UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA

TESIS DOCTORAL

Neuroimagen molecular preclínica en estimulación cerebral profunda

Preclinical Molecular Neuroimaging in Deep Brain Stimulation

MEMORIA PARA OPTAR AL GRADO DE DOCTORA

PRESENTADA POR

Marta Casquero Veiga

DIRECTORES

María Luisa Soto Montenegro
Manuel Desco Menéndez

Madrid

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Los Dres. María Luisa Soto Montenegro, Investigadora Principal del Instituto de Investigación Sanitaria Gregorio Marañón, y Manuel Desco Menéndez, Catedrático de la Universidad Carlos III de Madrid y Jefe de la Unidad de Medicina y Cirugía Experimental del Hospital Gregorio Marañón,

CERTIFICAN QUE:

El trabajo de investigación realizado bajo nuestra dirección por Marta Casquero Veiga, objeto de la presente tesis doctoral, y titulado "Neuroimagen molecular preclínica en estimulación cerebral profunda", reúne los requisitos de originalidad, metodológicos y científicos suficientes para optar al grado doctor ante el tribunal que legalmente proceda.

Y para que así conste, a los efectos oportunos, firman el presente certificado en Madrid, a 25 de septiembre de 2020.

Atentamente,

Dra. Mª Luisa Soto Montenegro **Dr. Manuel Desco Menéndez**

En todo estás e ti es todo,

pra min i en min mesma moras,

nin me abandonarás nunca,

sombra que sempre me asombras.

Fragmento de “Negra sombra” en *Follas Novas* (1880)

Rosalía de Castro

A tódolos seres, non humanos e humanos,
que fixeron posible a escritura destas liñas.

Graciñas.

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List of Abbreviations

[^{18}F]FDG / FDG: 2-deoxy-2-[^{18}F]fluoro-D-glucose

AA: Amygdala

AD: Anno Domini

AHiPM / AL: Amygdalohippocampal Area Posteriomedial / Anterolateral Part

ALIC: Anterior Limb of the Internal Capsule

ANCOVA: Analysis of Covariance

ANT: Anterior Nucleus of the Thalamus

ANOVA: Analysis of Variance

AP: Anteroposterior

Arc: Arcuate

ATP: Adenosine Triphosphate

Au: Auditory Cortex

BMI: Body Mass Index

BS / Bstm: Brainstem

Cb: Cerebellum

cc: corpus callosum

CE: Conformité Européenne

Cg: Cingulate

CPu: Caudate Putamen

CT: Computerized Tomography

DBS: Deep Brain Stimulation

DM: Dorsomedial Thalamus

DpME: Deep Mesencephalic Nucleus

DV: Dorsoventral

ECT: Electroconvulsive Therapy

Ect: Ectorhinal Cortex

Ent: Entorhinal Cortex

EMA: European Medicines Agency

FDA: Food and Drug Association

fMRI: Functional Magnetic Resonance Imaging

FMT: Fluorescence Molecular Tomography

FWHM: Full Width at Half Maximum

GABA: γ-Aminobutyric acid

GLUT: Glucose Transporter

GPe: External Globus Pallidus

GPi: Internal Globus Pallidus

HDE: humanitarian Device Exception

HF-diet: High Fat-diet model

Hi: Hippocampus

HTh / hypoth: Hypothalamus

I: Insular Cortex

IC: Inferior Colliculus

IP: Intraperitoneal

IPG: Implanted Pulse Generator

Lent: Lateral Enthorinal Cortex

LH: Lateral Hypothalamus

MDD: Major Depressive Disorder

MER: Microelectrode Recordings

MIS: Maternal Immune Stimulation

ML: Mesolateral

mPFC: medial Prefrontal Cortex

MRI: Magnetic Resonance Imaging

MW: Mann Whitney-U tests

NAcc: Nucleus Accumbens

NOR: Novel Object Recognition test

NSA: Non-Significant Area

Ob: Olfactory bulb

OCD: Obsessive Compulsive Disorder

PAG: Periaqueductal Gray Matter

PI: Preference Index

Pir / Pir C / PC: Piriform Cortex

PMCo: Posteromedial Cortical Amygdaloid nucleus

PRh: Perirhinal Cortex

PSA: Posterior Subthalamic Area

PSA-NCAM: Polysyalylated form of the Neural Cell Adhesion Molecule

PT: Pretectal Nucleus

PtA: Parietal Association Cortex

RM-ANOVA: Repeated Measurements ANOVA

RMC: Red Nucleus

RN: Reticular Nucleui

ROI: Region Of Interest

RS: Retrosplenial Cortex

S1 / 2: Primary / Secondary Somatosensory Cortex

SC: Superior Colliculus

SCC: Subcallosal Cingulate region

SEM: Standard Error of the Mean

SNc: Substantia Nigra *pars compacta*

SNr: Substantia Nigra *pars reticulata*

SPM: Statistical Parametric Mapping

SPECT: Single Photon Emission Computerized Tomography

SPIM: Selective Plane Illumination Microscopy

St / Str: Striatum

STN: Subthalamic nucleus

Syn: Synaptophysin

TeA: Temporal Association Cortex

Tem C: Temporal Cortex

Th: Thalamus

US: Ultrasound Imaging

VC/VS: Ventral Capsule / Ventral Striatum

VCx: Visual Cortex

VGAT: Vesigular GABA Transporter

VGLUT: Vesicular Glutamate Transporter

Vim: Ventral Intermediate Nucleus of the Thalamus

VTA: Ventral Tegmental Area

VMH: Ventromedial Hypothalamus

WHO: World Health Organization

Resumen

Introducción: La estimulación cerebral profunda (DBS) es una potente terapia de neuroestimualción dedicada al tratamiento paliativo de, en su mayoría, casos avanzados de patologías motoras. Los fructíferos resultados obtenidos dentro del ámbito de la neurología llevaron a explorar la posibilidad de extender la aplicación de la DBS a diferentes patologías psiquiátricas. Así, la DBS emerge como una alternativa a las tradicionales intervenciones neuroquirúrgicas, las cuales consisten en procedimientos de ablación, local o extensa, realizados en regiones cerebrales específicas involucradas en la sintomatología a paliar. En este sentido, aunque la DBS es de hecho una técnica invasiva, se trata de una terapia local, con potencial reversibilidad de los efectos inducidos por la estimulación eléctrica, y que proporciona la posibilidad de ajustar *in vivo* los parámetros de estimulación de acuerdo a las necesidades del paciente. Sin embargo, a pesar de los enormes esfuerzos y la extensa historia de investigación en el campo de la DBS, los mecanismos de acción específicos de esta terapia todavía se desconocen. Varias teorías han intentado explicar los efectos obtenidos bajo circunstancias patológicas y de estimulación concretas. No obstante, a pesar de la indudable eficacia de la DBS, un amplio número de preguntas sin responder permanecen abiertas en relación a los efectos fisiológicos específicos de la DBS, así como a las consecuencias a largo plazo de la misma. Por este motivo, la aplicación de la DBS en la mayoría de las patologías neuropsiquiátricas que han sido propuestas de poder potencialmente beneficiarse de su tratamiento, permanecen en una etapa de investigación.

Objetivos: La principal motivación de esta tesis consiste en evaluar los efectos de la DBS, por medio de técnicas de imagen funcional *in vivo*, en ratas sanas y en dos modelos murinos de obesidad. En concreto, la principal contribución de este trabajo radica en describir las consecuencias de la neuromodulación inducida por la DBS sobre el metabolismo cerebral, utilizando [^{18}F]FDG-PET en diferentes niveles: 1º) después de la inserción de los electrodos; 2º) durante la estimulación eléctrica; 3º) después de un protocolo crónico e intermitente de DBS; y 4º) tras un protocolo crónico y continuo de DBS. En este sentido, los objetivos específicos recogidos en esta tesis son:

1. Evaluar el efecto insercional del electrodo sobre el metabolismo cerebral, aislado de la modulación producida por un protocolo agudo de estimulación eléctrica, en ratas sanas con electrodos implantados en la corteza prefrontal medial (mPFC).
2. Identificar las redes neuronales moduladas por un protocolo agudo de DBS en tres dianas cerebrales diferentes (mPFC, tálamo dorsomedial -DM- y núcleo

accumbens -NAcc-), relacionadas con alteraciones funcionales en diferentes patologías neuropsiquiátricas, en ratas sanas.

3. Evaluar dos protocolos de DBS, a corto y largo plazo, haciendo diana en el NAcc y el hipotálamo lateral (LH), sobre el metabolismo cerebral, la ganancia de peso, el comportamiento y la neuroplasticidad, en un modelo animal de obesidad genético (rata Zucker) y uno alimentado bajo una dieta alta en grasa (HF-diet).

Métodos y Resultados: En primer lugar, encontramos que el efecto de la inserción del electrodo en la mPFC de ratas Wistar sanas macho (N=5) reduce específicamente el metabolismo cerebral en áreas corticales. Sin embargo, la DBS resultó en un patrón metabólico más extendido, involucrando a la misma red neuronal que la inserción del electrodo, en la cual la estructura diana juega un papel principal. Las consecuencias simultáneas del electrodo y de la DBS revelaron una combinación de ambos efectos por separado.

Por otro lado, la DBS indujo cambios metabólicos específicos en función con la diana de estimulación en ratas Wistar sanas macho (N=43). Sin embargo, un incremento de la actividad metabólica en la corteza piriforme apareció de forma paralela tras aplicar la DBS en las tres dianas bajo estudio. Además, la mPFC-DBS produjo un incremento de la actividad metabólica en el estriado, las cortezas temporales y la amígdala; una reducción en el cerebelo, el tronco cerebral y la sustancia gris periacueductal. Por otro lado, la NAcc-DBS incrementó la actividad metabólica en el subículum y el bulbo olfatorio, y la redujo en el tronco cerebral, la sustancia gris periacueductal, el septo y el hipotálamo. Finalmente, la DM-DBS incrementó la actividad metabólica en el estriado, el NAcc y el tálamo, y la redujo en las cortezas temporal y cingulada.

En relación al protocolo de estimulación intermitente (1 hora/día) aplicado sobre el NAcc-DBS durante 15 días en la rata Zucker (NAcc-DBS: N=6, NAcc-sham: N=9), encontramos que la estimulación produjo un incremento metabólico en las cortezas cingulada-retroplenial-parietal asociativa, así como una reducción del metabolismo en el NAcc, tálamo y núcleos pretectales. Sin embargo, este protocolo de estimulación sobre el NAcc no redujo ni la ganancia de peso ni la ingesta de comida. Además, los resultados longitudinales a largo plazo de este mismo protocolo de NAcc-DBS en la rata Zucker revelaron que, un mes después de la retirada de la estimulación, los animales NAcc-sham y NAcc-DBS mostraban un patrón metabólico común de modulación cerebral, probablemente derivado de la presencia de los electrodos. Una respuesta metabólica similar fue obtenida en el análisis longitudinal de las imágenes de PET del grupo de ratas

Zucker LH-sham (N=4). Sin embargo, las ratas Zucker LH-DBS (N=6) incluidas en el estudio reflejaron una relativa invariabilidad metabólica entre ambos puntos temporales (es decir, un día y un mes tras el final de la DBS), así como una reducción en los niveles de moléculas de plasticidad sináptica. Así, la estabilidad neural alcanzada tras el tratamiento con LH-DBS persistió un mes después de la retirada de la estimulación.

Finalmente, un protocolo de LH-DBS continuo (24 horas/día) durante 15 días no indujo una reducción en la ganancia de peso, ni en la rata Zucker (Zucker-sham: N=9, Zucker-DBS: N=11), ni en el modelo HF-diet (HF-diet-sham: N=15, HF-diet-DBS: N=7). No obstante, la inserción de los electrodos ralentizó la ganancia de peso en los animales HF-diet no estimulados. Además, la LH-DBS produjo una modulación metabólica a nivel de regiones relacionadas con el aprendizaje, la memoria y la recompensa, pero en diferente medida en función del modelo. Es de destacar el efecto hipermetabólico producido en el hipocampo en las ratas Zucker-DBS, unido con una promoción de los procesos de plasticidad sináptica en esta región. Sin embargo, los efectos fisiológicos observados a nivel hipocampal no se tradujeron en mejorías cognitivas, evaluadas a través del paradigma de reconocimiento de nuevo objeto (NOR), debidas a la LH-DBS. A pesar de ello, sí se observaron mejorías progresivas y a largo en ambos grupos de ratas Zucker operadas.

Conclusiones: La principal contribución de esta tesis consiste en la descripción de las consecuencias neuromoduladoras de diferentes estragegias de DBS sobre el metabolismo cerebral, por medio de estudios de PET preclínicos con [^{18}F]FDG, en ratas sanas y en dos modelos animales de obesidad. Además, se realiza un énfasis particular en el abordaje de hipótesis que incorporan, de forma secuencial, diferentes aspectos del tratamiento con DBS. En este sentido, las principales conclusiones alcanzadas en esta tesis son:

1. El efecto insercional del electrodo sobre el metabolismo cerebral difiere del inducido por un protocolo agudo de DBS de alta frecuencia en la misma diana cerebral, aunque ambos recaen sobre el mismo circuito cerebral en el que la diana de estimulación está integrada.
2. La estimulación aguda de alta frecuencia de mPFC, NAcc y DM inducen diferentes patrones de captación de [^{18}F]FDG, a pesar de interaccionar con los mismos circuitos cerebrales. En este sentido, la DBS en estas estructuras produce cambios significativos en la captación de [^{18}F]FDG en regiones cerebrales asociadas con el circuito de los ganglios basales-tálamo-corteza.

3. Un protocolo de NAcc-DBS intermitente aplicado durante 15 días induce cambios en el metabolismo cerebral de la rata Zucker en regiones asociadas con los sistemas cognitivo y de recompensa, cuyo deterioro ha sido descrito en obesidad. Sin embargo, la NAcc-DBS intermitente no reduce ni la ganancia de peso ni la ingesta de comida en este modelo animal.
4. La magnitud de la persistencia de la modulación inducida por la DBS sobre el metabolismo cerebral a largo plazo depende de la estructura diana (LH o NAcc), incluso a pesar de pertenecer al mismo circuito neuronal y en la misma patología (i.e. obesidad genética). Por tanto, la estabilidad funcional alcanzada durante LH-DBS determinaría la extensión de la huella de la DBS tras retirar el tratamiento.
5. Los mecanismos fisiopatológicos subyacentes a la obesidad, es decir, la genética o el ambiente, condicionan la respuesta terapéutica ante la LH-DBS. En consecuencia, protocolos personalizados adaptados al fondo patológico de cada condición producirían resultados más satisfactorios para el paciente.

Abstract

Introduction: Deep brain stimulation (DBS) is a very powerful neurostimulation therapy for the palliative treatment of, mainly, resistant cases of motor disorders. The fruitful results obtained in the neurological scenario led to explore the possibility of extending its application to different psychiatric pathologies. Thus, DBS emerges as a potential alternative to the traditional neurosurgical interventions, which consist of local or major ablative procedures, performed in specific brain regions involved in the symptomatology with the aim to palliate it. In this sense, although it is indeed an invasive technique, it is actually a focal therapy, with potential reversibility of the effects induced by the electric stimulation, and offering the possibility of adjusting the stimulation parameters according to the patient's needs. Nevertheless, despite the huge efforts and extensive research history in the DBS field, the specific mechanism of action of this therapy remains unknown. Several theories have tried to explain the effects obtained under specific pathologies and stimulation circumstances. However, regardless of DBS undoubted efficacy, a wide number of unanswered questions remain open regarding the specific physiological effects and long-term consequences. Therefore, the application of DBS in the majority of the proposed neuropsychiatric pathologies to potentially benefit from this treatment remains at a research level.

Objectives: The main aim of this thesis is to evaluate the effects of DBS, by means of *in vivo* functional neuroimaging techniques, in healthy rats and in two models of murine obesity. In particular, the main contribution of this thesis is to describe the neuromodulation consequences of DBS on brain metabolism using [^{18}F]FDG-PET at different stages: 1st) after the electrodes insertion, 2nd) during the electrical stimulation, 3rd) after a chronic and intermittent DBS protocol, and 4th) after a chronic and continuous DBS protocol. In this regard, the specific objectives of this thesis are:

1. To evaluate the electrode insertional effect on brain metabolism, isolated from the modulation induced by an acute protocol of electrical stimulation, in healthy rats with electrodes implanted in the medial prefrontal cortex (mPFC).
2. To identify the neural networks modulated by an acute protocol of DBS in three different brain targets (mPFC , medio dorsal thalamus -MD- and nucleus accumbens -NAcc-), related to functional alterations in different neuropsychiatric pathologies, in healthy rats.
3. To evaluate the short-term and long-term consequences of two DBS protocols, targeting the NAcc and the lateral hypothalamus (LH), on brain metabolism, weight

gain, behavior and neuroplasticity, in a genetic (Zucker rat) and a high-fat diet (HF-diet) animal models of obesity.

Methods and Results: First of all, we found that the electrode insertion in the mPFC of healthy male Wistar rats (N=5) proved to specifically reduced the brain metabolism in cortical areas. Nevertheless, DBS resulted in a more widespread metabolic pattern, involving the same neuronal network as the electrode insertion, and in which the target structure plays a key role. The consequences of simultaneous electrode and DBS factors revealed a combination of both separated effects.

Then, DBS induced site-specific metabolic changes in healthy male Wistar rats (N=43), although a common increased metabolic activity in the piriform cortex was found for the three brain targets. mPFC-DBS increased metabolic activity in the striatum, temporal cortices and amygdala, and reduced it in the cerebellum, brainstem and periaqueductal gray matter. NAcc-DBS increased metabolic activity in the subiculum and olfactory bulb, and decreased it in the brainstem, periaqueductal gray matter, septum and hypothalamus. DM-DBS increased metabolic activity in the striatum, NAcc and thalamus and decreased it in the temporal and cingulate cortices.

With regards to an intermittent (1 hour/day) protocol of NAcc-DBS applied during 15 days to the Zucker rat (NAcc-DBS: N=6, NAcc-sham: N=9), we found that NAcc-DBS led to increased metabolism in the cingulate-retrosplenial-parietal association cortices, and decreased metabolism in the NAcc, thalamic and pretectal nuclei. However, NAcc-DBS did not induce a decrease in either weight gain or food intake. Furthermore, longitudinal long-term results of this same NAcc-DBS protocol in the Zucker rat, one month after stimulation withdrawal, revealed that NAcc-sham and NAcc-DBS animals showed a common pattern of brain metabolic modulation, probably derived from the electrodes presence. A similar metabolic response was obtained in the longitudinal analisis of LH-sham (N=4) Zucker rats. However, the LH-DBS (N=6) Zucker rats showed a relative invariability between both time points (i.e. one day and one month after the end of DBS), as well as a reduction of neuroplasticity molecules. Thus, the neural steadiness achieved after LH-DBS persisted one month after stimulation withdrawal.

Finally, a 15-days continuous (24 hours/day) LH-DBS protocol did not reduce weight gain, neither in the Zucker rat (Zucker-sham: N=9, Zucker-DBS: N=11), nor in the HF-diet (HF-diet-sham: N=15, HF-diet-DBS: N=7) animal model. However, the insertion of the electrodes slowed down the weight gain in non-stimulated HF-diet animals. Also, LH-

DBS modulated brain metabolism in memory, learning and reward-related regions, but to a different extent in each model. Notably, the hypermetabolism produced in the hippocampus of stimulated Zucker rats, along with the enhanced synaptic plasticity processes in this region, did not translate into cognitive improvements in the novel object recognition (NOR) paradigm due to LH-DBS. However, progressive long-term memory and attentional benefits were obtained in both Zucker operated groups.

Conclusions: The main contribution of this thesis is to describe the neuromodulation consequences of different DBS approaches on brain metabolism, by means of preclinical PET studies with [^{18}F]FDG, in healthy rats and in two animal models of obesity. Furthermore, special emphasis is placed on addressing hypotheses which sequentially incorporate different aspects of the DBS treatment. Therefore, the main specific conclusions reached in this thesis are:

1. The electrode insertion effect on brain metabolism differs from that induced by acute high-frequency stimulation in the same brain target, although both are based on the same neural circuit in which the DBS target is integrated.
2. Acute high-frequency stimulation of mPFC, NAcc and DM induces different patterns of [^{18}F]FDG uptake, despite interacting with the same brain circuitries. In this sense, DBS applied to these structures produces significant changes in [^{18}F]FDG uptake in brain regions associated with the basal ganglia-thalamo-cortical circuitry.
3. A 15-days intermittent NAcc-DBS protocol induces changes in the brain metabolism of regions associated with cognitive and reward systems in the Zucker rat, whose impairment has been described in obesity. However, intermittent iNAcc-DBS does not induce a decrease in either weight gain or food intake in this animal model.
4. The magnitude of the long-term persistence of the DBS brain metabolic modulation depends on the brain target (LH or NAcc), even despite belonging to the same neural circuit and in the same disease (i.e. genetic obesity). Therefore, the functional steadiness reached during LH-DBS would determine the extent of the DBS footprint after removing the treatment.
5. The physiopathology mechanisms underlying obesity, namely genetics or environment, condition the therapeutic response to LH-DBS. Accordingly, personalized protocols adapted to each condition background would produce more successful results.

Outline of the document

This PhD thesis is based on five research works which have been carried out in the Laboratorio de Imagen Médica of the Instituto de Investigación Sanitaria Gregorio Marañón, leading the document organized in eight chapters. **Chapter 1** contains an introduction to the concepts of deep brain stimulation (DBS) and molecular neuroimaging, as well as an overview of the state of the art in the field of neuroimaging in DBS. **Chapter 2** poses the motivation and objectives of the present PhD thesis. **Chapter 3** explores the brain metabolic consequences of the two main neuromodulatory elements in DBS, namely the electrodes insertion and the electric stimulation, in healthy rats by means of voxel-wise analyses of [^{18}F]FDG PET images. After separately evaluating both effects, **Chapter 4** describes the effects on brain metabolism of acute high-frequency DBS in three brain targets with relevance in neuropsychiatric disorders in healthy rats, following a voxel-based approach. Going one step further, **Chapter 5** reports the brain metabolic effects of applying one daily hour of bilateral high-frequency DBS in the NAcc, during 15 days, in a genetic animal model of obesity. Then, **Chapter 6** presents a longitudinal study of brain metabolic changes which persist one month after finishing the same DBS protocol explained in Chapter 5. This time, both NAcc and lateral hypothalamus (LH) were assessed as DBS targets in the same genetic obesity model, and long-term neuroplasticity changes in the hippocampus and the entorhinal cortex were evaluated. Finally, **Chapter 7** presents the results of applying a continuous (i.e. 24 hours/day) DBS protocol in the LH during 15 days, in a genetic and a diet-induced models of obesity, reporting short and long-term brain metabolic changes as outcomes. This study also includes memory and learning behavioral tests, as well as an assessment of hippocampal neuroplastic long-term changes induced by LH-DBS. **Chapter 8** gathers the general discussion and conclusions of the thesis.

Ethics statement

All the experimental animal procedures conducted within this PhD thesis were performed according to the European Communities Council Directive 2010/63/EU and approved by the Ethics Committee for Animal Experimentation of Hospital General Universitario Gregorio Marañón (Comité de Ética de Experimentación Animal, CEEA; number ES280790000087).

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1. Introduction

1.1. Deep brain stimulation

The term *Neurostimulation* is defined as the chronic therapeutic stimulation of the central nervous system or special nerves with an implanted stimulating device (Gildenberg, 2009). It covers a variety of different techniques focused on the modulation of the neural system activity in order to overcome a functional impairment. These techniques can be classified according to three main features: 1) the invasiveness degree (internal or external application), 2) the nature of the applied stimulation (electric or magnetic), and 3) the place of application (central – i.e. the brain – or peripheric – i.e. the nerves). Thus, among the several neurostimulation techniques currently available, *deep brain stimulation* (DBS) appears to be as one of the most widespread neural modulatory approaches in the clinical practice. As its name suggests, DBS is an invasive neurosurgical technique that consists of placing electrodes targeting precise brain regions involved in the physiopathology of the patient, and whose activity is sought to be modulated by means of a continuous electric current. To this end, electrical pulses are delivered through the electrodes, which are connected to an external implantable pulses generator (IPG), similar to a pacemaker, usually placed in the infra-clavicular space. A remote programming device is used to set the stimulation parameters (i.e. intensity, amplitude, frequency and electrode contacts activation and polarity) (Volkmann et al., 2002). It is important to note that DBS is reserved for the intervention of treatment-resistant patients as a palliative approach, in an attempt to alleviate their symptoms and thus improve their quality of life.

A whole overview of the surgical procedure for DBS implantation can be reviewed in (Kasoff & Gross, 2016). Shortly, DBS systems are usually implanted in a two-steps surgical intervention, first placing the DBS lead and electrodes in the brain, and second implanting the IPG and the extension cable which connects the IPG to the lead. These two interventions can be performed during the same surgical procedure or after a period (several weeks) of recovery. Depending on the surgery planification and the brain target of interest, different medical imaging modalities are acquired from each patient at several time points of the procedure (e.g. before the surgery for planification, during the intervention for the confirmation of the stereotactic fiducials location, and after the surgery in order to ensure the correct electrodes placement and discard postoperative complications such as hemorrhage). The imaging techniques usually applied are magnetic resonance imaging (MRI) and computerized tomography (CT), in order to localize the specific coordinates of the brain target in the patient's own anatomy. Also,

specific MRI sequences or CT modalities (e.g. diffusion-tensor imaging, ventriculography or gadolinium-contrast images) are obtained in order to avoid blood vessels or white matter tracts across the electrode trajectories. At this point, all the gathered information is incorporated to a planning workstation which allows to co-register the patient's anatomic information to well-established brain atlas, such as the Tailarach-Tourneau or the Schaltenbrand-Wahren atlases (Schaltenbrand & Wahren, 1977; Talairach et al., 1988). Both provide the specific coordinates of the brain target of interest after deforming the 3D atlases grids to the patient's head.

Also relevant is the kind of stereotactic frame selected for implanting the DBS electrodes. In this sense, two main groups of devices can be distinguished:

1. Classic frames: These stereotactic apparatus contain the imaging fiducials in the own instrument holder platform. Therefore, the interventions which involved this kind of gadgets need to acquire the planification images before installing the stereotactic frame on the patient's head.
2. "Frameless" systems: Their instrument holders are separated from the imaging fiducials. These kind of approaches require optical cameras or tactile systems in order to localize the optical fiducials attached to the patient's head, and virtualize the placement information to register it to the virtual surgical planification.

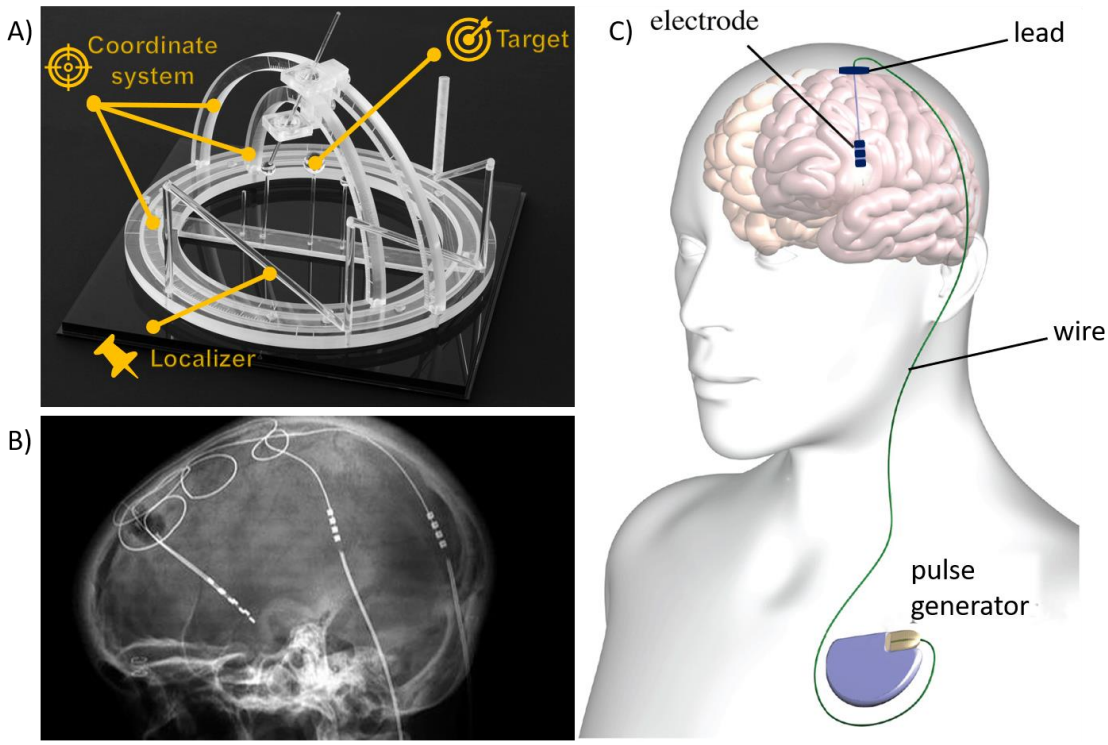


Figure 1 - DBS configuration elements. A) Arc-centered stereotactic frame with 3 N-localizers. **B)** X-Ray imaging of a patient implanted with DBS electrodes. **C)** Schematic view of the DBS system in the patient. *All the images are represented under a Creative Commons License.*

Besides imaging acquisitions, microelectrode recordings (MER) are usually performed during surgery, before electrode implantation, in order to confirm the precise location of the cannula which will drive the electrode insertion. Also, focal stimulation of the targeted region can be applied to confirm the location instead, and observe in real time the acute effects of the stimulation on the patients symptoms (particularly in movement disorders). In any case, either intraoperative MER-, stimulation- or imaging-guided surgeries provide similar success rates, and the methodology of choice depends on the hospital and surgeon availability and experience.

Normally, patients arrive at the hospital the same morning of the day of the operation. After 3-5 hours (unilateral) / 6-8 hours (bilateral) of surgical procedure, and provided that there are no complications, they are considered outpatients the same evening of the surgery. Furthermore, postoperative care is routine and, after healing of the skin wounds, there is no impediment which prevents the patients to return to their normal activities, which usually occurs in a 4-weeks period since the DBS surgery. In general, it is within this period that the stimulator is programmed and all the contacts implanted are tested, but maintaining active those which better target the brain structure of interest and provide the best therapeutic outcomes.

Finally, as every therapeutic procedure, DBS can lead to certain complications which occur in very rare occasions (Sugiyama, 2015). The first difficulties are the most health-threatening, and directly derive from the surgical procedure (e.g. intracranial hemorrhage, insertional edema, lead misplacement). They appeared in the 1.2-7.8% of the operated patients, depending on the surgical conditions and risk factors presented by the patient (Kasoff & Gross, 2016). Also, side effects of the stimulation (e.g. paresthesias, dysartria, psychiatric symptoms) may occur. They can be normally reversible by discontinuing the stimulation and adjusting the DBS programming. Lastly, hardware-related adverse events (e.g. infection of the IPG pocket, skin erosion, lead migration or fracture) are usually solved with antibiotic medication and system removal, when needed. In general, DBS presents good safety, reliability and tolerability profiles, with easy-to-manage side effects, which led to extent its application for the treatment of a wide spectrum of neuropsychiatric disorders.

1.1.1. Historical overview

The therapeutic potential of electrical stimulation was already recognized in Ancient Rome and Egypt. Thus, the first evidence that revealed a beneficial effect of an electric

current approximately dates from the year 15 AD, in which the physician Scribonius Largus of the Emperor Tiberius described the "miraculous" recovery of a freed slave suffering from gout after accidentally touching a torpedo fish (Gildenberg, 2009). Nevertheless, electricity as a physical phenomenon remains misunderstood until the middle of the eighteenth century, and it was not until 1799, with the creation of the electric battery by Alessandro Volta, that electricity started to become an essential element in our daily life. Thus, the medical application of this "new" phenomenon did not last, and the first register of neurostimulation in humans is attributed to Roberts Bartholow in 1874. Bartholow stimulated the parietal lobes of an awake patient with an erosive basal cell cancer, resulting in contralateral involuntary movements and seizures (Wojtasiewicz et al., 2019).

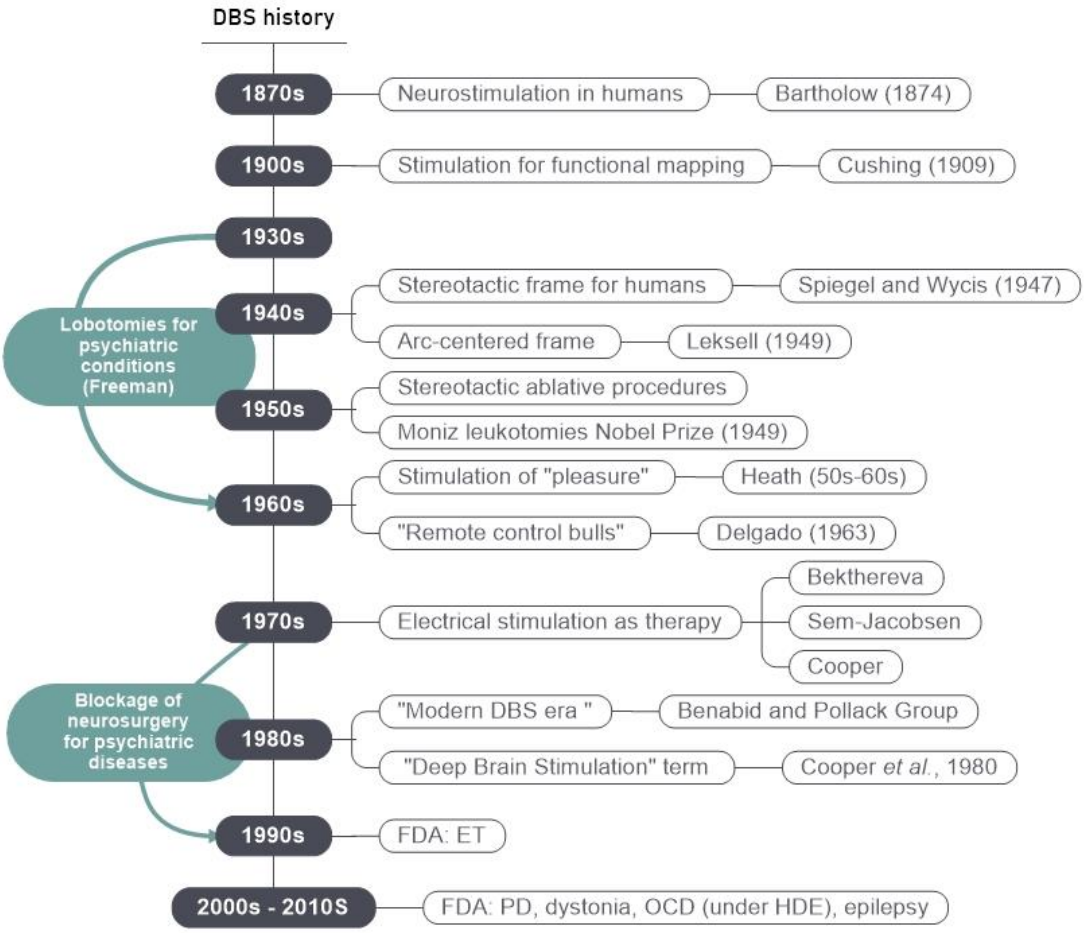


Figure 2 - DBS history. Overview of the most relevant events throughout the history of DBS, from the first brain stimulation procedure, to the present. *[HDE, humanitarian device exemption; OCD, obsessive compulsive disorder]*

Several other works followed this first approach, but they were mainly functional mapping studies, based on the different responses obtained after applying electrical stimulation in

precise areas of the brain. Thus, Harvey Cushing pioneered the intraoperative stimulation in order to localize these functional brain areas, among other neurosurgeons, in the early years of the 20th century (Cushing, 1909). In this sense, two main events set the bases for incorporating the electrical stimulation of deep brain structures into the clinical routine: 1st) advances in the knowledge of functional mapping of the brain, and 2nd) the development of the first stereotactic frame. Thus, Victor Horsley and Robert H. Clarke developed the first stereotactic apparatus for animal use in 1908 (Gildenberg, 2009). This cartesian frame was based on external cerebral landmarks, which avoids its application to human subjects given its unreliability of the measurements. Later, Ernest A. Spiegel and Henry T. Wycis recognized the potential of Drs. Horsley and Clarke's approach and created a new stereotactic apparatus similar to their predecessor's one. This new frame solved the previous drawbacks by introducing specific adaptations to the patient's anatomy observed by X-Ray images (Spiegel et al., 1947). Therefore, these adjustments allow the application of stereotactic surgery to be extended to various ablative interventions in human patients (e.g. pallidotomies, thalamotomies, etc.). After viewing the Spiegel-Wycis frame, Lars Leksell developed a novel arc-centered stereotactic frame, which used three polar coordinates in addition to the cartesian components implemented in the earlier head frame models (Leksell, 1949). The Leksell frame allowed to target almost any deep brain structure of interest from virtually any location on the skull and, despite very few modifications to the original design, it is used nowadays for human stereotactic neurosurgery (Wojtasiewicz et al., 2019).

Thus, Drs. Spiegel and Wycis gave rise to human stereotactic surgery and, as stated in the original report published in *Science* journal, "(stereotactic technique) *permits one to insert a wire or a cannula accurately into a desired subcortical area with minimal injury to the cerebral cortex or the white matter*", as a plus for the dissemination of the use of stereotactic surgery in clinical practice. Therefore, stereotactic surgery appeared in the early 1950s as an alternative to massive lesioning procedures conducted during the 1930s, such as the leukotomies that awarded António E. Moniz the Nobel Prize in 1949, by inducing targeted and precise lesions which avoided the side effects of the so far called psychosurgery (Kendall H. Lee et al., 2016). In fact, both Spiegel and Wycis and Leksell groups applied their own inventions to perform stereotactic ablative procedures in patients, as for the treatment of Parkinson's disease or obsessive compulsive disorders, respectively. Originally, these lesions were inferred by direct injection of

alcohol into the structure of interest, but this technique was soon replaced by inferring electrolytic lesions and, later, by radiofrequency (Gildenberg, 2009).

These early stereotactic interventions already included electrical stimulation so as to verify the electrode placement in the desired target, and to study the brain human physiology. Therefore, by stimulating the targeted area and observing the evoked physiological response, the surgical team confirmed the location of the electrode within the brain structure to be lesioned. Thus, the stimulation of deep brain structures during stereotactic surgery was initially considered only as a experimental technique for studying the brain, without obvious evident therapeutic interest. However, the neurophysiologist José M.R. Delgado, in the early 1950s, recognized the effects that subcortical brain stimulation could elicit on behavior, and then proposed its potential application to psychotic patients. Dr. Delgado became famous after a short movie in which he was shown avoiding the violent charge of a fighting bull, to which he had implanted a subcortical stimulation system, by pressing a remote control which activated the stimulation (Marzullo, 2017). He developed a vivid interest in discovering the mechanisms related to mind-control through electrical stimulation, which led him to carry out an extensive research career in deep brain stimulation including different animal species (rats, cats, monkeys and finally humans). His clear enthusiasm in brain electrical stimulation and behavior control led Dr. Delgado to write the book entitled "*Physical Control of the Mind: Towards a Psychocivilized Society*" (Delgado, 1969).

Parallel to Dr. Delgado's studies, Robert Heath, a psychiatrist from Tulane University, developed an extensive work from the 1950s to the 1970s, focused on the stimulation of the brain "pleasure" centers. He sought to alleviate physical pain and induce a sense of euphoria and joy through this approach. In fact, Dr. Heath suggested the application of septal area stimulation to patients with schizophrenia and cancer, in order to modulate their emotions and alleviate their pain (Baumeister, 2000). However, the research works headed by Dr. Heath have been accused of lacking from any scientific interest, and even their rigor, accuracy and ethics have been questioned (Hariz, 2016).

Far from Tulane University group, other researchers were exploring the DBS application in different modalities and pathologies. In this regard, DBS started to gain therapeutic interest after some serendipitous observations when using electrical stimulation to confirm the stereotactic target for ablative neurosurgery in the operation room. Indeed, improvement in symptoms was obtained after stimulation in Parkinson's disease (Spiegel

et al., 1964), as well as awake patients with dystonia (Alberts et al., 1966). Despite these initial steps, the main applications of DBS during the 1970s and 1980s were focused on chronic pain (Gildenberg, 2009), which led to a scarcity of studies involving movement disorders. Nonetheless, several researchers in very distant regions of the world set the basis for DBS therapy, as we understood it today. Among them, different names stand out as the pioneers of the DBS as a therapeutic approach: first, the Russian neurophysiologist Natalia P. Bekthereva, who applied chronic stimulation to study the physiology of mental activity and the treatment of motor disorders; then, the Norwegian psychiatrist Carl Wilhelm Sem-Jacobsen, who implanted recording and stimulating electrodes in patients with epilepsy and psychiatric disorders; and finally, the American neurosurgeon Irving S. Cooper, who led the first cerebellar stimulation studies for cerebral palsy, epilepsy and spasticity, as well as other neurosurgical techniques for movement disorders (Kendall H. Lee et al., 2016). In fact, Dr. Cooper was the first researcher to coin the term “deep brain stimulation” in the literature in a case report published in 1980 (Cooper et al., 1980).

While the application of DBS in pain and movement disorders continued evolving, research on neurosurgery in psychiatric diseases suffered a sudden blockage after the 1970s. In fact, not a single article on DBS in psychiatry was published from Dr. Dieckmann’s chapter on the application of DBS on phobias in 1979 (Dieckmann, 1979), until a brief article published in 1999 in *The Lancet* by Dr. Nuttin *et al.*, proposing the use of DBS in the internal capsules for obsessive compulsive disorder (OCD) (B. Nuttin et al., 1999). This impasse derived from two main events: First, the official recognition of the unethical and unprofitable nature, in terms of effectiveness and safety, of lobotomies (i.e. leukotomies) performed from the 1930s to the 1960s for the treatment of various psychiatric conditions (e.g. anxiety, psychosis). In this sense, Walter Jackson Freeman II stood out among the psychosurgery practitioners of that time, as he popularized the so-called “transorbital lobotomy”, and indiscriminately performed 2500 of these interventions in the United States between the mid-1940s to the early 1960s (Kendall H. Lee et al., 2016). And second, the discovery and standardization of the use of chlorpromazine and other antipsychotics for psychiatric disorders (Ban, 2007). In fact, the practice of previous psychosurgery techniques was initially extended due to the lack of effective psychopharmacology approaches and the dire situation in which psychiatric patients were, given that their only possible alternative was institutionalization (Heller et al., 2006). Therefore, psychosurgery was discouraged once the possibility of achieving

an effective treatment by a non-invasive pharmacological method for psychiatric patients was real. In fact, even the United State Congress considered banning the practice of psychosurgery in 1977, although the final verdict was limited to some caveats, recognizing the therapeutic value that psychosurgery could provide in certain cases (Correia, 2015).

The beginnings of contemporary DBS are attributed to studies conducted at Grenoble University in the late 1980s by Alim-Louis Benabid and Pierre Pollack's team. In particular, their publication entitled "*Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease*" (A L Benabid et al., 1987) inaugurates the conception of DBS as a therapeutic option for the amelioration of movement disorders symptoms. Since then, the amount of research in DBS for psychiatric and movement disorders has dramatically increased, particularly since the entry into the 21st century (D. J. Lee, Lozano, et al., 2019). Despite the frenetic growth in scientific articles on this topic, DBS application has been approved by the *Food and Drug Association* (FDA) for a very limited number of pathologies (i.e. essential tremor, Parkinson's disease, dystonia, OCD and medically refractory epilepsy), although it has been suggested that many other neuropsychiatric pathologies may potentially benefit from DBS (as will be discussed in the section *1.1.3 Therapeutic application in neuropsychiatry*). In addition, there is a large number of brain targets under research for different disorders, but none of them is considered as "established" for any specific condition. In this sense, special attention is now focused on avoiding to cross the ethical barriers overlooked in previous decades of psychosurgery. In fact, this interest in preventing the recurrence of "lessons learned from the past" led to the publication of several ethical guidelines for the performance of DBS in psychiatry, overlapping both in authorship and content, which were compiled and commented by Marwan Hariz in (Hariz, 2016).

In general, DBS is considered today as an invasive but adjustable and reversible brain stimulation technique, preferable to the previous ablative neurosurgery procedures, with great potential in the treatment of several various neuropsychiatric disorders, but still an experimental approach in the large majority of them. Thus, although its effectiveness has been clearly proved in various pathologies, the lack of understanding of its mechanism of action prevents DBS from achieving greater outreach in the clinical scenario. This brings us to the next section of this chapter, entitled *1.1.2. Mechanism of action*, in which

we will address the different theories proposed to explain the physiological and molecular effects observed after the application of DBS.

1.1.2. Mechanism of action

As stated above, the precise mechanism of action of DBS remains unknown. In this sense, DBS was first associated to an "ablative-like" effect in the targeted region. This first theory was based on the fact that similar improvements in motor symptoms (e.g. control of the muscular shaking) were obtained after intraoperative stimulation was applied for target confirmation in ablative stereotactic surgeries. Thus, the first theories about the potential mechanism of action of DBS stated that high frequency electrical stimulation would ameliorate motor symptoms, while low frequency DBS would promote them (Gildenberg, 2009).

Given that high frequency stimulation (over 100 Hz) is the most widespread modality in clinical treatment, the theories explained here refer to the effects induced by this high-frequency DBS, which also represents the frequency range used in the experimental chapters of the present thesis. Considering that the experiments included in this thesis show effects of neuromodulation on neuronal networks, both in local and distant regions to the electrode placement, general principles of the mechanism of action of DBS in the brain are gathered here (Mcintyre et al., 2004). Nevertheless, an excellent description of the different effects induced by cathode and anode stimulation applied in the different neuronal regions (i.e. soma, axon and dendrites) can be reviewed in (Matias et al., 2016). Also, the reviewed hypotheses are supported by experimental observations both in the clinical and preclinical scenarios (see (Blaha, 2016) for review). Of importance, they are not mutually exclusive in any case, meaning that the physiological effects observed after DBS may derived from a combination of the proposed mechanisms.

a) **Local inhibitory action:** This theory responds to the traditional perception of the DBS effects, which responds to the similarities between the ablative neurosurgery and the impact of high-frequency stimulation (A L Benabid et al., 1987). Thus, it stablishes that DBS would produce a temporary blockade of the voltage-dependent neuronal currents, preventing the required depolarization that leads to the firing of the action potential, and hence inducing an inhibition of the output signal from the stimulated area (Beurrier et al., 2001). Therefore, the activity in the downstream regions normally activated by the neuronal firing at the stimulated site would be cancelled.

b) **Synaptic depression:** In contrast to the local inhibitory theory, which defends an "ablative-like" effect of DBS due to a depolarization block, the synaptic depression theory explains the lack of neural transmission after DBS by means of neurotransmission affectation. Thus, it advocates that, after applying high-frequency electrical stimulation to a specific brain site, local neurons could not maintain the DBS-induced neural transmission at distal efferent terminals due to a rapid depletion of neurotransmitter vesicles stores in the afferent dendrites (L. Wang & Kaczmarek, 1998; Zucker & Regehr, 2002). Therefore, this phenomenon would lead to a synaptic blockade, which would inhibit the neuronal activity downstream of the stimulated terminal.

c) **Modulation of synaptic transmission:** Similar to the *synaptic depression* theory, this theoretical approach explains the effects of high-frequency DBS by means of the modulation of synaptic activity. However, instead of a depression in the availability of the neurotransmitter vesicles at the afferent terminals, DBS would activate the afferent axons that surround the electrode or are closely located (i.e. *en passage*), inducing the release of neurotransmitters in their respective dendrites (Chiken & Nambu, 2014, 2015). Thus, depending on the type of neural fibers stimulated, and hence the type of neurotransmitters released, the impact of the DBS on the neural activity would vary:

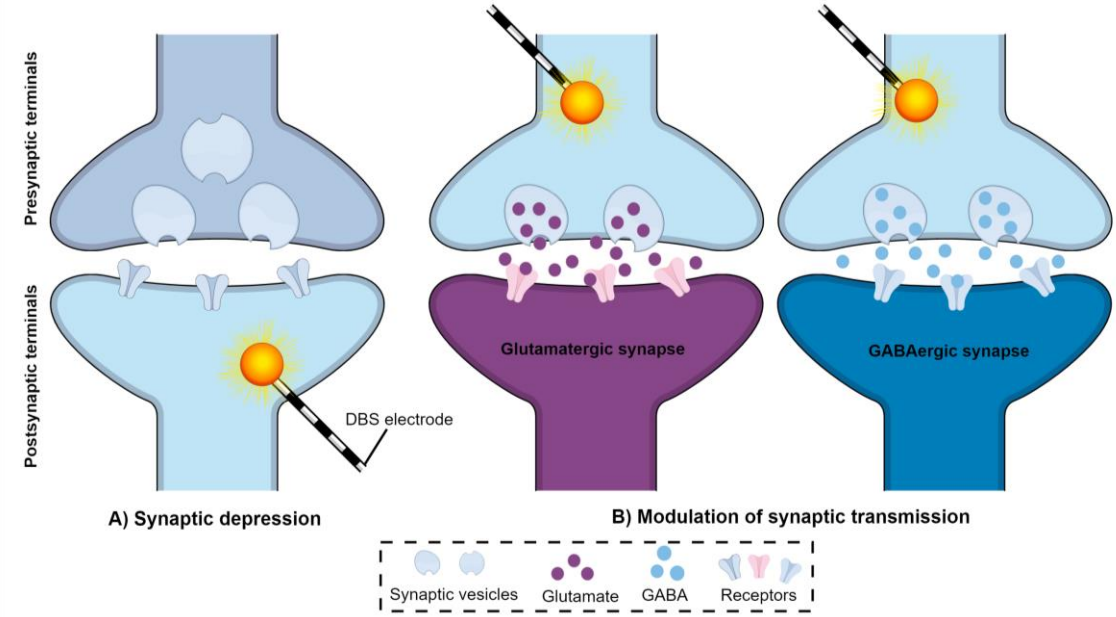


Figure 3 - Synaptic theories of DBS mechanism of action. A) Synaptic depression due to the unability of maintaining the local activity induced by DBS, inducing a rapid draining of the afferent synaptic vesicles. **B)** Modulation of synaptic transmission induced by DBS. Regarding the type of neurotransmitter released (excitatory or inhibitory), DBS would promote or decrease the neural efferent activity.

i. Whether the neurotransmitter released is inhibitory (e.g. GABA), the efferent neural activity would decrease.

ii. Whether the neurotransmitter released has an excitatory effect (e.g. glutamate), the derived neural activity would increase.

d) **Neural jamming:** Abnormal firing patterns of a certain brain target could lead to the onset of pathological symptoms. Therefore, this theory supports that high-frequency DBS would kidnap this aberrant neural transmission by replacing the pathological firing patterns with tonic high-frequency discharges induced by the stimulation in the surrounding neurons (McIntyre & Hahn, 2010; Montgomery & Gale, 2008). The new firing pattern generated would be normal. Therefore, it would help to recover a healthy firing activity. Another option would be that the induced pattern, although abnormal, would impact on the neural networks located downstream and, in this case, they would not be able to recognize it. Thus, the target structure would be isolated, creating an *informational lesion* (Carron et al., 2013; McIntyre & Anderson, 2016). Thus, the pathological transmission and, therefore, the derived symptoms would be blocked. Figure 4 shows a schematic representation of this hypothesis.

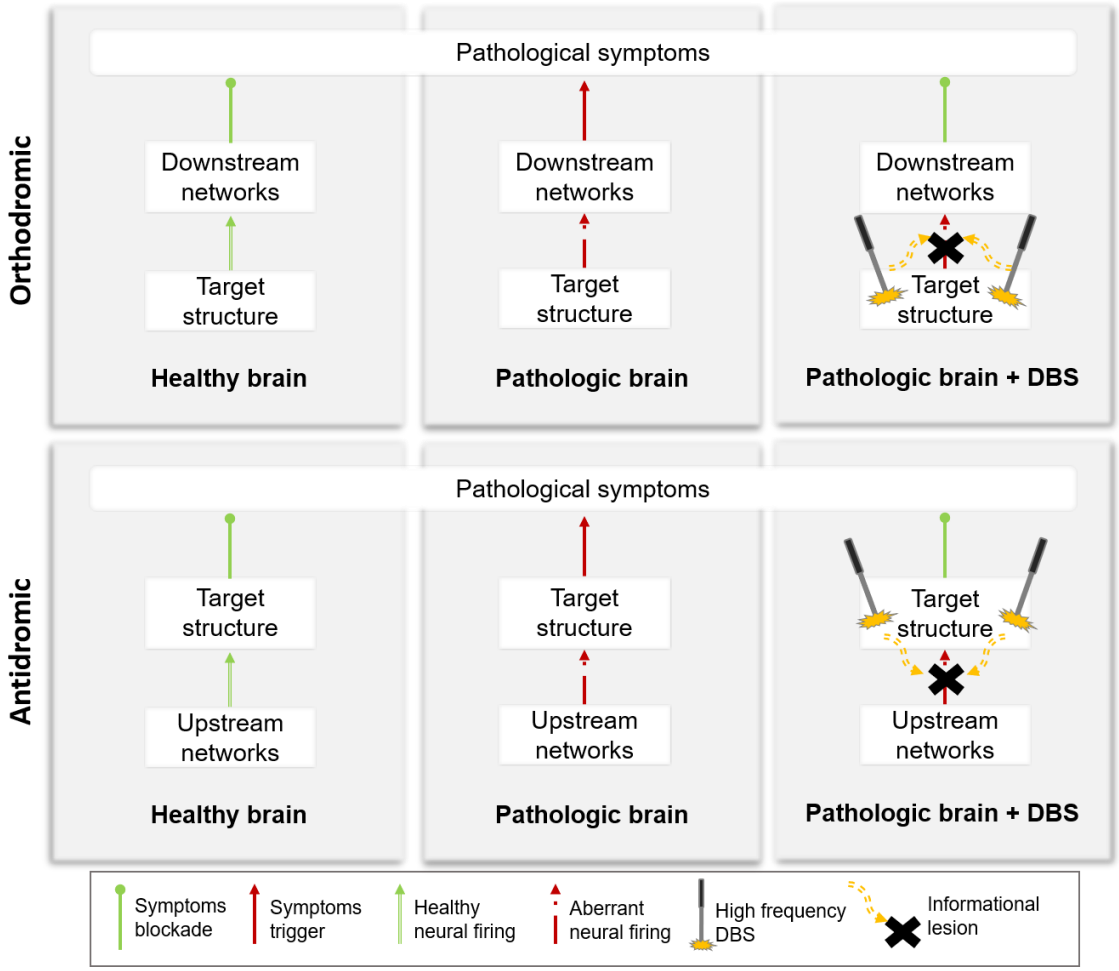


Figure 4 – Neural jamming hypohtesis. Schematic representation of the neural jamming hypothesis in the orthodromic (above) and the antidromic (below) directions..

Importantly, the consequences of DBS are not only evident in the downstream efferent connections from the target site. This means that, at a neuronal level, DBS modulation could occur in both directions, that is, from soma to dendrites (orthodromic) or from dendrites to soma (antidromic) (Carron et al., 2013; Hammond et al., 2008). Nonetheless, in both cases, the cancelation of the informational flow to and from the stimulated structure ultimately has similar consequences with respect to the physiological response (Fig. 4). On the one hand, in the orthodromic direction, firing de-coupling occurs from the stimulated soma to the axon, thus blocking its influence on downstream sites. On the other hand, antidromic currents collide with the orthodromic spikes originated upstream from the stimulation site. As a result, the stimulated nucleus remains isolated from the pathologic activity arising from the afferent connections.

Furthermore, since half of the brain cells are not neurons, other cellular components should also play a role in the complex functioning induced by DBS. Consequently, the participation of glial cells and, specifically, astrocytes in the neuromodulatory role of DBS has been demonstrated (Agnesi et al., 2013; Chang & Lee, 2017) and, although several authors have relied on these cells to explain the mechanism of DBS (Tawfik et al., 2010), its responsibility on the final therapeutic effect is not yet clear.

It becomes clear that the mechanism of action of DBS is explained by a combination of complex events induced by electrical stimulation, involving local and distant regions, and a host of different neurochemical, neuroplastic, oscillatory and synchronic processes of neural activity (Ashkan et al., 2017; Chiken & Nambu, 2015). Thus, the different mechanisms described may coincide in the stimulated brain, as they can be mutually explain each other, although not necessarily with the same predominance. In this sense, it seems unlikely that a unified theory explaining the mechanism of action of DBS will be soon proposed given the differential pathological circumstances to which this therapy is being applied. In fact, under the same DBS parameters and stimulation targets, the specific pathological circumstances will clearly interfere in the therapeutic results obtained. Moreover, current theories suggest that the conjunction of complex mechanisms begins with neuromodulatory processes that lead to subsequent neural plasticity modifications, and a final long-term neuronal reorganization, which would lead to anatomical restructuring (Agnesi et al., 2013; Herrington et al., 2016; Pérez-Caballero, 2018). Therefore, advances provided by anatomical and functional studies are now the best asset to uncover the major and minor physiological factors involved in the

therapeutic response of DBS, thus allowing to maximize the potential benefits that DBS provides in a more personalized way.

Then, the specific features of each disease condition the temporal onset of the different physiological effects of DBS, and therefore, of its therapeutic benefits (Agnesi et al., 2013; Ashkan et al., 2017; Herrington et al., 2016). Thus, while a reduction in tremor is evident seconds after turning on the stimulation in movement disorders, longer periods of stimulation (even months) are needed to obtain a therapeutic benefit in epilepsy (Ashkan et al., 2017). Nevertheless, these temporal frames can vary among patients, but are indicative of ongoing changes in brain networks due to DBS. Interestingly, in some patients an early therapeutic response has also been described in absence of stimulation (see (Pérez-Caballero, 2018) for review). In fact, the microlesion effect induced by the electrode insertion has been related with symptomatic improvement in several neurological diseases, such as epilepsy (Krishna et al., 2016) or chronic neuropathic pain (Shah et al., 2010), and even with a better therapeutic response to the stimulation in Parkinson's disease (Tykocki et al., 2013). Therefore, the insertion and presence of electrodes is a neural modulator factor itself, whose therapeutic effect has been suggested to depend on the inflammation derived from its implantation, as demonstrated in animal models of depression (Perez-Caballero et al., 2014).

1.1.3. Therapeutic application in neuropsychiatry

Since the beginning, the therapeutic potential of electrical stimulation has been recognized. In the particular case of DBS, the process was slowed down because it was originally conceived as a mere experimental approach. However, innovations with regard to the development of the stereotactic frames have allowed stereotactic ablative interventions to be largely refine. Thus, the use of electrical stimulation to confirm the target region led to early recognize its therapeutic potential, and therefore to promote its application to a wide variety of pathologies in the neurology and psychiatry fields (Hariz, 2016).

In this sense, despite the restricted number of pathological conditions for which the use of DBS has received FDA approval, its application to many other diseases is currently under research, specifically in the case of treatment-resistant patients. Thus, DBS was approved by the FDA for the treatment of patients with essential tremor in 1997, Parkinson's disease in 2002, dystonia in 2003, OCD in 2009 (under a humanitarian device exception, HDE) and epilepsy in 2018. In addition, the *European Medicines Agency*

(EMA) grants DBS with the CE mark (*Conformité Européenne*) for the treatment of essential tremor since 1993, parkinsonian tremor since 1995, advanced motor symptoms of Parkinson's disease since 1998, primary dystonia since 2003, OCD since 2009 and epilepsy since 2010.

This section presents a general overview of the most prominent pathologies for which the use of DBS has been therapeutically indicated, even at the research level. In addition, given the topic on which the last chapters of this thesis focus, a special section will be dedicated to the description of the use of DBS in obesity.

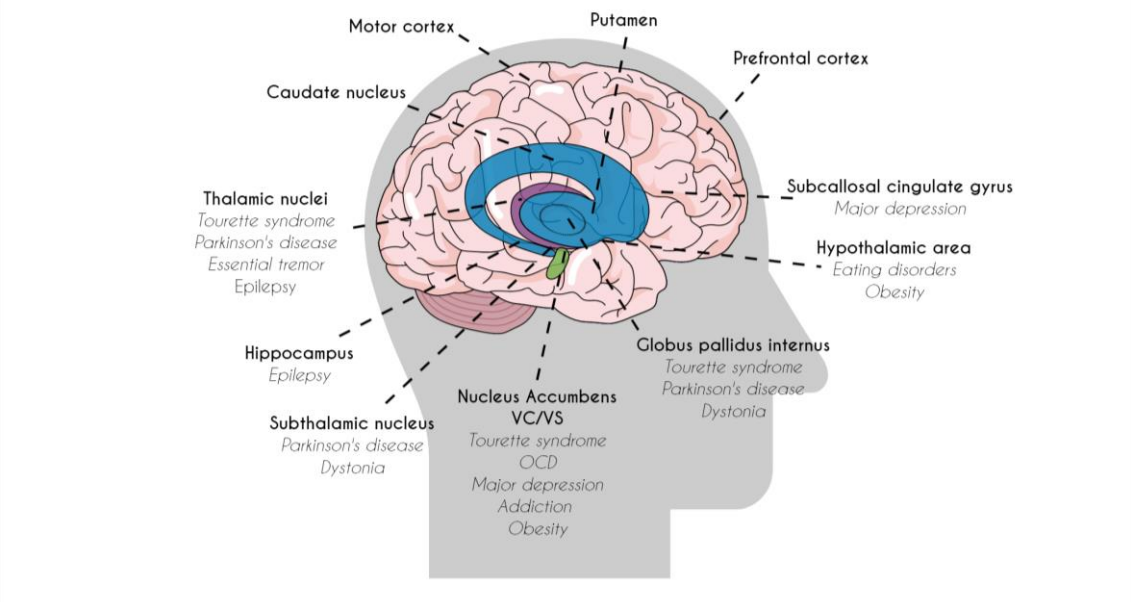


Figure 5 - DBS targets. Schematic representation of the brain structures considered as brain targets for DBS in neuropsychiatric disorders. Figure based on (Kuhn et al., 2009). *[OCD, obsessive compulsive disorder; VC/VS, ventral capsule / ventral striatum]*

1.1.3.1. Motor disorders

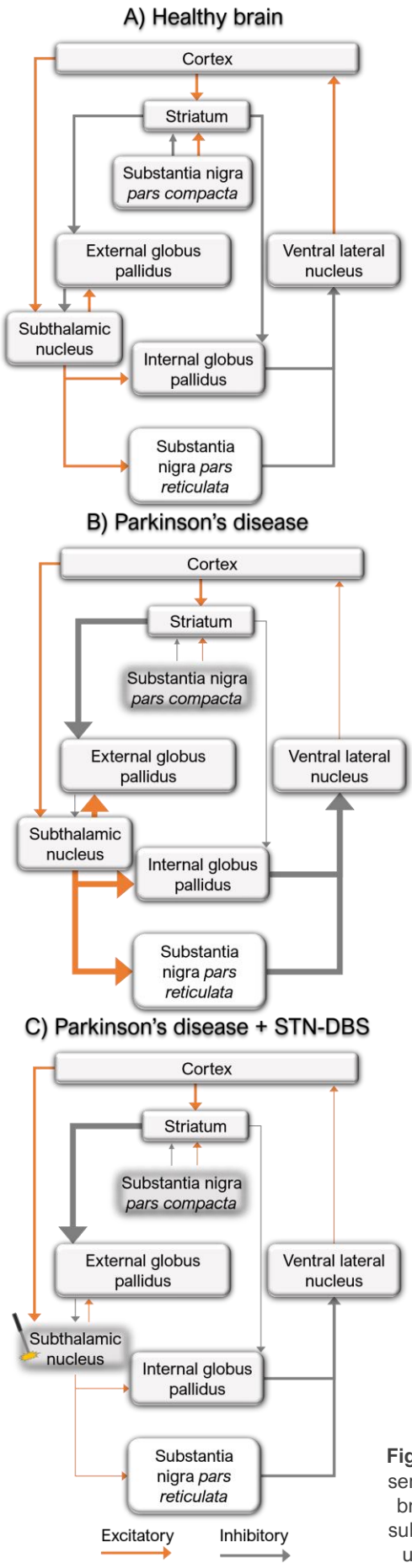
In contrast to psychiatric disorders, in which DBS is still investigational (despite FDA approval for OCD under a HDE) (Lipsman & Lozano, 2015), DBS is a well-established therapeutic option for treatment-resistant patients of several motor disorders. However, the development of effective pharmacological approaches, as in the case of levodopa for Parkinson's Disease in the early 1960s, led to a drastic decrease in the performance of neurosurgical interventions (Tasker et al., 1983). Nevertheless, 50% of parkinsonian tremors responded poorly to pharmacological treatment, ensuring the survival of stereotactic ablative interventions for these patients (Miyagi, 2015). As previously explained, the first approaches involving electrical stimulation of deep brain structures occurred in patients with movement disorders (Gildenberg, 2009), marking the beginning of a long history of DBS in this neurological field.

The involvement of the thalamic structures and basal ganglia in the pathophysiology of motor disorders, together with the reported benefits of their ablation, led to consider these brain structures as potential targets of stimulation (Agari & Date, 2015). Thus, despite specific differences between pathologies, all the tested and approved brain targets for DBS treatment belong to these circuitry. The specific characteristics of the use of DBS in the three motor pathologies officially approved (i.e. Parkinson's disease, dystonia and essential tremor) will be explained below.

Parkinson's disease:

Parkinson's disease is a neurodegenerative disorder which affects at least 1% of the population over 60 years old (Zafar & Yaddanapudi, 2020). The core feature of this pathology is bradykinesia (slowness), although the first manifested symptom to appear is usually tremor. In addition, rigidity, gait disturbances and postural instability appeared during the course of the pathology. This symptomatology is derived from the progressive degeneration of the dopaminergic fibers that innervate the striatum from the substantia nigra *pars compacta* (SNc), leading to a lack of inhibition on the basal ganglia activity (which involves the external and internal globus pallidus - GPe and GPi -, the subthalamic nucleus - STN - and the substantia nigra *pars reticulata* - SNr -), and therefore an exacerbated inhibition on the thalamocortical circuitry. Thus, the nuclei belonging to this network have been proposed as possible DBS targets given the previous efficacy of their ablation for the control of tremor. Among them, STN, GPi and the ventral intermediate nucleus of the thalamus (Vim) are the most important elements selected as DBS targets for Parkinson's disease due to their main role in the regulation of muscle tone and voluntary movement, as well as in the development of pathological involuntary movements (Pollak et al., 2002).

STN is the most frequently targeted brain structure for DBS treatment in Parkinson's disease. It has been proved to be safe and effective for the control of motor symptoms of the disease, as first reported by Benabid *et al.* (A. Benabid et al., 1994). In fact, STN-DBS is now an established therapy for the treatment of patients with advanced Parkinson's disease who manifest poor control of motor fluctuations and dyskinesia with drug therapy alone (Agari & Date, 2015). Thus, STN-DBS is normally initiated 10-15 years after disease onset, when significant motor symptoms become extremely difficult to control with drug therapy alone. Although it may seem paradoxical, a good response to levodopa is an important short-term predictor of the STN-DBS efficacy, as well as early age and short duration of the disease (Schuepbach et al., 2013), mild- or not cognitive



impairment and well-controlled or absence of psychiatric symptoms (Agari & Date, 2015). In fact, STN-DBS shows less effectivity on walking and postural disturbances (i.e. axial symptoms), which are indeed resistant to levodopa treatment (Agari & Date, 2015). Furthermore, STN-DBS makes it possible to reduce the dose of anti-parkinsonian drugs without affecting the therapeutic improvement (Tavella et al., 2002; Vingerhoets et al., 2002). Thus, sustained improvement of motor symptoms, rigidity, bradykinesia and daily quality of life has been reported in follow-up periods of 4-6 years since STN-DBS implantation (Moro et al., 2010; Østergaard & Sunde, 2006; Volkmann et al., 2009); and, although longer periods (8-10 years) also show good tremor control, the effect on axial symptoms begins to decline (Castrioto et al., 2011; Fasano et al., 2010).

However, STN-DBS treatment has been linked to certain mood, cognitive and mental dysfunctions which lead to the conception of other possible structures as DBS targets. In this sense, GPi-DBS has shown little difference with STN-DBS in improving motor symptoms, although it reported a significantly lower risk of inducing neuropsychological adverse events (Agari & Date, 2015; Follett et al., 2010; Moro et al., 2010). Furthermore, GPi-DBS is able to reduce levodopa-induced dyskinesia, although it fails to reduce the anti-parkinsonian drug dosage when applied.

Figure 6 - STN-DBS in Parkinson disease. Schematic representation of the basal ganglia circuitry functioning in a healthy brain **(A)**, a Parkinsonian brain, with the deterioration of the substantia nigra *pars compacta*, **(B)**, and a Parkinsonian brain under high-frequency DBS in the subthalamic nucleus **(C)**.

Finally, Vim-DBS appeared as the first target effective for treating intractable tremor in Parkinson's disease (A L Benabid et al., 1987). In fact, Vim-DBS replaced thalamotomy given the safety, reversibility and adaptability provided by this technique, both in the short- and long-term, despite its equivocal effects on other motor symptoms (which are effectively covered by STN- and GPi-DBS) (Miyagi, 2015). In fact, Vim-DBS is currently only suggested for those parkinsonian cases in which tremor is the only symptom, or at least the most predominant, and they show an unfavorable background (e.g. elderly patients, psychosis occurrence or cognitive decline) for targeting other stimulation regions (Miyagi, 2015).

Dystonia:

Dystonia ranks third in terms of prevalence among the neurological movement disorders. It is characterized by sustained muscle contractions which cause twisting, tremors and repetitive movements or abnormal postures (Pana & Saggu, 2019). Like the rest of the pathologies explained in this section, dystonia is caused by an abnormal functional organization of the thalamo-cortical-basal ganglia network, although its etiology is highly heterogeneous. In fact, dystonia can have a hereditary origin, although it can also be caused by physical trauma at birth or later in life, it can derived from an infection, or it may even respond to a reaction to pharmaceutical drugs, particularly neuroleptics (Taira, 2015). This broad pathological spectrum leads to a classification based on two main criteria: 1) etiology (primary or hereditary, and secondary or non-hereditary), and 2) other clinical features (age of onset, anatomical involvement - focal, generalized, segmental, multifocal, hemidystonia -, related pathological conditions) (Pana & Saggu, 2019).

In this sense, the prescription of DBS is mainly restricted to primary dystonias (Taira, 2015). Thus, pallidal-DBS has been a standard treatment for young-onset DYT-1-positive generalized dystonia since the past two decades (Moro et al., 2013; Pana & Saggu, 2019). However, several symptoms may respond worse or remain refractory to DBS, such as distal muscle control or speech difficulty, which prevent physicians from discontinuing routine drug therapy (e.g. botulin toxin) (Taira, 2015). The DBS gold-standard brain target for dystonia treatment is the sensorimotor area of the GPi, although GPi-DBS is sometimes associated with dysarthria. Furthermore, STN has also been proposed as an alternative DBS target by stimulating the Forel's H field (Kleiner-Fisman et al., 2007; Pahapill & O'Connell, 2010).

Remarkably, despite the mere palliative nature of DBS, there are some reports of a non-remission of the symptoms after depletion of the IPG battery in dystonic patients (Taira,

2015). Nonetheless, these are just anecdotal cases, and talking about complete cure or recovery from dystonia due to DBS at this time is far from real.

Essential tremor:

Essential tremor is the most common movement disorder. It affects the distal and proximal muscles of the trunk both in the postural and in the action-intention movement components (Morigaki & Goto, 2015). Thus, essential tremor is described as the presence of an involuntary rhythmic and oscillatory movement of a certain region of the body under a relatively constant frequency and amplitude. These involuntary movements are caused by the uncontrollable contraction of opposing muscles. Although its specific pathophysiology remains poorly understood, functional impairment of the olivocerebellar circuit has been proposed as the primary cause (Agarwal & Biagioni, 2020).

As for the other cases, the main medical treatment approach for essential tremor is pharmacotherapy. However, the rates of patients who remain resistant to treatment is dramatically high (25-50%) (Koller & Vetere-Overfield, 1989; Louis, 2001). In this sense, neurosurgical procedures, such as thalamotomies or DBS, belong to third-line therapies, have reported high effectiveness in the control of the upper limb tremor (Deuschl et al., 2011).

In relation to stimulated brain structures for the treatment of essential tremor, Vim has historically been the most explored (Miocinovic et al., 2013). However, despite the great enthusiasm that Vim-DBS initially elicited, this approach fails to overcome axial tremor, the action component of distal tremor and proximal tremor (Benabid et al., 1991; 1996), as well as it is related to high incidence of dysarthria, disequilibrium and tolerance (Morigaki & Goto, 2015; Schuurman et al., 2008; Xie et al., 2012). Given this unfavorable context, new DBS target options for essential tremor seem urgent. Thus, the posterior subthalamic area (PSA) has been recently proposed for this role, because it was previously considered a target region for ablation surgery (Lehman & Augustine, 2013), as well as STN (Lind et al., 2008). In this sense, although more evidence is needed, PSA-DBS seems to substantially improve tremor, particularly those components that remain uncontrolled by Vim-DBS, with mostly mild and transient side effects, and without inducing tolerance to treatment (Xie et al., 2012). Therefore, PSA emerges as a possible better brain target of DBS than Vim for the treatment of essential tremor.

1.1.3.2. Epilepsy

Epilepsy is a neurological disease that affects 50 million people worldwide. It is characterized by recurrent seizures and, despite available anti-epileptic drug therapy, 20% of patients suffered from a poor seizure control (Koch & Baltuch, 2015). Although resection surgery is an option for treatment-resistant patients, the presence of comorbidities or multifocal epilepsy, as well as the potential risk of permanent memory impairment, strongly limits its performance. In these cases, DBS is considered a potential alternative for refractory epilepsy.

Two are the brain structures proposed as DBS targets: the anterior nucleus of the thalamus (ANT) and the hippocampus (Fisher et al., 2010; Tellez-Zenteno et al., 2006). This targets selection responds to the current knowledge that the pathways that interconnect the hippocampus and subcortical limbic structures with the cortex are key in the propagation of seizures. These regions include the thalamus, amygdala, hippocampus and entorhinal cortex, and therefore, disruption of these networks by DBS, regardless of the structure in which the seizures originate, may frustrate the propagation of seizures (Koch & Baltuch, 2015). Thus, ANT-DBS was shown to induce a substantial reduction in seizure frequency after 3-6 years of stimulation (Fisher et al., 2010), suggesting that DBS may produce a chronic effect on the neural plasticity of the epileptogenic circuit that is needed to obtain the expected therapeutic effect (Ashkan et al., 2017). Furthermore, while ANT-DBS is conceived to disrupt this propagation, hippocampal-DBS aims to disrupt the epileptogenic focus itself (Tellez-Zenteno et al., 2006). However, other structures are being considered as DBS targets, such as the centromedial thalamic nucleus, the STN and the cerebellum (Klinger & Mittal, 2016).

1.1.3.3. Psychiatric disorders

Despite remaining on a research level, the potential of DBS in psychiatric conditions is gaining recognition. In fact, the high incidence of these disorders and the substantial proportion of treatment-resistant cases, explain the need to find alternative therapies, such as those provided by neurostimulation. In this sense, there is growing interest in DBS given its well-recognized safety and efficacy profiles, as well as the advances provided by anatomical and functional neuroimaging techniques in understanding the mechanisms underlying different psychiatric diseases (Lipsman & Lozano, 2015). Therefore, there is an emerging number of pathologies for which DBS is increasingly indicated. Among them, obsessive compulsive disorder (OCD) and major depressive disorder (MDD) stand out as the most common indications, although other pathologies

are gaining attention (D. J. Lee, Lozano, et al., 2019; Lozano et al., 2019), such as eating disorders (Dalton et al., 2017), addictions (Habelt et al., 2020), Tourette's syndrome (Xu et al., 2020) or bipolar disorder (Gippert et al., 2017). Unlike movement disorders, in which the response to DBS is almost simultaneous to the activation of the system, psychiatric pathologies usually require longer periods of stimulation to observe an evident improvement in the severity of symptoms.

Major depressive disorder:

MDD emerges as the third leading cause of the global burden of disease, with a lifetime prevalence of up to 16% in adult population (Kessler et al., 2003). It is a heterogeneous disease, whose symptomatology cannot be restricted to a depressed mood. Indeed, patients often suffer from a variable degree of apathy, lack of motivation and anhedonia, along with eating and sleep disturbances. Therefore, MDD should be conceived as a network disorder rather than a focal pathology, given the likely impairment of various systems involved in cognitive, reward and pleasure processing, and vegetative control; which denotes a main role of the limbic networks.

In this sense, the brain targets suggested for DBS are actually the main nuclei of these systems, which have been shown to be affected in MDD by functional imaging techniques (such as PET or fMRI). In fact, the subcallosal cingulate region (SCC) (Area 25) is metabolically overactive in depressed non-medicated patients and healthy subjects immersed in sadness (Mayberg et al., 1999). This hyperactivation normalizes after remission of the depression by pharmacological, psychotherapeutic or DBS treatment (Kennedy et al., 2001, 2007; Mayberg et al., 2005). Therefore, SCC has been functionally related to the regulation of negative emotions and depressive stages. Thus, it was hypothesized that SCC-DBS normalizes the aberrant activity throughout the depression circuit and, in consequence, it is the most explored DBS target in clinical and preclinical studies in MDD (Lipsman & Lozano, 2015). In fact, SCC-DBS showed a treatment response in up to 92% of patients, and a remission rate as high as the 58% of the included participants (Holtzheimer et al., 2012), after a 2-years follow-up period.

Another neural focus functionally altered in MDD is the reward and pleasure-related systems (Patel et al., 2012). Then, the high prevalence of anhedonia led to study the modulation of targets along the reward pathway, such as the nucleus accumbens (NAcc) (Bewernick et al., 2010, 2012; Schlaepfer et al., 2008a) and the ventral capsule/ventral striatum (VC/VS) (Dougherty et al., 2015). In this sense, the highest response rates were obtained during NAcc-DBS, compared to VC/VS-DBS (McInerney et al., 2015), in

treatment-resistant depression patients (up to 50% after 1 year) (Bewernick et al., 2012), suggesting that NAcc may be the preferred DBS target for the treatment of primarily anhedonic MDD (Lipsman & Lozano, 2015).

There are also other suggested targets for DBS in the treatment of MDD, such as the medial forebrain bundle (reward circuit), the lateral habenula or the inferior thalamic peduncle (wakefulness, attention and motorsensory behaviors). However, the number of clinical trials addressing their effectiveness in this disorder is considerably lower, hence requiring further studies in order to consider them as effective DBS targets for MDD (Mclnerney et al., 2015).

Obsessive compulsive disorder:

OCD is a chronic psychiatric pathology characterized by obtrusive, repetitive and anxiogenic thoughts (obsessions), along with intense anxiety and disproportionate ritualistic behaviors (compulsions). Its prevalence is up to 2-3% in general population, and almost 10% of patients remain unresponsive to pharmacological and behavioral therapy (Giffin et al., 2016; Lipsman & Lozano, 2015). In such a situation, alternative treatment options become a real urgency. Thus, neuroimaging studies have evidenced an abnormal functioning in the orbitofronto-striato-thalamo-cortical network, related to large increases in the orbitofrontal and ventral striatal connections. The clear identification of a specific network alteration makes OCD a perfect pathology to be managed by DBS in cases resistant to treatment. In fact, DBS was not only the first psychiatric condition in which OCD was investigated (B. Nuttin et al., 1999) but, as previously stated, OCD was the first psychiatric disorder for which official American and European health agencies considered approving DBS application.

The first target tested for OCD treatment was the anterior limb of the internal capsule (ALIC) (B. Nuttin et al., 1999). ALIC was recognized very early as a key structure in mood and anxiety disorders, and it was the standard structure for capsulotomy in the treatment of these conditions (Lipsman & Lozano, 2015). It consists of a portion of the internal capsule, containing fibers which connect the prefrontal cortex with subcortical structures, such as the thalamus. Nuttin *et al.* conducted the first ALIC-DBS trial in OCD. They reported beneficial effects in three of the four recruited patients during a 21-month follow-up period, and even a 90% reduction in compulsions in one of the patients (B. Nuttin et al., 1999).

These satisfactory results inspired other groups to increase the number of OCD patients treated by ALIC-DBS and, despite finding overall positive results regarding symptom severity, none of them were able to reproduce the initial high-rates shown by that first study (Giffin et al., 2016). In fact, due to the close location of ALIC to the VC/VS and NAcc, subsequent studies began to conceive these structures for targeting DBS in OCD. In fact, starting with Greenberg *et al.* studies (Greenberg et al., 2006), VC/VS is the DBS target with the most experience to date. That study shows evidence of a long-term efficacy of VC/VS-DBS in eight OCD patients followed for 3 years. They found a significant decrease in the severity of OCD symptoms in six of the eight candidates, as well as a reduction in anxiety and depressive behaviors (Greenberg et al., 2006). Several other groups followed the steps of Greenberg and colleagues to stimulate the VC/VS in patients with treatment-resistant OCD, and most of them found around a 50% of response rates after only one year of follow-up period. Besides, these response rates increased in longer studies (Senova et al., 2019). Therefore, despite some transient detrimental cognitive effects, VC/VS-DBS appears as a safe surgical approach for treatment-resistant OCD patients.

Furthermore, the motor component of OCD, with the repeated appearance of tics in many patients, reveals a clear involvement of motor circuitry in OCD. This fact led to consider the STN as a possible DBS target in this pathology, and this fact was confirmed by the reduction in OCD scores after STN-DBS in two patients with comorbid Parkinson's disease and OCD (Mallet et al., 2002). However, contrary to striatal targets, STN-DBS did not induce any significant effect on anxiety or mood symptoms. Interestingly, the unilateral combination of STN and NAcc DBS revealed more beneficial effects with respect to both affective and motor symptoms (Barcia et al., 2014).

Altogether, the assessed striatal, thalamic and STN structures addressed by DBS in this disorder reported similar efficacy profiles in reducing symptoms severity scores on the OCD scales (Senova et al., 2019). Although other structures have provided promising evidence as DBS targets, such as the inferior thalamic peduncle or the GPi, larger studies are needed to investigate their potential in OCD (Giffin et al., 2016; Lipsman & Lozano, 2015; Senova et al., 2019).

1.1.3.4. Obesity

The worldwide incidence of obesity has led many authors to coin it as a real epidemic in the 21st century. In fact, along with overweight, it affects a third of the population worldwide (Hruby & Hu, 2015). Obesity is defined by the *World Health Organization*

(WHO) as an abnormal or excessive fat accumulation which may impair health (D. J. Lee, Lozano, et al., 2019). It has a well-recognized multifactorial etiology, in which neuropsychiatric factors play a significant role. In fact, the mere metabolic conception of obesity leads health professionals to resort to dietary and physical routines in first place (Kushner, 2014), and only in severe, refractory cases to pharmacological (e.g. orlistat) and surgical interventions (e.g. bariatric surgery, gastric bypass) are indicated (Schernthaner & Morton, 2008). However, the serious adverse events elicit by these approaches, as well as the failure to control the disease in the long-term (Christou et al., 2006; Montan et al., 2019), evidence the need to explore alternative therapies for severe treatment-resistant patients. In this sense, given the neural basis of obesity, which involves the homeostatic and reward brain centers, DBS has been proposed as a potential therapeutic option for these patients (R. Franco et al., 2016; Halpern et al., 2008, 2011). To date, nine clinical trials have been registered in the U.S. National Library of Medicine evaluating the role of DBS in obesity (U.S. National Library of Medicine, 2020).

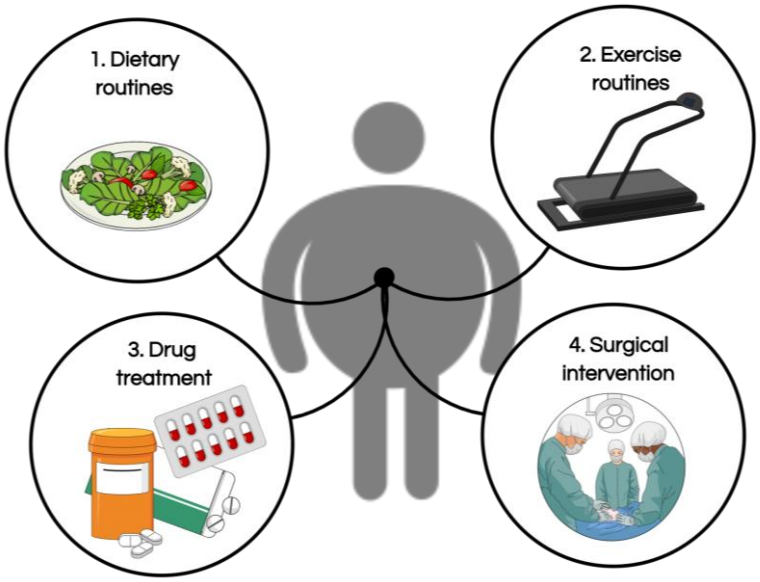


Figure 7 - Obesity treatment. Current therapeutic options for the treatment of obesity

The approach to obesity through DBS should be carried out evaluating the intervention at the level of homeostatic and hedonic nutrition. In this sense, the brain centers for satiety, feeding and reward have largely been the focus on obesity DBS studies, given the potential benefits that neuromodulation can provide in the control of food intake by targeting these regions (Formolo et al., 2019). Accordingly, the hypothalamic nuclei have received great attention due to their well-known association with eating behavior and metabolic control. Thus, the ventromedial hypothalamus (VMH) and the lateral hypothalamus (LH) host the brain centers of satiety and appetite, respectively. In addition, they are large enough to be targeted by DBS electrodes, which in turn makes them stand out from other thalamic nuclei as potential targets. (Formolo et al., 2019; Halpern et al., 2008, 2011; Hamani, Sankar, et al., 2012; Melega et al., 2012).

In this sense, the first clinical study performed to treat human obesity using DBS bilaterally targeted the VMH (Hamani et al., 2008), due to the positive results previously obtained in preclinical studies (Prinz & Stengel, 2018). Thus, high-frequency stimulation (130 Hz) did not provide significant effects on weight loss during the first 6 months of follow-up although, after adjusting the parameters to a lower frequency (50 Hz, among other settings), the patient experienced a sudden weight loss with no variations in diet and intake (Hamani et al., 2008). However, another study applying VMH-DBS (135 Hz) reported an unexpected panic attack, which forced to interrupt the treatment (Wilent et al., 2010). So far, one more clinical trial has been registered evaluating the role of VMH-DBS in morbid obesity, although results have not been reported yet (de Salles et al., 2018).

Extensive evidence has shown the potential of LH-DBS for obesity treatment. In this sense, although only two studies have evaluated the effect of high-frequency LH-DBS from the preclinical side (Sani et al., 2007; Soto-Montenegro et al., 2014), both reported weight-gain reductions in animal models of diet-induced and genetic obesity, respectively. In the field of human research, a trial evaluating LH-DBS recruited two women and one man with morbid obesity. Two of the patients showed a substantial weight reduction after 30-39 months of LH-DBS, and one of them reported a decrease in binge eating behavior (D. M. Whiting et al., 2013), thus providing the proof of concept for DBS application in obesity. Interestingly, Whiting *et al.* also found an increase in the resting metabolic rate, measured by respiratory chambers (A. C. Whiting et al., 2019). These authors have recently registered a new clinical trial with the objective of evaluating the safety and efficacy of chronic LH-DBS in six treatment-resistant adults with severe obesity (NCT04453020). This study is still in the recruiting phase, and they expect to complete the experimental phase by December, 2022 (D. M. Whiting, 2020). Other two clinical trials have evaluated weight outcomes in Parder Willi syndrome, a genetic condition characterized by low muscular tone, low levels of sex hormones and an overeating disorder that leads to hyperphagia and, ultimately, obesity. The first case report included a 19-year-old male patient who reported a feeling of fullness during active LH-DBS, although he continued to exhibit food craving behavior (Talakoub et al., 2017). In this case, although authors did not report changes in weight gain, they did find increases in the beta/low-gamma LH activity during exposure to food-related cues while feeling hungry, as well as prominent alpha rhythms during satiety. Their findings could help to better understand the functioning of LH, and therefore, guide neurostimulation therapies (Talakoub et al., 2017). The second clinical trial involved four obese patients

with Prader Willi syndrome, but LH-DBS reported no clinical benefit with respect to weight gain, neuropsychological function or hormone levels (R. R. Franco et al., 2018).

Besides the evident role of homeostatic nuclei in the control of food intake, the enhanced anticipation of the food rewarding and hedonic properties is an important mechanism leading to food consumption (Gearhardt et al., 2011). In fact, alterations in the neural networks involved in this phenomenon can lead to an excessive food intake, which may lead to food addiction and obesity (Joranby et al., 2005; Volkow et al., 2008, 2013; von Deneen & Liu, 2012; G.-J. Wang et al., 2004). Therefore, reward-related areas also suppose potential targets for obesity treatment with DBS. Among them, NAcc is an important brain core within this system. NAcc-DBS could potentially counteract the reward feeling related to food intake and, in theory, lead to weight reduction in treatment-refractory obesity (Halpern et al., 2008). Consistent with this hypothesis, three preclinical studies have observed significant reductions in weight and food intake in murine models of a high-fat diet and binge eating disorders (Doucette et al., 2015; Halpern et al., 2008; C. Zhang et al., 2015). Furthermore, four clinical trials have assessed the efficacy of NAcc-DBS and reported substantial weight reductions in those patients who completed the follow-up period. The first weight reductions observed after NAcc-DBS were really unexpected. In fact, Mantione *et al.* were using NAcc-DBS to treat an obese woman with OCD of (Mantione et al., 2010). During the stimulation phase, the patient reported a substantial decrease in the incidence of obsessions and compulsions. Surprisingly, after a 2-year follow-up, she reported having stopped smoking, as well as having stopped craving cigarettes, and she had lost 36 kg compared to her baseline weight at the beginning of the study (Mantione et al., 2010). The second clinical case report included a patient with hypothalamic obesity due to a craniopharyngioma surgery and, during 14 months of NAcc-DBS, she experienced a notable reduction in weight and body mass index (BMI), with no other apparent side effects (Harat et al., 2016). At the University of British Columbia, a similar clinical trial was initiated with the aim of applying DBS to six patients with hypothalamic obesity due to craniopharyngioma (Honey, 2018). However, this trial failed to recruit candidates who qualified for the study, and it was withdrawn already in the recruitment phase (Honey, 2018). In the third case report, NAcc-DBS was conceived for the treatment of the electroconvulsive-resistant depression in an obese patient (Tronnier et al., 2018). Thus, despite being a secondary outcome, the patient experienced a massive weight loss after 14-months of NAcc-DBS treatment accompanied by intense comforting feelings due to her self-efficacy in controlling food intake behavior (Tronnier et al., 2018). Finally, the latest clinical trial in NAcc-DBS for

morbid obesity was conducted at the Ohio State University (Rezai et al., 2018; Weichart et al., 2020). While all three patients lost weight during the study, only one of them successfully completed the 3-year trial. One patient abandoned the study at 13 months of NAcc-DBS, and the other one committed suicide after 27 months, although authors did not associate the event with NAcc-DBS (Rezai et al., 2018). The remaining participant was included in an additional research program exploring the cognitive task performance during the titration phase, revealing a potential mechanism to capture the acute effects of DBS and predict long-term outcomes in behavioral disorders (Weichart et al., 2020).

In view of the above, strong efforts are focused on reaching a therapeutic approach which helps to face the current epidemic situation of obesity through neurostimulation. Thus, there is a growing number of preclinical and human studies attempting to uncover the underlying mechanisms of the obesity cure. In this sense, although the indication of DBS is restricted to treatment-resistant patients, finding an effective strategy for severe cases would give hope to a currently unresolved situation. Consequently, the involvement of multidisciplinary researchers from around the world in this cause is undeniable, as evidenced by the continous registration of new clinical trials both testing new DBS approaches or devices (Beijing Pins Medical Co., 2016; Lozano, 2020; University Hospital, 2019).

1.2. Molecular imaging

The first radiographs obtained by Wilhelm Röntgen in 1895 marked the beginning of a new era in the medical imaging field. Thus, Dr. Röntgen produced electromagnetic radiation in the X-rays wavelengths for the first time, and interposed his hand between the electron beam tube and a photographic plate on which he clearly visualized his hand bones. Then, he repeated the process with his wife's hand, and obtained the famous picture of her left hand bones with the married ring (Fig. 8) (Scatliff & Morris, 2014). Since then, subsequent discoveries in the areas of the medical physics and engineering led to the development of a broad spectrum of imaging modalities with a great potential across all specialties of medicine and biomedical research.

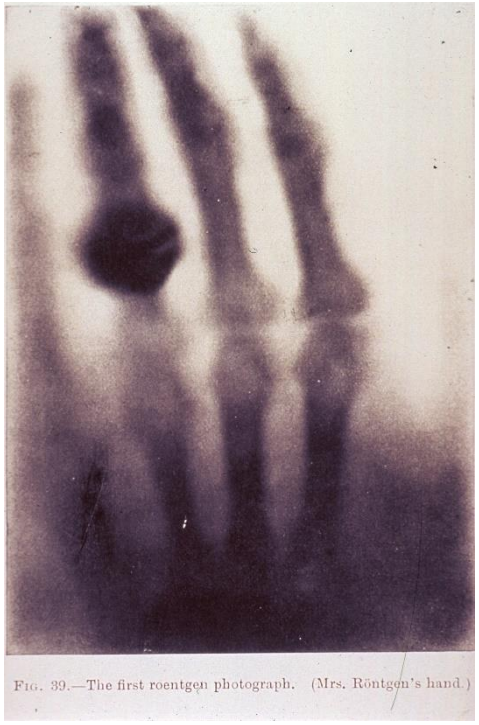


Figure 8 - **First radiography**, obtained by Wilhelm Röntgen of his wife's hand, Anna Bertha Ludwig, with her married ring (Public domain).

Medical imaging encompasses a variety of technologies used for visualizing the internal components of the body, both at a functional or structural level, in order to diagnose, monitor or treat different medical conditions (Food and Drug Association, 2018). Among them, molecular imaging refers to the group of techniques which allow to *in vivo* visualize cellular processes at the molecular level. Two key features of these techniques are that they are quantitative, in the sense that they provide measurements of the imaged phenomena in terms of numerical and quantifiable units; as well as non-invasive, since the acquisition procedures do not disturb the physiological systems of interest (Cussó, 2014).

According to the physical basis of the imaging, five groups of medical imaging modalities can be differentiated: nuclear, X-rays, magnetic resonance, optical and ultrasound imaging. In the next section, a deeper insight into each modality will be address, paying particular attention to the main relevant techniques for the development of this thesis (i.e. Optics and Nuclear Imaging).

1.2.1. Molecular imaging modalities

1.2.1.1. Nuclear imaging

Imaging modalities such as gamma-scan (two-dimensional images), single photon emission computerized tomography (SPECT) and positron emission tomography (PET) (three-dimensional techniques), are nuclear imaging modalities. This description responds to the use of radiotracers, molecules that include a radioactive isotope in their chemical structure, and serve as contrast agents to visualize the status or progression of a specific biological process. Radiotracers are usually biomolecules involved in the physiological process under study, labeled with a radionuclide that replaces one of their atoms. Radiotracers are injected into the subject in trace amounts, with the aim of minimizing the radioactivity side effects, and their distribution within the different tissues of interest is detected by radiation detectors placed on the scanner. Actually, these detectors detect γ-rays, which are produced during radioactivity decay. In this sense, the most remarkable advantages of nuclear imaging are: I) the almost limitless depth of penetration of nuclear imaging techniques due to the capability of the γ-rays to completely penetrate organic tissue; II) its great sensitivity, even reaching the nanomolar range of radiotracer concentration; and III) the wide variability of available radiotracers. Altogether, these techniques allow the study of a broad number of different biological processes *in situ* within organisms.

PET provides higher spatial and temporal resolution, providing the most practical features among nuclear imaging techniques for biomedical research and clinical practice. Given the relevance of PET imaging in the course of this thesis, a more detailed view of this technique will be develop in the next section.

1.2.1.2. X-rays imaging

Radiography (2D) and computerized tomography (CT) (3D) are among the most common imaging modalities performed in clinical practice, although the lesser known fluoroscopy (2D, in motion) also belongs to the X-rays imaging techniques. In these cases, the image is created according to the differential X-ray attenuation coefficient of the tissues, which is in direct relation to their densities. Thus, they provide anatomical images of great clinical value, with enhanced contrast at the bones level as it is the tissue with the greatest attenuation power. In the case of planar methods (2D), such as radiographic or fluoroscopic images, the final result can be understood as the projected “shadow” of the object interposed between the X-ray beam and the detector. Alternatively, tridimensional images (3D), such as CT, resolve this tissue superimposition by rotating the X-ray source

and detectors around the subject, and thus acquiring serial projections through the same object. Therefore, although it should not be strictly considered as a molecular imaging technique, CT provides high-resolution anatomical images, which have been of particular interest in hybrid systems (i.e. combined scanners of CT with other molecular functional techniques which normally provide lower spatial resolution, such as SPECT-CT or PET-CT) (Berg & Cherry, 2018).

However, X-ray imaging techniques are subjected to some limitations, such as the radiation side effects or the low contrast provided in soft tissues due to their low attenuation power (e.g. brain). In this sense, sophisticated radiopaque contrast agents have been developed in order to improve the visualization of the tissue of interest by CT.

1.2.1.3. Magnetic resonance imaging (MRI)

MRI is a non-ionizing molecular imaging technique, with great potential in the neuroimaging field due to the great variability of information it can provide (e.g. structure, function, diffusion, metabolism, etc.). It is based on the ability of specific atoms, usually included in organic tissues (such as hydrogen, phosphorus and carbon, among others), to absorb and emit electromagnetic energy at a very precise frequency (resonance). Briefly, the MRI scanner is a very powerful magnet inside which the subject is placed. Immediately, the strong magnetic field originated inside the scanner forces all the nuclei (usually protons) to align with the direction of the own magnetic field. Then, the application of a specific radiofrequency pulse, which coincides with the resonance frequency of the nucleus of interest, stimulates the nuclei and they spin out of equilibrium. When the radiofrequency energy is removed, the nuclei return to their equilibrium state (i.e. realign with the magnetic field) and the electromagnetic energy released in this process is detected by specific coils.

MRI provides an excellent spatial resolution due to its high soft-tissue contrast, which is obtained according to the differential speed at which the electromagnetic energy is released after turning off the radiofrequency pulse. Nevertheless, by adjusting the acquisition parameters, the operator can potentiate the signal emitted by a specific tissue (e.g. lipids, liquid, etc.), obtaining a higher contrast of it in the resulting image, even without the need of contrast agents (although some paramagnetic agents are available). Thus, MRI is a highly versatile technique with an excellent safety profile given that, up to date, no side effects that pose a health threat have been described. However, the long acquisition times, the huge size of the scanners and the elevated prices for the scanner

and image acquisition are major disadvantages of this technique, preventing MRI from being accessible to everyone.

1.2.1.4. Optical imaging

Optical imaging covers a variety of basic and highly sensitive techniques (reaching even picomolar concentration levels) based on the properties of the visible light in order to explore specific processes at the molecular level. Due to their low cost and extraordinary potential, they are the most widespread modalities in *in vitro* and *ex vivo* biomedical research. In fact, optical probes offer multiple opportunities as contrast agents depending on their nature. Thus, they can not only target receptors (as other contrast agents in previous modalities) but being activated once they have already reached the target tissue (as it is the case of activatable probes), or even being produced by the own cells in the form of bioluminescent enzymes of fluorescent proteins (Hoffman, 2005). From a very simplistic perspective, all optical imaging systems will need light sources to produce the signal of interest, filters to eliminate the background signal, and photon detectors to receive and interpret the originated signal (Schulz & Semmler, 2008). However, the continuous development of new acquisition systems and optimized technologies encourages the design of more sophisticated configurations, including different elements such as mirrors and lenses, in order to improve the image quality in terms of resolution and signal-to-noise ratio.

Two phenomena should be considered when talking about optical imaging: fluorescence and bioluminescence. On the one hand, fluorescence represents the physical process which occurs when an excited molecule emits a photon transiting to a relaxation state. These molecules are called fluorophores, and their excitation state is reached by the absorption of a short wavelength photon with higher energy than the difference between their excited and ground states. As a consequence, excited fluorophores will emit a longer wavelength photon when they return to their basal condition, which can be detected by fluorescence sensors (Schulz & Semmler, 2008). Fluorophores are used to label molecules, such as antibodies, which will be used as molecular probes to track the molecular target of interest. The prepared fluorescent probe is then exposed to the tissue sample or cell culture, where it will recognize and bind to the corresponding target. Subsequently, and after several washes to eliminate the free probe remnants, and hence the background signal, the sample is illuminated with a sufficiently energetic light source in order to excite the binded fluorophores, and thus detect the light signal that will be proportional to the amount of probe binded to the sample. Imaging techniques such as

fluorescence and confocal microscopy (planar), and selective plane illumination microscopy (SPIM) (tridimensional) benefit from this phenomenon for image acquisition. On the other hand, bioluminescence occurs when a bioluminescent enzyme (e.g. luciferase) metabolizes its respective substrate (e.g. luciferin) and, as a consequence, photons are emitted from the sample. Thus, although there is not background signal (neither from autofluorescence, nor from filter leakage), it is necessary to provide enough substrate to all the bioluminescent cells in order to ensure a correct evaluation of the desired process (Schulz & Semmler, 2008).

Optical imaging acquisitions are mainly limited by the low depth of penetration of the light in biological tissues, which are highly scattering and absorbing media (Cussó, 2014). In fact, the deeper the signal origin is within the tissue, the weaker is the detected signal. In addition, the large size, the relative instability and the certain degree of cytotoxicity of the fluorescent molecules are clear inconveniences to extend their application to *in vivo* organisms. Nevertheless, some techniques have been implemented in order to make the jump to the complex living world, and there are both planar and tridimensional (i.e. fluorescence molecular tomography, FMT) systems able to obtain *in vivo* small animal optical images.

1.2.1.5. Ultrasound imaging (US)

As it name implies, the physical phenomenon underlying the US technique is the sound. Specifically, high-frequency sound waves (higher than 1 MHz) are projected by a transducer directly to the subject. As some sound waves pass through the subject, some are reflected (echoes) and hit the transducer, which records them as electric signals that can be translated into images. In this sense, the time it takes for the echo to reach the transducer and the magnitude of the detected signal provide information about the depth of the tissue interface and the amount of reflection at this level (Philips et al., 2012). US is a planar structural technique which, like CT, cannot be purely considered a molecular imaging modality. However, the development of several targeted molecular imaging probes designed for US, such as microbubbles or liposomes, allows specific molecular processes to be tracked with this imaging technique (Jaffer & Weissleder, 2005).

Given the fact that US is a cheap, safe and non-ionizing imaging modality, its application has become greatly widespread in many different medical specialties. In fact, despite the difficulties involved in interpreting US images, advances in US technology have made this technology not only restricted to radiologist, being indeed of crucial relevance in the monitoring of pregnancy (Philips et al., 2012).

1.2.2. Positron emission tomography (PET)

PET is a molecular imaging technique that belongs to the nuclear imaging modalities, which imply the use of radioactivity in the process of image acquisition. Therefore, PET is a non-invasive, ionizing imaging technique which allows the *in vivo* visualization of biological and biochemical processes within the organisms. The wide variety of physiological phenomena susceptible to be studied with PET covers from glucose or dopaminergic metabolism to inflammation and perfusion. In fact, researchers and clinicians can potentially explore any physiological process within the imaging subject as long as they have the appropriate specific radiotracer. A deeper insight into the main features and applications of the PET technique is described below, as well as an overview of the most relevant radiotracers used in neuroimaging.

1.2.2.1. General features

Nuclear imaging techniques are based on the detection of γ-rays emitted from radioactive isotopes within the body of the subject to be scanned. These radionuclides reach the tissues via radiotracers injected into the subject. Radiotracers are hence biomolecules labeled with a radioactive isotope which, in the case of PET imaging, emits positrons during the decay process. Regarding the physiological role of the biomolecule that constitutes the radiotracer, different specific cellular processes can be addressed by PET imaging, such as metabolic routes or perfusion. Therefore, the greater the intensity of the process under study within a tissue, the greater the uptake of the radiotracer in that specific tissue.

In this sense, after injecting the radiotracer into the subject, the radiotracer is distributed throughout the body. As a consequence of the radioisotope decay, a positron is emitted and will collide with an electron located in the nearby area. This phenomenon is called annihilation, and it results in the emission of two 511 keV γ-photons in opposite directions. These photons are registered by the scanner's detector ring, which is positioned around the subject. The simultaneous detection of both photons by opposing detectors is called a coincidence event, and the number of coincidence events registered by a pair of detectors provides information of the amount of radiotracer uptaken by a specific tissue. The coincidence events information are transformed in electrical signals by photomultipliers, which will be used for the reconstruction of the image with the information of the distribution of the radiotracer within the body.

One of the main disadvantages of PET is the need of a cyclotron *in situ* or close to the imaging facility in order to obtain the PET radionuclides. In this sense, despite the initial enthusiasm raised by the fact that several PET radioisotopes are constituents of the organic matter, such as ^{11}C (20.39-minutes half life), ^{15}O (2.04-minutes half life), ^{13}N (9.97-minutes half life) or ^{18}F (109.77-minutes half life), their short half-life makes it neccesary to be close to a cyclotron for its application. This is essential for radiotracers with a low half-life as they provide limited temporal resolution given the fast decay of the emitted signal (Lu & Yuan, 2015). Therefore, PET radiotracers are usually best suited to address fast biological processes (Stoll et al., 2001). However, despite the time-limited signal, this rather rapid radiation decay provides PET radiotracers relatively favorable profiles according to the radiation absorbed by the patient. This supposes a clear benefit in safety terms in comparison with longer half-life SPECT radiotracers (Wahl et al., 2011).

1.2.2.2. Applications in neuroimaging: [^{18}F]-FDG-PET

PET is a highly versatile technique, since it allows the study of a wide spectrum of different physiological processes. Thus, any biological process could potentially be studied as long as there is an appropriate radiotracer available (Hooker & Carson, 2019). This feature allows its application to a wide variety of medical specialties, covering from oncology to neurology, cardiology and infectious diseases. Thus, the main contributions of PET imaging have been classically related to the oncology field given the great capacity of metabolic tracers, such as 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG), to detect and monitor the progression and treatment response of local or metastatic tumors. In this sense, FDG is the most widely available radiotracer in clinical and preclinical studies due to the relatively long half-life of [^{18}F]-fluorine (approximately 110 minutes), which provides enough time to transport the tracer to the imaging facility which do not have an *in situ* cyclotron. This fact makes FDG the most common tracer in oncology, cardiology and neuroimaging.

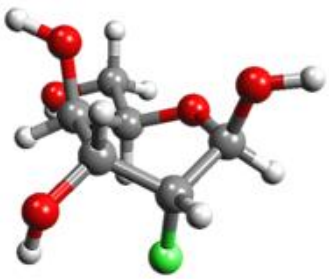


Figure 9 – [^{18}F]FDG. Atomic structure of [^{18}F]FDG (gray: carbon, red: oxygen, white: hydrogen, green: [^{18}F])

FDG is a glucose analogue which incorporates a [^{18}F]-fluorine at the C-2 position of the ring of glucose, instead of the hydroxy group (Fig. 9). Therefore, FDG participates in the glycolysis pathway (Fig. 10), i.e. the physiological process intended to obtain high-energy molecules (adenosine triphosphate, ATP) by catabolizing glucose. When FDG is injected into the subject, it travels through the bloodstream and it is internalized by cells through the membrane glucose transporter

GLUT. Once in the cytoplasm, FDG begins the glycolysis process and it is phosphorylated by a hexokinase, becoming FDG-6-P. In this step, a normal glucose-6-P would be processed by an isomerase and transformed in fructose-6-P in order to progress in the glycolysis pathway. However, the presence of the [^{18}F]-fluorine at the C-2 position impedes this transition, and FDG remains in form of FDG-6-P inside the cell. These process is known as *metabolic trapping*. Thus FDG-PET image allows to detect *in vivo* metabolic active tissues non-invasively. Once the radioactivity of the [^{18}F]-fluorine decays, it becomes a [^{16}O]-oxygen and this molecule is susceptible to be catabolized and continue the glycolysis pathway to obtain ATP and pyruvate.

The brain is a highly metabolic organ, which implies the detection of a large signal from

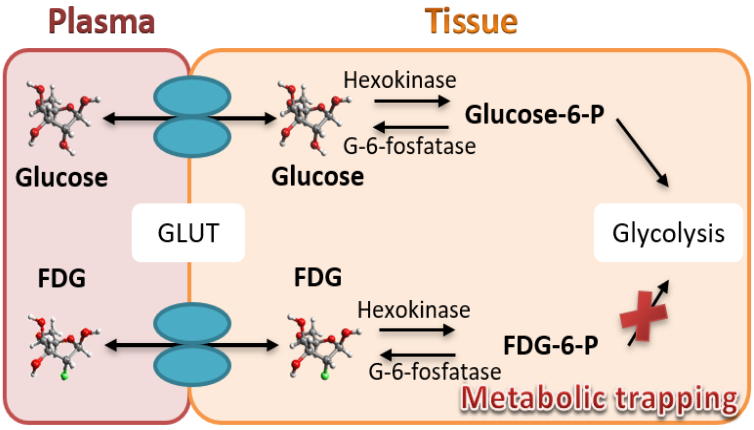


Figure 10 - **Metabolic trapping**. Mechanism of FDG uptake inside the cell.

this corporal area during FDG-PET studies since FDG can easily cross the brain-blood barrier. This is, in fact, a limiting feature for several radiotracers for neuroPET studies, since the brain-blood barrier supposes a very restrictive filter through which only small molecules can reach neural tissue. However, glycolysis in the brain occurs primarily in astrocytes (Magistretti & Allaman, 2015), and these glial cells are indeed the ones that mainly support the metabolic needs of neurons. In fact, the elements involved in the glycolysis cascade are particularly drained in neurons, while they are promoted in astrocytes. In addition, astrocytes have specialized processes that cover the surface of intraparenchymal capillaries, as well as other placed near the neuropils. Thus, when astrocytes detect high synaptic activity, they increase glucose consumption and, as result, they release lactate to the parenchyma. Under aerobic conditions, active neurons appear to prefer lactate as an energy fuel, which is converted into pyruvate in the cytoplasm and entered into the tricarboxylic acid cycle within the mitochondria in order to obtain energy. This process is called *metabolic coupling* (Magistretti, 2006) and

supports the basis for understanding the quantitative measurements provided by FDG-PET in brain studies as an indirect approach to quantify the neural activity.

This is particularly interesting in neuromodulation trials, such as DBS studies, in which the activation of neural networks by focal stimulation of a specific nucleus is a key mechanism to unravel the therapeutic effects induced by this treatment. However, this is not the only strategy to evaluate neural activity using PET. Thus, another technique to indirectly addressed neural activity is through perfusion PET studies with [^{15}O]-water. This approach is a direct measure of the cerebral blood flow given the hemodynamic changes induced by neural activity (i.e. increased blood flow). However, the reduce half-life of [^{15}O] is a main disadvantage for selecting this radiotracer instead of FDG or fMRI technique, which also studies the brain hemodynamics as patterns of neural activity (J. J. Chen et al., 2008).

Given that FDG is internalized by active metabolic cells, neural activity and tumor metabolism are not the only processes that can be indirectly quantified by FDG-PET. In fact, both inflammation and infections exhibit high metabolic profiles (Vaidyanathan et al., 2015). Therefore, areas affected by these pathological phenomena will also appear hyperintense in FDG-PET images, thus allowing to monitor their progression or response to a treatment. Furthermore, FDG-PET is also a great approach to evaluate cell viability within a tissue. Thus, dead or necrotic tissues, as those that die after a stroke, appear hypointense in FDG-PET images. Therefore, although these events are normally assessed by perfusion radiotracers (such as [^{13}N]-ammonia or [^{15}O]-water), particularly in cardiac events, FDG is a valuable alternative to avoid the reduced half-life limitation.

Finally, although FDG is the most common PET radiotracer in neuroimaging, other radiolabeled molecules are also used to explore specific physiological phenomena within the central nervous system. In fact, studies of neurotransmitters with, for instance, the dopamine receptors-binding radiotracers [^{11}C]-raclopride or [^{18}F]-fallypride (Sander & Hesse, 2017), the evaluation of the brain opioid system with the ligands [^{11}C]-carfentanil and [^{11}C]-diprenorphine (Sprenger et al., 2005), or the neuroinflammation assessment by TSPO-binding radiotracers (such as [^{11}C]-(R)PK11195) and new generation molecules (Jain et al., 2020), are just few examples of the great variability of specific functional processes susceptible to be studied by PET neuroimaging.

1.3. [^{18}F]-FDG-PET in Deep Brain Stimulation

DBS procedures strongly benefit from neuroimaging techniques (Gonzalez-Escamilla et al., 2020). Beginning from the surgery planification, both structural (CT, MRI) and functional (PET, fMRI) modalities are required to determine the more suitable stimulation target for each pathology, together with its precise location within the patient's brain. Furthermore, imaging techniques also play a major role in study designs aimed to uncover *in vivo* the mechanism of action underlying to DBS outcomes through pre- and post-surgery imaging. Finally, of particular clinical relevance are the imaging trials aiming to determine neural activation patterns that allow to predict the potential treatment response to the DBS treatment (Rodman & Dougherty, 2016). Several clinical studies have addressed the effects of DBS in different neuropsychiatric disorders and brain targets using PET imaging with various radiotracers (Ballanger et al., 2009; Ko et al., 2013; Rodman & Dougherty, 2016). In this sense, PET provides a particularly suitable technique to scan patients with neurotherapeutic implants since they are not susceptible to disruptions in electrical or magnetic fields, which may impair both the quality of the image and the functioning of the neuromodulation systems (Rodman & Dougherty, 2016). Here, an overview of clinical and preclinical studies applying FDG-PET to evaluate the effects of DBS on several brain conditions and targets is presented.

1.3.1. Clinical studies

The first clinical trials evaluating the neural networks activated by DBS with FDG-PET belong to the neurology field. Specifically, Fukuda *et al.* were pioneers in describing the effects induced by GPi-DBS on resting-state brain glucose metabolism in seven Parkinson's disease patients (Fukuda et al., 2001). They showed that GPi-DBS induced significant metabolic reductions in the pattern associated with Parkinson's disease (pallidal, thalamic and brainstem hypermetabolism), which correlated with clinical improvement. Later, similar results were obtained in this network after STN-DBS (Asanuma et al., 2006; Cao et al., 2017; Lyoo et al., 2007; Nagaoka et al., 2007; Trošt et al., 2006; J. Wang et al., 2010; Zhao et al., 2004), as well as a partially restored metabolism in limbic and associative regions of the basal ganglia (Hilker et al., 2004; le Jeune et al., 2009; le Jeune, Péron, et al., 2010). Furthermore, this metabolic modulation in the associative and limbic regions has been related to increases in weight gain observed in Parkinson's disease patients after four months of STN-DBS (Sauleau et al., 2014). Also, a common modulation of this Parkinson's disease-related network has been

seen with dopaminergic therapy, suggesting that effective symptomatic therapies in this disorder are based on common mechanisms (Asanuma et al., 2006).

Regarding neurological disorders, FDG-PET studies are mainly limited to Parkinson's disease with few recent exceptions, as in dystonia-deafness syndrome and in minimally conscious and vegetative state (Eskandar et al., 2018; Skogseid et al., 2018). Nevertheless, DBS has been evaluated in a variety of several psychiatric disorders by FDG-PET. In this sense, the first FDG-PET study performed in psychiatric patients aimed to evaluate the long-term effects of VC/VS-DBS in OCD patients (B. J. Nuttin et al., 2003). They included a functional imaging evaluation by FDG-PET and fMRI performed after three months of stimulation, and found a decreased metabolism in the frontal cortex. These results were later replicated by Le Jeune *et al.* after STN-DBS in ten OCD patients, suggesting that the therapeutic effect of STN-DBS would be related to an induced hypometabolism in the prefrontal cortex (le Jeune, Vrin, et al., 2010). Furthermore, other authors have addressed the modulatory effect on brain metabolism of VC/VS-DBS in OCD. Thus, Suetens *et al.* concluded that VC/VS-DBS induced similar metabolic changes in the cortico-striato-pallido-thalamo-cortical circuit to capsulotomy, although more pronounced and extended in the ablation cases, with a prominent metabolic decrease at the cingulate level (Suetens et al., 2014). Comparable improvements in clinical symptoms were obtained in both neurosurgical approaches (VC/VS-DBS and capsulotomy). Besides, a recent study in three patients showed local activation volumes at the stimulation sites (i.e. bilateral VC/VS) with DBS in ON condition, although different regional metabolic patterns were obtained across patients (Baldermann et al., 2019). Furthermore, metabolic patterns induced by DBS applied in other brain targets, such as inferior thalamic peduncle (D. J. Lee, Dallapiazza, et al., 2019), NAcc and ALIC (Park et al., 2019) have also been reported in OCD patients, resulting in reductions in FDG uptake in the striatum and cingulate, and limbic and frontal cortical regions, respectively.

Schlaepfer *et al.* carried out the first FDG-PET study evaluating the chronic effects of one week of NAcc-DBS in treatment-resistant depression, showing significant metabolic modulation in fronto-striatal networks (Schlaepfer et al., 2008b). Later, Millet *et al.* also tested NAcc-DBS neuromodulatory effects on depression and evidenced a regionalized effect on glucose metabolism, with decreases in the NAcc, cerebellum and posterior cingulate gyrus, and increases in the anterior cingulate gyrus (limbic lobe), among other structures (Millet et al., 2014). This group also attempted to stimulate the caudate nucleus when NAcc-DBS did not elicit the expected therapeutic response, but no clinical benefit

was obtained (Millet et al., 2014). Furthermore, few studies have evaluated SCC-DBS consequences on brain metabolism in treatment-resistant patients with depression. Thus, a reduction in SCC after 6 months of DBS correlated with improvement in symptoms (Brown et al., 2020). Remarkably, SCC metabolism was higher in responders than non-responders before starting DBS treatment. This finding is particularly interesting given that it is the first evidence of an imaging-based biomarker that predicts SCC-DBS response with 80% accuracy, based on machine learning analyses (Brown et al., 2020). Also, 48 hours of SCC-DBS discontinuation have been shown to induce metabolic reductions in areas involved in depression pathophysiology (i.e. cingulate, premotor region and putamen), which seem to precede clinical manifestations (Martín-Blanco et al., 2015).

In addition to OCD and treatment-resistant depression, FDG-PET studies have shown partial reversal of metabolic deficits in anorexia nervosa after NAcc-DBS (H.-W. Zhang et al., 2013) and SCC-DBS (Lipsman et al., 2017). Additionally, the second study demonstrated significant clinical improvements in depression, anxiety and weight after one year of SCC-DBS (Lipsman et al., 2017). No clinical outcomes were reported in (H.-W. Zhang et al., 2013). Scarce reports applying FDG-PET imaging have been published in other pathologies, such as two case reports of NAcc-DBS in a patient with autism spectrum disorder (Park et al., 2017), and VC/VS-DBS in a patient with explosive aggressive behavior (Giordano et al., 2017); as well as two clinical trials testing the effect of NAcc-DBS in schizophrenia (Roldán et al., 2020), and a combination of simultaneous NAcc- and ALIC-DBS in heroin addiction (L. Chen et al., 2019). In all cases, symptomatic improvement was observed, together with metabolic changes in the regions related with the pathology under study.

1.3.2. Preclinical studies

In comparison to clinical studies, the number of preclinical studies evaluating the neuromodulatory effects of DBS on brain metabolism by FDG-PET is particularly reduced. In this sense, only eight articles (excluding those included in this thesis) using this imaging approach have been identified, and they all date back to this last decade.

The first of them highlighted the innovation that this approach supposes in the preclinical field (Klein et al., 2011). Thus, Klein *et al.* unilaterally implanted DBS electrodes in the STN in six healthy rats, and performed two FDG-PET acquisitions (i.e. seven and nine days after surgery) (Klein et al., 2011). They explored the effects of acute high-frequency

DBS by applying the stimulation during the FDG uptake period of the second PET study. They found that STN-DBS increases the metabolism in the brainstem, cingulate cortex, mediodorsal thalamus, GP and caudate nucleus; as well as it induces a hypometabolism in the amygdala, entorhinal, somatosensory cortex, hippocampus and prelimbic cortex. However, this metabolic pattern was partially contradicted by *post mortem* c-Fos immunostaining although, as they explained, both techniques may complement each other in deciphering alterations in neuronal activity (Klein et al., 2011). In fact, as it is literally stated in their manuscript "*Whereas, FDG-uptake has been proposed to reflect metabolic activityat presynaptic terminals, c-Fos induction has been suggested to indicate metabolic activity within the post-synaptic cell body*" (Klein et al., 2011). Apetz *et al.* also studied the acute effects of high-frequency STN-DBS with FDG-PET but, this time, in a rat model of Parkinson's disease (Apetz et al., 2019). This model consists of performing a unilateral 6-hydroxydopamine (6-OHDA) injection into the medial forebrain bundle, inducing dopamine depletion. After applying similar DBS and imaging protocols to those decribed in (Klein et al., 2011), they reported a reversion of the pathological metabolic pattern described in this model by inducing a hypermetabolic effect in the ipsilesional ventrolateral striatum, together with a reduced metabolism in the contralesional hippocampus, thalamus and brainstem (Apetz et al., 2019).

Two animal studies have evaluated the DBS effects on memory-related regions. On the one hand, Van Den Berge *et al.* stimulated the right hippocampus in seven adult healthy rats by means of high-frequency DBS (van den Berge et al., 2015). Three PET scans of each animal were acquired: before surgery, after surgery and after one hour (30 minutes during FDG uptake) of hippocampal DBS. They studied both the stimulation and the electrode implantation effects alone and in combination, and observed significant metabolic reductions in both hippocampi and in other limbic structures. Of interest, the electrode implantation alone elicited a local hypometabolism at the brain target area (i.e. right hippocampus), suggesting that DBS might modulate the same regions as the electrode, but inducing a larger and more intense effect (van den Berge et al., 2015). On the other hand, Shin *et al.* selected the fornix as DBS target in fifteen healthy adult rats (Shin et al., 2019). They acquired two FDG-PET scans of each animal, with "on" and "off" stimulation conditions, respectively. High-frequency fornix-DBS was applied during the 30 minutes of FDG uptake, and generalized metabolic increases were observed in the medial subcortical structures which belong to the limbic circuit, as well as in the NAcc. They also reported significant reductions in different cortical regions and the cerebellum (Shin et al., 2019).

Furthermore, acute effects of medial prefrontal cortex (mPFC) and NAcc high-frequency stimulation have been tested on brain glucose metabolism in a maternal immune stimulation (MIS) rat model of schizophrenia (Bikovsky et al., 2016). This is a neurodevelopmental model which consists on the induction of an inflammatory response in the fetal brain by inoculating an immunogenic substance (e.g. Poly I:C acid) to the pregnant dam. As a result, there is a greater risk for the offspring to develop sensorimotor-gating and attentional-selectivity behavioral deficits, as well as altered metabolic, volumetric and oxidative stress patterns in the brain, at adulthood (Casquero-Veiga et al., 2019; Ravit Hadar et al., 2015). In this study, Bikovsky *et al.* performed the DBS surgeries at adulthood and, as in previous cases, the stimulation was applied during the FDG uptake period in order to evaluate which brain target was most appropriate in schizophrenia (Bikovsky et al., 2016). Thus, although NAcc- and mPFC-DBS similarly improved the behavioral deficits in the MIS model, substantial differences were shown at the brain metabolic level. In fact, while mPFC-DBS modulated the glucose metabolism in several limbic and cognitive regions, no remarkable changes were observed after NAcc-DBS in the MIS model (Bikovsky et al., 2016). The modulatory and behavioral results observed after mPFC-DBS led the authors to go one step further in this research work. Thus, they evaluated the preventive potential of mPFC-DBS applied during adolescence in the MIS model (R Hadar et al., 2017). To this end, they applied continuous stimulation (24 hours) of high-frequency during fifteen days at adolescence, and carried out the behavioral and imaging analyses at adulthood. Thus, Hadar *et al.* only reported minor effects of mPFC-DBS applied to MIS animals. As an explanation to these scarce results, authors suggest that the modulation inferred on brain metabolism during adolescence may be transient and, although effective in preventing the behavioral deficits of the MIS model, a FDG-PET study conducted two months after completion of stimulation would not be able to capture the induced changes (R Hadar et al., 2017).

As in humans, neuropathic pain has also been addressed using DBS in preclinical models. In this sense, one study evaluated the effects of motor cortex stimulation in a rat model of neuropathic pain (Kim et al., 2016). A partial counteraction of the neuropathic pain metabolic pattern was observed by stimulating the motor cortex (i.e. FDG uptake increases in cerebellum, right thalamus and right striatum), along with effective reduction of the neuropathic pain in this model (Kim et al., 2016). Finally, Soto-Montenegro *et al.* proved in 2014 that intermittent high-frequency LH-DBS (one hour) applied during fifteen consecutive days in a genetic model of resistant obesity was able to reduce the weight gain in the long-term (Soto-Montenegro et al., 2014). Remarkably, they also reported a

metabolic modulation in brain regions impaired in obesity, such as the brain areas involved in food intake control, brain reward system (cortico-striato-thalamic pathway) and memory functions (hippocampus), one day after finishing the LH-DBS treatment. Of importance, this study set the basis to the later development of the experimental works which conform the chapters 5, 6 and 7 of the present thesis.

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2. Rationale, Hypothesis and Objectives

2.1. Rationale

Deep brain stimulation (DBS) is a very powerful neurostimulation therapy for the palliative treatment of, mainly, advanced cases of motor disorders (Kuhn et al., 2009). The fruitful results obtained in the neurological scenario led to explore the possibility of spreading its application to different psychiatric pathologies (Hamani, Temel, et al., 2012; Kuhn et al., 2009; D. J. Lee, Lozano, et al., 2019). In fact, DBS received approval from the American *Food and Drug Association* (FDA) for the treatment of chronic, severe, treatment-resistant obsessive compulsive disorder (OCD) in 2008, under a Humanitarian Device Exemption (Blaha, 2016). However, this recent wave of thought is not lacking of controversy for two main reasons: first, the invasive nature of this technique; and second, the use of electrical currents for regulating the impaired brain activity. Given these characteristics, polemics were served due to the barbaric memories of previous experiences in mental asylums with electroconvulsive therapy (ECT) and lesioning psychosurgery back in the 50's (Chang & Lee, 2017). Thus, although both ECT and lesioning neurosurgery are still being applied today in the clinical scenario (Lozano et al., 2019), their techniques have been greatly refined, and their application is subjected to very strict ethical protocols and is restricted to exceptional clinical circumstances. In this sense, DBS emerges as a potential alternative to these interventions given that, although it is indeed an invasive technique, it is actually a focal therapy, with potential reversibility of the effects induced by the electric stimulation, and providing the possibility of *in vivo* adjusting the stimulation parameters according to the patient needs (Gonzalez-Escamilla et al., 2020). Furthermore, the safety of DBS application and the surgical intervention for DBS system implantation, although not free of possible surgical complications (e.g. cerebral bleeding, stroke, infections, etc.), has been widely reported in several neuropsychiatric pathologies. For these reasons, given the advances in the knowledge of the functioning of the brain and the neural networks involved in the physiopathology of neuropsychiatric disorders, a growing number of scientific works propose to extend the DBS application to a wide range of neurological-based pathologies (Klein et al., 2011).

In this sense, althought there are very precise and successful works reporting the usefulness of DBS, the specific mechanism of action of this therapy remains unknown. Several theories have tried to explain the effects obtained under specific pathological and stimulation circumstances (Magistretti, 2006). However, the reality is that the wide variety of DBS parameters applied, the different brain targets and mental pathologies evaluated, make extremely difficult to draw definitive conclusions. In fact, it is now

believed that several neuromodulation mechanisms may be simultaneously taking place in the stimulated region, leading to widespread effects in areas connected to the DBS target. Also, astrocytes and microglia cells have been proposed to have a role in the effects induced by electrical stimulation (Underwood, 2017). Therefore, despite the great efforts placed in deciphering the specific molecular mechanisms induced by DBS, there is not a definitive consensus, but the number of patients operated with DBS continues growing (Schnarr et al., 2015). Thus, electrodes are always implanted in brain regions known to have a substantial involvement in the patients' pathology, but the DBS parameters are generally stablished by a trial and error process. Consequently, understanding the *in vivo* effects of DBS on brain dynamics is an ongoing issue, which will make possible to adapt the stimulation setttings and protocols to the actual needs of the patient, and hence obtain greater success rate.

Given this background, the possibility of observing the *in vivo* neuromodulation effects induced by DBS supposes a powerful strategy to determine the impact of the stimulation during its application. These type of examinations would help to explain the nature of the desired and undesired side effects, prevent related clinical improvement, and ultimately adapt the stimulation parameters to the patients' needs (Fins, 2009). To this end, *in vivo* molecular imaging techniques are helpful tools for the acute and long-term follow-up of DBS patients. In particular, functional modalities, such as positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), provide real-time information about the activation status of the different brain regions, both in the resting state and in response to a specific task or stimulus (Desmoulin-Canselier & Moutaud, 2019). Specifically, PET imaging studies of the brain using 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) represent an indirect but accurate methodology to evaluate the neuronal activation based on the physiological principle of metabolic coupling, that occurs between neurons and glia cells in the brain (Desmoulin-Canselier & Moutaud, 2019). Shortly, the greater the neuronal synaptic activity is, the greater its metabolic needs, and therefore, the greater the [^{18}F]FDG uptake is by the surrounding astrocytes to supply the energetic needs of the neurons. In this sense, several clinical studies have proved the possibilities of [^{18}F]FDG-PET technique in evaluating brain activation due to DBS application (Klein et al., 2011; Shin et al., 2019).

Nevertheless, these clinical studies are mainly focused on patients and, for that reason, their research potential is limited due to several drawbacks. First, heterogeneity is unavoidable given the differences in pathological stages, medication and social

conditions. In addition, due to the invasive nature of DBS, its application is restricted to cases of advanced disease that are resistant to previous pharmacologic interventions. Therefore, the recruitment of patients who meet the criteria for inclusion is usually an arduous work, and hence these studies are mostly performed in a small number of patients. Additionally, there is an ethical dilemma about what to do with the implanted DBS device once the clinical trial has finished, given that large medical expenses would entail both to remove it or to maintain it (Desmoulin-Canselier & Moutaud, 2019) . This fact strongly limits the possibility of reaching definitive conclusions regarding the mechanism of action and the feasibility of DBS. Besides, PET is an ionizing imaging technique, as it requires the administration of a radiotracer, namely a biomolecule labeled with a positron-releasing radioactive isotope, to the patient to be scanned. This radiotracer is administered in a trace dose reducing the radiobiological effects (Desmoulin-Canselier & Moutaud, 2019), but the fact of using radioactivity limits the application of PET in clinical research. Finally, ethical criteria regarding human assays are always a matter of concern, particularly with invasive neurosurgical interventions (Desmoulin-Canselier & Moutaud, 2019), and *ex vivo* studies on brain tissue are normally not an option due to obvious reasons. Then, the opportunities to explore in depth the molecular mechanisms underlying the therapeutic effects of DBS are clearly relegated to indirect observations in clinical studies. In this context, animal models of human conditions provide an invaluable tool so as to test different biomedical approaches prior to their clinical translation or, if they are already applied in clinical practice, to confirm and explain both their beneficial results and side effects. Thus, although long distances between the human pathology and the mimicked condition in animal models usually exist, particularly in the psychiatric field (Desmoulin-Canselier & Moutaud, 2019), they suppose a necessary approach to first evaluate the different medical therapies nowadays, in order to make the subsequent leap to clinical practice in a safe and effective manner.

2.2. Hypothesis

In general terms, this thesis aims to help to understand the DBS consequences on brain activity. Specifically, based on the aforementioned context, the main motivation of this work is to contribute to the current knowledge about the *in vivo* effects of DBS by means of translatable and feasible techniques, routinely applied in the clinical scenario. Therefore, our hypothesis is that different DBS protocols produce specific modulations of brain activity according to the pathological condition, the treatment duration, the stimulation parameters and the brain target selected; which can be observed by using

real-time functional medical imaging techniques. Consequently, these brain effects determine the therapeutic outcomes and therefore, the knowledge generated from this work provides a suitable strategy to *in vivo* examine the stimulation effects and adapt the stimulation parameters to the patients' needs.

Based on this general consideration, we suggest three more specific hypotheses to be dealt with in the different chapters of this thesis:

Hypothesis 1. The mere insertion of the electrode into the brain tissue, targeting the structure of interest, would play a fundamental role in the mechanism of action and the DBS effects as a whole.

Hypothesis 2. The application of an acute DBS protocol in brain structures involved in the physiopathology of different psychiatric pathologies would induce the modulation of the activity of complex neural networks, which could be relevant when evaluating its potential use in pathological conditions.

Hypothesis 3. DBS applied in the lateral hypothalamus (LH) or nucleus accumbens (NAcc) should induce a weight reduction, a metabolic modulation of the brain regions related to food intake control and reward, an improvement in memory and learning processes, and neuroplasticity changes in the hippocampus.

2.3. Objectives

The main objective of this thesis is to evaluate the effects of deep brain stimulation (DBS), by means of *in vivo* functional neuroimaging techniques, in healthy rats and in two models of murine obesity. In particular, the main contribution of this thesis is to describe the neuromodulation consequences of DBS on brain metabolism using [^{18}F]FDG-PET 1st) after the electrodes insertion, 2nd) during the electrical stimulation, 3rd) after a chronic and intermittent DBS protocol, and 4th) after a chronic and continuous DBS protocol.

Therefore, the following specific objectives were established:

Objective 1. To evaluate the electrode insertional effect on brain metabolism, isolated from the modulation induced by an acute protocol of electrical stimulation, in healthy rats with electrodes implanted in the medial prefrontal cortex.

Objective 2. To identify the neural networks modulated by an acute protocol of DBS in three different brain targets (medial prefrontal cortex, medio dorsal thalamus and nucleus accumbens), related to functional alterations in different neuropsychiatric pathologies, in healthy rats.

Objective 3. To evaluate the effect of DBS in two animal models of obesity.

3.1. To evaluate the short-term effects of chronic and intermittent DBS applied in nucleus accumbens on brain metabolism, body weight gain and food intake in a genetic animal model of obesity.

3.2. To study the long-term effects of a chronic and intermittent DBS protocol applied in two different brain targets (LH and NAcc) on brain metabolism and neuroplasticity in a genetic animal model of obesity.

3.3. To evaluate the effects of chronic and continuous DBS in the lateral hypothalamus on brain metabolism, body weight gain, memory and learning, and neuroplasticity in two animal models of obesity (a genetic and a diet-induced models).

Each of the five specified objectives represents an article of the current thesis.

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3. Understanding Deep Brain Stimulation: *In Vivo* Metabolic Consequences of the Electrode Insertional Effect

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Research Article

Understanding Deep Brain Stimulation: *In Vivo* Metabolic Consequences of the Electrode Insertional Effect

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Deep brain stimulation (DBS) is a neurosurgery technique widely used in movement disorders, although its mechanism of action remains unclear. In fact, apart from the stimulation itself, the mechanical insertion of the electrode may play a crucial role. Here we aimed to distinguish between the insertional and the DBS effects on brain glucose metabolism. To this end, electrodes were implanted targeting the medial prefrontal cortex in five adult male Wistar rats. Positron Emission Tomography (PET) studies were performed before surgery (D0) and seven (D7) and nine days (D9) after that. DBS was applied during the ^{18}FDG uptake of the D9 study. PET data were analysed with statistical parametric mapping. We found an electrode insertional effect in cortical areas, while DBS resulted in a more widespread metabolic pattern. The consequences of simultaneous electrode and DBS factors revealed a combination of both effects. Therefore, the insertion metabolic effects differed from the stimulation ones, which should be considered when assessing DBS protocols.

1. Introduction

In recent years, brain stimulation techniques have emerged in bioscientific and clinical scenarios. Deep brain stimulation (DBS) is a technique that modulates neuronal discharge patterns through electrical current both locally, at the electrode implantation site, and also in remote brain areas associated with the deep brain target [1, 2]. The success and safety offered by DBS in movement disorders [3] have led to consider its potential application in other neurological and mental pathologies, such as psychiatric disorders [4–6], with the subsequent search for new DBS targets. However, the mechanism of action of DBS remains unclear and depends on two confounded factors: the electrode insertion *per se* and the electrical stimulation. Indeed, certain symptomatology improvement has been related to the mere insertion of the electrodes in the treatment of epilepsy [7] and chronic neuropathic pain in humans [8]. Also, antidepressant-like effects have been found in rats in which electrodes were implanted, but without applying electrical stimulation [9]. To our knowledge, these are the only studies that have shown this insertional effect, but none of them has studied the subsequent brain regional activity modulation. Thus, the aim of this study is to assess the insertional effect of the electrode, isolated from the acute electrical stimulation itself, on brain glucose metabolism studied by positron emission tomography (PET) and statistical parametric mapping (SPM) techniques in rats with electrodes placed in the medial prefrontal cortex (mPFC).

2. Materials and Methods

2.1. Animals. Adult male Wistar rats (~ 350 g) (N = 5) were housed in a temperature- and humidity-controlled vivarium,

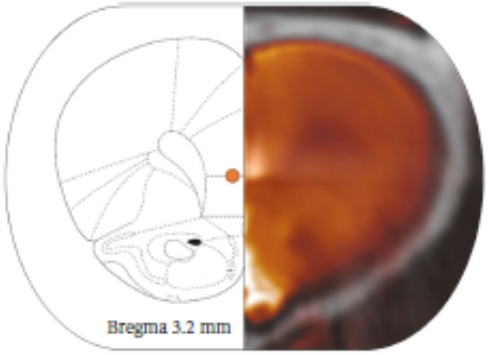

FIGURE 1: Electrode placement. Axial view of a CT scan registered to the MR template of an animal (right) and its correspondent slice in the Paxinos & Watson atlas [10] (left), to verify the correct electrode location in the mPFC. The bright and orange points represent the electrode tip in the CT and atlas images, respectively.

on a 12h light-dark cycle, with standard laboratory rat chow and water ad libitum. Animals were deprived of food 6-8 hours prior to the PET study. All experimental animal procedures were conducted according to the European Communities Council Directive 2010/63/EU and approved by the Ethics Committee for Animal Experimentation of the Hospital Gregorio Marañón.

This study was performed following the guidelines established by the principles of the 3Rs to minimize the number of animals included in this work [11]. Nevertheless, considering the longitudinal design of this research, the number of animals was sufficient to obtain enough statistically significant differences between time points.

2.2. Stereotaxic Surgery and DBS Protocol. Animals were anesthetized with ketamine/xylazine (100/10 mg/kg). Concentric bipolar platinum-iridium electrodes (Plastics One, Roanoke, USA) were bilaterally implanted targeting the mPFC (+3.5mm posterior, +0.6mm lateral from Bregma, -3.4mm ventral from Dura) [10]. Electrodes were fixed to the skull surface with dental acrylic cement (Technovit®, Germany). Antibiotic (ceftriaxone, 100mg/kg) and analgesic (buprenorphine, 0.1mg/kg) drugs were administered for 3 days as postoperative care.

DBS was applied during the radiotracer uptake period (45 min) with an isolated stimulator (CS 120 8i, CIBERTEC S.A., Spain) in a constant current mode at 130 Hz, 150 μA and a pulse width of 100 μs.

2.3. Imaging Studies. PET and computerized tomography (CT) scans were acquired just before surgery (D0, baseline) and 7 days (D7, without stimulation) and 9 days (D9, with stimulation) after that, in order to provide enough time for surgical recovery [12]. Additional CT scans were acquired at the end of the surgery to verify the correct placement of the electrodes (Figure 1). In addition, one magnetic resonance imaging (MRI) scan of a single nonoperated animal was acquired to be used as an anatomical template.

Animals were scanned using a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Madrid), under anaesthesia with isoflurane (3% induction, 1.5% maintenance in 100% O2). 2-Deoxy-2-[^{18}F]fluoro-D-glucose (FDG, ~ 37Mbq) was intravenously injected and, after 45 min of uptake, animals were scanned for 40 min. Images were reconstructed using a 2D-OSEM algorithm, with a spatial resolution of 1.45 mm Full Width Half Maximum (FWHM), a voxel size of 0.3875 x 0.3875 x 0.775 mm^3, and an energy window of 400-700 keV. Decay and dead-time corrections were applied.

CT studies were acquired with the same scanner, using the following parameters: 340 mA, 40 kV, 360 projections, 8 shots, and pixel size of 200 μm. Images were reconstructed using an FDK algorithm (isotropic voxel size of 0.124 mm) [13].

The MRI study was acquired with a 7-Tesla Biospec 70/20 scanner (Bruker, Ettlingen, Germany). A T2-weighted spin-echo sequence was acquired with TE=33 ms, TR=3732 ms, and a slice thickness of 0.8 mm (34 slices). The matrix size was 256 × 256 pixels with a FOV of 3.5 × 3.5 cm2.

2.4. Analysis of PET Data. PET images postprocessing and voxel value normalization were performed following the protocols previously described by our group [14, 15]. Briefly, PET images were spatially coregistered to a random reference CT scan (CTref) and smoothed with an isotropic Gaussian kernel of 2 mm FWHM. A brain mask segmented in the MRI, also registered to the CTref was applied to all PETs to exclude voxels outside the brain. Voxel values were normalized to average intensity of a brain region without statistically significant differences between groups [15].

The statistical analysis consisted on a voxel-wise analysis of PET data using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/) by means of paired T-tests, setting a significance threshold of $p<0.005$ uncorrected (voxel-level significance), but cluster-based corrected in order to avoid type II errors [16]. Only clusters higher than 50 adjacent voxels were considered aiming at reducing type I error.

We performed three different comparisons to evaluate the modulatory effect of the electrode insertion (D0 versus D7, study I), the stimulation (D7 versus D9, study II), and the combination of both the insertion and the stimulation (D0 versus D9, study III), on brain metabolism.

In this sense, we assume that the metabolic differences we show in the study II are almost completely due to the acute effect of the high-frequency electrical stimulation. Although the microlesional effect related to the electrode insertion is highly variable between subjects [8], the stimulation effect has been shown to be much stronger that the insertional one [17], and this latter tends to reduce over time. Furthermore, both the insertion (D7) and the stimulation (D9) PET acquisitions were separated by just two days, period in which no new relevant consequences derived from the electrode presence are expected. In this context, although a late effect of the insertion could have appeared, its influence on the study II would be minimum and possibly masked by the impact of a stronger stimulus represented by the application of high-frequency electrical stimulation.

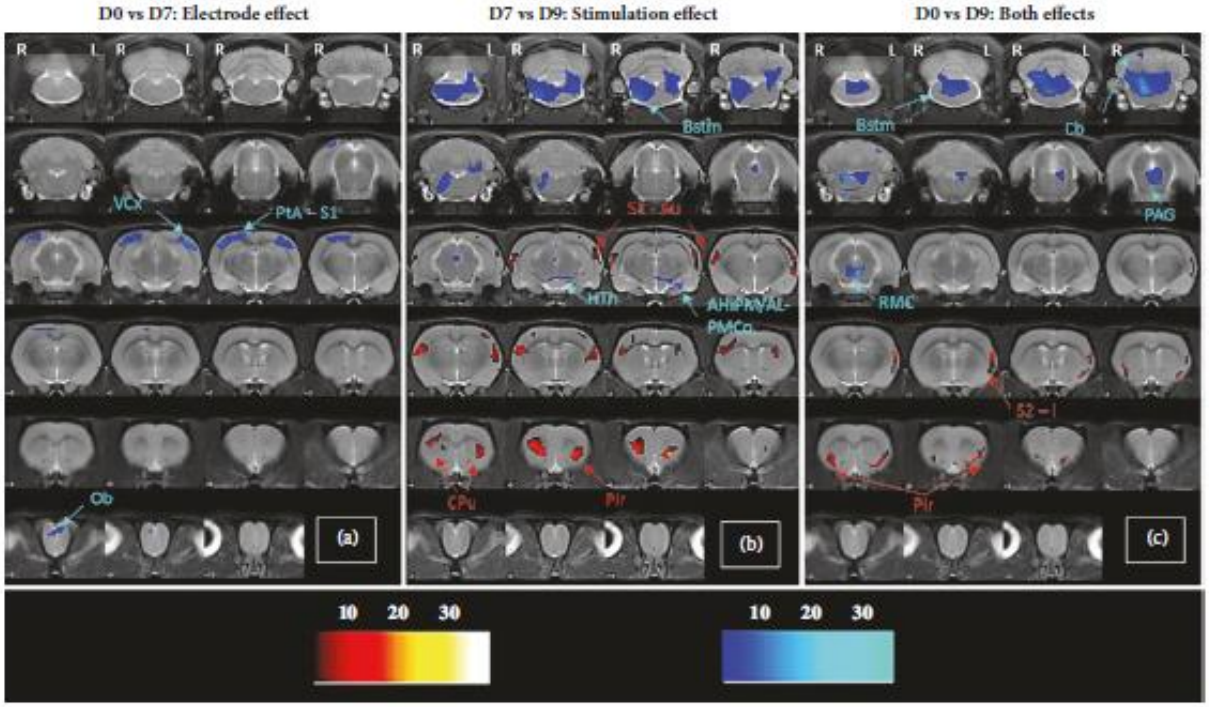

FIGURE 2: Changes in brain metabolic activity. Voxel based SPM results in T-maps overlaid on a T2 MR image, showing the changes in glucose metabolism due to electrodes insertion (a), stimulation (b), and both effects (c). The color bars in the right represent the T values corresponding to lower (blue) and higher (red) FDG uptake (p-value <0.005 (unc.); k= 50 voxels). Glucose metabolism: increase (hot colors); decrease (cold colors) [AHiPM/AL: amydalohippocampal area posteromedial/anterolateral part, Au: auditory cortex, Bstm: brainstem, Cb: cerebellum, CPu: caudate-putamen, HTh: hypothalamus, I: insular cortex, Ob: olfactory bulb, PAG: periaqueductal grey matter, Pir: piriform cortex, PMCo: posteromedial cortical amygdaloid nucleus, PtA: parietal association cortex, RMC: red nucleus, S1: primary somatosensory cortex, S2: secondary somatosensory cortex, and VCx: visual cortex].

3. Results

3.1. Electrode Insertion Effect. The presence of the electrodes (D0 versus D7, study I) led to a reduced FDG uptake in parietal association (PtA), primary somatosensory (S1), and visual cortices (Figure 2(a); Table 1(A)).

3.2. Stimulation Effect. The electrical stimulation (D7 versus D9, study II) led to a decreased FDG uptake in brainstem (Bstm), amygdaloid nuclei (AHiAL, AHiPM, and PMCo), and hypothalamus (HTh), together with an increased metabolism in caudate-putamen (CPu), piriform (Pir), S1, and auditory cortex (Au) (Figure 2(b); Table 1(B)).

3.3. Insertion and Stimulation Effect. The combination of both effects (D0 versus D9, study III) showed a decreased FDG uptake in Bstm, red nucleus (RMC), and periaqueductal grey matter (PAG) and higher metabolism in secondary somatosensory (S2), insular (I), and Pir cortices (Figure 2(c), Table 1(C)).

4. Discussion

First, we describe an insertional effect on brain glucose metabolism in sensory areas that are connected to mPFC [18]. Second, mPFC-DBS resulted in a distinct brain metabolic pattern, with more brain areas affected than in study I. Thus, DBS induced changes in circuits where the mPFC plays a key role, such as limbic (AHi, PMCo, and Pir) and reward (CPu and Bstm) systems [18]. Finally, the simultaneous consequences of the electrodes and the stimulation revealed lower cortical activation compared to the study II, showing a compensation of the hypometabolism derived from the electrodes presence (study I). Specifically, the absence of metabolic changes in S1 shown in study III exemplifies this mechanism, as this structure showed a metabolic reduction and an increase in studies I and II, respectively. Moreover, S1 is the only region in which there is an overlap between both effects.

The insertion effect could appear in response to the microlesion induced by the electrode in the mPFC [7] and the subsequent inflammation of the targeted area [9]. Thus, although the microlesion effect fades away over time [19], the clinical manifestations of the insertional effect could persist from days to months (exceptionally, years), or even being absent, despite comparing patients under the same surgical protocol and disease [8]. Besides, other authors have also provided evidence of its permanence on the healthy rat brain metabolism beyond one week after surgery [12]; although, in contrast to our findings, they showed similar effects of stimulation and insertion, being the latter of lower intensity.

Table 1: Changes in brain metabolism due to electrode (A), stimulation (B), and both effects (C).

ROI	Side	T	k	↓/↑	P_{unc} peak level	FWE peak level	FWE cluster level
(A) D0 vs D7: Electrode effect							
Ob	R & L	15.68	121	↓	<0.001	0.811	0.067
PtA - SI	R	14.97	365	↓	<0.001	0.880	<0.001
VCx	L	14.75	184	↓	<0.001	0.884	0.015
(B) D7 vs D9: Stimulation effect							
Bstm	R & L	18.39	1549	↓	<0.001	0.432	<0.001
AHiPM/AL-PMCo - HTh	L	10.39		↓	<0.001	0.949	
CPu	L	37.56	738	↑	<0.001	0.025	<0.001
SI-Au		10.53		↑	<0.001	0.947	
CPu-Pir	R	17.74	695	↑	<0.001	0.497	<0.001
SI-Au		10.45		↑	<0.001	0.948	
(C) D0 vs D9: Both effects							
RMC - PAG	R & L	26.24	1430	↓	<0.001	0.105	<0.001
Cb	R	5.90		↓	0.002	0.998	
S2 - I	L	15.20	475	↑	<0.001	0.892	<0.001
Pir	L	9.10		↑	<0.001	0.979	
Pir	R	12.96	152	↑	<0.001	0.929	0.026

Structures: AHiPM/AL: amydalohippocampal area posteromedial/anterolateral part, Au: auditory cortex, Bstm: brainstem, Cb: cerebellum, CPu: caudate-putamen, HTh: hypothalamus, I: insular cortex, Ob: olfactory bulb, PAG: periaqueductal gray matter, Pir: piriform cortex, PMCo: posteromedial cortical amygdaloid nucleus, PtA: parietal association cortex, RMC: red nucleus, SI: primary somatosensory cortex, S2: secondary somatosensory cortex, and VCx: visual cortex.
ROI: region of interest. Side: right (R) and left (L). T: t value; k: cluster size. Glucose metabolism: increase (↑) and decrease (↓). P_{unc}: p-value uncorrected; FWE: family wise error correction.

Furthermore, comparable results have been also shown in Parkinson disease (PD) patients after electrode insertion in the subthalamic nucleus, which resulted in similar but lower metabolic changes than subthalamotomy in PD-related pattern, while no significant clinical effect was observed due to the insertion [20]. Conversely, task-fMRI data found partial differences between the insertion and stimulation consequences in PD [19].

Therefore, the wide variability showed in relation to the clinical and physiological consequences of the electrode insertion could be highly dependent on several factors (e.g., the health state of the subjects, the DBS target selected, the number of microelectrode recording trajectories performed during the surgery [20], the time elapsed between the surgery and the test, etc.). In fact, PFC input and output connections shared with the sensory cortex occupy different locations and ordering [21], which is not common to other regions and could suppose a substantial difference regarding DBS effect. Thus, the opposed metabolism caused in the somatosensory cortex by the electrode placement and the stimulation alone could respond to the recent neural informative disruption theories of DBS mechanism of action [22, 23]. Importantly, these changes would have not been uncovered without a 3-times longitudinal design. Taken all together, both stimulation and insertion results seem to involve the same brain networks, although in a considerably different extent. This information would be helpful for adjusting the DBS protocols. Thus, understanding the regions affected by each involved factor (insertion; stimulation), together with the intensity and direction (activation; inhibition) of the produced modulation, could lead to more specific and efficient DBS protocols.

5. Limitations

Our work is subjected to several limitations. On the one hand, the small sample size selected, which responds to the aim of providing preliminary evidences that we considered to be important for understanding the DBS mechanism of action through a metabolic perspective. Therefore, we applied strict statistical thresholds in order to show more accurate results, which lead us to discard potentially important effects that do not reach statistical significance. In fact, the electrode insertion has been proved to produce lower metabolic changes than subthalamotomy or stimulation [12, 20], which could be masked in the present study. These points would explain why, in contrast with previous literature, the metabolic pattern observed with the insertion differs from that caused by the stimulation.

On the other hand, we have only included healthy animals in our study, which does not allow us to extrapolate the observed changes to a disease model due to the differences

related to a diseased brain. Nevertheless, we aimed to describe the metabolic consequences of electrode insertion and electrical stimulation, excluding any other intervening factors, in order to isolate those effects and improve their metabolic characterization.

6. Conclusions

In conclusion, our study highlights the importance of the design of appropriate protocols, particularly in neuroimaging, emphasizing the value of scanning the same subject with/without DBS, for a full understanding of the DBS mechanism of action and its clinical consequences. This will allow taking advantage of the electrodes and the stimulation consequences in order to optimize the DBS protocols for achieving the desired therapeutic effects.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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4. Response to Deep Brain Stimulation in Three Brain Targets with Implications in Mental Disorders: A PET Study in Rats.

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PLOS ONE

RESEARCH ARTICLE

Response to Deep Brain Stimulation in Three Brain Targets with Implications in Mental Disorders: A PET Study in Rats

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Abstract

Objective

To investigate metabolic changes in brain networks by deep brain stimulation (DBS) of the medial prefrontal cortex (mPFC), nucleus accumbens (NAcc) and dorsomedial thalamus (DM) using positron emission tomography (PET) in naïve rats.

Methods

43 male Wistar rats underwent stereotactic surgery and concentric bipolar platinum-iridium electrodes were bilaterally implanted into one of the three brain sites. [^{18}F]-fluoro-2-deoxy-glucose-PET (^{18}FDG-PET) and computed tomography (CT) scans were performed at the 7th (without DBS) and 9th day (with DBS) after surgery. Stimulation period matched tracer uptake period. Images were acquired with a small-animal PET-CT scanner. Differences in glucose uptake between groups were assessed with Statistical Parametric Mapping.

Results

DBS induced site-specific metabolic changes, although a common increased metabolic activity in the piriform cortex was found for the three brain targets. mPFC-DBS increased metabolic activity in the striatum, temporal and amygdala, and reduced it in the cerebellum, brainstem (BS) and periaqueductal gray matter (PAG). NAcc-DBS increased metabolic activity in the subiculum and olfactory bulb, and decreased it in the BS, PAG, septum and hypothalamus. DM-DBS increased metabolic activity in the striatum, NAcc and thalamus and decreased it in the temporal and cingulate cortex.

Conclusions

DBS induced significant changes in ^{18}FDG uptake in brain regions associated with the basal ganglia-thalamo-cortical circuitry. Stimulation of mPFC, NAcc and DM induced different patterns of ^{18}FDG uptake despite interacting with the same circuitries. This may have important

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implications to DBS research suggesting individualized target selection according to specific neural modulatory requirements.

Introduction

Mental disorders are the third leading cause of disability-adjusted life years (DALYs) loss and the first cause of years lived with disability (YLD) in Europe, accounting for 36.1% of those attributable to all causes[1]. Mental disorders greatly influence patients' overall health, economic situation and social integration. Even though effective treatment exist, 10–30% of the patients have little or no response to traditional treatment strategies and up to an additional 30% of the patients experience only partial relief [2], thus making it essential to explore other treatments. During the last decades, brain electrical stimulation techniques have emerged in the bio-scientific scenario. Among them, deep brain stimulation (DBS) constitutes a neurosurgery technique that modifies neural activity by means of an electrical current applied directly to specific brain targets. It has been licensed as a treatment option for several movement disorders [3]. The idea to extend DBS to the treatment of psychiatric disorders was based on the notion that psychiatric disorders are the clinical presentation of dysfunctional brain networks and the observation that DBS induces depressive and hypomanic states in Parkinson's disease patients [4]. Meanwhile, DBS in the ventral capsule/ventral striatum (VC/VS), which contains the nucleus accumbens (NAcc), has received FDA approval for treatment of obsessive compulsive disorders, is being tested for treatment of depressive disorders [5–7] and addiction [8–11] and the first preclinical report on successful DBS in the context of schizophrenia has just been published [12]. The only double-blind sham-controlled trials for chronic treatment-resistant depression stimulated the VC/VS[13] and Brodmann area 25[14], obtaining little success. Thus, it is noteworthy that, with exception of VC/VS-DBS for OCD, there is no much evidence yet supporting open loop DBS for psychiatric indications. Future research applying new study designs and DBS parameters (e.g. close-loop DBS[15]) are needed to confirm its clinical potential. On the other hand, DBS also holds scientific promise in the identification of interconnected functional networks and dysfunctional brain circuits underlying a physiological and pathological brain functions due to its capacity to specifically modify neural discharge patterns locally, at the electrode placement, and remotely, in associated brain areas [16, 17] and affect neural network activity[18–20]. Across the neuro-psychiatric disorders currently subjected to DBS treatment trials, the following DBS targets are being tested: medial prefrontal cortex (mPFC), globus pallidus internus, subthalamic nucleus, zona incerta, nucleus accumbens (NAcc)/ventral striatum, hippocampus and thalamus (centromedian/parafascicularis; anterior nucleus; periaqueductal gray/periventricular gray; ventrolateral intermedius; ventral posterolateral/ventro-posteromedial), lateral habenula, nucleus basali Meynert, medial forebrain bundle (MFB), and fornix/hypothalamus [21–24]. In addition, the mediodorsal thalamic nucleus (DM) structure has been suggested relevant in the context of psychiatric disorders as it interconnects with the dorsolateral PFC and limbic structures, including limbic cortex, hippocampus and basolateral amygdala [25]. Nevertheless, there is no consensus on which area is best for each disorder. Indeed, several areas are being investigated for the same pathology, i.e. mPFC, cingulum, MFB, ventral striatum or the NAcc for depression, STN, NAcc or ventral striatum for obsessive compulsive, mPFC or NAcc for future schizophrenia studies; more to that, some cases, the same area is being investigated for several disorders [26–29]. So far, targets have been selected upon assumptions about the pathophysiological relevance of the

respective brain site in the manifestation of the respective disorder but often enough lack a scientific framework that proves the selection. From a theoretical point of view, the optimal DBS target would be the one that mostly interconnects with circuits involved in the manifestation of the symptoms to be targeted.

In this context, functional neuroimaging is a powerful tool in terms of locating brain networks modulated by DBS and refining stimulation protocols [30]. Positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG) constitutes the traditional technique for in vivo direct quantification of regional brain glucose metabolism in humans and rodents [12, 18, 31–35]. The method has proven itself as an excellent tool for promoting our understanding of the neurobiological processes in healthy as well as diseased brains and allows for reliable comparative studies [36–40]. We used here ^{18}FDG-PET and statistical parametric mapping (SPM) techniques in rats to compare the metabolic modulation of neural networks by DBS applied to either the mPFC, NAcc or DM, all of which are linked to several known neuropsychiatric disorders [41–44].

Materials and Methods

Animals

Forty-three male Wistar rats (275–325 g) were housed in a temperature (24 ± 0.5˚C) and humidity controlled *vivarium* with a 12 h light-dark cycle. Commercial rodent laboratory chow and water were available *ad libitum* if not indicated differently. All experimental animal procedures were conducted according to the European Communities Council Directive 2010/63/EU and approved by the Ethics Committee for Animal Experimentation of our hospital (Comité de Ética de Experimentación Animal, CEEA; number ES280790000087).

Surgery and DBS protocol

Stereotaxic surgeries were carried out on animals anesthetized with a mixture of ketamine (100 mg kg^{-1}) and xylazine (10 mg kg^{-1}). Concentric bipolar platinum-iridium electrodes (Plastics One, Roanoke, USA) were bilaterally implanted in one of the following targets, according to the Paxinos and Watson rat brain atlas [45]: 1) mPFC; anteroposterior (AP) +3.5 (from Bregma), medio-lateral (ML) +0.6, dorso-ventral (DV) -3.4 (from Dura); 2) NAcc: AP +1.2, ML +1.8, DV -8.1; and 3) DM: AP -2.8, ML +0.75, DV -5.0. Electrodes were fixed to the skull with dental acrylic cement (Technovit^{2000}). Computed tomography (CT) scans of all the animals were obtained and co-registered to an MRI study of one non-operated animal (anatomical MRI template) to rule out errors in the placement of the electrodes. Only animals with correct electrodes positions were included in the PET study resulting in the following number of animals per group: 1) mPFC: 10, 2) NAcc: 10 and 3) DM: 11.

PET scans were acquired seven and nine days thereafter, preceded by either sham stimulation (baseline-condition) or DBS applied during ^{18}FDG-uptake period (DBS-condition) for 45 minutes. DBS was performed with an isolated stimulator (STG1004; Multi Channel Systems GmbH, Reutlingen, Germany) in a constant current mode at 130 Hz and 150 μA with a pulse width of 100 μs. These settings were chosen based on previous studies by our group [18, 19].

Imaging studies

All animals were scanned using a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Madrid) under anesthesia with isoflurane (3% induction and 1.5% maintenance in 100% O_2). ^{18}FDG (approximately 1 mCi) was injected into the tail vein and, after an uptake period of 45 minutes, animals were scanned for 45 minutes. Images were reconstructed using a

2D-OSEM (ordered subset expectation maximization) algorithm, which claims a spatial resolution for this scanner of 1.45 mm FWHM (full width at half maximum), with a voxel size of 0.3875 x 0.3875 x 0.7750 mm^3. The energy window was 400–700 keV. Decay and deadtime corrections were applied.

CT studies were acquired with the following parameters: 340 mA, 40 KV, 360 projections, 8 shots per projection, and 200 μm of resolution. CT images were reconstructed using a Feldkamp algorithm (isotropic voxel size of 0.121 mm).

In addition, one MRI scan of a non-operated animal was acquired with a 7-Tesla Biospec 70/20 scanner (Bruker, Ettlingen, Germany) under sevoflurane anesthesia (4.5% for induction and 2.5% for maintenance in 100% O_2). A T2-weighted spin echo sequence was acquired, with TE = 33 ms, TR = 3732 ms, and a slice thickness of 0.8 mm (34 slices). The matrix size was 256 × 256 pixels at an FOV of 3.5 × 3.5 cm^2. This single-animal study was used as an anatomical template in order to display the results of the SPM study.

Analysis of PET data

CT studies were co-registered to a random reference CT scan using an automatic rigid registration method based on mutual information, and the spatial transformation obtained for each CT image was subsequently applied to the corresponding PET[46]. The single MRI study was also spatially co-registered to the reference CT scan. A brain mask segmented on the MRI study was applied to all registered PET images and the resulting images were smoothed with an isotropic Gaussian filter (2 mm FWHM). Voxel values were normalized to the average white matter intensity in order to obtain the regional characterization of metabolic changes circumventing overall differences in animal brain metabolism. White matter normalization was used in accordance with the criteria of Shinohara et al.[47].

A region of interest (ROI) analysis was performed to determine the global metabolic differences. Whole brain and white matter masks segmented on the MR template were used for this analysis. Whole brain data were normalized to average white matter intensity.

Statistical analysis

Statistical analysis of regional PET data was performed using the software package SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK). Groups were compared by means of a paired *t* test with a significance threshold of $p<0.01$ ($T = 2.82$), uncorrected for multiple comparisons. To reduce type I error, a 50-voxel clustering threshold (spatial-extent) was applied. Global differences were assessed by means of a paired t-test with a threshold for statistical significance set at $p<0.01$.

Results

Fig 1 shows sagittal, coronal and axial views of a CT scan registered to the MR template of one animal to verify the correct electrode positioning. Only animals with electrodes placed correctly in the respective target were included in the study.

Measurements based on global differences for the whole brain metabolism displayed no significant differences across groups under either treatment, sham-stimulation or DBS. Values for DBS animals were normalized and expressed as a ratio of the average glucose metabolism in the basal time point for each animal: mPFC (0.99±0.020) ($p = 0.099$), NAcc (1.03±0.15) ($p = 0.631$) and DM (0.99±0.032) ($p = 0.185$).

mPFC-DBS treatment increased metabolic activity in the striatum, temporal and piriform cortex and amygdala (right: $T = 6.39$, $p<0.001$; left: $T = 4.98$, $p<0.001$), and reduced it in the cerebellum, brainstem and periaqueductal gray matter ($T = 11.52$, $p<0.001$) (Fig 2, Table 1).

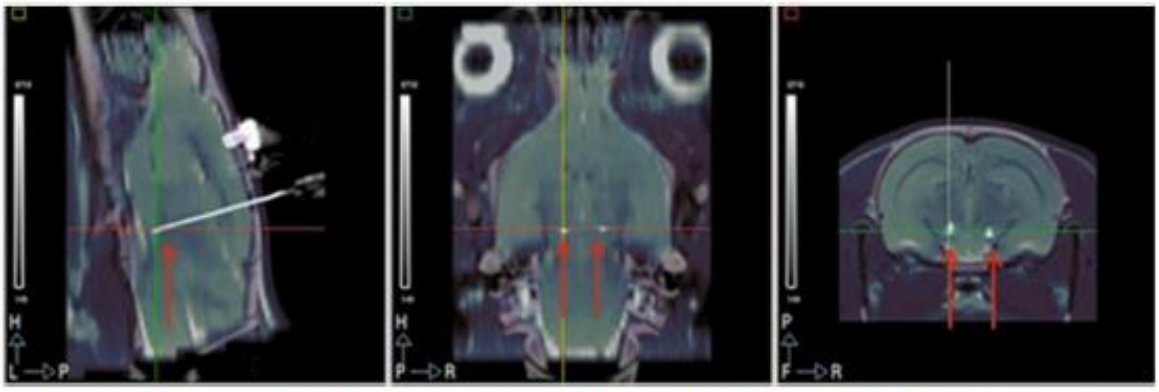

Fig 1. Correct electrode location verification. Sagittal, coronal and axial views of a CT scan registered to the MR template of an animal to verify the correct electrode location. Only animals with electrodes placed correctly in the respective target were included in the study.

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NAcc-DBS treatment increased metabolic activity in the left subiculum (T = 13.02, $p<0.001$), piriform cortex (right: T = 4.29, p = 0.001; left: T = 6.52, $p<0.001$) and olfactory bulb (T = 5.20, $p<0.001$), and decreased ^{18}FDG-uptake in the brainstem and PAG (T = 4.82, p = 0.001), septum (T = 5.27, $p<0.001$) and hypothalamus (T = 3.25, p = 0.005) (Fig 2, Table 1).

DM-DBS treatment increased metabolic activity in the striatum, NAcc and piriform cortex (right: T = 7.25, $p<0.001$; left: T = 3.73, p = 0.002) and thalamus (T = 7.78, $p<0.001$) and decreased ^{18}FDG-uptake in the temporal (right: T = 3.43, p = 0.003; left: T = 4.58, p = 0.001) and cingulate cortex (T = 3.64, p = 0.001) (Fig 2, Table 1).

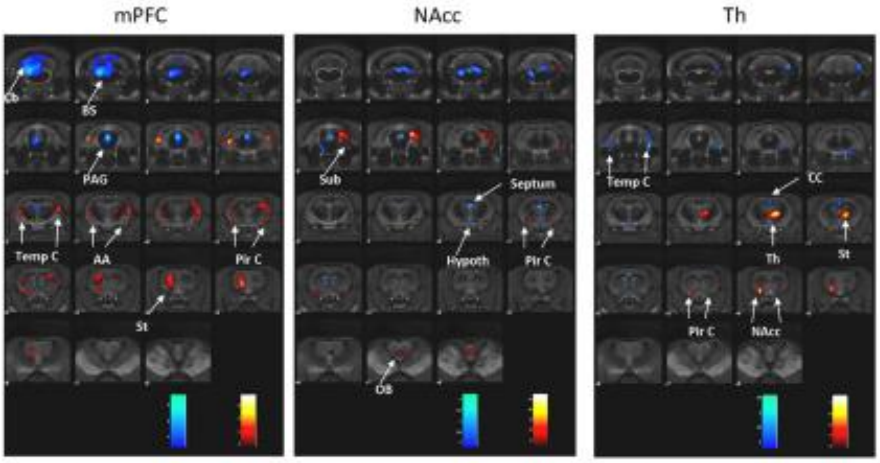

Fig 2. Brain glucose metabolism during DBS in the three brain targets. Effects depend on stimulation target. Colored PET overlays on MR reference indicate increased 18FDG uptake (hot colors) or decreased (cold colors). AA: amygdala; BS: brainstem, Cb: cerebellum, CC: cingulate cortex, Hypoth: hypothalamus, NAcc: nucleus accumbens, PAG: periaqueductal gray matter, Pir C: piriform cortex, Sub: subiculum hippocampal, Str: striatum, Temp C: temporal cortex, Th: thalamus.

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Table 1. Changes in brain metabolic activity during DBS in the three brain targets.

Target	ROI	Side	↑/↓	T	d	p value
mPFC	St, AA, Temp & Pir C	R	↑	6.39	2.13	< 0.001
	St, AA, Temp & Pir C	L	↑	4.98	1.66	< 0.001
	Hipp v	L	↓	7.07	2.57	< 0.001
	Cb, BS & PAG	R & L	↓	11.52	3.84	< 0.001
NAcc	Sub	L	↑	13.02	4.60	< 0.001
	Pir C	L	↑	6.52	2.31	< 0.001
	Pir C	R	↑	4.29	1.52	0.001
	Olfactory bulb	R & L	↑	5.20	1.83	< 0.001
	BS & PAG	R & L	↓	4.82	1.70	< 0.001
	Hypoth		↓	3.25	1.15	0.005
	Septum		↓	5.27	1.86	< 0.001
DM	St, NAcc & Pir C	R	↑	7.25	2.30	< 0.001
	St, NAcc & Pir C	L	↑	3.73	1.18	0.002
	Th	R & L	↑	7.78	2.46	<0.001
	Temp C	R	↓	3.43	1.10	0.003
	Temp C	L	↓	4.58	1.45	0.001
	Cing C	R & L	↓	3.64	1.15	0.002

Brain metabolic changes according to the stimulated target. Region of interest (ROI), side (left and right), glucose metabolism (increase: ↑ or decrease: ↓) t value (T), d Cohen (d) and statistical p value (p). AA: amygdala; BS: brainstem, Cb: cerebellum, CC: cingulate cortex, Hypoth: hypothalamus, NAcc: nucleus accumbens, PAG: periaqueductal gray matter, Pir C: piriform cortex, Sub: subiculum hippocampal, Str: striatum, Temp C: temporal cortex, Th: thalamus].

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Discussion

To the best of our knowledge, this is the first comparative report on the use of small animal ^{18}FDG-PET and SPM techniques in rats in an attempt to identify and compare the modulation of brain metabolic networks by DBS in the mPFC, NAcc and DM. We show that the effects of high frequency DBS on neuronal activity, reflected as the differences in regional glucose metabolism between DBS on and off conditions, involve modifications of complex networks rather than global or isolated regions. This is in agreement with our previous study for mPFC and NAcc stimulation in an animal model of schizophrenia [12]. Its capability to either increase or decrease activity supports the notion that DBS induces several mechanisms that lead to net inhibitory and excitatory effects irrespective of the function [48], suggesting a complex modulation of activity along cortico-basal ganglia-thalamo-cortical and the cerebello-thalamo-cortical circuits. Overall, stimulation in each brain target influenced a different set of structures at a distance from the target that might be relevant for addressing specific pathological conditions.

Common DBS effects across different targets

DBS to all three targets induced increased metabolic activity in the piriform cortex (PC). The PC is the largest area of the mammalian olfactory cortex, receives direct projections from the olfactory bulb and contains the most susceptible neural circuits of all forebrain regions for electrical (or chemical) stimulation [49, 50]. Thus, immunohistochemical studies have shown that during electrical stimulation of limbic brain regions, the PC exhibits the most consistent increase in glucose utilization [49], similar to our results.

Another interesting finding is that both mPFC-DBS and NAcc-DBS decreased glucose metabolism in the brainstem. The mPFC is reciprocally connected with the dorsal raphe nucleus, which contains most ascending serotonergic neurons, and the ventral tegmental area (VTA) which contains mesocortical dopaminergic (DA) neurons, which could account for the decreased glucose metabolism seen in the brainstem. The medium spiny neurons of NAcc receive input from both dopaminergic neurons in the VTA and the glutamatergic neurons of the hippocampus, amygdala and mPFC. Thus, stimulation of NAcc at high frequencies could lead to an inhibition of dopaminergic activity at the brainstem level, resulting in decreased glucose metabolism in the brainstem. Our results are in line with that reported with citalopram, an antidepressant medication, showing decreased blood oxygenation level dependent (BOLD) signal in the brainstem using pharmacological magnetic resonance imaging [51]. In this sense, both brain targets have been recently proposed as targets for DBS in resistant major depressive disorder [52, 53], and has been associated with antidepressant, anxiolytic, and precognitive properties.

mPFC-DBS increased brain metabolism in the temporal cortices

Hypofrontality is related to deficits in attention, memory and executive function, apathy, social withdrawal, restricted affection or anhedonia [54]. It has been suggested that the direct stimulation of the PFC may serve to modulate temporo-parietal attentional networks involved in the automatic processing of salient stimuli [30], playing a critical role in mood regulation [55]. In this sense, cortical stimulation for treatment-resistant depression constitutes a brain stimulation approach that has shown promise [56–58]. Here, we show that mPFC-DBS affected metabolic activity in the striatum, temporal and piriform cortices, the amygdala, cerebellum, brainstem and periaqueductal gray matter. This is in line with the PFC projecting to the ventral striatum and the head of the caudate, as well as other subcortical connections, including the amygdala [59]. Thus, our results showing an increased metabolism in temporal cortices support the notion that stimulation of mPFC could be explored for improving the attentional network. Moreover, behavioral experiments should be performed to corroborate these findings.

Cerebellar affectation has been commonly reported in schizophrenia, autism, and other developmental disorders [60–62]. Recent neuroanatomical evidence has also demonstrated closed-loop connectivity between prefrontal cortex and the cerebellum [63]. Moreover, electrophysiological and anatomical studies have demonstrated the existence of a prefrontal-olivo-cerebellar pathway in anesthetized mice [60], and the existence of disynaptic fronto-cerebellar connectivity in rats [64]. Our data showing that mPFC-DBS decreased glucose metabolism in the cerebellum, confirm the existence of a rodent prefrontal-cerebellar network [65, 66].

NAcc-DBS increased brain metabolism in the subiculum

The NAcc has traditionally been associated with reward, pleasure and addiction, behavioral categories/systems implicated in the pathophysiology of basically all psychiatric disorders [16, 67–69]. In fact, the ventral capsule/ventral striatum (VC/VS), which includes the NAcc, is the unique brain target with FDA approval for DBS treatment of a psychiatric condition (OCD). The NAcc receives major dopaminergic afferents from mesolimbic origin, and dopamine is the most important transmitter within these nuclei. Thus, NAcc stimulation may lead to direct interferences in the dopaminergic system, or possibly indirect influences on the synaptic efficiency of this neurotransmitter system, with a huge spread of metabolic changes in the brain. Given its vast pathophysiological implication, network effects of NAcc-DBS were less striking and limited to the subiculum, piriform cortex (PC), olfactory bulb (OB), and brainstem. Off

note, findings basically correspond to NAcc-DBS we recently reported using a functional MRI approach [70]. Of those effects, the increase of glucose metabolism in the subiculum is of particular interest. Neuroimaging and neuropsychological studies have shown an hippocampal dysfunction in Alzheimer's disease, cognitive ageing, post-traumatic stress disorder, obesity, schizophrenia, and depressive and anxiety disorders, among others [71]. Specifically in schizophrenia, there is robust evidence of hippocampal dysfunction, with impaired activation during memory tasks, increased baseline hippocampal perfusion, and reduced dentate gyrus neurogenesis and efferent signaling [72]. Moreover, obesity has been associated with defective hippocampal activity, which leads to cognitive deficiency in obese patients [73]. In this context and according to our results, it seems reasonable to explore the idea of applying NAcc-DBS in pathologies associated with hippocampal dysfunction.

DM-DBS increased brain metabolism in the thalamus

The dorsomedial thalamus (DM) has strong interconnections with the dorsolateral PFC and limbic structures, besides being a critical element in the attentional "selective engagement" system. The dysfunction of this "sensory gating apparatus" has been associated to hallucinations, a common symptom in psychosis [74, 75]. At present, DM-DBS has only been applied experimentally in animal models [21, 53, 76–79]. Here, we found that DM-DBS affected metabolic activity in the striatum, NAcc, piriform cortex, medial thalamus and temporal and cingulate cortices. This is in line with the DM projections to the dorsolateral prefrontal and orbitofrontal cortical areas, which together project to the anterior cingulate cortex [80] and to the dorsal and ventral striatum [81]. Among those effects, the increase of glucose metabolism in the thalamus is especially relevant from a translational point of view. Neuroimaging has shown abnormalities in the DM of schizophrenic patients, with decreases in the thalamic D2 receptor binding [82], less prominent thalamic glucose metabolism rate [83], decrease functional connectivity of DM to other circuit areas or decreases in the thalamic blood flow [84]. Patients with frontotemporal lobe degeneration associated with dementia also shown decreased glucose metabolism in the medial temporal region, the thalamus and striatum [85]. In Alzheimer disease, thalamic abnormalities at the anterior thalamic nuclei have been associated with cognitive deficits in memory and attention [86]. In view of these studies and our results, it seems essential to explore the idea of applying DM-DBS in pathologies associated with cognitive deficits in memory and attention and dementias.

Limitations of the study

Our study is subject to two limiting factors. The first is the use of naïve animals to study DBS' effects. Clearly, in the clinic, DBS is applied to diseased brains and its therapeutic effects are a function of its interaction with altered brain network. Another limitation is related to the temporal influence of DBS as studied here; we used an acute stimulation protocol preceding the PET scans acquisition. In the clinical scenario, DBS is applied chronically and usually therapeutic effects evolve over a timeline of stimulation.

Conclusion

In conclusion, we show that DBS in mPFC, NAcc and DM induced different patterns of ^{18}FDG uptake despite sharing interconnections with the same circuitry, and this may have important implications to DBS research suggesting individualized target selection according to specific neural modulatory requirements.

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5. Stimulating the nucleus accumbens in obesity: A positron emission tomography study after deep brain stimulation in a rodent model.

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RESEARCH ARTICLE

Stimulating the nucleus accumbens in obesity: A positron emission tomography study after deep brain stimulation in a rodent model

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Abstract

Purpose

The nucleus accumbens (NAcc) has been suggested as a possible target for deep brain stimulation (DBS) in the treatment of obesity. Our hypothesis was that NAcc-DBS would modulate brain regions related to reward and food intake regulation, consequently reducing the food intake and, finally, the weight gain. Therefore, we examined changes in brain glucose metabolism, weight gain and food intake after NAcc-DBS in a rat model of obesity.

Procedures

Electrodes were bilaterally implanted in 2 groups of obese Zucker rats targeting the NAcc. One group received stimulation one hour daily during 15 days, while the other remained as control. Weight and daily consumption of food and water were everyday registered the days of stimulation, and twice per week during the following month. Positron emission tomography (PET) studies with 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) were performed 1 day after the end of DBS. PET data was assessed by statistical parametric mapping (SPM12) software and region of interest (ROI) analyses.

Results

NAcc-DBS lead to increased metabolism in the cingulate-retrosplenial-parietal association cortices, and decreased metabolism in the NAcc, thalamic and pretectal nuclei. Furthermore, ROIs analyses confirmed these results by showing a significant striatal and thalamic hypometabolism, and a cortical hypermetabolic region. However, NAcc-DBS did not induce a decrease in either weight gain or food intake.

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Conclusions

NAcc-DBS led to changes in the metabolism of regions associated with cognitive and reward systems, whose impairment has been described in obesity.

Introduction

Obesity is defined as abnormal or excessive fat accumulation which may impair health [1]. The prevalence of obesity has reached epidemic levels, and its comorbid conditions cause at least 2.8 million deaths per year worldwide [2]. In addition, obesity is a risk factor for many other highly prevalent diseases [3].

Initial anti-obesity treatments focus on diet and exercise routines [4]. However, in refractory patients, specialists turn to pharmacological and surgical procedures, which can cause serious adverse effects and fail to control the disease in the long term [5,6]. Therefore, new therapeutic approaches must be found to reduce the prevalence of obesity. Among them, deep brain stimulation (DBS) seems promising for treatment-resistant obesity. This therapy involves sending electric stimuli from a pulse generator to brain nuclei via electrodes in order to modify impaired function. However, the mechanism of action of this approach remains unknown. In this sense, the traditional concept of an ablative effect of high-frequency (HF) DBS (100–160 Hz) [7,8] is now being replaced. In fact, there is growing interest in alternative proposals, such as the idea that HF-DBS could cause an 'informational lesion' [9] or disruption of the neural informational flow [10] in the target structure, which would produce therapeutic benefits. Therefore, inadequate signals from a specific nucleus could be isolated by stimulating a downstream target and hence correcting the malfunction of the neural circuit. In any case, DBS offers important benefits over ablative neurosurgery, such as reduced invasiveness, possible reversibility, and the possibility of *in vivo* adjustment of the stimulus applied [11]. Furthermore, several authors have shown stereotaxic surgery to be safe in clinical procedures [12–15], thus reinforcing its potential application to a broader range of diseases. In this sense, DBS has been validated as a palliative treatment in motor diseases [16] and obsessive-compulsive disorder [17], and its potential role has been investigated in other neuropsychiatric disorders [18].

The nucleus accumbens (NAcc) has received much attention as a key target structure of the reward system in the treatment of obesity [19–21]. Therefore, NAcc-DBS could modulate the reward processes related to food intake and lead to weight reduction [7,20,22]. Two case reports assessing bilateral NAcc-DBS in obese patients show significant weight loss [23,24]. Interestingly, the second report described a patient with pathological obesity due to craniopharyngioma surgery [24], thus highlighting the interaction between the homeostatic and reward mechanisms involved in feeding. The communication between these neural systems would be mediated by an interplay between the lateral hypothalamus (LH), ventral tegmental area (VTA), and NAcc, in which leptin would play a central role [20].

Neuroimaging offers a variety of powerful tools to study the regions involved in the pathophysiology of obesity, as well as those modulated by DBS. In particular, positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) is a suitable technique for characterizing functional neuronal networks in small animals, and has proven useful for elucidating the mechanism of action of DBS [25,26]. In fact, we previously showed that LH-DBS induced metabolic changes in brain regions related to the control of food intake and reduced weight gain in a leptin signal–deficient model of obesity (obese Zucker rat) [27].

Given this background, and considering the hypothesis that DBS can block the impaired signaling sent by VTA and LH in the absence of the influence of leptin, we assessed the

metabolic changes induced by NAcc-DBS in our previous animal model by applying an identical DBS protocol [20] (see Fig 1). As a result, the stimulation could reduce food intake and, hence, weight gain.

Material and methods

Animals

The obese Zucker rat was selected as an animal model of treatment resistant obesity, which is representative of the potential beneficiaries of this therapy. It is homozygous for a truncated form of the leptin receptor and hence has genetic resistance to this hormone. Leptin is released by adipose tissue in proportion to its extension and the amount of lipids ingested during meals. It targets the lateral LH, ventromedial hypothalamus and VTA [20,29], and acts as a signal to stop eating. Consequently, Zucker rats experience hyperphagia, hyperinsulinemia, and hyperlipidemia; which lead to spontaneous obesity [30].

In this work, fifteen adult male obese Zucker rats (fa/fa$^-$, Charles Rivers Laboratories, Spain) (10-week old) were housed individually in a temperature- and humidity-controlled room on a 12 h dark/light cycle with food (standard laboratory chow) and water available *ad libitum*. Weight, food and water consumption were monitored daily during the 15 days of stimulation, and twice per week during the following month. Measurements were always collected at the same time of the day. Prior to the PET study, animals were deprived of food but allowed free access to water for 6–8 hours. The study design is shown in Fig 2.

All experimental animal procedures were conducted according to European Communities Council Directive 2010/63/EU and approved by the Ethics Committee for Animal Experimentation of Hospital Gregorio Marañón.

Surgery

Stereotaxic procedures were performed at 10 weeks-age under a mixture of ketamine/xylazine (100/10 mg/kg). Concentric bipolar platinum-iridium electrodes (MS303/8-AIU/Spc, Bilaney Consultants GmbH, Germany) were bilaterally implanted to target the NAcc core (+1.2 mm posterior and +1.5 mm lateral from bregma, -8.2 mm ventral from the dura) [31]. Electrodes were fixed to the skull bone with acrylic dental cement (Technovit, Heraeus-Kulzer, Germany) reinforced with four small stainless steel screws attached to the skull. Ceftriaxone (100 mg/kg IM) and buprenorphine (0.1 mg/kg IP) were administered during 5 days as postoperative care. The correct electrode location verification is shown in Fig 3.

Remarkably, although the electrodes were implanted in the NAcc core, the selected stimulation protocol is expected to directly affect a wider area, including also the NAcc shell [32]. Therefore, we will refer to the NAcc as the target of stimulation, without distinguishing between subregions.

DBS protocol

DBS started 7 days after surgery to allow the animals sufficient recovery time. Animals were divided into 2 groups: NAcc-sham ($N = 9$) (surgery with electrodes implantation but no stimulation) and NAcc-DBS ($N = 6$) (surgery plus stimulation). As animals could freely move when receiving the stimulation into their cages, the stimulator wire was susceptible of snagging due to animals movements. Therefore, NAcc-sham animals were not plugged to the stimulator in "off position" in order to avoid losing any surgical implant.

DBS was performed with an isolated stimulator device (CS 120 8i, CIBERTEC S.A., Spain) set at a constant current of 150 μA (130 Hz) and a pulse width of 100 μs (biphasic stimulation

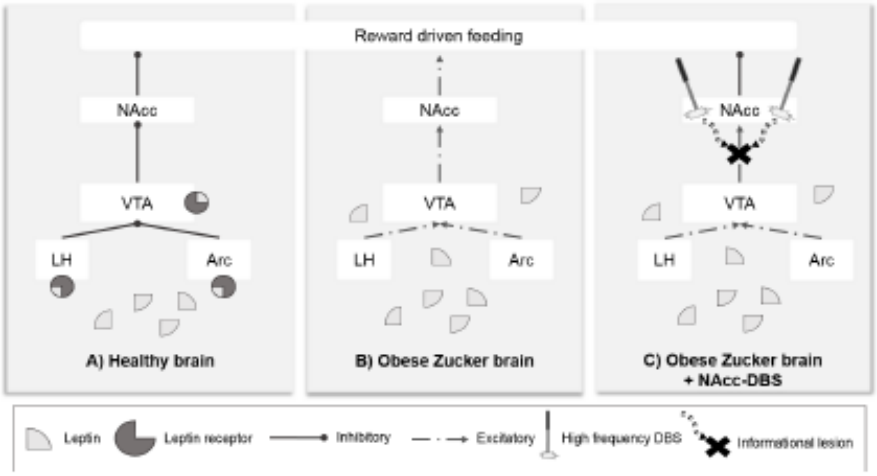

Fig 1. Study hypothesis. Schematic explanation of the hypothalamic-mesolimbic brain circuit state in the following: A) healthy brain; B) obese Zucker brain; and C) obese Zucker brain with NAcc-DBS, showing the disruptive mechanism of action theory [9,10]. Partially adapted from [20,28] [Arc: arcuate, LH: lateral hypothalamus, VTA: ventral tegmental area, NAcc: nucleus accumbens].

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mode). Stimulation was applied for 1 hour/day over 15 days. These settings were chosen based on previous preclinical and clinical studies [25,33,34].

Imaging studies

PET studies were acquired one day after the DBS protocol finished with a small-animal PET/CT scanner (ARGUS PET/CT, SEDECAL, Spain), under anesthesia with isoflurane (3% induction, 1.5% maintenance in 100% O_2). 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) (~37 MBq) was injected through the tail vein, and animals were scanned for 45 min. Images were reconstructed using a 2D-OSEM algorithm, Full Width at Half Maximum (FWHM) of 1.45 mm, with a voxel size of 0.3875 x 0.3875 x 0.775 mm^3 and an energy window of 400–700 keV. Decay and dead-time corrections were applied.

We obtained two CT scans for each animal: at the end of the surgery to check the correct placement of the electrodes, and simultaneously with the PET studies. CT studies were acquired with the same scanner (340 mA, 40 kV, 360 projections, 8 shots, and 200 μm of

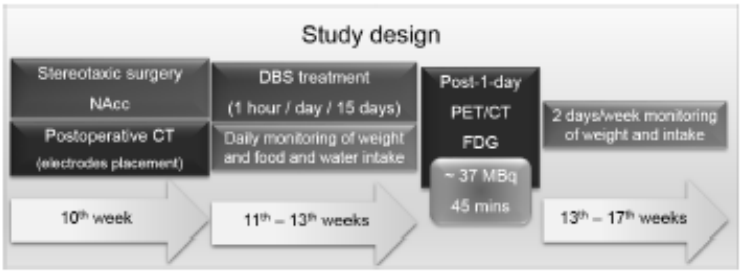

Fig 2. Study design. Design of the experimental procedures performed during the study in relation to the age of the animals.

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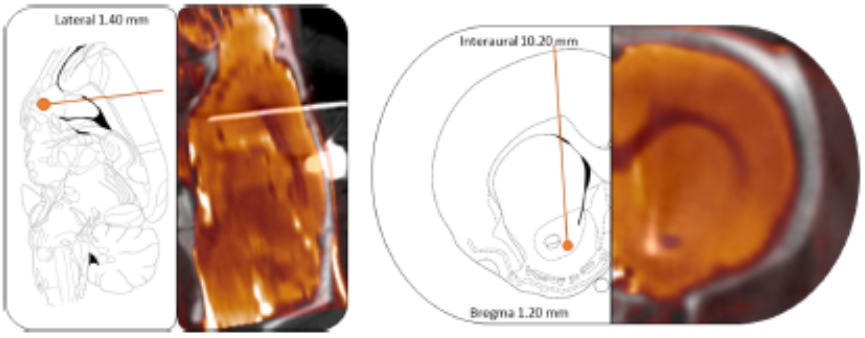

Fig 3. Electrodes placement verification. Representative sagittal (left) and axial (right) views of a CT scan registered to the MR template of an animal next to the correspondent slice from [31] to verify the correct electrode location in the NAcc.

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resolution) and reconstructed using an FDK algorithm (isotropic voxel size of 0.121 mm) [35]. Only animals with a correct placement of the electrodes were included in the study.

An MRI scan of a single non-operated animal was acquired with a 7-Tesla Biospec 70/20 scanner (Bruker, Germany) for use as anatomical template in the statistical analyses. A T2 spin-echo sequence was acquired, with TE = 33 ms and TR = 3732 ms. The scan parameters were as follows: 34 slices measuring 0.8 mm in thickness; matrix size 256x256 pixels; and FOV of 3.5x3.5 cm^2. The artifact caused by the surface coil was corrected.

Data processing and statistical analysis

Intake and body weight. Daily food and water intake during the DBS period, as well as average food ingested every 5 days, were used to evaluate the real consumption, as daily consumption is a very noisy variable. Body weight results are expressed as the difference in weight (%) with respect to baseline. Changes in weight and intakes were evaluated with GraphPad Prism version 5.00 (GraphPad Software, USA), using a 2-way ANOVA to compare both groups. Moreover, we used linear regression to evaluate the progression of weight changes from baseline, comparing the obtained slope for each group by an ANCOVA analysis.

PET data. PET data followed a preprocessing registration protocol previously described [27]. Briefly, PET scans were co-registered to a random reference CT scan by an automatic method based on mutual information [36]. The MRI scan was also registered to the same spatial frame with the same method. Images were studied by voxel-by-voxel and region of interest (ROI) analyses. For the former methodology, PET registered images were normalized to global mean brain intensity in accordance to Shinohara *et al.* criteria [37], and smoothed using a Gaussian kernel of 0.96875 x 0.96875 x 1.9375 mm^3 of FWHM. A whole brain (WB) mask was segmented from the registered MRI study and applied to all PET images in order to eliminate voxels outside the brain. Then, we performed a voxel-by-voxel analysis of data using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Groups were compared using a two sample T-test, setting a significance threshold of $p<0.005$ uncorrected (voxel-level significance), but cluster-based corrected in order to avoid type II errors [38]. Moreover, only significant regions larger than 50 activated connected voxels were accepted aiming at reducing type I error.

ROI analysis was performed to discard global differences in brain metabolism in order to confirm the validity of WB as normalization region. Moreover, we studied other brain areas with the aim of ratifying the previously observed group differences in the voxel-by-voxel

procedure. Thus, masks from WB, NAcc, caudate-putamen (CPu), thalamus (Th) and cortex (Cx) were segmented from the registered MRI. WB data was evaluated by means of standardized uptake values (SUV); while the remaining ROIs data were normalized to the mean intensity of the WB mask. Analyses were performed by a two sample T-test ($p<0.05$) in GraphPad Prism version 5.00.

Results

In vivo study of the DBS effect

DBS in the NAcc produced significant metabolic differences in several brain regions. In fact, voxel-by-voxel analysis revealed a decreased FDG uptake in NAcc, pretectal nucleus and thalamus ($T = 5.66$, $p_{FDR}<0.001$). Moreover, an increased uptake of the radiotracer is located in a cortical cluster that comprises different portions of the cingulate, retrosplenial and parietal association cortices ($T = 5.05$, $p_{FDR}<0.001$) (Fig 4, Table 1). Finally, there is also a slight hypermetabolic region in the ectorhinal–lateral entorhinal cortex ($T = 4.73$, $p_{unc}<0.001$, $p_{FDR} = 0.150$), although it does not overcome the cluster-based correction thresholds.

ROI analysis did not reveal statistically significant global differences in brain metabolism between sham and stimulated animals ($p_{SUV}>0.05$), which supports the validity of WB mean intensity as a normalization method in this study. Furthermore, we found significant changes in NAcc ($p<0.01$), CPu ($p<0.05$), Th ($p<0.01$) and Cx ($p<0.01$) (Table 2).

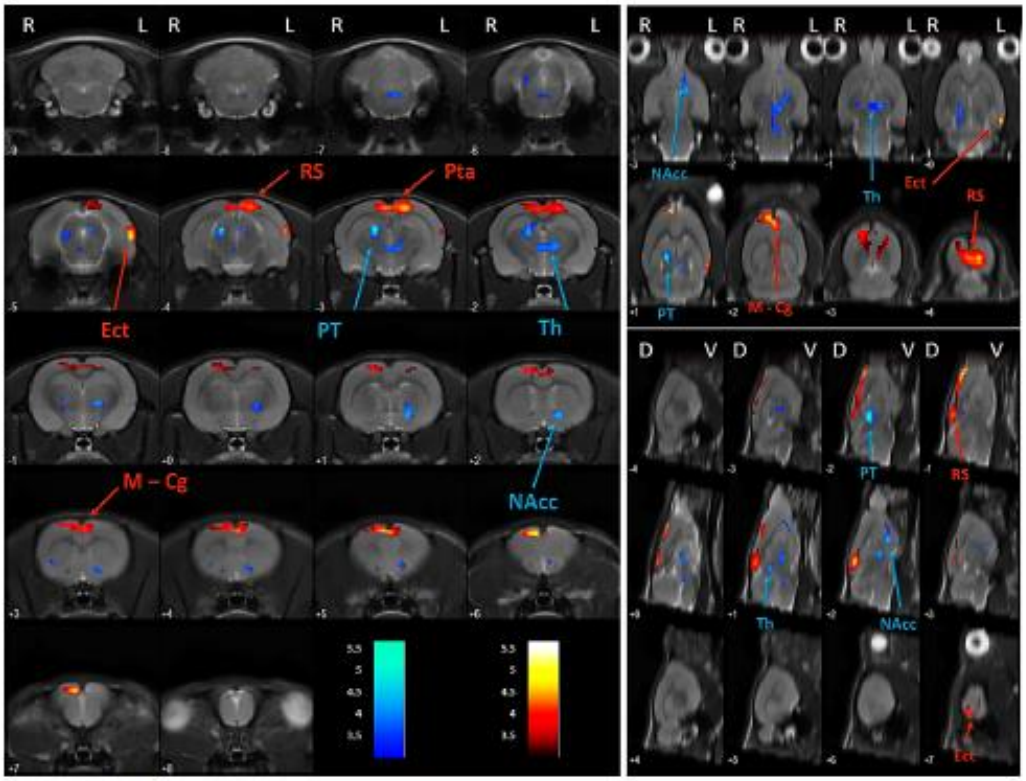

Fig 4. NAcc-DBS effects on brain metabolism. Axial (left), coronal (upper right) and sagittal (lower right) views of the brain. Colored PET overlays on the MR reference indicate increased FDG uptake (hot colors) or decreased FDG uptake (cold colors) 1 day after the end of the stimulation in NAcc. Left (L), right (R), dorsal (D), ventral (V).

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Table 1. Changes in brain metabolic activity following 15 days of NAcc-DBS.

ROI	Hemisphere	k	T	↑/↓	$p_{unc.}$	p_{FWE}	p_{FDR}
NAcc—PT—Th	L & R	654	5.66	↓	< 0.001	< 0.001	< 0.001
Cg—RS—Pta	L & R	923	5.05	↑	< 0.001	< 0.001	< 0.001
Ect—LEnt	L	101	4.73	↑	< 0.001	0.253	0.150

ROI: Region of interest (Cg: cingulate cortex, Ect: ectorhinal cortex, LEnt: lateral entorhinal cortex, NAcc: nucleus accumbens, PT: pretectal nucleus, PTa: parietal association cortex, RS: retrosplenial cortex, Th: thalamus). Hemisphere: left (L) and right (R). k: cluster size, T: T Student. Glucose metabolism: increase (↑) and decrease (↓). p: p value (unc: uncorrected, FWE: family wise error, FDR: false discovery rate).

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Weight

Neither significant differences in initial body weight were observed for the sham (382.20 ±19.78 g) or DBS (358.99±36.00 g) groups, nor in the weight gain between groups (Fig 5). Moreover, no statistically significant difference between slopes was found neither during the DBS treatment nor during the posterior month.

Food and water intake

No significant differences were found in food and water intake (daily or accumulated) between groups. (Fig 6). However, both food and water intake revealed a significant effect of the time (food: $F = 57.76$, $p<0.001$; water: $F = 44.02$, $p<0.001$) and the interaction between factors (food: $F = 14.54$, $p<0.001$; water: $F = 7.748$, $p<0.001$). Moreover, post-hoc test revealed differences between groups in food intake at the beginning of the DBS treatment (daily, day 3: $T = 3.01$, $p<0.05$; accumulated, days 0 to 5: $T = 3.02$, $p<0.05$), showing reduced accumulated food intake in the sham group compared to DBS animals.

Discussion

DBS has recently emerged as a potential therapy for treatment-resistant obesity. Thus, electrically modulating the impaired activity of brain nuclei involved in the pathophysiology of obesity, such as the NAcc, has proven to be effective in clinical studies. However, controversy regarding effectiveness can be found in the literature, and even the modulatory results for NAcc-DBS in the obese brain remain unclear. Therefore, identifying the functional consequences of NAcc-DBS could help to better understand the physiological effects of this approach and to decipher its mechanism of action.

Ours is the first study to apply small-animal FDG-PET to study brain networks undergoing 15 consecutive days of intermittent NAcc-DBS in an animal model of obesity. Thus, we showed that NAcc-DBS modulated glucose metabolism in neuronal networks related to

Table 2. ROIs analysis results.

ROI	WB_{SUV}	NAcc	CPu	Th	Cx
Sham	58.15 ± 8.28	1.30 ± 0.06	1.32 ± 0.05	1.20 ± 0.04	0.90 ± 0.11
DBS	51.38 ± 8.97	1.20 ± 0.02	1.24 ± 0.05	1.12 ± 0.01	1.08 ± 0.07
T	1.50	3.57**	2.91*	3.96**	3.78**

**p<0.01

*p<0.05

Data: mean ± SD, ROI: region of interest (WB: whole brain, NAcc: nucleus accumbens, CPu: caudate putamen, Th: thalamus, Cx: cortex), SUV: standardized uptake value, T: T Student

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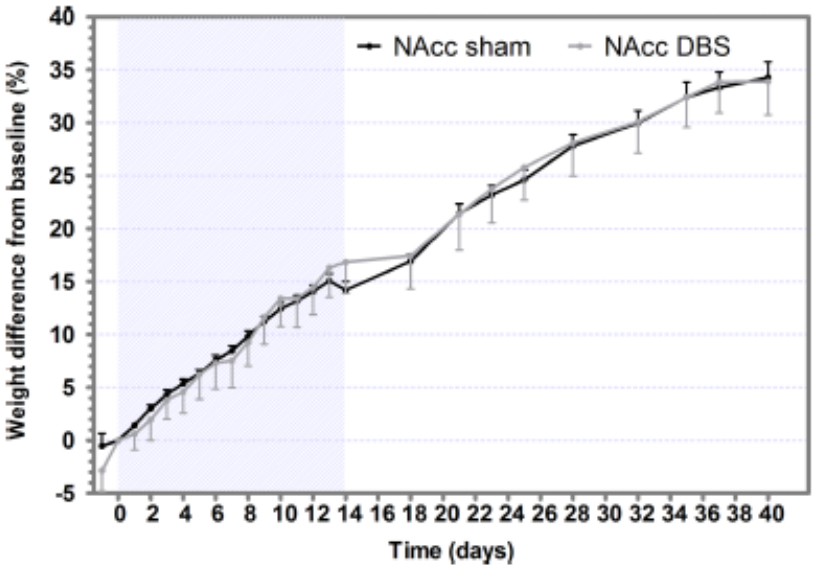

Fig 5. NAcc-DBS does not reduced weight gain. Weight difference (in percentage) with respect to weight recorded before DBS (baseline) in sham and DBS groups. Values are expressed as mean ± SEM. The gray-striped area indicates the DBS application period.

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reward and memory systems. However, we did not observe a significantly lower weight gain in animals that underwent NAcc-DBS.

Brain metabolism

Our PET data reveal a reduction in NAcc metabolism after 15 days of intermittent stimulation (1 hour per day). This finding could be consistent with reported neural informative disruption theories [9,10]. In this sense, the action potentials produced by NAcc would be governed by the stimulation pulses, with the result that DBS would 'capture' NAcc activity [9].

Moreover, one day after the DBS protocol had finished, NAcc-DBS produced a modulation pattern in brain metabolism that is similar to that observed with LH-DBS [27]. In fact, NAcc-DBS decreased glucose metabolism in the caudate-putamen, an effect which has also been

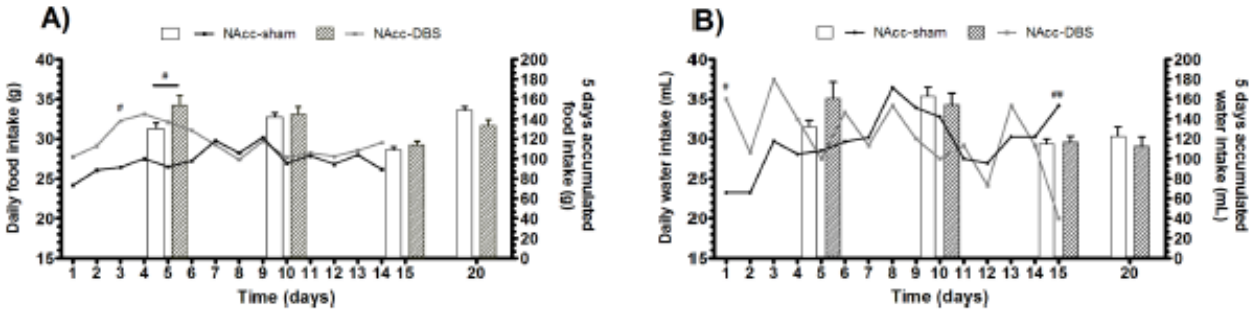

Fig 6. Neither food nor water intake are reduced by NAcc-DBS. Food (A) and water (B) consumption during DBS treatment in sham and DBS groups. Lines represent the mean daily intake of each group, while bars are referred to average consumption over 5 consecutive days. 5 days after stimulation are also shown for the accumulated intake (#$p<0.05$; ##$p<0.01$).

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shown in previous reports on major depression [39,40]. These nuclei are in close communication with the cortex and mediate motor and cognitive functions [41,42]. Indeed, patients with a tendency towards obesity exhibited hyperactivation of the cortico-striato-thalamic pathway in response to a food-dependent reward feeling [43]. Similarly, food images increased glucose metabolism in the striatum of obese patients, while lowering baseline D_2 receptor density [44]. This finding may reflect compensatory downregulation owing to the frequent transient increases in dopamine levels associated with recurrent overstimulation of the reward circuit by eating [45]. Consequently, this overstimulation might be counteracted by NAcc-DBS, as it reduced glucose metabolism in the striatum and thalamus.

NAcc-DBS also increased metabolism in the retrosplenial cortex, which plays a direct role in the consolidation of long-term memory owing to its association with the hippocampus, the parahippocampal region, and the thalamic nuclei [46–48]. Therefore, retrosplenial dysfunction could be caused by hippocampal damage and consequently contribute to the impact of hippocampal damage [49], thus supporting the idea that the thalamic nuclei depend on each other in memory and learning tasks [47,49]. Obesity has been related to defective hippocampal activity [48], which leads to cognitive deficiency in obese patients [50] and obese Zucker rats [51]. Given the strong connectivity between both structures [46], it seems reasonable that the increased metabolism observed in the cortical region might have an effect on defective hippocampal processes, thus improving the damaged memory function described in this animal model. However, behavioral experiments must be performed to corroborate these findings.

Of note, previous studies of the consequences of bilateral NAcc-DBS assessed by *in vivo* functional imaging were mainly focused on the acute effect of the NAcc-DBS (e.g. [52–55]) or applied a continuous stimulation protocol during prolonged periods [56,57], thus preventing them from being compared with the results we report here. To this end, further research should be carried out to uncover the benefits and modulatory consequences resulting from different stimulation protocols.

Body weight and food and water intake

Given the essential role of leptin in the mesolimbic circuit, the NAcc was selected as the DBS target for the obese Zucker rat [58,59]. Consequently, leptin regulates the mesolimbic reward centers, which include the NAcc, thus promoting dopamine (DA) synthesis [59] or release [60] and inducing a food-associated reward. However, the NAcc lacks leptin receptors, and its influence is mediated by VTA and LH [20]. Therefore, obese leptin-resistant animals present impaired feelings of satiety and reward, which lead them to increase their caloric intake [61,62].

Importantly, leptin receptor is present in dopaminergic neurons of the VTA, which directly project to the NAcc and receive afferent inputs from LH neurons expressing leptin receptor [58]. In fact, the increase in DA produced by food intake in the NAcc is inhibited by leptin signals in the VTA, which also induce cessation of food intake [59]. Neto *et al.* reported lower baseline DA and serotonin levels in Zucker rats than in Wistar rats and unchanged NAcc-DA flow when leptin is intranasally administered to Zucker rats, as opposed to a clear increase in Wistar rats [63]. Furthermore, these alterations seem to be exclusive to the obese Zucker rat strain, which exhibited lower striatal DA transporter levels than their lean littermates (+/fa) [64]. These findings reinforced the idea of DAergic modulation induced by leptin in the reward system, which is directly hampered in the Zucker rat.

Given the neural disruption theories applying to the mechanism of action of DBS [9,10], stimulating the NAcc would do the following: 1) isolate this structure from the VTA and LH signals, since they promote food intake owing to the absence of leptin influence; and 2) recover

normal functioning of the NAcc by directly taking charge of its activity. However, in contrast with our previous results with LH-DBS [27], NAcc-DBS did not reduce weight gain, possibly owing to the firm anorexigenic modulation of the LH and ventromedial hypothalamus by leptin [7]. In this sense, although NAcc-DBS was expected to modulate the impaired function of the reward system [65,66], it would not be able to resolve the imbalance caused by the lack of leptin signal in the hypothalamus.

In addition, van der Plasse *et al.* also reported absence of variation in average food intake when DBS was applied to the NAcc core of Wistar rats, whereas stimulation of the NAcc medial shell increased food intake [67]. Then, the fact that the stimulation could have affected both the core and the shell could explain the lack of anti-obesity effect in NAcc-DBS animals.

Importantly, the present study was based on a genetic model of obesity; in other words, a diet-induced model of obesity could show different effects. Zhang *et al.* reported that long-term DBS applied to the NAcc shell attenuated weight gain in rats with diet-induced obesity [68]. Similar results were also obtained in a mouse model of binge eating after NAcc shell DBS, although no related differences were observed after stimulating the NAcc core [22]. These results highlight the need to clarify the role of NAcc substructures before this nucleus can be considered a clinical target for DBS in obesity.

Limitations of the study

Our study is subject to limitations. On the one hand, we cannot extrapolate the effects observed in obese Zucker rats to lean Zucker rats or other animal models of obesity. Nevertheless, our animal model is representative of a particularly resistant kind of obesity, which could potentially benefit from NAcc-DBS depending on the genetic background. We selected the DBS parameters for three main reasons: the success obtained in previous approaches using bilateral DBS [23,24] and similar stimulation protocols [22,27,34,69]; the tolerance associated with continuous DBS treatments [70,71]; and the technical difficulties in obtaining portable rat stimulators in our facilities. Nevertheless, DBS protocols which were closer to the current clinical scenario may reveal larger differences in weight gain [22,68].

Conclusion

In conclusion, we describe an experimental approach to evaluate the neuromodulatory consequences of NAcc as a target of DBS in the treatment of obesity. Although no substantial effects in weight or intake parameters were observed, we proved that brain regions that were functionally impaired in obesity were modulated by an intermittent NAcc-DBS protocol.

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6. The footprint of deep brain stimulation after treatment: A PET and neuroplasticity study in an animal model of obesity.

The footprint of deep brain stimulation after treatment: A PET and neuroplasticity study in an animal model of obesity

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Running title: The footprint of DBS: a PET and neuroplasticity study

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6.1. Abstract

Deep brain stimulation (DBS) effects on brain dynamics continue to rise particular interest. Specifically, DBS reversibility is a major point of debate. In this sense, we previously showed that lateral hypothalamus (LH) and nucleus accumbens (NAcc) stimulation in an obesity rat model elicited metabolic changes in brain structures impaired in this pathology. Here, we examine which metabolic brain changes remained one month after 15 days of stimulation in these targets, together with the presence of neuroplastic modulations.

Positron emission tomography ([^{18}F]FDG-PET) studies were performed one day and one month after the end of DBS. Immunohistochemical analyses of PSA-NCAM, VGLUT1 and VGAT were performed in hippocampus and entorhinal cortex. As a result, NAcc-sham and NAcc-DBS animals showed a common pattern of brain metabolic modulation, probably derived from the electrodes presence. However, LH-DBS animals showed a relative invariability between both time points, and a reduction of neuroplasticity molecules in LH-DBS animals. Thus, the neural steadiness achieved after LH-DBS persisted one month after stimulation withdrawal.

In conclusion, despite applying the same DBS parameters, the long-term effects of stimulation depend on the brain target. Therefore, the functional steadiness reached during DBS would determine the extent of the DBS footprint after removing the stimulation.

Key words: Deep Brain Stimulation, Electrode, Nucleus Accumbens, Lateral Hypothalamus, Positron Emission Tomography, Neuroplasticity.

7. Two sides of the same coin: metabolic and synaptic plasticity imaging studies after lateral hypothalamus DBS in genetic and diet induced obesity.

Two sides of the same coin: metabolic and synaptic plasticity imaging studies after continuous lateral hypothalamus DBS in genetic and diet induced obesity

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Running title: DBS in obesity: PET and synaptic plasticity studies in two animal models

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Significance Statement

Deep brain stimulation (DBS) in the lateral hypothalamus (LH) has been suggested for the treatment of resistant obesity, although its molecular consequences remain unclear. As different responses to LH-DBS would be expected from different pathological etiologies, our aim is to assess the brain modulation derived from continuous LH-DBS in the genetic and high-fat-diet models of obesity. To our knowledge, this is the first study to evaluate the short and long-term consequences of continuous LH-DBS using *in vivo* functional imaging and behavioral approaches. Changes in hippocampal synaptic plasticity were also analyzed. Thus, while LH-DBS did not decrease the weight gain in any model, differences were found with respect to the obesity trigger: 1) the mere presence of the electrodes reduced weight gain on a high-fat-diet model, and 2) LH-DBS promoted the hippocampal functioning in the genetic model. Thus, our study highlights the need for adjusting the DBS protocols to the patients' pathology.

7.1. Abstract

Background: Deep brain stimulation (DBS) in the lateral hypothalamus (LH) has been proposed as a potential therapy for treatment-resistant obesity, given the LH involvement in energy homeostasis and appetite regulation. However, the role of the physiopathological background in LH-DBS therapeutic response is yet unclear.

Methods: Continuous bilateral LH-DBS was applied during 15 days in two animal models of obesity: a genetic model (obese Zucker rat) and an environmental model (high-fat diet-fed Sprague Dawley rat, HF-diet model). *In vivo* positron emission tomography ([^{18}F]FDG-PET) images were acquired one day and one month after the stimulation ending. Besides, animals performed the novel object recognition (NOR) test the day after each PET study. Finally, *ex vivo* immunohistochemical analyses of synaptophysin, VGLUT1 and VGAT were performed in the hippocampus.

Results: Continuous LH-DBS did not reduce weight gain. However, the mere insertion of the electrodes slowed down the weight gain in non-stimulated HF-diet animals. Also, LH-DBS modulated brain metabolism in memory, learning and reward-related regions, but to a different extent in each model. Notably, the hypermetabolism produced in the hippocampus of stimulated Zucker rats, together with the enhanced synaptic plasticity processes in this region, did not translate in cognitive improvements in NOR paradigms. However, long-term memory and attentional benefits were obtained in Zucker operated groups.

Conclusions: The physiopathological mechanisms underlying obesity determine the therapeutic response to LH-DBS. Thus, our results suggest that personalized protocols adapted to the pathological status of the subject would produce more successful results.

Key words: Zucker rat, high-fat diet, FDG-PET, synaptic plasticity, deep brain stimulation, lateral hypothalamus

8. General discussion and Conclusions

8.1. General discussion

This final chapter aims to summarize and contextualize the most relevant findings of the preclinical research works included in this thesis, so as to illustrate a general overview of the main contributions provided to the research field of deep brain stimulation (DBS). Therefore, this chapter offers a general discussion of the main results achieved along this thesis within the context of DBS neurophysiological research, rather than an exhaustive discussion of all the specific findings, which is included at the end of each chapter.

In this sense, the outline followed during the course of this manuscript denotes a progressive incorporation of elements to the complex DBS system under study. Besides, the proposed non-invasive *in vivo* approach, namely functional metabolic imaging using [^{18}F]FDG-PET, has proven to be a very powerful and adequate tool to assess the neurophysiological consequences of different aspects of high-frequency DBS therapy. In fact, the methodological strategies followed allowed us to evaluate the stimulation consequence from acute, chronic and longitudinal perspectives.

Thus, this thesis begins with the description of the most elementary aspect of the DBS neurosurgical technique, such as the electrode insertional effect in the medial prefrontal cortex (mPFC), on a healthy brain (**chapter 3**), thus minimizing the influence of confounding factors in the conclusions reached. Furthermore, the metabolic consequences of acute mPFC-DBS are also covered in this chapter. Then, the focus was on studying the acute DBS effects on three different brain targets with relevance in neuropsychiatric disorders, such as the mPFC, nucleus accumbens (NAcc) and dorsomedial thalamus DM, also in a healthy state (**chapter 4**). Subsequently, given the restricted application guidelines reserved for DBS, it became necessary to address its physiological consequences in a pathology that is potentially suggested to benefit from this therapy. Therefore, the short-term effects of a chronic (fifteen days) and intermittent (one daily hour) stimulation protocol applied in the NAcc on treatment-resistant obesity were evaluated (**chapter 5**). Furthermore, the long-term persistence of brain metabolic changes after DBS withdrawal have been barely explore in the literature. Then, a longitudinal study on the effects of the same intermittent DBS protocol addressed in **chapter 5**, applied to NAcc- and lateral hypothalamus- (LH), were explored one month after the stimulation withdrawal in the same genetic animal model of obesity (**chapter 6**) Finally, this thesis concludes with the evaluation of a more translational stimulation strategy, consisting of a paradigm of chronic (fifteen days) and continuous (twenty four

hours per day) stimulation, in two animal models of different obesogenic backgrounds (**chapter 7**), i.e. a genetic model (Zucker rat) and a high-fat diet-fed Sprague Dawley rat, (HF-diet model).

For the purpose of this discussion, this chapter is divided in two main sections which address, on the one hand, the results accounted by the mere effect of electrode microlesion and, on the other hand, the effects related to the differential stimulation protocols.

8.1.1. Electrode insertional effect

Our results reveal a substantial modulatory effect derived from the mere presence of the electrodes at different levels. In fact, the electrode-induced microlesion effect has been shown to greatly influence the physiological and symptomatic DBS outcomes. In this sense, coinciding with previous reports (van den Berge et al., 2015), we described an **impact of the electrode-induced microlesion on a neuronal network in which the target region (mPFC) plays a key role** (**chapter 3**). Besides, both the insertional effect and the stimulation itself modulated the same brain circuitry. In fact, van den Berge *et al.* have also provided evidence of the permanence of the electrode-derived consequences on brain metabolism in healthy rats beyond one week after surgery (van den Berge et al., 2015). However, in contrast to our findings, they showed a common directionality of the stimulation and insertion effects, the latter being less intense. In our case, the extension of the metabolic pattern obtained when considering the electrode insertion alone resulted considerably less than the brain modulation produced by the mPFC stimulation, although in the same cortico-limbic circuit. Evidence of this common modulatory pathway was the observation of opposite metabolic effects induced in the somatosensory cortex due to the electrode insertion (reduction) and the stimulation (increase) applied to the mPFC, when they were studied in parallel. Thus, the opposite metabolism caused in the somatosensory cortex by the electrode placement and the stimulation alone could respond to neural informative disruption theories of the mechanism of action of DBS (Mcintyre et al., 2004; McIntyre and Hahn, 2010; Chiken and Nambu, 2014, 2015; McIntyre and Anderson, 2016). In fact, these contrary functional patterns would not have been revealed without a dedicated study design, consisting of a longitudinal protocol comprised by three imaging acquisitions: before surgery, after surgery and during stimulation. Moreover, the functional comparison between pre-surgery and post-DBS stages masked the real effect of the stimulation, providing a lower cortical metabolism due to compensation of the possibly temporary hypometabolism induced by the

electrode. Thus, the third chapter of this thesis highlights the potential benefits of understanding the regions affected, together with the magnitude of the modulation induced, by each DBS component in order to personalize the DBS protocols according to the patient needs.

Despite the transitory feature related to the electrode insertional effect (Jech et al., 2012), its persistence on clinical symptoms is highly variable, since it varies from days to months (exceptionally years), or it may even be absent (Hamani et al., 2006). The great variability showed in relation to the clinical and physiological consequences of electrode insertion could largely depend on several factors (e.g. the health status of the subjects, the selected DBS target, the number of microelectrode recording trajectories performed during the surgery, (Pourfar et al., 2009), the time elapsed between the surgery and the test, etc.). Furthermore, the modulatory potential of the microlesion effect led to several clinical benefits in different pathologies, such as in epilepsy (Krishna et al., 2016), neuropathic pain (Hamani et al., 2006) and depression (Perez-Caballero et al., 2014). Of note, the inflammation derived from microlesion has been proposed as the underlying mechanism of these therapeutic outcomes (Perez-Caballero et al., 2014). In this sense, we demonstrated a sustained **slowdown of the weight gain in response to the insertion of electrodes in the LH**, during almost two months, **in a HF-diet model of obesity** (**chapter 7**). Outstandingly, this is the first time that such effects on weight gain are reported. However, no similar benefits were obtained neither with LH-DBS in the same model, nor in genetically-predisposed to obesity subjects (i.e. Zucker rats). In fact, DBS seems to counteract the effect of the electrode implantation in LH, reversing the slow progression in weight gain observed in the HF-diet sham group.

In the case of the Zucker rats, despite not having observed any influence on weight gain, we did observe a progressive cognitive improvement in the novel object recognition (NOR) task, unrelated to the stimulation (Huguet et al., 2009). Thus, Zucker rats suffer from insensitivity to leptin signaling due to the expression of a truncated form leptin receptor in homozygosis. Leptin is a hormone released by adipose tissue in proportion to its size and the amount of lipids ingested during meals. It targets the LH, ventromedial hypothalamus and ventral tegmental area (Arora and Anubhuti, 2006; Taghva et al., 2012), and acts as a signal to stop eating. In consequence, Zucker rats experience hyperphagia, hyperinsulinemia, and hyperlipidemia, leading to spontaneous obesity (Fulton et al., 2000). Furthermore, this lack of leptin signaling has been linked to deficits in hippocampal plasticity and spatial memory, together with an upregulation of molecular

biomarkers of Alzheimer's Disease (Doherty et al., 2013). Therefore, these animals showed a higher risk of suffering from cognitive and memory problems related to hippocampal neurodegeneration (Doherty et al., 2013). In this respect, the metabolic increase shown in the retrosplenial cortex and hippocampus in the longitudinal analysis of these Zucker-sham animals may be related to ongoing plasticity processes of the hippocampal-dependent memory network (Albasser et al., 2007; Vann et al., 2009; Grillo et al., 2011). Thus, these results are indicative of long-term modulatory changes induced by the presence of the electrodes in this circuit, which may induce a recovery from the consequences of neurodegeneration (Doherty et al., 2013). Indeed, both the sham and DBS Zucker groups showed poor performance in the NOR test performed one day after stimulation treatment, which significantly improved one month after stimulation withdrawal. Altogether, these changes might reflect a greater activation of this neuronal circuit which would lead to a recovery of cognitive abilities in the long-term (Huguet et al., 2009).

In accordance to these findings, we showed that the metabolic modulation induced by the presence of the electrodes evolves over time. In fact, **metabolic effects on remote regions but connected to the target structure** were evident even two months after electrode implantation in the NAcc and the LH of Zucker rats (**chapter 6**). In fact, a reduced metabolism in the striatum and somatosensory cortex, along with an increase in the brainstem and hypothalamus are evident at these long-term time points due to the presence of the electrodes and the resulting lesions (Pugh, 2019). Not surprisingly, all these structures are strongly interconnected through hypothalamic projections and belong to the limbic system (Pessoa and Hof, 2015), suggesting that the lesion of a brain structure in a given network evokes distant effects in functionally and physically connected regions. This ongoing modulation may be a reflection of underlying neuroplastic processes in the targeted circuitry (such as those observed in PSA-NCAM levels in the hippocampus and entorhinal cortex). Therefore, these effects might explain the sometimes the long-lasting therapeutic benefits associated with the electrode insertion. Furthermore, although the microlesion impact on brain metabolism related to the DBS implants has been previously demonstrated (Pourfar et al., 2009; van den Berge et al., 2015; Perez-Caballero et al., 2018; Baldermann et al., 2019), the **chapter 6** includes the first work in which continuous physiological changes derived from the electrode presence alone (i.e. without any influence of stimulation) are reported up to two months after implantation.

8.1.2. Deep brain stimulation effects

8.1.2.1. Acute DBS protocol

As anticipated, a recurrent conclusion stating that "the lesion and stimulation of a brain structure in a certain network evokes distant effects in functionally and physically connected regions" is repeatedly reached in functional neuroimaging works addressing the DBS physiological effects on the brain (Kuhn et al., 2010; Knight et al., 2013; Albaugh et al., 2016), as well as in those included in this thesis. Nevertheless, the acute stimulation of specific targets that share interconnections with the same neural circuitry, as mPFC, NAcc and MD (Mitchell and Chakraborty, 2013), evoked **differential brain metabolic patterns according to the stimulated structure** (**chapter 4**). This is of interest given that different brain structures have been proposed as potential DBS targets for the same neuropsychiatric disorder, as well as the same DBS target has been proposed for several pathological conditions (Kuhn et al., 2010). In fact, the hypofrontality reversion obtained during acute mPFC-DBS supports its potential role in major depressive disorder (Hamani et al., 2010; Hamani and Nobrega, 2012). Furthermore, NAcc-DBS has traditionally been related to reward and pleasure, which are affective levels impaired in mostly all psychiatric conditions (Kuhn et al., 2009, 2010; Halpern et al., 2011). Additionally, acute NAcc-DBS promoted the activity in the subiculum-hippocampal area, whose disfunction was related to memory and cognitive impairment in Alzheimer's disease, schizophrenia or obesity, among others (Small et al., 2011). Similarly, the metabolic promotion in the striato-thalamic network, evoked as a consequence of acute DM-DBS, supports its potential application for the treatment of cognitive and attentional deficits in schizophrenia (Lehrer et al., 2005), Alzheimer's disease (Aggleton et al., 2016) and dementias (Ishii, 2014). However, all the DBS approaches within the psychiatry scenario remain at a research level, implying that there is still no consensus on which area provides more benefits for each disorder (Lipsman and Lozano, 2015).

Despite the differential metabolic patterns obtained with acute DBS in mPFC, NAcc and DM, their stimulation also led to a common metabolic influence in specific brain regions. In fact, DBS to all three brain targets induced an increase in the metabolic activity of the piriform cortex. The piriform cortex is the largest area of the mammalian olfactory cortex, and contains the most susceptible neural circuits of all forebrain regions for electrical (or chemical) stimulation (Ebert and Lehmann, 1996; Boix-Trelis et al., 2009). Thus, immunohistochemical studies have shown that, during electrical stimulation of limbic brain regions, the piriform cortex exhibits the most consistent increase in glucose

utilization (Boix-Trelis et al., 2009), similar to our findings. Furthermore, in addition to this olfactory region, we found that the stimulation of both mPFC and NAcc decreased the glucose metabolism in the brainstem. This effect could respond to the reciprocal connection of the mPFC with the dorsal raphe nucleus and ventral tegmental area, both placed in the brainstem and origin of important serotonergic and dopaminergic connections, respectively. Therefore, both a synaptic depression (Wang and Kaczmarek, 1998; Zucker and Regehr, 2002) on distant efferences due to mPFC, and an orthodromic neural jamming (Hammond et al., 2008; Carron et al., 2013), may explain the observed metabolic effects on the brainstem. In the case of the NAcc, this nucleus receives strong dopaminergic afferences from the ventral tegmental area. In consequence, the stimulation of NAcc at high frequencies could lead to an inhibition of dopaminergic activity at the ventral tegmental area level by means of an antidromic neural jamming (Hammond et al., 2008; Carron et al., 2013), resulting in a decreased glucose metabolism in the brainstem. Consistent with these results, antidepressant medications, such as citalopram, have been shown to decreased the activity signal in the brainstem (Sekar et al., 2011). Thus, both mPFC and NAcc brain regions have been proposed as DBS targets for the treatment of resistant major depressive disorder due to their related antidepressant, anxiolytic and precognitive properties (Hamani et al., 2014; Millet et al., 2014).

8.1.2.2. Intermittent DBS in genetic obesity

When considering a chronic (fifteen days) and intermittent (one hour per day) **protocol of NAcc-DBS in the Zucker rat, we found a modulatory effect of the stimulation in regions related to reward** (hypometabolism in the striato-thalamic pathway) **and memory** (hypermetabolism in the retrosplenial cortex), which are indeed impaired in obesity patients (Wang et al., 2001; Winocur et al., 2005; Rada et al., 2010; Mueller et al., 2012) (**chapter 5**). In fact, patients with a tendency towards obesity exhibited an hyperactivation of the cortico-striato-thalamic circuitry in response to a food-dependent reward feeling (Stice et al., 2009, 2011). In this respect, NAcc-DBS could counteract the exacerbated functioning of this brain network. Regarding the hypermetabolism induced in the retrosplenial cortex, it is outstanding that initial metabolic increases in this network, at the level of the subiculum-hippocampal area, were already seen with the acute DBS protocol (**chapter 4**). In fact, the retrosplenial cortex plays a direct role in the consolidation of long-term memory due to its association with the hippocampus, the parahippocampal region and the thalamic nuclei (Vann et al., 2009; Miller et al., 2014).

Therefore, given the strong connectivity between hippocampus and retrosplenial cortex, it seems reasonable that the increased metabolism induced by NAcc-DBS, both during acute or chronic protocols, might have an effect on defective hippocampal processes, thus improving the damaged memory function described in obesity (Small et al., 2011).

Besides, we found similar results with intermittent (Soto-Montenegro et al., 2014) and continuous LH-DBS protocols, suggesting that both NAcc-DBS and LH-DBS would modulate the same limbic network. Furthermore, beyond the remote metabolic effects induced by NAcc-DBS, we did observe a focal decreased brain metabolism in the targeted region due to NAcc-DBS. This metabolic reduction may either respond to a reflection of the antidromic effect of DBS on ventral tegmental area (Hammond et al., 2008; Carron et al., 2013), or a focal inhibition in the targeted region due to the high-frequency stimulation applied (Beurrier et al., 2001). Altogether, these results are in line with the neural informative disruption theories proposed as a mechanism of action of DBS (Chiken and Nambu, 2014, 2015). Therefore, NAcc-DBS would theoretically recover NAcc function by isolating this nucleus from the food-intake promoting signals sent by the ventral tegmental area and LH due to the absence of leptin influence in this animal model. Thus, NAcc-DBS would supposedly improve activity patterns in the cognitive and reward systems. However, the lack of effect of NAcc-DBS on weight gain would indicate that this stimulation approach could not completely overcome the pathological brain functioning in this animal model.

In this sense, these results contrast with a previous article from our group, in which the application of the same intermittent DBS protocol in the LH of the Zucker rat was associated with a weight gain reduction in the long-term (Soto-Montenegro et al., 2014). Of importance, NAcc was selected as the DBS target for the obese Zucker rat given the essential role of leptin in the mesolimbic circuit, as it was previously introduced (Leinninger et al., 2009; Perry et al., 2010). Consequently, leptin regulates the mesolimbic reward centers, which include the NAcc, thus promoting the dopamine synthesis (Perry et al., 2010) or the release of this neurotransmitter (Dang et al., 2016). As a consequence, it induces a food-associated reward. However, NAcc lacks leptin receptors, and its influence is mediated by the ventral tegmental area and the LH. Therefore, leptin-resistant obese animals present impaired feelings of satiety and reward, which lead them to increase their caloric intake (Wang et al., 2001; Rada et al., 2010). In this sense, considering the neural disruption theories applied to the mechanism of action of DBS (Chiken and Nambu, 2015; McIntyre and Anderson, 2016), it was expected that NAcc-

DBS would modulate the impaired function of the reward system (Geiger et al., 2009; Green et al., 2011). Therefore, the absence of weight reduction may derive from the strong anorexigenic modulation of the LH and ventromedial hypothalamus by leptin (Halpern et al., 2008), thus being unable to resolve the hypothalamic imbalance caused.

Another plausible explanation for the lack of therapeutic improvement after NAcc-DBS would rely on the fact that the stimulation could have affected both the core and the shell of the NAcc. Thus, van der Plasse *et al.* reported an absence of variation in average food intake when DBS was applied to the NAcc core in Wistar rats, whereas the stimulation of the NAcc medial shell increased food intake (van der Plasse et al., 2012). Therefore, despite being the NAcc core the aimed target for DBS in this work, we could not ensure that the focal modulatory effects could have extended to adjacent regions. Furthermore, different results can be obtained in other animal models of obesity treated with NAcc-DBS protocols. In fact, Zhang *et al.* reported that long-term DBS applied to the NAcc shell attenuated weight gain in diet-induced obese rats (Zhang et al., 2015). Similar results were also observed in a mouse model of binge eating after stimulation of the NAcc shell, although no related differences were observed after stimulating the NAcc core (Halpern et al., 2013). These results highlight the need to clarify the role of NAcc substructures before this nucleus can be considered a clinical target for DBS in obesity.

Furthermore, when evaluating the long-term effects of DBS withdrawal in both NAcc and LH in this animal model, we found that, one month after the end of stimulation, the **magnitude of the long-term persistence of the DBS metabolic modulation** depended on the targeted brain region (**chapter 6**). In fact, at this time point, we found a common metabolic pattern in LH-sham, NAcc-sham and NAcc-DBS animals in limbic regions interconnected through hypothalamic projections (Pessoa and Hof, 2015) (as previously discussed). These results support the reversibility feature usually related to DBS stimulation consequences, but only in the case of NAcc-DBS. In fact, the similar modulation observed between NAcc-sham and NAcc-DBS groups one month after finishing the treatment, together with the lack of therapeutic effect on weight gain, revealed a predominance of the electrode insertional effect on brain metabolism over the stimulation consequences in the long-term. Together, both factors (i.e. absence of therapeutic effect and similarity with NAcc-sham metabolic modulation) would reflect a weak and transient impact on the brain network modulated in the long-term, unable to produce an evident clinical benefit. As a result, longer NAcc-DBS periods would be necessary to influence the subject symptoms (Ashkan et al., 2017). Furthermore, the

hypometabolism of the ectorhinal cortex observed in NAcc-DBS animals suggest a certain reversibility and rebound effect of the stimulation-derived metabolic modulation, in agreement with previous reports (Kuhn et al., 2009; Ewing and Grace, 2013; Ooms et al., 2014). In fact, the absence of metabolic changes in NAcc-sham animals in this region, together with the opposite effects induced by the stimulation alone one day after stimulation withdrawal, support this theory. Accordingly, Ewing and Grace reinforced this idea with their finding of a reduction in local field potentials towards baseline levels just forty eight hours after completion of high-frequency stimulation in the NAcc (Ewing and Grace, 2013). From the clinical side, a one week inactivation of the stimulation in obsessive compulsive disorder patients led to a relapse of the positive symptoms and a rebound of the negative ones (Ooms et al., 2014). Therefore, the same stimulation parameters would have exerted a differential degree of affectation regarding the specific structure, organization and connectivity of each brain region with the targeted nucleus.

In contrast, LH-DBS rats did exhibit greater permanence of the DBS effects induced immediately after the end of the stimulation treatment. Furthermore, this brain physiological invariance is supported by the reduced levels of PSA-NCAM observed in the hippocampus and the entorhinal cortex in LH-DBS animals, one month after stimulation withdrawal. This could explain the low levels of VGAT obtained in LH-DBS group, as PSA-NCAM is a potent regulator of inhibitory cortical networks (Castillo-Gómez et al., 2011). Additionally, the most rostral region of the entorhinal cortex, as the piriform cortex layer II, harbors immature PSA-NCAM expressing neurons that progressively mature into excitatory neurons during adult life (Bonfanti and Nacher, 2012). In this respect, the decrease in the expression of PSA-NCAM may boost their differentiation. Furthermore, LH-DBS proved to produce a clinical benefit in these animals in the long-term, namely a weight gain reduction (Soto-Montenegro et al., 2014), suggesting that the preservation of the DBS consequences on brain metabolism may lead to a symptomatic improvement in the treated subject. In agreement with this, a maintenance of clinical symptoms has been already observed after DBS withdrawal in patients with dystonia (Ruge et al., 2011). In this work, Ruge *et al.* described a great neurophysiological instability after DBS withdrawal under the apparent clinical calm. This finding led them to hypothesize that the stimulated system would be seeking a balance which would make the pathological symptoms evident when it was reached (Ruge et al., 2011). Therefore, the long-term DBS effects in the brain could be consequence of a functional stability achieved during stimulation, which would underlie a prolonged positive outcome for the subject, as in LH-DBS Zucker rats (Soto-Montenegro et al., 2014). At this respect, DBS

modulation should overcome a certain affectation threshold in order to elicit long-term and persistent clinical benefits.

In addition to the regional differences in brain metabolism, DBS induced changes in **neuronal plasticity patterns**. The effects of DBS on the expression of PSA-NCAM support the previously introduced idea that the targeted circuitry is subjected to continuos long-term neuroplastic changes. Particularly interesting are the changes observed in the hippocampus after NAcc stimulation, since both limbic regions are intensely interconnected (LeGates et al., 2018; Perez and Lodge, 2018). Closely related to our results, Schmuckermaier *et al.* found that intermittent NAcc-DBS (one daily hour, as in our study) during only seven consecutive days promoted the neural activity and neurogenesis in the hippocampus (Schmuckermair et al., 2013). In addition, they also described antidepressant effects, obtained by improvements in the performance of the forced swimming and tail suspension tests, in a murine model of enhanced anxiety and depression (Schmuckermair et al., 2013). Thus, these findings support the potential role of NAcc-DBS in major depressive disorder (Hamani et al., 2014; Millet et al., 2014). In our study, the increase in PSA-NCAM expression in the hippocampus could be related both to changes in excitatory or inhibitory neurons, since in this limbic region, and especially in the CA1 strata where the neuroplastic changes were evaluated, PSA-NCAM is expressed by both cell types (Gómez-Climent et al., 2011). In fact, there is a tendency towards an increase in the levels of VGLUT1 and VGAT in the hippocampus of NAcc-DBS groups.

Taken together, our results support the evidence that the physiological and clinical outcomes after DBS, not only in the short- but in the long-terms, are strongly dependent on the brain target selected for stimulation, even under identical stimulation protocols and pathological conditions. Therefore, under fixed stimulation conditions, DBS elicits the simultaneous activation of several molecular mechanisms, both at local and distant levels, which might interact but could not be gathered under a simple theoretical explanation (Blaha, 2016). In this sense, the duration of the modulation induced by the impact of the electrical stimulation on a certain brain target would determine the persistence of the derived clinical benefits.

8.1.2.3. Continuous LH-DBS in genetic and diet-derived obesity

The pathological background (environmental vs. genetic) and degree have proven to be decisive in relation to the DBS outcomes. In fact, as previously introduced, we obtained a significant weight-gain slowdown in the HF-diet model of obesity due to electrodes

implantation itself in the LH (**chapter 7**). However, **no similar impacts on weight progression were induced after 15 days of continuous LH-DBS**, neither in the same animal model, nor in the Zucker rat. In the case of the HF-diet animals, our results strongly contrast with Sani *et al.* findings, who reported a sustained absence of weight gain in HF-diet rats treated with continuous LH-DBS (Sani et al., 2007). In that case, the higher stimulation frequencies used (185 Hz) may have greater impact on hypothalamic activity, thus resulting in an effective weight gain reduction (Sani et al., 2007). Nonetheless, previously reported reductions in weight gain are normally unrelated to changes in food intake after effective hypothalamic DBS in various animal models (Sani et al., 2007; Lehmkuhle et al., 2010; Melega et al., 2012; Torres et al., 2012; Soto-Montenegro et al., 2014). Therefore, other physiological mechanisms should underlie the observed weight reductions, such as the increase in metabolic rate (Whiting et al., 2019). Moreover, as presented above, Zucker-DBS and Zucker-sham groups showed an overlapping weight-gain progression during the whole study. In fact, a similar lack of effectivity was obtained after long-term LH-DBS in patients with Prader Willy syndrome (Franco et al., 2018). Prader Willi syndrome is a rare genetic condition in which the affected patients suffer from obesity due to a hypothalamic dysfunction that causes hyperphagia (Franco et al., 2018), similar to our genetic rat model. Curiously, our results contrast with previous findings, where intermittent LH-DBS was effective in reducing body weight gain in the Zucker rat (Soto-Montenegro et al., 2014). In this regard, our continuous stimulation approach would not be enough to trigger the necessary physiological mechanisms, underlying an effective weight reduction, in any of the studied models in this thesis. Therefore, at least in the genetic model, an intermittent LH-DBS protocol seems to be more effective in reducing the weight-gain in the long-term (Soto-Montenegro et al., 2014). This is not an isolated case, since intermittent stimulation protocols have proved to be beneficial in other neuropsychiatric disorders, such as in schizophrenia (Shiozawa et al., 2016).

As presented in the previous section of this discussion, imaging studies in obese individuals have also reported functional abnormalities in the striatum (Rothemund et al., 2007). In this sense, one day after the end of stimulation, LH-DBS diminished the glucose metabolism in the dorsal striatum in the HF-diet model, coinciding with previous modulatory evidences in the same DBS target (Soto-Montenegro et al., 2014). Thus, this modulation may revert the striatal overactivation observed in obese individuals, which has been considered a main mechanism to explain the enhanced sensitivity to food cues and overeating (Nummenmaa et al., 2012; Val-Laillet et al., 2015). This is remarkable

given that the increased activity in the striatum has been related to a greater reward response and food addiction (Farr et al., 2016). Therefore, these network abnormalities describe a potential physiological mechanism which could underlie the improvements in the urge to eat obtained in previous clinical studies (Whiting et al., 2013, 2019). Nonetheless, LH-DBS did not affect these areas in the genetic model. However, in the Zucker strain, the insatiability that underpins the compulsive eating behavior manifested in these animals derives from an insensitivity to leptin (as it was previously explained) (Wang et al., 2014). Therefore, the beneficial mechanisms motivated by the modulation of the brain reward system would be relegated to a secondary place in this model, being overshadowed by the consequences of the lack of leptin signaling.

Furthermore, we observed contradictory modulation patterns due to LH-DBS in the HF-diet and the Zucker rat at the hippocampal level. In fact, this opposite hippocampal response may derive from the differential leptin status in both animal models. Thus, an increase and a decrease in the hippocampus metabolism were shown by Zucker-DBS and HF-diet-DBS groups, respectively. While the obesogenic trigger in the case of the Zucker rat is precisely the lack of leptin signaling, the high-fat diet protocol responsible for the obesogenic condition in HF-diet animals would not be sufficiently strong to alter the leptin signaling. In this respect, hippocampal-dependent memory and learning deficits have been described in both animal models (Winocur et al., 2005; Cordner and Tamashiro, 2015), as well as in obese patients (Elias et al., 2003). However, these deficits have been related to an impaired leptin signaling in the hippocampus, which is crucial to induce synaptic plasticity processes involved in learning and memory (Doherty et al., 2013; van Doorn et al., 2017). Therefore, the opposite hippocampal response obtained in HF-diet DBS group may derive from a still-healthy leptin signaling in these animals and in their sham group. In fact, this lack of altered leptin functioning in HF-diet animals would prevent to see a favorable therapeutic effect of LH-DBS at the hippocampal-dependent memory network.

Accordingly, these metabolic findings are supported by the results obtained in the hippocampal synaptic plasticity. On the one hand, no significant effect of LH-DBS was observed in synaptophysin levels in the HF-diet model. However, LH-DBS induced a sustained metabolic reduction in this area, which is in line with the reduction of synaptophysin levels observed after fornix-DBS in healthy rats (Aldehri et al., 2019). Therefore, although high-fat diets have been related with synaptic defects (Page et al., 2014; Nam et al., 2017), longer and earlier periods of exposure to these obesogenic diets

may be needed to impact on leptin signaling and hippocampal synaptic state. This reasoning would explain the lack of DBS-related synaptic changes in the HF-diet model. On the other hand, LH-DBS produced a great significant increase in synaptophysin levels in almost all the CA1 hippocampal layers studied in the Zucker groups. In fact, similar synaptogenic increases were obtained after DBS applied in the hippocampal-memory network (Gondard et al., 2015, 2019). However, the metabolic and synaptic modulation obtained did not translate into DBS-derived cognitive improvements, as evaluated using the NOR paradigm. In fact, as discussed above, both sham and DBS Zucker groups significantly improve their performance in the NOR test one month after stimulation withdrawal. Therefore, the cognitive benefit observed in Zucker animals was apparently unrelated to the LH-DBS applied.

In this regard, the initial and transient metabolic increase induced by LH-DBS in the hippocampus of Zucker animals may indicate an incipient growth in synaptic plasticity, also supported by the higher levels of synaptophysin obtained in the Zucker-DBS group. However, the lack of DBS-derived cognitive improvement, together with the absence in neurotransmitters levels, suggested that the synaptogenic effect induced by DBS in the hippocampus would not be enough to elicit a better memory-learning performance. Therefore, the effective maturation of the newly generated synapses, reflected in an increase of neurotransmitters activity, and a consequently neuronal reorganization, would be necessary in order to produce the expected behavioral benefits (Mayford et al., 2012; Ashkan et al., 2017).

Of note, we have found that fifteen days of continuous stimulation in the LH produce an important impact on neuronal plasticity in a remote but connected area, such as the hippocampus. This process could be explained through the activation of the hypothalamic-hippocampal circuit (U'ylings et al., 2000; Bouret et al., 2004), being these hypothalamic afferents responsible for the stimulation of the granular neurons of the dentate gyrus, and in turn transfer an excitatory signal to CA3 (Leranth and Nitsch, 1994) and CA1 (Freund and Buzsaki, 1988) (our region of study in the hippocampus).

Finally, the longitudinal analyses of the metabolic brain imaging studies also reinforce the evident physiological differences manifested by both animal models. Thus, while very few changes appeared over time in HF-diet animals, both Zucker groups (sham and DBS groups) presented a common metabolic pattern one month after stimulation ending, in comparison to the [^{18}F]FDG-PET studies acquired one day after the end of DBS treatment. This pattern involved increases in thalamus and superior

colliculi/hippocampus, together with reductions in several prefrontal cortex subregions, such as the cingulate and the insular cortices. Of interest, these results coincide with the brain metabolic consequences of exposing obese Zucker rats to food cues (Thanos et al., 2008). Also, these regions are interconnected between them and the hypothalamus in order to control the energy balance (Farr et al., 2016). Therefore, the functioning of these neuronal circuitry is affected in obese subjects given the hormonal disequilibrium derived from the impairment of the orexigenic and anorexigenic signals, and their influence on brain dynamics (Winocur et al., 2005; Leinninger et al., 2009; Fernández-Galaz et al., 2010; Wallner-Liebmann et al., 2010; van Zessen et al., 2012; van Swieten et al., 2014; van Doorn et al., 2017). Thus, the longitudinal changes observed in both Zucker groups prove the occurrence of ongoing alterations on the hypothalamic circuit, which were not halted neither with LH-DBS nor with electrode implantation. However, the absence of similar long-term changes in the HF-diet groups suggests a lower influence of their respective obesogenic trigger, namely the high-fat diet ingested, over the critical brain deficits related to obesity.

8.1.3. Limitations and possible future lines of research

The studies included in this thesis are subjected to certain limitations which, altogether, provide the basis for possible future lines of research.

First, the small sample size included in some of the experimental procedures, which responds to the aim of complying with the international 3-R's principle in animal research. In this sense, we prioritized the dissemination of preliminary, although relevant, results that would serve as a basis for the development of future, more extensive projects. Therefore, we applied strict statistical thresholds in order to show more accurate results, which lead us to discard potentially important effects that do not reach statistical significance. Furthermore, the number of animals in each group proved to be large enough to detect statistically significant changes in glucose metabolism and neuroplasticity markers derived from the DBS consequences.

Second, each DBS approach was only tested in healthy or pathological models, which prevent us to extend the observed changes to other animal models of different diseases or healthy animals of the same strain, respectively. Furthermore, only males were considered in the different experiments carried out. Therefore, extending the experimental strategies developed in this thesis to the study of the DBS effects in females and in other animal models (pathological or naïve), provides a great opportunity to

increase the current knowledge on DBS-derived neuromodulation, and bring the experimental strategies, followed in this thesis, closer to the clinic.

Third, the voxel-based [^{18}F]FDG-PET imaging analyses along the different chapters of this thesis were not corrected for multiple comparisons at voxel level due to the impossibility of assuming independence between adjacent voxels (Stephan, 2017). Instead, only significant regions larger than 50 activated connected voxels were accepted with the aim of reducing type I error. Also, a cluster-based correction was applied in order to minimize type II error.

Furthermore, specific DBS parameters and protocols were evaluated in this thesis. Therefore, it would be interesting to carry out a "dose-response" study for each brain target and condition examined. Thus, testing different combinations of DBS parameters (e.g. modifying the current intensity, the frequency levels or the duration of the stimulation treatment) according to the pathophysiology and brain target under study, would allow to obtain a more suitable and personalized protocol, with greater clinical impact, in relation to the treatment response.

Also, although [18F]FDG-PET is a really useful technique to study the brain dynamics in vivo, its application in the clinical field is being more and more substituted by other non-ionizing techniques, such as functional magnetic imaging (fMRI). Therefore, the evaluation of the DBS modulatory effects on brain activity using fMRI in animal models would suppose a promising future line of research, with great translational perspective.

Finally, DBS stimulates the target region in an unspecific way, namely involving all the cell types and fibers surrounding the tip of the electrode, and without necessarily involving common molecular mechanisms through the neural network. Furthermore, the lack of a complete understanding of the physiopathology of several neuropsychiatric disorders, highlights the challenge of applying DBS to these patients. In this context, optogenetics and chemogenetics appear as revolutionary techniques, which allow to selectively manipulate the functioning of a specific cell subpopulation, and study the molecular and behavioral consequences (Vlasov et al., 2018). Despite being still far from reaching a clinical application, these new experimental methods offer very powerful tools to study specific pathological and therapeutic mechanisms in the neural circuits and systems involved. Therefore, the selective manipulation of specific brain networks during the course of the disease, or during the application of DBS, represents an innovative and elegant possibility for future research.

8.1.4. Concluding remarks

In summary, all the presented original papers highlight the need to fully understand the physiological and symptomatic consequences of DBS in order to minimize the current "trial and error" procedures to adjust DBS parameters to the patients' needs. Furthermore, [^{18}F]FDG-PET serves as a methodological link throughout this thesis, allowing the evaluation of the most elementary effects of DBS, such as the electrodes presence, to more complex DBS paradigms in different animal models. As a result, we provided evidence that I) both the electrode itself and the applied stimulation on a brain area modulate the same neuronal network, but to a significantly different extension or degree; II) the acute stimulation of different brain targets involved in the same neuronal circuitry induce distinct metabolic patterns, highlighting their potential role in different psychiatric pathologies; III) intermittent and chronic NAcc-DBS in an animal model of genetic obesity modulates the brain metabolism in regions functionally impaired in this condition (i.e. reward and memory systems), without affecting weight gain; IV) LH-DBS, but not NAcc-DBS, induces sustained and persistent metabolic and neuroplastic effects in the same genetic model of obesity, which may explain the differential long-term benefits on pathological symptomatology; V) the underlying pathophysiological environment of a specific condition strongly determines the symptomatology, brain metabolism, behavioral and synaptic effects induced by LH-DBS. Therefore, there is a need to recognize the influence of each patient's physiopathological background in the final therapeutic results obtained with DBS treatment, which can be fully addressed with specific study designs.

In this sense, the studies included in the present thesis are original contributions to the DBS and neuropsychiatric research fields from a preclinical perspective. Thus, we have described several *in vivo* experimental approaches to evaluate the neuromodulatory consequences of different DBS protocols, applied to distinct pathological conditions, by means of translational and feasible techniques routinely applied in the clinical scenario. Therefore, the present thesis aims to provide new and original strategies to evaluate the *in vivo* DBS consequences and serve as baseline for future clinical and preclinical studies in the field.

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8.2. Conclusions

The main contribution of this thesis is to describe the neuromodulation consequences of different DBS approaches on brain metabolism, by means of preclinical PET studies with [^{18}F]FDG, in healthy rats and in two animal models of obesity. Furthermore, special emphasis was placed on addressing hypotheses that sequentially incorporate different aspects of the DBS treatment.

The specific conclusions derived from the studies included in this thesis are:

1. Regarding the differential metabolic consequences of the electrode insertion and the acute stimulation in the mPFC:
   1.1. The mere electrode insertion induced a metabolic reduction in sensory areas connected to the targeted region.
   1.2. The mPFC-DBS resulted in a distinct brain metabolic pattern than the electrode insertion, with more brain areas affected, including circuits in which the mPFC plays a key role, such as limbic and reward systems.
   1.3. The simultaneous consequences of the electrodes and the stimulation revealed lower cortical activation compared to the stimulation alone, showing a compensation of the cortical hypometabolism derived from the presence of the electrodes.
2. Regarding the DBS response in three brain targets (i.e. mPFC, NAcc and DM) with implications in mental disorders:
   2.1. The effects of high-frequency DBS on neuronal activity involve modifications of complex networks rather than global or isolated regions.
   2.2. DBS induces several mechanisms that lead to net inhibitory and excitatory effects irrespective of the function, suggesting a complex modulation of activity along cortico-basal ganglia-thalamo-cortical and the cerebello-thalamo-cortical circuits.
   2.3. DBS in each brain target influenced a different set of structures at a distance from the target despite sharing interconnections with the same circuitry. This might be relevant for addressing specific pathophysiological conditions, suggesting the need for individualizing the target selection according to the specific neural modulatory requirements of the patients.

3. Regarding the effects of fifteen days of intermittent NAcc-DBS protocol in a genetic animal model of obesity:
   3.1. NAcc-DBS modulates glucose metabolism in neuronal networks related to reward and memory systems, which are indeed functionally impaired in obesity.
   3.2. NAcc-DBS does not induce neither a significantly lower weight gain nor decreases in food intake in this animal model, probably due to not being able to counteract the pathophysiological mechanisms underlying this obesity condition.
4. Regarding the long-term effects after 15-days of intermittent LH-DBS or NAcc-DBS in a genetic model of obesity:
   4.1. The magnitude of the long-term persistence of the DBS brain metabolic modulation depends on the brain target (LH or NAcc), even despite belonging to the same neural circuit and in the same disease.
   4.2. The NAcc-DBS results supported the well-known reversibility related to DBS, only obtaining changes in brain metabolism in those structures modulated by the electrode presence, although an increase in hippocampal neural plasticity due to NAcc-DBS was found.
   4.3. Scarce changes in brain metabolism were obtained one month after LH-DBS withdrawal, suggesting a preservation of the stimulation effects, also supported by the reduction in neural plasticity molecules in the hippocampus and entorhinal cortex.
5. Regarding the short and long-term effects after 15 days of continuous LH-DBS in two animal models of obesity (genetic and diet induced models).
   5.1. In the high-fat diet model, LH-DBS does not affect food intake, weight gain, learning-memory abilities or hippocampal synaptic plasticity, while it reduces the metabolism in the hippocampus and striatum.
   5.2. In the genetic model, LH-DBS does not elicit any changes in food intake or weight gain, while it increases the metabolism and the synaptogenic activity in the hippocampus. However, LH-DBS could not recover the hippocampal-dependent memory deficits of this model.
   5.3. The electrodes presence induces a weight gain slowdown in the sham group of the high-fat diet model while, in the genetic model, it produces a long-term improvement in learning-memory abilities.