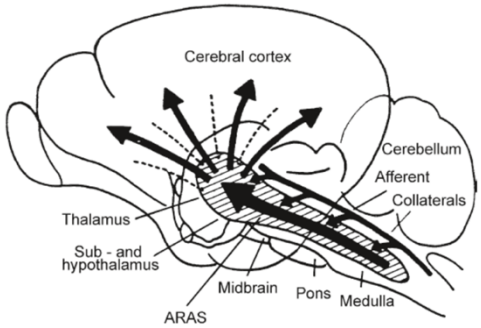
numerous functions, such as the processing of emotions, motivation, long-term memory, olfaction, socio-sexual behavior, and survival.



[**Fig 4.** Limbic system structures.]

Secondly, we find the cerebellum located at the posterior part of the brain, under the cerebrum and above the brainstem. Therefore, the cerebellum connects the cerebrum with the brainstem and the spinal cord. The cerebellum performs higher functions like controlling the movement initiation and voluntary muscle movements (motor abilities), and helps to maintain posture, balance and equilibrium. It also controls thought, temperature, touch, vision, hearing, speech, sense of reality, reasoning, problem-solving, emotions, learning, hunger and each process that regulates the functioning of the body.

Thirdly, there is the brainstem placed in the middle of the brain which connects the cerebrum and cerebellum with the spinal cord. The brainstem performs many automatic functions like controlling the movement of the eyes, face and mouth. Also regulates breathing, cardiac function, body temperature, digestion, swallowing, sneezing, coughing, involuntary movements, vomiting, and also transmits sensorial messages like heat, pain or noise. The brainstem is like an interface that processes descending and ascending information. Moreover, it is formed by the midbrain, the pons and the medulla.



[**Fig 5.** Parts of the brainstem: midbrain, pons and medulla.]

- ***Neuroplasticity***

Neuroplasticity is the way that humans adapt to changing conditions, learn new things, and develop new skills. In addition, neuroplasticity occurs constantly, which means that the brain is constantly changing. So, neural plasticity is a durable change in the nervous system associated with learning and development.

The mechanisms of neuroplasticity consist of recovering the function without restoring the original damaged structure. Therefore, the objective is to obtain functional improvements without structural repairs. After an injury, the nervous system suffers a reorganization of neural circuits, trying to replace the injured ones and enhance the remnants. Therefore, neuroplasticity increases after nervous system's injuries. Nevertheless, it is important to mention that neuroplasticity is not specific of the injury response mechanism. When the lesion is produced in the cortex, it will have a subcortical effect and normally it should be easy to detect it. Even so, sometimes there is an absence of changes, so we speak of a silent injury. If there is a recovery of the lost function thanks to neuroplasticity, we talk about adaptive changes. On the other hand, if there are deficits after neuroplasticity, we call it maladaptive changes.

Furthermore, neural plasticity can be modulated with training or pharmacological therapy in order to be used as a rehabilitation technique to recover or modify impaired functions.

It is also important to note that adult neurons lack mitotic capacity. Moreover, the creation of new neurons does not exist, except in very specific areas. Therefore, enhancing neuroplasticity can have great advantages to recover a lost function.

Last but not least, there are different mechanisms of neuroplasticity like:

- Neuronal phenotypic changes: it consists of increasing excitability in the membrane and modulating existing synaptic connections. There exist invasive techniques and non-invasive techniques in order to modulate cortical excitability.
- Growth of new connections thanks to neurotrophic factors. Neurotrophic factors stimulate axonal growth and intervene in connections with the target tissue for the establishment of synaptic connections.[6]
- Neurogenesis: generation and maturation of new neurons that can be incorporated into a specific neural network.

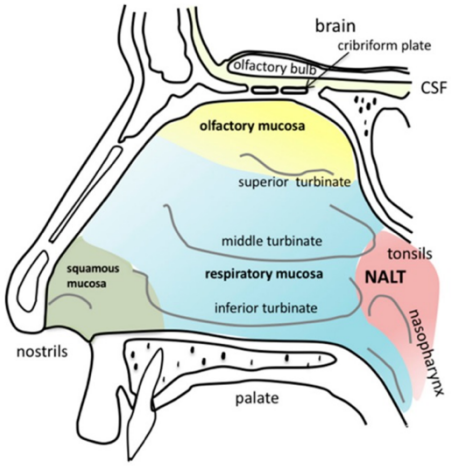
- Changes in the excitatory-inhibitory balance. Such as the excitation of cancelled inputs or the enhancement of previously silent inputs. An applied therapeutic case of this technique is to treat depression. Depressed people have been shown to have a tonic hypoactivation of the left frontal cortex [7]. Hypoactivation means less than normal activation. Therefore, the greater the activation of the left prefrontal cortex, the severity of depression decreases. Then, TMS (transcranial magnetic stimulation) is used to apply excitatory stimulation (10Hz) to the left prefrontal zone and inhibitory stimulation (1Hz) to the right prefrontal zone. In this way, it is possible to reduce depression. [8]

3.1.2 Olfactory system

The smell stimulus is called odorant which is a volatile and small substance ($< 6*10^{-22}$ g). Smell contributes to capturing information about substances found in the environment, it contributes to capturing the flavor of nutrients (complementary to taste), it detects dangerous environments and food in bad condition, and it also has an important role in emotional life and relations.

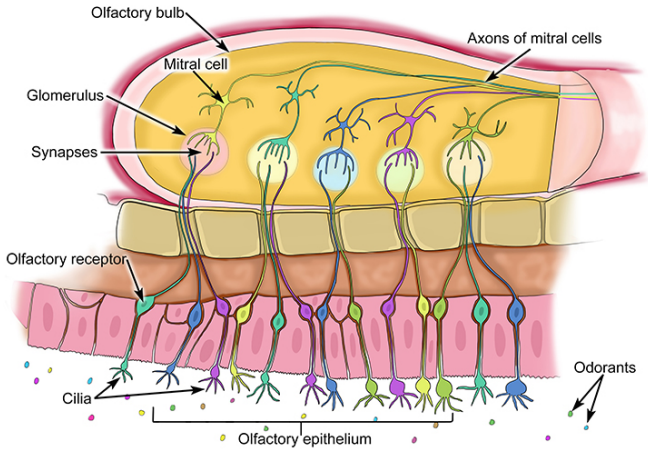
Olfactory perceptions have a detection threshold, that is a threshold from which the person is able to detect an odor. Olfactory perceptions also have a recognition threshold, that is a threshold from which the person is able to detect what type of smell it is. Depending on the odorant, these thresholds will be one or another. Furthermore, olfactory perceptions also have an intensity that is the concentration of the substance in the inspired air. This intensity increases when sniffing.

The basic mechanism for processing olfactory signals is explained down below. The odor particles (odorant) that are suspended in the air, travel in the form of vapor to the nostrils. There is placed the olfactory mucosa, which is located in the upper part of each nasal cavity. In the olfactory mucosa can be found the olfactory receptor cells which are bipolar primary sensory neurons. There, the odorants bind to receptors. Each olfactory receptor cell has several membrane receptors, being excited by different substances at a variable level. An odorant is recognized by the activity pattern of various receptors with different levels of stimulation. Different scents activate a different set of olfactory receptors. Then, the olfactory receptor cells are activated and send electric signals through their axons that go to NCS.



[**Fig 6.** Parts of the nasal cavity.]

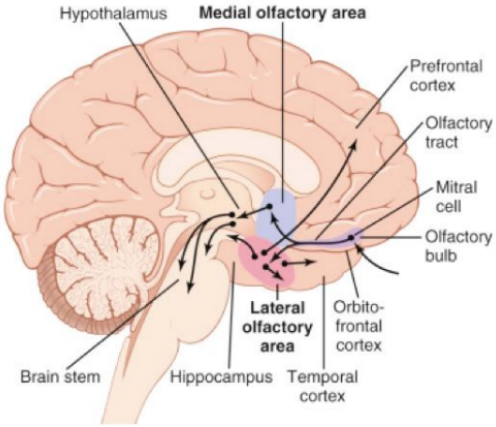
The axons of the olfactory receptor neurons end at the glomerulus of the olfactory bulb where the signals are relayed. A glomerulus receives approximately 25,000 olfactory afferences from receptors of the same type of smell. Afterwards, the afferent axons synapses with mitral neuron dendrites, which transmit signals to higher regions of the brain through axons that travel across the olfactory tract.



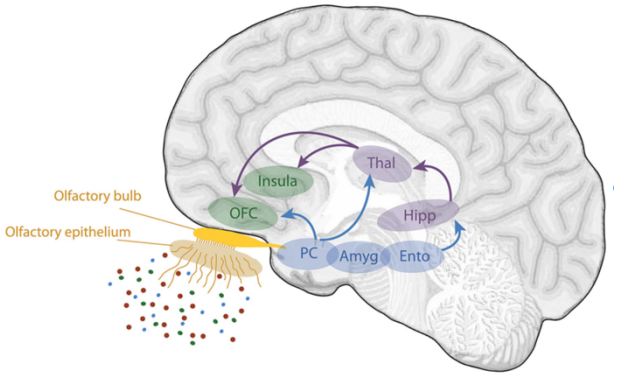
[**Fig 7.** Parts of the olfactory bulb.]

The olfactory bulb sends signals to the olfactory cortex, concretely it sends signals to the lateral olfactory area and the medial olfactory area. On one hand, the lateral olfactory area sends the information to the olfactory tubercle and the piriform cortex. On the other hand, the medial olfactory area sends information to the amygdala and entorhinal cortex (in which the olfactory memory is found). Then, signals from the piriform cortex and the amygdala travel to the thalamus and from there to the orbitofrontal cortex, which gives humans the ability to learn and to perceive consciously. Moreover, the amygdala also sends signals to the hypothalamus where

autonomic responses such as salivation will occur. Finally, other signals from the amygdala and entorhinal cortex travel to the hippocampus where the affective components take place.



[**Fig 8.** Olfactory cortex.]



[**Fig 9.** Olfactory cortex.]

3.2 Pathologies involved

The COVID-19 disease and anosmia disease will be explained in more detail in this section, as the subjects from which the medical images have been obtained have suffered from these diseases. In this way, the origin of the project and the results obtained will be better understood.

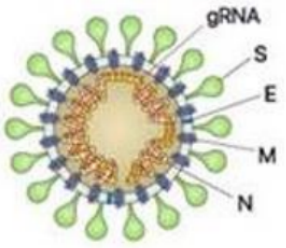
3.2.1 COVID-19

COVID-19 is an infectious disease caused by the new coronavirus SARS-CoV-2. Coronavirus are a type of viruses that can cause common infectious respiratory diseases like a cold, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Therefore, the virus SARS-CoV-2 is a coronavirus of type 2 of the Severe Acute Respiratory Syndrome which causes the COVID-19 disease.

WHO detected the existence of COVID-19 in December 2019 in Wuhan (public republic of china) for the first time, which was detected as a group of cases of viral pneumonia. Later, in march 2020 WHO declared COVID-19 as a pandemic. [10]

Focusing on the coronavirus structure, coronaviruses are spherical viruses of 100-160 nm in diameter. They contain single-stranded RNA (ssRNA) of positive polarity. Moreover, the SARS-

CoV-2 genome encodes 4 structural proteins: protein S (spike protein), protein E (envelope), protein M (membrane) and protein N (nucleocapsid). [9]



[**Fig 10.** Genomic structure of SARS-CoV-2.]

- ***Causes***

[11][12] The cause of COVID-19 disease is clear. It can only be acquired when someone is infected with the SARS-CoV-2 coronavirus. This virus spreads easily among people. According to the data, it is spread primarily from person-to-person between those who are in close contact (within approximately 2 meters or less of distance). When someone who has the virus coughs, sneezes, breathes or talks, respiratory droplets are released and the virus spreads. Then, people nearby may inhale these droplets, or they may land in their mouth, eyes or nose. This is how the virus is transmitted.

In addition, the SARS-CoV-2 can also be transmitted when a person touches a surface or an object where the virus is present and then touches his or her mouth, nose or eyes, although the risk of transmission is low.

Last but not least, it is important to mention that asymptomatic people, that is people who are infected but have no symptoms, can also transmit the virus that causes COVID-19. Moreover, people who are pre-symptomatic, that is people who are infected but do not yet have symptoms, can also transmit the virus, what is called pre-symptomatic transmission.

- ***Symptoms***

[11][12] The symptoms of COVID-19 can vary from mild to very severe. However, there are also asymptomatic people, that is people who may have no symptoms while they suffer COVID-19. The COVID-19 symptoms usually appear between four and seven days after exposure to the

virus, although this period can be extended up to 2 weeks. This period is called incubation period.

The most common symptoms are fever, dry cough and tiredness. In addition, many people also experience loss of taste or smell [32].

Other symptoms that may appear are shortness of breath or difficulty breathing (dyspnea), muscle pain, chills, sore throat, nasal congestion, headache, chest pain, conjunctivitis, nausea, vomiting, diarrhea or rash. In some cases, cardiological problems also arose with symptoms related to heart failure. In other cases, some patients had ophthalmological symptoms such as dry eyes, blurred vision and conjunctival congestion. Neurological symptoms such as dizziness, altered consciousness, stroke, epilepsy or neuralgia have also been observed. Finally, hematological symptoms have also been observed because there was an increased incidence of thrombotic phenomena associated with COVID-19 cases manifesting as stroke, cardiac ischemia, sudden death, embolisms or deep vein thrombosis.

Furthermore, it is important to mention that older adults have a higher risk to have more severe symptoms and the risk increases with age. The risk to have severe symptoms also increases in people who already have other medical pathologies.

In addition, there are cases of patients with medical sequels after passing COVID-19, these persistent symptoms are variable and usually disappear with time but can be maintained for months which affects the quality of life of patients. Some of these sequels are: altered body temperature, chills, dizziness, fatigue, conjunctivitis, shortness of breath, dry cough, chest tightness, discomfort during urination, muscle and joint pain, headache, impaired concentration and memory, loss of smell or taste, tachycardia, nasal congestion, voice changes, ear pain, nausea or dry skin. In addition to these persistent symptoms mentioned above, many people also suffer psychological symptoms such as anxiety and depression as a consequence of the situation experienced.

- ***Diagnosis***

[11][12] There are different diagnostic tests for COVID-19, however, the main criterion for diagnosis of the disease is the assessment by a healthcare professional. Therefore, the

healthcare professional will decide if the test is necessary and which of the tests the patient should undergo. Afterwards, it will also be the healthcare professional who will be in charge of interpreting the test results and indicating the next steps whether the patient's result is positive or negative.

As mentioned above, there are several types of tests for detecting COVID-19. The most common are PCR (polymerase chain reaction), LFTs (lateral flow tests) and serology test.

First, PCR is a test that detects the genetic material of the coronavirus SARS-CoV-2. In addition, PCR is able to detect the virus in both symptomatic and asymptomatic individuals. This test involves the extraction of a sample from the patient's nasal and/or buccal mucosa by the healthcare professional with the aid of a stick. Then, these samples are analyzed in the laboratory and after 24 to 72 hours the results are obtained. Moreover, the PCR test is highly reliable as it is very specific and sensitive. For this reason, PCR is considered to be the reference test.

Second, the LFTs is a test to detect virus proteins. It is also performed by the health professional with the help of a stick to extract a nasal and a buccal sample from the patient. Nevertheless, in this case the results are obtained after 20 minutes and can be analyzed at the same place where the sample was taken. Furthermore, the lateral flow test can identify individuals who have COVID-19 especially within the first five days of presenting symptoms. Eventually, this test is less reliable than PCR in detecting the infection. Therefore, it is important to know that a negative result does not rule out the presence of COVID-19 in the patient and the he or she should still follow all the preventive measures indicated by the health professional.

Third, the serology test is used to detect antibodies that fight the SARS-CoV-2 coronavirus in the blood. In this case, the healthcare professional obtains a blood sample from the patient for examination. Then, the results can take from a few minutes to a few hours to be obtained. Moreover, these tests are usually performed as a complement to the PCR test because their results are not as significant in diagnosing whether a person has COVID-19 or not. However, it is a useful test to detect if a person has already suffered COVID-19 and has antibodies.

- ***Treatment***

[11][12] There is currently no effective treatment to cure COVID-19 disease. But there are treatments to reduce or control its symptoms.

On the one hand, if the patient with COVID-19 has mild symptoms, it is important to rest as much as possible and the necessary until the person feels better. It is also very important to drink plenty of water, and it may help to take antipyretics such as paracetamol or any other medication recommended by health professionals.

On the other hand, if the patient is in a more critical case, treatment with advanced life support in the intensive care units of hospitals will be required where oxygen supply will be necessary. In some more critical cases, more advanced respiratory support methods such as ventilators will be applied. In addition, dexamethasone which is a corticosteroid can help to reduce the time patients spend on a ventilator and save the lives of critically ill patients.

3.2.2 Anosmia

Anosmia is the total loss of smell due to an alteration in the olfactory system. There are times when the loss of smell is not total, but partial, then it is called hyposmia. In this case, people can only smell certain odors or smell differently than they used to do before. [13]

In the case of anosmia, not only the smell is lost completely, but also the sense of taste may be altered and the ability to perceive flavors may be diminished. Moreover, anosmia can be a health problem by itself or a symptom of another health problem as in happens with COVID-19. When anosmia occurs after a viral infection, we call it post-viral anosmia. Post-viral anosmia is the most common cause of anosmia and in most cases the anosmia resolves once the patient is cured of the viral infection. Although there are times when anosmia remains after the infection due to virus-induced neuronal impairment. Even so, patients with post-viral anosmia have been observed to have spontaneous recovery after 1-3 years. [19]

- ***Causes***

[13][14][15] Anosmia occurs when there is an intranasal inflammation or obstruction that prevents odorant molecules from reaching the olfactory area. It can also cause anosmia when the olfactory neuroepithelium, filaments, bulbs or central connections of the olfactory nerve are destroyed. There are several ways that can cause this damage: it can be congenital, infectious or inflammatory, structural or neurological. Although the most common cause is upper respiratory tract infection such as cold or flu. The causes of anosmia mentioned above are explained in more detail below.

First, in some cases, anosmia may be congenital. Although in most cases anosmia is acquired.

Second, the cause can be infectious and inflammatory, which as mentioned above it is the most common cause. It can be caused by a flu, rhinitis, acute or chronic sinusitis or due to COVID-19. In addition, there seem to be two probable causes of post-viral anosmia [19]:

- loss of smell as a result of nasal inflammation, mucosal edema and airflow obstruction during upper respiratory tract infection,
- and/or that there is neurodegeneration of the olfactory neuroepithelium produced by direct infection and inflammation of the olfactory mucosa.

Third, the cause may be structural. Due to nasal septum deviations, nasal surgeries (rhinoplasty), due to nasal polyps or intranasal tumors.

Fourth, the cause of anosmia can also be neurological. It can be an aging impairment as there is people that lose some degree of smell with age. Degenerative neurological disorders such as multiple sclerosis, Parkinson's, Alzheimer's, traumatic brain injury, intracranial surgery, head tumor or radiotherapy treatment can also destroy nerve pathways and generate anosmia. In addition, other diseases such as obesity, malnutrition, diabetes or hypertension can also cause anosmia.

Finally, toxins such as nicotine in tobacco can also weaken sensory cells and olfactory receptors. Exposure to other toxins such as cadmium or magnesium, or the use of drugs such as cocaine can also cause anosmia. Furthermore, some chemicals such as acetone, hydrogen sulfide, acrylate or methacrylate can be harmful too. Moreover, the prolonged use of some drugs such as nasal decongestants, amphetamines or estrogens can also cause the pathology.

- ***Symptoms***

[14] The main symptom is the loss of the ability to smell. In addition, anosmia of infectious cause is often accompanied by increased mucus, cough, general malaise, fever and a feeling of fullness in the nostrils.

Moreover, the sense of smell is related to the sense of taste. And the patient with anosmia is also likely to have problems when tasting food. This may cause the patient to eat less and loose or gain weight in an uncontrolled manner. This can also cause the patient to not be able to get the nutrients they need, or if they unintentionally add too much salt to their food, they may develop hypertension or kidney damage.

Furthermore, loss of smell can impair the patient's mood and affect social interactions, leading to depression.

Finally, anosmia can also put the patient in dangerous situations as he or she will not be able to smell a gas leak or smoke from a fire.

- ***Diagnosis***

[13][15] The doctor can diagnose the lack of sense of smell by means of subjective tests or objective tests.

On one hand, subjective tests are olfactory threshold tests, in which it is observed whether the patient is able to identify an odorant and evaluate his or her olfactory memory. The most widely used subjective test in the world is the olfactometry of the *University of Pennsylvania Smell Identification Test* (UPSIT) in which 40 different odorants are used to classify the patient as having anosmia, hyposmia (mild, moderate or severe) or normosmia (normal olfaction). In addition, the VAS (visual analog scale) tool is used, where the patient marks the number that seems most appropriate on a scale ranging from 0 (no loss of smell) to 10 (total loss of smell). [18] Clinical tests are based on thresholding, discrimination and identification.

On the other hand, there are objective tests. They basically consist of electrophysiological explorations and structural and functional imaging tests. These include the electroolfactogram,

which consists of applying olfactory stimuli and recording the electrical activity of the nasal olfactory epithelium by means of intranasal electrodes. Another similar technique is olfactory evoked potentials where olfactory stimuli are presented and the electrical activity of the olfactory bulb and frontal cortex is recorded by means of external electrodes. Another diagnostic technique is computed tomography (CT) to evaluate inflammation of the nasosinusal tract. Moreover, magnetic resonance imaging (MRI) is also used to evaluate the bulb and the olfactory tract. Positron emission tomography (PET) is also used to measure brain function using radioisotopes. Finally, functional magnetic resonance imaging (fMRI) is also used to detect the activity of different areas of the brain in response to an olfactory stimuli. In following sections, the medical imaging technique MRI and fMRI will be explained in more detail as they have been used for this project.

- ***Treatment (Rehabilitation)***

[13][14] There is no single specific treatment for anosmia. In fact, treatment often depends on the cause of the anosmia. Many times, patients with anosmia recover naturally where the sense of smell returns spontaneously (especially after common colds or viral infections). However, there are other times when this does not occur and the anosmia becomes permanent. Moreover, if the sense of smell is partially preserved, taste and odorant enhancers can be added to food so that the patient can detect more taste when eating.

First, the treatment in case of anosmia of infectious origin is treated with anti-inflammatory drugs, antihistamines and, if necessary, intranasal corticosteroids and antibiotics. Some of these substances used for post-viral anosmia include intranasal sodium citrate which modulates olfactory receptor transduction cascades, intranasal vitamin A which acts to promote olfactory neurogenesis, and omega-3 which may act by neuroregenerative or anti-inflammatory means.

There are also rehabilitation therapies for olfactory training in cases of persistent anosmia or hyposmia. For example, at the Clínica Universidad de Navarra there was already a treatment for patients suffering from hyposmia before the arrival of COVID-19 [17], where 4 odorants (well differentiated between them) are offered to patients several times a day for twelve weeks. Afterwards, patients write down their experience of smelling the odorant in a notebook. Then, after twelve weeks, a subjective evaluation of what the patient has written down is made and olfaction tests are done to observe if there has been an improvement. Furthermore, there are

variations of olfactory training that also consist of repeated exposure to odorants for a prolonged period of time, however, they may differ in the type of odorants used for instance or the number of times per day the odorant is presented. Another example is the olfactory rehabilitation treatment performed at the University Hospital Quironsalud Madrid in collaboration with the URJC where an evaluation is made on the olfactory stimuli to be prioritized for the patient to start the therapeutic treatment. [32] [33]

Second, when there is a physical obstruction of the nasal cavities leading to loss of smell, surgery is performed to correct the problem.

Third, psychological or psychiatric therapy is offered in those patients where the loss of smell has most affected their quality of life.

Finally, patients with anosmia are advised to take safety measures such as smoke detectors or alarms for added protection against dangerous situations.

3.3 Neuroimaging techniques

Neuroimaging is the use of different techniques to either directly or indirectly image the structure, function, or dynamics of the nervous system. Some of these techniques are computed tomography (CT), diffuse optical imaging, event-related optical signal, magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography, single-photon emission computed tomography, cranial ultrasound, and functional ultrasound imaging.

The neuroimaging techniques can be divided in structural or functional. The structural techniques allow us to know how the brain is conformed and the functional techniques allow us to know how the brain and its areas are working between each other by measuring activity while performing tasks.

On one hand, a structural technique is CT which obtains several slices of Xray in order to reconstruct a 3D image of the brain. CT has a disadvantage because Xray can harm the tissue. Another structural technique is MRI which does not give radiation to the body. The principle of MRI technique is to apply a magnetic field to the body and see how the water molecules are aligned. Then, the image of the brain can be reconstructed because the different type of tissues

(fat, bone, water…) react different to the magnetic field. Therefore, MRI can differentiate diverse areas of the brain. Another structural technique that came from MRI is DWI (diffusion weight imaging). Inside DWI we have a specific type called DTI (diffusion tensor imaging) where magnetic fields are applied in different directions and it can be observed which is the preferent direction of the magnetic field representing nerve tracts. Then, with this information, the white-matter tractography can be reconstructed representing the anatomical connectivity in the human brain.

On the other hand, a functional technique is fMRI which measures the changes on the blood flow of the brain. The main drawback of fMRI is that it is not possible to see fast changes. Other functional techniques are EEG or MEG which both measure the electrical activity of the brain. MEG measures magnetic fields created by neurons because when neurons create an electrical interaction, they also create a magnetic field. The advantage of MEG is that the magnetic field do not get affected by the skull, because the skull can distort the electric field but not the magnetic field. The drawback of MEG is that it is a very expensive machine. Lastly, EEG measures the activity in the scalp. Afterwards, source modelling methods can be applied in order to know which part of the brain is getting this activation.

In this section, MRI and fMRI are going to be presented in more detail as this project uses these techniques in order to compare subject's brain images.

3.3.1 MRI

Magnetic resonance imaging is a structural imaging technique that measures the alignment of water molecules to strong magnetic fields. Moreover, magnetic field intensity is measured in Teslas (T).

Basically, in MRI a strong magnetic field is applied which aligns hydrogen atoms from H_2O molecules. Then, when the magnetic field is deactivated, the energy that takes to go back to equilibrium is measured (step called relaxation). Afterwards, the times that take to go back to equilibrium are measured. This time can be measured on two orientations of the magnetic field and, depending on the orientation, T1 or T2 will be obtained.

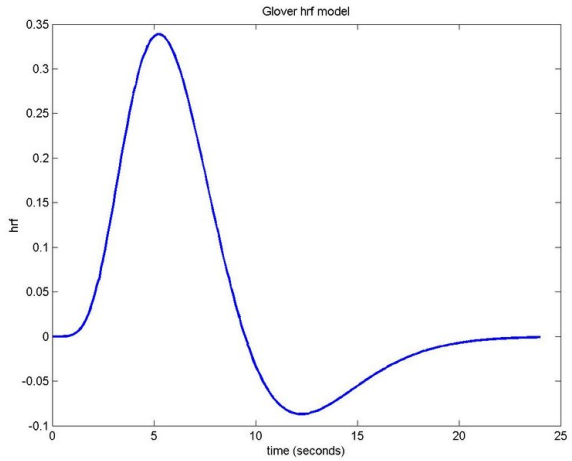
The advantages of MRI are that a good level of resolution can be achieved and does not emit radiation for the body. The disadvantages are that it is an expensive device and it requires the patient to be quiet to obtain non-blurry images. [20]

3.3.2 fMRI

Functional magnetic resonance imaging is a functional imaging technique that measures the cognitive or affective processes of the brain. The main goals of fMRI are localizing which areas are activated during a task, measuring connectivity of different brain areas, and decoding and predicting activity of the brain (from the brain activation, using maching learining, we can predict what is the subject thinking or seeing for example). In addition, fMRI is measured with the same MRI machine where a sequence of MRI images is acquired along time. Sometimes, and most of the time, subjects perform a set of tasks while recording. Moreover, in the fMRI technique, the intensity of each voxel of the brain is measured which varies through time.

Unlike MRI that uses several contrasts (like proton density, T1 or T2), fMRI uses the Blood Oxygenation Level Dependent (BOLD) contrast. BOLD measures the ratio of oxygenated and deoxygenated hemoglobin in the blood. Therefore, fMRI does not measure the neuronal activation of the brain (as with EEG or MEG), fMRI measures the metabolic demand of the area of the brain performing a task instead. On one hand, when fMRI measures oxyhemoglobin, it means there is low interaction with the magnetic resonance signal called diamagnetic. On the other hand, when fMRI measures deoxyhemoglobin, it means there is higher interaction with the magnetic resonance signal called paramagnetic.

Furthermore, it is important to know that when a subject performs a task, the brain needs oxygen. This is represented with the hemodynamic response function (HRF) where the area of the brain that needs oxygen will have a peak around 5-6 seconds and will decrease around 10-15 seconds.

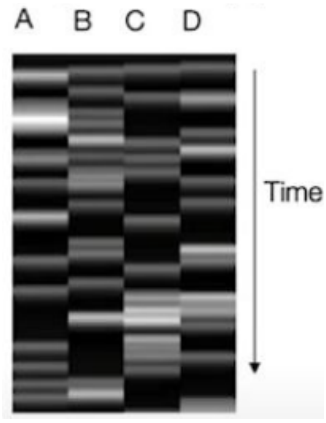


[**Fig 11.** Hemodynamic response function.]

Last but not least, the common fMRI study design will be briefly explained. As it is very difficult to extract the activation from the noise with the obtained images, in most of the cases, a statistical comparison is made (where the basal state can be compared, or a group analysis can be done) because the noise will be random but the activation will be always the same. In this way, the noise can be removed or reduced. Then, it is important to differentiate between the two type of study designs:

- Block design: a design where the stimulus are presented in blocks of an incidence structure where the frequency of appearance is constant.
- Event-related design: brief events of different types are intermixed randomly.

After reviewing these concepts, the general linear model (GLM) can be explained. First, each one of the onsets of the event-related design or block design are separated into a spiking temporal function. Then, each one of these conditions is convolved with the HRF function to obtain representation of signal activation in time. This model of activation can be put in a matrix (called design matrix) where one color will be the maximum value and the other will be the lowest value. Finally, the design matrix is correlated with the observed time series for each voxel where higher correlation means higher activations. Afterwards, connectivity measures can be observed through atlases if desired.



[**Fig 12.** Example of design matrix with four conditions (A, B, C, D), representing activation through time.]

3.4 Previous work on COVID-19 patients with anosmia

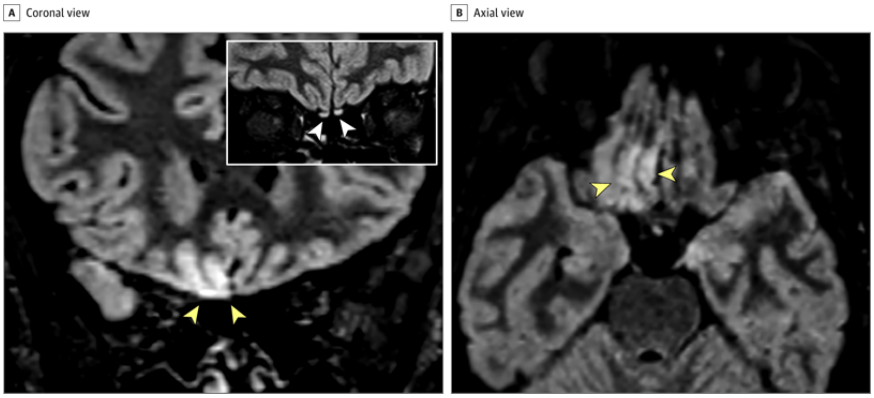
Several studies were reviewed in order to obtain information about the state of art of anosmia disease in patients who had suffered COVID-19. In order to achieve that, the key words searched were anosmia, COVID-19 and fMRI.

The first article published that mentioned the presence of anosmia in patients with COVID-19 was the one by Ling Mao et al. posted on February 25, 2020 called *Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China: a retrospective case series study* **[21]**. The article studied the neurological manifestations of 214 patients with COVID-19 disease hospitalized in a hospital in China where central and peripheral nervous system symptoms were differentiated. Moreover, hyposmia was found in 11 patients (5.1% of cases). However, no mention is made of how the presence of hyposmia was assessed, neither if all patients were systematically asked about these symptoms. Furthermore, the possibility that the alterations in taste and smell may be due to a conduction mechanism is not considered at any time, and neither are other rhinological symptoms such as nasal obstruction evaluated simultaneously.

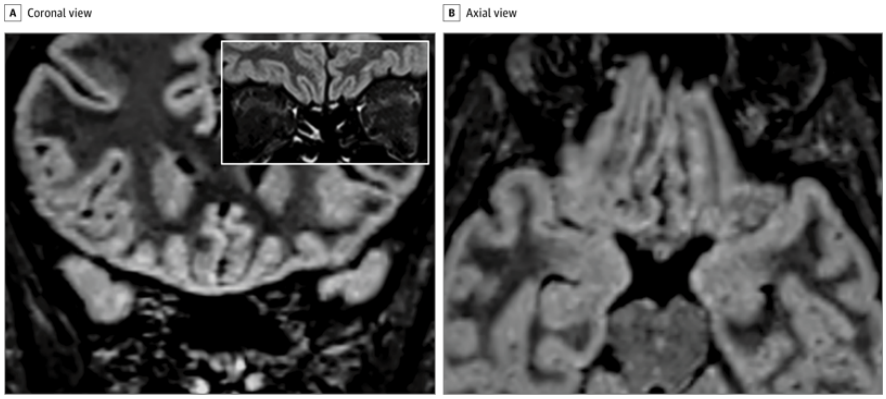
There is another article focused on anosmia disease and dysgeusia (distortion of the sense of taste) in patients who suffered COVID-19. The article was published by Nishanth Dev et al. on January 8, 2021 named *COVID-19 with and without anosmia or dysgeusia: A case-control study* **[22]**. The objective of this study is to identify factors associated with anosmia or dysgeusia in patients positive by SARS-CoV-2 and also to determine which is the clinical prevalence of these symptoms in COVID-19 patients. The study took into account 261 COVID-19 patients which 55 (21%) resulted to have anosmia or dysgeusia. Moreover, between these patients, anosmia (96%, n=53) was more common than dysgeusia (75%, n=41). Furthermore, the presence of both anosmia and dysgeusia was noted in 39 patients (71%). Therefore, the study demonstrates that anosmia and dysgeusia are fairly frequent in patients with SARS-CoV-2 infection because were noted in more than 1/5th of the cases. In addition, comparing the cases with a control group, fever, rhinitis, thrombocytopenia, elevated creatinine and bilirubin were significantly associated with anosmia or dysgeusia. The limitation of the study is that in does not include a validated tool to assess theses dysfunctions. And a larger sample size would be required in order to analyze better the clinical presentations.

Then, systematic reviews on anosmia disease have also been published lately. For instance, Abdul K. Saltagi et al. published *Diagnosis of Anosmia and Hyposmia: A Systematic Review* on July 5, 2021 **[23]**. This review examined 226 abstracts checking the diagnostic evaluation of anosmia and hyposmia. The publication concluded that the diagnosis of anosmia and hyposmia includes diagnostic smell tests and imaging modalities. Moreover, it mentions that the main method to diagnose anosmia has to be a thorough physical examination followed by smell tests such as UPSIT. Then, the study reports that the feasibility of imaging modalities is questionable in cases of patients without a neurological disorder, as well as not being cost-effective.

Afterwards, articles have also been searched where imaging techniques are used to evaluate and draw conclusions in patients with covid and anosmia. Articles have been checked in order of publication. The first article checked is *Magnetic Resonance Imaging Alteration of the Brain in Patient With Coronavirus Disease 2019 (COVID-19) and Anosmia* by Letterio S. Politi published on May 29, 2020 **[24]**. This publication studies the case of a 25-year-old woman who presented COVID-19 with anosmia. The woman had no fever and did not present any trauma, seizure or hypoglycemic event. She underwent various tests such as a nasal fibroscopic evaluation and a CT scan of the thorax and maxillofacial, none of which yielded significant results. On the other hand, a brain magnetic resonance imaging was also performed, showing cortical hyperintensity in the right gyrus rectus and a subtle hyperintensity in the olfactory bulbs (figure 13.1). Then, the patient recovered from anosmia and, 28 days later, another MRI was performed where the signal alteration completely disappeared. Moreover, the olfactory bulbs were thinner and less hypertense (figure 13.2). In addition, a brain MRI was taken from two other patients with COVID-19 and anosmia, 12 and 25 days from symptom initiation and no brain impairments were seen. The article concluded mentioning that SARS-CoV-2 might cause an olfactory dysfunction of sensorineural origin. However, brain imaging in other patients with COVID-19 and olfactory dysfunctions did not show cortical MRI abnormalities. Therefore, imaging changes might not always be present in COVID-19 or might be limited to the early phase of the infection.



[**Fig 13.1** Brain MRI in a patient with COVID-19 and anosmia (4 days from symptom onset).]



[**Fig 13.2** Brain MRI in the same patient 28 days from symptom onset.]

The second article checked is *Structural and metabolic brain abnormalities in COVID-19 patients with sudden loss of smell* by Maxime Niesen et al. published on October 20, 2020 **[25]**. This article evaluates the structural and metabolic brain factors of COVID-19 patients with dysomia[1]. Twelve patients with COVID-19 and dysomia took part in the study in which MRI and PET with [18F]-fluorodeoxyglucose (FDG-PET) were acquired. In the results, 6 patients had a bilateral blocking of the olfactory cleft, and 3 patients presented olfactory bulb asymmetry. Moreover, no signal abnormality was found in MRI images. However, core olfactory and high-order neocortical areas presented heterogeneous glucose metabolism abnormalities which were decreased or increased. Finally, this study concludes by mentioning that loss of smell may not be related to SARS-CoV-2 neuroinvasiveness, but it is related to heterogeneous cerebral metabolic changes in core olfactory and high-order cortical areas associated with active functional reorganization due to the lack of olfactory stimulation.

The third article checked is *Absent Blood Oxygen Level-Dependent Functional Magnetic Resonance Imaging Activation of the Orbitofrontal Cortex in a Patient With Persistent Cacosmia and Cacogeusia After COOVID-19 Infection* by Ismail Ibrahim Ismail et al. published on January 22, 2020 **[26]**. In this article also a 25-year-old woman had COVID-19 with anosmia and ageusia[2]. Although her COVID-19 evolution was not complicated and the anosmia and ageusia improved after a month, she started to feel an offensive odor (cacosmia) and taste (cacogeusia). Cacosmia and cacogeusia persisted for three months, so doctors did a neurological examination. MRI of the brain revealed normal results without structural abnormalities in the olfactory bulbs and sulci. However, a task-based functional MRI (fMRI), with alternating blocks of smell activation and periods of rest, was performed and showed that there was missing activation of the orbitofrontal cortex area (OFC), while the piriform cortex showed strong BOLD signal. The study compared these findings with the normal cases, where fMRI of normal individuals show a consistent BOLD activation of primary (piriform cortex, amygdala, and entorhinal cortex) and secondary (OFC, hypothalamus, and insula) olfactory areas. Therefore, the study concluded that OFC is mainly affected and may be involved in patients with impaired olfactory and gustatory function after a viral infection.

The last article checked is *A Comparative Olfactory MRI, DTI and fMRI Study of COVID-19 Related Anosmia and Post Viral Olfactory Dysfuncton* by Dazgun Yildirim published on October 2021 **[27]**.

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[1] Dysomia: dysfunction of the sense of smell.

[2] Ageusia: loss or impairment of the sense of taste.

This article compares COVID-19 anosmia with post-infectious olfactory disfunction (OD) using MRI, DTI and fMRI imaging techniques. There were 31 patients with COVID-19 and a related OD, and 97 patients with post-infectious OD. After the analysis of the imaging results, it was concluded that olfactory bulb may be an area related to COVID-19 anosmia, because in many cases of COVID-19 with anosmia the olfactory bulb was observed with abnormal shape and abnormal signal intensity. Moreover, post-infectious OD cases resulted to have a greater decrease in olfactory bulb volume and a greater decrease in the integrity of the white matter tract of the olfactory regions than COVID-19-related anosmia. Finally, it was also observed that trigeminal nerve[1] activity of patients with COVID-19 and OD was higher than patients with post-infectious OD, which may reflect that the olfactory system is better preserved in COVID-19 with OD cases.

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[1] Trigeminal nerve: Cranial nerve that transmits sensory information to the skin, sinuses, and mucous membranes in the face.

4. PROJECT PLANNING

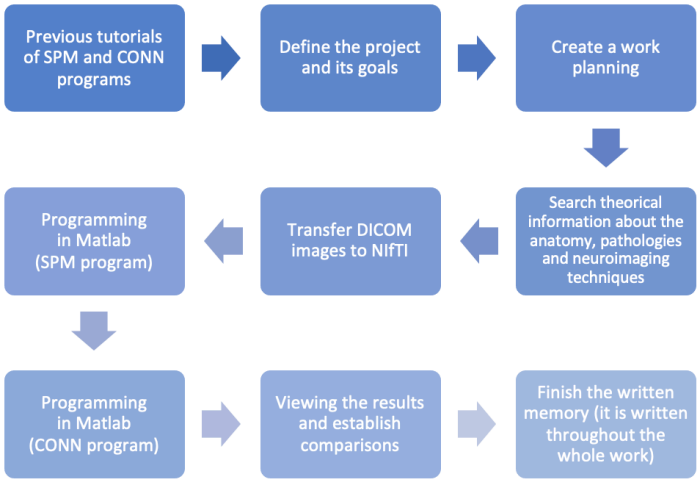
4.1 Workplan

The workplan is represented from the beginning to the end of the processing of functional images affected by real artifacts, in order to extract knowledge and validate the hypothesis in a real clinical project. Therefore, it is necessary to implement some tasks to make this project a good one. This modus operandi is quite common nowadays in every single company when working in any project, so this project follows the same strategy they usually do.

It is important to take care about the planning before starting the project, the control during the process and the presentation at the end. To make that possible there are some requirements that must be completed:

- Optimize times: do not spend too much time in a single section when it gets stuck, just skip it and it will be reviewed and fixed in the future.
- Efficient work: being well organized is quite necessary to create more content and not be redundant.
- Meetings: a very important part in a control process. There must be communication at all times from the student to her project partners and tutors in order to obtain better results.
- Presentation: the final result needs to be well presented to the audience. That is when everything matters and when everybody will judge this work. Therefore, it is essential to show this work as well as possible, getting a good explanation in writing and orally.

The diagram below shows the general sequence of how the whole process has been carried out.



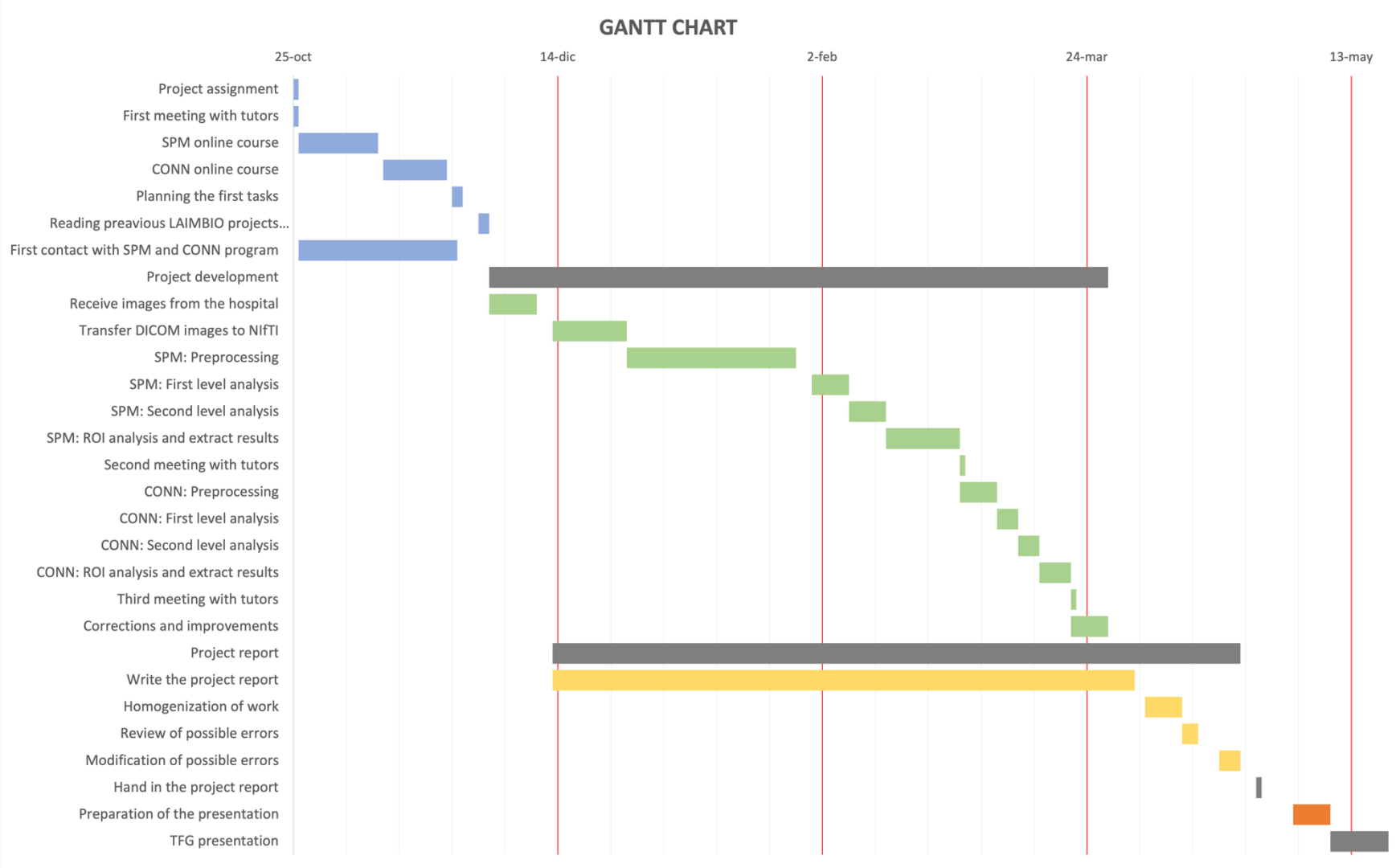
[**Fig 14.** Workflow during the final work degree.]

4.2 Gantt Chart

All tasks of the project have been planned with the Gantt chart tool. This graphic tool has provided a general view of the programmed assignments. It shows all the activities realized, the start date, the end date and the duration of each activity measured in days.

Activity	Start date	Duration (in days)	End date
Project assignment	25-oct	1	26-oct
First meeting with tutors	25-oct	1	26-oct
SPM online course	26-oct	15	10-nov
CONN online course	11-nov	12	23-nov
Planning the first tasks	24-nov	2	26-nov
Reading preavious LAIMBIO projects on this topic (TFGs, TFMs)	29-nov	2	1-dic
First contact with SPM and CONN program	26-oct	30	25-nov
Project development	1-dic	117	28-mar
Receive images from the hospital	1-dic	9	10-dic
Transfer DICOM images to NIfTI	13-dic	14	27-dic
SPM: Preprocessing	27-dic	32	28-ene
SPM: First level analysis	31-ene	7	7-feb
SPM: Second level analysis	7-feb	7	14-feb
SPM: ROI analysis and extract results	14-feb	14	28-feb
Second meeting with tutors	28-feb	1	1-mar
CONN: Preprocessing	28-feb	7	7-mar
CONN: First level analysis	7-mar	4	11-mar
CONN: Second level analysis	11-mar	4	15-mar
CONN: ROI analysis and extract results	15-mar	6	21-mar
Third meeting with tutors	21-mar	1	22-mar
Corrections and improvements	21-mar	7	28-mar
Project report	13-dic	130	22-abr
Write the project report	13-dic	110	2-abr
Homogenization of work	4-abr	7	11-abr
Review of possible errors	11-abr	3	14-abr
Modification of possible errors	18-abr	4	22-abr
Hand in the project report	25-abr	1	26-abr
Preparation of the presentation	2-may	7	9-may
TFG presentation	9-may	11	20-may

[**Fig 15.** Activities schedule.]



[**Fig 16.** Project Workplan: Gantt Chart.]

4.3 Functional and non-functional requirements

A requirement describes the services to be provided by the system and its constraints. It is a characteristic that the system must have or a restriction that the system must satisfy and can be thought of in terms of how the system responds to inputs.

In this case, the system is referred to the Matlab programming code. The functional and non-functional requirements related to the project must accomplish some factors. The needs of the programmer and researcher, the current system and the current organization must be reviewed. In addition, the current version of the programming commands for neuroimaging processes must be known and existing documents and similar background must be reviewed.

4.3.1 Functional requirements

A functional requirement is what is expected from a system to do, and in the case of this project, what is expected from the Matlab code to do. Functional requirements detail the services or functions to be provided by the system, as well as the inputs and outputs of the processes, and the data to be stored in the system. These inputs may come from users or may be the result of interactions with other systems. Therefore, a functional requirement explains how the product should react to particular inputs and particular situations.

The specific functional requirement for this code is to process the images with these steps and the corresponding parameters: preprocessing, first level analysis, second level analysis and ROI analysis.

4.3.2 Non-functional requirements

A non-functional requirement defines how should the system be in relation to quality properties of the service. Non-functional requirements refer to restrictions of some system properties such as reliability, response time, robustness, efficiency, security, storage capacity, performance, availability, or delivery time. There are different types of non-requirements and they are classified according to their implications:

- Product requirements: Requirements on the speed of the system execution and the amount of memory required, the reliability requirements that establish the failure rate for the system to be acceptable, the portability requirements and the usability requirements.
- Organizational requirements: Standards in the processes, implementation requirements such as programming languages or the design method, and delivery requirements that specify when the product will be delivered and its documentation.
- External needs: Include the requirements that define how the system interacts with the other systems in the organization, the legal requirements that must be followed to guarantee that the system works within the law, and ethical requirements.

The specific non-functional requirements for this project are:

- Software needed: Matlab, SPM12, CONN, Horos, MRIcron and Xjview programs. They have to be suitable in the compatible version of the computer's operating systems to work well.
- Hardware needed: a computer with at least 8 GB of RAM (more RAM means less time spent in processing).
- Matlab code must be able to process the four subjects at every run (or the subjects specified previously).
- Matlab code must interact correctly with SPM program, where windows should pop up while each preprocessing and statistical module is run.
- The speed of the code execution must be around 3 hours for every subject, with the characteristics of the computer used.
- The programming language used is Matlab (an abbreviation of "MATrix LABoratory").
- The programming commands used do not have to be obsolete or outdated.
- The code documentation will be summarized in this project report.
- As a legal requirement, patients used for this study must be anonymized.
- Compatibility with LAIMBIO's working procedures and tools used in their projects.

5. TOOLS USED

Hardware and software used for this project are explained in more detail in this section. On one hand, hardware devices are referred to the physical parts used to achieve the data processing. Therefore, in this project, hardware consists on the computer and the devices used for image acquisition. On the other hand, software is the set of programs or applications, instructions and computer rules that make the operation of the equipment possible.

5.1 Hardware

On one hand, the main electronic device to carry out this project has been a computer. Particularly, high computing power is required for imaging processing projects. Depending on the type of fMRI study, some could need intensive visualizations or running a lot of processes in parallel. This is related with the computer's processor (CPU) and RAM. The best idea would be to have as much CPU and RAM in the computer as possible in order to be faster and waste less time waiting for processes to finish. However, it is not always possible, or at least, it is not the case of this project. If there is not a lot of intensive visualization, 8 GB of RAM would be the minimum acceptable. The computer used is a MacBook Pro with a CPU processor of 2,7 GHz dual-core Intel Core i5 and a RAM memory of 8 GB.

On the other hand, the device used for fMRI image acquisition was a Signa Premier 3T (General Electric Healthcare) Magnetic Resonance scanner located in University Hospital Quironsalud Madrid. However, the imaging acquisition was not performed by the author of this project; the images were provided by the healthcare professionals of the hospital and the members of LAIMBIO.

5.2 Software

Several computer programs have been used in this project in order to process fMRI images: Matlab, SPM12 and CONN. Moreover, the *Horos* software has been used to convert raw medical images into DICOM format images and then, the *MRIcron* software was used to convert these DICOM format images into NifTi images with BIDS format to be able to process them. Finally,

Xjview software was used as an image result viewer. Each of these softwares are common working tools for LAIMBIO projects and they are explained in more detail below.

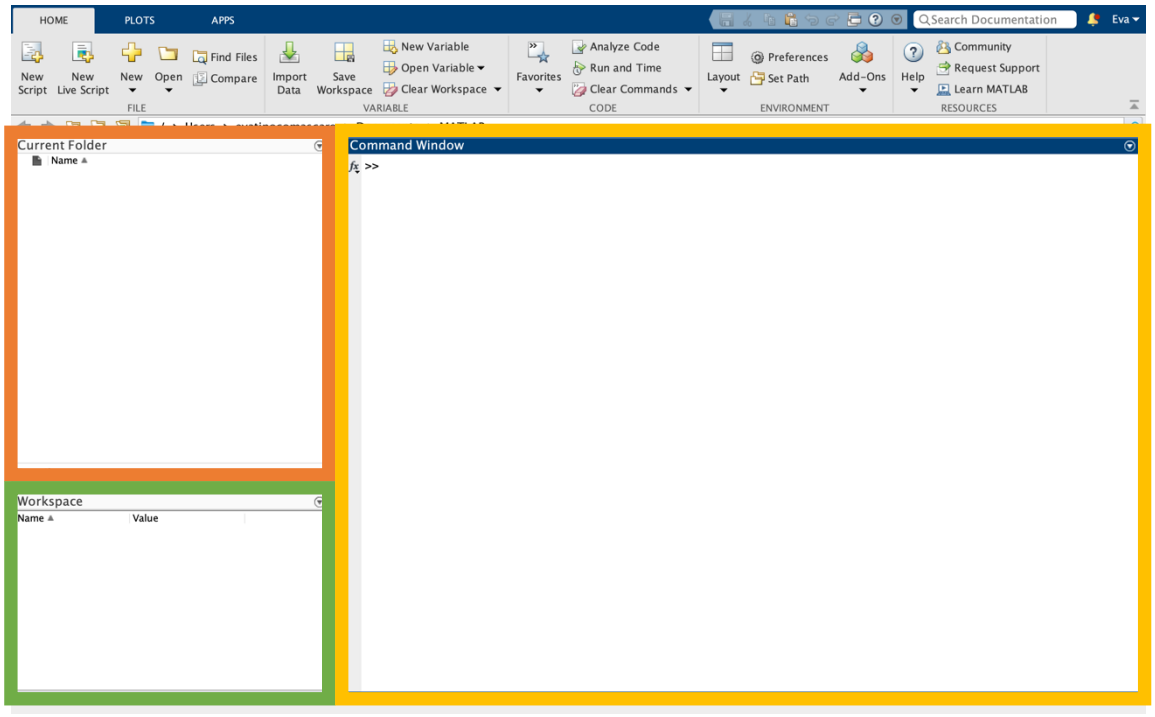
5.2.1 Matlab

The Matlab program is the main motor to run this project and extract the final conclusions. Matlab stands for matrix laboratory, and is developed by MathWorks. Matlab combines a powerful desktop environment for iterative analysis and design processes with a programming language that expresses the mathematics of arrays and matrices directly. It helps with mathematical calculations, generating plots, and performing numerical methods.[28] Furthermore, it has complementary toolboxes for a wide variety of scientific and engineering applications, it has tools for creating applications with custom user interfaces, and it also has interfaces for C/C++, Java, .NET, Python, SQL, Hadoop and Microsoft Excel. Moreover, Matlab program can be used in different applications like:

- Control systems
- Machine learning
- Signal processing
- Deep learning
- Predictive maintenance
- Test and measurement
- Image processing and computer vision
- Robotics
- Wireless communications

Then, the Matlab programmer works with a set of tools and facilities called “Matlab working environment” which allows to manage the variables in the workspace and to import and export data. This Matlab interface is composed by three main panels:

- *Current Folder*: to access the files. It represents the orange color in figure 17.
- *Workspace*: to browse data you create or import from files. It represents the green color in figure 17.
- *Command Window*: to enter commands in the command line, identified by the indicator (>>). It represents the yellow color in figure 17.

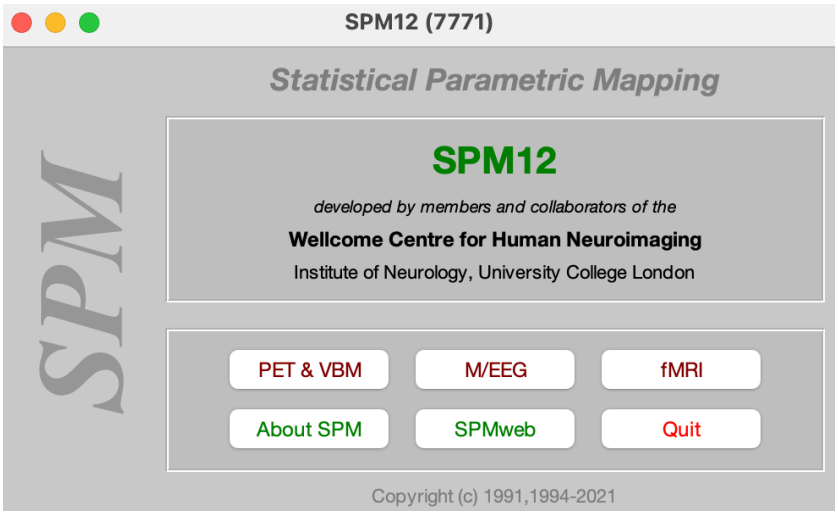


[**Fig 17.** Matlab working environment.]

5.2.2 SPM12

SPM stands for Statistical Parametric Mapping. It is a software package that is run in Matlab, designed for the analysis of brain imaging data sequences. The version used is SPM12 which is designed for the analysis of fMRI, PET, SPECT, EEG, and MEG. However, in this project, SPM will only work with fMRI images. Thanks to SPM software, not only fMRI images will be analyzed, but also SPM contains toolboxes for performing volume-based morphometry and effective connectivity. Therefore, SPM will allow fMRI preprocessing, creation of a statistical model to estimate brain activity in response to different conditions, group analyses, and region of interest (ROI) analyses.

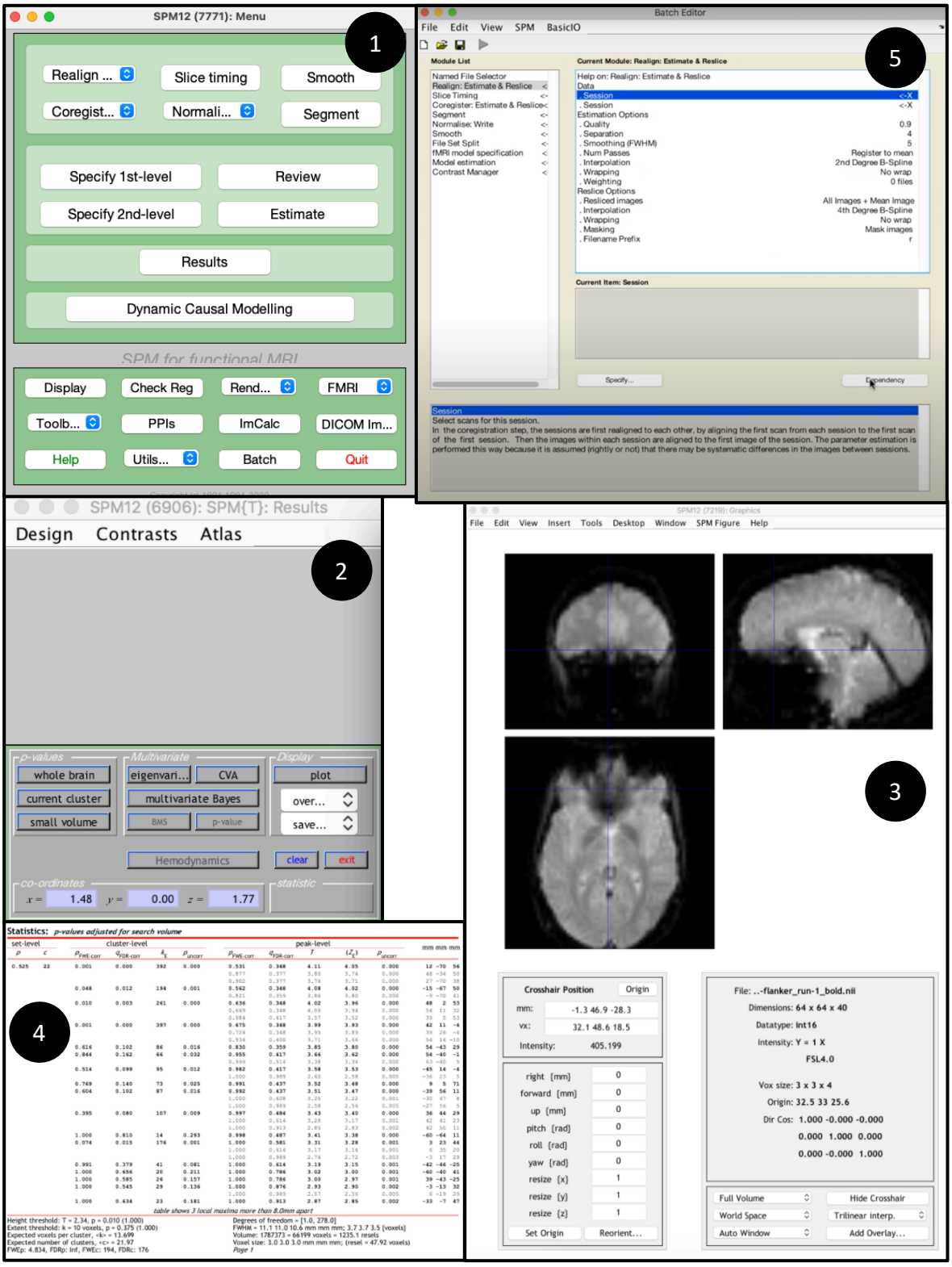
Once SPM12 software is installed, the command *>>> spm* is written in the *Command Window* of Matlab to open the program. Then, the initial window of SPM software opens (figure 18) and fMRI button has to be clicked in order to process this type of images.



[**Fig 18.** SPM12 initial window.]

After pressing the fMRI button, the windows of SPM for functional MRI are opened. The interface of the SPM12 software for fMRI modality consist on:

- Menu window (1): window where the user can decide between different functions of SPM.
- Interactive window (2): window used when SPM functions require additional information from the user when they are applied.
- Graphics window (3): window where results are shown.
- Satellite Results window (4): window where extra results can be displayed.
- Batch editor interface (5): interface that allows to apply different functions of SPM consecutively.

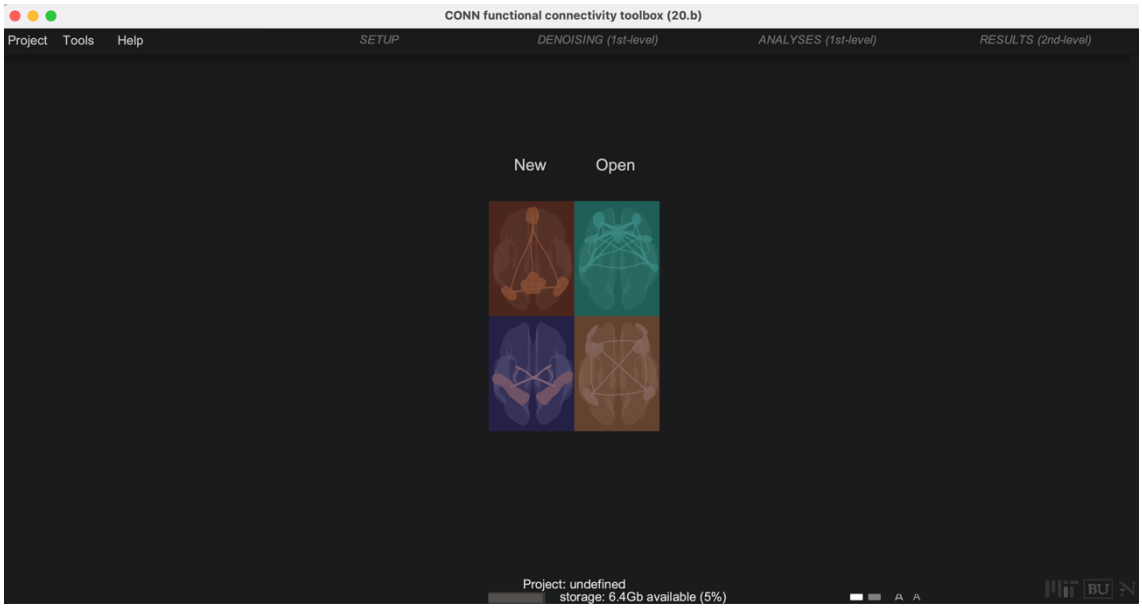


[**Fig 19.** SPM12 fMRI main windows: (1) menu window, (2) interactive window, (3) graphics window, (4) satellite results window, (5) batch editor interface.]

5.2.3 CONN

CONN software is used for functional connectivity analysis in fMRI images. Moreover, the correlation between different brain regions can be done when the subject is at resting state (resting-state connectivity) or when the subject is performing a task (task-based connectivity). It is also important to mention that CONN toolbox needs Matlab and SPM to be run.

The CONN version downloaded is 20.b. Once CONN software is installed, the command *>>> conn* is written in the *Command Window* of Matlab to open the program. Then, the initial window of CONN software opens (figure 20). A new project can be created, or an older one can be opened.



[**Fig 20.** CONN initial window.]

Inside CONN GUI it can be found four tabs along the top of the window (setup, denoising (1^{st}-level), analyses (1^{st}-level), results (2^{nd}-level)) which represent the steps that need to be done to analyze a functional dataset. Once some of these tabs is pressed, there appear more buttons on the left side of the screen from top to bottom. All these tabs and buttons control what is seen in the working space.

5.2.4 Horos

Horos software is a medical image viewer available only for apple computers, designed by Horos Project. It is based on *OsiriX* program which is also a very powerful diagnosis tool and medical

image viewer. *Horos* is a totally free and open-source software which means it is available for anyone who wants to use it. Furthermore, *Horos* allows to export all medical images of the same sequence at once into different formats: DICOM, JPG, PNG, TIFF.

The main tools of *Horus* appear on the top toolbar of the program window. There, the user has access to Cloud Service shortcuts (Cloud Dashboard, Cloud Report and Cloud Sharing), the user can also import and export the images, as well as emailing or sending data. Moreover, there is information about the images, the subjects or the machine used, on the *Meta-Data* button.

[**Fig 21.** Toolbar of *Horus* software.]

5.2.5 MRIcron

MRIcron software is NifTi format image viewer. It also incorporates a DICOM to NifTi format conversion tool (*dcm2nii*). It is available for OSX, Linux and Windows PCs. This program can load various layers of images, ROIs and generate volume renderings. [30]

5.2.5 Xjview

Xjview software is a viewing program for SPM12. This useful tool makes easier to plot the results and it saves a lot of time when viewing fMRI images, it can display several images at the same time, it includes a p-value slider and can also be used to build ROI masks. [29]

6. MATERIALS AND METHODS

6.1 Database

The database of this project is formed by fMRI images which were acquired using a Signa Premier 3T (General Electric Healthcare) Magnetic Resonance scanner located in University Hospital Quironsalud Madrid. Four volunteers with permanent viral anosmia, developed after having COVID-19, were studied. The patients were all females with ages between 44 and 54 years. In the table below, information about the subjects studied is shown (table 1).

SUBJECT	SEX	AGE	WEIGHT	GROUP	DATE ACQUIRED
Subj 1	F	45	60	Pre	24/4/21
			58	Post	30/6/21
Subj 2	F	44	58	Pre	24/4/21
				Post	23/9/21
Subj 3	F	52	75	Pre	8/5/21
			76	Post	25/6/21
Subj 4	F	53	72	Pre	8/5/21
		54	70	Post	1/7/21

[**Table 1.** Subjects, sex, age group type (pre or post rehabilitation therapy), and date acquired.]

6.2 Rehabilitation therapy

Olfactory rehabilitation therapies consist of two types of sessions: face-to-face sessions with medical specialists in the area, and home sessions to be completed by the patient. The home sessions are interesting because the patient has to write down all the situations he or she faces in relation to smell in his or her daily life. In this case, there was a first phase of clinical evaluation by the therapist and then, patients had ten face-to-face sessions (one session per week) with a duration of one hour per session which were carried out by occupational therapists.

Olfactory rehabilitation therapies usually have three phases for the formation of a correct olfactory association pathway.

The first phase consists of habituation to a daily life odorant stimulus that is previously chosen by the patient with the help of the therapist. At the end of this first phase, the patient should be able to differentiate the odor among odors with opposite characteristics to the chosen one.

In the second phase, the patient is already able to recognize the odor among others. Then, this phase consists of the patient having to find a characteristic of the previously chosen odor that he or she can relate to, as it will help the patient to retain this learned odor in his or her memory.

Finally, in the third phase, the patient can work with more than one odor. At the end of this phase the patient should be able to name and differentiate the odors worked among other odors. In addition, the patient will also be able to recognize two or more odors when they are presented at the same time.

6.3 Stimulation paradigm

During the acquisition of the functional images, patients were stimulated with two different odorants (vanilla and mint). These aromatic stimulants are delivered by the olfactometer, a stimulation device that is placed inside MRI scanner. This olfactometer has the capacity to deliver up to eight different types of odorants through its eight valves. In this study, valve number 4 was used to deliver vanilla odorant and valve number 6 was used to deliver mint odorant. These two odorants were chosen because they are pleasant odors and thus the patient does not have a bad experience while they are inside the scanner. In addition, vanilla is a primary odor which only stimulates the olfactory nerves (I) and mint, which is a bimodal odor, stimulates olfactory nerves (I) and the trigeminal nerve (V).

The study consists of an event-related design synchronized with patient’s breathing, which means that the different stimulus do not have a constant frequency of occurrence. Moreover, the same stimulation sequence was used in all patients in both the pre- and post-rehabilitation therapy acquisition. This sequence consists of ten minutes of stimulation between 12 and 14 stimuli depending on the patient's breathing rate. The olfactometer follows the patient's breathing and patient 2 breathes slower than the other subjects. The stimulation sequences used are shown in the following table.

SUBJECT	SESSION	STIMULATION SEQUENCE
1, 3 and 4	Pre and post rehabilitation	mint – vanilla – vanilla – vanilla – mint – mint – mint – vanilla – vanilla – mint – vanilla – mint – mint – mint
2	Pre rehabilitation	mint – vanilla – vanilla – vanilla – mint – mint – mint – vanilla – vanilla – mint – vanilla – mint – mint
	Post rehabilitation	mint – vanilla – vanilla – vanilla – mint – mint – mint – vanilla – vanilla – mint – vanilla – mint

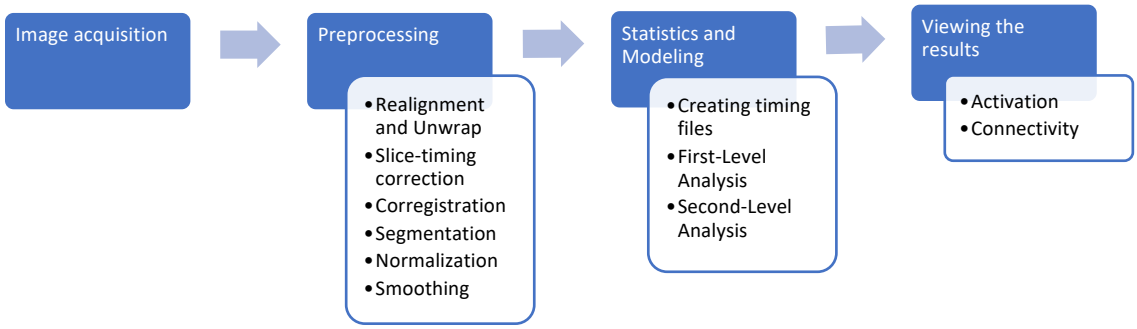
[**Table 2.** Stimulation sequences between subjects and sessions.]

Furthermore, the onset and offset of each valve will also be needed to make a correct functional analysis; the olfactometer saves these values automatically in a .txt document.

6.4 Procedure

The procedure for the analysis of the functional images of the different patients was as follows. First, the images were registered with the magnetic resonance scanner. Once obtained the subject's raw data, they have to be imported in *Horos* program to obtain the images in a DICOM format. Then, the DICOM images will be transferred to NIfTI images with BIDS format. Then, the preprocessing of functional images, the first level analysis and the second level analysis with SPM will be done. Then, the functional images will be analyzed with CONN software. Finally, the results will be displayed and analyzed to draw conclusions.

Below, there is the pipeline of the fMRI data processing used for this project.



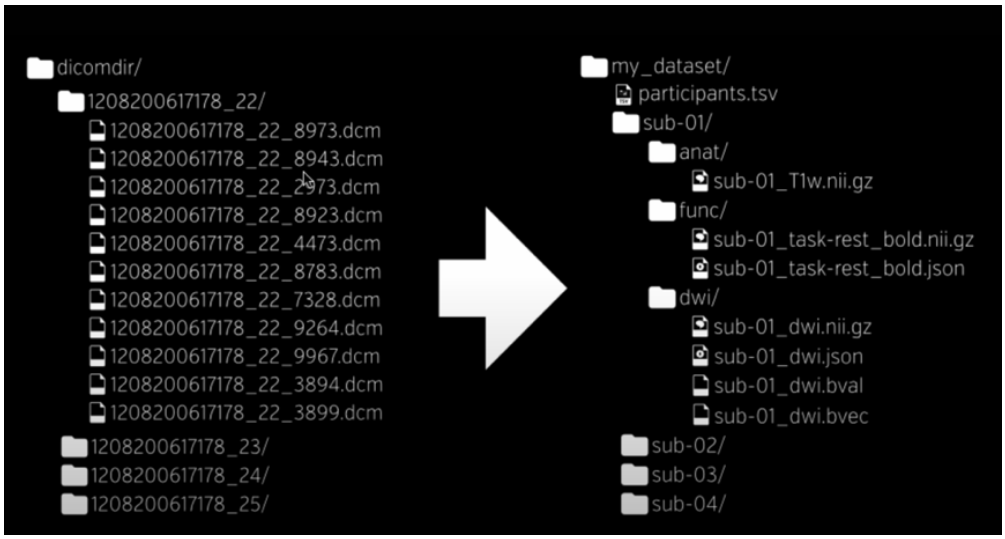
[**Fig 22.** Diagram of fMRI processing steps.]

These processes are explained in more detail in the following subsections.

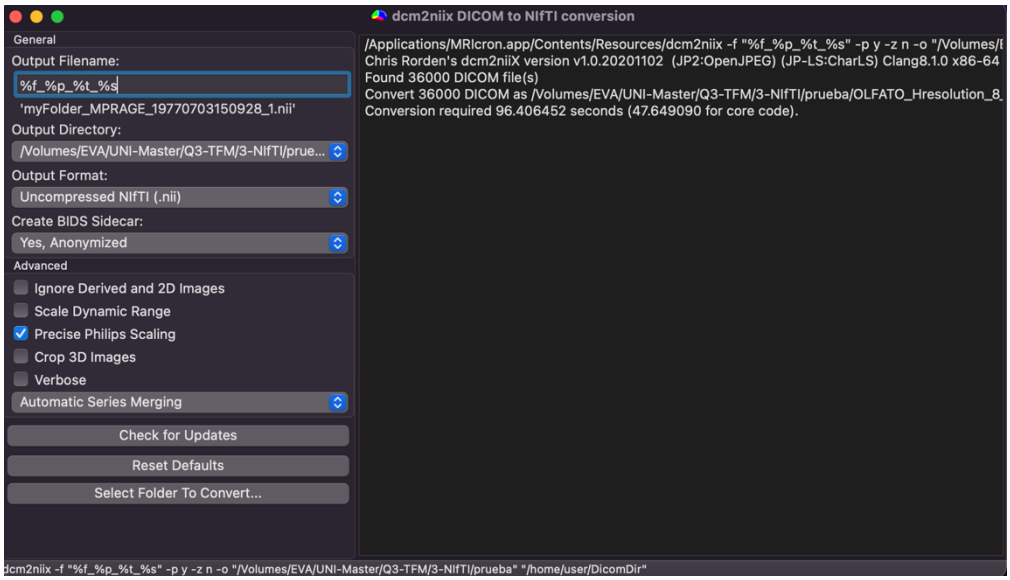
6.4.1 Image acquisition

First, the healthcare professionals of University Hospital Quironsalud Madrid and LAIMBIO members performed the tests on the patients with the Signa Premier 3T (General Electric Healthcare) Magnetic Resonance scanner. Then, they sent the images obtained from the MRI scanner and the first thing to do is to import these raw images in *Horos* program, which will allow to export all medical images of the same sequence at once into DICOM format. In addition, if the metadata tab in *Horos* menu is clicked, information about the device used to take the images and subjects' information can be extracted.

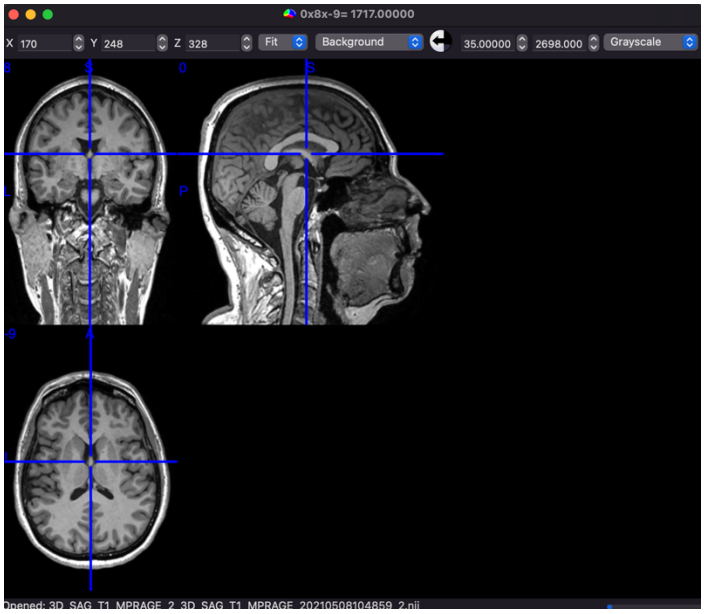
Once we have the images in DICOM format, we must sort them in subfolders of our interest, which means to pass them in BIDS format. The BIDS format is a standard for organizing and describing the data collected by neuroimaging tests. To do this, first the DICOM images have to be passed in NIfTI format, which can be done with the MRIcron software. MRIcron uses the library *dcm2niiX*, which allows to convert DICOM files into NifTi files either uncompressed or gz-compressed files. Afterwards, the folders will be sorted in BIDS format. A figure of an example of format change between the raw images obtained from the hospital, and the BIDS structure containing NIfTI format images, is shown below.



[**Fig 23.** Raw images obtained from the hospital (left). BIDS structure containing NIfTI images (right).]



[**Fig 24.** Step of transferring DICOM images into NIfTI images in MRIcron program.]

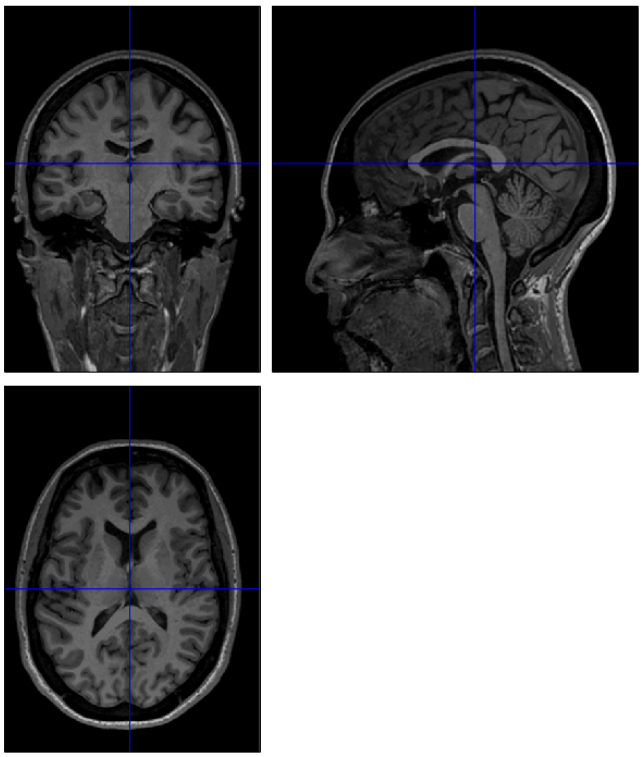


[**Fig 25.** Viewer tool of MRIcron program.]

6.4.2 Image preprocessing

The image preprocessing step will be performed in SPM program. SPM can read NIFTI format images which are not compressed. However, before doing the preprocessing, it has to be checked if the images are well aligned. If the origin is not well set, it will cause the failure of later preprocessing steps. SPM requires to set the origin manually. The crosshairs need to be dragged until they are placed on the anterior commissure. This thin band of white matter fibers can be

found at the base of the fornix, an arch-like band of white matter extending downward from the corpus callosum. In the figure below there is a correct representation of the origin situation.



[**Fig 26.** Correct setting of the origin in subject's 1 brain.]

After setting well the origin in all subjects, the image preprocessing can start. The preprocessing steps consist on: realigning and unwarping the data, slice-timing correction, coregistration, segmentation, normalization, and smoothing.

- ***Realigning and unwarping the data***

First of all, the functional images need to be realigned. This will set all images in the same orientation and the sides will be line up. There are several options in SPM program to do that. In this case, the tool "Realign (Estimate and Reslice)" has been used. First, the amount that each volume is out of alignment with a reference volume is estimated. This reference volume can be the mean of all volumes or the first volume; for this project, the mean has been selected. Afterwards, these estimates are used to align all volumes with the reference volume.

- ***Slice-timing correction***

The next step is the slice-timing correction because fMRI images are acquired in slices at different times as they can not be obtained all at once at a single moment. Therefore, the differences caused by these delays in time during the acquisition have to be corrected. In this way, all slices will be temporally aligned to a reference time-point thanks to slice-timing correction. The parameters needed for slice-timing preprocessing are number of slices, repetition time (TR), acquisition time (TA), slice order and reference slice:

  - Number of slices: 60
  - Repetition time (TR): 1
  - Acquisition time (TA): 0
    - As the next two items are entered in milliseconds, this entry will not be used and can be set to 0.
  - Slice order:

```
0     533.3333    66.66667    600     133.3333    666.6667    200     733.3333    266.6667    800     333.3333    866.6667    400
933.3333    466.6667    0     533.3333    66.66667    600     133.3333    666.6667    200     733.3333    266.6667    800     333.3333
866.6667    400     933.3333    466.6667    0     533.3333    66.66667    600     133.3333    666.6667    200     733.3333
266.6667    800     333.3333    866.6667    400     933.3333    466.6667    0     533.3333    66.66667    600     133.3333
666.6667    200     733.3333    266.6667    800     333.3333    866.6667    400     933.3333    466.6667
```

    - The slice timing is entered in ms for each slice individually. To obtain these values, Pablo Garcia from General Electrics provided me two Matlab functions (GE_PlotSliceTiming.m and GE_SliceTiming.m). Then, the scipt called ObtainSliceTimingInfo.m was created (which uses the two General Electrics' functions) and where it can be obtained the variable called *slicetime* that contains the values to enter in Slice Order parameter in SPM12. These values are:

      (The codes ObtainSliceTimingInfo.m, GE_PlotSliceTiming.m and GE_SliceTiming.m can be found at Annex section).
  - Reference slice: 533,3333
    - The value closest to TR/2 is put in Reference Slice. In this case is 533,3333.

- ***Coregistration***

The following step is coregistration. It consists on aligning the functional images to the anatomical images as much as possible. First, the anatomical image is normalized (or warped) to a template and the transformations done are recorded. Then, these transformations will be

applied to the functional images. Instead of using directly the functional images, the anatomical image is warped because it has a higher resolution, so will match better with the anatomical details of the template. Afterwards, we can obtain correct results when applying the same transformations on the functional data. This alignment between the functional and anatomical images is called registration.

- ***Segmentation***

The segmentation step is used to identify which voxels of the images belong to which tissue type. SPM can compare the voxels with images of six tissue priors (white matter, gray matter, cerebrospinal fluid, soft tissue, skull, and other tissues that are not classified in any other type) and identify which type of tissue it is. In this way, mapping the tissues of the anatomical image to the tissues of the template will increase the accuracy of registration.

- ***Normalization***

The results obtained from segmentation are used to normalize the image, which means that the images are moved to a standardized space.

- ***Smoothing***

The last preprocessing step is to smooth the functional images in order to increase signal and cancel out noise. This step consist on replacing the signal at each voxel with a weighted average of that voxel's neighbors.

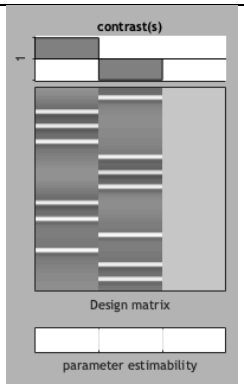
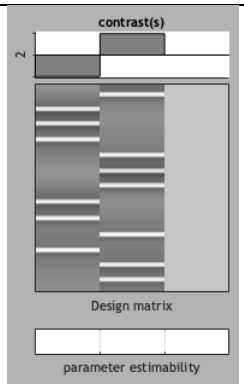
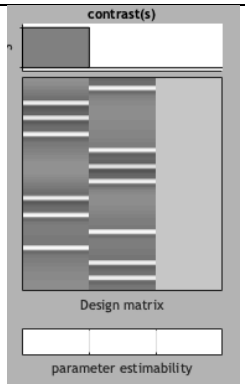
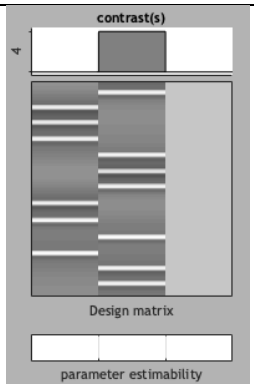
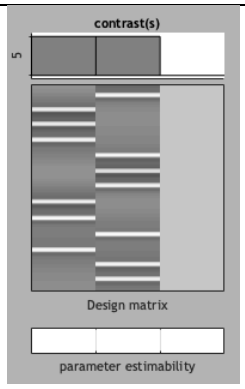
6.4.3 Statistical analysis

After correctly preprocessing all subject's brain images, a statistical analysis will be performed. The statistical analysis consists on a first-level analysis and a second-level (or group-level) analysis. In the first-level analysis, the evidence per subject is summarized in a linear contrast of the parameters in order to model the study design. Then, these contrast images are passed to the second-level analysis in which the evidence is weighted over subjects. Therefore, in the first-level analysis each session (pre therapy and post therapy) of each subject is analyzed individually.

As mentioned, the first-level analysis consists on fitting a model to the data. In this step, first, the model will be specified, then, the model will be estimated, and last, contrasts will be created.

In order to specify the model, a time series file has to be created. A Matlab code has been developed for that purpose, which is added in the Annexes section. This code called *convertOnsetTimes.m*, converts timing files from BIDS format into a two-column format that can be read by SPM. For each patient and each session (pre-therapy images and post-therapy images), two files are created: one for vanilla stimulus (*vainilla.txt*) and the other for mint (*menta.txt*). These files have two columns: the first the onset times of each stimulus and the second for the duration of the stimulus.

Once timing files are created, they can be used to fit the General Linear Model (GLM) to the fMRI data. To do this, statistical parametric maps are created which indicate the correlation between the ideal time-series (which are the onset times convolved with the HRF) and the time-series collected during the experiment. The contrasts created are t-statistic type and five contrasts were created in total:

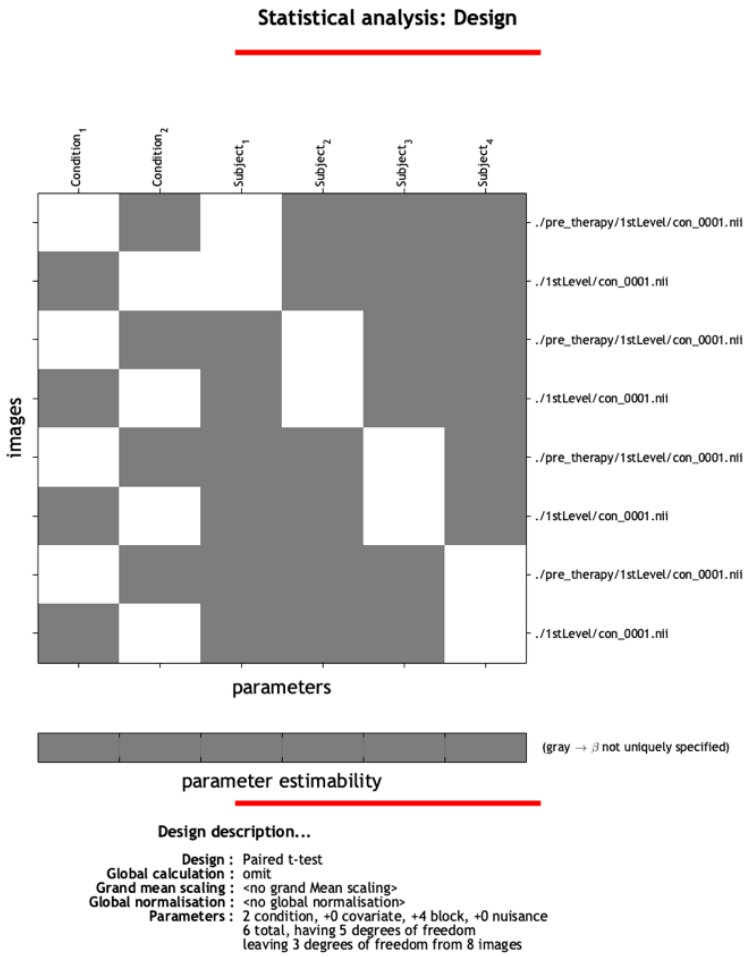
Vanilla – mint	Mint – vanilla	Vanilla	Mint	Stimulation – Non stimulation
				

[**Table 3.** The five different contrasts created are represented.]

A Matlab code has been used to make the preprocessing and first level analysis step in a more optimal way. In this code, the parameters of interest of each step have been selected and applied. Thanks to the interface of the SPM program, the different stages of preprocessing and first level analysis can be done all in a row without the need to do each of them manually. In addition, a for-loop has been used to run all the subjects at once. This code named *RunPreproc_1stLevel_job.m* can be found in the Annex section. To use it correctly, first the SPM

program has to be executed writing >>> *spm fmri* in MATLAB command window, and then, click run to the *RunPreproc_1stLevel_job.m* script.

Afterwards, a group level analysis (or second level analysis) was carried out to check if the changes observed in one subject, can be seen in the total population of the study as well. The group level analysis consisted on a paired t-test which calculates differences between paired observations like before and after images. In this case, pre-therapy and post-therapy acquisitions were compared for each contrast. To do it, the standard error and the mean were calculated for each contrast estimate (created before in the first level analysis). All subjects are taken into account in each contrast type. And then, a t-test is performed over the mean parameters from each subject.



[**Fig 27.** Design matrix of the paired t-test for the second level analysis.]

The second level analysis was also performed tanks to the SPM interface. A Matlab code was created to perform the analysis in a more optimal way. This code named *Run2ndLevel_job.m* can be found in the Annex section.

6.4.4 Connectivity ROI-to-ROI

The connectivity between the different brain areas has been measured with CONN software which is a functional connectivity toolbox. CONN toolbox can generate several graphs like ROI-to-ROI connectivity maps, seed-to-voxel, and voxel-to-voxel.

Images already preprocessed with the SPM12 program were loaded into CONN. There, a group-level analysis was performed too in order to view the results. The Results tab of CONN program includes multiple options to view the results: they can be plotted for each subject individually or as a group analysis including all subjects. Moreover, they can be displayed on a glass brain, inflated brain, cortical surface… The pValue and cluster threshold can be selected, and a panel with the coordinates of the most relevant clusters activated is shown.

However, all mentioned above can also be done with SPM12 (as it has been done in previous sections). In contrast, CONN software has been used to plot the functional connectivity results as a ROI-to-ROI plot, so, ROIs that are significant correlated with other ROIs can be seen.

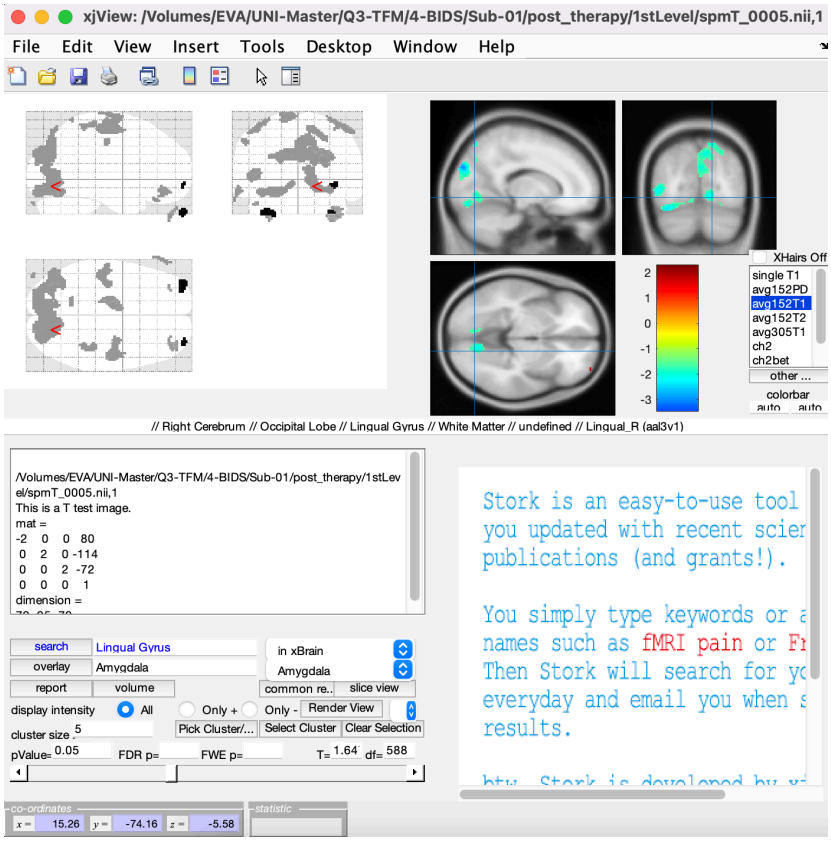
7. RESULTS

The Xjview software has been used to view and export better the results. In this section, the results from 1st level analysis, 2nd level analysis and connectivity will be displayed.

7.1 First level analysis results

As mentioned in section 6.4.3, five types of contrasts or conditions were created. However, only the result of four conditions will be presented as the first contrast (vainilla-menta) and the second (menta-vainilla) show the same results.

To ensure a statistically significant result, a pValue of 0.05 and cluster size higher than 5 have been established in all cases.



[**Fig 28.** Interface of Xjview].

Xjview software has been used to plot the results in the interactive glass brain. Moreover, the 'Slice view' and 'Render view' are useful tools to have an overall view of the results from all over the brain.

In the following table, the main clusters activated for each condition (vainilla-menta, vainilla, menta, or Estim-NoEstim) are presented for each subject and each session (pre-therapy images and post-therapy images) individually.

If there is --- means that no significant results were found. It could be that with the pValue of 0,05 no clusters were found, the structures are not well defined, or may be only clusters outside brain like movement of the eyes.

Contrast	Subj1 pre	Subj1 post	Subj2 pre	Subj2 post	Subj3 pre	Subj3 post	Subj4 pre	Subj4 post
Vainilla-menta	------	Frontal Lobe Left Cerebrum Frontal_Sup_2_L (aal3v1) Superior Frontal Gyrus brodmann area 10 Gray Matter Middle Frontal Gyrus White Matter Frontal_Mid_2_L (aal3v1)	------	Occipital Lobe Right Cerebrum Lingual Gyrus Gray Matter brodmann area 18 White Matter Cerebellum_Crus1_R (aal3v1) brodmann area 17 Inter-Hemispheric Left Cerebrum Occipital Lobe Cuneus brodmann area 19 Gray Matter Cuneus_L (aal3v1) Precuneus_R (aal3v1) Parietal Lobe Precuneus brodmann area 7 Parietal_Sup_R (aal3v1) Superior Parietal Lobule	------	------	------	Frontal Lobe Gray Matter Right Cerebrum brodmann area 11 Superior Frontal Gyrus OFCmed_R (aal3v1) Rectus_R (aal3v1) Orbital Gyrus Cuneus Cuneus_L (aal3v1) Left Cerebrum Occipital Lobe brodmann area 18 White Matter

[**Table 4.** Clusters detected using contrast Vainilla-menta for each subject and session individually in the first level analysis.]

Contrast	Subj1 pre	Subj1 post	Subj2 pre	Subj2 post	Subj3 pre	Subj3 post	Subj4 pre	Subj4 post
Vainilla	------	Frontal Lobe Right Cerebrum Gray Matter brodmann area 10 Middle Frontal Gyrus Frontal_Mid_2_R (aal3v1) Frontal_Sup_2_R (aal3v1) Superior Frontal Gyrus brodmann area 11 White Matter Left Cerebrum Occipital Lobe Occipital_Sup_R (aal3v1) Cuneus brodmann area 19 brodmann area 7 Cuneus_R (aal3v1) brodmann area 31 Precuneus brodmann area 18	Left Cerebrum Frontal Lobe brodmann area 6 Gray Matter Precentral Gyrus Superior Frontal Gyrus Paracentral_Lobule_L (aal3v1) White Matter Medial Frontal Gyrus	------	------	------	Right Cerebrum Frontal Lobe brodmann area 10 Gray Matter Frontal_Mid_2_R (aal3v1) Superior Frontal Gyrus Middle Frontal Gyrus Frontal_Sup_2_R (aal3v1) Frontal_Sup_Medial_L (aal3v1) Parietal_Sup_L (aal3v1) Left Cerebrum Parietal Lobe Parietal_Inf_L (aal3v1) Superior Parietal Lobule brodmann area 7 Inferior Parietal Lobule brodmann area 40 Angular_L (aal3v1) White Matter	Cerebellum_Crus1_R (aal3v1) Right Cerebrum, Left Cerebrum Fusiform Gyrus Temporal Lobe, Parietal Lobe, Occipital Lobe Gray Matter, White Matter Cerebellum Posterior Lobe Declive Right Cerebellum Fusiform_R (aal3v1) Temporal_Inf_R (aal3v1) brodmann area 37, 18, 17, 19, 7, 6, 4, 3 Lingual Gyrus Inferior Occipital Gyrus Calcarine_L (aal3v1) Cuneus, Cuneus_L (aal3v1) Inter-Hemispheric Precuneus, Precuneus_L (aal3v1), Precuneus_R (aal3v1) Postcentral Gyrus Frontal Lobe Superior Frontal Gyrus Supp_Motor_Area_L (aal3v1), Supp_Motor_Area_R (aal3v1) Medial Frontal Gyrus Paracentral_Lobule_L (aal3v1), Paracentral_Lobule_R (aal3v1) Precentral Gyrus Paracentral Lobule

[**Table 5.** Clusters detected using contrast Vainilla for each subject and session individually in the first level analysis.]

Contrast	Subj1 pre	Subj1 post	Subj2 pre	Subj2 post	Subj3 pre	Subj3 post	Subj4 pre	Subj4 post
Menta	Gray Matter Inferior Parietal Lobule Left Cerebrum Parietal Lobe Parietal_Inf_L (aal3v1) brodmann area 40	Left Cerebrum, Right Cerebrum Occipital Lobe Fusiform_L (aal3v1), Fusiform_R (aal3v1) White Matter Fusiform Gyrus Gray Matter Middle Occipital Gyrus brodmann area 18, 19, 37, 39, 21, 7, 31, 6, 5, 4, 3, 2, 1, 40 Inferior Occipital Gyrus Cerebellum_Crus1_L (aal3v1), Cerebellum_Crus1_R (aal3v1) Sub-Gyral Lingual_L (aal3v1), Lingual_R (aal3v1) Lingual Gyrus Declive Left Cerebellum, Right Cerebellum Cerebellum Posterior Lobe, Cerebellum Anterior Lobe Cerebellum_6_L (aal3v1) Temporal Lobe Occipital_Inf_L (aal3v1), Occipital_Inf_R (aal3v1) Culmen Occipital_Mid_L (aal3v1), Occipital_Mid_R (aal3v1) Middle Temporal Gyrus Temporal_Mid_L (aal3v1), Temporal_Mid_R (aal3v1) Inferior Temporal Gyrus Cuneus, Cuneus_L (aal3v1), Cuneus_R (aal3v1) Precuneus, Precuneus_R (aal3v1), Precuneus_L (aal3v1) Parietal Lobe Occipital_Sup_R (aal3v1), Occipital_Sup_L (aal3v1) Inter-Hemispheric Parietal_Sup_R (aal3v1), Parietal_Sup_L (aal3v1) Superior Parietal Lobule Superior Occipital Gyrus Sub-Gyral Frontal lobe Precentral_L (aal3v1), Precentral_R (aal3v1) Precentral Gyrus Postcentral Gyrus Postcentral_L (aal3v1), Postcentral_R (aal3v1) Superior Frontal Gyrus Supp_Motor_Area_L (aal3v1), Supp_Motor_Area_R (aal3v1) Middle Frontal Gyrus Frontal_Sup_2_L (aal3v1), Frontal_Sup_2_R (aal3v1) SupraMarginal_L (aal3v1) Frontal_Mid_2_L (aal3v1), Frontal_Mid_2_R (aal3v1) Parietal_Inf_L (aal3v1) Paracentral Lobule Paracentral_Lobule_L (aal3v1), Paracentral_Lobule_R (aal3v1) Postcentral_L (aal3v1), Postcentral_R (aal3v1) Inferior Parietal Lobule	Cerebellum_Crus1_L (aal3v1), Cerebellum_Crus2_L (aal3v1) Left Cerebrum, Right Cerebrum Occipital Lobe White Matter, Gray Matter Fusiform Gyrus Declive Cerebellum Posterior Lobe Supp_Motor_Area_R (aal3v1), Supp_Motor_Area_L (aal3v1) brodmann area 17, 18, 7, 40, 8, 6, 3, 7, 5, 4 Lingual Gyrus Inferior Occipital Gyrus Inter-Hemispheric Precuneus, Precuneus_L (aal3v1), Precuneus_R (aal3v1) Inferior Parietal Lobule Parietal Lobe Parietal_Inf_R (aal3v1) Parietal_Sup_R (aal3v1), Parietal_Sup_L (aal3v1) Paracentral Lobule Precuneus_L (aal3v1) Precentral Gyrus Medial Frontal Gyrus Paracentral_Lobule_L (aal3v1), Paracentral_Lobule_R (aal3v1) Postcentral Gyrus Frontal_Sup_2_L (aal3v1) Frontal Lobe Superior Frontal Gyrus	Cerebellum_Crus2_L (aal3v1) Left Cerebellum, Right Cerebellum Cerebellum Posterior Lobe Uvula Declive Cerebellum_Crus1_L (aal3v1) Tuber Right Cerebrum, Left Cerebrum Occipital Lobe Lingual Gyrus Gray Matter Cerebellum_Crus1_R (aal3v1) Cerebellum_Crus2_R (aal3v1) White Matter brodmann area 18 Inter-Hemispheric brodmann area 17 Frontal Lobe Superior Frontal Gyrus Frontal_Sup_2_R (aal3v1) brodmann area 10 Inferior Frontal Gyrus brodmann area 6 Precuneus_R (aal3v1), Precuneus_L (aal3v1) Precuneus Precuneus_L (aal3v1) Paracentral_Lobule_R (aal3v1) Postcentral Gyrus brodmann area 5 Parietal_Sup_R (aal3v1) Parietal_Sup_L (aal3v1) Superior Parietal Lobule Parietal Lobe brodmann area 7	------	Cuneus_L (aal3v1) Inter-Hemispheric Precentral_R (aal3v1) Right Cerebrum Frontal Lobe Precentral Gyrus Gray Matter brodmann area 6 brodmann area 4 White Matter Parietal Lobe Postcentral Gyrus brodmann area 3	Precuneus_L (aal3v1) Left Cerebrum, Right Cerebrum Parietal Lobe Precuneus brodmann area 7 Gray Matter Inter-Hemispheric White Matter Parietal_Sup_L (aal3v1) Superior Parietal Lobule Postcentral Gyrus brodmann area 4 Superior Frontal Gyrus Frontal Lobe brodmann area 6 brodmann area 3 Supp_Motor_Area_L (aal3v1) Supp_Motor_Area_R (aal3v1) Paracentral_Lobule_R (aal3v1) Paracentral_Lobule_L (aal3v1) Paracentral Lobule	**pValue = 0.02** Frontal Lobe Right Cerebrum, Left Cerebrum Gray Matter brodmann area 11, 39, 19, 17 Inter-Hemispheric Frontal_Med_Orb_R (aal3v1) Medial Frontal Gyrus Superior Frontal Gyrus Rectus_R (aal3v1) Frontal_Med_Orb_L (aal3v1) White Matter Cerebellum_6_L (aal3v1) Declive Left Cerebellum Cerebellum Posterior Lobe Occipital Lobe Cuneus, Cuneus_L (aal3v1) Sub-Gyral Fusiform Gyrus Lingual Gyrus Temporal Lobe Middle Temporal Gyrus Precuneus Precuneus_L (aal3v1) Precuneus_R (aal3v1) Parietal Lobe

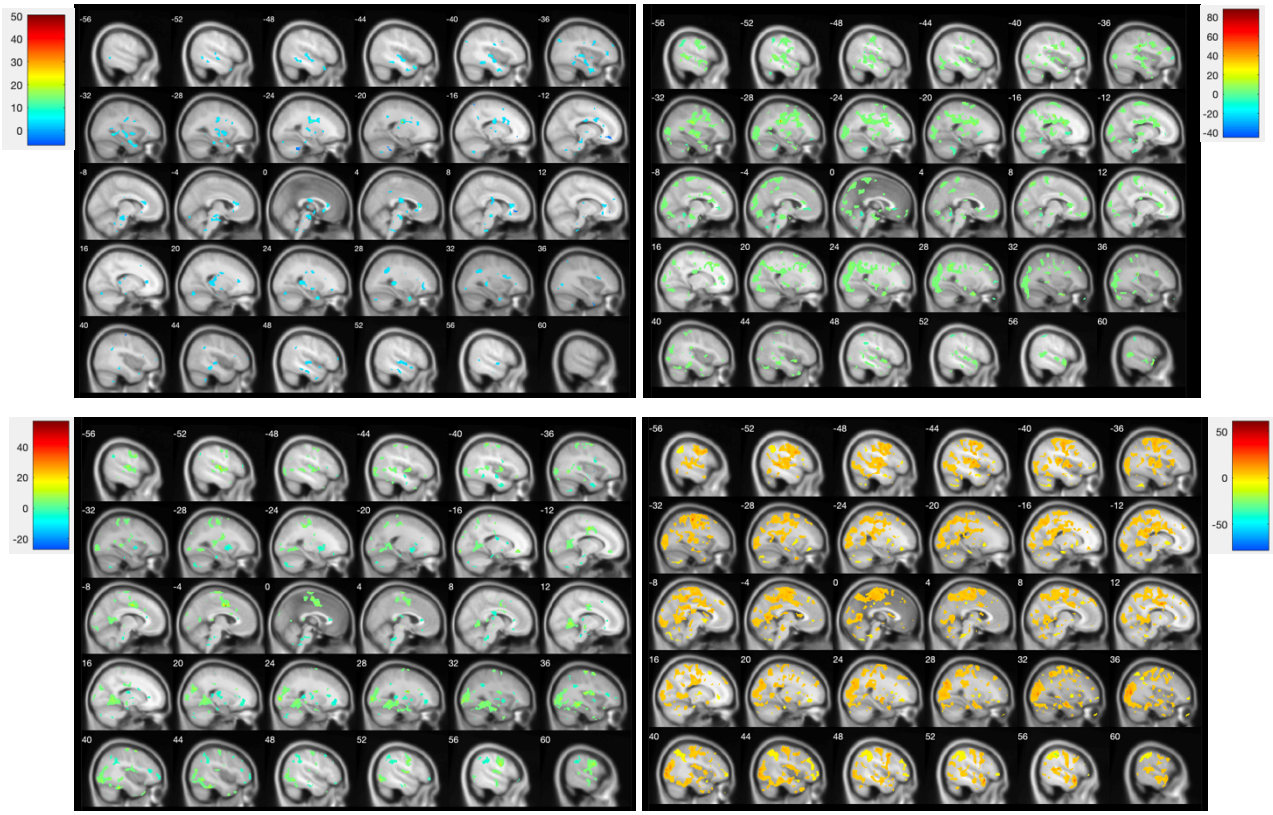
[**Table 6.** Clusters detected using contrast Menta for each subject and session individually in the first level analysis.]

Contrast	Subj1 pre	Subj1 post	Subj2 pre	Subj2 post	Subj3 pre	Subj3 post	Subj4 pre	Subj4 post
Estim-NoEstim	------	**pValue = 0.02** Left Cerebrum, Right Cerebrum Occipital Lobe Fusiform_L (aal3v1) White Matter Fusiform Gyrus Gray Matter Middle Occipital Gyrus brodmann area 19, 18, 37, 7, 31, 6 Cerebellum_Crus1_L (aal3v1) Lingual_L (aal3v1), Lingual_R (aal3v1) Inferior Occipital Gyrus Sub-Gyral Lingual Gyrus Cerebellum_6_L (aal3v1) Occipital_Mid_L (aal3v1), Occipital_Mid_R (aal3v1) Temporal_Mid_L (aal3v1) Temporal Lobe Middle Temporal Gyrus Cuneus Occipital_Sup_R (aal3v1), Occipital_Sup_L (aal3v1) Cuneus_R (aal3v1) Cuneus_L (aal3v1) Precuneus Inter-Hemispheric Superior Occipital Gyrus Parietal Lobe Precuneus_R (aal3v1) Precentral Gyrus Frontal Lobe Supp_Motor_Area_L (aal3v1)	Cerebellum_Crus2_L (aal3v1) Cerebellum_Crus1_L (aal3v1) Declive Fusiform Gyrus Left Cerebellum Left Cerebrum, Right Cerebrum Occipital Lobe Cerebellum Posterior Lobe brodmann area 18, 7, 6 Gray Matter White Matter Lingual gyrus Temporal Lobe Inter-Hemispheric Precuneus_L (aal3v1) Precuneus_R (aal3v1) Precuneus Frontal Lobe Parietal Lobe Postcentral Gyrus Superior Frontal Gyrus Medial Frontal Gyrus	Cerebellum_Crus2_L (aal3v1) Left Cerebellum, Right Cerebellum Cerebellum Posterior Lobe Uvula Declive Cerebellum_Crus1_L (aal3v1) Tuber Cerebellum_Crus2_R (aal3v1) Cerebellum_Crus2_L (aal3v1) Cerebellum_Crus1_R (aal3v1) Right Cerebrum, Left Cerebrum Occipital Lobe Lingual Gyrus Cerebellum_Crus2_L (aal3v1) brodmann area 18, 19, 10, 6, 4 Gray Matter Fusiform_R (aal3v1) Limbic Lobe Frontal Lobe Inter-Hemispheric White Matter Culmen Cerebellum Anterior Lobe Superior Frontal Gyrus Precentral Gyrus	------	------	Right Cerebrum Frontal Lobe brodmann area 10 Gray Matter Middle Frontal Gyrus Frontal_Mid_2_R (aal3v1) Superior Frontal Gyrus Frontal_Sup_2_R (aal3v1)	**pValue = 0.02** Right Cerebrum, Left Cerebrum Fusiform Gyrus Cerebellum_Crus1_R (aal3v1) Occipital Lobe Temporal Lobe Gray Matter Fusiform_R (aal3v1) brodmann area 19, 17, 18, 7, 6, 37 White Matter Middle Occipital Gyrus Occipital_Inf_R (aal3v1) Temporal_Inf_R (aal3v1) Cerebellum Posterior Lobe Declive Right Cerebellum Lingual Gyrus Parietal Lobe Postcentral_R (aal3v1) Postcentral Gyrus Lingual_R (aal3v1) Middle Temporal Gyrus Occipital_Mid_L (aal3v1) Inferior Occipital Gyrus Precuneus_L (aal3v1), Precuneus_R (aal3v1) Inter-Hemispheric Precuneus Paracentral_Lobule_R (aal3v1) Supp_Motor_Area_R (aal3v1) Paracentral Lobule Paracentral_Lobule_L (aal3v1) Calcarine_L (aal3v1) Precentral_L (aal3v1) Precentral Gyrus Frontal Lobe Inferior Temporal Gyrus Cuneus Medial Frontal Gyrus Superior Frontal Gyrus Supp_Motor_Area_L (aal3v1)

[**Table 7.** Clusters detected using contrast Estim-NoEstim for each subject and session individually in the first level analysis.]

7.2 Second level analysis results

Four different results have been obtained from the group analysis (paired t-test); one for each contrast created. Xjview program has been used to plot the results. Xjview allows to plot the results in a glass brain or in a brain image, it also allows to have a Slice View of the results in different brain slices, and a Render View to plot the results in different brain sections in 3D. Below, there is the Slice View of the group analysis of all subjects for each contrast used comparing pre-acquisition vs. post-acquisition images.



[**Fig 29.** Sagittal slice view of a paired t-test comparing all subject's images pre-therapy vs. post-therapy. Top-left image using contrast con_0001 (vainilla-menta). Top-right image using contrast con_0003 (vainilla). Lower left corner using contrast con_0004 (menta). Lower right corner using contrast con_0005 (Estim-NoEstim).]

In the paired t-test the weights applied were [1 -1] comparing pre-therapy vs. post-therapy images. So, the positive results will belong to a major activation in the pre-therapy acquisitions, and the negative results will belong to a major activation in post-therapy acquisitions. In general, the correlation between subjects in pre-therapy acquisitions show an activation of different brain areas. However, in the post-therapy acquisitions the activation is more focalized in the

same brain areas. In the following table, the main clusters activated in post-therapy acquisitions are shown for each condition. A pValue of 0.05 and cluster size higher than 5 have been established in all cases.

Contrasts	Vainilla-menta	Vainilla	Menta	Estim-NoEstim
post-therapy clusters activated	Precuneus_L (aal3v1) Frontal Lobe Precentral Gyrus Right Cerebrum White Matter brodmann area 6, 22, 10, 32 Gray Matter Temporal Lobe Superior Temporal Gyrus Limbic Lobe Anterior Cingulate Frontal_Med_Orb_R (aal3v1) Medial Frontal Gyrus Sub-Gyral Left Cerebellum Cerebellum Anterior Lobe Cerebellar Tonsil Cerebellum Posterior Lobe Cerebellum_10_L (aal3v1) Cerebellum_4_5_L (aal3v1) Middle Temporal Gyrus	Cerebellum Posterior Lobe Left Cerebellum Cerebellar Tonsil Left Cerebellum Cerebellum Anterior Lobe Left Brainstem, Right Brainstem Cerebellum Posterior Lobe Cerebellar Tonsil Pons Cerebellum_10_L (aal3v1) Culmen Cerebellum_4_5_L (aal3v1) Medulla Dentate Uvula Cerebellar Lingual Limbic Lobe Sub-Gyral Hippocampus_L (aal3v1) Frontal Lobe Putamen Insula_L (aal3v1) brodmann area 24, 32, 10, 40 Inter-Hemispheric Corpus Callosum Medial Frontal Gyrus Olfactory_L (aal3v1) Sub-lobar Extra-Nuclear Sub-lobar Thal_AV_L (aal3v1) Thalamus Extra-Nuclear Transverse Temporal Gyrus Insula Parietal Lobe Inferior Parietal Lobule Right Cerebrum SupraMarginal_R (aal3v1) Postcentral Gyrus	Medulla Left Brainstem, Right Brainstem Cerebellar Tonsil Pyramis Inferior Semi-Lunar Lobule Declive Uvula Tuber Pons Culmen Temporal Lobe Sub-Gyral Middle Temporal Gyrus brodmann area 21, 20, 35, 37, 36, 11, 47, 46, 10 Inferior Temporal Gyrus Fusiform Gyrus Limbic Lobe Uncus Left Brainstem ParaHippocampal_L (aal3v1) Midbrain Hippocampus_L (aal3v1), Hippocampus_R (aal3v1) ParaHippocampal_R (aal3v1) Occipital Lobe Sub-lobar Insula OFCant_L (aal3v1), OFCant_R (aal3v1) OFCmed_L (aal3v1) Extra-Nuclear OFClat_R (aal3v1) Middle and Inferior Frontal Gyrus Sub-Gyral Olfactory_R (aal3v1) Right Cerebrum Frontal Lobe Corpus Callosum Caudate_R (aal3v1), Caudate_L (aal3v1) Inter-Hemispheric ACC_sup_R (aal3v1), ACC_sup_L (aal3v1) Lentiform Nucleus Lateral Globus Pallidus Pallidum_R (aal3v1), Pallidum_L (aal3v1) Putamen_R (aal3v1) Medial Globus Pallidus Amygdala_R (aal3v1) Caudate, Caudate Body Claustrum Lateral Globus Pallidus Parietal Lobe Thalamus Putamen, Pulvinar	Cerebellar Tonsil Inferior Semi-Lunar Lobule Pyramis Right Brainstem, Left Brainstem Medulla Uvula Declive Pons Tuber Cerebellum Posterior Lobe Culmen Parahippocampa Gyrus Limbic Lobe Midbrain brodmann area 35, 28, 38, 10, 47, 11, 40, 8 Fusiform_R (aal3v1) ParaHippocampal_R (aal3v1), ParaHippocampal_L (aal3v1) Hippocampus_L (aal3v1) Temporal Lobe Superior, Middle and Inferior Temporal Gyrus Olfactory_L (aal3v1) Uncus Subcallosal Gyrus Sub-Gyral Insula_R (aal3v1), Insula_L (aal3v1) OFCant_L (aal3v1), OFCant_R (aal3v1) OFClat_R (aal3v1) OFCpost_L (aal3v1) Frontal Lobe Anterior Cingulate ACC_pre_R (aal3v1) Corpus Callosum Sub-lobar Putamen_L (aal3v1), Putamen_R (aal3v1) Lentiform Nucleus Lentiform Nucleus Putamen Caudate, Caudate Head Extra-Nuclear Medial and Lateral Globus Pallidus Claustrum Right Cerebrum Insula Thalamus Frontal_Inf_Tri_R (aal3v1) Angular Gyrus Inter-Hemispheric Cingulate Gyrus

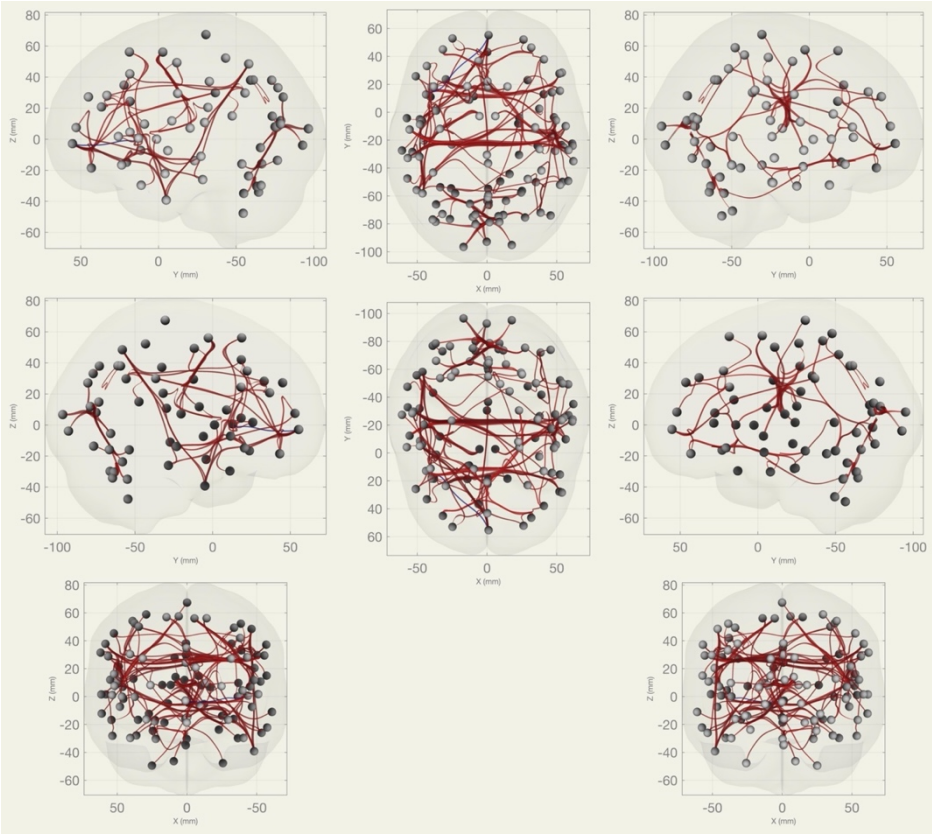
[**Table 8.** Clusters activated on the post-therapy acquisitions in the group analysis of the four subjects for each contrast (Vainilla-Menta, Vainilla, Menta, and Estim-NoEstim).]

7.3 Connectivity results

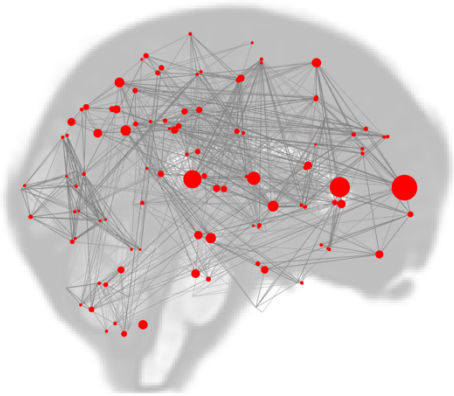
CONN software was used to measure the connectivity between ROIs. The images already processed with SPM12 have been loaded and CONN has been used to visualize the connection between clusters with the ROI-to-ROI tool. This tool allows a 3D display of the results and indicates the coordinates and anatomical name of each cluster.

On the one hand, the connectivity has been visualized with a group analysis considering all the subjects' acquisitions before the therapy, and on the other hand, considering all the subjects' acquisitions after the therapy. A statistical correction limit (p-value) of 0.001 and a cluster threshold of 0.05 were selected.

- Pre-therapy connectivity results:



[**Fig 30.** 8-view mosaic of ROI's connections in a group-level analysis of the pre-therapy images.]



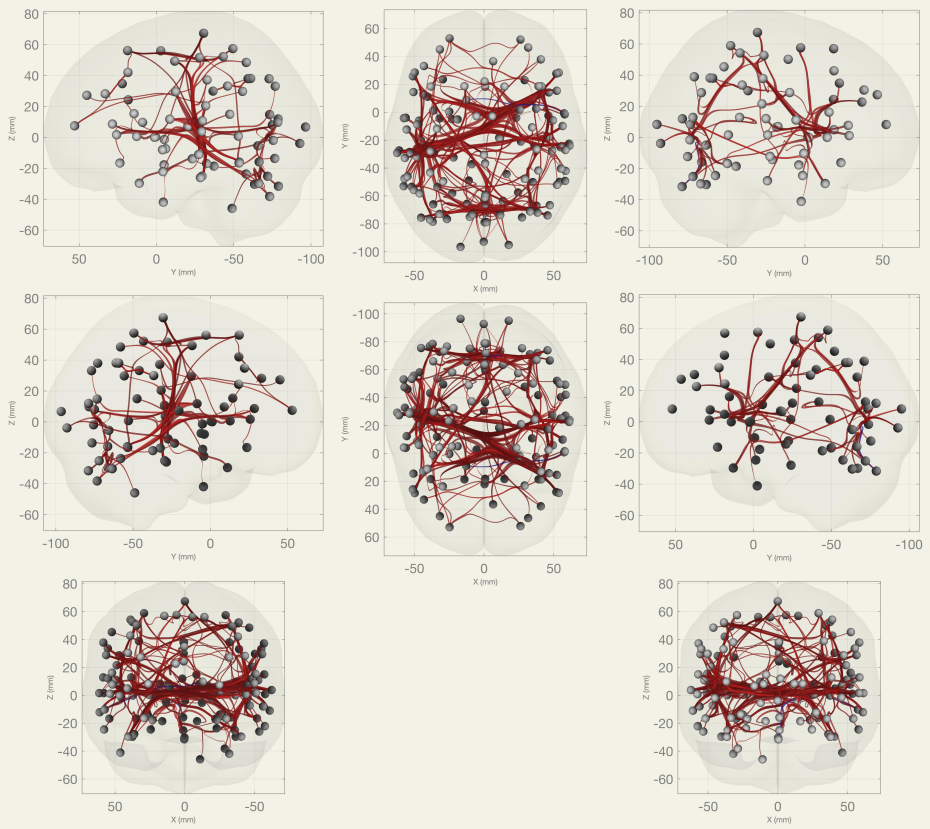
Network nodes: Network of all ROIs | **Network edges (adjacency matrix**: 0.1 | cost | one-side...

Analysis measure: GlobalEfficiency | **Analysis threshold (p-value)**: 0.001 | p-FDR ... | two-sided

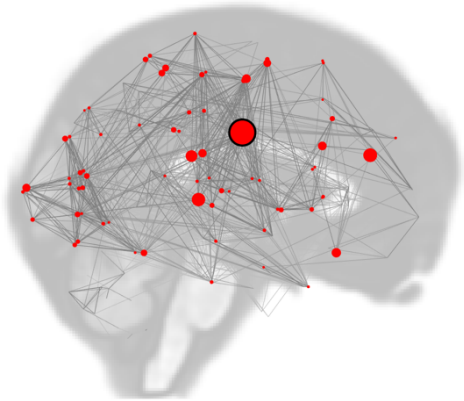
ROI	beta	T	dof	p-unc	p-FDR
network	**0.39**	**34.82**	**3**	**0.000052**	
FP l	0.46	148.03	3	0.000001	0.000104
IFG tri r	0.44	116.05	3	0.000001	0.000104
PT l	0.49	104.96	3	0.000002	0.000104
CO r	0.43	76.04	3	0.000005	0.000204
Putamen r	0.41	62.65	3	0.000009	0.000211
pMTG r	0.47	59.96	3	0.000010	0.000211
AG l	0.47	59.85	3	0.000010	0.000211
FrontoParietal.PP...	0.48	56.88	3	0.000012	0.000211
SFG l	0.49	53.65	3	0.000014	0.000211
Cereb9 l	0.31	53.14	3	0.000015	0.000211
pITG l	0.41	50.40	3	0.000017	0.000211
DefaultMode.LP r	0.43	50.22	3	0.000017	0.000211
Language.IFG r	0.46	48.67	3	0.000019	0.000211
pMTG l	0.49	47.92	3	0.000020	0.000211
Precuneous	0.41	46.38	3	0.000022	0.000211
MedFC	0.41	46.03	3	0.000023	0.000211
IFG oper r	0.48	45.56	3	0.000023	0.000211
IFG oper r	0.48	45.56	3	0.000023	0.000211
DefaultMode.LP l	0.43	45.54	3	0.000023	0.000211
aMTG r	0.41	44.09	3	0.000026	0.000220
PC	0.44	42.65	3	0.000028	0.000222
PreCG r	0.53	42.52	3	0.000029	0.000222
HG l	0.43	41.15	3	0.000032	0.000234
FP r	0.45	40.52	3	0.000033	0.000234
Cereb6 r	0.37	39.88	3	0.000035	0.000236
HG r	0.45	37.33	3	0.000042	0.000276
Language.pSTG r	0.49	36.66	3	0.000045	0.000280
aSMG l	0.48	35.91	3	0.000048	0.000283
aSMG r	0.48	35.62	3	0.000049	0.000283
DefaultMode.PCC	0.41	34.58	3	0.000053	0.000295
sLOC r	0.45	34.19	3	0.000055	0.000295
Cereb8 r	0.39	33.96	3	0.000056	0.000295
DefaultMode.MPFC	0.43	33.51	3	0.000058	0.000298
Cereb2 r	0.43	32.84	3	0.000062	0.000303
Salience.SMG r	0.46	32.62	3	0.000063	0.000303
Salience.SMG r	0.46	32.62	3	0.000063	0.000303
PT r	0.45	31.27	3	0.000072	0.000309
DorsalAttention.I...	0.47	31.15	3	0.000073	0.000309
SPL r	0.44	30.99	3	0.000074	0.000309
PO r	0.46	30.83	3	0.000075	0.000309
SensoriMotor.Late...	0.51	30.81	3	0.000075	0.000309
FrontoParietal.PP...	0.44	30.71	3	0.000076	0.000309
IFG oper l	0.45	30.22	3	0.000080	0.000311
Language.IFG l	0.46	30.15	3	0.000080	0.000311
AG r	0.46	29.10	3	0.000089	0.000333
DorsalAttention.I...	0.48	29.01	3	0.000090	0.000333
MidFG l	0.48	28.39	3	0.000096	0.000340
pITG r	0.44	28.24	3	0.000097	0.000340
PreCG l	0.53	28.18	3	0.000098	0.000340
Cerebellar.Anterior	0.45	27.25	3	0.000109	0.000369
aMTG l	0.41	27.03	3	0.000111	0.000370
OFusG l	0.39	26.68	3	0.000116	0.000377
pSMG r	0.46	26.41	3	0.000119	0.000381
Putamen l	0.38	18.75	3	0.000331	0.000633
Cereb7 r	0.38	18.70	3	0.000334	0.000633
OP r	0.35	18.20	3	0.000362	0.000674
toITG l	0.39	18.17	3	0.000364	0.000674
Language.pSTG l	0.49	18.01	3	0.000373	0.000683
Cereb2 l	0.41	17.62	3	0.000398	0.000721
Salience.SMG l	0.45	17.56	3	0.000402	0.000721
SFG r	0.48	17.39	3	0.000414	0.000734
FOrb r	0.38	17.18	3	0.000430	0.000753
Cereb9 r	0.34	16.99	3	0.000444	0.000771
DorsalAttention.F...	0.41	16.68	3	0.000470	0.000806
SMA L	0.46	16.27	3	0.000505	0.000858
SPL l	0.44	16.06	3	0.000525	0.000881
LG l	0.42	16.01	3	0.000530	0.000881
Visual.Medial	0.41	15.91	3	0.000540	0.000883
IC l	0.48	15.89	3	0.000542	0.000883
AC	0.44	15.49	3	0.000585	0.000943
LG r	0.40	15.38	3	0.000597	0.000954
PO l	0.47	20.94	3	0.000238	0.000570
SensoriMotor.Supe...	0.46	20.75	3	0.000245	0.000570
TP r	0.45	20.48	3	0.000255	0.000570
ICC l	0.39	20.44	3	0.000256	0.000570
PaCiG r	0.45	20.43	3	0.000256	0.000570
pSMG l	0.46	20.30	3	0.000262	0.000570
Salience.RPFC l	0.45	20.29	3	0.000262	0.000570
Cereb1 l	0.40	20.26	3	0.000263	0.000570
Cuneal l	0.43	20.19	3	0.000266	0.000570
PostCG r	0.49	20.01	3	0.000273	0.000571
Cuneal r	0.39	19.99	3	0.000274	0.000571
SubCalC	0.38	19.85	3	0.000280	0.000571
FOrb l	0.43	19.83	3	0.000280	0.000571
CO l	0.43	19.69	3	0.000286	0.000576
iLOC l	0.45	19.26	3	0.000306	0.000608
PostCG l	0.50	19.05	3	0.000316	0.000621
OFusG r	0.41	18.78	3	0.000330	0.000633
Putamen l	0.38	18.75	3	0.000331	0.000633
pSMG r	0.46	26.41	3	0.000119	0.000381
Visual.Occipital	0.39	25.70	3	0.000129	0.000400
PP r	0.45	25.65	3	0.000130	0.000400
FrontoParietal.LP...	0.44	25.42	3	0.000134	0.000403
FrontoParietal.LP...	0.44	25.10	3	0.000139	0.000411
toMTG r	0.45	24.36	3	0.000152	0.000442
MidFG r	0.48	22.99	3	0.000180	0.000516
Salience.AInsula r	0.47	22.63	3	0.000189	0.000531
IFG tri l	0.45	22.36	3	0.000196	0.000541
sLOC l	0.45	22.08	3	0.000203	0.000553
Cereb7 l	0.41	21.77	3	0.000212	0.000567
Salience.AInsula l	0.47	21.54	3	0.000219	0.000570
Salience.RPFC r	0.43	21.32	3	0.000226	0.000570
PaCiG l	0.44	21.30	3	0.000226	0.000570
Visual.Lateral r	0.45	21.11	3	0.000233	0.000570
SMA r	0.49	21.07	3	0.000234	0.000570
SensoriMotor.Late...	0.50	21.02	3	0.000236	0.000570
PO l	0.47	20.94	3	0.000238	0.000570
LG r	0.40	15.38	3	0.000597	0.000954
PP l	0.39	15.32	3	0.000605	0.000957
TOFusC r	0.41	15.20	3	0.000619	0.000970
iLOC r	0.44	15.13	3	0.000627	0.000971
aSTG l	0.41	15.09	3	0.000632	0.000971

[**Fig 31.** Network connection of group-level analysis results of the pre-therapy images.]

- Post-therapy connectivity results:



[**Fig 32.** 8-view mosaic of ROI's connections in a group-level analysis of the post-therapy images.]



ROI	beta	T	dof	p-unc	p-FDR
network	**0.35**	**18.82**	**3**	**0.000327**	
SensoriMotor.Late...	0.53	180.69	3	0.000000	0.000061
PaCiG l	0.40	95.11	3	0.000003	0.000150
pSTG l	0.48	92.61	3	0.000003	0.000150
PO l	0.48	81.00	3	0.000004	0.000168
FOrb l	0.43	64.82	3	0.000008	0.000228
PreCG r	0.52	59.99	3	0.000010	0.000228
AC	0.40	59.67	3	0.000010	0.000228
PO r	0.49	56.94	3	0.000012	0.000228
OP r	0.40	55.85	3	0.000013	0.000228
SMA L	0.43	52.85	3	0.000015	0.000242
DorsalAttention.I...	0.46	47.45	3	0.000021	0.000267
SensoriMotor.Late...	0.52	47.12	3	0.000021	0.000267
DorsalAttention.I...	0.47	46.03	3	0.000023	0.000267
TOFusC r	0.48	45.68	3	0.000023	0.000267
Cuneal l	0.44	41.13	3	0.000032	0.000340

ROI	beta	T	dof	p-unc	p-FDR
Cuneal l	0.44	41.13	3	0.000032	0.000340
iLOC l	0.50	38.80	3	0.000038	0.000340
Visual.Lateral r	0.50	38.74	3	0.000038	0.000340
SPL l	0.45	38.03	3	0.000040	0.000340
Salience.SMG l	0.39	37.27	3	0.000042	0.000340
PostCG l	0.53	36.38	3	0.000046	0.000340
HG l	0.46	36.12	3	0.000047	0.000340
Salience.ACC	0.41	35.67	3	0.000048	0.000340
SCC r	0.39	35.14	3	0.000051	0.000340
pSTG r	0.46	34.53	3	0.000053	0.000340
PreCG l	0.53	34.47	3	0.000054	0.000340
IC r	0.45	34.29	3	0.000055	0.000340
ICC r	0.40	32.43	3	0.000064	0.000387
OFusG l	0.45	31.72	3	0.000069	0.000394
SMA r	0.46	31.46	3	0.000071	0.000394
Salience.AInsula r	0.42	30.95	3	0.000074	0.000400

ROI	beta	T	dof	p-unc	p-FDR
Salience.AInsula r	0.42	30.95	3	0.000074	0.000400
Visual.Occipital	0.41	30.59	3	0.000077	0.000401
OFusG r	0.44	30.12	3	0.000080	0.000407
aSMG l	0.43	29.70	3	0.000084	0.000411
SPL r	0.48	29.03	3	0.000090	0.000428
aSMG r	0.44	27.72	3	0.000103	0.000474
ICC l	0.42	27.53	3	0.000105	0.000474
LG l	0.48	25.98	3	0.000125	0.000548
pTFusC r	0.45	25.40	3	0.000134	0.000568
FO r	0.39	25.12	3	0.000138	0.000568
Visual.Lateral l	0.49	24.82	3	0.000143	0.000568
SensoriMotor.Supe...	0.49	24.79	3	0.000144	0.000568
SCC l	0.42	24.44	3	0.000150	0.000570
aSTG l	0.47	24.28	3	0.000153	0.000570
Cuneal r	0.44	24.19	3	0.000155	0.000570
OP l	0.43	23.61	3	0.000167	0.000600

ROI	beta	T	dof	p-unc	p-FDR
OP l	0.43	23.61	3	0.000167	0.000600
pMTG r	0.46	23.28	3	0.000174	0.000602
IC l	0.46	23.23	3	0.000175	0.000602
PC	0.39	22.84	3	0.000184	0.000613
DefaultMode.LP r	0.42	22.63	3	0.000189	0.000613
PT l	0.50	22.62	3	0.000189	0.000613
FOrb r	0.39	21.66	3	0.000215	0.000664
Visual.Medial	0.45	21.65	3	0.000216	0.000664
CO r	0.47	21.50	3	0.000220	0.000664
sLOC r	0.46	21.32	3	0.000226	0.000664
AG r	0.41	21.32	3	0.000226	0.000664
PostCG r	0.50	21.21	3	0.000229	0.000664
IFG oper l	0.41	20.87	3	0.000241	0.000684
CO l	0.43	20.72	3	0.000246	0.000687
SFG l	0.43	20.31	3	0.000261	0.000716
PT r	0.46	20.12	3	0.000268	0.000725

ROI	beta	T	dof	p-unc	p-FDR
iLOC r	0.48	19.95	3	0.000275	0.000731
TP r	0.46	19.56	3	0.000292	0.000762
SFG r	0.42	19.42	3	0.000298	0.000762
IFG oper r	0.46	19.36	3	0.000301	0.000762
Language.pSTG r	0.48	19.03	3	0.000317	0.000789
Salience.RPFC r	0.38	18.94	3	0.000321	0.000789
toITG l	0.40	18.77	3	0.000330	0.000798
PaCiG r	0.38	18.52	3	0.000344	0.000818
HG r	0.42	18.27	3	0.000358	0.000840
aMTG l	0.40	17.97	3	0.000376	0.000870
FO l	0.37	17.48	3	0.000408	0.000918
LG r	0.45	17.48	3	0.000408	0.000918
sLOC l	0.44	17.27	3	0.000423	0.000932
MidFG l	0.38	17.23	3	0.000426	0.000932

[**Fig 33.** Network connection of group-level analysis results of the post-therapy images.]

8. DISCUSSION

Analyzing the fMRI results from the first-level analysis and the second-level analysis, it can be observed that more brain areas are activated in post-olfactory rehabilitation images than in pre-olfactory rehabilitation images. Moreover, the variability of brain areas activated between subjects on pre-therapy images is higher than in the post-therapy images. In addition, using the menta stimulus showed better results because it activates more areas than the vanilla stimulus. This has coherence with the last paper explained in the state of the art section [27] where it explains that mint stimulus also activates the trigeminal nerves. Also, comparing figures 30 and 32 from the connectivity analysis, can be seen that more connections are established on post-therapy images than on pre-therapy images.

Furthermore, these brain areas that are more activated in post-olfactory rehabilitation acquisitions coincide with the areas described in section *3.1.2 Olfactory system*, which are mentioned below.

- The amygdala is activated as it analyses emotional, endocrine and visceral consequences of smells. Also, several anatomical brain parts of the anterior cortex nucleus show activation as it processes stimulus intensity. In addition, the amygdala works with orbitofrontal cortex to connect language area and giving meaning to the olfactory stimuli learned in the therapy.
- The orbitofrontal cortex is activated to give a name to a smell and to codify olfactory features.
- The occipital and temporal lobes are activated because they are related to the linguistic information that the patients perceive from the smell. This is because in the olfactory rehabilitation sessions they have to denominate and describe the smells in order to learn them. Also, the fusiform gyrus participates when memorizing the stimulus as it is related to perceptive and semantic information of olfactory stimulus.
- The caudate nucleus which is involved in learning and memorizing.
- The insular cortex which processes the sense of disgust to smells.
- The occipital lobe that is related to visual stimuli processing and analysis, and interpretation of images. That is because in the olfactory rehabilitation, the patient has to imagine a visual image of the smell to memorize it better.

- Other areas related to the olfactory cortex which are activated in post-therapy images are the cerebellum, putamen, thalamus, hypothalamus, olfactory tubercle, or hippocampus, between others.

Last but not least, it is interesting to comment that in the first-level analysis results, the patient number 3 has no detected clusters activated for the established pValue of 0,05. It could be that the activation is very small or null. Moreover, it could be related to the fact that patient 3 commented that she had not smelled anything during the MRI acquisition. Also, the set-up was checked to discard that it was an equipment error, and everything was correct. Therefore, these results confirm the lack of activation in this subject.

9. LIMITATIONS AND FUTURE WORK

9.1 Limitations

During the development of the project, two main limitations or problems were detected in this study. On one hand, the most obvious limitation is the low number of subjects who participated in the study, since taking into account only 4 patients offers a very low statistical significance and, consequently, it is not possible to make a good interpretation of the results.

On the other hand, there is no control group in this study. The project did not have budget to acquire more images of healthy subjects within this age range. So, it was only possible to work with the images of the patients. LAIMBIO's intention is to continue with this study and acquire images for a control group.

Finally, it is also worth mentioning that anosmia does not have a single known cause but there are multiple causes of this disease (congenital, infectious, inflammatory, structural, neurological impairments, toxins...). Then, depending on the cause, it could generate different effects on the brain and different responses, so, different olfactory therapies will be applied. As the therapy is different for each type of anosmia, the results of this study cannot be generalized to the effects of all cases of anosmia. The interesting thing is to see if the evolution is the same with COVID as with viral anosmias.

9.2 Future work

As a consequence of the limitations mentioned above, future lines are summarized in the following points:

- Observe if the evolution is the same with COVID patients as with other viral anosmias patients.
- Perform the study with more subjects for a better statistical significance and thus, to have a more reliable interpretation of the results.
- Take into account a control group in the study to compare the brain activation between patients and healthy subjects.
- This study is focused on women in the 40-55 age range. However, it would also be interesting to observe the same study with men patients and/or with patients of other age ranges to observe the effects of olfactory therapy in these groups.
- Finally, it would be interesting to follow up these patients and carry out a long-term study to better evaluate the olfactory rehabilitation process.

10. CONCLUSIONS

In this project it has been achieved the initial objective to identify which brain areas are activated and which connections are established in patients' brains that have suffered COVID-19 with anosmia, before and after an olfactory rehabilitation therapy. The objective of establishing comparations between subjects and acquisitions (pre vs. posts) has been fulfilled and the respective results have been obtained and discussed.

Moreover, the initial hypothesis which was that post-therapy acquisitions will show more activation on the olfactory area than pre-therapy acquisitions, has been confirmed. In addition, it has been discovered that also other areas are activated which are not only related with olfactory system, but also related with memory and with the olfactory rehabilitation technique to learn new smells. Therefore, fMRI allows to validate the olfactory rehabilitation used. On the other hand, on the initial hypothesis was also thought that the results will not differ a lot between subjects of the same condition (pre or post). It can be seen that on post-therapy images that is true, but on pre-therapy images there exists more variability between subjects. However, in exception of subject 3 as very few clusters were detected because no smell improvement was done.

Furthermore, after developing this project, I have realized the importance of rehabilitation therapies and the work of occupational therapists, and how brain neuroplasticity can be modulated allowing a person to learn again.

Last but not least, learning new image processing and analysis programs has been satisfactory for the student. As well as learning to interpret brain activation maps and identify brain areas with an acceptable statistical result. Also, it has been discovered the potential of the neuroimaging technique fMRI to use it as a tool for neurorehabilitation therapy in olfaction.

REFERENCES

[1] 2022. [Online]. Available: https://www.svneurologia.org/libro%20ictus%20capitulos/cap2.pdf

[2] 2022. [Online]. Available: http://www.untumbes.edu.pe/vcs/biblioteca/document/varioslibros/1382.%20Neuroanatom%C3%ADa.%20Texto%20y%20atlas%20en%20color.pdf

[3]"Anatomía del cerebro", *Myhealth.ucsd.edu*, 2022. [Online]. Available: https://myhealth.ucsd.edu/Spanish/RelatedItems/85,P03866

[4]"Brain Anatomy and How the Brain Works", *Hopkinsmedicine.org*, 2022. [Online]. Available: https://www.hopkinsmedicine.org/health/conditions-and-diseases/anatomy-of-the-brain

[5]"Mayfield Brain & Spine", *Mayfieldclinic.com*, 2022. [Online]. Available: https://mayfieldclinic.com/pe-anatbrain.htm

[6]2. S.L.U., "Factores neurotróficos y su trascendencia en el trastorno por déficit de atención/hiperactividad : Neurología.com", *Neurologia.com*, 2022. [Online]. Available: https://www.neurologia.com/articulo/2013555

[7] J. Silva C and A. Slachevsky C, "Modulación de la actividad de la corteza prefrontal como mecanismo putativo del cambio en psicoterapia", AEPC, 2005. [Online]. Available: https://aepc.es/ijchp/articulos_pdf/ijchp-162.pdf

[8]Garcia-Toro M, Salva J, Daumal J, Andres J, Romera M, Lafau O, Echevarría M, Mestre M, Bosch C, Collado C, Ibarra O, Aguirre I. High (20-Hz) and low (1-Hz) frequency transcranial magnetic stimulation as adjuvant treatment in medication-resistant depression. Psychiatry Res. 2006 Jan 30;146(1):53-7. doi: 10.1016/j.pscychresns.2004.08.005. Epub 2005 Dec 13. PMID: 16356697

[9]*Mscbs.gob.es*, 2022. [Online]. Available: https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos/ITCoronavirus.pdf

[10]"Información basíca sobre la COVID-19", *Who.int*, 2022. [Online]. Available: https://www.who.int/es/news-room/questions-and-answers/item/coronavirus-disease-covid-19

[11]"COVID-19", *Canal Salut*, 2022. [Online]. Available: https://canalsalut.gencat.cat/ca/salut-a-z/c/covid-19/

[12]"Enfermedad del coronavirus 2019 (COVID-19) - Síntomas y causas - Mayo Clinic", *Mayoclinic.org*, 2022. [Online]. Available: https://www.mayoclinic.org/es-es/diseases-conditions/coronavirus/symptoms-causes/syc-20479963

[13]"¿Qué es la anosmia? Síntomas y tratamiento | CinfaSalud", *Cinfasalud*, 2022. [Online]. Available: https://cinfasalud.cinfa.com/p/anosmia/

[14]D. médico, "Anosmia: qué es, síntomas y tratamiento | Top Doctors", *Top Doctors*, 2022. [Online]. Available: https://www.topdoctors.es/diccionario-medico/anosmia

[15]*Salud.mapfre.es*, 2022. [Online]. Available: https://www.salud.mapfre.es/enfermedades/otorrinolaringologicas/anosmia/
[16]"Anosmia | Cigna", *Cigna.com*, 2022. [Online]. Available: https://www.cigna.com/es-us/individuals-families/health-wellness/hw/anosmia-abl0388

[17]"Entrenamiento olfativo para recuperarse de la anosmia por COVID-19", *Cun.es*, 2022. [Online]. Available: https://www.cun.es/actualidad/noticias/entrenamiento-olfativo-recuperarse-anosmia-covid

[18]Izquierdo-Domínguez A, Rojas-Lechuga MJ, Mullol J, Alobid I. Olfactory dysfunction during COVID-19 pandemic. Pérdida del sentido del olfato durante la pandemia COVID-19. *Med Clin (Barc)*. 2020;155(9):403-408. doi:10.1016/j.medcli.2020.06.006

[19] V. Sepúlveda, S. Waissbluth and C. González, "Anosmia y enfermedad por Coronavirus 2019 (COVID-19): ¿Qué debemos saber?", SciELO, 2020. [Online]. Available: https://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0718-48162020000200247

[20]*Youtube.com*, 2022. [Online]. Available: https://www.youtube.com/watch?v=MiL0wCZr0Mw&ab_channel=ominhs

[21]L. Mao et al., "Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China: a retrospective case series study", medRxiv, 2020. [Online]. Available: https://www.medrxiv.org/content/10.1101/2020.02.22.20026500v1.full.pdf

[22] [3]N. Dev et al., "COVID-19 with and without anosmia or dysgeusia: A case-control study", Wiley Online Library, 2021. [Online]. Available: https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.26784

[23] Saltagi AK, Saltagi MZ, Nag AK, et al. Diagnosis of Anosmia and Hyposmia: A Systematic Review. Allergy & Rhinology. January 2021. doi:10.1177/21526567211026568

[24]Politi LS, Salsano E, Grimaldi M. Magnetic Resonance Imaging Alteration of the Brain in a Patient With Coronavirus Disease 2019 (COVID-19) and Anosmia. *JAMA Neurol.* 2020;77(8):1028–1029. doi:10.1001/jamaneurol.2020.2125

[25]M. Niesen et al., "Structural and metabolic brain abnormalities in COVID-19 patients with sudden loss of smell", medRxiv, 2020. [Online]. Available: https://www.medrxiv.org/content/10.1101/2020.10.18.20214221v1.full.pdf

[26]Ismail II, Gad KA. Absent Blood Oxygen Level–Dependent Functional Magnetic Resonance Imaging Activation of the Orbitofrontal Cortex in a Patient With Persistent Cacosmia and Cacogeusia After COVID-19 Infection. *JAMA Neurol.* 2021;78(5):609–610. doi:10.1001/jamaneurol.2021.0009

[27]D. Yildirim, S. Giray Kandemirli, D. Tekcan Sanli, O. Akinci and A. Altundag, A Comparative Olfactory MRI, DTI and fMRI Study of COVID-19 Related Anosmia and Post Viral Olfactory Dysfunction. Academic Radiology, 2021

[28]"What is Matlab", *Cimss.ssec.wisc.edu*, 2022. [Online]. Available: https://cimss.ssec.wisc.edu/wxwise/class/aos340/spr00/whatismatlab.htm

[29]*Alivelearn.net*, 2022. [Online]. Available: https://www.alivelearn.net/xjview/manual.pdf

[30]P. Reyes, "Conversion NIFTI - Manual Neuroimágenes de Pablo", *Scrips-to-mri.readthedocs.io*, 2022. [Online]. Available: https://scrips-to-mri.readthedocs.io/es/latest/dcm2nii/

[31]"ENECOVID", *Portalcne.isciii.es*, 2022. [Online]. Available: https://portalcne.isciii.es/enecovid19/

[32]"Recuperar el olfato tras el coronavirus", *Quirónsalud*, 2022. [Online]. Available: https://www.quironsalud.es/es/comunicacion/notas-prensa/recuperar-olfato-tras-coronavirus

[33]"La URJC estudia la pérdida de olfato y gusto como consecuencia de la COVID-19 - Universidad Rey Juan Carlos", *Urjc.es*, 2022. [Online]. Available: https://www.urjc.es/todas-las-noticias-de-actualidad-cientifica/5327-la-urjc-estudia-la-perdida-de-olfato-y-gusto-como-consecuencia-de-la-covid-19

Author's notes of the lessons of the master's degree in Neuroengineering and Rehabilitation (UPC) have also been consulted. Specifically, notes of the subjects of anatomy and physiopathology, neuromodulation and neurostimulation, and neuroimaging.

IMAGE REFERENCES

Figure 1: "Anatomía del cerebro", *Myhealth.ucsd.edu*, 2022. [Online]. Available: https://myhealth.ucsd.edu/Spanish/RelatedItems/85,P03866.

Figure 2: "Redirect Notice", *Google.com*, 2022. [Online]. Available: https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.pinterest.com%2Fpin%2F822188475694139850%2F&psig=AOvVaw29FlclF3xvUnpsY95hZYq6&ust=1636560051899000&source=images&cd=vfe&ved=0CAsQjRxqFwoTCIiSprjTi_QCFQAAAAAdAAAAABAJ.

Figure 3: "Mayfield Brain & Spine", *Mayfieldclinic.com*, 2022. [Online]. Available: https://mayfieldclinic.com/pe-anatbrain.htm.

Figure 4: "Brain Anatomy and How the Brain Works", *Hopkinsmedicine.org*, 2022. [Online]. Available: https://www.hopkinsmedicine.org/health/conditions-and-diseases/anatomy-of-the-brain.

Figure 6: "Epitelio olfativo - Wikipedia, la enciclopedia libre", *Es.wikipedia.org*, 2022. [Online]. Available: https://es.wikipedia.org/wiki/Epitelio_olfativo#/media/Archivo:Anatomy_of_the_human_nasal_cavity.png.

Figure 7: "The olfactory system is the sensory system used to smell", *Mammothmemory.net*, 2022. [Online]. Available: https://mammothmemory.net/biology/coordination-and-response/sensory-receptors/olfactory-bulb.html.

Figure 8: *Zenodo.org*, 2022. [Online]. Available: https://zenodo.org/record/3407886/files/El%20olfato.pdf?download=1.

Figure 9: 2022. [Online]. Available: https://www.researchgate.net/figure/Schematic-view-of-the-human-olfactory-system-The-primary-and-secondary-olfactory_fig2_264433979.

Figure 10: *Mscbs.gob.es*, 2022. [Online]. Available: https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos/ITCoronavirus.pdf.

Figure 11: 2022. [Online]. Available: https://www.researchgate.net/figure/Glover-haemodynamic-response-function-HRF_fig2_45918345.

Figure 13: Politi LS, Salsano E, Grimaldi M. Magnetic Resonance Imaging Alteration of the Brain in a Patient With Coronavirus Disease 2019 (COVID-19) and Anosmia. *JAMA Neurol.* 2020;77(8):1028–1029. doi:10.1001/jamaneurol.2020.2125. [Online] Available: https://jamanetwork.com/journals/jamaneurology/fullarticle/2766765?guestAccessKey=ce736188-4120-47d7-ab95-

7d72220ccafb&utm_source=For_The_Media&utm_medium=referral&utm_campaign=ftm_links&utm_content=tfl&utm_term=052920

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