Impact d'une stimulation transcrânienne par courant continu (tDCS) frontal sur le réseau dopaminergique chez le sujet sain

Clara Fonteneau

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Clara Fonteneau

Impact of a single frontal transcranial direct current stimulation on the dopaminergic network in healthy subjects

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Plaze Marion, Praticien hospitalier, Hôpital Sainte Anne, Paris	Examinatrice
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Résumé

La stimulation transcrânienne par courant continu (tDCS) sert à moduler l'activité neuronale. Elle consiste à appliquer un faible courant constant entre deux électrodes placées sur le cuir chevelu. Deux montages semblent efficaces pour moduler les capacités cognitives et/ou soulager des symptômes cliniques. Cependant, les effets neurobiologiques de la tDCS sont encore mal connus. Ce travail de thèse a tenté de clarifier les mécanismes cérébraux de la tDCS chez les sujets sains, en particulier en lien avec le système dopaminergique. En utilisant un design randomisé en double aveugle, nous avons combiné une session de tDCS online avec plusieurs modalités d'imagerie (PET ou PET-IRM simultanée) chez le sujet au repos. Une première étude (n=32, 2mA, 20min) a montré que la tDCS bifrontale induit une augmentation de la dopamine extracellulaire dans le striatum ventral, impliqué dans le réseau de récompense-motivation, après la stimulation. Une seconde étude (n=30, 1mA, 30min) a montré que la tDCS fronto-temporale induit une augmentation de la dopamine extracellulaire dans la partie exécutive du striatum et une diminution de la perfusion dans une région du réseau du default mode (DMN), après la stimulation. L'analyse des données de cette étude est toujours en cours. Dans l'ensemble, ce travail fournit la preuve qu'une seule session de tDCS frontale peut impacter le système dopaminergique dans des régions connectées aux zones corticales stimulées. Par conséquent, les niveaux d'activité et réactivité dopaminergique doivent être de nouveaux éléments à considérer dans l'hypothèse globale de modulation de l'activité cérébrale par la tDCS frontale.

Mots clés

tDCS, homme, TEP, ASL, IRM fonctionnelle, DTI, dopamine, cortex frontal

Abstract

Transcranial direct current stimulation (tDCS) is used to modulate neuronal activity in the brain. It consists in applying a small constant current between two electrodes placed over the scalp. Two frontal tDCS montages have shown promises in modulating cognitive abilities and/or helping to alleviate clinical symptoms. However, the effects of tDCS on brain physiology are still poorly understood. The aim of this thesis work was to clarify brain mechanisms underlying frontal tDCS in healthy subjects, specifically in relation to the dopaminergic system. Using a double blind sham-controlled design, we combined a single session of tDCS online with several imaging techniques (PET or simultaneous PET-MRI) with the subject at rest. A first study (n=32, 2mA, 20min) showed that bifrontal tDCS induced an increase in extracellular dopamine in the ventral striatum, involved in the reward-motivation network, after the stimulation period. A second study (n=30, 1mA, 30min) showed that fronto-temporal tDCS induced an increase in extracellular dopamine in the executive part of striatum as well as a decrease in perfusion in a region part of the default mode network (DMN), after the stimulation period. The data analysis of this study is still ongoing. Overall, the present work provides evidence that a single session of frontal tDCS impacts the dopaminergic system in regions connected to the stimulated cortical areas. Therefore, levels of dopamine activity and reactivity should be new elements to consider for a general hypothesis of brain modulation by frontal tDCS.

Keywords

tDCS, healthy human, PET, ASL, functional MRI, DTI, dopamine, frontal cortex

Résumé

La dopamine est impliquée dans de nombreux processus cognitifs tels que les processus liés à la récompense, la motivation et les fonctions exécutives, via la voie méso-cortico-limbique. Cette voie dopaminergique majeure relie l'aire tegmentale ventrale (ATV), le système limbique (y compris le striatum ventral) et le cortex frontal. De plus, des anomalies dopaminergiques de cette voie ont été rapportées dans de multiples conditions telles que le trouble dépressif majeur, le trouble de la consommation de substances addictives, la schizophrénie et la maladie de Parkinson. De façon intéressante, les processus cognitifs et la symptomatologie des maladies impliquant la dopamine se sont révélés sensibles aux techniques de stimulation cérébrale non invasive (NIBS) appliquées sur le cortex préfrontal dorsolatéral (DLPFC). Parmi les NIBS actuels, la stimulation transcrânienne par courant continu (tDCS) consiste en l'application d'un faible courant continu entre deux électrodes placées sur le cuir chevelu (une cathode et une anode) et est supposée moduler l'activité cérébrale. En tant que tel, la tDCS est une technique émergente ayant des effets pro-cognitifs chez l'homme en bonne santé et est utilisé comme une thérapie prospective pour diminuer certains symptômes et améliorer la cognition chez les patients souffrant de troubles neurologiques et psychiatriques. Récemment, les études de neuroimagerie et les études de modélisation ont montré que les effets neurobiologiques de la tDCS ne se limitaient pas aux zones cérébrales situées sous les électrodes mais se propageaient largement à travers des réseaux corticaux fonctionnellement connectés et pouvaient atteindre des zones sous-corticales telles que les régions dopaminergiques. Cependant, des études contradictoires existent concernant les effets exacts de la tDCS et ce potentiellement du à la conception des études et la variabilité inter-individuelle des participants. Ainsi, les effets neurobiologiques spatiaux et temporels de la tDCS sont loin d'être complètement compris, notamment en ce qui concerne le système dopaminergique.

Le travail présenté ici s'inscrit dans un axe de recherche visant à clarifier les mécanismes cérébraux sous-jacents de la tDCS frontale. Dans un premier temps, le but spécifique de cette thèse était d'étudier cette question chez des sujets sains, en particulier en relation avec le système dopaminergique, dans le but de passer aux patients dans des études ultérieures. Dans ce contexte, deux études ont été réalisées en utilisant différents montages d'électrodes de plus en plus utilisés dans la littérature (tDCS bifrontale et tDCS frontotemporale). Nous avons conceptualisé des études avec le sujet au repos avec la tDCS online cela avec plusieurs techniques d'imagerie. L'administration online de la stimulation a permis d'étudier les changements induits non seulement après mais aussi pendant la stimulation.

Dans une première étude, nous avons évalué l'effet d'une seule session de tDCS bifrontale, avec l'anode au dessus du DLPFC gauche et la cathode au dessus du DLPFC droit, sur la transmission dopaminergique sous-corticale, pendant et immédiatement après la stimulation. Dans cette étude randomisée en double aveugle, 32 sujets sains ont reçu aléatoirement une seule séance de tDCS bifrontale active (20min, 2mA, n=14) ou sham

(n=18) au cours d'une tomographie par émission de positrons (TEP) dynamique en utilisant la disponibilité du récepteur D2 dopaminergique via la liaison du [^{11}C] raclopride. Durant la stimulation, aucun effet significatif de la tDCS n'a été observé. Après la période de stimulation, comparée à la tDCS sham, la tDCS active a induit une diminution significative du rapport de liaison du [^{11}C]Raclopride (BP_R) dans une partie spécifique du striatum, le striatum ventral. Cette diminution du BP_R suggère une augmentation de la dopamine extracellulaire dans cette partie du striatum impliquée dans le réseau de récompense/motivation. Cette étude fournit la preuve directe que la tDCS bifrontale induit une libération de neurotransmetteurs dans des zones sous-corticales connectées polysynaptiquement.

De plus, les effets neurobiologiques de la tDCS ont été décrits, à ce jour, à différents niveaux indépendant les uns des autres, en utilisant plusieurs techniques d'imagerie. Ainsi, la création d'un ensemble cohérent semble une étape obligatoire et essentielle pour comprendre les mécanismes d'action de la tDCS. De plus, les effets neurobiologiques spécifiques de la tDCS fronto-temporale ont rarement été étudiés. Dans cette ligne, la deuxième étude a étudié l'impact neurobiologique d'une tDCS fronto-temporale en utilisant une approche combinée d'imagerie multimodale simultanée (PET-MR), afin de créer un ensemble cohérent pour mieux comprendre les mécanismes d'action de la tDCS. Dans une étude contrôlée en double aveugle, 30 sujets sains ont reçu aléatoirement une seule séance online de tDCS fronto-temporale active (30min, 1mA, n=15) ou sham (n=15) au cours d'un scanner PET-MR simultané. Cette acquisition simultanée de données TEP et IRM offre de nouvelles possibilités de compréhension pour de nombreux aspects du fonctionnement cérébral, tels que l'intégration des composantes spatiales et temporelles de la neurotransmission, de la connectivité et de l'activité cérébrale. Dans notre étude, nous avons exploré les changements distribués dans le système dopaminergique au repos à travers 1) la transmission dopaminergique spécifique et localisée évaluée par TEP en utilisant la disponibilité du récepteur D2 dopaminergique via la liaison du [^{11}C] raclopride; 2) la connectivité fonctionnelle spontanée évaluée par imagerie par résonance magnétique fonctionnelle (IRMf); 3) l'activité cérébrale évaluée par la quantification du débit sanguin cérébral et directement mesuré par le marquage de spin artériel (ASL); 4) la connectivité structurale évaluée par imagerie du tenseur de diffusion (DTI). Cependant, l'utilisation d'un scanner novateur et la combinaison de ces différentes modalités ne sont pas exemptes de difficultés techniques et une partie de ce travail de thèse a également été de les aborder en collaboration avec le CERMEP. Avant d'analyser les données TEP, nous avons optimisé la correction d'atténuation (MaxProb, Merida et al, 2017) et la correction de mouvement (EBER, Reilhac et al, 2017) afin d'avoir une bonne quantification de nos résultats. L'analyse TEP a montré une diminution du rapport de liaison du [^{11}C]Raclopride (BP_{ND}), en utilisant l'approche Logan Plot, reflétant une augmentation de la dopamine extracellulaire induite par une libération de dopamine évoquée par une seule session tDCS. L'impact sur la transmission dopaminergique est significatif au cours de la période de 15 à 30 minutes

après la fin de la stimulation, dans la partie exécutive du striatum. De plus, l'analyse ASL a suggéré un impact significatif sur la perfusion cérébrale dans une région interconnectée avec les sites de stimulation. En effet, nous avons montré une diminution de la perfusion dans le gyrus pariétal supérieur bilatéral, notamment dans la région precuneus, par rapport au groupe sham. Cependant, aucun effet significatif sur la perfusion cérébrale n'a été signalé dans les régions situées sous les électrodes. A ce jour, les analyses rs-fMRI et DTI sont toujours en cours. Tous les prétraitements ont été effectués. Nous nous attendons à ce qu'ils apportent une nouvelle pièce au puzzle afin de mieux comprendre les effets neurophysiologiques de la tDCS fronto-temporale spécifiquement sur les réseaux structurels et fonctionnels liés au système dopaminergique. Finalement, en utilisant une imagerie PET-MR simultanée, le but sera de combiner ces modalités afin de créer un ensemble cohérent des effets neurobiologiques sous-jacents d'une seule session de tDCS fronto-temporale.

De manière intéressante, lorsque l'on considère les deux études, la tDCS bifrontale et fronto-temporale, avec une anode sur le DLPFC gauche comme un montage commun, induisent des effets similaires concernant la localisation de la libération de dopamine dans une sous-région du striatum impliqué dans le réseau de récompense/motivation. Ainsi, dans le contexte du débat en cours dans la littérature sur la tDCS et au-delà du simple modèle «inhibiteur-excitateur», les niveaux d'activité et de réactivité dopaminergique devraient être un nouvel élément de la mosaïque, s'ajoutant à d'autres paramètres, tels que la position des électrodes et l'état du cerveau au moment de la stimulation, dans la formulation d’une hypothèse générale de modulation du cerveau par tDCS frontale.

Avec ces notions en tête, d'autres projets, notamment l'extension de ce protocole aux patients atteints de maladies neuropsychiatriques, serait intéressant pour traduire nos résultats dans le domaine clinique. Par exemple, la mise en place d’un programme de recherche clinique multi-centrique (PHRC-STIMZO-2015, 138 patients) va permettre à notre équipe d'étendre l'application de tDCS aux patients atteints de schizophrénie en utilisant le même montage fronto-temporal et plusieurs séquences d'imagerie telles que l'IRMf et le DTI au repos.

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List of publications

Research publications

Fonteneau Clara, Redouté Jérome, Haesebaert Frédéric, Le Bars Didier, Costes Nicolas, Suaud-Chagny Marie-Françoise, Brunelin Jérome; Frontal transcranial direct current stimulation induces dopamine release in the ventral striatum in human, *Cerebral Cortex,* Volume 28, Issue 7, 1 July 2018, Pages 2636–2646, https://doi.org/10.1093/cercor/bhy093

Fonteneau Clara, Merida Ines, Redouté Jérome, Haesebaert Frédéric, Le Bars Didier, Costes Nicolas, Brunelin Jérome, Suaud-Chagny Marie-Françoise; Neurophysiological impact of a fronto-temporal transcranial direct current stimulation (tDCS) in healthy humans: a simultaneous PET-MR approach**,** *in preparation*

Fonteneau Clara, Mondino Marine, Suaud-Chagny Marie-Françoise, Brunelin Jérome; Blinding or real biological effect? Thoughts around sham transcranial direct current stimulation, Letter to Editors, *in preparation*

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Reviews

Psomiades Marion*, Fonteneau Clara*, Suaud-Chagny Marie-Françoise, Haesebaert Frédéric, Brunelin Jérôme. Neurostimulation du cortex préfrontal dorsolatéral : quels effets sur la symptomatologie et les émotions dans la dépression et la schizophrénie ? ; Santé mentale au Québec *;* *co-author

Chapters

Mondino M, Fonteneau C, Brunelin J. Schizophrenia. In “Transcranial Direct Current Stimulation in Neuropsychiatric Disorders: Clinical Principles and Management », by Brunoni AR, Nitsche MA, Loo CK. Springer NY, USA, *in press*

List of abbreviations

3-MT	3-methoxytyramine
AADC	L-aromatic-amino-acid-decarboxylase
ACC	anterior cingulate cortex
AD	aldehyde dehydrogenase
ASL	arterial spin labeling
BLA	basolateral amygdala
BOLD	blood oxygenation level dependent
BP	binding potential
cAMP	cyclic adenosine monophosphate
CBF	cerebral blood flow
CEN	central executive network
COMT	cathol-O-methyltransferase
CON	cingulo-opercular network
CSF	cerebral spinal fluid
DAN	dorsal attention network
DAT	dopamine transporter
DBS	Deep brain stimulation
DMN	default mode network
DOPAC	dihydroxyphenylacetic acid
DLPFC	Dorsolateral prefrontal cortex
DTI	diffusion tensor imaging
ECT	electroconvulsivotherapy
EEG	electroencephalography
FA	fraction anisotropy

FEM	finite element method
fNIRS	functional near-infrared spectroscopy
FPN	frontal parietal network
GABA	gamma-Aminobutyric acid
GM	grey matter
GP	globus pallidus
HVA	homovanillic acid
LTD	long term depression
LTP	long term potentiation
M1	primary motor cortex
MAO	monoamine oxidase
MEP	motor evoked potential
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MSN	medium spiny neurons
NIBS	non invasive brain stimulation
NMDA	N-Methyl-D-aspartate
OFC	orbital frontal cortex
PCC	posterior cingulate cortex
PET	positron emission tomography
PFC	prefrontal cortex
rs-fMRI	resting-state functional MRI
sg25	subgenual cingulate
SN	substantia nigra
STN	subthalamic nucleus

SUD	substance use disorder
TBSS	tract based spatial statistics
tDCS	transcranial direct current stimulation
TH	tyrosine hydroxylase
TMS	transcranial magnetic stimulation
TNN	task-negative network
TPJ	temporal parietal junction
TPN	task-positive network
VAN	ventral attention network
VBM	voxel-based morphometry
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
WM	white matter

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Introduction

Dopamine is involved in numerous cognitive processes such as reward, motivation and executive functions, via the meso-cortico-limbic pathway. This major dopaminergic pathway links the ventral tegmental area (VTA) of the midbrain, the limbic system (including the ventral striatum) and the frontal cortex.

Moreover, dopamine anomalies of this pathway have been reported in multiple conditions such as major depressive disorder, substance use disorder, schizophrenia and Parkinson's disease. Interestingly, cognitive processes and symptomatology of diseases involving dopamine have been shown sensitive to non-invasive brain stimulation techniques (NIBS) applied over the dorsolateral prefrontal cortex (DLPFC). Among current NIBS, transcranial direct current stimulation (tDCS) consists in applying a weak direct current between two electrodes placed above the scalp (a cathode and an anode) and is thought to modulate brain activity. As such, tDCS is a technique emerging as having pro-cognitive effects in healthy humans and a prospective therapy to decrease symptoms and improve cognition in patients with neurologic and psychiatric disorders. In the last decade, neuroimaging studies and computational model analyses highlighted that the neurobiological effects of tDCS are not restricted to the brain areas located under the stimulating electrodes but spread through distributed cortical networks functionally connected and could reach subcortical areas, such as dopaminergic regions. However, contradictory studies exist due to design and individual variability. This reinforces the fact that the spatial and temporal neurobiological effects of tDCS are far from being completely understood, notably regarding the dopaminergic system.

The work presented here fits into a broad research axis intended to clarify brain mechanisms underlying frontal tDCS. As a first step, the specific aim of this thesis was to investigate this question in healthy subjects, specifically in relation to the dopaminergic system (Chapter 5 and 6). These studies are first put into perspective in the literature (Chapter 1 to 4). A synthesis of the results of this work is then proposed and discussed in terms of brain mechanisms related to tDCS and possible implications of repeated stimulation sessions. Indeed, repeated tDCS sessions may induce clinical improvements in several neuropsychiatric conditions, but inconsistencies remain. Furthermore, the easy installation, growing media attention, commercialized head-sets and the online do-it-yourself tutorials favor the uncontrolled use of tDCS. However little or no warning to the interaction with medication or psycho-stimulant is provided (Chapter 7).

Thus, this work should be of interest for readers in areas of therapeutic development and more broadly in the area of non-invasive brain stimulation since tDCS is increasingly used in controlled conditions as a therapeutic solution and as a tool to modulate brain activity. Furthermore, it should also be of interest to the scientific community preoccupied by the increasing use of tDCS in uncontrolled/recreational conditions, notably as a neuroenhancer.

Part I - Context / State of the art

1. Chapter 1 - The Dopamine System

1.1. Introduction and History

Dopamine, an organic molecule, part of the catecholamine family, was at first better known for its role as the metabolic precursor of the neurotransmitter norepinephrine. In the late 1950s, pioneering studies by Arvid Carlsson first supported the idea that dopamine served a function as a neuromodulator in the mammalian brain (Carlsson et al., 1957, 1958; Carlsson, 1959). In the same line, Greengard (2001) led to the understanding that dopamine and other monoamines do not mediate fast synaptic transmission in the central nervous system but rather modulate it. Since then, neuroscientists have sought to understand the role of dopamine in neural circuits and behavior, as well as the mechanisms underpinning such roles.

Dopamine was shown to regulate electrical and biochemical aspects of neuronal function such as excitability and synaptic transmission (Gu, 2002; Tritsch and Sabatini, 2012). Their long axons, originating from nuclei in subcortical centers, innervate wide cortical regions and their actions can either be excitatory or inhibitory depending on the features of the dopaminergic receptors present on the targeted cell types and their intracellular pathways. To better understand these mechanisms, the use of specific agonists and antagonists have been developed to target these receptors and influence dopaminergic transmission thus enhancing or blocking the actions of dopamine. These methods have put forward the major involvement of dopamine in the control of motor actions and higher cognitive functions as well as in many neuropsychiatric diseases, such as schizophrenia, depression, substance-use-disorders (SUD) and Parkinson's disease (Price and Drevets, 2012; Brunelin et al., 2013; Hanganu et al., 2015; Nutt et al., 2015; Maia and Frank, 2017).

However, the exact mechanisms used by dopamine to exert its control over behavior are still not fully understood due to their complexity. Indeed, due to the numerous variables involved, replicable results across experiments are difficult to obtain. In addition, dopamine's action is also dependent on the strength and duration of receptor stimulation and influenced by brain-state (Nicola et al., 2000; Seamans and Yang, 2004). Nowadays, an important part of the dopamine research focuses on the interactions between the subcortical nuclei and the frontal cortex via a complex cortico-basal ganglia network in order to carry out complex behaviors (Haber, 2016).

Here, we present the basic knowledge of dopamine: dopaminergic pathways, dopamine cycle (from synthesis to degradation), and give a brief glance at the key involvement of dopamine in higher cognitive functions (reward and motivation, executive processes and placebo effect).

1.2. Dopaminergic pathways

1.2.1. Anatomy of the dopamine system

1.2.1.1. Dopaminergic tracts

Major dopaminergic pathways have been identified in the human brain and can be classified depending on the origin and length of their dopaminergic projections (Bentivoglio and

Morelli, 2005; Arias-Carrión and Pöppel, 2007). In this thesis, we will focus on the 'long' dopaminergic pathways: nigrostriatal, mesolimbic and mesocortical (**Figure 1**)

The nigrostriatal circuit originates from the substantia nigra pars compacta (SNc; A9 nucleus) and projects primarily to the dorsal striatum (caudate nucleus and putamen). This pathway is traditionally involved in the regulation of movement.

The mesolimbic and mesocortical pathways are often grouped into one main ascending dopaminergic pathway: the mesocorticolimbic pathway, originating from the ventral tegmental area (VTA; A10 nucleus). The mesolimbic part of this system projects to regions such as the nucleus accumbens, olfactory tubercule of the ventral striatum, septum, amygdala and hippocampus. The mesocortical part of this system projects to the prefrontal (PFC) and anterior cingulate (ACC) cortex. This mesocorticolimbic system is implicated in motivated behaviors and reward processing, as well as executive processes (emotional processing and learning) (Ikemoto, 2007).

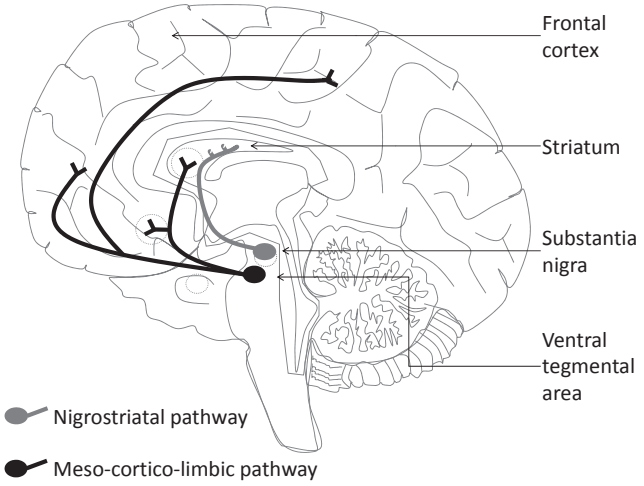


Figure 1: Major dopamine pathways - Schematic drawing of the 'long' dopaminergic pathways: nigrostriatal and meso-cortico-limbic pathways. Adapted from Brunelin et al., 2013

In recent years, studies have put forth a complex cortico-basal ganglia network (Haber, 2016) making the comprehension of these dopaminergic pathways the foundation to understand the interaction of the subcortical nuclei and the frontal cortex. The main actors of this network are the dopamine-producing nuclei in the midbrain, the frontal cortex and the basal ganglia (which includes the main dopamine projection: the striatum).

1.2.1.2. Dopamine-producing nuclei: VTA-SN

In regards to the dopaminergic pathways, the production of dopamine is located in groups of neurons termed nuclei. With the help of modern techniques, 10 dopamine-producing nuclei have been put forth in the human brain (A8-A17) (Tritsch and Sabatini, 2012). These nuclei present different characteristics depending on their projection sites, as well as their electrophysiological and chemical properties.

Some of these neurons are located in the midbrain and more specifically in the the substantia nigra pars compacta (SNc; A9 nuclei) and in the ventral tegmental area (VTA;

A10 nuclei). These two nuclei are the most studied due to their implication in many aspects of motion and behavior (Bentivoglio and Morelli, 2005).

1.2.1.3. Major dopamine projection: The striatum

Dopamine-neurons mainly project onto a subcortical region: the striatum. Studies have put forward two general groups of neurons in this region: projections neurons (with the most common being the medium spiny neurons (MSN), GABAergic) and interneurons (GABAergic and aspiny cholinergic). Thus, depending on which neurons are recruited, dopamine can initiate a wide range of intracellular cascade in the striatum (Tritsch and Sabatini, 2012). Interestingly, it has been shown that clusters of striatal neurons formed cells called interface islands (or cell islands), thought to contain quiescent immature cells that remain in the adult brain (Goldman-Rakic, 1982; Heimer, 2000).

Concerning its architecture, the striatum has been extensively studied both at the anatomical and functional level. Anatomically, the human striatum is subdivided into three separate parts: the nucleus accumbens, the caudate nucleus and the putamen (**Figure 2 A**). From an anatomical standpoint, the only delineation between the caudate nucleus and the putamen is the internal capsule. However, in the majority of studies, the striatum is divided into two parts: the ventral and dorsal striatum (**Figure 2 B**). In human and non-human primates, the ventral striatum includes the nucleus accumbens, the medial caudate nucleus and rostralventral portions of the putamen. The dorsal striatum is comprised of rest of the caudate nucleus and putamen. Nevertheless, it should be noted that no clear boundaries (cytoarchitectonic or histochemical) exist to dissociate the ventral and dorsal striatum. More recent studies have started defining the ventral and dorsal striatum by their afferent projections from cortical areas. Indeed, it has been shown that the ventral striatum alone receives projections from the amygdala and hippocampus (Haber and McFARLAND, 1999; Martinez et al., 2003; Haber and Knutson, 2010; Haber, 2016). In addition, one can also find reference to a central striatum which references the part of the striatum involved in associative functions. Functionally and in regards to its cortical projections, the striatum has been divided in limbic, associative and sensorimotor subareas (**Figure 2 C**) (Martinez et al., 2003; Tziortzi et al., 2014; Haber, 2016). This will be discussed in detail in §1.2.1.4 Basal Ganglia: Extrinsic Projections.

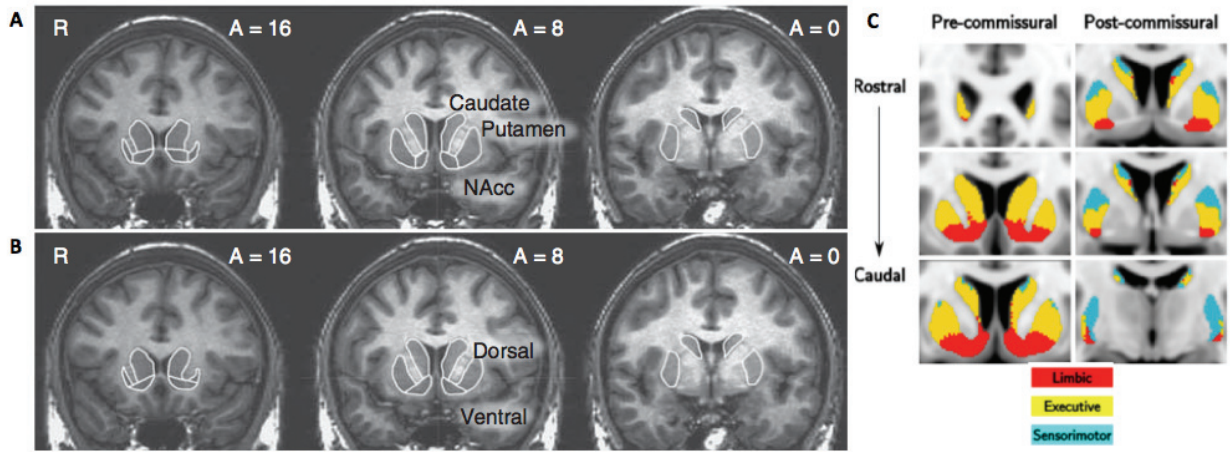


Figure 2: Organization of the striatum - Parcellations based on structural landmarks. (**A**) Tripartite anatomical division (caudate nucleus, putamen and nucleus accumbens) (**B**) Ventral and Dorsal Striatum (**C**) Tripartite functional division (limbic, executive and sensorimotor subareas). Adapted from Haber & Knutson, 2010 and Tziortzi et al, 2014

Due to its complexity and polyvalent nature, the striatum has been shown to be the key region for dopamine action and release in the basal ganglia (Bentivoglio and Morelli, 2005).

1.2.1.4. Basal Ganglia

In human and non-human primates, the basal ganglia is a group of structures involved not only in purely sensory-motor functions but has been increasingly linked to more complex ones, mediating goal-directed behaviors, including reward, motivation and cognition.
Nowadays the main structures of the basal ganglia include the striatum, the Globus Pallidus (GP), the subthalamic nucleus (STN), and the midbrain dopaminergic nuclei (SN and VTA) (**Figure 3 A**). This system finds its strength in both anatomically interconnected and functionally related centers and circuits (Haber and Knutson, 2010; Nelson and Kreitzer, 2014; Haber, 2016). More specifically, the system has a unique set of intrinsic and extrinsic connections, which are described below.

Intrinsic connections

Throughout the years, different models of connections between the various structures of the basal ganglia (**Figure 3 B**) have been developed. The first functional model was proposed in the late 1980s (Albin et al., 1989). This model suggested a balance between two pathways: a direct “monosynaptic” and indirect “polysynaptic”. On the one hand, the direct pathway involves direct striato-nigral inhibitory (GABAergic) connections, which would allow a behavioral response. On the other hand, the indirect pathway involves relays in the external globus pallidus (GPe) and subthalamic nucleus (STN), which would suppress behavior. These pathways were thought to be regulated by afferent dopaminergic signals from the SNc and the VTA, depending on the receptor subtype in the striatum (Bentivoglio and Morelli, 2005). However, due to new technological advancements notably in neuroimaging, a

more complex model emerges, in which the behavioral outputs were less easy to predict (Redgrave et al., 2010).

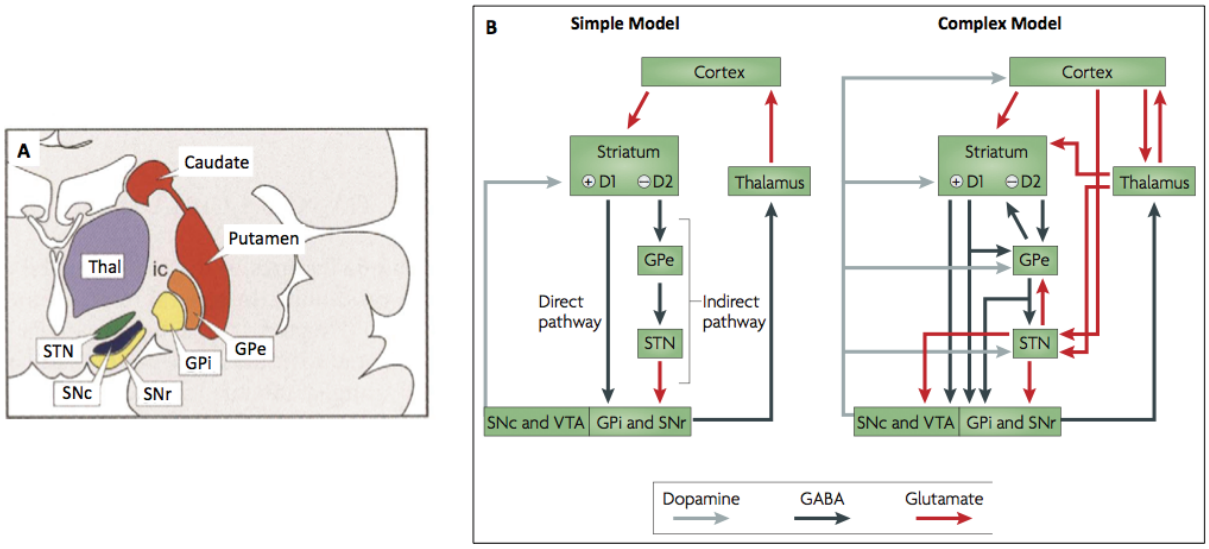


Figure 3: Intrinsic connections within the basal ganglia - (**A**) Structures of basal ganglia in a section through the primate brain. The striatum is represented in red and the output nuclei are in yellow. (**B**) Models of basal ganglia architecture (simple and complex). Organization of intrinsic connections within the basal ganglia are represented, as well as the type of signal: Dopamine (grey), GABA inhibitory (blue) and Glutamate excitatory (red) - *Abbreviations: GPi: internal globus pallidus; GPe: external globus pallidus; ic: internal capsule; STN: subthalamic nucleus; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticula; Thal: thalamus; VTA: ventral tegmental area* - Adapted from Bentivoglio & Morelli, 2005 and Redgrave et al, 2010

Extrinsic connections

In the late 1980s, the concept of parallel-projecting anatomical cortico-subcortical loops has been put forth to explain the main connections between the basal ganglia and external structures (Alexander et al., 1986; Alexander and Crutcher, 1990). According to this model (**Figure 4**), each loop includes projections from the cerebral cortex through the basal ganglia to the thalamus and back to the cerebral cortex. More interestingly, each striatal subarea receives input from a different cortical area, thus specializing the output response (motor, associative or limbic). This concept of parallel-projecting cortico-subcortical loops was comforted in humans by a meta-analysis of corticostriatal coactivation in task-based functional studies (Postuma and Dagher, 2006).

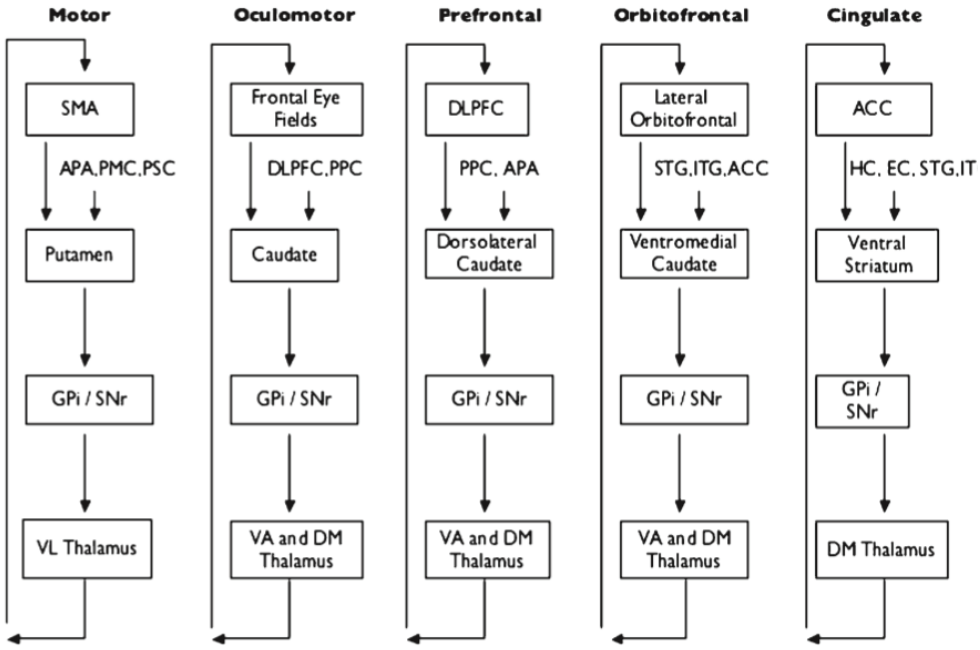


Figure 4: Cortico-subcortical loops - Schematic representation of the parallel organization of the five basal ganglia-thalamocortical circuits. Each circuit integrates regions from the cortex, striatum, pallidum, substantia nigra and the thalamus. Adapted from Alexander, 1986

On a side note, the term “prefrontal cortex”, defined as the anterior portion of the frontal lobe, will be used throughout this thesis work as it is now widely used in the literature. I acknowledge that the prefix “pre” could be thought of as in front of the frontal cortex. “Nevertheless, that designation has been condoned by so much usage that is seems unwarranted to discard it for semantic reasons” (Fuster, 2015). In relation to this model, the frontal cortex is known to be divided into several subregions: the orbital and medial prefrontal cortex (respectively OFC and mPFC), involved in reward and motivation; the dorsolateral PFC (DLPFC), involved in executive functions; the premotor areas, involved in motor planning; and motor cortex, involved in the execution of those plans. These cortical regions are in line with the concept of parallel cortico-subcortical loops (Haber, 2016) (**Figure 5 A**).

With this in mind and novel noninvasive neuroimaging techniques, recent studies have explored and refined these corticostriatal projections. These methods include intrinsic functional connectivity (Di Martino et al., 2008; Zhang et al., 2008; Barnes, 2010; Choi et al., 2012), diffusion tensor imaging (DTI; Leh et al., 2007; Draganski et al., 2008; Tziortzi et al., 2014), T1-weighted voxel-based morphometry (VBM; Cohen et al., 2008). These studies are consistent with a functional topographic organization of the striatum. Indeed, the dorsolateral striatum receives cortical input from sensory-motor areas, the central striatum from associative cortical areas, and the ventral striatum from limbic cortical areas. Thus, based on anatomical and functional connectivity, a model has emerged in the literature of a tripartite division of the striatum into motor, associative and limbic subareas (Redgrave et al., 2010;

Haber, 2016) (**Figure 5 B**). This fronto-striatal organization could be the substrate of behavioral flexibility (Morris et al., 2016).

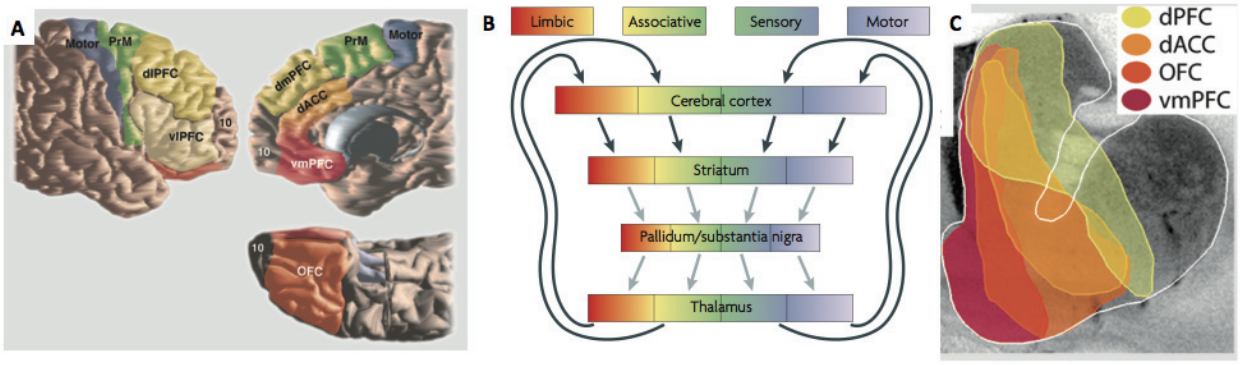


Figure 5: Extrinsic connections within the basal ganglia - (**A**) Anatomical delineation of frontal regions (**B**) Cortical-subcortical parallel-projecting loops with specific information (limbic, associative and sensori-motor) (**C**) Projections from the frontal cortical regions in the striatum. Adapted from Haber et al, 2016 and Redgrave et al, 2010

When looking more closely, the cortical projections onto the striatum show a clear overlap, creating a complex interface between inputs from functionally distinct regions. The idea of broad cortical networks featuring nodes (i.e connective hubs) that integrate and distribute information across multiple systems is of growing interest in the literature (Power et al., 2013). These networks, also called resting-state networks, are consistently found in healthy subjects and represent specific patterns of synchronous activity at rest (high level of correlated BOLD signal) (Fox and Raichle, 2007; Power et al., 2011). The major networks are named depending on their impact: Cognitive processes (Task-Negative (default mode network (DMN) or Task-Positive (fronto-parietal network (FPN), dorsal attention network (DAN), salience or cingulo-opercular network (SN/CON)), Sensory processes (sensorimotor, auditory, visual). When looking at the dopamine literature, it seems that this architecture involves subcortical structures such as the striatum. In particular, the rostral striatal region may serve as a hub for the vmPFC, OFC, and dACC to connect with dorsal and lateral PFC regions that integrate motivational, reward, and cognitive control information (Haber, 2016) (**Figure 5 C**). Choi and colleagues (2012) have examined the coupling between striatal and cortical regions and its link to resting state networks. They found that the coupling between the DLPFC and the central striatum is linked with the FPN (**Figure 6 A**). In addition, they found that regions linked to the DMN - PFCm and posterior cingulate cortex (PCC) - are linked with striatal regions between the nucleus accumbens and the central caudate (linked to the DLPFC) (**Figure 6 B**). In the same line, other studies have investigated the impact of pharmacological challenges (agonist and antagonist) in healthy subjects on these resting-state networks, in order to map the dopamine-dependent architecture of subcortical functional connectivity. Agonist administration increased cortico-subcortical network connectivity, whereas the opposite effects were reported with antagonist administration. The neuromodulatory effects were seen in the functional connectivity between 1) the midbrain and the DMN; 2) the right caudate and right FPN; 3) the ventral striatum and CON (Cole et

al., 2013). Thus, these studies reinforce the fact that distinct large-scale networks can provide an indirect measure of dopamine neurotransmission and are coupled to distinct zones of the striatum.

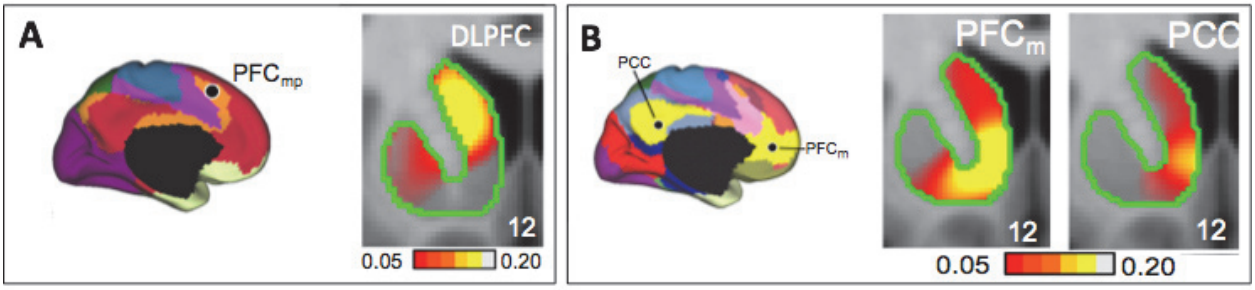


Figure 6: Distributed cortical regions within resting-state networks show specific overlaps in different regions of the striatum - (**A**) Functional connectivity patterns show distributed overlaps within the fronto-parietal network (FPN) and the dorsal striatum (shown on a coronal slice y=12) (**B**) Functional connectivity patterns show distributed overlaps within the default mode network (DMN) and the ventral striatum (shown on a coronal slice y=12). Adapted from Choi et al, 2012

1.3. Cycle of dopamine

The dopamine cycle is comprised of several steps (**Figure 7**) which will be addressed in this section: 1) Synthesis; 2) Storage and Release; 3) Reuptake and Degradation; 4) Dopamine receptor; 5) Regulation of dopamine release (phasic and tonic firing properties); 6) Modulation of synaptic transmission.

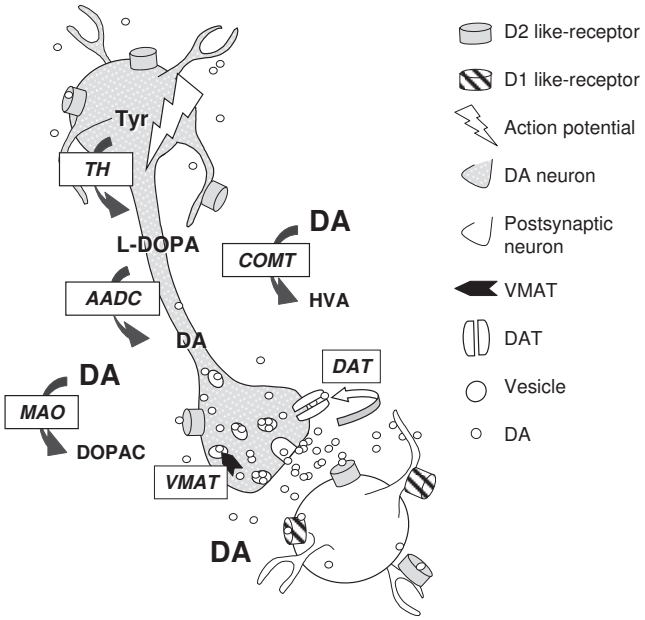


FIGURE 7: Typical dopaminergic neuron - Summarizing the different steps in the cycle of dopamine: Synthesis, Storage, Release, Reuptake and Degradation. Adapted from Brunelin et al, 2013

1.3.1. Synthesis

The synthesis of dopamine occurs from circulating tyrosine in several steps as shown in **Figure 8** (Meyer and Quenzer, 2005). Tyrosine is transported via amino acid transporters through the blood-brain barrier and cell membranes. Once in the intracellular domain and

with the help of the enzyme Tyrosine Hydroxylase (TH), L-tyrosine is converted into L-DOPA (Nagatsu et al., 1964). This conversion is relatively a slow conversion, rendering TH a rate-limiting enzyme in the synthesis of dopamine. Next, L-DOPA is decarboxylated into Dopamine by L-aromatic-amino-acid-decarboxylase (AADC) (Holtz, 1939). This step being quite fast, the levels of L-DOPA in the brain are very low in normal conditions. Thus, increasing the levels of L-DOPA would increase dopamine synthesis, which is one of the reasons why it is a target of choice in multiple clinical conditions (e.g Parkinson's disease).

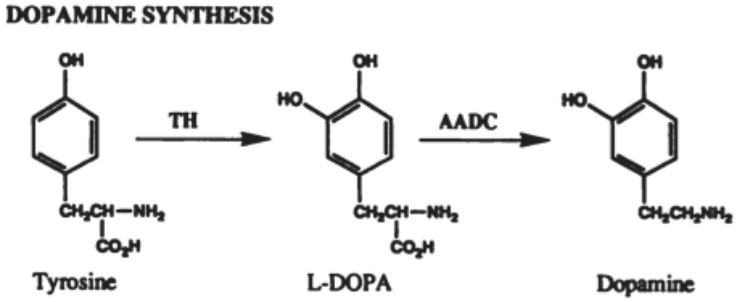


Figure 8: Dopamine synthesis - Multistep pathway in the central nervous system. *Abbreviations: TH: tyrosine hydroxylase; AADC: L-aromatic-amino-acid-decarboxylase.* Adapted from Volkow et al., 1996

1.3.2. Release

After synthesis, dopamine is transported into storage vesicles for later release by the vesicular monoamine transporter (VMAT). In normal conditions, dopamine contained in the vesicles is released into the synaptic cleft in response to an action potential and via the process of calcium-dependent exocytosis (Staal et al., 2004). See **FIGURE 7**

1.3.3. Reuptake and Degradation

At the level of the synaptic cleft, once dopamine is released its clearance depends on two processes: re-uptake and enzymatic degradation (Meyer and Quenzer, 2005).

The re-uptake process transports dopamine back into the dopaminergic neuron, where it is recycled in vesicles (for re-release) or metabolized in the presynaptic cytoplasm. This process enables the end of dopamine action and helps maintain homeostasis. Re-uptake is made possible by a membrane carrier called the dopamine transporter (DAT).

In parallel, to prevent an excessive dopamine accumulation, a degradation process occurs using primarily three enzymes (**Figure 9**): the catechol-O-methyltransferase (COMT), monoamine oxidase (MAO) and aldehyde dehydrogenase (AD). They give rise to several metabolites (the major one called homovanillic acid (HVA)). This process can occur in the synaptic cleft, in the cytoplasm of the presynaptic terminal and in glial cells. However, the role of each enzyme depends on both their location in the synapse and the brain area. Thus, in the synaptic cleft, dopamine will be converted into 3-methoxytyramine (3-MT) by COMT then into HVA by MAO and AD. At the presynaptic level, dopamine will be metabolized into dihydroxyphenylacetic acid (DOPAC) by intraneuronal MAO and AD then converted into HVA by COMT.

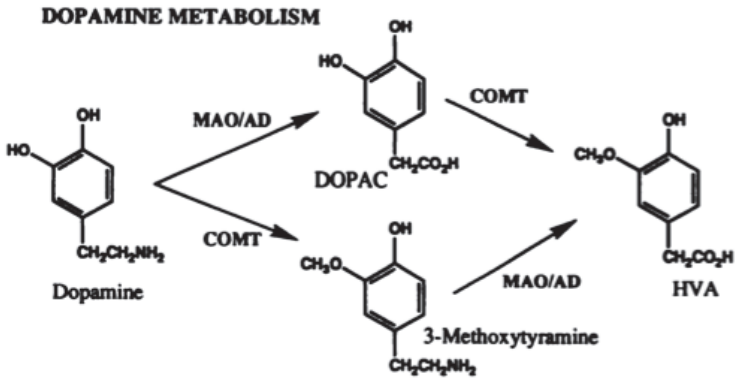


Figure 9: Dopamine metabolism - Multistep pathway in the central nervous system. *Abbreviations: COMT: cathol-O-methyltransferase; DOPAC: dihydroxyphenylacetic acid; HVA: homovanillic acid; MAO/AD: monoamine oxidase/aldehyde dehydrogenase.* Adapted from Volkow et al, 1996

In addition, DAT and COMT have been reported to differ in location throughout the dopaminergic system. Indeed, the highest density of DAT is seen in the striatum, whereas COMT is an important regulator in the prefrontal cortex. Hence, dopamine metabolism in the prefrontal cortex depends primarily on enzymatic degradation by COMT rather than on transport and reuptake by the DAT, and inversely in the striatum. More specifically, it should also be noted that the DAT is relatively low throughout the ventral striatum (Haber, 2016).

1.3.4. Dopamine receptor

The literature provides extensive knowledge on dopamine receptors (Beaulieu and Gainetdinov, 2011; Tritsch and Sabatini, 2012). Once in the synaptic cleft, dopamine interacts with a family of metabotropic receptors (G-protein coupled receptors). They are present both presynaptically on dopaminergic neurons and postsynaptically on dopaminergic targeted cells (Bentivoglio and Morelli, 2005). To date, five main subtypes are described (D1 to D5), and they are commonly divided into two functional subgroups: D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors. These two subgroups are based on their coupling with adenylyl cyclase and can be distinguished using pharmacological agonists and antagonists of dopamine receptors. The affinity of D2-like receptors for dopamine in usually reported greater than that of D1-like receptors. In addition, D1-like receptor activation requires high levels of phasic dopamine release, whereas the D2-like receptors, which have a higher affinity for dopamine, are continuously activated by lower tonic levels of dopamine (Grace, 1991). However, studies have shown that both subgroups exist in high and low affinity states (Beaulieu and Gainetdinov, 2011).

Furthermore, distribution and concentration of dopamine receptors differ between brain areas. The density of both subgroups of receptors is lower in extrastriatal regions (Volkow et al., 1996). However, D1-like receptors predominate largely in the PFC, whereas D2-like receptors predominate in the striatum. It should also be noted that dopamine autoreceptors, located on the dopamine neuron (soma, dendrites, nerve terminal) are primarily D2-like type (Beaulieu and Gainetdinov, 2011).

When looking at their functional contribution, D1- and D2-like receptors modulate differently the intracellular signaling pathways. Indeed, D1-like receptors stimulate adenylyl cyclase activity inducing the formation of cyclic adenosine monophosphate (cAMP), while D2-like receptors exert the opposite effect (Kebabian and Calne, 1979; Beaulieu and Gainetdinov, 2011). Interestingly, cAMP increases the phosphorylation of a substrate known as DARPP-32, which has shown a key role in mediating the actions of dopamine (Greengard, 2001) (**Figure 10**).

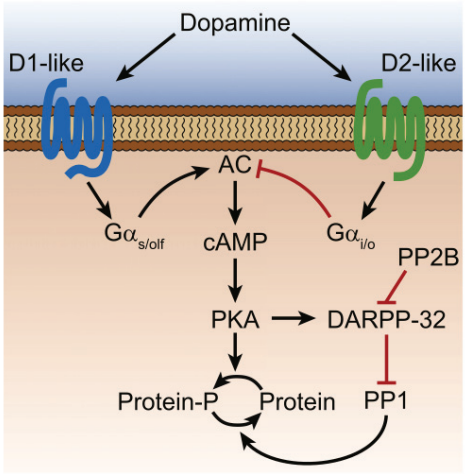


Figure 10: Intracellular dopamine signaling pathways. These pathways are differentially modulated by D1 and D2 receptors. Indeed, D1-like receptors stimulate (activating black) the cAMP/PKA dependent pathway, while D2-like receptors exert the opposite effect (inhibitory red). *Abbreviations: AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A.* Adapted from Tritsch et al, 2012.

1.3.5. Electrophysiological properties

Furthermore, dopamine release is also a function of the firing pattern of the dopamine neurons. Indeed, dopamine neurons exhibit several activity patterns: hyperpolarized state (inactive, non firing), tonic (slow single-spike) and phasic (burst) firing (Floresco et al., 2003; Beaulieu and Gainetdinov, 2011). However, the terms tonic and phasic also include the relationship between a dopamine neuron's activity and dopamine release states. Indeed, a tonic dopamine neuron activity is related to tonic extrasynaptic dopamine levels, whereas a phasic activity - also called burst firing - is related to a rapid high-amplitude intrasynaptic dopamine release (Grace, 1991, 2016).

On the one hand, a tonic firing pattern (**Figure 11-A**) represents the state-dependent baseline level of extrasynaptic dopamine and is mediated by tonic (spike) firing of dopamine midbrain neurons. It is defined as a slow-regular (2-10Hz) firing pattern (Grace and Bunney, 1984 p.198; Chergui et al., 1994) and can induce a change in dopamine concentration lasting from tens of seconds to hours or days (Ikemoto, 2007). Tonic dopamine levels primarily stimulate extrasynaptic receptors including dopamine autoreceptors, which exert feedback-inhibition of phasic release.

On the other hand, a phasic firing pattern (bursting activity; **Figure 11-B**) is defined as a transient high-amplitude dopamine intrasynaptic release, resulting from a burst firing of one

or more midbrain neurons (Suaud-Chagny et al., 1992; Chergui et al., 1994; Ikemoto, 2007). The burst is comprised of a rapid series of action potentials (3-10) with a short interspike interval (40-80ms) and followed by a prolonged post-burst inhibition (Grace and Bunney, 1984). This type of firing happens when exposed to behaviorally salient stimuli and results in a stimulation of post-synaptic dopamine receptors (Schultz, 2016).

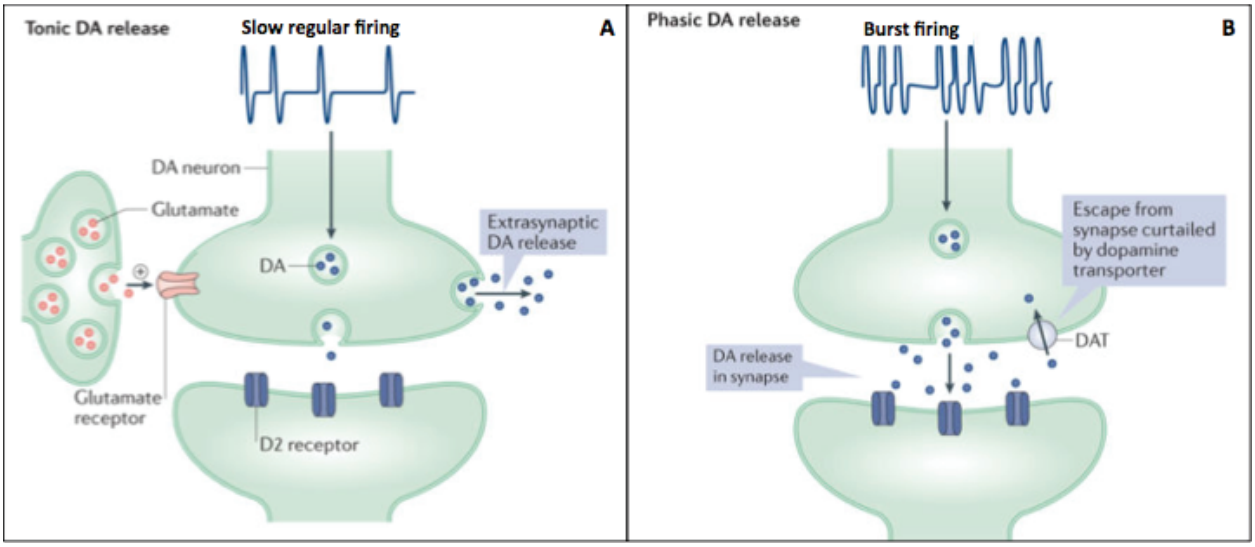


Figure 11: Dopamine neuron firing and dopamine release - (**A**) Tonic firing pattern induced extrasynaptic dopamine release (**B**) Phasic firing pattern induced a dopamine release in the synapse. Adapted from Grace et al, 2016

In addition, only dopamine neurons that are already firing (i.e. tonic), that is when the magnesium block is removed from the NMDA channel, are capable of switching to a burst firing pattern. Therefore, hyperpolarized neurons (non firing neurons) exhibiting a magnesium block of the NMDA channel will not be able to transition to burst pattern. Thus, the number of tonic dopamine neurons in the midbrain sets the dopaminergic system level of responsiveness (Lodge and Grace, 2006; Grace, 2012; Tritsch and Sabatini, 2012). Based on this model, with an increase in tonic dopamine neuron firing, the amplitude of the phasic response would also increase, since more dopamine neurons can transition from tonic to phasic firing. However, it should be noted that this relationship also depends on the action of dopamine released extrasynaptically. Indeed, tonic extracellular dopamine would predominantly stimulate extrasynaptic receptors including dopamine auto-receptors, which could exert feedback-inhibition of dopamine release (tonic and phasic) (Suaud-Chagny et al., 1992; Floresco et al., 2003; Suaud-Chagny, 2004).

Furthermore, the transition between hyperpolarized non-firing neurons and spontaneous firing neurons could be modulated by a combination of frontal and limbic projections. More specifically, neuronal firing in the striatum is regulated by two consecutive mechanisms, one from the hippocampus and/or the basolateral amygdala providing a gating influence over information flow, allowing for a possible modulation in neuronal activity from the prefrontal cortex at the level of the striatum (Grace, 2000, 2016) (**Figure 12**).

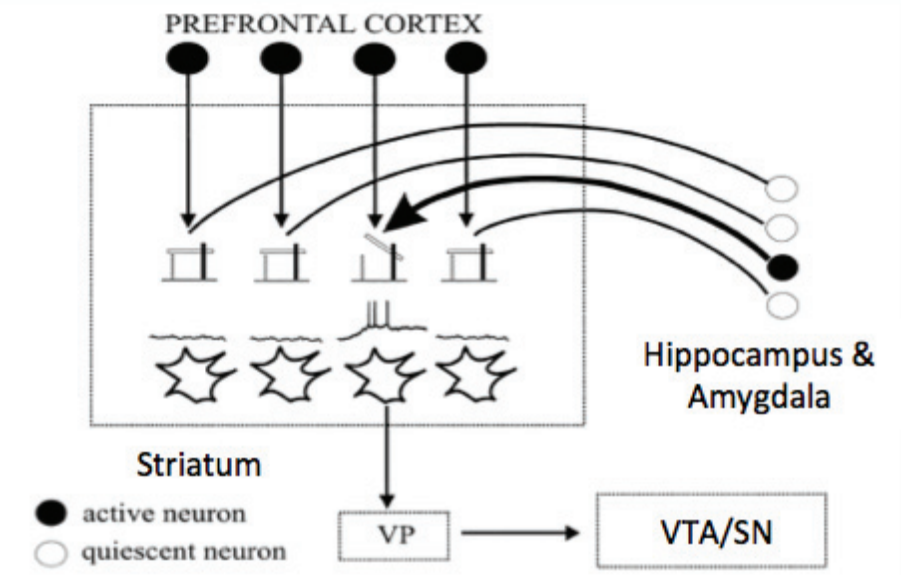


Figure 12: **Modulation of neuronal activity in the striatum via consecutive action of both the frontal cortex and limbic regions (hippocampus and amygdala)** - Adapted from Grace et al, 2000 - *Abbreviations: SN: Substantia Nigra; VP: ventral pallidum; VTA: Ventral Tegmental Area*

1.4. Dopamine and cognition: Key involvement

1.4.1. Reward-Motivation

Dopamine has been shown to be of critical importance in reward-related behaviors, which involves different components such as motivation, learning and emotion/affect. This reward-motivation pathway seems involved in incentive-based learning and in giving an appropriate response to stimuli (Berridge and Robinson, 2003; Wise, 2004; Bromberg-Martin et al., 2010). Neuroimaging studies have put forth a combined action of the ventral cortico-basal ganglia network with frontal activity (especially the vmPFC, OFC, dACC and DLPFC) in reward processing and report an increased activity in frontal and striatal regions (Phillips et al., 2008; Haber and Knutson, 2010; Duverne and Koechlin, 2017). Furthermore, studies have reported that phasic activity is involved in reward processing (Schultz, 1998, 2013; Schultz et al., 2017) as well as in switching attention towards salient cues in the environment. In contrast, tonic activity supports relatively stable affective states and motivation (Ikemoto, 2007). In addition, to transform the basic reward response into action planning and associative learning, models of reward processing point towards ventral to dorsolateral transfer of information.

1.4.2. Executive processes

Executive processes, such as working memory, planning and cognitive control, appear mediated by dopamine. This is well documented in a rhesus monkey study, where at the same time, cognitive deficits during a spatial delayed response task were seen after regional depletion of dopamine in the prefrontal cortex and the administration of dopamine receptor agonist reversed these impairments (Brozoski et al., 1979). In the same line, agonists of

dopamine receptors were shown to improve performance on cognitive tasks, while the opposite effect was observed with antagonists (Cools, 2011).

However, across individuals and depending on the task, dopamine modulations vary greatly. This concept has been increasingly studied this past decade, with the models developed pointing towards an optimum dopamine level for cognitive function. Indeed, dopamine seems to contribute to the balance between maintenance of task-relevant information (cognitive stability) and distracting interference (flexibility). More specifically, high dopamine levels in the prefrontal cortex would promote cognitive stability and conversely, high dopamine levels in the striatum would promote flexible updating (Cools, 2006, 2016; Boot et al., 2017).

This optimum dopamine level fits in the 'old' notion of an inverted U-shape dose-response curve supporting the relationship between dopamine levels and behavioral performance (Goldman-Rakic et al., 2000; Vijayraghavan et al., 2007; Cools and D'Esposito, 2011). Indeed, more and more studies put forth that both too little and too much dopamine impairs performance (**Figure 13**).

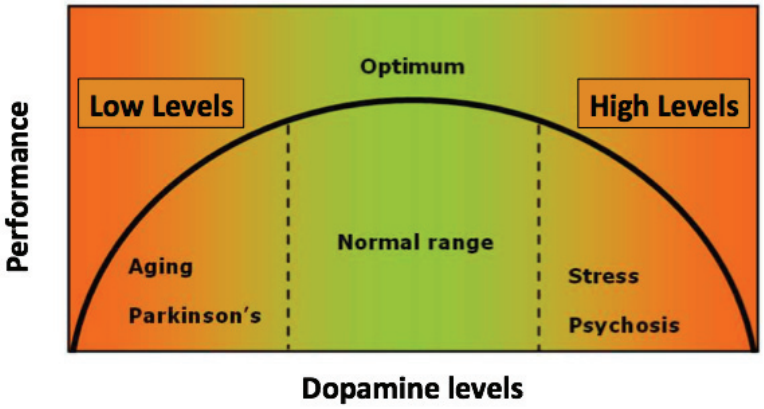


Figure 13: Inverted U-Shape dose-response dopamine curve - Adapted from Goldman-Rakic et al, 2000

With this in mind, dopamine is shown to be an essential neuromodulator of the central nervous system involved in numerous processes, such as reward-motivation and executive processes, which engage cortico-subcortical networks. In addition, these past few years, new tools have been developed in order to modulate these processes in healthy subjects and clinical populations, notably by acting as a neuroenhancer. One of these tools, of interest in this thesis, is transcranial direct current stimulation (tDCS).

2. Chapter 2 - Transcranial direct current stimulation (tDCS)

2.1. History - Why use tDCS?

Brain function can be modified by applying stimulation over the scalp. Non invasive brain stimulation (NIBS) methods are being developed in order to modulate brain activity and to restore altered functions in specific diseases. Indeed, NIBS can be used to enhance or inhibit a specific neuronal pattern to show that it is necessary (though may not be sufficient) for a certain brain function.

To date, the simplest technique, transcranial direct current stimulation (tDCS) consists in applying a constant unidirectional low current stimulation between two scalp electrodes, a cathode and an anode, placed above two cortical areas. The current flows inward under the anode and outward under the cathode.

First described in the late 1950's and 60's (Terzuolo and Bullock, 1956; Bindman et al., 1962; Creutzfeldt et al., 1962; Purpura and McMurtry, 1965), tDCS has been shown to be effective in many therapeutic trials (Been et al., 2007). This technique was introduced as tDCS in clinical neurophysiology studies 20 years ago (Nitsche and Paulus, 2000) and serves as a non-invasive and painless tool for neuromodulation (Brunoni et al., 2012; Aparício et al., 2016; Bikson et al., 2016; Nikolin et al., 2018). It is generating increasing interest among neuroscientists and clinical practitioners (Tremblay et al., 2014; Bestmann et al., 2015; Parkin et al., 2015; Lefaucheur, 2016; Wörsching et al., 2016), due to its use as a prospective therapy for neurologic (Schultz, 2013; Flöel, 2014), psychiatric and addictive disorders (Kuo et al., 2014; Mondino et al., 2014; Tortella et al., 2014; Kekic et al., 2016). Notably, there is promising evidence suggesting a positive therapeutic effect in patients with major depressive disorder (Brunoni et al., 2012, 2013b), addiction to substance abuse and compulsive eating disorders (Jansen et al., 2013; Dunlop et al., 2016) as well as schizophrenia (Brunelin et al., 2012). However, despite an increasing use in clinical settings, tDCS suffers from limitations, especially regarding the strength and the duration of therapeutic effects (Brunoni et al., 2012).

In addition, a lack of consensus on tDCS effects among studies needs to be addressed (Antal et al., 2015; Horvath et al., 2015a). As problematic as this is however, these debates surrounding tDCS have helped to improve the field, especially with new reproducible considerations trying to standardize and individualize tDCS studies. It is now even possible to combine NIBS and neuroimaging studies (Venkatakrishnan and Sandrini, 2012; Saiote et al., 2013). These advancements will enable this booming field to better understand and use tDCS (Klooster et al., 2016; Woods et al., 2016; Thair et al., 2017).

Moreover, the use of tDCS is currently not restricted to the field of care. Based on scientific articles showing a potential (in a controlled environment) for frontal tDCS to improve certain cognitive functions (e.g working memory, reaction time or learning speed), the use of tDCS in a recreational context is encouraged. Indeed, as the interest grows, more and more websites emerge allowing everyone to buy or build tDCS apparatus for at home use.

Examples of this use are:

- accelerated training (HRL, department of defense)
- electrodoping (US SKIteam, HALO neuroscience)
- electroceuticals - Brain Healing (NIH)
- videogames : Foc.us : http://www.foc.us/index.php/
- youtube tutorials (https://www.youtube.com/watch?v=hgFWEBwT6BE)

This private use worries neuroscientists and health authorities because the risks and benefits of this approach in uncontrolled environments are far for being completely understood (Dubljević et al., 2014; Bikson et al., 2017).

To date (March 2018), 4805 total publications studying tDCS, within which 631 studied healthy humans and within these studies 18 investigated the link between DCS and dopamine (https://app.webofknowledge.com).

2.2. Applications - How Do I use it?

2.2.1. Stimulation parameters

A specific and simple montage has been developed in order to deliver tDCS. This system is comprised of a 9-volt battery, scalp electrodes, stimulation cables and a conducting agent (**FIGURE 14**).

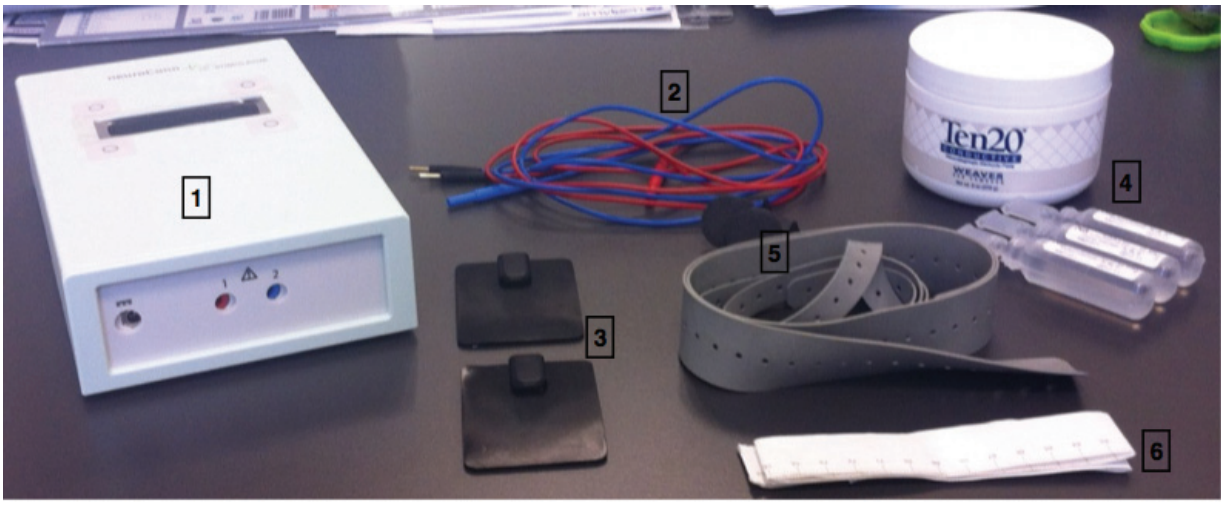


Figure 14: tDCS Material - **1**: Stimulator **2**: Stimulation cables **3**: Scalp Electrodes **4**: Conducting agents (Ten20 paste or NaCl) **5**: Rubber straps **6**: Measuring tape

The delivered current has different parameters that must be set at the start of every experiment and should be reported for reproducibility purposes: Intensity (~1-2 mA), Duration (~15-30 minutes), Ramp-up and Ramp-down (~30 seconds) (**FIGURE 15)**.

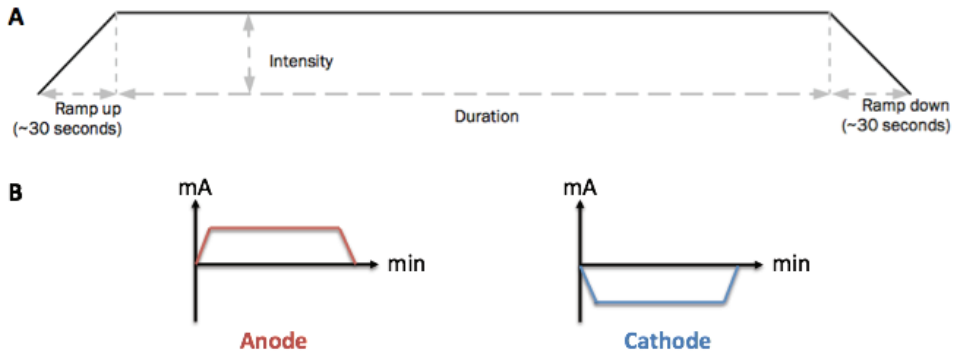


Figure 15: Active tDCS waveform - (**A**) Typical active waveform and the different parameters that can be modulated (ramp-up; ramp-down; intensity; duration). (**B**) Schematic representation of the different waveforms for anode and cathode electrode.

2.2.2. Electrodes

The electrodes are non-metallic, and made of conductive rubber. To create a good conduction between scalp and stimulation electrodes two solutions exist: Conducting gel (directly on the scalp, no sponges needed; Ten20 paste) or saline solution (NaCl 0.9%, saline-soaked synthetic sponges). This helps avoid skin damage. They hold in place thanks to adjustable rubber bands.

In most studies, at least 2 electrodes are needed for the stimulation: an anode (inward current) and a cathode (outward current) (Merrill et al., 2005).

The size of the electrode can range from $1cm^2$ to $100cm^2$ (Antal et al., 2017) and they should be placed at least 5cm apart to avoid the shunting effect (Miranda et al., 2006). However, it is important to keep in mind that the distance between electrodes, the position of both electrodes as well as individual anatomy under the electrodes could impact the current density entering the brain (Bikson et al., 2010; Seibt et al., 2015).

In this line, different methods have been developed in order to correctly place the tDCS electrodes. The most common is to position the electrodes over the scalp according to the International 10-20 System (**FIGURE 16**), usually used in EEG studies. Different electrode montages have been developed (unilateral or bilateral) in regard to the target sites (Nasseri et al., 2015).

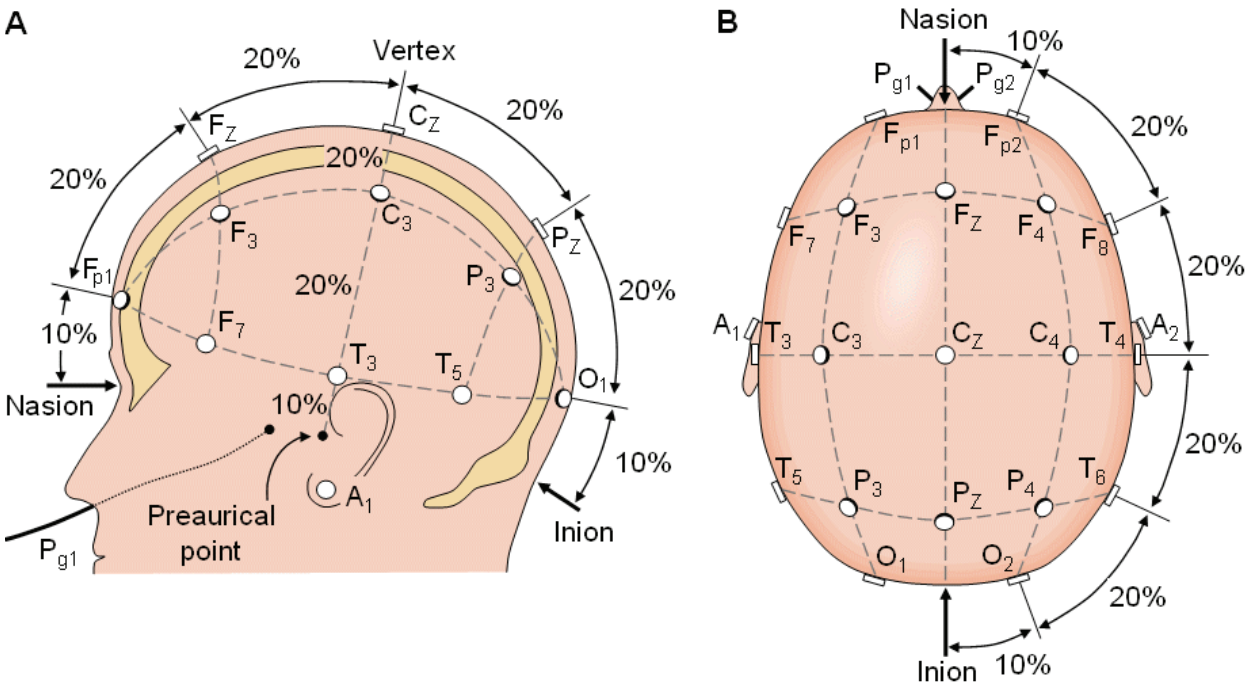


Figure 16: Electrode positioning - International 10-20 system

2.2.3. Sham-Placebo Mode

As with every scientific experiment, controls are crucial for a correct data interpretation. With this in mind, how to develop a good control for tDCS? To date, stimulators, such as the NeuroConn DC stimulator (used in our studies) have implemented a sham mode. A randomization code is assigned for each subject, corresponding to the code to be entered into the tDCS device. This system allows the person who administers the tDCS to also be blind (Gandiga et al., 2006; Palm et al., 2013b). During placebo stimulation, the active direct current is applied for the first 30 seconds only (if active duration is 15min; $Duration_{Sham}$ (s) = $Duration_{Active}$ (s) / 30), then no stimulation is performed for the rest of the active stimulation time except for brief pulses of 110µA over 15ms every 550ms to control impedance (which reliably detects bad electrode contact or electrode disconnection) and keep the manipulator blind of the condition. The peak current lasts for 3ms and the average current over time is not more than 2µA, which is considered to have no therapeutic effect (**FIGURE 17**). However, it should be noted that the sham-placebo mode varies across stimulator brands, which be a confounding factor when comparing studies. Thus, more research seems needed to make sure this sham is reliable and does not induce unexpected effects.

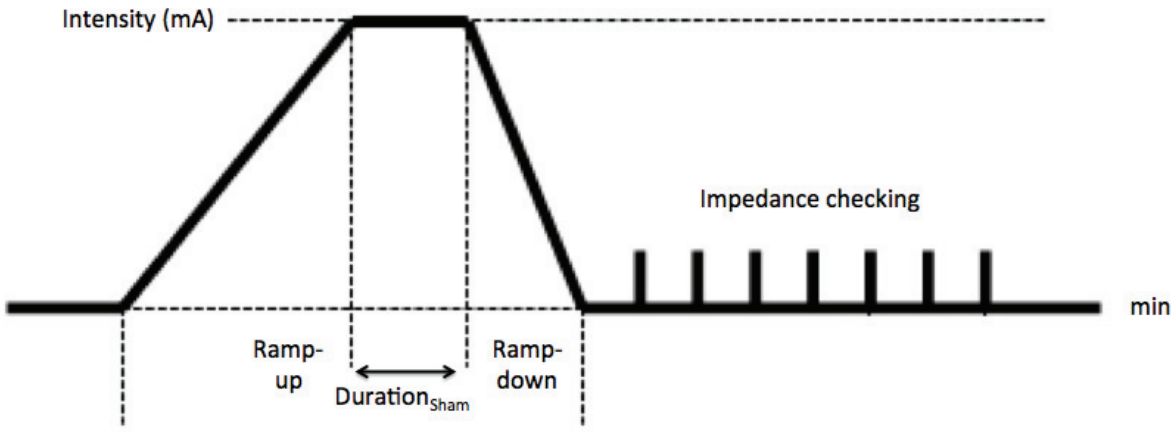


Figure 17: Sham tDCS waveform. Adapted from NeuroConn Manual

2.2.4. Security

tDCS is considered a safe and well tolerated technique when proper protocols are followed (Liebetanz et al., 2009; Brunoni et al., 2011; Bikson et al., 2016; Woods et al., 2016; Antal et al., 2017; Jackson et al., 2017). The main parameters to consider are current density, total charge and stimulation duration:

$$\text{Current Density}(A/m^2) = \frac{\text{Stimulation Intensity}(A)}{\text{Electrode Size}(m^2)}$$

$$\text{Total Charge}(C/m^2) = \frac{\text{Stimulation Intensity}(A)}{\text{Electrode Size}(m^2)} \times \text{Total Stimulation Duration}(ms)$$

In human studies, conventional and safe tDCS was defined with a stimulation intensity inferior to 4mA, a stimulation duration up to 60min per day and using electrode size between 1cm^2 and 100cm^2 (Antal et al., 2017). Evidence of brain injury by tDCS occurs at much higher current densities than the conventional tDCS in humans (for example, 2mA with 35cm^2 electrode size = 0.057 A/m^2). Indeed, animal and modeling studies reported that tissue damage could occur at much higher current densities: 6.3-13 A/m^2 or even 20 A/m^2 after anodal stimulation (Jackson et al., 2017), which is below the reference threshold of 142.9 A/m^2 after cathodal stimulation (Liebetanz et al, 2009). In addition, the impedance is controlled by the device throughout each tDCS session. An excess of limits (e.g., an increase of impedance by drying up or electrode slip) would lead to an automatic termination of the stimulation.

Studies investigating adverse events that may occur during a tDCS session have not reported adverse effects or major risks, when using proper stimulation protocols. The most commonly reported effects were stinging, itching, or burning sensations under the electrodes during the tDCS session (Bikson et al, 2016; Antal et al, 2017). However, these effects were transient and not severe.

However, from a regulatory point of view, a record of undesirable effects needs to be kept after each tDCS session. Several questionnaires have been developed. Recently, a

standardized questionnaire has reached consensus in the literature (Brunoni et al, 2011; Antal et al, 2017). This questionnaire assesses the side effects of tDCS such as itching, pain, burning, warmth/heat, metallic/iron taste, fatigue/decreased alertness on a scale of 0 to 4 (none, mild, moderate and strong).

2.2.5. MR-compatible

The biological effects of tDCS can be studied online through MR imaging by applying the stimulation under the scanner using a specific compatible stimulator (Eldith DC stimulator MR stimulator, NeuroConn GmbH, Germany, http://www.neuroconn.de/dcstimulator_plus_en/). The direct current is applied via a pair of rubber electrodes (7*5cm) specific to MRI acquisition. The basic material of these rubber electrodes is silicone with certain electrical conductor inside (carbon graphite) with a conductivity volume of 2.8 Ω / cm. This allows a resistance of the electrodes of 50 Ω.

Stimulation cables are equipped with a 5 kΩ resistor to reduce induction voltage due to high radio-frequency (RF) impulses during scanning. Electrodes are connected to the stimulator placed outside the magnet room via a cable running trough an RF filter tube in the cabin wall (MRI waveguide cable - 20m length). Two RF filter boxes are placed between the stimulator and the electrodes, to suppress artifacts by absorbing RF mostly between 50 to 140MHz. Stimulator, electrodes, cables and boxes are specifically MRI compatible (CE 0118 for medical applications). This montage is commonly used in published studies and was locally tested under real conditions and no artifacts were detected during EPI sequences (**FIGURE 18**).

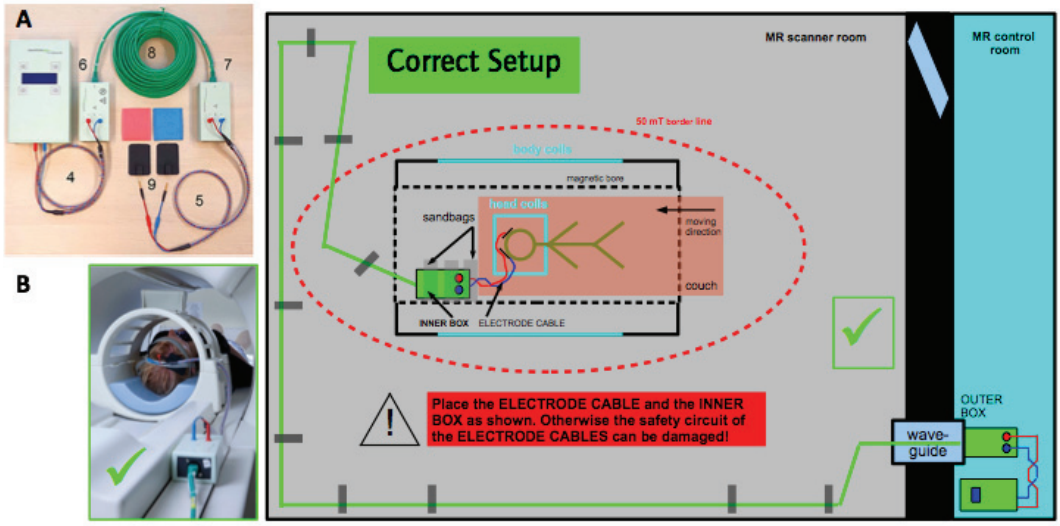


Figure 18: MRI compatible - Experimental setup and tDCS equipment - Adapted from Meinzer et al, 2014 and Neuroconn Manual - (**A**) Equipment required for intra-scanner tDCS (**B**) Correct setup to stimulate inside the MR scanner and concurrently with fMRI

To date, few studies have used this device but the numbers are increasing due to availability and interest in combining tDCS with neuroimaging techniques (Venkatakrishnan and

Sandrini, 2012; Saoite et al, 2013). Methodological studies have also verified that introducing an electric current with this device in the scanner provides analyzable results. Indeed, careful considerations should be taken into account due to the possibility of introducing field artifacts for BOLD fMRI protocols notably. These artifacts are dependent on the experimental setup and need to be verified at the beginning of each protocol and may vary from center to center (Holland et al., 2011; Antal et al., 2014a; Woods et al., 2016).

2.3. Mechanism of action

2.3.1. Animal studies (in vitro and in vivo)

The increasing use of tDCS in humans has motivated animal research, which is more and more needed to rapidly test various stimulation protocols and investigate the underlying cellular and molecular mechanisms of tDCS. The goal of this translational research is to test for efficacy and safety as well as optimizing protocols (optimum parameters and reproducibility across experiments). Animal studies investigate tDCS mechanisms with different protocols such as transcranial stimulation in animals, intracranial stimulation in vivo with one electrode on the cortex or stimulation of tissue in vitro (brain slices). Thus, reviews have emerged to try and make sense of these informative studies (Jackson et al 2016). To date, the research can be subdivided in three sections: 1) the acute effect (single neuron level); 2) the lasting effects (synaptic processing and plasticity); 3) the network level.

Acute effect - Single neuron level

From early animal studies, it has been hypothesized that tDCS-mediated effects are related to a shift in neuronal resting membrane potential toward depolarization and increased spontaneous neuronal firing at the anodal level and toward hyperpolarization and decreased firing at the cathodal level (**Figure 19**) (Bindman et al., 1964).

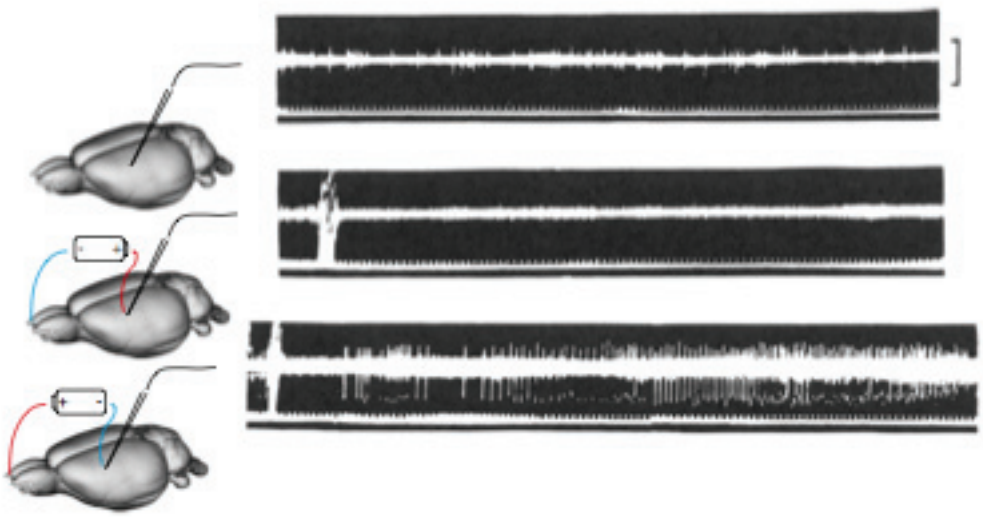


Figure 19: Effect of polarizing current - (**A**) Control period (baseline recording from an anesthetized rat) (**B**) Activity recorded after cathodal stimulation (**C**) Activity recorded after anodal stimulation. Adapted from Bindman et al, 1964

However, the polarizing differences are not so clear. Indeed, direct current stimulation (DCS) has been shown in vitro to result in both depolarization and hyperpolarization depending on the neuron compartment (soma, dendrite, axone) (for review see: Rahman et al., 2013). Radman and colleagues (2009) reported that in typical cortical pyramidal neurons, the anode induced a cortical inward current leading to a somatic and basal dendrite depolarization in addition to a hyper polarization of the apical dendrite. The opposite effects were seen when the cathode was applied instead (outward current flow). Moreover, these effects are reported dependent on the position of the electric field. Notably, the maximum depolarization is seen when the electric field is parallel to the somatodendritic axis, i.e radial to the cortical surface (Bikson et al., 2004). In the same line, the presence of CSF can distort the pattern of current flow and locally invert the current direction (Creutzfeldt et al., 1962).

However, effects observed at the single neuron level may not support alone the clinical or behavioral effects seen in human studies. A strong theory enabling these effects is the process of amplification of ongoing activity by tDCS. Indeed, studies have shown that amplifying the effects of weak membrane polarization can be done via spike timing modulation (firing latency of single neurons) (Radman et al., 2007). In addition, polarization of afferent neurons in interconnected brain regions could modulate activity in the targeted brain region, via increasing synaptic integration and thus inducing perceivable effects (Rahman et al., 2017). However, further studies need to be conducted to better understand how prolonged polarization triggers the induction of plasticity, and how to integrate the different neuronal compartments in this model.

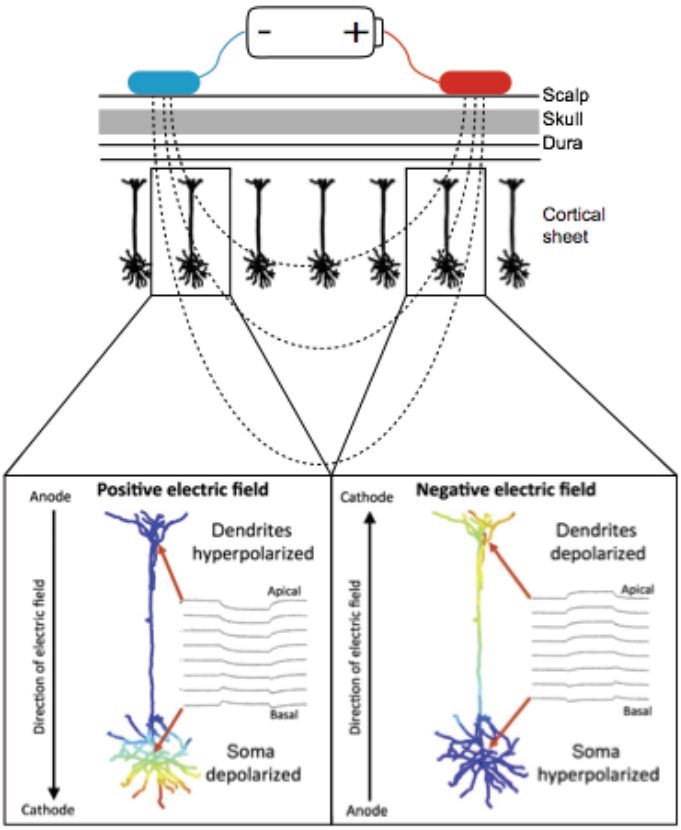


Figure 20: Schematic representation of the effect of a bipolar electric field - Illustration of the influence of tDCS on the electric fields of neurons close to the anode (blue) and the cathode (red). This schematic representation shows the differences between anodal and cathode stimulation on the near ionic gradients. Adapted from Rahman et al, 2013; Reinhart et al., 2017

With this is mind, the next step would be to incorporate these notions in a more complex picture, notably the cortex as a whole. Indeed, the cortex, being convoluted, the current would not stay unidirectional nor would the flow be dominantly radial. Thus, the simplistic model proposed (**Figure 20**), with a radial and inward current flow under the anode, and a radial and outward current flow underneath the cathode, needs to be revisited to take into account not only the somatic polarization but also a more complex integration of the information from compartments such as the dendrites and the axon (Rahman et al, 2013).

Lasting effects - Synaptic processing and plasticity

The notion that tDCS can have lasting changes in brain excitability at the membrane neuronal level dates from the 1960's (Bindman et al, 1964). In line with these observation, other studies have confirmed these changes in brain slices (Fritsch et al., 2010), coupling membrane polarization with ongoing synaptic activity (Kronberg et al., 2017; Lafon et al., 2017; Rahman et al., 2017), and changes in network dynamics (Reato et al., 2013, 2015). In addition, DCS-induced plasticity has been shown to share molecular mechanisms with LTP/LTD processes. The lasting effects of tDCS are hypothesized to be dependent on dendrite activity, via NMDA receptors (Kronberg et al, 2017). In addition, the orientation of the current flow, either radial or tangential, seems effective in modulating synaptic efficacy

(Bikson et al., 2004; Radman et al., 2009; Kabakov et al., 2012; Rahman et al., 2013). Thus, it seems as though the lasting effects are dependent on the orientation of synaptic terminals (i.e they are potentiated if pointing towards the anode; and inhibited if pointing towards the cathode).

In addition, an important notion to keep in mind when investigating the effects of tDCS, is the impact at the network level. Indeed, during network activities, neurons are near threshold and primed for firing. Thus, a small depolarization only is needed to trigger an action potential in the neuron, as well as in interconnected neurons (Reato et al., 2010). Furthermore, this network involvement has been put forward in a recent mice tDCS study looking at the dopaminergic network. It reported a long lasting release of subcortical dopamine in the striatum while stimulating the frontal cortex (Tanaka et al., 2013).

In sum, animal studies have improved our understanding of the cellular mechanisms underlying tDCS. However, new questions arise such as the involvement of interneurons and non-neuronal cells (glial and endothelial cells) (Radman et al., 2009; Ruohonen and Karhu, 2012). In addition, few animal studies have investigated tDCS mechanisms and up to now, only one study investigated the effects of tDCS in psychiatric animal models (Peanlikhit et al., 2017). Moreover, two studies investigated the cumulative effect of multiple tDCS sessions (Rueger et al., 2012; Rushmore et al., 2013). These studies seem important to decipher biological tDCS effects in impaired conditions such as those present in pathological conditions. These next steps in animal studies would help us to better understand the underlying tDCS mechanisms in human tDCS clinical studies. Nevertheless, human studies also need to account for the complexity of normal and pathological brain function.

2.3.2. Cortical Excitability

With these notions in mind, tDCS could impact cortical excitability by modulating resting membrane potential. Moreover, depending on the stimulation polarity, tDCS may induce excitatory or inhibitory effects on motor cortex excitability.

Indeed, stimulation of the motor cortex was the prime focus of early tDCS studies, due to a measurable output, called TMS-evoked motor evoked potentials (MEP). Early 2000s, the first studies using this technique detailed tDCS effects on polarization in humans, with the anode over the left primary motor cortex (M1) and the cathode over the contralateral orbit. MEPs of the right first dorsal interosseous muscle were elicited from M1. They explored the effects of on the peak-to-peak MEP amplitude, duration and latency of MEPs and found that anodal tDCS induced excitatory effects, whereas cathodal stimulation resulted in inhibitory effects on motor cortex excitability (Nitsche and Paulus, 2000). When the stimulation was applied continually during several minutes, the induced excitability changes lasted for up to an hour (Nitsche and Paulus, 2001; Nitsche et al., 2003b) (**FIGURE 21**).

These effects were also observed when tDCS was applied over the visual cortex (Antal et al., 2003). Anodal tDCS reduced the phosphene threshold (i.e increased excitability of the occipital cortex) while cathodal tDCS resulted in an opposite effect.

Therefore, these observations are in accordance with tDCS effects observed in animal studies, and point toward important parameters involved in the design of tDCS studies, such as intensity and timing of the stimulation (duration, number of sessions and time between sessions).

Effects of intensity

The intensity was shown to be an important parameter in tDCS study design. Indeed, anodal tDCS excitatory effects are obtained only when stimulating at least at 0.6mA, with 5 minutes duration. In addition, increasing the intensity leads to an increase in MEP amplitude and prolonged after-effect (Nitsche and Paulus, 2000). However, an intensity increase could also lead to opposite effects. A study reported that 2mA cathodal stimulation for 20 minutes resulted in cortical excitability enhancement instead of inhibition (observed after 1mA-20min) (Batsikadze et al., 2013).

It seems that intensity is not the only parameter involved in mediating tDCS effects. Indeed, other studies also suggest an impact of the timing of the stimulation.

Effects of timing

Timing effects are reported in the literature at different scales such as, the duration of the stimulation, the number of session and the time between sessions.

Concerning the duration of the stimulation, studies have suggested that, when stimulating with the same intensity, the longer the stimulation the more durable the effects are (Nitsche and Paulus, 2001; Nitsche et al, 2003). Indeed, with 1mA tDCS stimulation, an anodal stimulation duration of 13 minutes and a cathodal stimulation duration of 9 minutes induce long-lasting effects on cortical excitability (60-90 minutes) (**FIGURE 21**). In the same line, a cathodal stimulation of 18 minutes would result in more durable inhibitory effects (up to 90 minutes) (Monte-Silva et al., 2010).

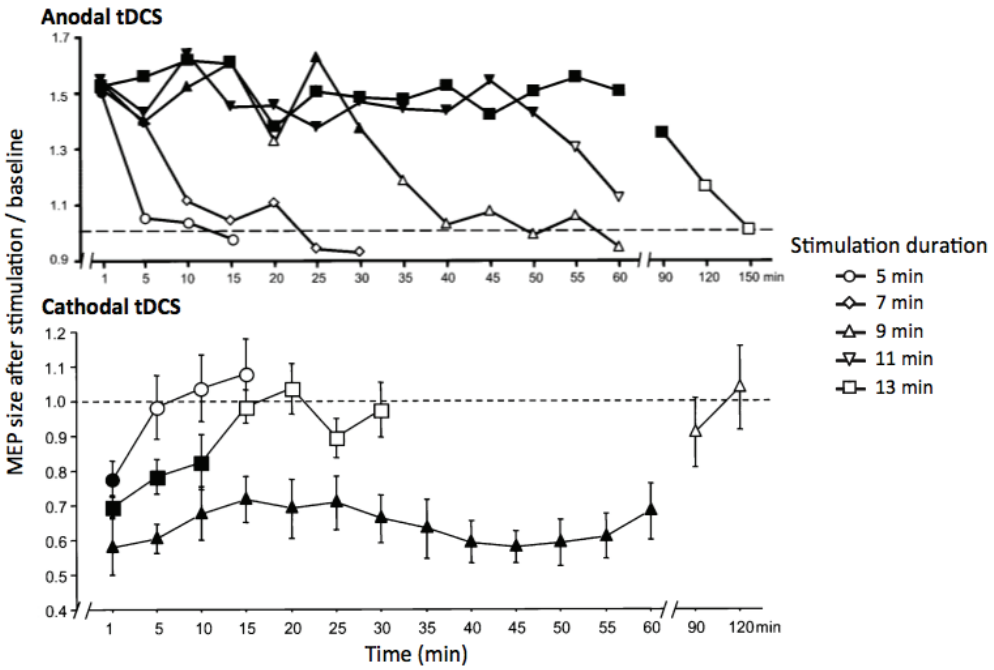


Figure 21: Effect of tDCS duration on MEP size after anodal and cathodal stimulation (1mA). Here are reported the after effects on cortical excitability seen after anodal and cathode stimulation of the motor cortex: with longer stimulation duration, the after effects could last up to 90minutes. Filled symbols indicate a significant difference compared to baseline. Adapted from Nitsche and Paulus, 2001 and Nitsche et al, 2003b.

However, longer stimulation duration does not always mean long-lasting effects. Indeed, an opposite effect on cortical excitability was reported with different duration parameters. For example, an anodal stimulation applied for 26 minutes induces an inhibition of MEP amplitudes instead of an increase, which could be due to differential effects on LTP-like plasticity induction (Monte-Silva et al., 2013).

Concerning the number of sessions, due to the clinical efficacy of tDCS cures (repeated tDCS sessions), studies have supported that stimulation over consecutive days would lead to a cumulative and larger excitability effects (Alonzo et al., 2012; Gálvez et al., 2013; Ho et al., 2016).

The timing between sessions seems to also play a key role in the stimulation effects on cortical excitability. Indeed, a prolonged effect is reported when the second session is done between 3 to 20 minutes after the first one, for both anodal and cathodal stimulations. However, if the second session is done after the after-effects of the first session are gone (3h or 24h after) then the effects of the second session are decreased and delayed or even abolished (**FIGURE 22**). However, these results seem to be dependent on the stimulation duration (Fricke et al., 2011).

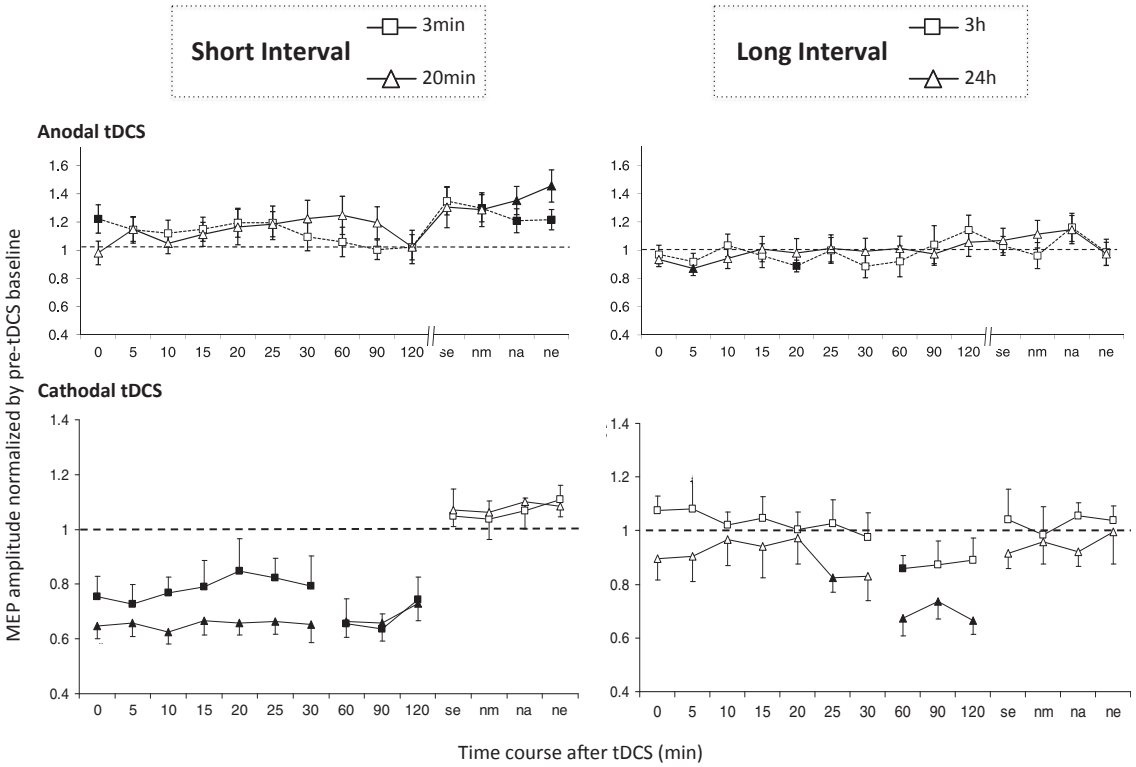


Figure 22: Effect of time between tDCS sessions. Here is reported the after effects on cortical excitability seen after anodal and cathode stimulation of the motor cortex. The inter-stimulus duration determines these after effects. Filled symbols = significant difference compared to baseline. *Abbreviations: se = same evening; nm = next morning; na = next afternoon; ne = next evening.* Adapted from Monte-Silva et al 2010 and 2013

With this in mind, all of these variables make it hard to reliably compare stimulations studies. This underlines the importance of carefully choosing the combination of stimulation parameters relevant for the study. However, one should keep in mind that these studies are done in healthy subjects and assess the reactivity of the motor cortex, not the entirety of the neurobiological effects.

2.3.3. Neurobiological effects

To date, tDCS has shown effects on cortical excitability leading to the question: How does the stimulation impact such a process? Multiple groups have reported that tDCS could influence both excitatory and inhibitory pathways, and translate to modulations in functional brain networks (for review see, Nitsche et al., 2008; Stagg and Nitsche, 2011; Medeiros et al., 2012; Cirillo et al., 2017). With this in mind, different tDCS montages have been developed.

Motor tDCS montage

tDCS research has focused on stimulation of the motor cortex due to its measurable output, using a montage called “motor tDCS montage” with the anode generally placed over the left M1 and the cathode over contralateral orbit.

Using this montage, pharmacological studies have sought to understand the relationship between cortical excitability and neurobiological changes. Glutamate, specifically via NMDA receptors, and GABAergic transmission seem to play an important part in tDCS effects (Liebetanz et al., 2002; Nitsche et al., 2003a, 2004a, 2004b; Monte-Silva et al., 2013). In addition, other, neuromodulatory, systems such as norepinephrine (NE) (Nitsche, 2004; Kuo et al., 2017), serotonin (5HT) (Nitsche et al., 2009; Kuo et al., 2016), acetylcholine (Kuo et al., 2007) and dopamine (Nitsche et al., 2006; Kuo et al., 2008; Monte-Silva et al., 2009; Fresnoza et al., 2014a, 2014b; Jongkees et al., 2017) are implicated in the effects of tDCS on cortical excitability. For more details, see **Table 1** and Chapter 4 §4.1 (Dopamine).

Neurotransmitter System	Paper	Drug	Dose	Main Effects Under the Anode & Cathode
Glutamate	Liebetanz et al., 2002 Lugon et al., 2017 Monte-Silva et al., 2013 Nitsche et al., 2003	Dextromethorphane (NMDA antagonist)	50mg (low) 100mg (med) 150mg (high)	Anode: Dose dependent effects. No impact on excitability enhancement with **low** doses. Eliminated excitability enhancing after-effects with **medium** and **high** doses. Cathode: Eliminated after-effects at high dosages.
	Nitsche et al., 2004	d-Cycloserine (partial-NMDA agonist)	100mg	Anode: Prolonged excitability enhancing after-effects. Cathode: Increased intensity of excitability reduction 10-minutes post-stimulation.
GABA	Nitsche et al., 2004	Lorazepam ($GABA_A$ receptor agonist)	2mg	Anode: Initially delayed then prolonged excitability enhancing after-effects, following both short (5 mins) and long (11 mins) stimulation. Cathode: No impact the excitability reduction response.
Norepinephrine/ Epinephrine	Kuo et al., 2016	Reboxetine (Selective NE reuptake inhibitor)	8mg	Anode: Increased and prolonged excitability enhancement with chronic administration Cathode: Reversed excitability reducing into excitability enhancing after-effects
	Nitsche et al., 2004	Propranolol (Unselective Beta-adrenergic antagonist)	80mg	Anode: Shortened duration of excitability enhancing after-effect. Cathode: Shortened duration of excitability reducing after-effects.
Serotonin (5-HT)	Kuo et al., 2016 Nitsche et al., 2009	Citalopram (SSRI)	20mg	Anode: Increased and prolonged excitability enhancing after-effects. Cathode: Reversed excitability reduction response to excitability enhancement and prolonged after-effects.
Acetylcholine	Kuo et al., 2007	Rivastigmine (Cholinesterase inhibitor)	3mg	Anode: Initially blocked excitability enhancing after-effects. Cathode: Initially blocked then prolonged excitability reducing after-effects.

Table 1: tDCS and Pharmacological studies in humans - From McLaren et al., 2018

Some imaging reports suggest that tDCS effects are not restricted to the brain areas located under the electrodes, but spread through distributed cortical networks functionally connected with the targets and reach subcortical areas including modulations of cortico-striatal and thalamo-cortical functional connectivity. These studies have investigated these effects with an online or offline design and using different imaging modalities such as fMRI (Baudewig et al., 2001; Kwon et al., 2008; Stagg et al., 2009b; Antal et al., 2011; Kwon and Jang, 2011; Polanía et al., 2011, 2012; Sehm et al., 2013; Amadi et al., 2014; Stagg et al., 2014; Bachtiar et al., 2015), EEG (Polanía et al., 2011), ASL (Zheng et al., 2011), MRS (Stagg et al., 2009a; Clark et al., 2011; Stagg and Nitsche, 2011) and PET (Lang et al., 2005; Paquette et al., 2011). In general, these studies report an increased excitability after anode stimulation and a decrease after cathode stimulation, as well as possible intra- and interhemispheric changes.

Frontal tDCS montage

Besides M1, other regions such as the frontal cortex are of major interest as targets for cognitive and neuropsychiatric conditions. Recently, frontal tDCS montage received a booming interest due to its possible effects, notably cognitive, in healthy volunteers (Tremblay et al., 2014; Mondino et al., 2015) and for various clinical applications (Jansen et

al., 2013; Mondino et al., 2014; Tortella et al., 2014; Brunoni et al., 2016; Lefaucheur et al., 2017).

With this montage, most of the studies have focused on the impact on cortical and subcortical networks interconnected with the stimulation sites. As seen with the motor tDCS montage, the stimulation is reported to have an effect in local and distributed areas in relation to the stimulation site with different imaging modalities: ASL (Stagg et al., 2013), fNIRS (Merzagora et al., 2010), fMRI (Keeser et al., 2011; Peña-Gómez et al., 2012; Park et al., 2013; Clemens et al., 2014; Weber et al., 2014; Hunter et al., 2015) and MRS (Hone-Blanchet et al., 2016).

However, other studies also point an inter- and intra-individual variability in response to tDCS (López-Alonso et al., 2014; Chew et al., 2015; López-Alonso et al., 2015; Wörsching et al., 2017).

2.3.4. Computational Models of tDCS

In parallel, the use of computational models is generating interests in that they are able to simulate the electric field resulting from tDCS. Computational models have been developed since the early 2000s and are increasingly used to help understand and predict the mechanisms of action of tDCS (for review see Bikson et al., 2012, 2015; Bonaiuto and Bestmann, 2015).

They started from simple spherical models with only 3 tissue compartments (skull, CSF, brain; Miranda et al., 2006; Datta et al., 2008), to more complex and realistic ones, investigating the MRI-derived finite element head models (FEM), taking into account more tissue compartments (skin, layers of the skull, CSF, WM, GM), the gyrification, high conductivity of CSF, white matter anisotropy and subcortical regions (Holdefer et al., 2006; Wagner et al., 2007; Datta et al., 2009; Suh et al., 2009; Datta et al., 2011; Parazzini et al., 2011; Suh et al., 2012; Miranda et al., 2013; Rampersad et al., 2013; Wagner et al., 2014; Opitz et al., 2015; Huang et al., 2017b). These studies put forth several important notions. The conductivity of specific compartments (skin, skull spongiosa and the CSF) has a major impact on tDCS electric field. Indeed, current flow tends to be oriented towards the closest higher conducting region, and thus surface currents are shunted by the skin, attenuated by the thick skull and dispersed in the CSF. In addition, even if the current density decreases with increasing distance from the electrodes, the electric current does not necessarily peak under the stimulation sites. Indeed, studies have demonstrated that the current flow could reach deep targets. To add to the complexity, is seems important to point out that with this information, the anode and cathode cortical stimulation may inevitably result in mixed polarity underlying tDCS effects (**FIGURE 23**). This is interesting when looking at a network involvement that may lead to different tDCS effects at the cortical and subcortical level (Datta et al., 2009; Rahman et al., 2013; Brunoni et al., 2014; Huang et al., 2017b; Lee et al., 2018).

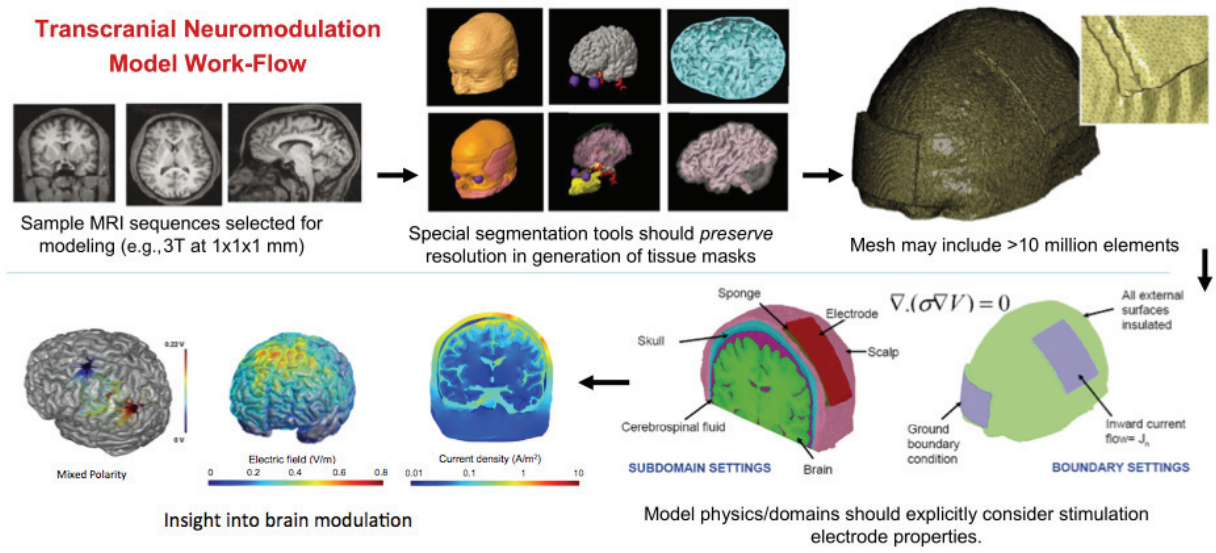


Figure 23: Imaging and Computational workflow - Individualized model of the electric field and current during tDCS - Adapted from Bikson et al., 2012; Peterchev, 2017; Rawji et al., 2018. This workflow represents the general steps involved in the generation of realistic MRI-derived finite-element head models. It should be noted that this workflow can vary depending on the software used.

Different electrode montages can result in distinct brain current flow patterns across the brain. Using computational models, one can modulate controllable parameters, such as electrode number, position, size, shape and intensity, in order to target or avoid specific brain regions. An increasing number of studies put forth the importance of customizing tDCS not only for potentially vulnerable populations (e.g skull defects, brain damage, age) but also in regards to the variability of individual head anatomy.

Indeed, concerning the electrode placement, the relative placement of the cathode in respects to the anode seems to determine the amount of current reaching the brain. Thus, tDCS studies should really keep in mind that the current flows between electrodes and influences all intermediary regions (Datta et al., 2008, 2009; Bikson et al., 2010; Datta et al., 2011; Bai et al., 2014). In this line, the direction of current flow is an important part of the study design, with electrode montages oriented orthogonally to M1 creating a consistent oriented current in the targeted region and thus allowing tDCS effects (TMS-MEPs variations) (Rawji et al, 2017).

In sum, brain activity modulation could result from both direct effects from the general current flow distribution and indirect effects linked to the position of the electrodes and underlying brain connectivity. Thus, tDCS is an interesting tool to modulate brain activity and is increasing used in general and clinical populations. In this line, our thesis work has focused on the dopaminergic system, in order to better understand the mechanisms of tDCS.

3. Chapter 3 - Studying the impact of tDCS on the dopaminergic system

Several neuroimaging techniques are available to investigate the impact of tDCS on the dopaminergic system in humans, at different levels (biological and anatomical, temporal and spatial). We will focus here on several neuroimaging techniques (that are used in our studies) such as Positron Emission Tomography (PET), and Magnetic Resonance Imaging (MRI). More specifically, different MRI sequences are used to assess different components of the brain: resting state functional MRI (rs-fMRI), Arterial Spin Labeling (ASL) and Diffusion Tensor Imaging (DTI) (**FIGURE 24**).

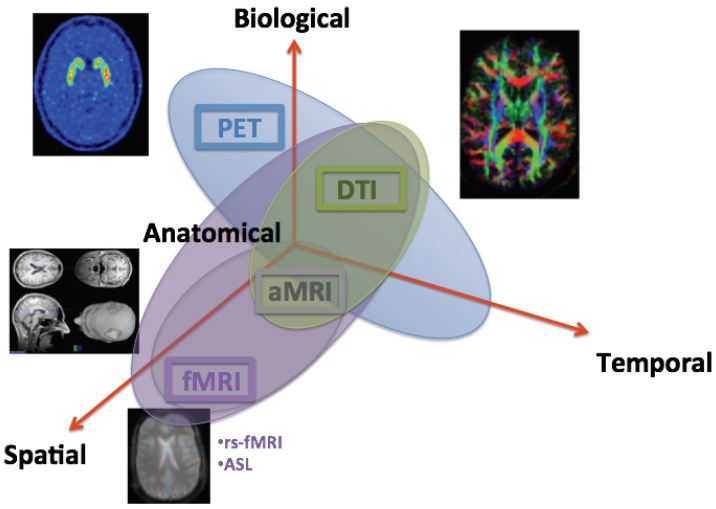


Figure 24: Neuroimaging methods - Investigating the dopamine system at different levels: Biological: PET; Spatial: rs-fMRI and ASL; Anatomical: structural MRI and DTI.

3.1. Positron emission tomography (PET)

Positron emission tomography (PET) is a nuclear imaging technique that measures physiological function by looking at blood flow, metabolism and neurotransmitters via radiolabelled molecules. The technique is based on the detection of radioactivity emitted after a small amount of a radioactive tracer is injected into a peripheral vein. The total radioactive injection is used at very low dose in order to not trigger any physiological reaction in the body. This allows the detection of the tracer by the PET scanner, following the disintegration process of the isotope. Thus, PET offers quantitative analyses, allowing us to monitor relative changes over time and space, using different isotopes with varying half-life. However, to obtain a quantitative image, multiple preprocessing steps are required: radiotracer synthesis, venous injection, data acquisition, image reconstruction and motion/attenuation corrections.

Radiotracer

The radiotracer injected consists of a chemical compound labeled with a radioactive isotope. These radioactive isotopes are produced by a nuclear reaction in a small circular particle accelerator (i.e cyclotron). The most common isotopes used in PET studies are: oxygen-15(^{15}O), fluorine-18 (^{18}F), carbon-11(^{11}C), or nitrogen-13(^{13}N). Their use depends on the molecules targeted and the half-life of these isotopes.

The next step, radiosynthesis, is the incorporation of the radioactive isotope into the chemical compound (specific to the molecules targeted). Concerning the dopaminergic system, as mentioned in Chapter 1, PET radiotracers have been developed in order to target different compartments: pre-synaptic and post-synaptic (**FIGURE 25**).

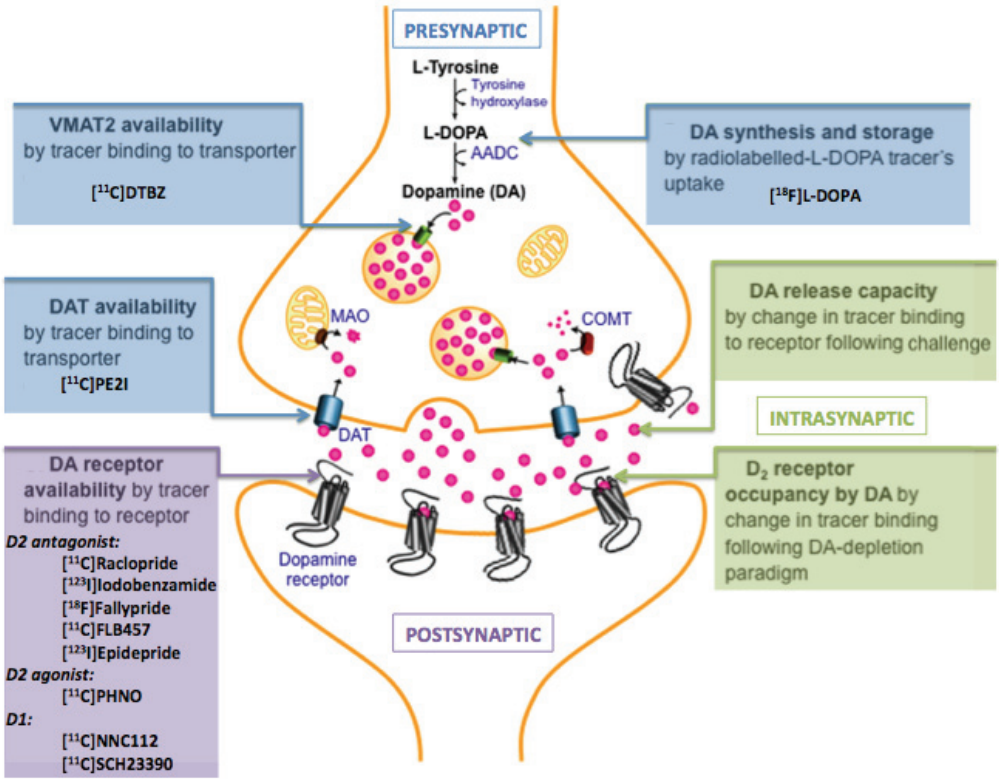


Figure 25: Dopaminergic imaging targets and PET radiotracers - Most common PET radiotracers to assess the dopaminergic system in the presynaptic and post-synaptic compartments. Adapted from Weinstein et al., 2017

In our studies, we used [^{11}C]Raclopride, a benzamide showing selective and moderate affinity as an antagonist to D2 receptor, commonly used in PET studies, aiming to analyze dopaminergic transmission (Hall et al., 1988) (**FIGURE 26**). Due to its modest affinity, accurate quantification of this radiotracer is mostly possible in high D2 density regions such as the striatum. This has been confirmed with test-retest studies reporting high reliability (Volkow et al., 1994). Its half-life of 20min is well adapted for a 2 hour study design, meaning that at the end of the protocol, only residual radioactivity will be left in the subject's system.

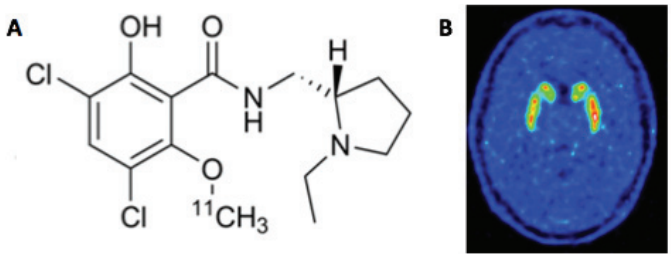


Figure 26: [^{11}C]Raclopride - (**A**) Radiotracer composition (**B**) Example of radiotracer distribution in the human brain. Image clearly reveals high accumulation of the radiotracer in the striatum and low non-specific accumulation in the rest of the brain.

Furthermore, dopamine changes measured with PET are observed because the radiotracers bind reversibly to receptors. This has led to theorize a model called the 'occupancy model'. This is based on a the competitive interaction between the radiotracer and endogenous dopamine binding, enabling studies to derive a parameter such as the binding potential (BP), sensitive to changes in dopamine levels. Specifically, tracers with moderate affinity, such as [^{11}C]Raclopride, have provided reliable BP in the striatum (Slifstein and Laruelle, 2001; Innis et al., 2007). This model predicts that when a challenge increases synaptic dopamine levels, a decrease in BP would be observed (due to an increased occupancy of D2 receptors by endogenous dopamine), whereas a reduced dopamine activity would be reflected by an increased BP (Laruelle, 2000). Thus with this technique, PET is a reliable technique to investigate the neurophysiological impact of tDCS on the dopaminergic system.

Tracer delivery method

Two types of methods are available for tracer delivery: bolus or bolus infusion design. Both designs are used to measure changes in the distribution of the tracer in response to a challenge condition. The main difference between the two is that bolus studies need two syntheses on two separate days and the bolus/infusion studies only require one synthesis (**FIGURE 27**). However, limitations exist for both methods. Indeed, bolus studies are more susceptible to physiological variations between scans, whereas bolus/infusion studies are more susceptible to equilibrium problems: the equilibrium might not be reached at the same time in all brain regions.

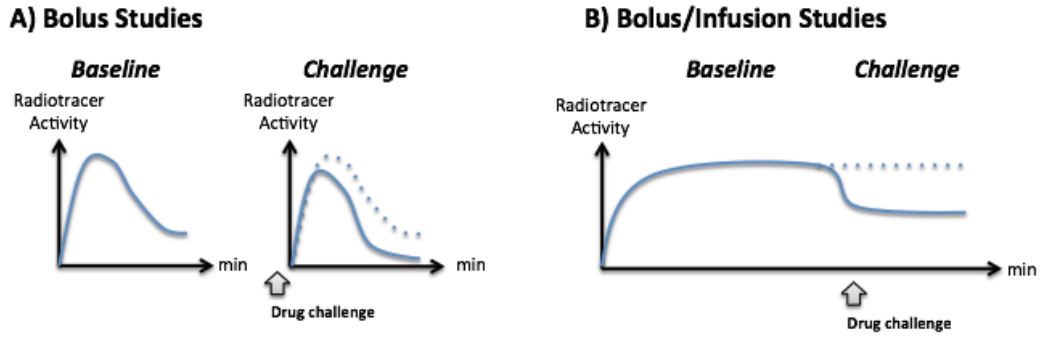


Figure 27: Tracer delivery method - 2 designs in challenging condition - (**A**) Bolus design (**B**) Bolus/Infusion design

Data acquisition and Image reconstruction

To detect and subsequently form an image of the radiotracer concentration and distribution, the primary principles are an annihilation reaction and the detection of coincidences.

Indeed, once injected, the isotope disintegrates into a more stable state, i.e. a positron and a neutrino are emitted from the nucleus. The ejected positron travels until it collides with an electron, which creates an annihilation reaction leading to the emission of a pair of 511keV gamma-ray photons in opposite direction. These photons are then detected simultaneously by the PET scanner. This process is called the detection of coincidences and helps to

determine the spatial source of the annihilation by recording these events and storing them into a list mode file or sinogram. With this information, two types of image reconstructions are possible: analytic (filtered back projection, FBP, inconsistent results with noisy data) and iterative (ordered subset expectation maximization, OSEM, more realistic model) reconstruction (**FIGURE 28**).

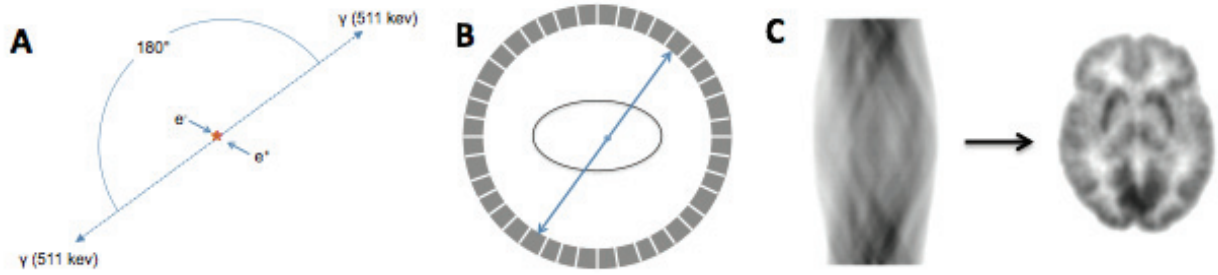


Figure 28: PET Principle - (**A**) Annihilation reaction (**B**) Detection of coincidences (**C**) Reconstruction from the sinogram. Adapted with permission from Ines Merida

However, these reconstructions need to be corrected for factors such as photon attenuation by the tissues and motion, in order to have a more accurate quantitative measure of the distribution and concentration of the radiotracer and thus the underlying biological processes (**FIGURE 29)**.

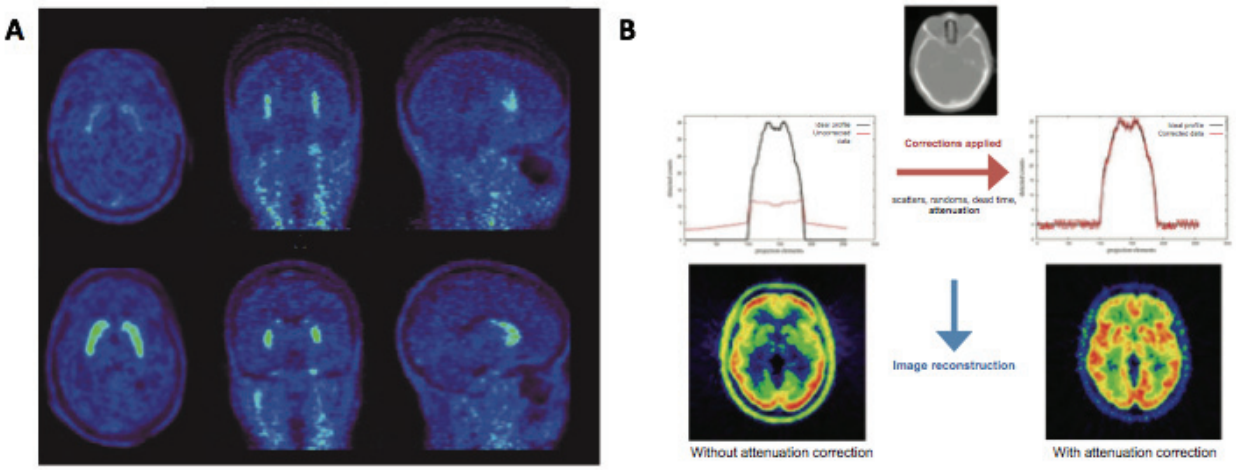


Figure 29: Correction (**A**) Motion correction with EBER method: Uncorrected (*top*) and corrected (*bottom)* mean images (**B**) Attenuation correction with MaxProb method: uncorrected (left) and corrected (*right*) mean images. Adapted with permission from Ines Merida and Anthonin Reilhac.

3.2. Magnetic Resonance Imaging (MRI)

MRI is an imaging technique used in clinical and research settings in order to produce high quality images of the inside of the human body. MRI is based on the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum. More specifically, the human body is composed mainly of hydrogen atoms, whose nuclei have a nuclear magnetic resonance signal. Without going into details of the physics behind it, an MR sequence is based on repetition of radio-frequency (RF) pulses that excite the system. The signal recorded is sampled at several time intervals. Thus, contrasts of the MR images

depend on two main parameters: the echo time (TE; time between the RF pulse and the MR signal sampling) and the repetition time (TR; time between two excitation pulses). Here, we are interested in MRI sequences investigating different but complementary biological processes: resting-state functional MRI (rs-fMRI), diffusion tensor imaging (DTI) and arterial spin labeling (ASL).

3.2.1. Resting state functional magnetic resonance imaging (rs-fMRI)

Functional magnetic resonance imaging (fMRI) provides an indirect measure of neural activity across brain regions via changes in blood oxygenation. Indeed, these changes are thought to generate a blood oxygenation level dependent (BOLD) signal, due to a change in the magnetic susceptibility between oxyhemoglobin and deoxyhemoglobin. In general, an increase in neural activity induces an increase in oxyhemoglobin leading to an increased BOLD signal in a specific region, called a hemodynamic response. Based on this principle, different designs can be used in fMRI studies: activation designs (induced activity; where a task induces blood oxygenation changes) and resting-state designs (spontaneous activity; where the subject is at "rest"). Here, we will focus on this last design: resting-state fMRI (rs-fMRI).

One way to analyze rs-MRI is to assess functional connectivity between regions in the brain. Thus, resting state functional connectivity measures the functional connections in the brain via the temporal correlation of low frequency fluctuations (0.01-0.08Hz) in the MRI signal. These fluctuations are thought to reflect synchronized variations in spontaneous neuronal firing within specific neuroanatomical systems. This organized functional activity in the resting brain involves cortical and subcortical set of areas, grouped into what is now universally termed 'resting state networks' (Biswal et al., 1995; Fox et al., 2005; Damoiseaux et al., 2006; Fox and Raichle, 2007; Mantini et al., 2007; Di Martino et al., 2008; Vincent et al., 2008; Zhang et al., 2008). It is now widely accepted that these specific networks increase or decrease in activity during task performance (activation/deactivation) and represent a new view of the organization of the brain intrinsic activity. These networks appear to be relatively consistent across individuals while showing positive or negative correlated spontaneous activity over time (Fox et al., 2005; Dosenbach et al., 2007; Seeley et al., 2007; Power et al., 2011; Yeo et al., 2011; Sadaghiani and D'Esposito, 2015); **FIGURE 30 A**). To date, an extensive literature has surfaced trying to grasp the role of major networks such as a task-negative network (TNN; active when subjects are not engaged in goal-directed task performance; Greicius et al., 2003; Raichle, 2015); a task-positive network (TPN; when engaged in cognitive processes; Fox and Raichle, 2007; Bressler and Menon, 2010) and other networks restricted to somatomotor, visual or auditory brain regions (Yeo et al., 2011).

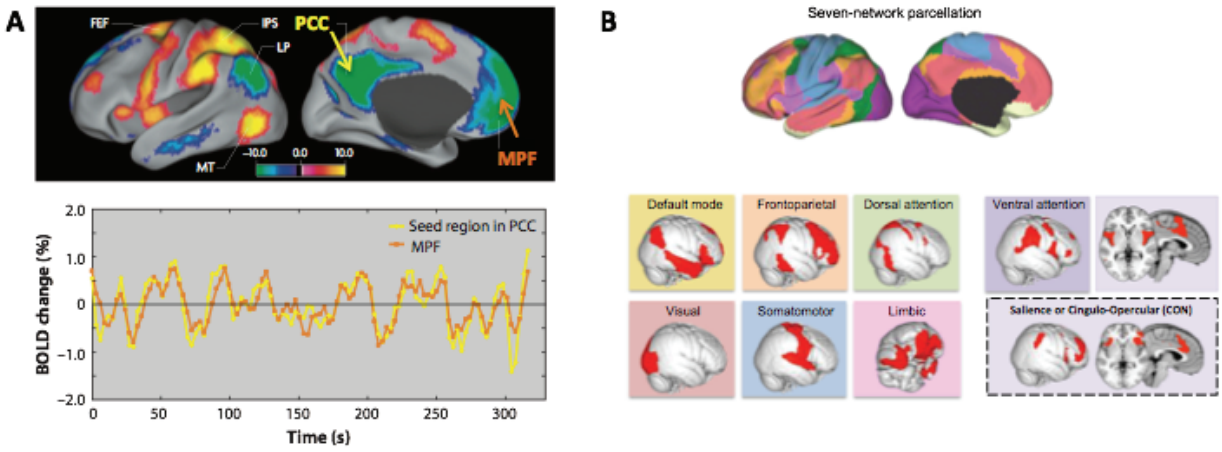


FIGURE 30: resting-state fMRI Principle - (**A**) Two main resting state networks TNN (cool colors) and TPN (warm colors) (*Top*) and Synchronized BOLD signal changes within 2 seeds of the TNN (bottom) (**B**) Subnetworks parcellation: DMN, FPN, DAN/CEN, VAN (SN/CON). *Abbreviations: TNN= task negative network; TPN = task positive network; DMN= default mode network; FPN= fronto-parietal network; DAN= dorsal attention network; CEN= central executive network; VAN = ventral attention network; SN = salience network; CON = cingulo-opercular network.* Adapted from Raichle, 2015; Yeo et al, 2011; Downar et al., 2016.

Of interest to us in this thesis, based on their anatomical architecture, are the TNN and TPN as they involve cortical and subcortical regions. More specifically, the TNN is better known as the default mode network (DMN), thought to be involved in emotion processing, self-referential mental activity and recollection of prior events (Raichle, 2015). With an opposite effect, the TPN is divided in 3 subnetworks better known in the literature as the fronto-parietal network (FPN), the dorsal attention network (DAN or central executive network (CEN)) and the ventral attention network (VAN). More specifically, the VAN is shown to include the salience or cingulo-opercular network (SN/CON; Dosenbach et al, 2007; Seeley et al, 2007). These three networks are thought to act on different time scales relating to cognitive control processes (Sadaghiani & D'Espostio, 2014). The dopaminergic system involves notably the SN/CON network, playing a role in the stable maintenance of tasks sets. Indeed, its connectivity robustly implicates a cortico-striato-thalamic loop (Peters et al., 2016; Choi et al., 2017) (**FIGURE 30 B**). Moreover, dopamine levels have also been shown to impact the functional connectivity within multiple networks (DMN, FPN, SN/CON) (Cole et al., 2013).

Thus, functional connectivity within resting-state networks seems a relevant indicator of the neurophysiological impact of tDCS specifically on networks related to the dopaminergic system.

3.2.2. Perfusion - Arterial Spin Labeling (ASL)

Arterial spin labeling (ASL) is a technique developed in order to non-invasively give a direct and quantitative measure of cerebral blood flow (CBF). Basically, CBF can be measured with magnetically labeled arterial blood water, which generates an arterial spin labeling (ASL) signal (Borogovac and Asllani, 2012; Wong, 2014; Alsop et al., 2015; Grade et al.,

2015). More specifically, ASL sequences produce two images: a flow-sensitized or 'labeled' image and an 'unlabeled' or 'control' image. Both measurements have a labeling period, an inversion delay (time for the labeled blood to reach capillaries in the region of interest) and an acquisition period. Thus, for both images, the static tissue signals are identical and they only differ concerning the magnetization of the inflowing blood. Then, in order to create the perfusion image, a subtraction of the labeled and unlabeled blood is generated (Detre et al., 2012) (**FIGURE 31**). However, the signal difference is relatively small, thus in order to ensure sufficient signal-to-noise ratio (SNR), multiple repetitions are needed. In addition, the analysis of ASL data typically requires the use of a model of perfusion signal to quantify the perfusion, along with the calculation of the equilibrium magnetization of arterial blood (M0). Furthermore, there are multiple types of ASL acquisition: Pulsed ASL (pASL), Continuous ASL (CASL) and pseudo-continuous ASL (pCASL). The main differences between pASL or CASL compared to pCASL is the size of the labeling volume (pASL) and the inversion delay (CASL). Indeed, in pCASL sequences, a train of short RF pulses is applied in a thin volume to invert the arterial blood. In addition, the inversion time being longer which contributes to an increased SNR in perfusion images and higher labeling efficiency (Wu et al., 2007).

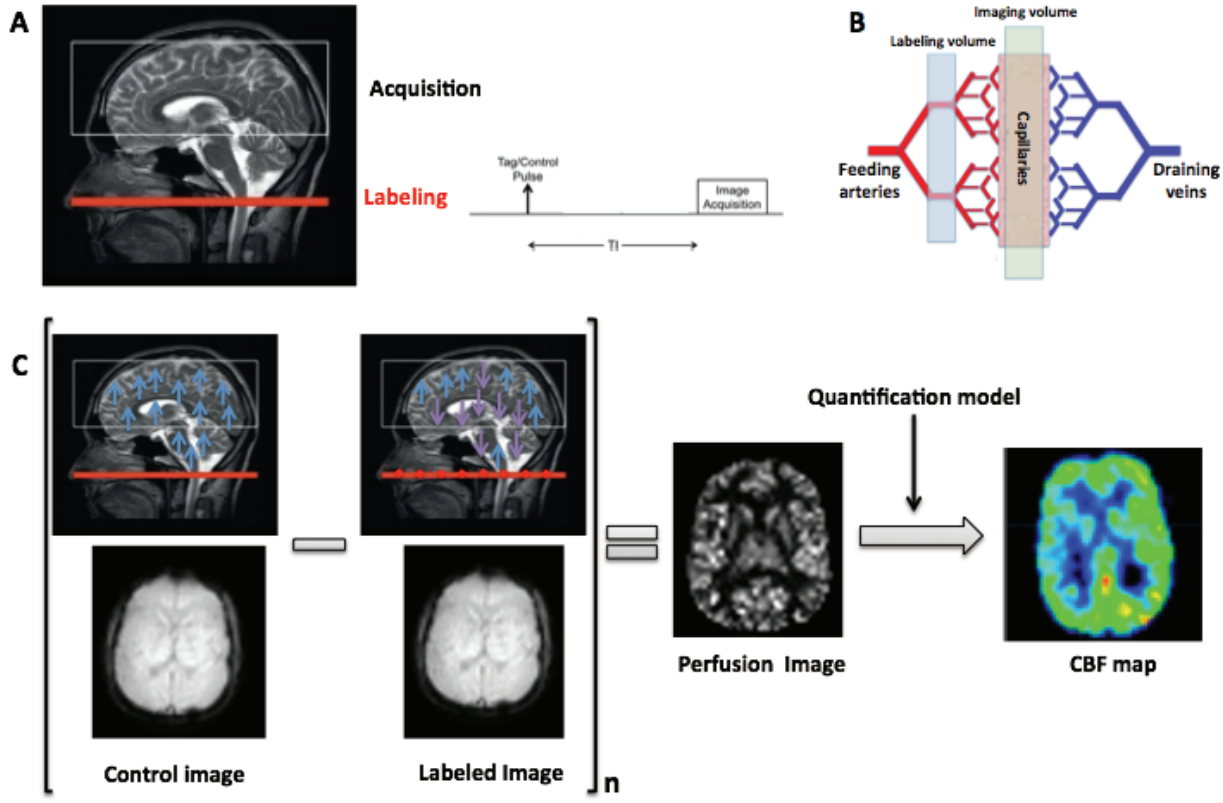


Figure 31: ASL principle -(**A**) Schematic diagram of pCASL sequence (**B**) Quantification of cerebral blood flow - Adapted from Alsop et al, 2015 and Grade et al, 2015

In sum, ASL has been shown to be a reliable and reproducible technique for measuring CBF (Xu et al., 2009; Petersen et al., 2010) even if artifacts need to be carefully recognized and investigated (Amukotuwa et al., 2016).

Furthermore, studies have tried to understand whether and how perfusion reflects underlying neurotransmitter activity such as dopamine. Interestingly, there seems to be a link between the changes in CBF measured with ASL and the underlying receptor density/activity (Dukart et al., 2018). Indeed, studies have shown that the use of dopamine agonist or antagonist would increase striatal CBF in the same regions (i.e. the striatum) (Fernández-Seara et al., 2011; Handley et al., 2013; Schouw et al., 2013).
Thus, CBF perfusion could be a relevant indicator of the neurophysiological impact of tDCS specifically on networks related to the dopaminergic system.

3.2.3. Diffusion Tensor Imaging (DTI)

Diffusion MRI has been developed in order to quantify the diffusion process of water molecules in biological tissues, as the human brain white matter. The concept is based on the random motion of these water molecules or Brownian motion. Indeed, depending on the underlying microstructure, the diffusion can be either free (i.e isotropic) or constrained (i.e anisotropy). It has been reported that diffusion tends to be anisotropic in white matter fibers (WM) whereas it tends to be less anisotropic in grey matter (GM) and isotropic in CSF (Hagmann et al., 2006). This pattern of anisotropy is the core process behind DTI (Basser et al., 1994). This technique is thus capable of accurately describing the geometry, such as position and orientation, of the underlying white matter axonal microstructure in each voxel.
In the brain, this process can be represented with spheres deformed depending on the orientation of water molecules. Their behavior will depend on the cell type and integrity of the surrounding architecture (Beaulieu, 2002). To estimate the underlying microstructure, DTI is based on the resolution of an equation in order to obtain a diffusion tensor (3*3 matrix) in each voxel of the brain. From this tensor, an eigenvalue decomposition can be performed to obtain three eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) coupled to three eigenvectors (e_1, e_2, e_3), with λ_1 giving the principal direction. These six parameters integrate the geometric and diffusion properties of the tensor from which we can infer, in each voxel, properties such as the molecular diffusion rate (Mean diffusivity (MD) or Apparent Diffusion Coefficient (ADC)), the fractional anisotropy (FA), the axial (parallel) and radial (perpendicular) diffusivity (Mori and Zhang, 2006). For visualization purposes, a color code is given depending on the principal direction (e_1) observed in each voxel (sagittal (x; red), coronal (y; green), axial (z; blue)) (**FIGURE 32**).
In most studies, the FA map is reported in order to represent, in each voxel, the organization with a value between 0 (isotropic) and 1 (anisotropic). For example, high FA values (>0.7) would reflect a highly organized fiber bundle in a single direction, whereas low FA values (<0.15) would reflect isotropic diffusion. To correctly interpret FA values, it is possible to combine the FA values with the axial and radial diffusivity.

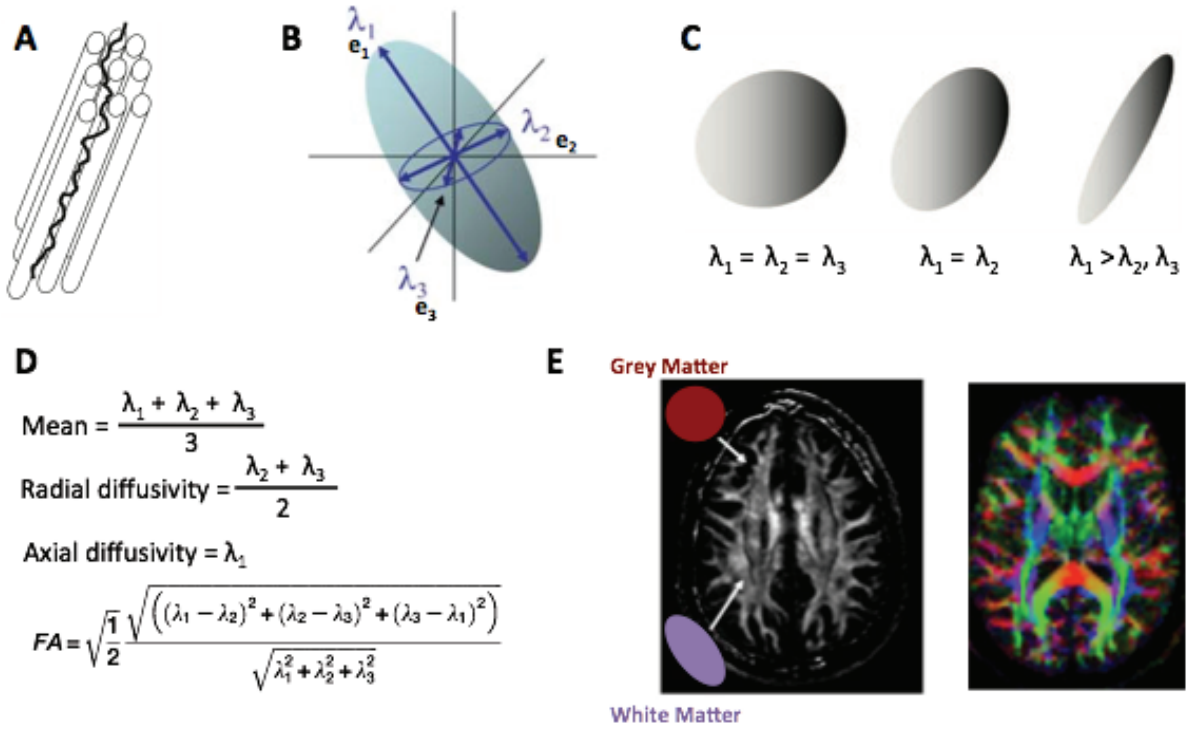


Figure 32: DTI principle - (**A**) Schematic illustration of water molecule diffusion within a fiber bundle (**B**) Diffusion tensor model in each voxel (3 eigenvectors and associated eigenvalues) (**C**) Schematic representation of the geometric shape and diffusion properties of the tensor (**D**) Measures derived from the tensor, in each voxel (**E**) Fractional anisotropy maps (with and without RGB color code depending on λ_1) - Adapted from Mori et al, 2006; Berg and Rushworth, 2009

However, one limitation of this technique is the fact that only one direction per voxel can be assessed, thus this might be a problem concerning fiber crossing. In this line, new techniques are developed to help this problem (e.g. HARDI; High Angular Resolution Diffusion Imaging; Descoteaux, 2015).

With this in mind, several techniques have been developed in order to analyze specific anatomical tracts such as region of interest, Tract-Based Spatial Statistics (TBSS; Smith et al., 2006) or tractography (Calamante, 2017).

Thus, DTI could be a relevant indicator of the neurophysiological impact of tDCS specifically on structural pathways related to the dopaminergic system.

3.3. Simultaneous PET-MRI

In addition to the different techniques that can be used to investigate the dopaminergic system, a new type of scanner has been developed recently: Simultaneous PET-MR. These machines combine PET and MR within the same field of view, in order to acquire both imaging modalities simultaneously. This is a promising tool in order to investigate the relationship between changes in neuroreceptor occupancy measured with PET and changes in brain activity detected with fMRI (Sander et al., 2013; Wey et al., 2014).

Thus, these imaging techniques will enable us to investigate the impact of tDCS on the dopaminergic system at different levels (neurotransmitter release, functional connectivity, perfusion, structural connectivity).

However, combining tDCS with these techniques can be done with two study designs: either 'online' (during the stimulation) or 'offline' (after the stimulation period), depending on the aim of the study. Offline studies investigate the changes occurring only after the end of the stimulation whereas online studies will be able to investigate the changes occurring both during and after the stimulation.

4. Chapter 4 - tDCS and Dopamine

Using either offline or online study designs, the NIBS community has been very interested in investigating the relation between tDCS and dopamine. As the cortex is densely connected with basal ganglia areas, NIBS are probably capable to reach subcortical dopaminergic areas and inversely dopamine agents could modulate its effects.

4.1. Non-Invasive Brain Stimulation and Dopamine

Effect of NIBS on dopamine transmission

As explained in Chapter 2, recent fMRI studies highlighted subcortical effects of tDCS applied at the cortical level including modulations of cortico-striatal and thalamo-cortical functional connectivity. However, to date, no study has directly assessed the direct impact of tDCS on dopamine transmission in humans. Nevertheless, we can hypothesize the impact of tDCS on dopamine transmission based on other human and animal NIBS studies. Indeed, some stimulation approaches, such as transcranial magnetic stimulation (TMS), have been shown to modulate dopaminergic transmission in human and animals (for review see Ko and Strafella, 2012; Cirillo et al., 2017).

When looking at in vivo animal studies, TMS of the frontal cortex was reported to increase extracellular dopamine levels in the rat dorsolateral striatum (Keck et al., 2002; Kanno et al., 2004) and hippocampus (Ben-Shachar et al., 1997; Keck et al., 2000, 2002). Electrical stimulation of the frontal cortex was also shown to increase dopamine levels in the striatum (Taber and Fibiger, 1995; You et al., 1998; Tanaka et al., 2013; Leffa et al., 2016). In the same line, frontal TMS in monkeys also showed a release in the left and right striatum (Ohnishi et al., 2004).

Moreover, in humans, neuroimaging studies (PET or SPECT) reported that, when stimulating the DLPFC, rTMS would increase endogenous dopamine release in the striatum in healthy subjects (Strafella et al., 2001; Ko et al., 2008), and in patients with depression (Pogarell et al., 2006, 2007), Parkinson's disease (Strafella et al., 2005) and schizophrenia (Brunelin et al., 2011). In addition, TMS of the frontal cortex has been reported to evoke a dopamine release in extrastriatal areas such as the anterior cingulate cortex (ACC) and medial orbitofrontal cortex (mOFC) (Cho and Strafella, 2009).

From these studies, we can hypothesize that stimulation of the frontal cortex can modulate the frontostriatal network, by releasing dopamine in regions such as the striatum. However, this could be dependent on the stimulated region and the interconnected cortical and subcortical regions. Indeed, when stimulating the left frontal cortex, rTMS induces dopamine release specifically in the ipsilateral frontostriatal network (Strafella et al, 2001 (left caudate nucleus), Cho and Strafella, 2009 (left ACC and OFC)). However, other studies showed bilateral release in the striatum (Ohnishi et al, 2004; Pogarell et al, 2006; Pogarell et al, 2007; Ko et al, 2008). Furthermore, these modulation in the frontostriatal network have been linked to modulation in executive functions (Ko et al, 2008).

Dopamine modulates tDCS effects

The impact of baseline dopamine on tDCS after-effects has been explored primarily using pharmacological studies, based on the measurement of TMS-evoked MEPs, However very few studies have examined the impact of baseline dopamine on the behavioral effects of frontal tDCS (i.e. working memory). These studies, either blocking or stimulating specific dopamine receptors, suggest a direct involvement of dopaminergic transmission in the effects of tDCS (**TABLE 2**; for review see McLaren et al, 2017).

Table 2: Dopamine modulates tDCS effects

Study				tDCS parameters / Results				
Author Date	**Population**	**n**	**tDCS sham**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Dopamine precusor (L-DOPA)								
Monte-Silva et al (2010)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Low dosage (25mg): eliminated excitability with anode and cathode Medium dosage (100mg): Turned excitatory anode into inhibitory. Prolonged cathodal effect. High dosage (200mg): eliminated excitability with anode and cathode				
Kuo et al (2008)	Healthy	18	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Medium dosage (100mg): Turned excitatory anode into inhibitory and prolonged cathodal effect.				
D2 agonist (Bromocriptine)								
Fresnova et al (2014a)				Left M1 / right orbit	1	1	anode:13 cathode: 9	35
	Healthy	12	N	Low dosage (2.5mg): eliminated excitability with anode and cathode Medium dosage (10mg): abolished anodal effect (trend towards inhibitory), no effect on cathode. Prolonged cathodal effect. High dosage (20mg): eliminated excitability with anode and cathode				
D2/D3 agonist (Ropinirole)								
Monte-Silva et al (2009)	Healthy	12	Y	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Low dosage (0.125 - 0.25mg): eliminated excitability with anode and cathode Medium dosage (0.5mg): no effect on anode. Prolonged cathodal effect. High dosage (1mg): Decrease excitability with anode and turned reduction into excitability with cathode.				
D1/D2 agonist (Pergolide)								
Nitsche et al (2006)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Medium dosage (0.025mg): no effect for anode and prolonged effects of cathode				
Selective D2/D3 antagonist (Sulpiride)								
Nitsche et al (2006)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Medium dosage (400mg): reduced both anode and cathode after effects				
D1 transmission (Sulpiride + L-DOPA or Pergolide)								
Nitsche et al (2006)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35

Table 2: Dopamine modulates tDCS effects

Study				tDCS parameters / Results				
Author Date	**Population**	**n**	**tDCS sham**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
				Pergolide Medium dosage (0.025mg): effects under anode and cathode abolished by sulpiride were not restored				
Nitsche et al (2009)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				L-DOPA Medium dosage (100mg): restored excitability under anode (excitatory) and cathode (inhibitory)				
Fresnova et al (2014b)	Healthy	12	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				L-DOPA Low dosage (25mg): elimination of excitability enhancement under the anode, turned inhibitory cathode into excitatory Medium dosage (100mg): no effect on anode, abolished effect of cathode High dosage (200mg): elimination of excitability enhancement under the anode, turned inhibitory cathode into excitatory				
Precusor of amphetamine (Amphretaminil)								
Nitsche et al (2004)	Healthy	15	N	Left M1 / right orbit	1	1	anode:13 cathode: 9	35
				Amphetamine: Prolonged excitability enhancing under the anode and diminished reduction under the cathode.				
Tyrosine								
Jongkees et al (2017)	Healthy	18	N	F3/F4 or F4/F3	1	1	15	35
				Tyrosine (2mg): F4/F3 reported higher working memory performance than F3/F4 stimulation				

Interestingly, early studies suggested that blocking D2 transmission would suppress tDCS after effect for both the anode and cathode (Nitsche et al, 2006), whereas activating D1 transmission would restore the excitatory and inhibitory effects of tDCS (Nitsche et al, 2009). However, further studies have now reported an inverted U-shaped dose response curve underlying tDCS effects, as well as receptor specific effects. More specifically, it seems that when combining a dopamine agonist, at medium dosage, with anodal stimulation, the excitatory effects on plasticity invert into inhibitory ones, whereas combining it with cathode stimulation, the inhibitory effects would be prolonged (Kuo et al, 2008; Monte-Silva et al, 2010; Fresnova et al, 2014a). In this line, low and high dosage seem to abolish tDCS excitability effects. In addition, it would seem that anodal facilitatory effect could be dependent on baseline D1 transmission (Fresnova et al, 2014b). These results, in general, suggest that dopamine levels can influence tDCS effects on cortical excitability, in a dose and receptor dependent manner. New studies emerge also showing that dopamine levels seem to impact the effects of tDCS on behavioral performances (Jongkees et al, 2017).

4.2. Frontal tDCS

Based on the positive reports from the motor tDCS montage, frontal tDCS montages have been developed in order to modulate frontal networks involved in numerous behavioral and clinical observations. In the literature, two frontal montages stand out: the bifrontal montage (bilateral electrode montage, with one on the left hemisphere and the other on the right hemisphere) and the fronto-temporal montage (unilateral electrode montage, with one electrode on the frontal cortex and the other on the temporal cortex).

4.2.1. Bifrontal montage

4.2.1.1. Montage

This montage has been developed based on the role of the frontal cortex in cognitive functions (i.e reward-motivation, executive processes), and with the hope that stimulation of the frontal cortex would improve cognitive function in both healthy and clinical population (Filmer et al., 2014; Wörsching et al., 2016).

The standard bifrontal montage consists of one anode placed over the left DLPFC (F3; Brodmann areas 9, 46) and one cathode placed symmetrically over the right DLPFC (F4). At the behavioral level, these bifrontal montages seem promising for pro-cognitive studies (Tremblay et al, 2014) and psychiatric disorders (Kekic et al., 2016; Lefaucheur et al., 2017), such as major depressive disorder (Brunoni et al., 2016, 2017; Loo et al., 2018; Pavlova et al., 2018; Sampaio-Junior et al., 2018), negative symptoms in schizophrenia (Palm et al., 2013a; Kennedy et al., 2018; Osoegawa et al., 2018; Pontillo et al., 2018), substance-use-disorders (SUD; Jansen et al., 2013; Coles et al., 2018), and cognitive alterations in Parkinson's disease (Leite et al., 2014). Studies report similar effects of the stimulation when the cathode is placed either over F4, FP2 or F8. Computational models have reported that these montages are suitable for frontal cortex stimulation (Bai et al., 2014; Laakso et al., 2016), but also that they exert similar effects beyond the cortex regions underneath the stimulation electrodes (**FIGURE 33**). Other montages using extra-cephalic electrodes (deltoid, mastoid, upper-arm, chin) or targeting other cortical regions (occipital, parietal, central) are not considered in our review, due to the difference in current distribution generated by the stimulation.

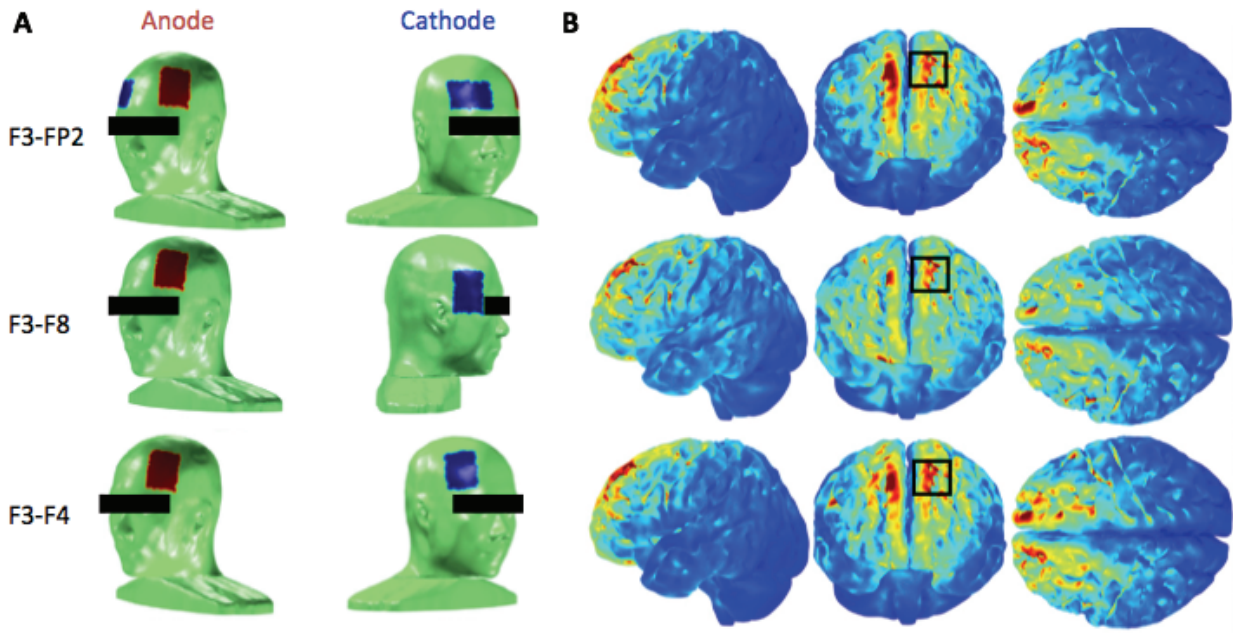


Figure 33: Bifrontal tDCS Montages and Electric Field distribution - (**A**) Electrode position: F3/FP2; with the anode over the left DLPFC and the cathode over the right supraorbital area; F3/F8, with the anode over the left DLPFC and the cathode over the right lateral supraorbital area; F3/F4; with the anode over the left DLFC and the cathode over the right DLPFC. (**B**) Electric field magnitude distribution for the 3 montages. Adapted from Bai et al, 2014.

As reported in Chapter 2 §2.3.3, several studies (n=7) have focused on the neurobiological effect of these bifrontal montages (**TABLE 3**). Using different imaging modalities, they report an impact locally under the electrodes and in distributed areas linked to the stimulation site: ASL (Stagg et al, 2013), fMRI (Keeser et al, 2011; Pena-Gomez et al, 2012; Park et al, 2013; Weber et al, 2014) and MRS (Hone-Blanchet et al, 2016). Based on these studies, an interesting hypothesis is that, in clinical population showing altered connectivity patterns, tDCS could restore unbalanced brain networks connected to the stimulation sites. However, these results should be discussed with caution because other studies also point to inter- and intra-individual variability in response to tDCS (Worsching et al, 2017) Thus, uncertainty still remains on the specific neurobiological effects underlying bifrontal tDCS and its effect on the brain. Nevertheless, the certainty remains that bifrontal tDCS induces changes in regions close to the stimulation sites as well as in distant cortical and subcortical regions.

Thus, as dopamine and the frontal cortex are tightly linked together, it seems worthwhile to investigate conditions associated with dopamine networks, such as pro-cognitive effects in healthy subjects, and/or dopamine disruptions as seen in depression, negative symptoms of schizophrenia, cognitive alterations in Parkinson's disease and SUD.

4.2.1.2. Implication of bifrontal tDCS

4.2.1.2.1. Pro-cognitive effects in healthy humans

In the last 20 years, groups have sought to decipher the impact of frontal tDCS in cognition. The hypothesis was that because the frontal cortex is implicated in different cognitive

functions, modulating frontal cortex activity using tDCS could impact these functions. Indeed, depending on the type of stimulation (excitatory anode or inhibitory cathode), cognitive function could be differently impacted. To date, effects of bifrontal tDCS seem to affect a wide range of high-order cognitive processes with some discrepancies (i.e: stimulation enhancing, inhibiting or having no effects) (Jacobson et al., 2012; Bennabi et al., 2014; Brunoni et al., 2014; Filmer et al., 2014; Tremblay et al., 2014; Horvath et al., 2015b; Santarnecchi et al., 2015; Dedoncker et al., 2016; Hill et al., 2016). Here, we report 40 single session tDCS studies (**TABLE 3**) that looked at how cognitive functions were impacted by tDCS. Amongst others, working memory, verbal fluency, planning, attention, emotion processing and decision-making are affected by a single session of bifrontal tDCS. The latest review and meta-analysis reported that after single-session of anodal tDCS, but not cathodal tDCS, participants responded faster and more accurately to cognitive tasks. However, others report mixed or no effects (Hill et al, 2015; Horvath et al, 2015). Differences between studies may arise from inhomogeneity of task and stimulation parameters. Moreover, only a single study investigated the impact of repeated bifrontal tDCS, which reported no changes in mood and cognitive function in healthy subjects (Motohashi et al., 2013, 11 subjects, 4 sessions).

As presented in Chapter 1§1.4, those cognitive functions, and more precisely reward-motivation and executive processes involve dopamine and its related cortico-subcortical networks. Indeed, dopamine receptor agonists were shown to improve performance on cognitive tasks whereas the opposite effect was observed with antagonists (Cools, 2011). However, when looking into more details, models point towards an optimum dopamine level for cognitive function following an inverted U-shape dose response curve (Cools and D'Esposito, 2011). This notion could be a starting point to explain the differences observed among tDCS studies. Interestingly, one study reported an improvement in performance dependent on the subject's motivation state, which could be underlain by dopamine levels (Morgan et al., 2014).

In sum, the potential is here but further studies are needed to understand the exact underlying neurobiological effects of both tDCS and its discrepancies between studies.

4.2.1.2.2. Depression

Based on the pathophysiology of depression, the bifrontal montage seems like a very promising therapeutic tool. Indeed, major depression is associated with a hypo-activity of the left DLPFC (George et al., 1994) and studies, treating patients with other tools such as antidepressants, ECT and TMS, report a return to normal activity levels in the prefrontal cortex which correlates with mood improvement. Thus, positioning the excitatory anode over this region would help restore this hypoactivity imbalance.

Here, we reported 6 single session tDCS studies and 19 repeated session tDCS studies (**TABLE 3**), focusing on the cognitive and antidepressant effect of bifrontal tDCS in patients with depression. Mixed results were reported. The majority of the single session bifrontal

tDCS studies reported an improvement in cognitive processes such as working memory, negative bias and attention in patients with depression.

Concerning the antidepressant effect after repeated bifrontal tDCS sessions (with varying number of sessions), the latest meta-analysis reported that the effect size of tDCS antidepressant treatment was comparable to those of rTMS and antidepressant drug treatment (Brunoni et al, 2016). However, previous reviews and meta-analysis report no significant difference between active and sham tDCS for both response and remission (Berlim et al., 2013; Meron et al., 2015). Moreover, the latest multi-centric trials reported mixed results with bifrontal tDCS: either no antidepressant effect (Loo et al, 2018) or an effective add-on antidepressant effect (Sampaio-Junior et al, 2018; Pavlova et al, 2018). However, these multi-centric studies have different patient profiles, i.e. mild to moderate depression (Pavlova et al, 2018); MDD bipolar (Sampaio-Junior et al, 2018); MDD unipolar and bipolar (Loo et a, 2018). Moreover, the stimulation parameters were very different from past studies, notably the sham parameters (stimulation of 0.034mA throughout the entire stimulation; Loo et al, 2018). Thus, the clinical efficacy of bifrontal tDCS as a treatment for depression remains unclear, but as of 2017 from the latest guidelines this treatment for depression is at a level B evidence (probable efficacy) (Lefaucheur et al, 2017).

In addition, amongst many symptoms, the most prevalent one in depression is anhedonia (i.e loss of interest or pleasure). This core feature has been consistently associated with a down-regulation of the reward-motivation dopaminergic networks and is particularly difficult to treat with antidepressants (Berton and Nestler, 2006; Yadid and Friedman, 2008; Price and Drevets, 2012; Grace, 2016; Belujon and Grace, 2017). For example, depression and anhedonia have been associated with a reduced striatal activity, notably in response to rewarding stimuli. Interestingly, the reward-motivation network has been targeted with different therapies such as DBS. This invasive technique reports improvement in symptoms such as anhedonia in patients with depression (Nauczyciel et al., 2013; Schlaepfer et al., 2014). This is in accordance with studies showing a dopamine release (via amphetamine administration) correlated with a mood improvement in depressed patients. Thus, with this information, one hypothesis would be that bifrontal tDCS clinical efficacy in depression could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network.

4.2.1.2.3. Schizophrenia -Negative symptoms

Negative symptoms of schizophrenia are characterized by conditions such as affective flattening, poverty of speech, and motivational deficits. Despite the development of antipsychotic medications effective for the positive symptoms (i.e hallucinations), these symptoms remain a persistent feature of the illness. Recent studies have highlighted the potential link between negative symptoms and the reward-motivational network (Foussias et al., 2015; Owen et al., 2016; Dollfus and Lyne, 2017). With this in mind, research groups

have seen the potential benefit of bifrontal tDCS on the negative symptoms in patients with schizophrenia.

Here, we reported 13 studies (7 single sessions and 6 repeated sessions) (**TABLE 3**) that investigated the effects of bifrontal tDCS on negative symptom improvements in patients with schizophrenia. Single session tDCS studies explored the impact of bifrontal tDCS on cognitive processes. They reported an improvement in working memory and probabilistic association learning in patients with schizophrenia. The repeated bifrontal tDCS sessions, usually 10 sessions in 2 weeks (1 per weekday), reported improvements on the negative symptoms in patients with schizophrenia. These improvements were associated with changes in functional connectivity in fronto-thalamic-temporo-parietal networks and notably a reduced functional connectivity in the anterior parts of the DMN as well as a deactivation of clusters in the striatum and the subgenual cortex. With the low number of studies, recent guidelines could not give any recommendation regarding the efficacy of this treatment for patient with schizophrenia (Lefaucheur et al, 2017). Since then, several reviews and meta-analyses have suggested that negative symptoms in schizophrenia can be treated with tDCS over the DLPFC but that larger randomized-controlled studies are needed to establish its effectiveness (Pontillo et al, 2018; Osoegawa et al, 2018; Kennedy et al, 2018).

In addition, as stated above, symptoms of schizophrenia seem underlain by dopaminergic alterations (Brunelin et al, 2013; Weinstein et al, 2017). A striatal dopaminergic hyper-reactivity, especially in the associative striatum, is reported present regardless of prior antipsychotic treatment and seems a reliable index of dopamine impairment (Kegeles et al., 2010; Howes et al., 2012). This dysregulation involves an excessive presynaptic release in the striatum and functional super-sensitivity of striatal D2 receptors. Moreover, in the frontal cortex, a recent study, using [^{11}C]FLB457, reported a link between blunted dopamine release and cognitive deficits. This decreased dopamine release capacity was also seen in other extrastriatal regions (Slifstein et al., 2015). However, no detectable alterations in postsynaptic receptors and transporters are reported (Howes et al., 2012; Kambeitz et al., 2014). More specifically, studies report an increased intrinsic activity in the ventral striatum, correlated with negative symptoms such as emotional withdrawal and blunted-effect (Sorg et al., 2013). Furthermore, negative symptoms are associated with cortical dopaminergic hypo function (Weinberger, 1987; Guillin et al., 2007). Thus, with this information, one hypothesis would be that bifrontal tDCS clinical efficacy could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network of patients with schizophrenia.

4.2.1.2.4. Parkinson's disease - Cognitive alterations

Patients with Parkinson's disease, in addition to motor dysfunctions, present neuropsychiatric symptoms such as depression, apathy, psychosis and impulse control disorders (Weintraub and Burn, 2011; Zahodne et al., 2012). Based on these impairments, studies hypothesized that bifrontal tDCS could have a potential beneficial impact on these cognitive alterations (Leite et al, 2014).

Here, we reported 4 studies (3 single session and 1 repeated session) (**TABLE 3**) focusing of cognitive changes with bifrontal tDCS in patients with Parkinson's disease. The majority reported significant improvements in cognition processes such as working memory, task fluency, executive function, balance and functional mobility. With the low number of studies, the recent guidelines could not give any recommendation regarding the efficacy of the treatment of non-motor parkinsonian symptoms (Lefaucheur et al, 2017).

The cognitive alterations stated above are thought to be underlain by dopaminergic impairments in fronto-striatal circuits, specifically in the reward-motivation pathway, and especially with apathy symptoms thought to be due to a hypo-dopaminergic state (Robbins and Cools, 2014). Thus, one hypothesis would be that bifrontal tDCS could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network.

4.2.1.2.5. Substance-use-disorders (SUD)

Based on the pathophysiology of SUD, consisting in decreased frontal activity and impaired reward-motivation network (Cooper et al., 2017), the bifrontal montage seems very promising.

Here, we reported 6 studies (5 single session and 1 repeated session) (**TABLE 3**) investigating the effect of bifrontal tDCS in patients with SUD. The majority reported a significant reduction in cue-induced craving (alcohol, cannabis, food and smoking).

In this line, the latests reviews and meta-analysis reported that the tDCS treatment was comparable to rTMS and decreased craving levels (Jansen et al., 2013; Hone-Blanchet et al., 2015; Coles et al., 2018). In addition, the latest guidelines recommended this treatment for craving at level B evidence (probable efficacy), but with the anode placed over the right DLPFC and the cathode over the left DLPFC (Lefaucheur et al, 2017).

In addition, as stated above, the prevailing theory is to view SUD as a disorder of the dopamine system. Indeed, addictive drugs are shown to induce a release of dopamine in the striatum and studies report diminished striatal dopamine receptor availability coupled with a decreased dopamine release in SUD patients. However, the role of dopamine in addiction seems more complex than proposed due to possibly different mechanisms depending on the substance abused (i.e stimulant, alcohol, opiate, nicotine and cannabis) (Nutt et al., 2015). Furthermore, stimulation techniques such as TMS or DBS could offer a promising new avenue in treatment of addiction, via alterations of the rewards circuit (Cooper et al, 2017). In this line, the clinical efficacy of bifrontal tDCS could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network.

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Neurobiological studies								
Single session								
Hone-Blanchet et al (2016)	Healthy	15	Y	F3/F4	1	1	30	35
				During stimulation, elevated prefrontal NAA and striatal glutamate/glutamine. No GABA difference (prefrontal or striatal). After active stimulation: no difference glutamate/glutamine, NAA, or GABA levels in the left DLPFC. No information on the striata levels.				
Keeser et al (2011)	Healthy	13	Y	F3/FP2	1	2	20	35
				After active stimulation, increased intrinsic FC within the frontal node of the DMN, the left FPN and the right PCC, as well as parts of the right FPN.				
Park et al (2013)	Healthy	25	Y (14)	F3/FP2	1	1	20	25
				After active stimulation, increased interhemispheric connectivity at rest (increased connectivity of the left DLPFC with the right hemisphere, decreased connectivity with the left hemisphere)				
Pena-Gomez et al (2012)	Healthy	10	Y	F3/FP2 F4/FP1	1	2	20	35
				After active stimulation, - AN increased synchrony - DMN reduced synchrony				
Stagg et al (2013)	Healthy	24	Y	F3/FP2 F4/FP1	1	1	20	35
				During stimulation: Anodal tDCS led to an increase and cathode tDCS to a decrease in perfusion in widespread regions, stable across the 20min of stimulation. After active stimulation: Widespread decrease in perfusion after anodal and cathodal tDCS.				
Weber et al (2014)	Healthy	11	Y (11)	F4 / F3	1	1.5	15	25
				After active stimulation: Via connections from right DLPFC: - disconnects right ACC from the rest of the brain - reduces resting rCBF in OFC and right caudate				
Repeated session								
Wörsching et al (2017)	Healthy	20	Y	F3/F4	3 (1 per week)	2	20	35
				Test-Retest (3 sessions) - tDCS & fMRI: low reliability of resting state fMRI after active tDCS; reliability of resting state fMRI baselines and resting state fMRI responses to sham tDCS was low to moderate				
Procognitive studies								
Single session								
Andrews et al (2011)	Healthy	10	Y	F3/FP2	1	1	10	35
				improvement in performance on digit span forward when tDCS applied during the n-back task, compared with tDCS applied while at rest and sham tDCS during the n-back task.				
Axelrod et al (2015)	Healthy	45	Y	F3/FP2	1	1	20	16 (anode) / 35 (cathode)
				tDCS over the DLPFC increased the propensity to mind-wander				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Cerruti et al (2009)	Healthy	30	Y	F3/FP2	1	1	20	16.3 (anode) / 30 (cathode)
				Improvement after F3/FP2 tDCS in verbal problem solving. No cathodal effect.				
Conson et al (2015)	Healthy	16	Y	F3/F4 or F4/F3	1	1	15	35
				F4/F3 montage induced faster explicit recognition of fearful facial expressions in male subjects and negatively affected both male and female participants' tendency to adopt another's point of view in the visual perspective taking task				
Dockery et al (2009)	Healthy	24	Y	F3/FP2	1	1	15	35
				Both anodal and cathodal tDCS can improve planning performance with the Tower of London test, but in a time dependent manner.				
Fecteau et al (2007)	Healthy	35 12	Y	F3/F4 or F4/F3 F3/FP2 or F4/FP1	1	2	15-20	35
				Adopted with both bilateral montage only a risk averse response to the Balloon Analog Risk task (decrease in risk taking)				
Filmer et al (2014)	Healthy	18	Y	F3/FP2	1	0.7	8	25
				The cost of responding to two tasks (i.e., the reduction in performance for dual- versus single-task trials) was significantly reduced by cathodal stimulation, but not by anodal or sham stimulation.				
Fregni et al (2005)	Healthy	15	Y	F3/FP2 or F4/FP1 or left M1/FP2	1	1	10	35
				3-back working memory improvement after anodal tDCS of DLPFC				
Gladwin et al (2012)	Healthy	20	Y	F3/FP2	1	1	10	35
				tDCS did improve reaction times, but in the congruent rather than incongruent mapping condition, during an implicit association test.				
Gladwin et al (2012)	Healthy	14	Y	F3/FP2	1	1	10	35
				improve reaction time significantly only when the incorrect choice had been a distracter stimulus, during a Sternberg task				
Hammer et al, (2011)	Healthy	36	Y	F3/FP2	1	1	30	35
				Left cathodal: hampered memory performance after errorful learning				
Hecht et al, (2010)	Healthy	28	N	F3/F4 or F4/F3	1	2	22	9
				active tDCS (F3/F4) modified strategies (Probabilistic Guessing Task) (quicker to select the most frequent strategies)				
Hortensius et al, 2012	Healthy	60	Y	F3/F4 F4/F3	1	2	15	35
				F3/F4 tDCS increased aggressive behaviours and anger				
Hoy et al, (2013)	Healthy	18	Y	F3/FP2	1	1 or 2	20	35
				active tDCS can enhance behavioral performance in working memory. They showed that 1 mA produced the most significant effects.				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Ironside et al (2015)	Healthy	60	Y	F3/F4	1	2	20	25
				reduced vigilance to threatening stimuli. no effects of tDCS on other measures of emotional processing. No effect when cathode over F8.				
Iuculano and Cohen Kadosh et al (2013)	Healthy		Y	F3/F4	1	1	20	3
				stimulation to the dorsolateral prefrontal cortex impaired the learning process, whereas automaticity for the learned material was enhanced				
Iyer et al (2005)	Healthy	103	Y	F3/FP2	1	1 or 2	20	25
				anodal tDCS applied to the left DLFC enhances verbal fluency with 2mA				
Javadi et al, (2012)	Healthy	13	N	F3/FP2	1	1.5	1.6s	12.25 (target) / 30.25 (ref)
				Short duration tDCS modulates verbal memory and highlight the importance of period of stimulation				
Javadi et al, (2013)	Healthy	30	Y	F3/FP2	1	1.5	20	12.25 (target) / 30.25 (ref)
				anodal tDCS over the left DLPFC can enhance the reconsolidation of long-term memory only when the memory has been reactivated				
Jeon et al, (2012)	Healthy	32	Y	F3/F4 F4/F3	1	1	20	35
				tDCS can induce verbal working memory improvement and naming facilitation by stimulating the left prefrontal cortex. It can also improve the visuospatial working memory by stimulating the right prefrontal cortex.				
Keshvari et al, (2013)	Healthy	60	Y	F3/F4 F4/F3	1	2	20	25
				Left anodal/ right cathodal stimulation of DLPFC could impair working memory, while the reverser stimulation had no effect.				
Knechtel et al (2014)	Healthy	16	Y	F3/FP2	1	2	20	35
				increase of N1 amplitudes while smaller P3b amplitudes correlated with higher cortical Glu and Glx levels in the stimulated brain area when performing an auditory go/no-go discrimination task. No change in MMN or task performance or Glu/Glx levels				
Knoch et al (2007)	Healthy	64	Y	FP1/F4	1	1.5	14	35 (cathode) 100 (anode)
				Less propensity to punish unfair behavior with cathode of right DLPFC				
Leite et al (2011)	Healthy	30	Y	F3/FP2	1	1	15	35
				Left anodal: increased performance (set switching task). Left cathodal: decreased performance (set switching task)				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Leite et al (2013)	Healthy	16	Y	F3/F4	1	2	duration of experiment (30min max)	35
				Left anodal: increased switching performance in the letter/digit naming task but decreased switching performance and improved accuracy in the vowel-consonant/parity task. Left cathodal: improved accuracy in the letter/digit naming task				
Maeoka et al (2012)	Healthy	15	Y	F3/FP2	1	1	20	35
				Beta band power was significantly increased, and the alpha band power was significantly decreased during unpleasant pictures after anodal tDCS compared with sham tDCS as well as a decrease in unpleasantness ratings.				
Manenti et al (2013)	Healthy	32	Y	F3/FP2	1	1.5	6	35
				Anodal tDCS applied during the retrieval phase facilitates verbal episodic memory				
Metuki et al (2012)	Healthy	21	Y	F3/FP2	1	1	11	35
				enhanced solution recognition for difficult problems. This effect was modulated by trait motivation, i.e. was larger for participants with lower approach motivation. No effects were found for easy problems, or solution generation.				
Morgan et al (2014)	Healthy	18	N	F3/F4	1	1	12	9
				F4/F3				
				The polarity of bifrontal tDCS influenced performance on a stimulus-response compatibility task and this effect was dependent on participants' prior motivational state. No change in mood.				
Mulquiney et al (2011)	Healthy	10	Y	F3/FP2	1	1	10	35
				F3/FP2 improved speed of working memory performance (2- back task).				
Mylius et al (2012)	Healthy	12	Y	F3/F4 or F4/F3	1	2	20	35
				Involvement of the DLPFC in the processing of pain and WM				
Nelson et al (2014)	Healthy	19	Y	F3/F4	1	1	10	35
				tDCS decreased less time-on-task blood flow velocity and increased cerebral oxygenation. Significant improvement in target detection performance.				
Nitsche et al (2012)	Healthy	14-17	Y	F3/FP2	1	1	20 or 10	35
				No effect on subjective emotions. However, emotional face identification was improved with anodal tDCS over the left DLPFC, for positive emotional content.				
Nozari et al (2013)	Healthy	24	Y	F3/F4	1	1.5	20	25
				consistent with the focus hypothesis: prefrontal stimulation caused a reliable increase in the benefit and a marginal increase in the cost of selective attention.				
Ohn et al (2008)	Healthy	15	Y	F3/FP2	1	1	30	25

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
				Improved performance of 3 n-back task after tDCS for at least 30min.				
Plazier et al (2012)	Healthy	17	Y	F3/F4 F4/F3	1	1.5	20	35
				No significant effects on mood after tDCDS				
Plewnia et al, (2013)	Healthy	46	Y	F3/FP2	1	1	20	35
				In COMT Met/Met allele carrier anodal tDCS of the dlPFC was associated with a deterioration of set-shifting ability, which is assessed by the most challenging level of the performance of a parametric Go/No-Go test.				
Sela et al, (2012)	Healthy	22	Y	F3/F4 or F4/F3	1	1.5	15	35
				the neural enhancement of a left lateralized prefrontal network improved performance when participants had to make decisions to figurative targets of highly predictable idioms, whereas the neural enhancement of the opposite network improved participants' performance to literal targets of unpredictable idioms.				
Teo et al, (2011)	Healthy	12	Y	F3/FP2	1	1 or 2	20	35
				no improvement of accuracy for WM performance But improvement reaction time in the 3-back WM task with 2mA tDCS.				
Vanderhasselt et al, (2013)	Healthy	25	Y	F3/FP2	1	2	20	35
				more negative N450 amplitudes along with faster reaction times when inhibiting a habitual response to happy compared to sad facial expressions				
Repeated session								
Motohashi et al (2013)	Healthy	11	Y	F3/FP2	4	1	20	35
				Repeated anodal tDCS over left DLPFC may not change mood and cognitive function in healthy subjects				
Depression studies								
Single session								
Boggio et al (2007)	Depression	26	Y (7)	F3/FP2	1	2	20	35
				Significant improvement in Go-no-go task performance after 1 anodal tDCS session, specific to figures with positive emotional content. Not correlated with mood improvements (after cure of 10 days).				
Brunoni et al (2012)	Depression	28	Y	F3/F4	1	2	30	25
				Procedural or implicit learning acquisition between tasks also occurred only for sham. No improvement in implicit learning after real stimulation.				
Brunoni et al (2014b)	Depression	12	Y(12)	F3/F4	1	2	30	25
				Active tDCS, significantly modified the negative attentional bias, abolishing slower reaction time for negative words				
Oliveira et al (2013)	Depression	28	Y	F3/F4	1	2	30	25
				Enhancement in working memory assessed by the *n*-back task after active stimulation.				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Powell et al (2014)	Depression	18	Y	F3/F8	1	2	20	NA
				Significant reduction in the N2 amplitude and reduced theta activity over frontal areas during memory retrieval				
Segrave et al (2014)	Depression	27	Y	F3/F8	1	2	20	35
				Concurrent CCT enhances antidepressant outcomes from tDCS				
Repeated session								
Bennabi et al (2015)	Depression	24	Y	F3/Fp2	10 sessions (2per day, 1 week)	2mA	30	35
				No significant antidepressant effect				
Blumberger et al (2012)	Depression	13	Y (11)	F3/F4	15 (3weeks, 1per day)	2	20	35
				No significant difference in depression scores between active and sham tDCS groups (TRD).				
Boggio et al (2008)	Depression	21	Y (10)	F3/FP2	10 (2 weeks)	2	20	35
				Larger reductions in depression scores after DLPFC tDCS compared to occipital and sham tDCS, and persisted 1 month after the end of the treatment.				
Brunoni et al (2011b)	Depression	31	N	F3/F4	10 (5 days, 2per day)	2	20	35
				Significant mood improvement in both study groups, persisting one week and one month after treatment.				
Brunoni et al (2013)	Depression	60	Y (60)	F3/F4	12 (1per day)	2	30	25
				The combination of tDCS and sertraline increases the efficacy of each treatment. Comparable efficacy between tDCS and sertraline.				
Brunoni et al (2014a)	Depression	20	Y(17)	F3/F4	10 (1per day)	2	30	25
				Both CCT alone and combined with tDCS ameliorated depressive symptoms. CCT and tDCS combined might be beneficial for older depressed patients				
Brunoni et al (2017)	Depression	91 + 94	Y (60)	F3/F4	15 (1per day) + 7 (1per week)	2	30	25
				Escitalopram and tDCS were both superior to placebo. tDCS for the treatment of depression did not show non- inferiority to escitalopram over a 10-week period				
Dell'Osso et al (2012)	Depression	23	N	F3/F4	10 (5 days)	2	20	32
				Significant mood improvement				
Ferrucci et al (2009)	Depression	14	N	F3/F4	10 (5 days)	2	20	32
				Significant mood improvement and maintained at one month in TRD. No effect on cognition.				
Fregni et al (2006)	Depression	18	Y	F3/FP2	5 (5days, 1per day)	1	20	35
				Beneficial effect on cognitive and mood improvement. However no correlation between both cognitive and mood improvements				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Knotkova et al (2012)	Depression	10	N	F3/FP2	10 (5 days)	2	20	25
				Improvement in depressive symptoms post-tDCS, maintained for at least 2 weeks (further improvement in MADRS scores only).				
Loo et al (2010)	Depression	20	Y (20)	F3/FP2	10 (3/week)	1	20	35
				Significant mood improvement after active tDCS and sham tDCS.				
Loo et al (2012)	Depression	33	Y (31)	F3/F8	15 (3weeks)	2	20	35
				Significant improvement of mood after real compared with sham. No difference in response rate. Attention and working memory improved after a single session of active				
Loo et al (2018)	Depression	130	Y	F3/F8	20 (4/weeks)	2.5	30	35
				Mood improved significantly over the 4-week treatment period in both unipolar and bipolar groups. No antidepressant difference between active and sham stimulation for unipolar or bipolar depression				
Palm et al (2012)	Depression	11	Y (11)	F3/FP2	20 (3/weeks)	1 or 2	20	35
				No significant difference in depression scores and cognitive performances after active compared with sham tDCS (TRD).				
Pavlova et al (2018)	Depression	69	Y	F3/FP2	10 (1per day)	0.5	20 or 30	17.5 (anode) / 35 (cathode)
				Combined with sertraline, significant improvement after tDCS in HDRS scale, greater improvement with 30 minutes of stimulation. For mild and moderate depression.				
Rigonatti et al (2008)	Depression	42	Y	F3/FP2	10 (1per day)	2	20	35
				Antidepressant effects of bifrontal tDCS are similar to a 6-week course of fluoxetine. However, the benefits of tDCS appear faster.				
Salehinejad et al (2017)	Depression	24	Y	F3/F4	10 (1per day)	2	20	35
				Improved executive dysfunction in patients and improvement on depression scores				
Sampaio-Junior et al (2018)	Depression	59	Y	F3/F4	12 (1per day + 2 over 2 weeks)	2	30	35
				Active tDCS condition showed significantly superior improvement compared with those receiving sham. For bipolar depression.				
Schizophrenia								
Single session								
Hoy et al (2014)	Schizophrenia	18	Y	F3/FP2	1	0, 1 or 2	20	35
				2mA tDCS improves working memory performances				
Hoy et al (2015)	Schizophrenia	16	Y	F3/FP2	1	0, 1 or 2	20	35
				Increase in gamma event-related synchronization in the left DLPFC, when working memory was improved, whereas sham stimulation reported a decrease in gamma event-related synchronization and no changes in WM.				

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Knechtel et al (2014)	Schizophrenia	14	Y	F3/FP2	1	2	20	35
				Single application of tDCS has no acute effects on ERPs and associated auditory information processing				
Nienow et al (2016)	Schizophrenia	10	Y	F3/FP2	1	1	20	35
				Addition of tDCS concurrent with working memory training enhances cognitive performance in patients with schizophrenia				
Orlov et al,(2016)	Schizophrenia	24	Y (25)	F3/FP2	1	2	30	35
				Working memory task demonstrated a significant mean difference in performance in the tDCS treatment group				
Orlov et al, (2017)	Schizophrenia	28	Y	F3/FP2	1	2	30	35
				The 'real' and 'sham' groups did not differ in online working memory task performance, but the transcranial direct current stimulation group demonstrated significant improvement in performance at 24 h post-transcranial direct current stimulation. Transcranial direct current stimulation modulated functional activation in local task-related regions, and in more distal nodes in the network				
Vercammen et al (2011)	Schizophrenia	20	Y	F3/FP2	1	2	20	35
				Differential effects of baseline performance on active tDCS and sham treatment Facilitate probabilistic association learning in a subgroup with active tDCS				
Repeated session								
Gomes et al (2015)	Schizophrenia	7	Y (8)	F3/F4	10 (2 weeks)	2	20	35
				Improvement in general and negative symptoms.				
Palm et al (2013a)	Schizophrenia	1	N	F3/FP2	10 (2 weeks)	2	20	35
				Improvement in positive and negative symptoms. Reduced FC in the anterior part of the DMN				
Palm et al (2014)	Schizophrenia	10	Y (10)	F3/FP2	10 (2 weeks)	2	20	35
				Improvement in positive and negative symptoms. FC showed a significant deactivated cluster in striatum and subgenual cortex.				
Palm et al (2016)	Schizophrenia	20	Y	F3/F4	10 (2 weeks)	2	20	35
				Improvement in general and negative symptoms. Changes in subgenual cortex and DLPFC connectivity within frontal-thalamic-temporo-parietal networks were reported.				
Shiozawa et al (2016)	Schizophrenia	10	Y	F3/F4	10 (5 days)	2	20	35
				No effect of concomitant use of tDS and cognitive training in patients with schizophrenia.				
Smith et al (2015)	Schizophrenia	30	Y	F3/FP2	5	2	20	5
				Improvement in cognitive performance with active tDCS				
Parkinson's disease studies								

Table 3: Bifrontal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Single session								
Boggio et al (2006)	Parkinson	18	Y	F3/Fp2	1	1 or 2	20	35
				Working memory improvement with active tDCS over DLPFC and with 2mA.				
Pereira et al (2013)	Parkinson	16	N	F3/Fp2	1	2	20	35
				DLPFC tDCS increased performance on the fluency task and enhanced FC in verbal fluency and deactivation of task-related networks.				
Lattari et al (2017)	Parkinson	17	Y	F3/Fp2	1	2	20	35
				active DCS improves balance and functional mobility				
Repeated session								
Doruk et al (2014)	Parkinson	18	Y	F3/Fp2	10 (2 weeks)	2	20	35
				Active tDCS prolonged improvements in executive functions in parkinson's disease patients.				
Substance use disorders (SUD) studies								
Single session								
Boggio et al (2008)	SUD (alcohol)	13	Y	F3/F4 F4/F3	1	2	20	35
				Decreased alcohol craving for both active conditions				
Boggio et al (2010)	SUD (cannabis)	25	Y	F3/F4 F4/F3	1	2	20	35
				Craving reduction with anode-left/ cathode-right tDCS. Increased risk taking in decision making task with both active conditions				
Fregni et al (2008)	SUD (food)	23	Y	F3/F4 F4/F3	1	2	20	35
				Reduction in cue-induced craving with anode-right/cathode-left tDCS vs. sham.				
Fregni et al (2008a)	SUD (nicotine)	24	Y	F3/F4 F4/F3	1	2	20	35
				Decrease smoking craving for both active conditions				
Gorini et al (2014)	SUD (cocaine)	18	Y	F3/F4 or F4/F3	1	1.5	20	32
				Reduction of risky behaviors at the BART task both in controls subjects and cocaine dependent users. Cocaine users increased safe behavior after right DLPFC anodal stimulation, while risk-taking behavior increased after left DLPFC anodal stimulation, on GDT,				
Repeated session								
Boggio et al (2009)	SUD (nicotine)	27	Y	F3/F4 F4/F3	5	2	20	35 (anode) 100 (cathode)
				Reduction in cue-induced craving with both active tDCS conditions. Decreased number of cigarettes smoked in active group.				

4.2.2. Fronto-temporal montage

4.2.2.1. Montage

This montage has been developed, in a therapeutic context, based on schizophrenia-associated imaging evidence of a fronto-temporal dysconnectivity with a hypo-activation of the left DLPFC (Sanfilipo et al., 2000; Lawrie et al., 2002) and a hyper-activation of the left temporo-parietal junction (TPJ) (Silbersweig et al., 1995).

The standard electrode montage consists of the anode placed at equidistance between F3 and FP1 (over the left DLPFC, Brodmann areas 8, 9, 10 and 46) and the cathode placed at equidistance between T3 and P3 (over the left TPJ, Brodmann areas 22, 38, 40, 41 and 42). This fronto-temporal montage seems promising for psychiatric conditions, mainly schizophrenia (Brunelin et al., 2012; Ponde et al., 2017). Computational models have reported an impact of this montage under the stimulating electrodes, as well as in cortical and subcortical interconnected regions, involved in the pathophysiology of schizophrenia (Brunoni et al., 2014) (**FIGURE 34**).

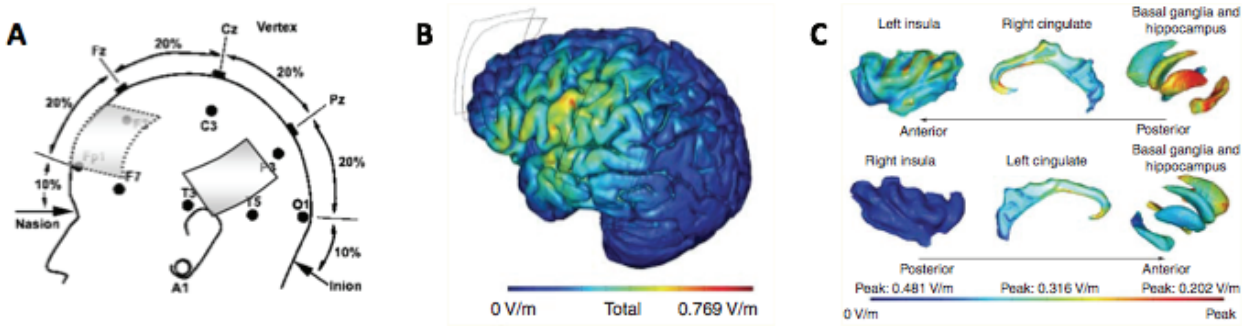


Figure 34: Fronto-temporal tDCS Montage and Electric Field distribution - (**A**) Electrode position: F3FP1/T3P3; with the anode over the left DLPFC and the cathode over the left TPJ (**B**) Electric field magnitude distribution in cortical regions (**C**) Electric field magnitude distribution in subcortical regions. Adapted from Brunoni et al, 2014

4.2.2.2. Implication of fronto-temporal tDCS

4.2.2.2.1. Schizophrenia - Dopamine

As stated above, the fronto-temporal tDCS montage is used specifically for patients with schizophrenia, notably with predominant positive symptoms (auditory hallucinations) (Koops et al., 2015; Ponde et al., 2017). Here, we reported 12 case studies and 9 controlled studies (**TABLE 4**) focusing on the effect of frontal-temporal tDCS in patients with schizophrenia. The majority reported significant reduction of positive symptoms, notably auditory hallucinations. With the low number of studies and small sample size, the recent guidelines could not give any recommendation regarding the efficacy of this treatment for patient with schizophrenia (Lefaucheur et al, 2017).

According to the therapeutic effects of fronto-temporal tDCS on schizophrenia and the dopaminergic pathophysiological hypothesis of schizophrenia (Brunelin et al., 2012; Grace, 2016; Weinstein et al., 2017), the effect of fronto-temporal tDCS on dopaminergic transmission is of major interest. As stated above (§4.2.1.2.3), studies report an imbalance between a striatal dopamine hyper-reactivity, linked to positive symptoms (i.e delusions and

hallucinations) and a hypo-dopaminergic state in cortical regions, leading to inappropriate attribution of salience to unimportant stimuli (Kapur, 2003; Howes et al., 2012; Slifstein et al., 2015). Interestingly, striatal hyper-reactivity can predict the response to antipsychotics (i.e. the higher the excess of striatal dopamine the more likely the patient will respond to antipsychotics) (Demjaha et al., 2012). More specifically, studies report an increased intrinsic activity in the dorsal part of striatum, correlated with positive symptoms such as delusions and hallucinations (Sorg et al, 2013). Thus, with this information, one hypothesis would be that fronto-temporal tDCS clinical efficacy could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network.

Table 4: Frontotemporal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
Case studies								
Andrade et al (2013)	Schizophrenia	1	N	F3/T3P3	once to twice daily	1 to 3	20 to 30	25
				At-home deliverability of tDCS until near-normal functioning during 3years.				
Bose et al (2015)	Schizophrenia	1	N	F3FP1/T3P3	18 (2per day)	2	20	35
				No changes in auditory hallucinations. However, patient responded to right sided stimulation.				
Brunelin et al (2012)	Schizophrenia	2	N	F3FP1/T3P3	10	2	20	35
				Improvement of hallucinations and global symptoms				
Jacks et al (2014)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35
				tDCS as an adjunctive treatment to ECT and clozapine provided an improvement in positive and negative symptoms.				
Narayanaswamy et al (2014)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Sustained reduction of auditory hallucinations and negative symptoms				
Nawani et al (2014)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Reduction of auditory hallucinations.Increase in N1 amplitude after tDCS.				
Nawani et al (2014)	Schizophrenia	1	N	F3/T3P3	10 (2per day)	2	20	35
				Reduction of auditory hallucinations. Modulates the corollary discharge dysfunction in patients with AVH				
Praharaj et al (2015)	Schizophrenia	1	N	F3FP1/T3P3	5 (1per day)	2	20	ND
				Improvement in AH and negative symptoms, however lasted less than a week.				
Rakesh et al (2013)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Complete remission of hallucinations and improvement in insight into illness				
Shenoy et al (2015)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35

Table 4: Frontotemporal tDCS studies

Study				tDCS parameters				
Author Date	**Population**	**n**	**sham (n)**	**Anode/Cathode placement**	**n session**	**I (mA)**	**Duration (min)**	**Electrode size (cm^2)**
				Reduction of auditory hallucinations, long lasting (4 months after tDCS), sage during pregnancy				
Shivakumar et al (2013)	Schizophrenia	1	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Complete remission of auditory hallucinations. Improvement of insight. Last until 4-week follow-up.				
Shivakumar et al (2014)	Schizophrenia	1	N	F3FP1/T3P3	2 (1 per month)			
				After a relapse, 2 tDCS booster sessions sustained improvement in a schizophrenia patient with AVH for a period of one year.				
Controlled studies								
Bose et al (2014)	Schizophrenia	21	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Significant improvement in insight with concurrent significant reduction in auditory hallucination severity. Correlation between both.				
Bose et al (2017)	Schizophrenia	25	Y	F3FP1/T3P3	10 (2per day)	2	20	35
				Significant reduction of AVH score in the active group				
Brunelin et al (2012)	Schizophrenia	15	Y (15)	F3FP1/T3P3	10	2	20	35
				Improvement of hallucinations and global symptoms in adults patients with schizophrenia				
Brunelin et al (2015)	Schizophrenia	16	N	F3FP1/T3P3	10	2	20	35
				Improvement of hallucinations after tDCS in non-smokers. No effect of tCDS on smokers.				
Ferrucci et al (2014)	Schizophrenia	6	N	F3FP1/T3P3	10 (2per day)	2	20	35
				Improvement of hallucinations and negative symptoms				
Fitzgerald et al (2014)	Schizophrenia	24	Y	F3/T3P3	15 (3 weeks)	2	20	35
				No changes reported after tDCS				
Mondino et al (2014)	Schizophrenia	28	Y	F3FP1/T3P3	10 (2per day)	2	20	35
				Reduced the externalization bias and AVH frequency in patients with schizophrenia				
Mondino et al (2015)	Schizophrenia	23	Y	F3FP1/T3P3	10 (2per day)	2	20	35
				Active tDCS significantly reduced AVH and the negative symptoms. These changes are associated with a modulation of the FC within a network, involved in inner speech production and monitoring.				
Shivakumar et al (2015)	Schizophrenia	23	Y	F3FP1/T3P3	10 (2per day)	2	20	35
				Reduction of auditory hallucinations in both groups. However, patients in Val group showed greater reductions that those in the Met group				

Critical issue and thesis statement

As mentioned above, dopamine is involved in numerous cognitive processes such as reward-motivation processes and executive functions, via the meso-cortico-limbic pathway. This major dopaminergic pathway links the ventral tegmental area (VTA) of the midbrain, the limbic system (including the ventral striatum) and the prefrontal cortex. Moreover, dopamine anomalies of this pathway have been reported in multiple neuropsychiatric conditions.

In addition, we have seen that using a tool such as tDCS applied over the frontal cortex, these cognitive processes and symptomatology of diseases involving the dopaminergic system can be improved. More specifically, different techniques can be used to assess the impact of tDCS of the dopaminergic system, such as PET, functional and structural MRI. In the last decade, neuroimaging studies and computational model analyses have highlighted that the neurobiological effects of tDCS are not restricted to the brain areas located under the stimulating electrodes but spread through distributed cortical networks functionally connected and could reach subcortical areas, such as dopaminergic regions. In addition, other NIBS approaches such as TMS reported a subcortical dopamine release in the striatum following a single session applied over the DLPFC. However, inconsistencies surrounding the neurobiological effects of tDCS emerge and information about biological effects of tDCS is scattered. This reinforces the fact that the spatial and temporal neurobiological effects of frontal tDCS are far from being completely understood, notably regarding the dopaminergic system.

Thus, the aim of this thesis work is to study the neurophysiological effect of a single session of frontal tDCS on the dopaminergic system. To investigate this question, we used two different electrode montages, which are being increasingly used in the literature (bifrontal tDCS and frontotemporal tDCS). We used an online study design and combined the stimulation with several imaging techniques, with the subject at rest. The online implementation of the stimulation allowed for deciphering changes induced not only after but also during the stimulation. As a first step, healthy subjects were involved in the present work, with the goal to move on to patients in subsequent studies.

In this line, the aim of our first study was to test, in healthy subjects, the effect of a single-session of bifrontal tDCS with the anode over the left DLPFC and the cathode over the right DLPFC on the subcortical dopaminergic transmission. A simple study was designed to investigate the online effects of tDCS using positron emission tomography (PET) via dopaminergic D2 subtype receptor availability via [^{11}C]Raclopride binding. We expected this study to answer several unanswered questions in the literature: **Does tDCS have an impact in subcortical structures?** If so, **is the effect specifically distributed in a temporal and spatial manner across subcortical dopaminergic areas?**

In addition, the neurobiological effects of tDCS have been described, to date, at different independent levels using several imaging techniques. Thus, creating a coherent ensemble seemed a mandatory and critical step to understand the mechanisms of action of tDCS. Furthermore, the specific effects of fronto-temporal tDCS have rarely being investigated. In this line, according to the hypothesis that fronto-temporal tDCS modulates brain activity, connectivity and dopaminergic transmission, the aim of this project was to reveal the combined neurobiological impact of an online single session of fronto-temporal tDCS in a unique experiment by developing a simultaneous multimodal imaging approach (PET-MR). The distributed changes in the dopaminergic system were explored at rest through 1) Specific and localized dopaminergic transmission evaluated by PET using dopaminergic D2 subtype receptor availability via [^{11}C]Raclopride binding. 2) Spontaneous functional connectivity assessed by functional magnetic resonance imagery (fMRI). 3) Brain activity assessed by cerebral blood flow quantitatively and directly measured by arterial spin labeling (ASL). 4) Connectivity assessed by diffusion tensor imaging (DTI).

Using this innovative simultaneous multimodal imaging system, we expected that our unique approach would provide an imaging biomarker essential to improve our understanding of the neurobiological effects of tDCS, notably regarding the dopaminergic system. More specifically, 1) **Does fronto-temporal tDCS have an impact on dopaminergic subcortical structures?** If so, **is the distributed effect similar or different than that of the bifrontal montage?**; 2) **Does tDCS have an impact on brain perfusion in local and interconnected regions?**; 3) **Does tDCS have an impact on the functional connectivity throughout dopaminergic distributed networks?**; 4) **Does a single session of tDCS have an impact on structural connectivity?**; 5) **Are the changes observed at different levels integrated in a comprehensive model?**; 6) **Can electric field modeling and structural connectivity explain the effects of tDCS on the dopaminergic networks?**

In this thesis, we answered the first two questions, while the others are still currently being investigated using the data acquired during the thesis. Moreover, using a novel scanner and combining these different imaging modalities is not without its challenges and part of this thesis work was also to address them. Notably, before analyzing the PET-MRI data, we investigated several questions in collaboration with the CERMEP: 7) **Without a CT scanner, how could we correct for attenuation in PET data?**; 8) **How to correct for movement in order to have good quantification of our results?**

Part II - Experimental studies

5. Chapter 5 - Impact of bifrontal transcranial direct current stimulation (tDCS) on the dopaminergic transmission in healthy humans

Article 1

Fonteneau Clara, Redouté Jérome, Haesebaert Frédéric, Le Bars Didier, Costes Nicolas, Suaud-Chagny Marie-Françoise, Brunelin Jérome; Frontal transcranial direct current stimulation induces dopamine release in the ventral striatum in human, *Cerebral Cortex,* Volume 28, Issue 7, 1 July 2018, Pages 2636–2646, https://doi.org/10.1093/cercor/bhy093

TITLE

FRONTAL TRANSCRANIAL DIRECT CURRENT STIMULATION INDUCES DOPAMINE RELEASE IN THE VENTRAL STRIATUM IN HUMANS

RUNNING TITLE

tDCS INDUCES DOPAMINE RELEASE IN HEALTHY HUMANS

AUTHORS

Clara FONTENEAU;MSc [a,b,c], Jérome REDOUTE;PhD [d], Frédéric HAESEBAERT;MD-PhD [a,b,c], Didier LE BARS;PharmD-PhD[d,e,f], Nicolas COSTES;PhD [d], Marie-Françoise SUAUD-CHAGNY ;PhD * [a,b], Jérome BRUNELIN;PhD * [a,b,c]

* authors contributed equally to this work

AFFILIATION

a) INSERM, U1028; CNRS, UMR5292; Lyon Neuroscience Research Center, Psychiatric Disorders: from Resistance to Response Team, Lyon, F-69000, France ;

b) University Lyon 1, Villeurbanne, F-69000, France

c) Centre Hospitalier Le Vinatier, Lyon, F-69000, France

d) CERMEP—Imagerie du vivant, Lyon, F-69000, France

e) ICBMS, Université de Lyon, F-69000, France

f) Hospices Civils de Lyon, F-69000, France

CORRESPONDING AUTHOR

Marie-Françoise SUAUD-CHAGNY

Centre Hospitalier Le Vinatier

Equipe de Recherche PSYR2

Pôle Est - Bâtiment 416 – 1er étage

BP 300 39 – 95 boulevard Pinel

69678 BRON Cedex – FRANCE

Phone Number : +33(0)437915565

e-mail: Marie-Francoise.SUAUD-CHAGNY@ch-le-vinatier.fr

ABSTRACT

A single transcranial direct current stimulation (tDCS) session applied over the dorsolateral prefrontal cortex (DLFPC) can be associated with pro-cognitive effects. Furthermore, repeated DLPFC tDCS sessions are under investigation as a new therapeutic tool for a range of neuropsychiatric conditions. A possible mechanism explaining such beneficial effects is a modulation of meso-cortico-limbic dopamine transmission.

We explored the spatial and temporal neurobiological effects of bifrontal tDCS on subcortical dopamine transmission during and immediately after the stimulation. In a double blind sham-controlled study, 32 healthy subjects randomly received a single-session of either active (20min, 2mA; n=14) or sham (n=18) tDCS during a dynamic positron emission tomography scan using [^{11}C]raclopride binding.

During the stimulation period, no significant effect of tDCS was observed. After the stimulation period, compared with sham tDCS, active tDCS induced a significant decrease in [^{11}C]raclopride binding-potential ratio (BP_R) in the striatum, suggesting an increase in extracellular dopamine in a part of the striatum involved in the reward-motivation network.

The present study provides the first evidence that bifrontal tDCS induces neurotransmitter release in polysynaptic connected subcortical areas. Therefore, levels of dopamine activity and reactivity should be a new element to consider for a general hypothesis of brain modulation by bifrontal tDCS.

Keywords: Dopamine, Dorsolateral prefrontal cortex, Positron emission tomography, Striatum, Transcranial direct current stimulation

INTRODUCTION

Dopamine is involved in various cognitive processes such as reward-related processes (Bromberg-Martin et al. 2010; Haber and Knutson 2010), emotion regulation (Lindquist et al. 2012) and executive functions (Wise 2004; Monchi et al. 2006; Cools 2011), via the meso-cortico-limbic pathway. This major dopaminergic pathway links the ventral tegmental area (VTA) of the midbrain, the limbic system (including the ventral striatum) and the prefrontal cortex (Haber and Knutson 2010). Moreover, dopamine abnormalities in this pathway have been shown in multiple conditions such as major depressive disorder (Price and Drevets 2012), substance-related and addictive disorder (Nutt et al. 2015), schizophrenia (Brunelin et al. 2013; Maia and Frank 2017) and in Parkinson's disease (Hanganu et al. 2015).

Interestingly, cognitive processes and symptomatology of diseases involving dopamine have been shown sensitive to non-invasive brain stimulations techniques (NIBS) applied over the dorsolateral prefrontal cortex (DLPFC). Among current NIBS, transcranial direct current stimulation (tDCS) consists in applying a weak direct current between two electrodes, a cathode and an anode, placed above the subject's scalp. Applied over the primary motor cortex, anodal tDCS induces excitatory effects, whereas cathodal stimulation results in inhibitory effects on motor cortex excitability. When the stimulation is applied continually during several minutes, the induced excitability changes last for up to an hour (Nitsche et al. 2005). From animal studies, it has been hypothesized that tDCS-mediated effects are related to a shift in neuronal resting membrane potential either toward depolarization and increased spontaneous neuronal firing at the anodal level and toward hyperpolarization and decreased firing at the cathode level (Bindman et al. 1964).

As such, tDCS is a technique emerging as having pro-cognitive effects in healthy humans (Levasseur-Moreau et al. 2013) and a prospective therapy to decrease symptoms and improve cognition in patients with neurologic and psychiatric disorders (Kuo et al. 2014; Lefaucheur et al. 2017). Specifically, bifrontal tDCS, with the anode applied over the left DLPFC coupled with the cathode placed over the right DLPFC may induce beneficial emotional and attentional processing in healthy subjects (Mondino et al. 2015), as well as clinical improvements in several psychiatric conditions

involving dopamine transmission abnormalities, such as major depressive disorder (Brunoni et al. 2016; Sampaio-Junior et al. 2018), substance-related and addictive disorder (Jansen et al. 2013), schizophrenia with predominant negative symptoms (Palm et al. 2016) and the cognitive alterations in Parkinson's disease (Leite et al. 2014). However, contradictory studies exist putting forward the importance of the study design, the individual variability and the brain state dependency in the results obtained in both cognitive (Horvath et al. 2015; Wörsching et al. 2017) and clinical studies (Brunoni et al. 2017; Loo et al. 2018). These discrepancies reinforce the need to better understand the spatial and temporal neurobiological effects of bifrontal tDCS. In the last decade, fMRI studies (Keeser et al. 2011; Pena-Gomez et al. 2012) and computational model analysis (Bai et al. 2014) highlighted subcortical effects of bifrontal tDCS reaching subcortical areas, such as dopaminergic areas. Offline studies also suggest that cortical stimulation by other NIBS approaches, such as a single session of high frequency transcranial magnetic stimulation (TMS) applied over the left DLPFC may evoke a dopamine release in the striatum (Strafella et al. 2001; Brunelin et al. 2011). However, the effect of a bifrontal tDCS on dopamine transmission is unknown.

The aim of this study was to test, in healthy subjects in a randomized placebo controlled double blind study, the effects of a single-session of bifrontal tDCS with the anode over the left DLPFC and the cathode over the right DLPFC on the subcortical dopaminergic transmission. These effects were explored online by positron emission tomography (PET) using dopaminergic D2 subtype receptor availability via [^{11}C]raclopride binding. We hypothesized that bifrontal tDCS can modulate subcortical dopaminergic transmission during and after the stimulation.

METHODS AND MATERIALS

Subjects.

Thirty-six healthy adults were included. Exclusion criteria were smoking, history of neurological and/or psychiatric illness, medical treatments (except for oral contraceptive), contraindications to MRI or tDCS and pregnancy. Volunteers were asked not to have caffeine on the day of scanning. Procedures were reviewed, approved by the standing ethics committee (CPP SUD EST 6, AU1148;

ANSM, A01405-42) and registered on ClinicalTrials.gov (NCT02402101). All subjects gave written informed consent after a detailed description of the study by the recruiting psychiatrist. Subjects were compensated 100 euros. Four subjects were excluded due to technical problems (see CONSORT Flow Diagram). Thirty-two subjects (mean age = 25.25 ±3.55 years, n=16 females) completed the study.

Experimental Design.

This study is randomized, double blind and with 2-arm parallel groups, active (n=14) vs sham (n=18) bifrontal tDCS (Figure 1.A). The experiment visit at the CERMEP imaging center consisted in an anatomical MRI and a PET scan during which subjects received a single tDCS session. At baseline, subjects completed personality questionnaires: Life Orientation Test-Revised (LOT-R, (Trottier et al. 2008), Motivation (Guay et al. 2003), Big Five Inventory (Plaisant et al. 2010). During the experiment visit, subjects completed before and after the PET scan a State-Trait Anxiety Inventory (STAI-YA, (Spielberger et al. 1970) and a structured adverse effect of tDCS questionnaire (Brunoni et al. 2011). Blinding integrity was assessed by having subjects guess the nature of the received stimulation (active or sham). Results are provided in table 1.

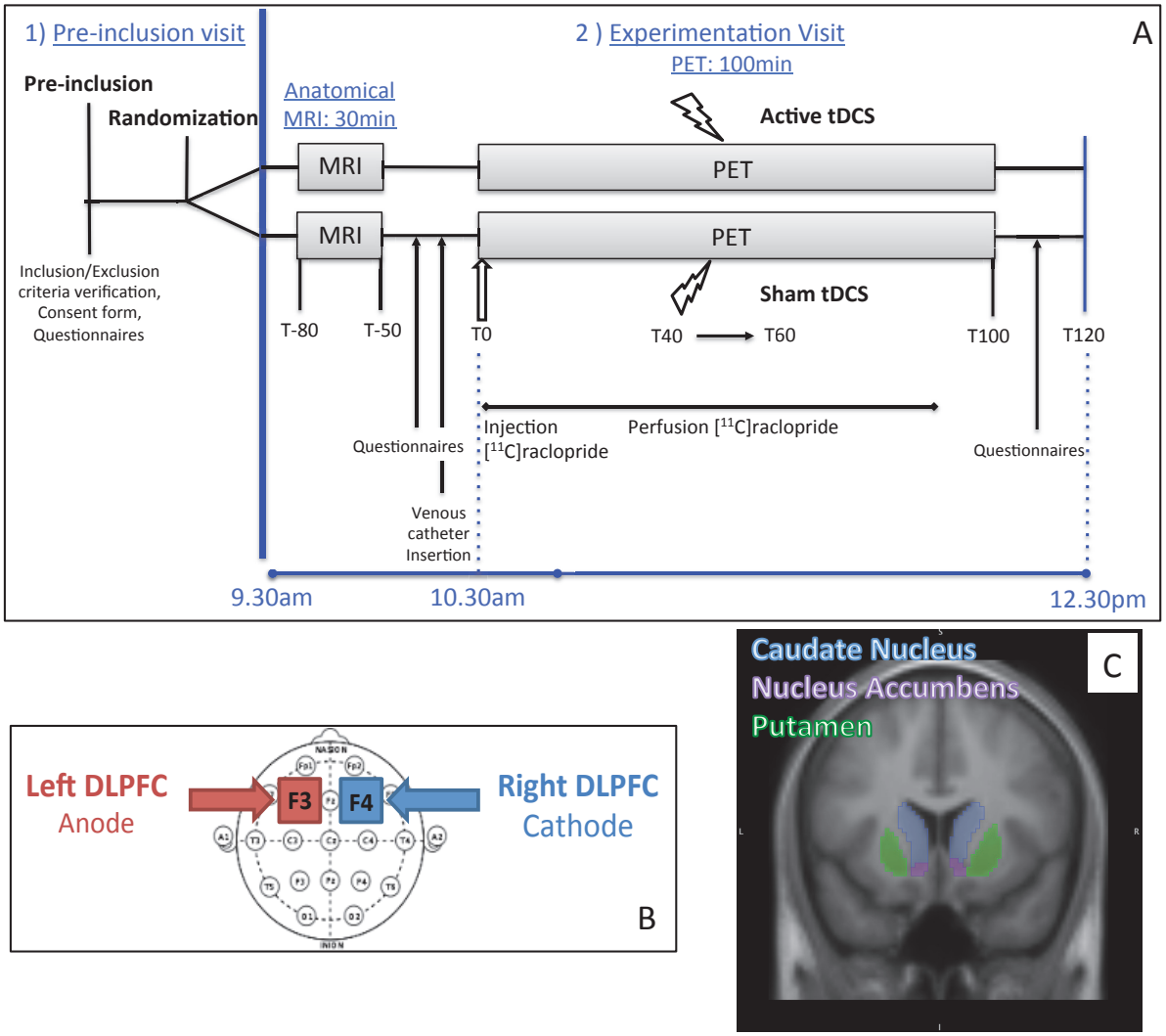


Figure 1 - (**A**) Study design (**B**) Transcranial direct current stimulation bifrontal montage (**C**) Hammersmith maximum probability brain atlas used as a striatal mask including the caudate nucleus, putamen and nucleus accumbens. Abbreviations: DLPFC, Dorsolateral prefrontal cortex; tDCS, transcranial direct current stimulation

Transcranial direct current stimulation.

tDCS was applied using a standard equipment (NeuroConn DC Stimulator Plus, GmbH). The anode was placed with the center of the electrode over F3 (left DLPFC) and the cathode was located over F4 (right DLPFC), according to international 10/20 EEG electrodes placement system (Figure 1.B). Electrode size was 7*5, 35cm^2. tDCS (either active of sham) was delivered at rest in a single-session during a dynamic PET scan. The stimulation started 40min after the injection of the tracer, lasted

20min, with 30s fade in/fade out periods, and was set at 2mA in active mode. For sham stimulation, the built-in sham mode mimicked the somatosensory artifact of active tDCS (30s fade in/fade out, 40s of active tDCS delivered at the beginning of stimulation).

Anatomical MRI.

All subjects underwent an anatomical MRI examination performed on a 1.5-T Magnetom scanner (Siemens), including a 3D anatomic T1-weighted sequence covering the whole brain volume, with 1mm^3 cubic voxels and 176 1mm-thick slices (TR=1970ms, TE=3.93ms). This scan was done before PET scan to control subject anatomy, electrode position and was further used for spatial normalization and to define the regions of interest (ROI).

Positron Emission Tomography.

PET scan session always started around 10.30am. During the 100min PET acquisition, subjects were lying at rest in the machine.

Radiochemistry.

Raclopride is a benzamide, a selective D2 receptor antagonist labeled with carbon-11, commonly used in PET studies (Hall et al. 1988). After synthesis at the CERMEP (1 synthesis per subject), [^{11}C]raclopride was purified, formulated and sterilized. The specific radioactivity obtained was around 3.7-18.5GBq/μmol (100 to 500mCi/μmol) at the time of injection.

Data Acquisition.

PET scans were conducted on a Biograph mCT PET-CT tomograph (Siemens). Subjects were positioned in the scanner such that acquired planes would be parallel to the orbital-meatal line. Head movement was minimized with an airbag. A camera allowed visual control of the head's position during acquisition. Measures for tissue and head support attenuation were performed with a 1min low-dose CT scan acquired before emission data acquisition. A bolus of [^{11}C]raclopride (18MBq+2.6MBq/kg) for 30s followed by a constant infusion of 57% of the initial dose (i.e. 10MBq+1.5MBq/kg) over 100min, was injected through an intravenous catheter (see doses in Table

1). This bolus-plus-continuous-infusion method is currently used when measuring dopamine release in challenging conditions (Adler et al. 2000; Brunelin et al. 2011). A dynamic emission scan was acquired in list mode during the 100min after injection. A total of 20 successive frames (5min each) were reconstructed by using 3D-ordinary Poisson-ordered subset expectation maximization iterative algorithm incorporating point spread function and time of flight (with a Gaussian filter of 3mm) after correction for scatter and attenuation. Reconstructed volumes consisted of 109 contiguous slices (2.03mm thickness) of 128×128 voxels (2.12x2.12mm^2). Actual resolutions for reconstructed images were approximately 2.6mm in full width at half maximum in the axial direction and 3.1mm in full width at half maximum in the transaxial direction measured for a source located 1cm from the field of view (Jakoby et al. 2011).

Data preprocessing and binding parametric imaging.

All preprocessing were carried out by a single individual blind to group status (active or sham). For each subject, preprocessing of MRI and PET data was done using an in-house script combining functions of Statistical Parametric Mapping 12 (SPM12, Wellcome Trust Centre of Neuroimaging), the MINC Tool Kit (Mc Connell brain Imaging centre, McGill university) and the Turku PET analysis software (Turku PET Centre). PET dynamic was corrected for between-frame motion with a 3D rigid body model using SPM12. This realigned dynamic PET scan was used hereon out. T1 was coregistered to the mean PET image for each subject and then spatially normalized into standard MNI space (Montreal Neurological Institute/International Consortium for Brain Mapping stereotactic space) with the *segment* function of SPM12. This step provided a classification of the T1 MRI into 6 tissue classes and generation of MNI to subject space deformation fields. The atlas was then back normalized into the subject space, and combined with the grey matter image. The assessment of free and nonspecific [^{11}C]raclopride ligand kinetics was based on the time–activity curve of a reference region (i.e., the cerebellum, without vermis) devoid of specific dopamine D2-like receptors (Pinborg et al. 2007). Thus, extracellular dopamine concentration was assessed using simple pseudo-equilibrium 5min ratios of ROI (striatum) to cerebellum activities (BP_R), computed with the *imgratio* function of the Turku PET library. BP_R images were spatially normalized into the standard MNI space

and smoothed using an isotropic 8mm full width half maximum Gaussian kernel. ROIs were selected from the Hammersmith maximum probability brain atlas (Hammers et al. 2003; Gousias et al. 2008). The a-priori ROI used in this study is the striatum. This ROI was used for subsequent regional BP_R and used as mask in the SPM analysis. The anatomical subparts of the striatum (i.e. caudate nucleus, putamen and nucleus accumbens; Figure 1.C) were used only after the analysis to name the significant clusters accordingly.

Statistical analyses.

A voxel-based SPM analysis was performed, using a flexible factorial design based on repeated measures ANOVA, to assess the effect of active tDCS compared with sham tDCS on BP_R for each time period: Baseline period (30-40min), Stimulation period (45-60min, effects during stimulation), Post1 period (65-80min, acute after-effects), Post2 period (80-95min, subsequent after-effects). This analysis was restricted to voxels belonging to the striatum mask (a-priori ROI). In SPM12, used in the present study, contrasts (post-hoc) can be performed only when the omnibus ANOVA created with the model is significant (Friston et al. 1991). Post-hoc Student t-score (SPM-{t}) maps were computed to elucidate the increase or decrease of [^{11}C]raclopride uptake during (Stimulation time period) or after (Post1 and Post2 time periods) tDCS, by comparing active and sham groups. The following contrasts were computed: [(Stimulation-Baseline)$_{sham}$ vs (Stimulation-Baseline)$_{active}$], [(Post1-Baseline)$_{sham}$ vs (Post1-Baseline)$_{active}$], [(Post2-Baseline)$_{sham}$ vs (Post2-Baseline)$_{active}$], [((Post1+Post2)-Baseline)$_{sham}$ vs ((Post1+Post2)-Baseline)$_{active}$], [((Stimulation+Post1+Post2)-Baseline)$_{sham}$ vs ((Stimulation+Post1+Post2)-Baseline)$_{active}$], [(Post1-Stimulation)$_{sham}$ vs (Post1-Stimulation)$_{active}$], [(Post2-Stimulation)$_{sham}$ vs (Post2-Stimulation)$_{active}$], [(Post1-Post2)$_{sham}$ vs (Post1-Post2)$_{active}$], [((Post1+Post2)-Stimulation)$_{sham}$ vs ((Post1+Post2)-Stimulation)$_{active}$]. SPM maps were thresholded at $P_{uncorr} < 0.001$ at the voxel level, with a minimum of 10 contiguous voxels (80 mm^3), which is the expected number of voxels per cluster in the 3D gaussian space. Then, only clusters with $P_{FWE} < 0.05$ (SPM Family wised error correction for multiple comparisons) at the cluster level were considered significant. Reported coordinates (Table 2) conform to the MNI space, for each cluster. Time-activity curves were extracted for each cluster and the BP_R value computed in each time period

and for each group. BP_R were also expressed as the relative difference between groups at each time periods (Table 3). A secondary analysis was performed in order to investigate the potential impact of dopamine baseline levels (BPR) on the relative changes (Delta (%)) observed in the significant cluster, with a correlation analysis (Pearson r correlation coefficient). $P<0.05$ was considered significant. Demographic and clinical characteristics were examined using descriptive statistics. Normality was assessed by the Shapiro–Wilk test. Statistical analyses were done between groups using the Welch two sample t-test (Injected dose/kg, Years of education, Motivation score, LOT-R score, BFI-N score, STAI-difference, BP_R baseline) or the Wilcoxon rank sum test with continuity correction (Age, STAI-A scores baseline and post, Movement translation) and the Chi2 test for handedness, sex variables and blinding integrity. These analyses were done using in-house scripts in R (https://cran.r-project.org/).

Data and code Availability.

The data and custom-written analysis code that support the findings of this study are available on request from the corresponding author.

RESULTS

Subjects' characteristics. The subjects' characteristics are shown in Table 1 for both active and sham tDCS groups. No statistical differences were found between the two groups. As no distribution differences between groups (gender and age) were observed, we did not us them as co-variables for the statistical calculations. No adverse effects were reported either due to the tDCS stimulation, the MRI or the PET scans.

Table 1 | Characteristics of subjects among the two groups

Variable	Active tDCS (N=14)	Sham tDCS (N=18)	P value
Demographic			
Age [years]	24.86 (±4.05)	25.56 (±3.18)	0.3886
Sex [Male:Female]	6 : 8	10 : 8	0.7216
Years of education [years]	3.92 (±1.86)	4.78 (±1.83)	0.2077
Handedness [Right:Left]	12 : 2	13 : 5	0.6278

Table 1 I Characteristics of subjects among the two groups			
Variable	**Active tDCS (N=14)**	**Sham tDCS (N=18)**	**P value**
PET scan			
Injected dose [MBq/kg]	320.86 (±35.02)	320.33 (±59.10)	0.7812
Movement translation [mm]	-0.20 (±1.38)	-0.13 (±1.79)	0.2792
Movement rotation [degrees]	0.004 (±0.011)	0.004 (±0.012)	0.8879
Psychological assessment			
Motivation score	123.79 (±21.00)	124.06 (±15.90)	0.9684
LOT-R score	17.86 (±3.35)	15.72 (±14.86)	0.1525
BFI N score	17.64 (±5.57)	20.72 (±7.06)	0.1781
Baseline STAI-A score	25.5 (±4.07)	26.61 (±5.25)	0.5794
Post-tDCS STAI-A score	24.71 (±4.34)	26.56 (±3.49)	0.1173
STAI-A score difference	0.79 (±3.47)	0.05 (±3.90)	0.5803
Blinding [Active:Sham:None]	6 : 6 : 2	5 : 13 : 0	0.1203

Table 1 - Characteristics of subjects among the two groups (active and sham tDCS; mean (±sd). Abbreviations: BFI N, Big Five Inventory Neuroticism; LOT-R, Life Orientation Test-Revised; STAI-YA, State-Trait Anxiety Inventory (form Y-A, anxiety state); tDCS, transcranial Direct Current Stimulation. Welch two sample t-test or Wilcoxon rank sum test with continuity correction and the chi-square tests were conducted to assess group differences for continuous and discrete variables, respectively.

Kinetic analysis. The extraction of the [^{11}C]raclopride binding potential ratio (BP_R) in the region of interest (striatum) (Figure 2.A) enabled us to determine a baseline time period during which BP_R reached a state close to equilibrium. The other time periods, that have been be used to create the mean ratio images for the parametric analysis, were of 15min each. Thus, the effects of the stimulation have been examined over 4 time periods: Baseline period (30-40min after tracer injection), Stimulation period (45-60min, effects during stimulation), Post1 period (65-80min, acute after-effects), Post2 period (80-95min, subsequent after-effects). A baseline BP_R difference between the active and sham group in the striatum was reported (p=0.018; Active 5.23±0.51; Sham: 4.79±0.46; mean ± sd).

Therefore, subsequent analysis took this difference into account with comparisons of relative variations in each contrast.

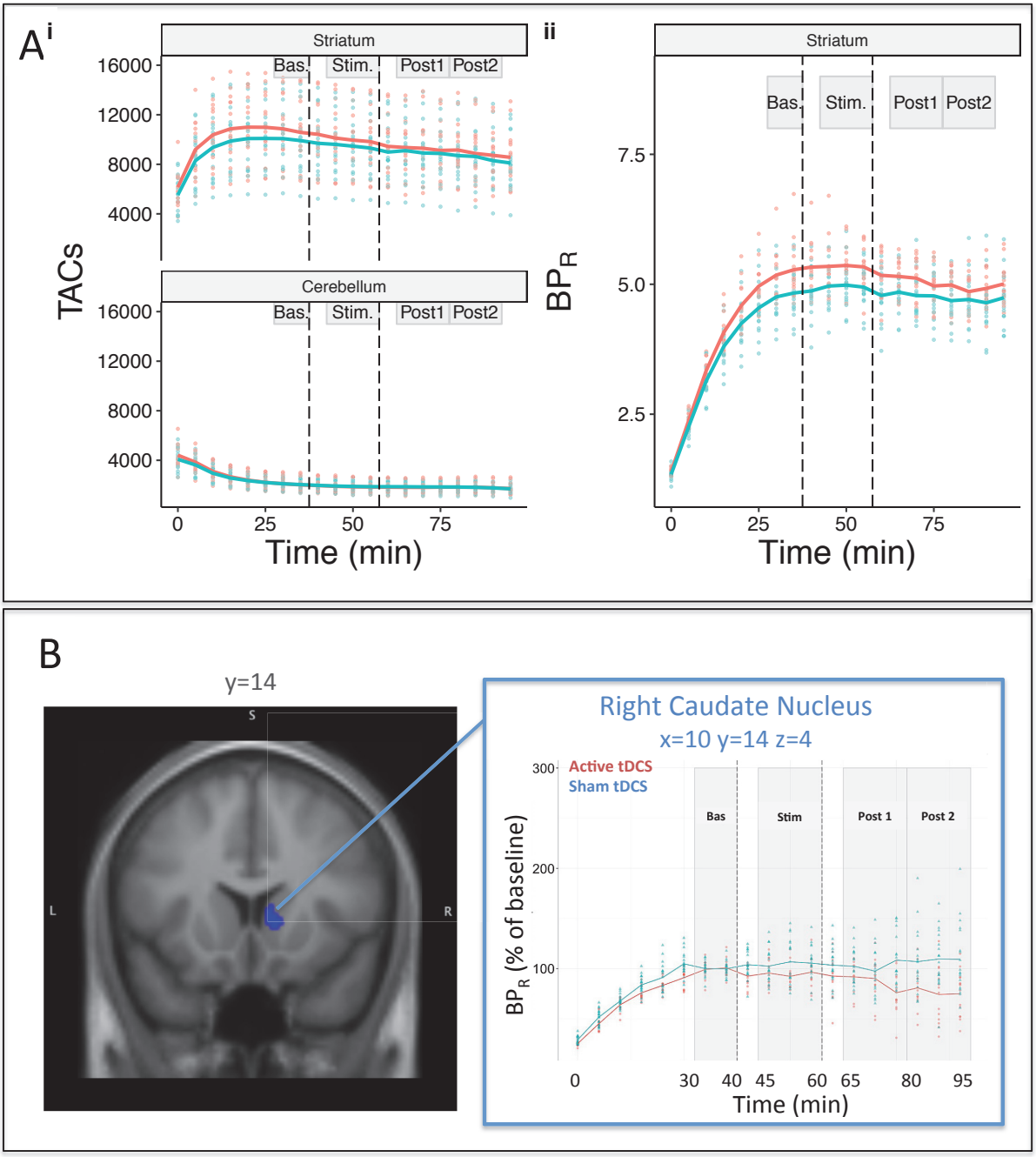


Figure 2 - (**A**) Kinetic analysis. (**i**) Time activity curve in the striatum and the cerebellum (**ii**) Binding potential ratio in the striatum; for both groups across the 20 PET frames (5 minutes per frame). (**B**) Parametric analysis - Subsequent after-effects of the stimulation in different clusters when comparing the baseline period to the Post-2 period ($P_{uncorr} < 0.001$ at the voxel level, with a minimum of 10 contiguous voxels (80 mm^3)). For the significant cluster ($P_{FWE} < 0.05$ at the cluster level), the

BP_R kinetic curves for both groups across the 20 PET frames (5 minutes per frame) are extracted and are expressed as BP_R ratio (% of baseline). The anatomical subparts of the striatum (i.e. caudate nucleus, putamen and nucleus accumbens) were named based on the Hammersmith maximum probability brain atlas. Abbreviations: Bas., Baseline period; Stim, Stimulation period; Red curve, Active tDCS; Blue curve, Sham tDCS, BP_R, Binding Potential Ratio; TACs, Time Activity Curves

Parametric analysis. The analysis was performed using a mask of the whole striatum (a-priori ROI). The voxel-based analysis showed significant clusters in the striatum when comparing the time periods determined between groups (Table 2). More specifically, when comparing active and sham tDCS groups, areas of significant changes in dopaminergic activity showed BP_R decreases in the striatum (Figure 2.B). After the complete analysis, the position of the significant clusters have been identified according to the anatomical subparts of the striatum delineation of Hammersmith maximum probability brain atlas, i.e. the caudate nucleus, putamen and nucleus accumbens. The [^{11}C]raclopride BP_R in clusters and their relative difference in the active tDCS group compared to the sham group are summarized in Table 3.

Effects of tDCS during the stimulation (Stimulation period) No significant differences in BP_R were observed in the striatum when comparing stimulation and baseline periods, between groups [$(\text{Stimulation-Baseline})_{sham}$ vs $(\text{Stimulation-Baseline})_{active}$].

After effects of tDCS

- *During the 5 to 35min period following the stimulation (Post1 + Post2 period)* Significant differences in BP_R were reported in the active group compared to sham group when comparing the baseline period with the 5 to 35min period following the stimulation [$(\text{(Post1+Post2)-Baseline})_{sham}$ vs $(\text{(Post1+Post2)-Baseline})_{active}$], specifically in the right caudate nucleus (-25.4%). Accordingly, a trend towards significance was also reported when comparing the stimulation period with the 5 to 35min period following the stimulation [$(\text{(Post1+Post2)-Stimulation})_{sham}$ vs $(\text{(Post1+Post2)-Stimulation})_{active}$], specifically in the left putamen (-16.5%) and in the right caudate nucleus (-20.3%)

- *Acute after-effects: During the 5 to 20min period following the stimulation (Post 1 period)* No significant differences in BP_R were observed between groups in the striatum when comparing the baseline period with the 5 to 20min period immediately following the stimulation [$(Post1\text{-}Baseline)_{sham}$ vs $(Post1\text{-}Baseline)_{active}$].

However, when comparing the 5 to 20min period immediately following the stimulation to the stimulation period [$(Post1\text{-}Stimulation)_{sham}$ vs $(Post1\text{-}Stimulation)_{active}$], a trend towards a significant BP_R decrease was observed in the active group compared to sham group specifically in the left putamen (-14.0%) and in the right accumbens and caudate nuclei (-33.8%).

- *Subsequent after-effects: During the 20-35min period following the stimulation (Post2 period) (Figure 2.B)* Differences in BP_R were reported in the active group compared to sham group when comparing the baseline period with the 20-35min period following the stimulation [$(Post2\text{-}Baseline)_{sham}$ vs $(Post2\text{-}Baseline)_{active}$], specifically significant in the right caudate nucleus (-32.0%) and trending significance in the left putamen (-25.9%). Furthermore, a trend towards a significant differences was reported specifically in the right caudate nucleus (-29.3%) when comparing the stimulation period with the 20-35min period following the stimulation [$(Post2\text{-}Stimulation)_{sham}$ vs $(Post2\text{-}Stimulation)_{active}$].

Table 2 I Parametric Analysis: Group comparison

	MNI coordinates (mm)				Cluster	
Contrast/Region	**x**	**y**	**z**	**Z-score**	**P_{FWE}**	**Volume (mm^3)**
(Stimulation + Post 1 + Post 2)-Baseline						
right caudate nucleus	10	14	4	4.06	0.037	272
(Post 1 + Post 2) - Baseline						
right caudate nucleus	10	14	4	4.28	0.023	352
(Post 1+ Post 2) - Stimulation						
right caudate nucleus	6	16	-4	3.49	0.092	144
left putamen	-28	2	-2	3.65	0.081	160
Post 1 - Stimulation						
right caudate and accumbens nuclei	6	12	-4	4.17	0.092	144
Left putamen	-30	2	-6	3.53	0.064	192

Post 2 - Baseline						
right caudate nucleus	10	14	4	4.40	0.022	360
left putamen	-22	10	-12	3.47	0.105	128
Post 2 - Stimulation						
right caudate nucleus	10	14	2	3.63	0.105	128

Table 2 - Clusters of the parametric analysis. Effect of tDCS in the striatum using a flexible factorial design (time periods*groups). SPM maps were thresholded at $P_{uncorr} < 0.001$ at the voxel level, with a minimum of 10 contiguous voxels (80 mm^3). Only clusters with $P_{FWE} < 0.05$ at the cluster level were considered significant. We also reported the z-score at the peak level. The contrast reported here are [Active tDCS<Sham tDCS]. No significant clusters were reported with the contrast [Active tDCS>Sham tDCS]. The anatomical subparts of the striatum (i.e. caudate nucleus, putamen and nucleus accumbens) were named based on the Hammersmith maximum probability brain atlas.

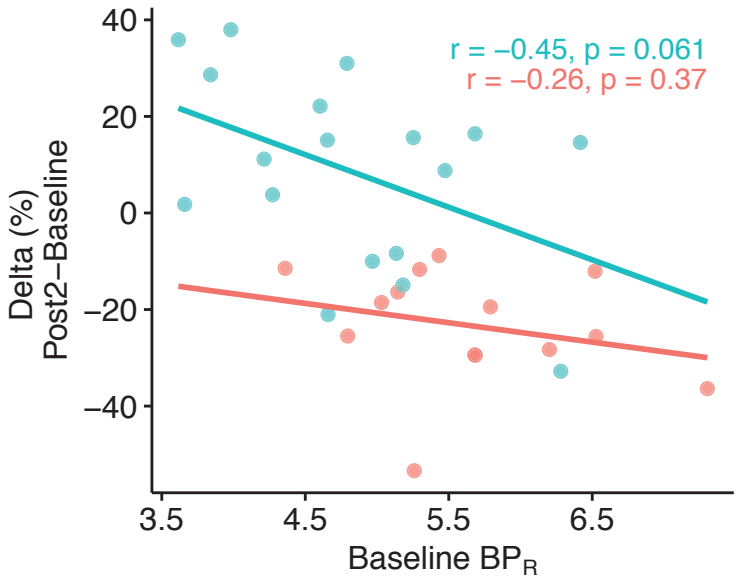
Table 3 I [^{11}C]raclopride binding potential ratio in clusters

Cluster volume (mm^3) /tDCS group	Raclopride BP_R, mean (±sd) Condition 1	Condition 2	Relative variation, %	Difference Active - Sham Group
Right Caudate Nucleus				
272	Baseline	Stimulation+Post1+Post 2		
active tDCS	5.78 (±0.88)	4.89 (±1.40)	-15.05 (±9.47)	-22.46
sham tDCS	4.93 (±1.01)	5.23 (±1.48)	7.41 (±14.79)	
352	Baseline	Post 1 + Post 2		
active tDCS	5.66 (±0.83)	4.55 (±1.28)	-19.05 (±9.86)	-25.36
sham tDCS	4.78 (±0.91)	5.02 (±1.39)	6.31 (±15.57)	
144	Stimulation	Post 1 + Post 2		
active tDCS	4.77 (±1.19)	4.18 (±1.74)	-11.73 (±21.18)	-20.33
sham tDCS	4.46 (±1.49)	4.84 (±2.05)	8.60 (±15.48)	
144	Stimulation	Post 1		
active tDCS	3.99 (±1.30)	3.39 (±1.43)	-13.34 (±18.71)	-33.82
sham tDCS	3.35 (±1.09)	3.94 (±1.57)	20.48 (±26.03)	
360	Baseline	Post 2		
active tDCS	5.64 (±0.92)	4.31 (±1.36)	-23.32 (±12.03)	-31.97
sham tDCS	4.82 (±0.89)	5.16 (±1.55)	8.65 (±19.90)	

Table 3 I [^{11}C]raclopride binding potential ratio in clusters					
		Raclopride BP_R, mean (±sd)			
Cluster volume (mm^3) /tDCS group		Condition 1	Condition 2	Relative variation, %	Difference Active - Sham Group
128		Stimulation	Post 2		
	active tDCS	5.64 (±1.47)	4.52 (±2.04)	-19.02 (±25.83)	-29.32
	sham tDCS	5.00 (±1.62)	5.47 (±2.45)	10.30 (±22.03)	
Left Putamen					
160		Stimulation	Post 1 + Post 2		
	active tDCS	7.91 (±2.13)	6.58 (±2.00)	-13.89 (±18.07)	-16.49
	sham tDCS	6.81 (±2.03)	6.80 (±1.88)	2.60 (±19.96)	
192		Stimulation	Post 1		
	active tDCS	6.86 (±1.53)	5.78 (±1.52)	-13.99 (±21.78)	-13.99
	sham tDCS	6.04 (±1.39)	6.00 (±1.55)	-0.00 (±18.03)	
128		Baseline	Post 2		
	active tDCS	4.89 (±0.94)	4.44 (±2.13)	-6.95 (±25.21)	-25.9
	sham tDCS	4.43 (±0.86)	5.28 (±2.10)	18.95 (±23.71)	

Table 3 - [^{11}C]raclopride binding potential in clusters revealed by the parametric analysis - BP_R variations during and after the stimulation compared to baseline (Relative variation, %). The anatomical subparts of the striatum (i.e. caudate nucleus, putamen and nucleus accumbens) were named based on the Hammersmith maximum probability brain atlas.

Correlation analysis. An analysis was performed in the cluster reported significant with the voxel-based parametric analysis, i.e. in the right caudate nucleus, to investigate the impact of baseline dopamine BP_R levels on the relative BP_R difference (Delta (%)) between Post 2 and Baseline time periods. This correlation analysis was not significant (active group: r=0.26, p=0.37; sham group: r=0.45, p=0.061) (Supplementary Figure 1).



Supplementary Figure 1 - Correlation between the baseline dopamine BP_R levels and the relative BP_R difference (Delta (%)) between Post 2 and Baseline time periods. No impact of the baseline dopamine levels on the dopamine release induced by tDCS in the right caudate nucleus (Pearson r correlation coefficient) was observed in sham and active groups. P<0.05 was considered significant. Red curve, Active tDCS; Blue curve, Sham tDCS; BP_R, Binding Potential Ratio

DISCUSSION

Here, we present the first direct evidence of temporally and spatially distributed effects of bifrontal tDCS on dopamine transmission in the striatum after one session of 20min at 2mA, in healthy subjects. These results provide the first proof of a decrease in [^{11}C]raclopride BP_R suggesting an increase in extracellular dopamine induced by a dopamine release evoked by a tDCS session.

The impact on dopamine transmission seems progressive during the stimulation and reaches significance during the 5 to 35min period following the end of stimulation. The absence of significant effects during the stimulation could be considered as contrasting with some previous online stimulation studies. In this line, glutamate/glutamine variations have been observed in the left striatum during a single bifrontal tDCS session using online MRS (Hone-Blanchet et al. 2016). The discrepancy with our results could be explained by several factors. First, regarding technical features, MRS measures a mixture of compounds involved in neurotransmission and metabolism in every cellular compartment of the voxel. Here, with [^{11}C]raclopride PET, we addressed dopamine

neurotransmission in terms of extracellular dopamine. Second, regarding physiological features, it cannot be ruled out that the time-scale of glutamate and dopamine variations is different. With this hypothesis, changes in the macro- and microenvironment induced during tDCS could trigger dopamine release only when the stimulation ends. Another explanation could be a matter of significant threshold. Indeed, the BP_R curve over time (Figure 2.B) shows a continuous decrease starting at the beginning of the stimulation, only for the active tDCS group. However, this decrease reaches significance only after the end of the stimulation compared to the sham group.

The increase in dopamine is in line with studies exploring TMS impact on dopamine transmission in animals, healthy subjects and in pathological conditions, as well as tDCS in animals (Ko and Strafella 2012). These studies conducted offline showed modulations of dopamine transmission in the striatum after stimulation protocols applied over the prefrontal cortex. For example, an increase in extracellular dopamine specifically in the left dorsal caudate nucleus was shown after a repetitive TMS stimulation with the coil over the left DLPFC (Strafella et al. 2001). In the same line, an animal tDCS study reported an increase in dopamine concentration in rat basal ganglia after cathodal tDCS compared to sham and anodal conditions. The effect was significant from 120min after the stimulation (Tanaka et al. 2013).

The significant clusters identified in our study are localized specifically in the ventral regions of the striatum. This spatially distributed after-effect of bifrontal tDCS is supported by the notion that complex organized behavior is made possible by the connectivity between several striato-thalamo-cortical circuits, including distinct striatal and cortical regions (Haber and Knutson 2010). Several studies have highlighted the model of a tripartite division of the striatum (Postuma 2005; Di Martino et al. 2008; Draganski et al. 2008; Pauli et al. 2016), using noninvasive neuroimaging methods. This striatal parcellation corresponds to three functionally distinct regions: a motor region which includes the dorsal part of the putamen and caudate nucleus, a cognitive region including the ventral rostral putamen, dorsal caudate, superior ventral striatum corresponding to the ventral caudate and an affective region composed of the inferior ventral striatum or nucleus accumbens. In addition, connectivity studies have traced the structural and functional coupling between individual striatal and

cortical regions and have shown that the DLPFC projects extensively to the ventral striatum, an overlap between regions involved in affective and cognitive processes, corresponding to the reward/motivation network. According to these studies, our clusters are located in the cognitive and affective regions of the striatum, regions anatomically linked to the DLPFC targeted by the tDCS montage.

Our findings of an increase in extracellular dopamine spatially located in the ventral striatum, obtained after a single session of bifrontal tDCS, are in line with the possible pro-cognitive effects seen after frontal tDCS in healthy subjects and in pathological conditions (Kuo and Nitsche 2015; Lefaucheur et al. 2017). Indeed, multiple studies focused on the reciprocal influence of cognitive functions and variations in dopamine. Imaging studies have detected increases in ventral striatal extracellular dopamine concentrations during task components such as motor learning and execution, reward-related processes, stress and cognitive performance (Egerton et al. 2009). Moreover, predictions about anticipated future rewarding have been shown to be encoded by dopamine concentration of the ventral striatum, and that the amount of dopamine itself encodes the distance from the reward (Howe et al. 2013). In the same line, manipulations that enhance dopamine transmission, such as addictive drugs and dopamine agonists, often act as neuroenhancers (Wise 2004; Nutt et al. 2015). However, as with pharmacological neuroenhancers, tDCS could also be linked to a direct dopamine release within the prefrontal cortex. We acknowledge that our study did not allow for an evaluation of the tDCS effects on dopamine release in the prefrontal cortex. Using a high affinity radioligand ([^{11}C]FLB-457), Cho and collaborators have shown that TMS over the left DLPFC induces a reduction of BP in the ipsilateral pre and subgenual anterior cingulate cortex, and medial orbitofrontal cortex (Cho and Strafella 2009). Further studies are needed to evaluate the direct effect of tDCS on the prefrontal cortex.

The significant after-effects of bifrontal tDCS beg the question of possible mechanisms leading to the dopamine release in the striatum. The literature supports two possible mechanisms, involving glutamatergic cortical projections: a direct pathway, *via* corticostriatal projections and an indirect pathway, involving cortical projections on mesostriatal dopamine neurons in the midbrain. Both

mechanisms could be involved in tDCS effects, according to animal studies showing that stimulation of the PFC could promote activation in both striatal and ventral tegmental regions (Taber and Fibiger 1995; Peanlikhit et al. n.d.). With the notion that tDCS modulates glutamatergic and GABAergic activity under the electrodes (Stagg et al. 2009), bifrontal tDCS may impact the glutamatergic projections from the DLPFC, and consequently modify subcortical activity, as shown by a recent MR-spectroscopy study reporting that bifrontal tDCS had fast excitatory effects in the left striatum (Hone-Blanchet et al. 2016). To further investigate the exact mechanism, future studies could explore the impact of bifrontal tDCS on the relation between blood flow and dopamine transmission variations.

Based on our results and according to the increasing use of tDCS in various populations, the level of dopamine signaling should be considered for each tDCS application. Indeed, it is important to note that the subject's brain-state at the time of stimulation plays an important role in the response (Silvanto and Pascual-Leone 2008). Accordingly, effects of bifrontal tDCS could be sensitive to the level of dopamine activity at baseline. An inverted U-shape hypothesis has been put forth describing a non-linear relationship between cognitive performance and dopamine concentration. Both too high as well as too low concentrations of dopamine are associated with suboptimal cognitive processing (Cools and D'Esposito 2011). Pharmacological studies suggest a similar relationship between dopaminergic activity and neuroplastic changes induced by tDCS applied over the human motor cortex (Kuo et al. 2008; Monte-Silva et al. 2010; Fresnoza et al. 2014). A recent review has reported that the modulation of dopamine D1 and D2 signaling by agonist and antagonist administration has a significant dose and receptor-dependent impact on tDCS after-effects (McLaren et al. 2018). Combined with our results, a reciprocal interaction between dopaminergic systems and tDCS can be suggested.

Therefore, exploring the effects of bifrontal tDCS under conditions where basal dopamine activity is altered could be of major relevance. First, in psychiatric conditions such as depression, stimulation studies robustly report groups of responders and non-responders to repeated bifrontal tDCS while physiological levels of dopamine activity have been shown heterogeneous across subjects (Seamans and Yang 2004). According to our results, it can be hypothesized that the basal dopamine activity

level or the change in extracellular dopamine evoked by a first bifrontal tDCS session could be a predictive marker of the therapeutic response obtained after applying multiple tDCS sessions on several days, protocol used in studies developing tDCS as a treatment for psychiatric disorders. Second, tDCS devices are being increasingly used in a recreational manner with little or no warning to interaction with medication or psycho-stimulant, in particular those interacting with the dopamine transmission. However in our study, we did not observe any impact of the baseline dopamine levels on the release induced by tDCS. Nevertheless, our study included only healthy subjects at rest and free of treatment interfering with dopaminergic transmission. From this, it could be suggested that, in these specific population and conditions, the inter-subjects difference in dopamine activity may not impact tDCS effects. Overall, our work shows the ongoing importance of controlled studies when using tDCS and should boost the research in this field to prevent the unsafe use of tDCS in uninformed people.

One limitation of this study is that PET results were not associated with behavioral findings (e.g., improvement of working memory performances), hence no pro-cognitive effects were in fact inspected in the present study. The second limitation is that dopamine has also been shown to be involved in placebo responsiveness (Benedetti 2014). The placebo-controlled study design developed here overcame in part this problem. Moreover, the psychological assessment conducted did not reveal differences between active and sham groups regarding personality traits, motivation and anxiety.

To conclude, the present study provides first direct evidence that bifrontal tDCS induces neurotransmitter release in polysynaptic connected subcortical areas. Our findings offer new insights for innovative use of tDCS as a therapeutic solution in neuropsychiatric conditions involving dopamine transmission impairments in the reward-motivation network. In the context of the ongoing debate surrounding tDCS in the literature and beyond the simple 'excitatory-inhibitory' model, levels of dopamine activity and reactivity should be a new element of the mosaic, adding to other parameters such as individual head anatomy variability, electrode position and brain state dependency for a general hypothesis of brain modulation by bifrontal tDCS (Krause and Cohen Kadosh 2014; Opitz et al. 2015; Wörsching et al. 2016).

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6. Chapter 6 - Neurophysiological impact of a fronto-temporal transcranial direct current stimulation (tDCS) in healthy humans: a simultaneous PET-MR approach

Contributing authors

Clara FONTENEAU *;MSc [a,b,c], Ines MERIDA;PhD [d], Jérome REDOUTE;PhD [d], Frédéric HAESEBAERT;MD-PhD [a,b,c], Didier LE BARS;PharmD-PhD[d,e,f], Nicolas COSTES;PhD [d], Jérome BRUNELIN;PhD [a,b,c], Marie-Françoise SUAUD-CHAGNY ;PhD [a,b]

Affiliation

a) INSERM, U1028; CNRS, UMR5292; Lyon Neuroscience Research Center, Psychiatric Disorders: from Resistance to Response Team, Lyon, F-69000, France ;
b) University Lyon 1, Villeurbanne, F-69000, France
c) Centre Hospitalier Le Vinatier, Lyon, F-69000, France
d) CERMEP—Imagerie du vivant, Lyon, F-69000, France
e) ICBMS, Université de Lyon, F-69000, France
f) Hospices Civils de Lyon, F-69000, France

Corresponding Author

Clara FONTENEAU
Centre Hospitalier Le Vinatier
Equipe de Recherche PSYR2
Pôle Est - Bâtiment 416 – 1er étage
BP 300 39 – 95 boulevard Pinel
69678 BRON Cedex – FRANCE
Phone Number : +33(0)437915565
e-mail:

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6.1. General aspects of the study

6.1.1. Introduction

Brain function can be modified by applying stimulation over the scalp. To date, the simplest technique, transcranial direct current stimulation (tDCS), consists in applying a weak constant current between two electrodes, a cathode and an anode, placed above two cortical areas. Applied over the primary motor cortex, anodal tDCS is thought to induce excitatory effects, whereas cathodal stimulation is thought to result in inhibitory effects on motor cortex excitability (Nitsche and Paulus, 2000). When the stimulation is applied continually during several minutes, the induced excitability changes last for up to an hour (Nitsche et al., 2005). From animal studies, it has been hypothesized that tDCS-mediated effects are related to a shift in neuronal resting membrane potential either toward depolarization and increased spontaneous neuronal firing (anodal) or toward hyperpolarization and decreased firing (cathodal) (Bindman et al., 1964).

tDCS is a technique emerging as a prospective therapy for neurologic, psychiatric and addictive disorders (Lefaucheur et al., 2017). Specifically, fronto-temporal tDCS, with anodal stimulation over the left dorsolateral prefrontal cortex (DLPFC) and cathodal stimulation over the left temporo-parietal junction (TPJ), has been suggested to reduce treatment-resistant auditory hallucinations (AH), negative symptoms and increase insight of the illness in schizophrenia (Brunelin et al., 2012; Bose et al., 2017; Ponde et al., 2017). However, despite an increasing use in clinical settings, there is not enough proof to suggest a clear clinical efficacy of fronto-temporal tDCS due to the small sample size of the studies (mostly case studies) (Lefaucheur et al., 2017; Ponde et al., 2017).

Furthermore, strategies to optimize the conditions for fronto-temporal tDCS application lack knowledge about tDCS neurophysiological impact. Indeed, to date, no neuroimaging studies in humans have investigated the neurobiological effects of fronto-temporal tDCS.

When looking at other tDCS montages, studies have reported that frontal tDCS effects are not restricted to the brain areas located under the electrodes, but spread through distributed cortical networks functionally connected with the targets and reach subcortical areas (for review see Wörsching et al., 2016). Overall, these studies suggest that tDCS modulates functional connectivity within and across resting-state networks and brain activity. More specifically, fMRI studies highlighted subcortical effects of tDCS applied at the cortical level including modulations of cortico-striatal and thalamo-cortical functional connectivity. A recent study reported that bifrontal tDCS induced a release of dopamine in the striatum after the end of the stimulation (Fonteneau et al, 2018).

Moreover, frontal and temporal regions are tightly connected with the dopamine networks (Haber et al, 2016). These networks have been shown to be altered in disorders such as schizophrenia, may it be relating to negative or positive symptoms (Grace, 2016). More specifically, a fronto-temporal dysconnectivity was reported with a hypo-activation of the left DLPFC (Sanfilipo et al., 2000; Lawrie et al., 2002) and a hyper-activation of the left temporo-parietal junction (TPJ) (Silbersweig et al., 1995). Thus, according to the therapeutic effects of fronto-temporal tDCS on schizophrenia and the pathophysiological hypothesis of an altered dopamine transmission in patients with schizophrenia, the effect of fronto-temporal tDCS on dopaminergic networks is of major interest.

These effects are currently described at different levels depending on the imaging technique used. More recently, some have coupled several imaging modalities but acquired on separate days and with different montages (Stagg et al., 2014; Hunter et al., 2015). However, the brain state of the subject could differ between the imaging modalities and could result in differential effects of tDCS. Finally, effects of the stimulation applied online are rarely inspected. Thus, information about biological effects of tDCS is scattered and creating a coherent ensemble is a mandatory and critical step to better understand the mechanisms of action of tDCS.

The aim of this project is to reveal the combined neurobiological impact of an online single session of fronto-temporal tDCS in a unique experiment by developing a simultaneous multimodal imaging approach (PET-MRI). The online implementation of the stimulation will allow deciphering changes induced during and after stimulation. As a first step, before investigating patients with schizophrenia, healthy subjects will be involved in the present study. The distributed changes will be explored at rest through: 1) Specific and localized dopaminergic transmission evaluated by positron emission tomography (PET) using dopaminergic D2 subtype receptor availability via [^{11}C]Raclopride binding. 2) Brain activity assessed by cerebral blood flow quantitatively and directly measured by arterial spin labelling (ASL). 3) Spontaneous functional connectivity assessed by functional magnetic resonance imagery (fMRI). 4) Connectivity assessed by diffusion tensor imaging (DTI). We hypothesized that fronto-temporal tDCS can modulate brain activity, connectivity and dopaminergic transmission during and after the stimulation.

In this thesis, I will report the spatial and temporal effects of fronto-temporal tDCS on dopaminergic subcortical structures (PET data) and on brain perfusion in local and interconnected regions (ASL data). Moreover, using a novel scanner is not without its challenges. Notably, concerning the PET analysis, we have detailed several preprocessing steps developed and optimized during my thesis such as the attenuation correction and the movement correction enabling a good quantification of our results. I have also detailed the analysis planned for the remaining parameters of interest (rs-fMRI; DTI) with the aim to finish these analyses after the thesis.

6.1.2. Materials

Subjects

Thirty-seven healthy adults were included. Exclusion criteria were smoking, history of neurological and/or psychiatric illness, medical treatments (except for oral contraceptive), contraindications to MRI or tDCS and pregnancy. All volunteers are right handed. Volunteers were asked not to have caffeine on day of scanning. Procedures were reviewed, approved by the standing ethics committee (CPP 2015-064B; ANSM 2015-A01281-48) and registered on ClinicalTrials.gov (NCT03056170). All subjects gave written informed consent after a detailed description of the study by the recruiting psychiatrist. Subjects were compensated 100 euros. Seven subjects were excluded due to technical problems. Thirty subjects (mean age = 25.67 ±2.57 years, n=15 females) completed the study.

Experimental design

This study was randomized, double blind and with 2-arm parallel groups, active (n=15) vs sham (n=15) fronto-temporal tDCS. The experiment visit at the CERMEP imaging center consisted in a PET-MR (fMRI, ASL, DTI) scan during which subjects received a single tDCS session (**Figure 1**). At baseline, subjects completed personality questionnaires: Life Orientation Test-Revised (LOT-R, Motivation, Big Five Inventory). During the experiment visit, subjects completed before and after the PET scan a State-Trait Anxiety Inventory (STAI-YA) and a structured adverse effect of tDCS questionnaire. Blinding integrity was assessed by having subjects guess the nature of the received stimulation (active or sham). Psychometric results are provided in **Table 1**.

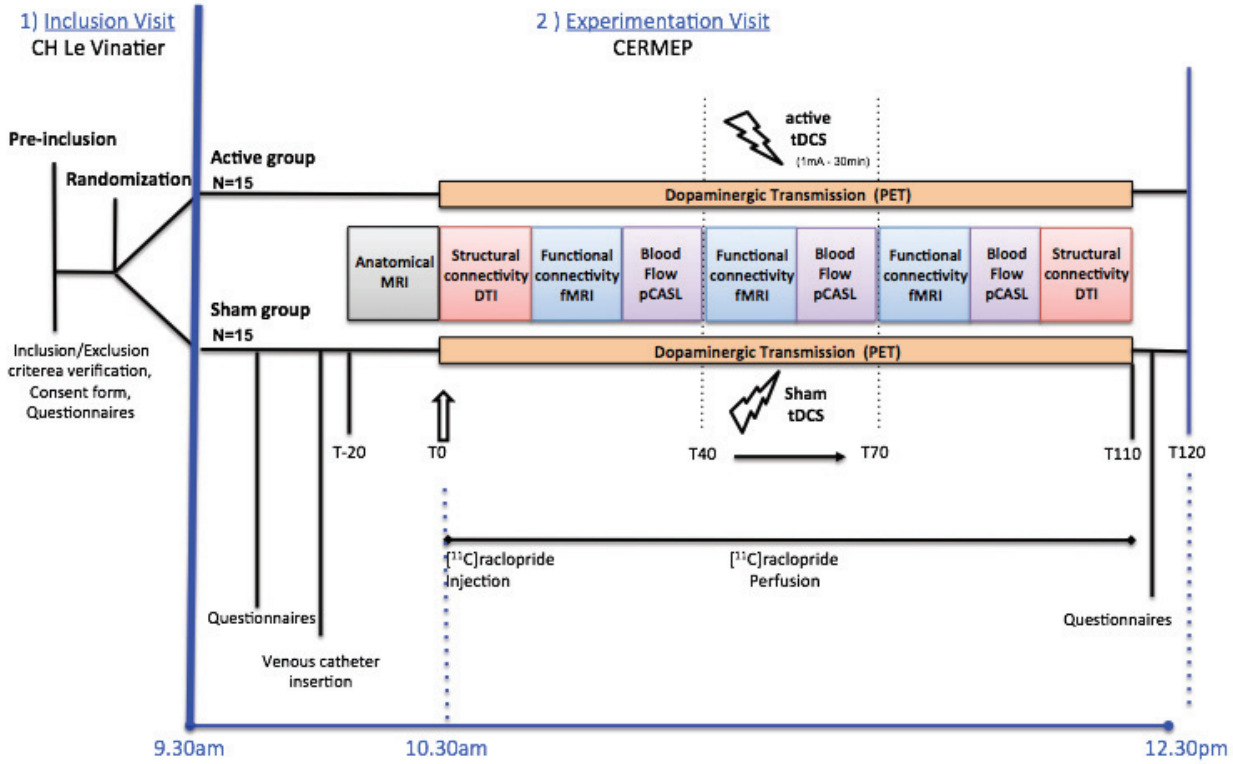


Figure 1: Experimental design - At rest, several imaging sequences have been acquired 1) Specific and localized dopaminergic transmission evaluated by PET using dopaminergic D2 subtype receptor availability via [^{11}C]raclopride binding; 2) Spontaneous functional connectivity assessed by fMRI acquired before, during and after the stimulation; 3) Brain activity assessed by cerebral blood flow quantitatively and directly measured by ASL acquired before, during and after the stimulation; 4) Connectivity assessed by DTI acquired before and after the stimulation

Transcranial direct current stimulation (tDCS)

tDCS was applied using a standard MR compatible equipment (NeuroConn DC-Stimulator Plus MR). The anode was placed with the center of the electrode midway between F3 and FP1 (left DLPFC) and the cathode was located midway between T3 and P3 (left TPJ), according to international 10/20 EEG electrode placement system (**Figure 2**). Electrode size was 7*5, 35cm^2. tDCS (either active of sham) was delivered at rest in a single session during a dynamic PET-MR scan. The stimulation started 40min after the injection

of the tracer, lasted 30min, with 30s fade in/fade out periods, and was set at 1mA in active mode. For sham stimulation, the built-in sham mode mimicked the somatosensory artifact of active tDCS (30s fade in/fade out, 1min of active tDCS delivered at the beginning of stimulation).

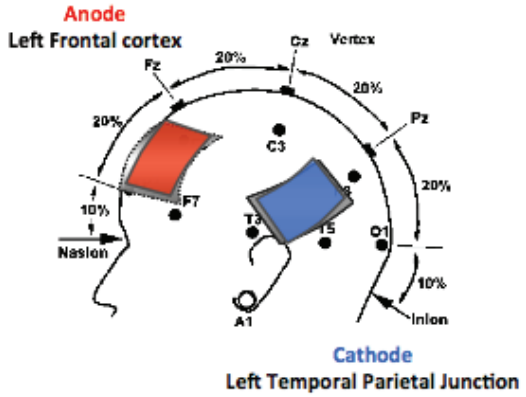


Figure 2: Electrode design - Frontal temporal tDCS - Anode (red) positioned over the left frontal cortex and the cathode (blue) over the left temporal parietal junction.

Anatomical MRI

All subjects underwent an anatomical MRI examination, including a 3D anatomic T1-weighted sequence covering the whole brain volume, with 1mm^3 cubic voxels and 176 1mm-thick slices (TR=2300ms, TE=2.34ms). This scan was done before the simultaneous PET-MR acquisition to control subject anatomy, electrode position, and was further used for spatial normalization and define regions of interest (ROI).

6.2. PET-MRI study

6.2.1. Subjects' characteristics

6.2.1.1. Methods - Statistical analysis

Demographic and social characteristics were examined using descriptive statistics. Analyses were performed using JASP and R studio. Assumption checks were conducted using Shapiro-Wilk test and Levene's equality of variance test. To assess the difference between groups (active and sham tDCS), independent t-tests were performed for age, injected dose/kg, years of education, motivation, LOT-R, BFI-N and STAI-difference. Chi2 tests were performed for handedness, gender and blinding integrity. When $p<0.05$, we considered the difference between groups to be significant.

6.2.1.2. Results

The subjects' characteristics (mean and standard deviation) are shown in **Table 1** for both active and sham groups. No statistical differences were found between the two groups. No adverse effects were reported either due to the tDCS stimulation, MR and PET scans.

Table 1 | Characteristics of subjects among the two groups			
Variable	**Active tDCS (N=15)**	**Sham tDCS (N=15)**	**P value**
Demographic			
Age [years]	25.00 (±2.07)	26.13(±2.94)	0.2238
Sex [Male:Female]	5 : 10	10 : 5	0.1441
Years of education [years]	4.87 (±1.96)	5.60 (±2.44)	0.28
tDCS			
Impedance	13.66 (±1.11)	13.63 (±0.77)	0.6524
Sensation [Yes:No]	7 : 8	8 : 7	1
Blinding [Active:None:Sham]	6 : 4 : 5	7 : 6 : 2	0.4142
PET			
Injected dose [MBq/kg]	4.77 (±0.58)	4.31 (±0.80)	0.116
Psychological assessment			
Motivation score	128.53(±17.08)	125.93(±20.17)	0.7087
LOT-R score	15.33(±3.96)	17.33(±4.27)	0.1753
BFI N score	20.66 (±6.59)	17.06 (±5.62)	0.1241
STAI-Y-A score difference	-0.20(±5.01)	0.66(±4.47)	0.9336

Table 1 - Characteristics of subjects among the two groups (active and sham tDCS; mean (±sd). Abbreviations: BFI N, Big Five Inventory Neuroticism; LOT-R, Life Orientation Test-Revised; PET, Positron Emisson Tomography; STAI-Y-A, State-Trait Anxiety Inventory (form Y-A, anxiety state); tDCS, transcranial Direct Current Stimulation. Welch two sample t-test or Wilcoxon rank sum test with continuity correction and the chi-square tests were conducted to assess group differences for continuous and discrete variables, respectively.

6.2.2. Positron Emission Tomography (PET)

6.2.2.1. Methods

PET scan session always started around 10.30am. During the 110min PET acquisition, subjects were lying at rest in the scanner.

6.2.2.1.1. Radiochemistry

Raclopride is a benzamide, a selective D2 receptor antagonist labeled with carbon-11, commonly used in PET studies (Hall et al., 1988). After synthesis at the CERMEP (1 synthesis per subject), [^{11}C]Raclopride was purified, formulated and sterilized.

6.2.2.1.2. Data acquisition

PET scans were conducted on the Biograph mMR PET-MR system (Siemens). Subjects were positioned in the scanner such that acquired planes would be parallel to the orbital-meatal line. A bolus of

[^{11}C]Raclopride (18MBq+2.6MBq/kg) for 30s followed by a constant infusion of 57% of the initial dose (i.e. 10MBq+1.5MBq/kg) over 110min, was injected through an intravenous catheter (see doses in **Table 1**). This bolus-plus-continuous-infusion method is currently used when measuring dopamine release in challenging conditions (Adler et al., 2000; Brunelin et al., 2011). A dynamic emission scan was acquired in list mode during the 110min after injection. A total of 21 successive frames (5min each) were reconstructed by using 3D-ordinary Poisson-ordered subset expectation maximization iterative algorithm incorporating point spread function using 12 iterations of 21 subsets and a zoom of 3 after correction for scatter and attenuation (Mérida et al., 2017). Gaussian post-reconstruction filtering (FWHM = 2mm) was applied to PET images. Reconstructed volumes consisted in 127 contiguous slices (2.03mm thickness) of 256×256 voxels (0.93x0.93mm^2). The 10 first seconds of PET acquisition, preceding the tracer injection, were excluded for PET image reconstruction. Each acquisition was visually inspected before any preprocessing.

6.2.2.1.3. Data preprocessing

Motion correction

In PET acquisition, head motion can lead to image blurring, inaccurate localization of structures and thus to errors in activity measures. To better account for between and intra frame motion, we used a novel approach to motion correction called EBER (Event-by-event rebinner motion correction) (Reilhac et al, 2017; PSMR and RITS, **Annexe 6**). This technique is based on the correction of the listmode data directly by rebinning the detected events according to the estimated inter-frame motion. For motion estimation, dynamic PET data were first reconstructed without attenuation correction in 63 frames of 100s each. Then the 63 motion correction matrices were applied to the listmode data, rebinned in sinograms of 21 regular 5-minute frames (**Figure 3**).

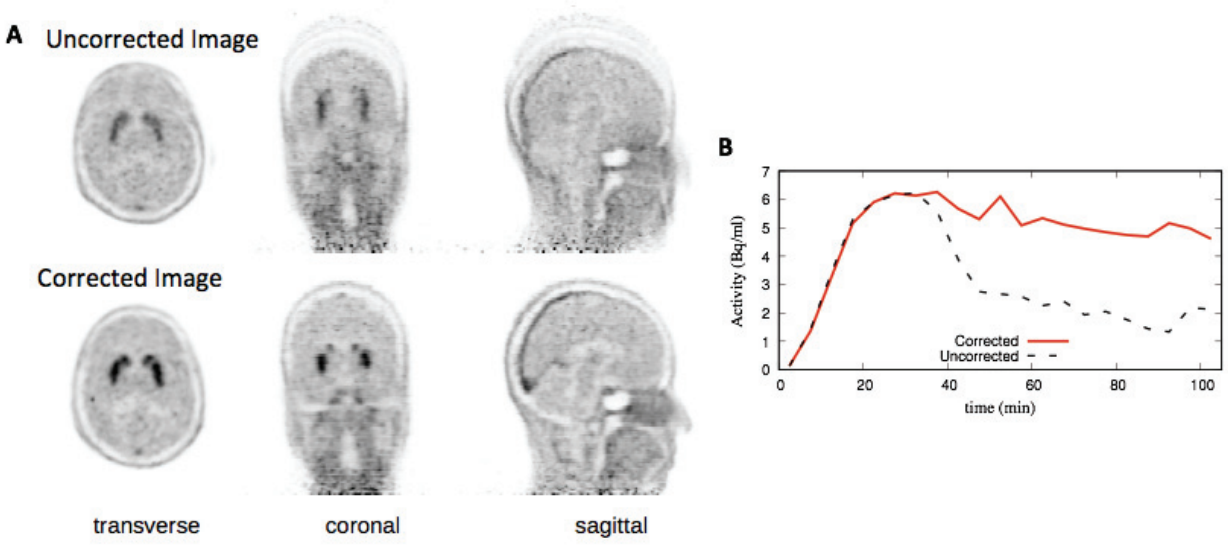


Figure 3: Motion correction with EBER – Example for one subject of the study - (**A**) Uncorrected and corrected mean images (**B**) Time activity curves extracted in the striatum from the uncorrected (black) or corrected dynamic (red) PET image. Adapted from A. Reilhac and Ines Merida

This approach is being developed in the CERMEP imaging facility. In order to help validate this technique, we have explored it with the results of this study, in addition to simulation studies. This has led to two conference presentations (oral and poster) (Merida et al, 2017, PSMR; Reilhac et al, 2017, RITS; **Annexes 6 and 8**)

Attenuation correction

Attenuation correction is important for the quantification as it corrects for photon attenuation by the tissues in PET studies. In classic PET/CT systems, attenuation correction can be done in a reproducible manner using the CT image. Unlike the PET/CT, PET/MR systems cannot provide CT images. Thus, an MR-based alternative to estimate the μ-maps has been implemented in the scanner by the vendor (UTE-based approach, Keereman et al., 2010). However, inaccurate MR-based attenuation maps, such as UTE-based μ-maps, can induce quantification errors (~10%) on dynamic PET data that depend on tracer distribution and also vary over time (Andersen et al., 2014; Ladefoged et al., 2015; Mérida et al., 2017). Thus, new methods have been developed in order to correct for photon attenuation. To date, MaxProb, based on multi atlas maximum probability attenuation correction, generates pseudo-CT images with high accuracy. This technique showed an enhanced sensitivity to detect physiological variations compared to the standard UTE approach, with fewer than 2% bias in quantification (Merida et al, 2015; Merida et al, 2017, Merida et al, 2017 BrainPET) (**Figure 4**).

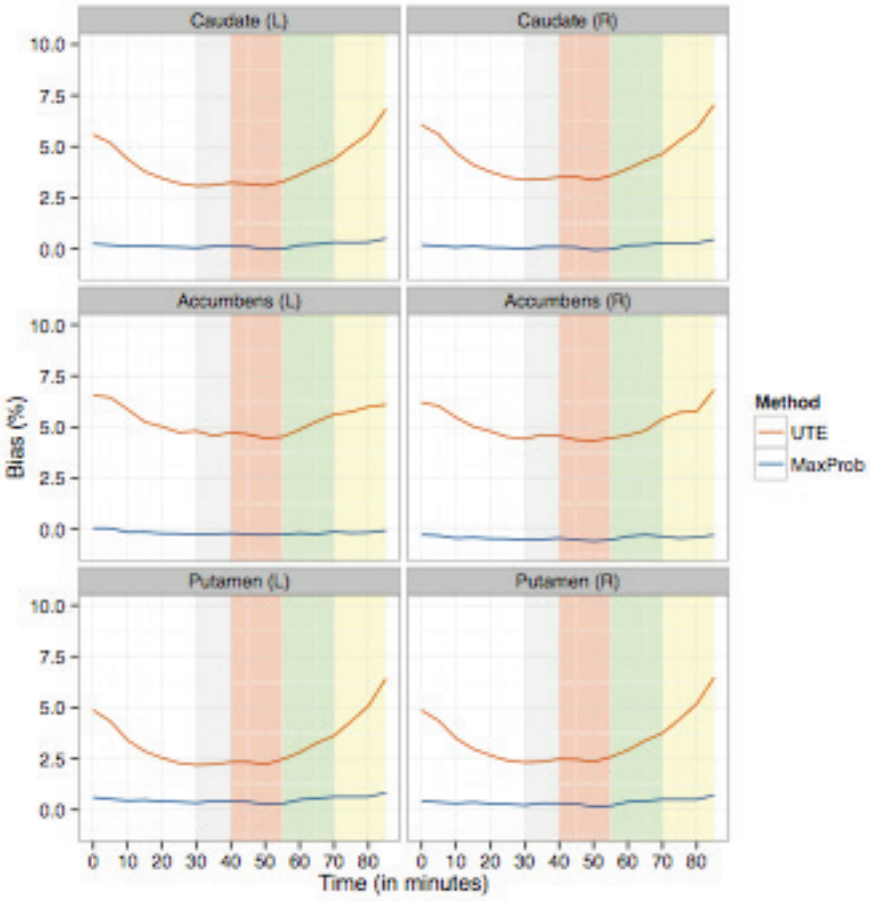


Figure 4: Attenuation correction - MR-based approaches - Mean bias (%) over time for the binding potential ratio in subregions of the striatum (caudate, accumbens and putamen) and in the reference region (cerebellum), in both hemispheres with 2 different attenuation correction methods: UTE (orange) and MaxProb (blue). Background colors indicate specific time periods: Grey: Baseline; Red: Stimulation1; Green: Stimulation2; Yellow: Post1. Adapted from Ines Merida

This approach is being developed in the CERMEP imaging facility. In order to help validate this technique, we have explored it with the results of this study, in addition to simulation studies. This has led to two conference presentations (oral and poster) (Merida et al, 2017, PSMR and BrainPET; **Annexes 7 and 8**).

Preprocessing

All preprocessing were carried out by a single individual blind to group status (active or sham). For each subject, PET and anatomical MRI preprocessing was done using an in-house script combining functions of Statistical Parametric Mapping 12 (SPM12, Wellcome Trust Centre of Neuroimaging), the MINC Tool Kit (McConnell brain Imaging centre, McGill university) and the Turku PET analysis software (Turku PET Centre). The realigned and corrected dynamic PET scan was used hereafter. T1 was coregistered to the mean PET image for each subject and then spatially normalized into standard MNI space (Montreal Neurological Institute/International Consortium for Brain Mapping stereotactic space) with the *segment*

function of SPM12. This step provided a classification of the T1 MRI into 6 tissue classes and the generation of MNI to subject space deformation fields. The atlas was then back normalized into the subject space, and combined with the grey matter image.

From these images, regions of interest (ROIs) were selected from the Hammersmith maximum probability brain atlas (Hammers et al., 2003; Gousias et al., 2008) (anatomical ROIs, cerebellum) and the Oxford-GSK-Imanova connectivity striatal atlas (functional ROIs). The functional subregions of the striatum (limbic, associative and sensorimotor) were chosen as ROIs according to the known dopamine D2 receptor specific binding. The assessment of free and nonspecific [^{11}C]Raclopride ligand kinetics was based on the time–activity curve of a reference region (i.e., the cerebellum, without vermis) devoid of specific dopamine D2-like receptors (Pinborg et al., 2007). These ROIs were used for subsequent analyses (**Figure 5**).

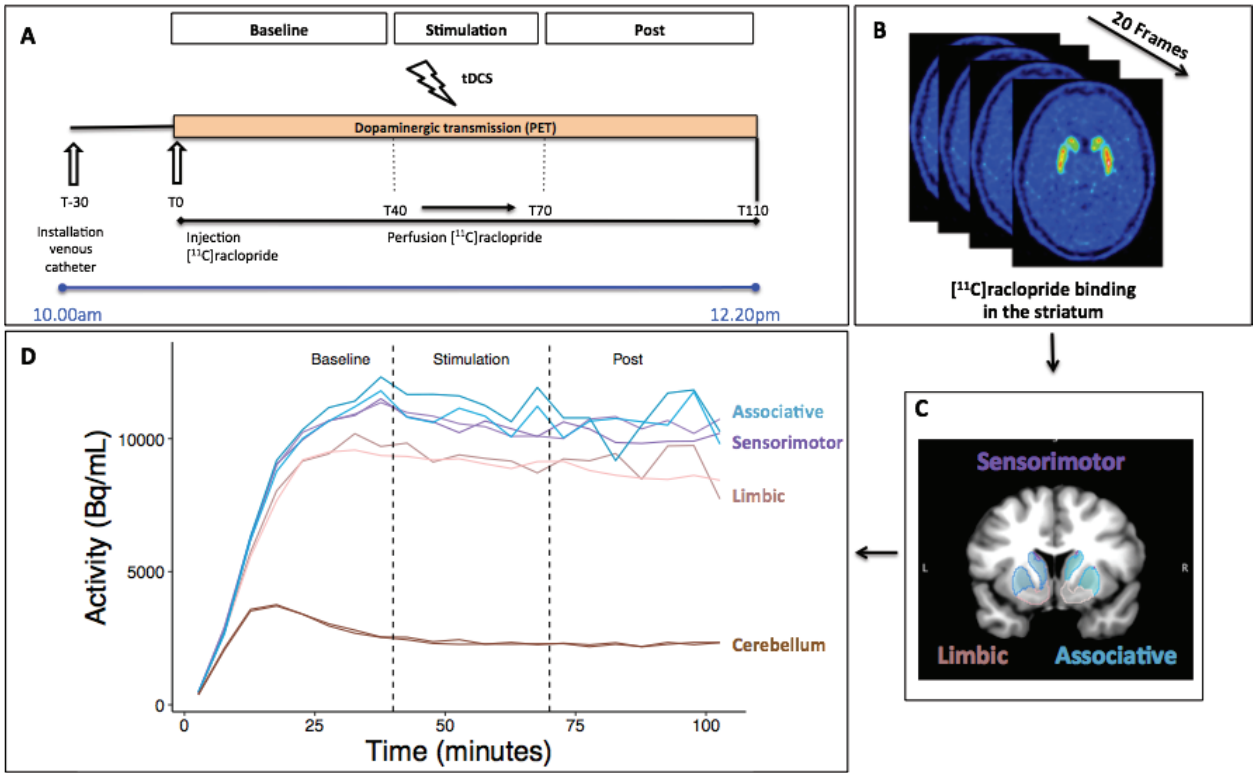


Figure 5: PET analysis - Example for one standard subject - (**A**) PET design (**B**) Dynamic PET reconstructed with a total of 20 successive frames (5min each), after motion and attenuation correction (**C**) Atlas used for the analyses: Combination of anatomical ROIs (cerebellum, in brown), using the Hammersmith maximum probability brain atlas and functional ROIs (limbic, in pink; associative in blue; sensorimotor, in purple), using the Oxford-GSK-Imanova connectivity striatal atlas. (**D**) Extraction of the time-activity curves in each ROI

6.2.2.2. Analysis - Results

6.2.2.2.1. Binding potential ratio (BP_R)

Principle

With the extraction of the time-activity curves (TACs) from the PET dynamic series in striatal subregions (limbic, associative/executive, sensorimotor) and in the cerebellum (grey matter excluding the vermis, considered as the reference region), we can estimate the changes in extracellular dopamine levels induced

by tDCS. To estimate the binding potential of the radiotracer, tissue-to-reference 5min ratios were computed (BP_R) with the *imgratio* function of the Turku PET library, for each striatal subregion, following the equation:

$$BP_R = \frac{Activity_{\text{Region of interest}}}{Activity_{\text{Cerebellum}}}$$

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

Results

From this extraction, we can observe a baseline problem in both groups. Indeed, when normalizing to the baseline, BP_R seems to increase continually during the stimulation period in the entire striatum and in both groups (**Figure 6**). Thus, with our results, this technique is not suitable to investigate the changes in BP_R linked to the stimulation effect.

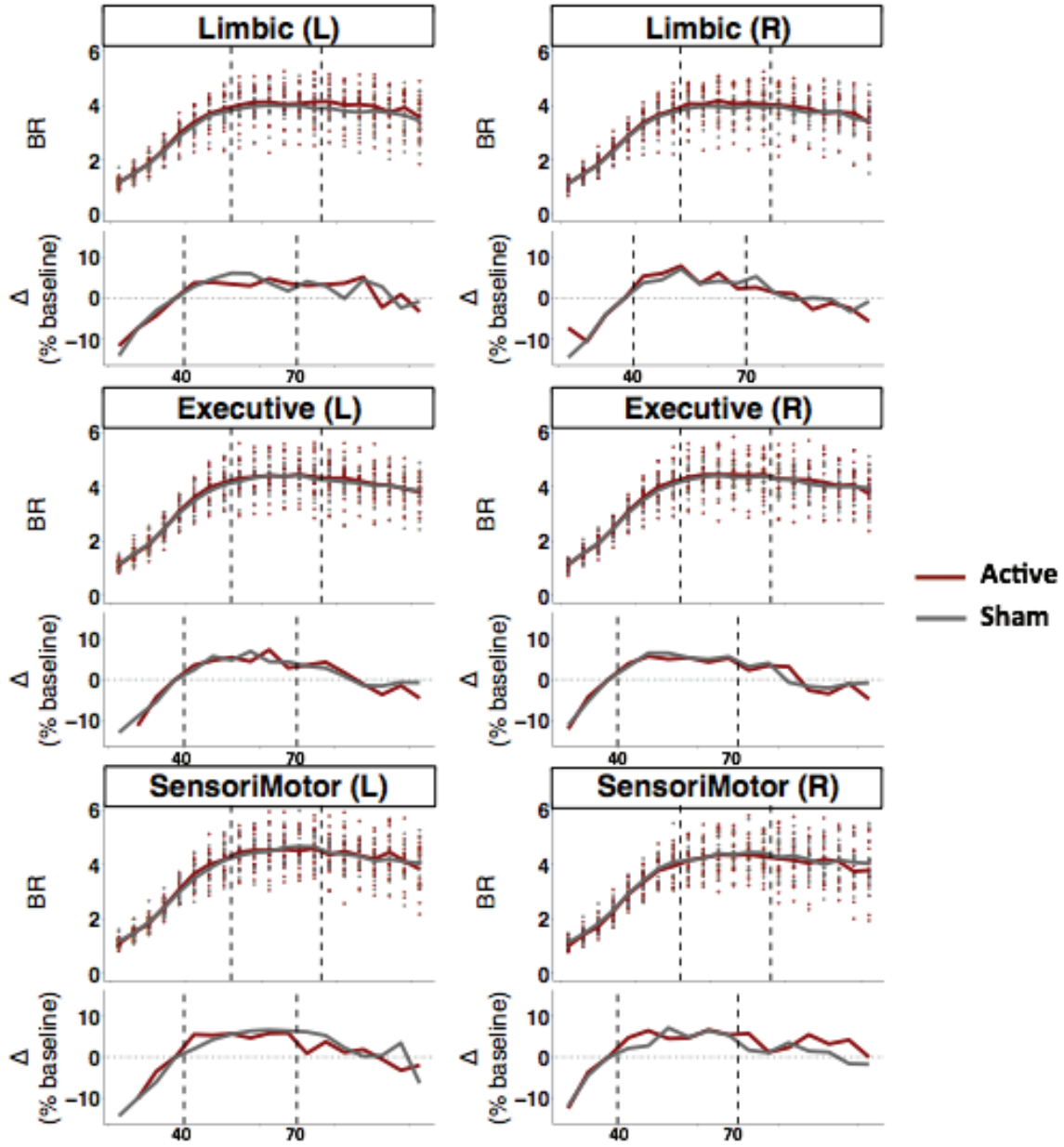


Figure 6: Binding potential ratio (BP_R) analysis - BP_R variations in each functional ROI of the striatum (Limbic, Executive, SensoriMotor) in both hemispheres and for both groups. For each ROI are presented the raw BP_R (top) and the normalized to baseline (%) BP_R (bottom). Dots represent the subject variability. Red: Active group; Grey: Sham group.

With this in mind, we turned to different analyses that would be less sensitive to the baseline equilibrium.

6.2.2.2.2. Logan plot

Principle

The Logan plot is an interesting method as is it robust and has fast computation time. In addition, it can either be used for regional analysis or at the individual voxel level for parametric analysis. This technique is based on the compartment model that uses linear regression to analyze pharmacokinetics of tracers

involving reversible uptake. As [^{11}C]Raclopride is a reversible tracer, we can use the multiple-time graphical analysis with reference region input (Logan plot) to determine the distribution volume ration (DVR) of the PET tracer. This has been validated for studies involving [^{11}C]Raclopride (Logan et al., 1996).
DVR is calculated by plotting Logan's equation and determining the slope of the regression. Indeed, DVR represents the ratio of specific (ROI) to non-specific (REF) binding of the radiotracer in tissue.

$$\frac{\int_0^t \mathrm{ROI}(t')dt'}{\mathrm{ROI}(t)} = \mathrm{DVR}\frac{\int_0^t \mathrm{REF}(t')dt'}{\mathrm{ROI}(t)} + b$$

From regional PET TACs, one can determine the DVR for different time periods of interest, using the *logan* function of the Turku PET library. The time intervals are based on the timing after the injection of the radiotracer and regroup 3 data points for the regression (i.e time period of 15minutes). The different periods of interest were determined as: Baseline (25-40min), Stim1 (40-55min), Stim2 (55-70min), Post1 (70-85min) and Post2 (85-100min). The Logan plots were drawn and controlled visually for linearity and quality of the data. Values below or above 3 times the interquartile range were considered outliers and removed from analysis (**Figure 7**).

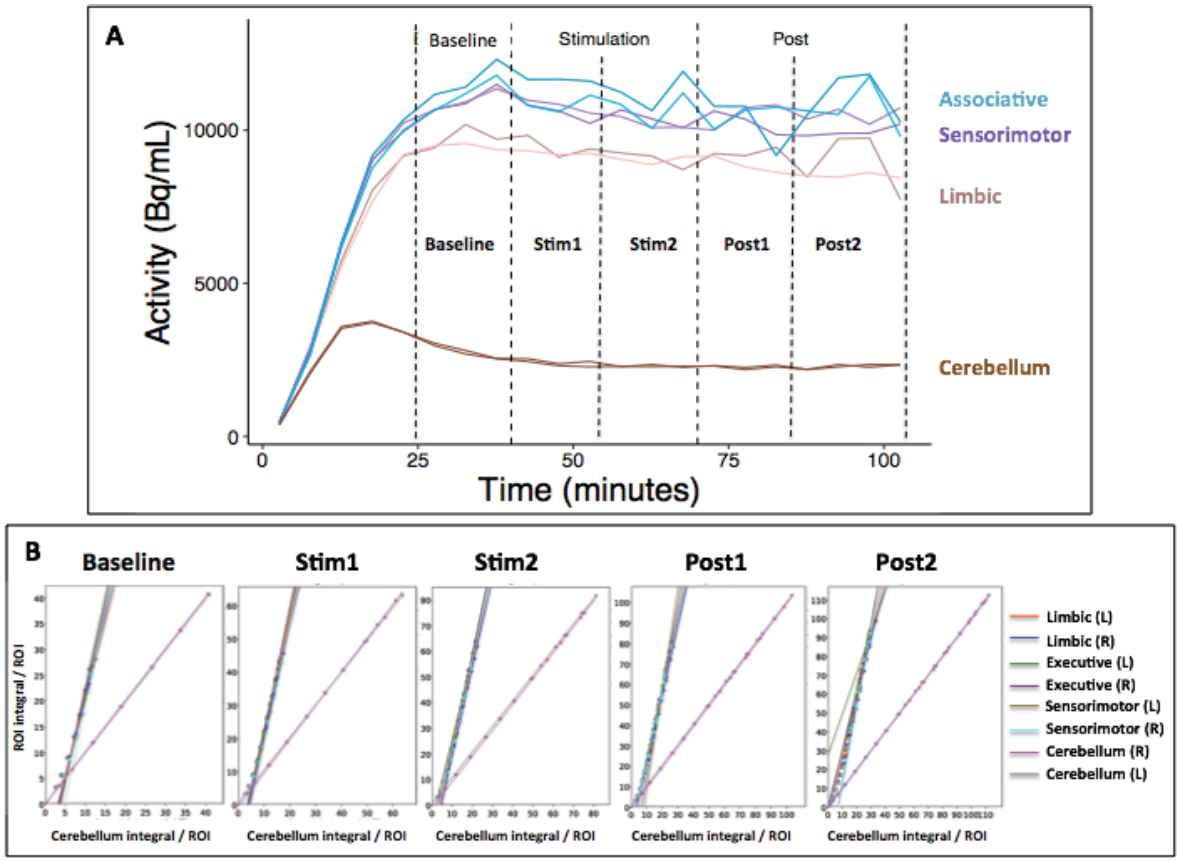


Figure 7: Logan plot analysis (**A**) Time periods of interest are positioned on the time-activity curves (TACs): Baseline (25-40min); Stim1 (40-55min); Stim2 (55-70min); Post1 (70-85min) and Post2 (85-100min). (**B**) Example of 5 Logan plots (1 per time period). The slope represents the DVR.

With this information, we can determine the non-displaceable binding potential (BP_{ND}), which describes how much radiotracer can potentially bind to its target (Innis et al 2007), with the equation:

$$BP_{ND} = DVR - 1.$$

To quantify dopamine displacement induced by tDCS we reported the relative variations of the BP_{ND} (□) for the 4 time periods: Stim1, Stim2, Post1, Post2; compared to Baseline period.

$$\boldsymbol{\Delta}(\%) = \frac{(BP_{ND})_{\text{time period}} - (BP_{ND})_{\text{baseline}}}{(BP_{ND})_{\text{baseline}}} \times 100$$

Statistical analysis

Regional analyses were performed on JASP and R studio. Intraregional mean and standard deviation of BP_{ND} were assessed for each time period in each region. A mixed model analysis of variance (ANOVA; time and group as factors) was performed, in each subregion of the striatum, with Bonferroni post-hoc tests for each time period intra and inter groups. When $p<0.05$, we considered the difference to be significant. The effect size is also reported with η^2_p for ANOVA and Cohen's d for post-hoc tests. Assumption checks were conducted prior to ANOVA, using Mauchly's W sphericity test and Levene's equality of variance test.

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

Results

From the multiple-time graphical analysis (Logan Plot), we extracted raw BP_{ND} values in each functional subregion of the striatum (limbic, executive and sensorimotor) for each time period (Baseline, Stim1, Stim2, Post1 and Post2). In order to quantitatively assess changes compared to the baseline period, we computed normalized BP_{ND} values (□ (%)). Both curves are reported in **Figure 8** for both groups.

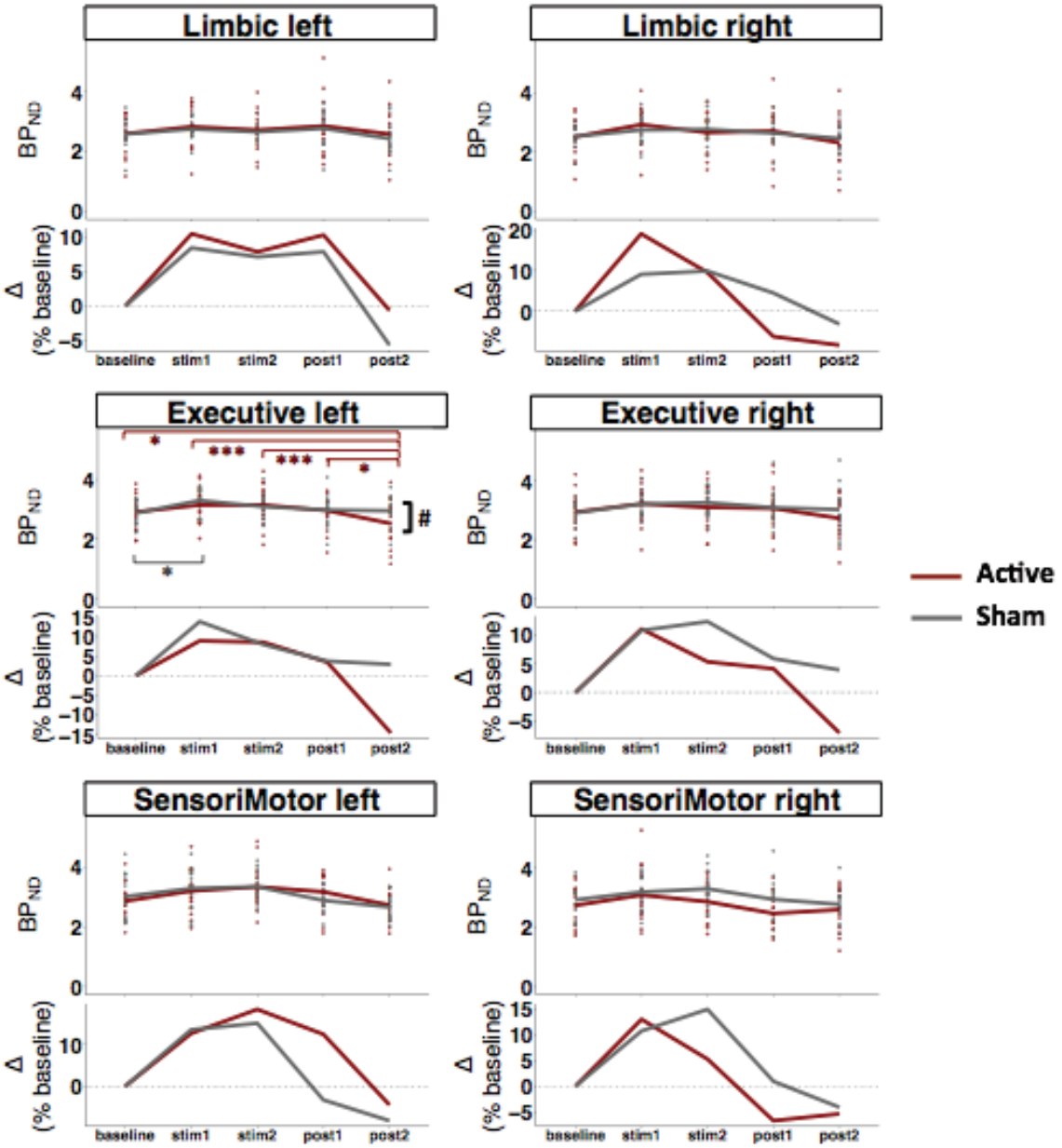


Figure 8: Logan Plot analysis - BP_{ND} - *Top*: Raw BP_{ND} values for each time point, in each subregion of the striatum. *Bottom*: Normalized BP_{ND} to baseline values. *Left*: Left hemisphere. *Right*: Right Hemisphere. N_{active}=14; N_{sham}=14. Values above or below 3*IQR were considered outliers and removed from analysis. Dots represent the subject variability. Red: Active group; Grey: Sham group. # p<0.01; * p<0.05; *** p<0.001

A significant effect was seen only in the left executive functional subregion of the striatum (*time effect*: $F_{(4,104)}$=11.787, p<0.001, η^2_p=0.312; *group effect*: $F_{(1,26)}$=1.130, p=0.298, η^2_p=0.042; *interaction*: $F_{(4,104)}$=4.37, p=0.003; η^2_p=0.144).

Effects during stimulation

No effects were seen in the active group during the stimulation period, may it be Stim1 or Stim2 period. However, intragroup analysis revealed significant BP_{ND} increase during the Stim1 period compared to the Baseline period (p_{bonf}=0.029, Cohen's d=0.976), in the sham group. No intergroup effects were seen.

Effects after stimulation

Intragroup analysis revealed a significant BP_{ND} diminution in the Post2 period, compared to Baseline (p_{bonf}=0.025, Cohen's d=0.999), Stim1 (p_{bonf}<0.001, Cohen's d=1.714), Stim2 (p_{bonf}=0.001, Cohen's d=1.431) and Post1 (p_{bonf}=0.016, Cohen's d=1.063) periods, in the active group. While there was no clear intergroup effect for the Post2 period (p_{bonf}=0.085, Cohen's d=0.652), no significant intragroup effect was reported in the sham group (at least p_{bonf}>0.5).

Table 2 | BP_{ND} variations

ROI /tDCS group	BP_{ND}, mean (±sd) Condition 1	Condition 2	Relative variation, %	Difference Active - Sham Group
Executive left				
	Baseline	Stimulation 1		
active tDCS	2.84 (±0.56)	3.08 (±0.58)	8.85 (±11.69)	-4.91
sham tDCS	2.92 (±0.35)	3.30 (±0.40)	13.76 (±14.38)	
	Baseline	Stimulation 2		
active tDCS	2.84 (±0.56)	3.09 (±0.69)	8.49 (±15.53)	0.59
sham tDCS	2.92 (±0.35)	3.13 (±0.40)	7.90 (±9.15)	
	Baseline	Post 1		
active tDCS	2.84 (±0.56)	2.96 (±0.76)	3.43 (±13.1)	-0.25
sham tDCS	2.92 (±0.35)	3.06 (±0.41)	3.68 (±11.36)	
	Baseline	Post 2		
active tDCS	2.84 (±0.56)	2.464 (±0.78)	-14.64 (±14.37)	-17.49
sham tDCS	2.92 (±0.35)	3.03 (±0.40)	2.85 (±12.99)	

Table 2: BP_{ND} variation in significant regions of the striatum - BP_{ND} variations between groups during (stim1 and stim2) and after (post1, post2) the stimulation, compared to the baseline, in significant regions, i.e left executive/associative subregion of the striatum. N_{active}=14; N_{sham}=14. Values above or below 3*IQR were considered outliers and removed from analysis.

However, several studies have highlighted that these conventional methods can reproduce sustained decreases in $BP_{ND,}$ but are found unreliable when quantifying short-lived variations in endogenous dopamine (Sullivan et al., 2013). Thus, other models are now been proposed, such as lp-nt PET.

6.2.2.2.3. Lp-nt PET

Principle

To observe highly localized transient dopamine changes, a new model has been developed: lp-nt PET (linear and parametric neurotransmitter PET; Normandin et al., 2012; Morris et al., 2013; Sullivan et al., 2013; Cosgrove et al., 2014). The principle of this model and the extension proposed by the CERMEP imaging center is presented in **Figure 9**.

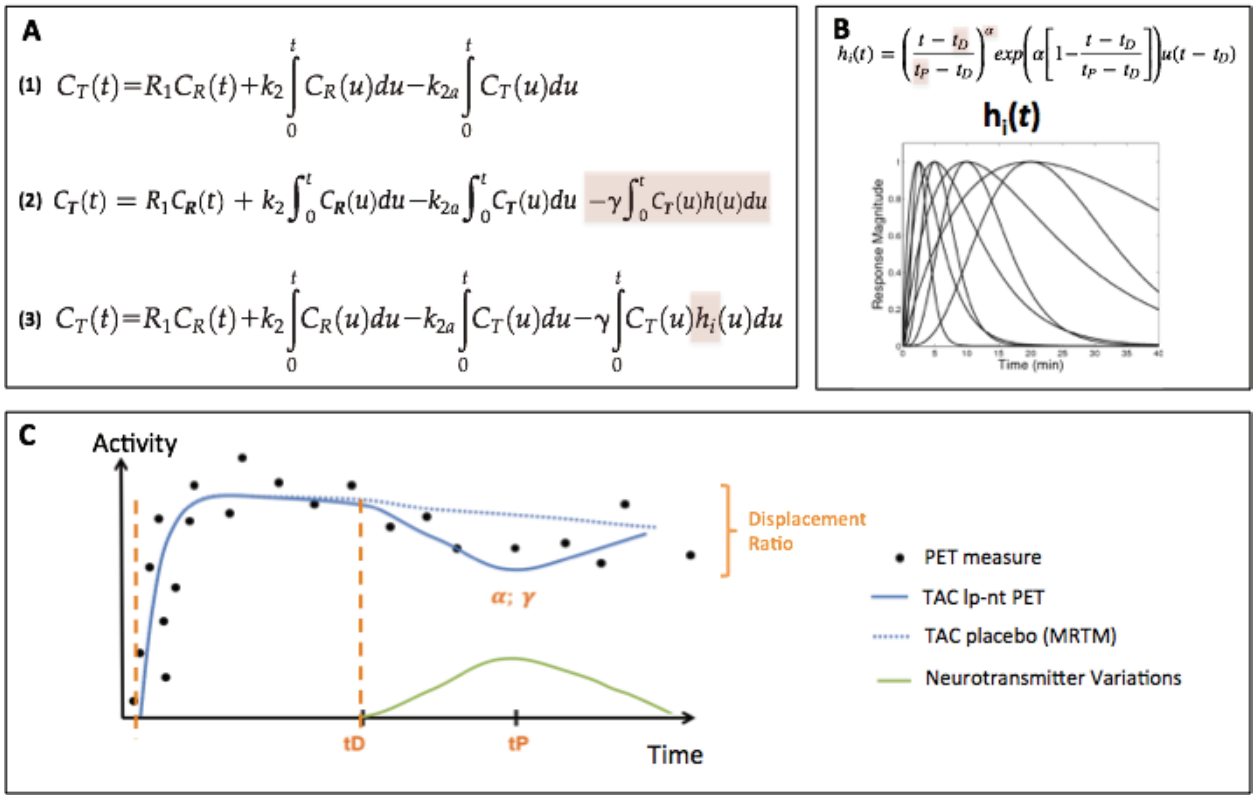


Figure 9: lp-nt PET Analysis - (**A**) Principle: The lp-nt PET technique is derived in three steps. (1) MRTM (Ichise et al., 2003); (2) LSSRM (Alpert et al., 2003), which adds the time varying dopamine component, however with a fixed function response (h); (3) lp-nt PET (Normandin et al., 2012) with h_i function which enables a flexible response function. (**B**) Response function: $h_i(t)$ represents the family of activating responses. The form depends on 3 parameters: alpha (α; sharpness of the responses), indeed, the higher the alpha the sharper the response; tD: expected interval including changes in dopaminergic transmission; tP: expected interval including the peak of the response. (**C**) Example of a parameter extraction - The model needs a time activity curve (TAC) in the ROI and reference region (cerebellum), as well as a set of response functions which will extract several parameters from this model (R1, k2, k2a, tD, tP, α, γ and displacement ratio). Adapted from Normandin et al, 2012 and Ines Merida

To assess the transient variations of dopamine in each ROI, three main steps are needed. The first step estimates several kinetic parameters (R1, k2, k2a) in a 2-step manner. This 2-step method estimates the parameters on the baseline period rather than on the entire TAC. The second step tests different combinations of {alpha, tD, tP} with a gamma estimation and selects the combination best fitted to the data. Finally, the third step measures a displacement ratio at specific time frames, which takes into account the placebo (MRTM) TAC and the lp-nt PET TAC, for each subject in each region of interest. This

displacement ratio is thought to be a macro-parameter and represents the release of dopamine in the ROI; i.e a positive displacement ratio suggests a increase in dopamine in the ROI.

$$\text{Displacement Ratio} = \frac{\int_{t_{tD}}^{t_{end}} C_{MRTM} - \int_{t_{tD}}^{t_{end}} C_{lp-ntPET}}{\int_{t_{tD}}^{t_{end}} C_{MRTM}} \times 100$$

Therefore, we focused on this parameter and measured this displacement ratio at specific time periods, for each subject in each region of interest.

Statistical analysis

Analyses were performed using JASP and R studio. Intraregional mean and standard deviation of the ratio parameter were assessed for each time period in each subregion of the striatum (limbic, executive and sensorimotor) of both hemispheres.

A first analysis was done using 1 interval to investigate the global impact of tDCS (Stimulation + Post periods; i.e 40 to 100 minutes). Independent t-tests were performed to compare active and sham groups, in each functional subregion of the striatum. When $p<0.05$, we considered the difference between groups to be significant. The effect size was also reported with Cohen's d. Assumption checks were conducted prior to t-test, using Shapiro-Wilk test and Levene's equality of variance test.

A second analysis was done to investigate the temporal impact of tDCS, using 5 intervals: Baseline (25-40min), Stim1 (40-55min), Stim2 (55-70min), Post1 (70-85min), Post2 (85-100min). A mixed model analysis of variance (ANOVA; time and group as factors) was performed, in each subregion of the striatum, with Bonferroni post-hoc tests for each time period intra and inter groups. Assumption checks were conducted prior to ANOVA, using Mauchly's W sphericity test and Levene's equality of variance test. When the sphericity was violated ($p<0.001$) Huynh-Feldt sphericity correction were applied.

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

Results

When exploring the general effect of the stimulation (40-100min period, including during and after stimulation effects), a near significant difference between both groups was observed in the left limbic striatal region ($p=0.067$; Cohen's $d=-0.708$) (**Figure 10**). No other subregions of the striatum showed a significant difference linked to tDCS.

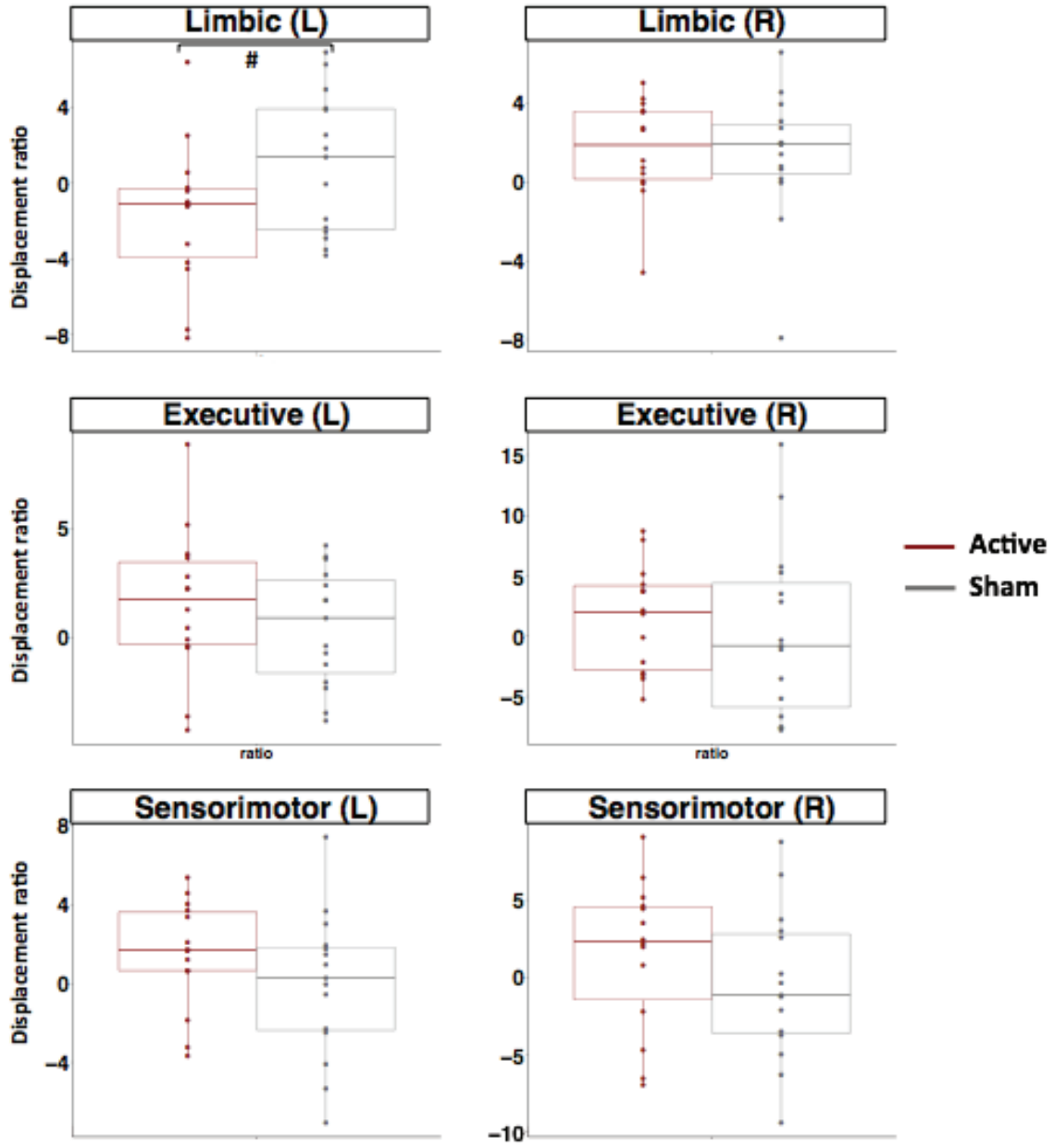


Figure 10: General effect of tDCS on the displacement ratio measured with lp-nt PET - Ratio parameter for the general time period including during and after the stimulation, in each subregion of the striatum. The only subregion showing a significant effect is the left limbic striatal area suggesting a transient dopamine change in this area. N_{active}=14; N_{sham}=15. Dots represent the subject variability. # p>0.01

When exploring the temporal tDCS effects, that is comparing 5 intervals (Baseline, Stim1, Stim2, Post1, Post2 periods), a significant difference between both groups was also observed in the left limbic striatal region (*time effect*: $F_{(1.674,45.195)}$=2.398, p=0.111, η^2_p=0.082; *group effect*: $F_{(1,27)}$=3.708, p=0.065, η^2_p=0.121; *interaction*: $F_{(1.674,45.195)}$=3.650, p=0.041; η^2_p=0.119). The difference between both active and sham groups is progressive and reaches significance during the Post2 period (p_{Bonf}=0.034; Cohen's d=-0.830). The ratio in the active group is reported as a decrease over time (*time effect*: $F_{(1.378,17.919,)}$=4.881, p=0.031,

η^2_p=0.273), and specifically significant between Stim1 and Stim2 period (p_{Bonf}=0.036; Cohen's d=0.949). No effect was reported in the sham group (*time effect*: $F_{(2.234,31.279)}$=0.848, p=0.449, η^2_p=0.057) (**Figure 11**). In addition, no other subregions of the striatum showed a significant difference linked to tDCS.

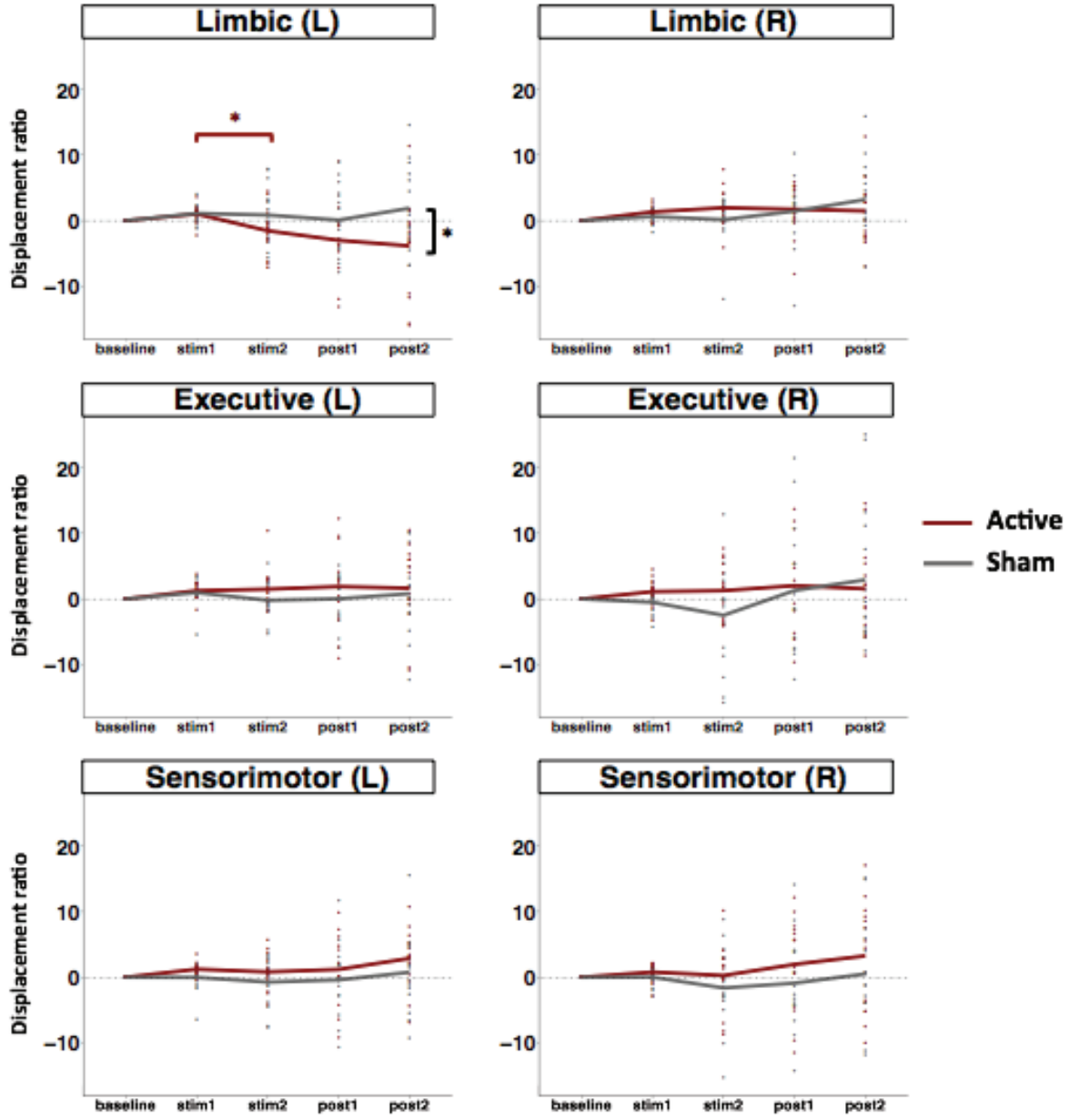


Figure 11: Temporal effect of tDCS on the displacement ratio measured with lp-nt PET - Ratio parameter for the 5 time periods (Baseline, Stim1, Stim2, Post1, Post2), in each subregion of the striatum. The only subregion showing a significant effect is the left limbic striatal area suggesting a transient dopamine change in this area. N_{active}=14; N_{sham}=15. Dots represent the subject variability. Red: Active group; Grey: Sham group. # p>0.01; * p>0.05; *** p>0.001

Table 3 | lp-nt PET variations					
		Displacement Ratio, mean (±sd)			
ROI /tDCS group		**Stim1**	**Stim2**	**Post1**	**Post2**
Limbic left					
	active tDCS	1.01 (±1.35)	-1.54 (±3.54)	-3.00 (±5.54)	-3.85 (±7.47)
	sham tDCS	1.10 (±1.33)	0.85 (±4.38)	0.09 (±5.30)	1.89 (±6.37)
	Difference	-0.09	-2.39	-3.09	-5.74

Table 3: Displacement ratio variation in the significant subregion of the striatum : the left limbic striatal area - Displacement ratio variations for each groups during (stim1 and stim2) and after (post1, post2) the stimulation, compared to the baseline, in significant regions, i.e left executive/associative subregion of the striatum. The difference between groups for each time period is also reported. N_{active}=14; N_{sham}=15.

6.2.2.3. Discussion

Here, we present the first direct evidence of temporally and spatially distributed effects of fronto-temporal tDCS on dopamine transmission in the striatum after one session of 30min at 1mA, in healthy subjects. The results show a decrease in [^{11}C]Raclopride BP_{ND}, using the Logan Plot approach, suggesting an increase in extracellular dopamine in the left executive region, induced by the tDCS session. The impact on dopamine transmission reaches statistical significance during the 15 to 30min period following the end of stimulation. However, this effect in the left executive region was not confirmed using the lp-nt PET approach. We only visually observe an increase, though not significant, in the left executive region. Moreover, preliminary results using the lp-nt PET approach, reported a significant and progressive decrease in displacement ratio suggesting a decrease in dopamine levels in the left limbic region. Based on the different nature of these approaches, it could suggest that tDCS impacts the dopaminergic transmission in a sustained manner in the left executive region, while in a more transient manner in the left limbic region. Nevertheless, the results presented here concerning the lp-nt PET approach are very preliminary as this is an ongoing project with the CERMEP. Indeed, one thing to keep in mind is that we are using the 2-step method of lp-nt PET to estimate the model parameters (i.e. estimation during the baseline period), thus a baseline not quite at equilibrium could influence the model. In the rest of this discussion, we will focus more thoroughly on our results obtained with the Logan plot approach.

The increase in dopamine in subparts of the striatum is in line with studies exploring NIBS impact on dopamine transmission in animals, healthy subjects and in pathological conditions (Ko and Strafella, 2012; Cirillo et al., 2017). Studies conducted offline showed modulations of dopamine transmission in the striatum after stimulation protocols applied over the prefrontal cortex. For example, an increase in extracellular dopamine specifically in the left dorsal caudate nucleus was shown after a repetitive TMS stimulation with the coil over the left DLPFC (Strafella et al., 2001). In the same line, an animal tDCS study reported an increase in dopamine concentration in the rat basal ganglia after cathodal tDCS compared to sham and anodal conditions. The effect was significant from 120min after the stimulation (Tanaka et al., 2013). These

results are also in accordance with those of another tDCS study, conducted by our lab, using a different frontal electrode montage, the bifrontal montage (anode over the left DLPFC and cathode over the right DLPFC) (Fonteneau et al, 2018).

The effect identified in our study is localized specifically in the executive part of the striatum. This spatially distributed after-effect of fronto-temporal tDCS is supported by the notion that complex organized behavior is made possible by the connectivity between several striato-thalamo-cortical circuits, including distinct striatal and cortical regions (Haber and Knutson, 2010; Tziortzi et al., 2014; Haber, 2016). This striatal subregion includes the ventral rostral putamen, dorsal caudate, superior ventral striatum corresponding to the ventral caudate. In addition, connectivity studies, which have traced the structural and functional coupling between individual striatal and cortical regions, have shown that the DLPFC projects extensively to the ventral striatum, an overlap between regions involved in affective and executive processes, corresponding to the reward/motivation network. According to these studies, our cluster is located in the executive of the striatum, region anatomically linked to the DLPFC targeted by the tDCS montage. Thus, the effect of fronto-temporal tDCS on the dopaminergic transmission may be due to the stimulation of the DLPFC. This is supported by the fact that we observe dopamine changes in the same region as the one obtained after bifrontal tDCS (Fonteneau et al, 2018). However, the TPJ could also contribute to those effects. Indeed, localized at the intersection of the posterior temporal sulcus, inferior parietal lobule and lateral occipital cortex, it is thought to be linked functionally to subcortical structures such as the striatum via its involvement in the resting-state networks (Mars et al., 2012). Furthermore, the lateralized effect in the left hemisphere seen in our study is in line with a recent computational study reporting that our fronto-temporal tDCS montage exerts lower peak electric field strengths in right-sided AVH-ROIs (Lee et al., 2018).

Our findings of an increase in extracellular dopamine in the executive striatum after a single session of fronto-temporal tDCS, should be put into perspective relative to the physiopathology of schizophrenia. Indeed, this fronto-temporal montage has been developed in order to specifically alleviate positive symptoms in patients with schizophrenia, such as auditory verbal hallucinations (AVH) (Brunelin et al., 2012; Bose et al., 2017). It was based on studies investigating the AVH in patients with schizophrenia which reported an increased functional connectivity between the TPJ and striatal regions (Hoffman and Hampson, 2012; Sorg et al., 2013; Alderson-Day et al., 2015; Rolland et al., 2015), possibly indirectly inducing aberrant salience processes, which would alter sensory integration (Heinz and Schlagenhauf, 2010). Moreover, most studies in patients with schizophrenia report a dysregulation from afferent structures such as a hippocampal hyperactivity induced possibly by stress. This hyperactive afferent could lead to an increase in tonic firing of dopaminergic neurons of the VTA reflecting a hyper-responsive dopaminergic system (Grace, 2016). To date, most antipsychotic drugs block the dopaminergic system, notably the D2 receptors.
This could appear contradictory to our result showing an increase in dopamine levels in the associative part of the striatum after tDCS. However, several hypotheses could be formulated to explain this discrepancy.

One thing to keep in mind is that both antipsychotics drugs and fronto-temporal tDCS do not have an immediate clinical effect but need several weeks of exposure in order to obtain a clinical improvement. Here, we only investigated an immediate effect of a single session of fronto-temporal tDCS in healthy subjects without any ongoing medication. Thus, our acute effect could be thought of as contradictory with the physiopathology of schizophrenia but maybe repeated tDCS would have a different impact notably involving adaptive downstream mechanisms, such as changes in dopamine receptor expression or neurotrophic factors-associated plasticity (Gershon et al., 2007; Fritsch et al., 2010). Another hypothesis, concerning the increase of dopamine reported after tDCS possibly reflecting a decrease in symptoms, could be that those changes in basal extracellular level induce changes in tonic versus phasic dopamine signaling mode, notably perhaps by the recruitment of dopamine D2 autoreceptors (Grace, 1991; Suaud-Chagny, 2004; Brunelin et al., 2013). In turn, this could perhaps decrease the hyper-reactive state seen in patients with schizophrenia. Furthermore, clinical studies deliver fronto-temporal tDCS concurrently to antipsychotic medication. Thus, perhaps this combination helps lead to decrease reactivity and improve positive symptoms. In this line, a recent study reported that the affinity of the antipsychotic drug could predict tDCS effects. More specifically, patients with a high affinity antipsychotic showed less clinical improvement after add-on tDCS compared to patients with medication with lower affinity (Agarwal et al., 2016). Another possibility could be that the dopamine release observed here may be linked to the improvement of cognitive symptoms of schizophrenia rather that the positive symptoms also reported after fronto-temporal tDCS (Brunelin et al., 2012). Thus, the decrease of positive symptoms could be due to modifications of other brain areas.

On another front, the significant after-effects of fronto-temporal tDCS beg the question of possible mechanisms leading to the dopamine release in the striatum. The literature supports two possible mechanisms: a direct pathway, via voltage dependent calcium channels in the striatum (Christie et al., 2011; Das et al., 2016) or an indirect pathway, involving glutamatergic and GABAergic cortical projections on mesostriatal dopamine neurons in the midbrain (Taber and Fibiger, 1995; Stagg and Nitsche, 2011; Hone-Blanchet et al., 2016; Peanlikhit et al., 2017). We acknowledge that our study did not allow for an evaluation of the tDCS effects on dopamine release in the cortex. Interestingly, using a high affinity radioligand ([^{11}C]FLB-457), Cho and collaborators have shown that TMS over the left DLPFC induces a reduction of BP in the ipsilateral pre and subgenual anterior cingulate cortex, and medial orbitofrontal cortex (Cho and Strafella, 2009). Thus, further studies are needed to evaluate the direct effect of tDCS on the frontal cortex.

To conclude, the present study provides the first evidence that fronto-temporal tDCS induces neurotransmitter release in polysynaptic connected subcortical areas. Therefore, levels of dopamine activity and dopamine reactivity should be a new element to consider for a general hypothesis of brain modulation by fronto-temporal tDCS.

6.2.3. Arterial spin labeling (ASL)

6.2.3.1. Methods

During the 110min of PET acquisition, three ASL scans were done: 1 before the stimulation (Baseline period; 30-36min after tracer injection), 1 during the stimulation (Stimulation period; 60-66min after tracer injection) and 1 after the end of the stimulation (Post period; 91-97min after tracer injection). The times of scans were the same across subjects. During the three scans, subjects were lying at rest in the machine with their eyes fixated on a cross (**Figure 12 A**).

Data acquisition

Pseudocontinuous ASL was acquired on the Biograph mMR PET-MR system (Siemens; 3T MRI), using a 12-channel head coil. Head stabilization was achieved with cushioning, and all participants wore earplugs to attenuate noise. The ASL sequence used GRAPPA (with a factor of 2) and applied a gradient-echo EPI readout (TR, 4000ms; TE, 12ms). Twenty-two axial slices were acquired in interleaved (multislice) order ($3.4*3.4*5mm^3$ voxels) with distance factor of 20% to ensure whole brain coverage. The labeling slice was placed for each subject at 7 cm inferior to the center of the axial slice (label offset 70mm). Each labeling pulse lasted 1.5s and was followed by a post-labeling delay of 1.5s. Each ASL scan required approximately 5min and the acquisition of a reference image (M0) needed for the CBF quantification added an additional minute to the acquisition. In total, each ASL image was composed of 90 volumes (45 control and 45 tag volumes). The M0 image was acquired with a post-labeling delay of 8s, TR=12000ms, TE=12ms and was composed of 4 volumes. Each acquisition was visually inspected before any preprocessing.

Data preprocessing and quantitative analysis

All preprocessing were carried out by a single individual blind to group status (active or sham). For each subject, ASL and anatomical MRI preprocessing was done using an in-house script combining functions of the ASL perfusion fMRI data processing toolbox (ASLtbx; http://www.cfn.upenn.edu; Wang et al, 2008) and Statistical Parametric Mapping 12 (SPM12, Wellcome Trust Centre of Neuroimaging). All functional images (ASL and M0) were corrected for between-frame motion with a 3D rigid body model (Wang, 2012). These realigned images were used hereafter. T1 and functional images were coregistered to the mean ASL at baseline for each subject. T1 image was segmented with the *batch_nsegment* in the ASLtbx. This step provided a classification of the T1 MRI into 3 tissue classes: the grey matter, white matter and CSF images. These three images were used to create a subject specific brainmask using *imcalc* in SPM12. This mask was used for the next steps leading to CBF quantification. Denoising included temporal filtering using a high-pass Butterworth filter (cutoff frequency of 0.04Hz), temporal nuisance cleaning, to regress out from functional images at each voxel, head motion (3 translations and 3 rotations), global signal, white matter signal and CSF signal time courses (Wang, 2012), as well as a spatial smoothing with a Gaussian kernel of 6mm full-width at half-maximum, using *batch_filtering* and *batch_smooth* in ASLtbx. Using these preprocessed images, a quantitative analysis was done using *batch_perf_substract* in ASLtbx to calculate

perfusion and CBF signals, for each subject and for each session (Baseline, Stimulation, Post). The detailed model parameters can be found in Wang and colleagues (2008). These perfusion images were corrected for partial volume effects using *batch_pve_corr* in ASLtbx and then spatially normalized into standard MNI space (Montreal Neurological Institute/International Consortium for Brain Mapping stereotactic space) with *batch_norm_spm12* in ASLtbx as well as a spatially smoothed with a Gaussian kernel of 6mm full-width at half-maximum, using *batch_smooth* in ASLtbx (**Figure 12 B**).

From these images, two complementary approaches were performed. First, a whole brain analysis using the Hammersmith maximum probability brain atlas (Hammers et al., 2003; Gousias et al., 2008). Second, regional CBF quantification using different regions of interest (ROIs) selected in the regions targeted by the stimulation (left DLPFC and left TPJ). The left DLPFC ROI was determined using the Sallet atlas (Sallet et al., 2013), which combined three different areas: area 46/9 dorsal, area 9 and area 10. The left TPJ ROI was determined using the Mars atlas (Mars et al., 2012). To assess only CBF quantification in grey matter, theses atlases were combined with the grey matter image. For whole brain analysis, this combination was done with the standard grey matter template in the MNI space. For regional analysis, the atlases were back normalized into the subject space and then combined to individual subject's grey matter image. Theses atlases were used for subsequent analyses (**Figure 12 C**).

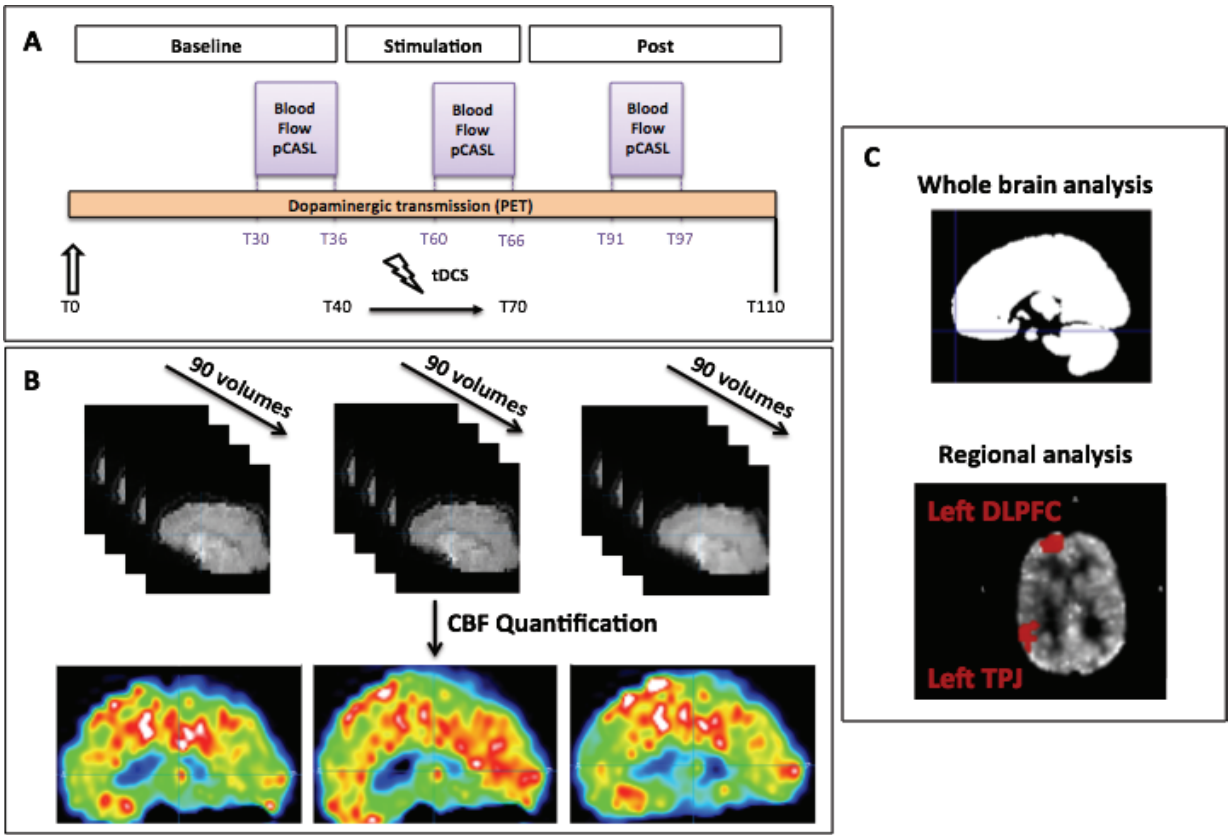


Figure 12: ASL analysis - Example for one standard subject - (**A**) ASL Design (**B**) ASL preprocessing (90 volumes: 45 control and 45 tag volumes) into CBF quantification (45 volumes into 1 mean CBF image used for parametric and regional analysis) (**C**) Atlas used for the analyses. Whole brain analysis used the binarized Hammersmith maximum probability brain atlas as mask. Region analyses used different regions of interest: Left DLPFC and Left TPJ.

Statistical analysis

A first analysis was done to investigate the spatial and temporal impact of tDCS in the whole brain. A voxel-based SPM analysis was performed, using a flexible factorial design based on repeated measure ANOVA, to assess the effect of active tDCS compared with sham tDCS on CBF signal for and across each time period: Baseline (30-36min), Stimulation (60-66min; effects during the stimulation) and Post (91-97min; after-effects of the stimulation). This analysis was done using a whole brain mask (Hammersmith maximum probability brain atlas). Post-hoc Student t-score (SPM-{t}) maps were computed to elucidate the increase or decrease in CBF during (Stimulation) or after (Post) tDCS, by comparing both groups. The following contrasts were computed: [(Stimulation - Baseline)$_{sham}$ vs (Stimulation - Baseline)$_{active}$], [(Post - Baseline)$_{sham}$ vs (Post - Baseline)$_{active}$], [(Post - Stimulation)$_{sham}$ vs (Post - Stimulation)$_{active}$]. SPM maps were thresholded at $P_{uncorr} < 0.001$ at the voxel level, with a minimum of 69 contiguous voxels (3988 mm^3), which is the expected number of voxels per cluster in the 3D Gaussian space. Then, only clusters with $P_{FWE} < 0.05$ (SPM Family wise error correction for multiple comparisons) at the cluster level were considered significant. Reported coordinates (**TABLE 4**) conform to the MNI space.

A second analysis was done to investigate the temporal impact of tDCS in a-priori ROIs; i.e regions under the stimulating electrodes (left DLPFC and left TPJ), using the same time intervals as in the whole brain analysis. Analyses were performed using JASP and R studio. Intraregional mean and standard deviation of CBF were assessed for each time period in each ROI. A mixed model analysis of variance (ANOVA; time and group as factors) was performed, in each ROI, with Bonferroni correction on *post-hoc* t-tests for each time period intra and inter groups. Assumption checks were conducted prior to ANOVA, using Mauchly's W sphericity test and Levene's equality of variance test. As the sphericity was violated ($p<0.001$) Huynh-Feldt sphericity correction was applied.

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

6.2.3.2. Results

Whole brain - Parametric analysis

Using the whole brain mask, the voxel-based analysis showed significant clusters when comparing the time periods determined between groups (**TABLE 4**). More specifically, when comparing active and sham tDCS groups, areas of significant changes showed CBF decreases after the end of the stimulation (**Figure 13**). After the complete analysis, the position of the significant clusters has been identified according to the anatomical delineation of Hammersmith maximum probability brain atlas. The CBF in clusters and their relative difference in the active tDCS group compared to the sham group are summarized in **TABLE 5**.

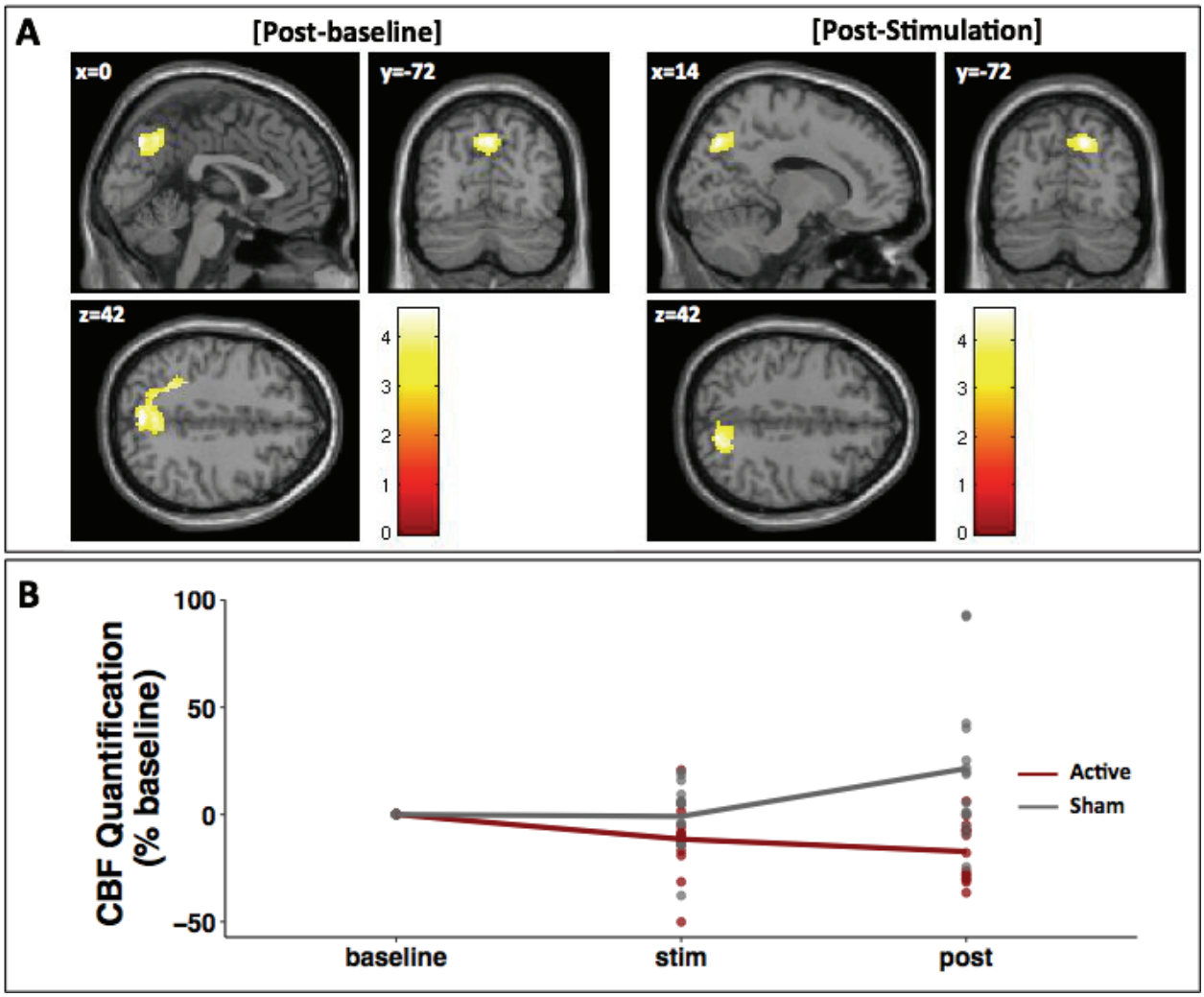


Figure 13- Whole brain parametric analysis - CBF quantification - Brain perfusion changes during and after the stimulation are reported here when comparing the active and the sham group. Clusters shown here are corrected with $P_{uncorr} < 0.001$ at the voxel level, with a minimum of 69 contiguous voxels (3988 mm^3) and with $P_{FWE} < 0.05$ at the cluster level. (**A**) Regions of decreased perfusion after fronto-temporal tDCS, when comparing the Post period to the Baseline period (*Left*) Regions of decreased perfusion after fronto-temporal tDCS, when comparing the Post period to the Stimulation period (*Right*). No variations are reported during the stimulation compared to baseline. (**B**) CBF values were extracted in these significant clusters and expressed as the relative variations compared to baseline (%). As the clusters from both contrasts overlap, the CBF quantification values were only shown here for the first cluster [Post-Baseline]. Dots represent the subject variability. $N_{active}=15$; $N_{sham}=15$. Red: Active group; Grey: Sham group.

Effects of tDCS during the stimulation (Stimulation period)

No significant differences in CBF were observed between groups in the whole brain when comparing stimulation and baseline periods [$(Stimulation-Baseline)_{sham}$ vs $(Stimulation-Baseline)_{active}$].

After effects of tDCS (Post period)

Significant decrease in CBF was reported in the active group compared to sham group when comparing the Baseline period with the Post period [$(Post\text{-}Baseline)_{sham}$ vs $(Post\text{-}Baseline)_{active}$], specifically in a part of the superior parietal gyrus (Active group: -17.29% (±13.49); Sham group: 21.42%(±34.13)).

Accordingly, similar decrease in CBF was observed in the active group compared to sham group when comparing the Stimulation period with the Post period [$(Post\text{-}Stimulation)_{sham}$ vs $(Post\text{-}Stimulation)_{active}$], specifically in a part of the superior parietal gyrus (Active group: -12.91% (±17.49); Sham group: 26.49%(±33.62)).

Table 4 | Voxel-based Analysis: CBF Quantification

		MNI coordinates (mm)				Cluster	
Contrast/Region		**x**	**y**	**z**	**Z-score**	**P_{FWE}**	**k**
Post - Baseline							
	Superior Parietal Gyrus	0	-72	42	4.20	0.004	894
		2	-62	42	3.87		
		-26	-48	44	3.83		
		18	-64	46	3.89	0.404	136
		30	-58	50	3.37		
Post - Stimulation							
	Superior Parietal Gyrus	14	-72	42	4.26	0.039	490
		18	-64	44	3.94		
		24	-66	34	3.48		

Table 4 - Clusters of the voxel-based analysis. Effect of tDCS on CBF in the whole brain using a flexible factorial design (time periods*groups). Clusters were considered significant with $P_{uncorr}<0.001$ uncorrected for multiple comparisons and a minimum of 69 contiguous voxels (3988 mm^3) at the voxel level and with multiple comparisons (P_{FWE})<0.05 at the cluster level. We reported the z-scores at the peak level, the P_{FWE} at the cluster level and the number of contiguous voxels (k). The clusters reported here are from the contrast [Sham tDCS>Active tDCS] and with $P_{uncorr}<0.001$ and k>69 contiguous voxels. No significant clusters were reported with the contrast [Sham tDCS<Active tDCS].

Regional analysis

Using the specific ROI mask under the anode (left DLPFC) and cathode (left TPJ) electrode location, we extracted the CBF quantification for each subject. When comparing active and sham tDCS groups, no change in CBF quantification was observed either under the left DLPFC (*time effect*: $F_{(1,19)}=0.056$, p=0.816, $\eta^2_p=0.003$; *group effect*: $F_{(1,19)}=0.263$, p=0.614, $\eta^2_p=0.014$; *interaction*: $F_{(1,19)}=2.858$, p=0.107, $\eta^2_p=0.131$) or the left TPJ (*time effect*: $F_{(1,19)}=0.620$, p=0.441, $\eta^2_p=0.032$; *group effect*: $F_{(1,19)}=0.258$, p=0.618, $\eta^2_p=0.013$; *interaction*: $F_{(1,19)}=0.201$, p=0.659, $\eta^2_p=0.032$) (**Figure 14**). The CBF values and their relative difference in the active tDCS group compared to the sham group are summarized in **TABLE 5**.

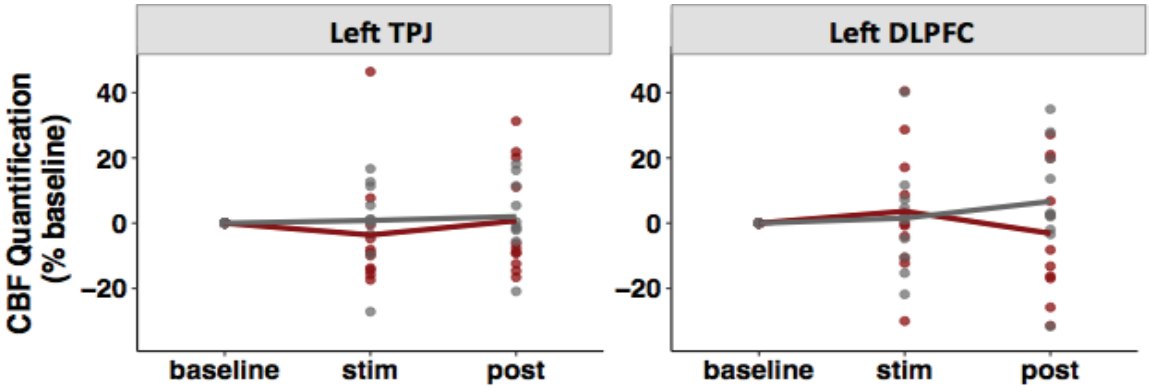


Figure 14 - Regional analysis - CBF quantification - CBF values were extracted in both ROIs located under the stimulating electrode (anode, left DLPFC; cathode, left TPJ) and expressed as the relative variations compared to baseline (%). N_{active}=11; N_{sham}=10. Values above or below 1.5*IQR and under 0 were considered outliers and removed from analysis. Dots represents the subject variability. Red: Active group; Grey: Sham group.

Table 5 | CBF variations

ROI /tDCS group	CBF quantification (mL/100g/min), mean (±sd) Condition 1	Condition 2	Relative variation, %	Difference Active - Sham Group
Superior parietal gyrus				
	Baseline	Post		
active tDCS	66.23 (±19.77)	54.31 (±16.91)	-17.29 (±13.49)	-38.71
sham tDCS	52.14 (±16.09)	60.59 (±17.45)	21.42 (±34.13)	
	Stimulation	Post		
active tDCS	57.20 (±19.12)	48.97 (±17.67)	-12.91 (±17.49)	-39.40
sham tDCS	45.85 (±12.07)	56.08 (±13.69)	26.49 (±33.62)	
Left DLPFC				
	Baseline	Stimulation		
active tDCS	60.02 (±15.34)	62.57 (±21.63)	3.64 (±19.68)	2.10
sham tDCS	52.58 (±12.44)	53.75 (±16.06)	1.54 (±17.15)	
	Baseline	Post		
active tDCS	60.02 (±15.34)	57.74 (±17.59)	-3.14 (±19.91)	-9.80
sham tDCS	52.58 (±12.44)	56.10 (±15.59)	6.66 (±18.78)	
Left TPJ				
	Baseline	Stimulation		
active tDCS	57.80 (±15.72)	55.53 (±17.95)	-3.619 (±18.15)	-4.44
sham tDCS	53.41 (±15.79)	53.62 (±16.22)	0.82 (±12.62)	
	Baseline	Post		
active tDCS	57.80 (±15.72)	57.30 (±14.82)	0.74 (±17.01)	-1.28
sham tDCS	53.41 (±15.79)	54.09 (±15.49)	2.023 (±11.48)	

Table 5 - CBF in ROIs - CBF variations during and after the stimulation in the different ROIs revealed by the parametric (Active n=15; Sham n=15) and used for the regional analysis (Active n=11; Sham n=10).

6.2.3.3. Discussion

Here, we present the first direct evidence of temporally and spatially distributed effects of fronto-temporal tDCS on brain perfusion after one session of 30min at 1mA, in healthy subjects. No significant effect on brain perfusion was seen in regions located underneath the electrodes, i.e left DLPFC (anode) and left TPJ (cathode). However, the results showed a significant impact on brain perfusion in a region interconnected with the stimulation sites: a decreased perfusion in the bilateral superior parietal gyrus, notably the precuneus region, compared to the sham group.

This is in line with increasing evidence suggesting that tDCS influences activity in brain regions distant from the stimulation site. Notably, Stagg and colleagues (2013) also reported a widespread perfusion decrease induced by another frontal tDCS montage (bifrontal tDCS) after the stimulation compared to baseline and during the stimulation, in regions such as the precuneus. In contrast with our study, they showed an increased perfusion in regions closely connected to the stimulating electrode using a bifrontal montage with the anode placed over the left DLPFC. However, it is important to note that they were less statistically conservative than in our study. These caveats were reported in a commentary published by Nord and colleagues (2013) and could potentially explain the discrepancies between our online effects. Other studies using different montages reported a polarity-specific effect on perfusion, i.e increased perfusion after anodal tDCS and decreased perfusion after cathodal tDCS (Lang et al., 2005; Wachter et al., 2011). These changes in rCBF remained stable throughout the 50-min period of PET scanning (Lang et al., 2005).

Furthermore, the precuneus region is part of the superior parietal gyrus and is engaged in reflective, self-related processing, conscious processing, episodic memory and visuospatial processing (Cavanna and Trimble, 2006). The activity of this region was reported to be increased at rest compared to during a cognitive task and is notably an important node in a resting-state network called the default mode network (DMN) (Raichle et al., 2001; Zhang and Li, 2012). This network is anti-correlated with networks involved in attention and cognitive engagement, such as the frontal-parietal network (FPN) or cingular-opercular/salience network (CON). Thus, one hypothesis of the decreased perfusion in the precuneus after the end of the stimulation would be that tDCS induces a disturbance of the integrity of the DMN, favoring the activation of these anti-correlated networks and facilitating reallocation of cerebral resources for various cognitive tasks.

The impact of tDCS on spontaneous network organization could be of interest concerning the pathophysiology of schizophrenia. Indeed, studies involving patients with schizophrenia reported alterations in the DMN (Nekovarova et al., 2014) and suggested that it plays a role in self-monitoring and the generation of auditory verbal hallucinations (AVH) (Rotarska-Jagiela et al., 2010; Jardri et al., 2012). More specifically, the DMN suppression usually reported in healthy subjects during a cognitive task is reduced in patients with schizophrenia (Nekovarova et al., 2014). Furthermore, studies involving patients with schizophrenia reported that reducing the left temporo-parietal junction (TPJ) activity using fronto-temporal tDCS improves source-monitoring performance as well as auditory verbal hallucinations (Mondino et al., 2015). Thus, our finding of a decreased perfusion in a hub of the DMN after tDCS could support potential clinical improvement after fronto-temporal tDCS.

The exact mechanism underlying these variations remains unclear, however a possibility is that this change in perfusion after a single session of fronto-temporal tDCS could be related to the neuro-vascular coupling, reflecting underlying activity. Neuro-vascular coupling is the ability of neurons to modulate cerebral blood flow in regions of increased synaptic activity. However, it should be noted that a similar increase in regional synaptic activity may be excitatory or inhibitory, which would then lead to differential excitability changes depending on the subpopulation of neuronal or non-neuronal cells involved. In this context, it has been shown that repeated fronto-temporal tDCS increases resting-state functional connectivity between the left TPJ and the precuneus, suggesting a change in activity in these areas (Mondino et al., 2015). Interestingly, we observe a decrease in perfusion after the stimulation. One hypothesis could be that this underlies the reallocation process between different resting-state networks. Indeed, as supported by other studies (Peña-Gómez et al., 2012), a reallocation of resources could be done from the DMN to other task-positive networks, such as FPN or CON, in order to improve cognitive performance. However, besides a decrease in DMN perfusion, we do not observe increases in these networks. One possibility would be that as the subjects are not engaged in a cognitive task, the resources could be reallocated equally in a widespread manner.
Furthermore, we do not see any significant changes directly underneath the electrodes but significant changes in interconnected regions. This could be due to the possible action of tDCS on sub-threshold somatic depolarization sufficient to activate axonal voltage dependent calcium channels. Thus, tDCS could potentialize neurons via changes in calcium levels locally (Christie et al., 2011; Das et al., 2016) and induce significant effects in interconnected regions.

In all, fronto-temporal tDCS seems able to modulate perfusion in the human brain, supporting the fact that tDCS is not focal and acts in a network manner.

6.2.4. Resting-state functional MRI (rs-fMRI)

6.2.4.1. Methods

During the 110min of PET acquisition, three rsMRI scans were done: 1 before the stimulation (Baseline period; 15-28min after tracer injection), 1 during the stimulation (Stimulation period; 45-58min after tracer injection) and 1 after the end of the stimulation (Post period; 76-89min after tracer injection). The times of scans were the same across subjects. During the three scans, subjects were lying at rest in the machine with their eyes fixated on a cross (**Figure 15 A**).

Data acquisition

Resting-state functional images were acquired on the Biograph mMR PET-MR system (Siemens; 3T MRI), using a 12-channel head coil. Head stabilization was achieved with cushioning, and all participants wore earplugs to attenuate noise. Images were collected using a gradient echo T2* weighted sequence (TR = 2000ms; TE = 30ms; Flip angle = 90°). Thirty-one slices, parallel to the inter-commissural plane (voxel size: 2.4×2.4×4mm), were acquired in an interleaved manner. During acquisition, subjects were instructed to remain awake with the eyes open fixed on a visual crosshair centered on a screen. No other instructions were given. Each rs-fMRI acquisition required approximately 13 minutes. In total, each rs-fMRI image was composed of 390 volumes. Prior to functional images, we acquired a sequence of gre_field_mapping needed to correct for distortions (31 slices interleaved; TR=400ms; TE 1 = 4.92ms; TE 2 = 7.38ms; Flip angle = 60°; bandwidth = 261Hz/Px). Each acquisition was visually inspected before any preprocessing.

Data preprocessing

Images were preprocessed using Statistical Parametric Mapping 12 (SPM12, Wellcome Trust Centre of Neuroimaging) and in-house scripts. rs-fMRI data were corrected for head motion, using rigid body motion correction, and slice-timing correction. Outliers were then detected with the ART toolbox using z-value threshold of 9 and composite motion threshold of 2mm. Subject with more than 5 consecutive outlier volumes were excluded from the analysis. At this point, 7 subjects were excluded from the analysis. The images were then normalized to the Montreal Neurological Institute (MNI) altas and spatially smoothed using a Gaussian kernel (6mm full width at half-maximum). The normalized and smoothed images obtained were used hereafter.

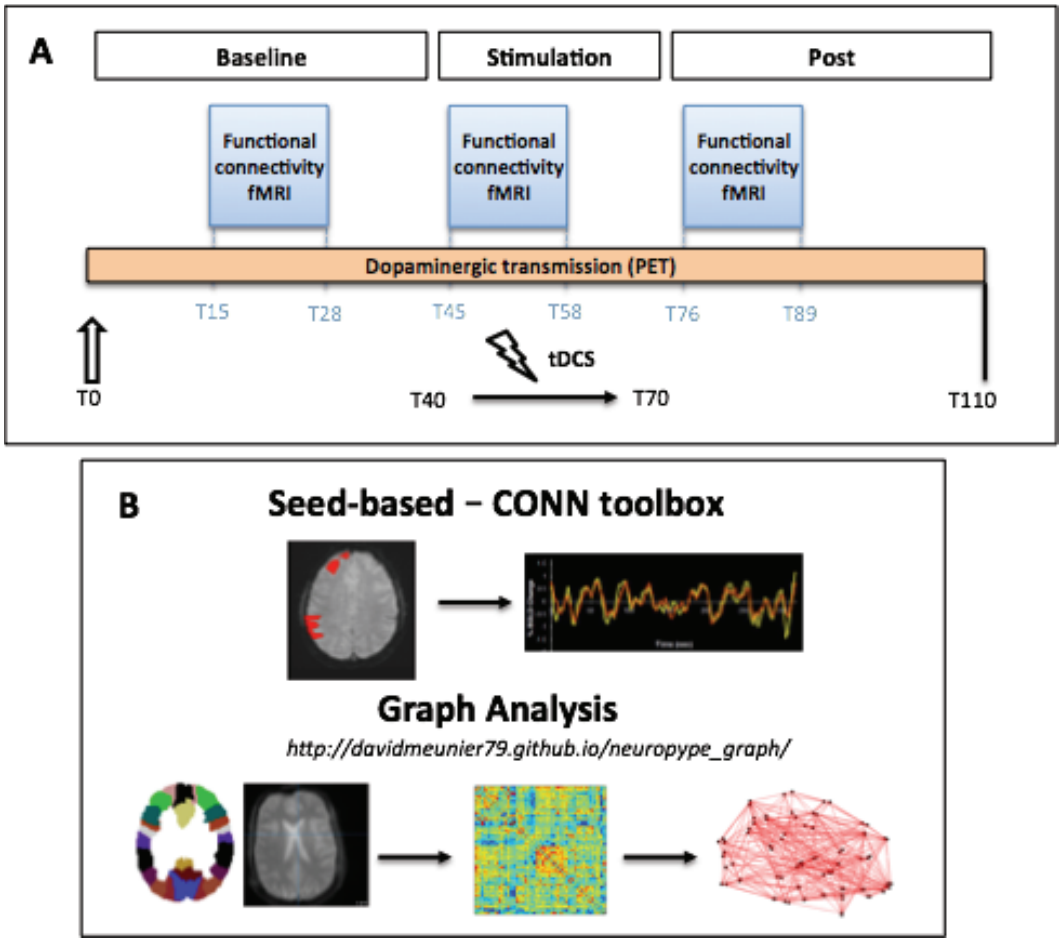


Figure 15: Resting-State MRI analysis - Example for one standard subject - (**A**) Resting-State functional MRI (rs-fMRI) Design (**B**) rs-fMRI analysis; Top: Seed-based analysis using region-of-interest located underneath the electrodes (Left DLPFC, Left TPJ). Bottom: Graph analysis, using the neuropype_graph pipeline, in collaboration with David Meunier (CRNL) (http://davidmeunier79.github.io/neuropype_graph/). Adapted from Wang, 2010; Raichle, 2015.

Data analysis

Seed based functional connectivity analysis (CONN toolbox)

To prepare the data for functional connectivity analysis, denoising included nuisance cleaning to regress out from functional images at each voxel, head motion (3 translations and 3 rotations), WM signal, CSF signal time courses and scrubbing (ART), temporal filtering using a band-pass filter (0.09Hz - 0.008Hz).

For each subject, correlations between the time-course extracted from a priori selected seeds (ROI) and other brain voxels were computed. Seed ROIs were the left DLPFC (Sallet et al., 2013) and left TPJ (Mars et al., 2012), regions located under the stimulating electrodes (anode and cathode) (**Figure 15 B**).

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

6.2.4.2. Perspectives

To date this part of the analysis is still ongoing. All the preprocessing has been performed. The next step will consist in group analysis of the seed-based analysis in order to assess the difference in functional connectivity between both stimulated regions (left DLPFC and left TPJ) when comparing the active and sham groups. We hypothesize that we could find similar results to those reported after repeated fronto-temporal tDCS (2mA, 20min, 2 sessions/d for 5d) in patients with schizophrenia (Mondino et al., 2015), where they found increased connectivity of the left TPJ with the left angular gyrus, the left DLPFC and the precuneus as well as reduced functional connectivity with the left anterior insula and the right inferior frontal gyrus. However, our results might differ due to the different conditions between our study and the one published by Mondino and colleagues (2015). Indeed, we applied only 1 session of tDCS in healthy humans instead of 10 sessions in patients with schizophrenia. The connectivity changes observed in patients with schizophrenia after repeated tDCS concern regions known to be involved in schizophrenia. Thus, one question concerning the action of tDCS is: Will a single session of tDCS have the same effect on functional connectivity if the pathological impairments are absent? In addition, as those sequences are acquired simultaneously to the PET, do functional connectivity changes, if any, explain the changes observed in dopamine levels in the striatum?

Furthermore, we hypothesize that fronto-temporal tDCS impacts spontaneous network organizations. Thus, a complementary approach is ongoing using the neuropype_graph pipeline (http://davidmeunier79.github.io/neuropype_graph/) in order to analyze the data with graph analysis. This work is done in collaboration with David Meunier of the CRNL. Using graph analysis allows us to observe how tDCS impacts the networks in a general manner and how, if any, functional reorganisation occurs after a single tDCS session. More specifically, we hope to focus on networks involved in the dopaminergic system, such as the interplay between the default mode network (DMN), fronto-parietal network (FPN) and the cingulo-opercular or salience network (CON). Indeed, these three networks are thought to recruit subcortical structures such as the striatum and act on different time scales relating to cognitive control processes (Sadaghiani and D'Esposito, 2015). More specifically, studies using pharmacological challenges in healthy volunteers reported that, compared to placebo, a dopamine agonist increased functional connectivity between the subcortical dopaminergic areas and resting-state networks, while a dopamine antagonist decreased the functional connectivity in those regions (Kelly et al., 2009; Cole et al., 2013). Thus, the question of interest is: Does the stimulation change the interactions between those networks? This is supported by several studies in the literature. Indeed, tDCS using other montages report after-effects on resting-state connectivity in the selected networks. Two studies reported an increased connectivity in the FPN (Keeser et al., 2011; Peña-Gómez et al., 2012) after bifrontal tDCS. Concerning the effect of tDCS on the default mode network (DMN) results are not so clear. Keeser and colleagues (2011) reported an increased coactivation in frontal parts of the DMN, whereas Pena-Gomez and colleagues (2012) reported a diminished spatial robustness of the DMN. Thus, tDCS seems to be able to modulate resting-state connectivity in the human brain, supporting the fact that tDCS is not focal and acts in a

network manner. Moreover, resting-state network analysis is increasingly used in clinical settings, such as schizophrenia (Fornito et al., 2012; Tu et al., 2012, 2013; Manoliu et al., 2014; Sheffield et al., 2015), showing disturbances in the functional interaction between the DMN, FPN and CON/salience networks. Moreover, reviews and meta-analysis point to a possible core node involved in most psychiatric disorders centered on the dorsal anterior cingulate cortex (dACC) and anterior insula. This core is one of the central nodes of the CON/salience network (Goodkind et al., 2015; Downar et al., 2016; Peters et al., 2016). Thus, this node could also be a potentially interesting target for further tDCS studies in clinical populations.
Another interesting aspect of using graph analysis in our study is that most of these studies have been conducted using ICA or seed-based approaches. Graph analysis could provide a more general vision of the effect of tDCS on a network level. Based on graph theory, this method, strictly exploratory requiring no prior assumptions, can describe large-scale human brain networks during resting-state as a small-world organization (Sporns et al., 2004; Achard et al., 2006; Bassett and Bullmore, 2006) with the simultaneous presence of high local clustering (functional segregation) and short path lengths (functional integration) between nodes. A simple way to visualize this approach is to think of a graph of nodes, representing brain regions, linked by edges, reflecting measures of structural or functional interaction between nodes. To date, only few studies using tDCS montages applied over different cortical region have used this approach (*left inferior frontal gyrus:* Meinzer et al., 2012, 2013, 2014; *F4/F3 frontal:* Weber et al., 2014; *Motor:* Polanía et al., 2011) and reported that connectivity between distant brain areas can change after a single session of tDCS.

Thus, we expect to that these analyses will bring a new piece to the puzzle to better understand the neurophysiological effects of fronto-temporal tDCS specifically on functional networks related to the dopaminergic system.

6.2.5. Diffusion Tensor Imaging (DTI)

6.2.5.1. Methods

During the 110min of PET acquisition, two DTI scans were done: 1 before the stimulation (Baseline period; 0-10min after tracer injection) and 1 after the end of the stimulation (Post period; 100-110min after tracer injection). The times of scans were the same across subjects. During the two scans, subjects were lying at rest in the machine (**Figure 16 A**).

Data acquisition

DTI images were acquired on the Biograph mMR PET-MR system (Siemens; 3T MRI), using a 12-channel head coil. Head stabilization was achieved with cushioning, and all participants wore earplugs to attenuate noise. Images were collected using GRAPPA (with a factor of 2) with a single shot echo-planar imaging (EPI) sequence (TR = 6600ms; TE = 91ms; FOV 240mm; phase encoding in the A-P direction; 7/8 partial Fourier). Thirty-five slices (voxel size: 1.8×1.8×3mm; distance factor 30%) were acquired in an interleaved manner. Each DTI acquisition required approximately 10 minutes. In total, each DTI image was composed of 93 volumes, 3 without diffusion weighting (b-value = 0 s/mm^2) and 90 volumes with diffusion weighting (b-value of 1000 s/mm^2) along 30 directions. 3 DTI acquisitions were done (1 B0 and 30 directions) in order to improve signal-to-noise ratio. Prior to functional images, we acquired a sequence of gre_field_mapping needed to correct for distortions (31 slices interleaved; TR=400ms; TE 1 = 4.92ms; TE 2 = 7.38ms; Flip angle = 60°; bandwidth = 261Hz/Px). Each acquisition was visually inspected before any preprocessing.

Data preprocessing

Images were preprocessed using FSL (FMRIB Software Library, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl/index.html) and in-house scripts. DTI data were corrected for head motion and eddy currents, respectively, by aligning all images onto the mean reference volume, using FSL *eddy* tool. The three DTI repetitions were merged to obtain a single DTI images composed of 1 B0 and 30 direction volumes. Subsequently, brain tissue was extracted using FSL *brain extraction tool (BET)*. The diffusion tensor and FA-maps were estimated for each voxel using FSL *DTIFIT* tool. The maps obtained were used hereafter.

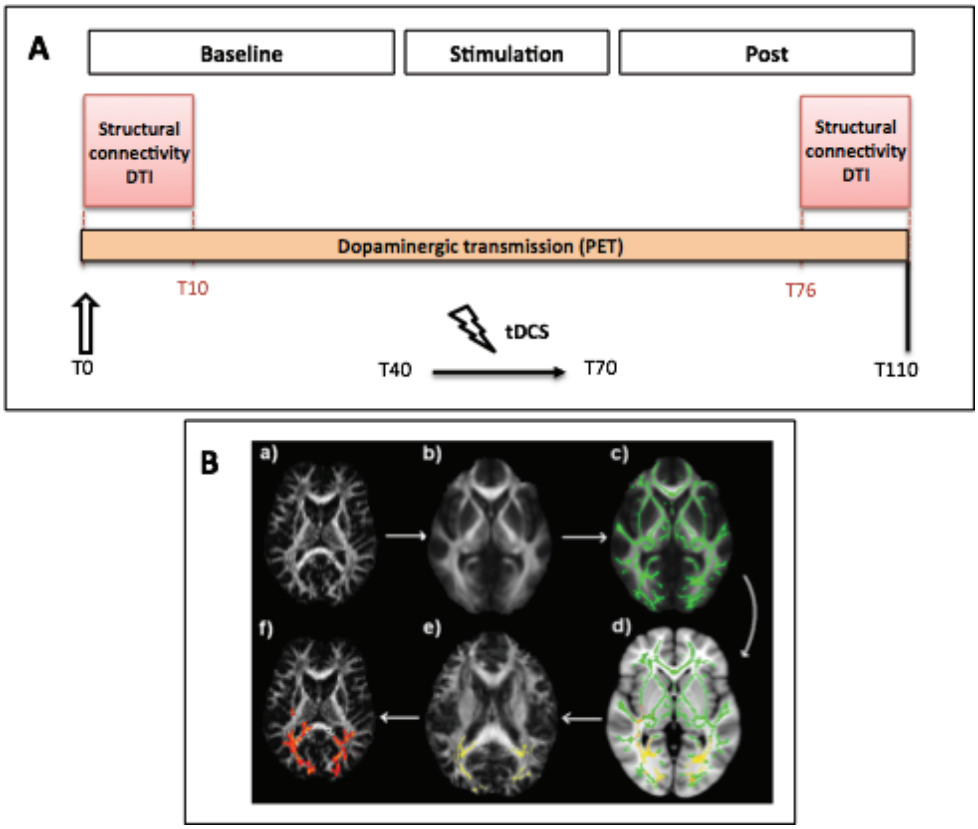


Figure 16: Diffusion tensor imaging analysis - Example for one standard subject - (**A**) DTI Design (**B**) DTI analysis. a) FA map in the subject space b) FA map align non-linearly to the target map c) Skeleton projection d) Voxelwise statistics on the skeleton at the group level e) Back-projection into the standard space for each subject for post-hoc analysis f) Back-projection into the subject space to extract anatomical location. Adapted from Dietrich et al., 2015.

Data analysis - TBSS

Voxel-vise statistical analysis of FA-maps will be performed using Tract-based spatial statistics (TBSS) in FSL (Smith et al., 2006). FA maps will be aligned and normalized in the standard Montreal Neurological Institute (MNI) space, using non-linear registration (FNIRT), which uses a b-spline representation of the registration warp field and thus be resampled to a spatial resolution of 1 x 1 x 1mm^3. Subsequently, a mean FA image will be created, generating a mean FA skeleton. Each subject's normalized FA maps will be projected onto the mean FA skeleton using an FA threshold of 0.2, to limit the subject variability and to restrict the analysis to white matter. The resulting maps will then be used for voxel-wise cross subjects' and between groups analysis (**Figure 16 B**).

Data and code availability

The data and custom-written analysis code that support the findings of this study will be available on request from the corresponding author.

6.2.5.2. Perspectives

To date this part of the analysis is still ongoing. All the preprocessing has been performed. The next step will consist in running the TBSS analysis and subsequently the voxel-wise statistical analysis. We will assess the group difference between active and sham tDCS, as well as within the active group, on the whole-brain mean FA skeleton, in order to identify significantly modulate white matter tract. This will be done using non-parametric permutation tests with the FSL Randomize Tool (Nichols and Holmes, 2002; Winkler et al., 2014). Each contrast will be analyzed according to permutation-based non-parametric inference, using threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009), allowing for multiple comparisons with a significance level of $p<0.05$. To identify impacted white matter tracts, the resulting clusters will be labeled using the Jülich DTI tracts or John Hopkins University (JHU) White Matter Tractography Atlas (Wakana et al., 2007; Hua et al., 2008).

One mechanism of interest to us in this thesis work would be to assess the effect on FA values of a single session of fronto-temporal tDCS, usually reported in clinical setting for patients with schizophrenia. However, the link between changes in FA values and structural remodeling is complex. In general, higher FA values are associated with more aligned fibers, possible myelination and overall fiber integrity increase, while lower FA values with less alignment and possible axonal sprouting (Berg and Rushworth, 2009). With this in mind, it is a possibility that a single-session of tDCS only may not be able to induce changes in FA values, but have more undetectable subtle effects.

To date, only one study has investigated the impact of tDCS on structural white matter changes, using DTI (Zheng and Schlaug, 2015). They reported an increase in FA (1.5mA, 30min, 10 sessions) in the treated group in descending motor tracts and the changes correlated with improvements in motor impairment after undergoing a treatment course of tDCS and physical therapy. However, this study differs from ours in regards to the location (tDCS over the motor cortex) and the design of the stimulation (repeated stimulation). The main question is the following: Is a single session of tDCS (1mA, 30min) sufficient to modulate the structural connectivity, notably changes in FA values.

Several studies reported that cognitive training induces modification of structural connectivity, measured with DTI. Some report FA increases (Scholz et al., 2009; Engvig et al., 2012) while other report FA decreases (Elmer et al., 2011; Halwani et al., 2011). These studies report remodeling of the brain tissue after several weeks of training. Interestingly, a study reported microstructural changes (increased FA) after only 2h of training, using DTI both in human and in rat (Sagi et al., 2012). These discrepancies may reflect different underlying mechanisms. Thus, our hypothesis fits in the line of possible microstructural changes over a short-time scale.

Futhermore, studies investigating structural integrity in patients with schizophrenia reported abnormalities in several fiber tracts, some dependent on the clinical status of patients (Kyriakopoulos and Frangou, 2009; Luck et al., 2011; Ellison-Wright et al., 2014; Samartzis et al., 2014). Compared with healthy controls, some studies reported decreased FA within tracts such as the uncinate fasciculus, the inferior longitudinal

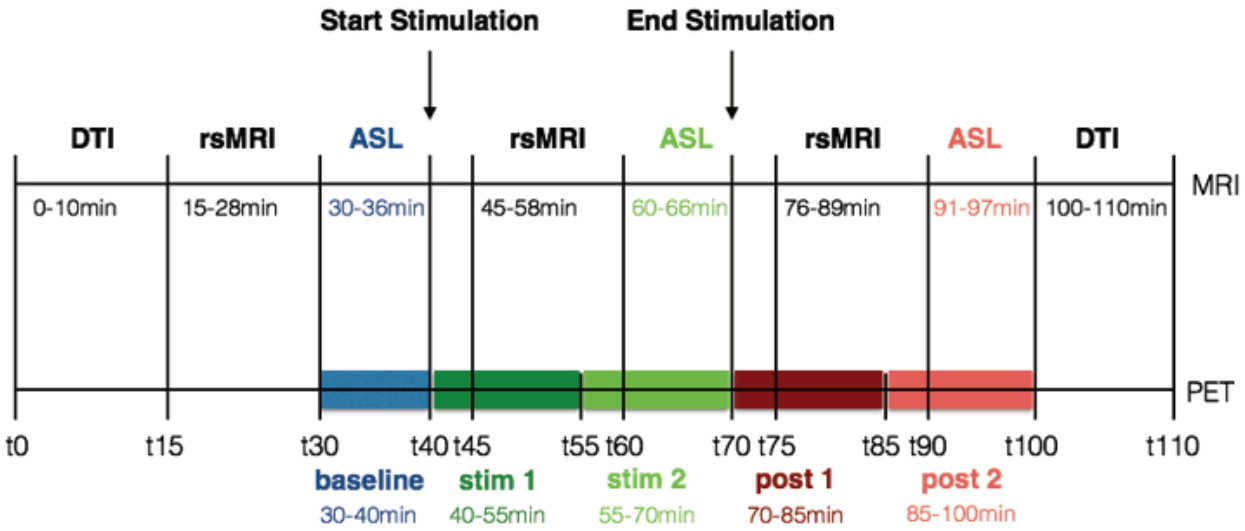
fasciculi, the cingulum bundle and the arcuate fasciculus in patients with schizophrenia, whereas some reported increased FA in fibers such as the arcuate fasciculus (for review see Kubicki et al., 2007).

In all, we expect to that these analyses will bring a new piece to the puzzle to better understand the neurophysiological effects of fronto-temporal tDCS specifically on structural pathways related to the dopaminergic system.

6.3. Discussion and Perspectives

This study, using simultaneous PET-MR imaging, enabled us to put forward the first direct evidence of temporally and spatially distributed effects of a single session of fronto-temporal tDCS (1mA, 30min) regarding the dopaminergic system, in healthy subjects. PET analysis suggests a decrease in [^{11}C]Raclopride BP_{ND}, using the Logan Plot approach, reflecting an increase in extracellular dopamine induced by a dopamine release evoked by a single tDCS session. We see an impact on dopamine transmission during the 15 to 30min period following the end of stimulation, in the associative/executive part of the striatum. Furthermore, ASL analysis suggests a significant impact on brain perfusion in a region interconnected with the stimulation sites. Indeed, we showed a decreased perfusion in the bilateral superior parietal gyrus, notably the precuneus region, compared to the sham group. However, no significant effect on brain perfusion was reported in regions located underneath the electrodes, i.e. left DLPFC (anode) and left TPJ (cathode). As of today, the rs-MRI and DTI analyses are still ongoing. All the preprocessing has been performed. We expect to that they will bring a new piece to the puzzle in order to better understand the neurophysiological effects of fronto-temporal tDCS specifically on structural and functional networks related to the dopaminergic system.

However, this analysis in an independent modality manner is just the tip of the iceberg. Using simultaneous PET-MR imaging, the goal will be to combine these modalities in order to create a coherent ensemble of the underlying neurobiological effects of a single-session of fronto-temporal tDCS. Indeed, this unique approach will enable us to combine ASL and rs-MRI data with simultaneous PET acquisitions as represented below. One possibility would be to use toolboxes such as the Biological Parametric Mapping (BPM; Casanova et al., 2007), VoxelStats (Mathotaarachchi et al., 2016) or Multimodal Imaging Brain Connectivity Analysis (MIBCA; Ribeiro et al., 2015) for voxel-wise multimodal correlation between the specified metrics.



Combining multiple imaging levels could improve our understanding of the tDCS effects. This is one of the advantages of the PET-MRI machine. This fits in the innovative aspects of using multimodal imaging to

perform individualized predictions, notably in clinical settings in order to find potential imaging biomarkers (Meng et al., 2017).
However, one of the relevant questions in the field, is “Is there an advantage to simultaneous PET-MR? Besides timing, how does it differ to multimodal imaging?” In regards to our study design and based on the notion that tDCS effects have been reported to be dependent on the subject’s brain-state at the moment of the stimulation, simultaneous PET-MR study gives us the possibility to acquire different and complementary imaging modalities simultaneously, with the subject being in the same state of mind. Thus, when we combine the imaging modalities, we will be able to assess the neurophysiological impact of tDCS across multiple modalities with no differential impact of brain state. Moreover, the simultaneous acquisition also gives us the opportunity to explore at baseline the coupling of the neuronal energetics to the CBF perfusion or BOLD signal in regards to the dopaminergic system. In this line, studies are starting to emerge taking advantage of this simultaneous PET-MR scanner (Riedl et al., 2014; Aiello et al., 2015). For example, recent studies explored the relationship between the glucose metabolism provided by PET-FDG and functional brain activity using resting-state fMRI, in the healthy subject. Using voxel-wise comparison, they reported a significant correlation between functional connectivity and glucose uptake across the brain (Aiello et al., 2015) and more specifically that the local activity of a region at rest determines its resting-state functional connectivity (Riedl et al., 2014). In our study, however, as the baseline dopamine has not quite reached the equilibrium, we fear this investigation during baseline could not be possible.

Furthermore, several limitations of this study should also be pointed out. Notably, the results were not associated with behavioral findings (e.g., self-monitoring task), hence no behavioral or clinical effects were in fact inspected in the present study. The second limitation is that dopamine has also been shown to be involved in placebo responsiveness (Benedetti, 2014). The placebo-controlled study design developed here overcame in part this problem. Moreover, the psychological assessment conducted did not reveal differences between active and sham groups regarding personality traits, motivation and anxiety. However, it should also be noted that we used the standardized blinding protocol provided by the NeuroConn device, thus the sham group still received a active stimulation of 1min (NeuroConn Manual). To date, this sham mode is thought only as a way to minimize any potential neuromodulatory effects unrelated to the stimulation itself. However, more research seems needed to make sure sham protocols are reliable and do not induce unexpected neurobiological effects (Fonteneau et al, Letter to Editor, in preparation, Annexe 5).
Moreover, this montage is exclusively used in clinical population, where clinical effects are reported after repeated tDCS. The implication of our results on the clinical effect put forth several questions: Would the effects on the dopaminergic system after repeated tDCS be the same as after single tDCS session? As patients with schizophrenia could have altered dopamine baseline activity, would this affected baseline have a differential impact on tDCS after-effects? Indeed, studies have reported that dopamine baseline could induce different effects of tDCS in an inverted U-shape manner (Jongkees et al., 2017). Furthermore, do the pharmacological medication (antipsychotics) affect and/or play a role in the clinical efficacy of tDCS? Indeed, pharmacological challenges, administrating dopaminergic agonist or antagonist are reported to

impact differently the after-effect of tDCS (Monte-Silva et al., 2010; Fresnoza et al., 2014a, 2014b; McLaren et al., 2018).

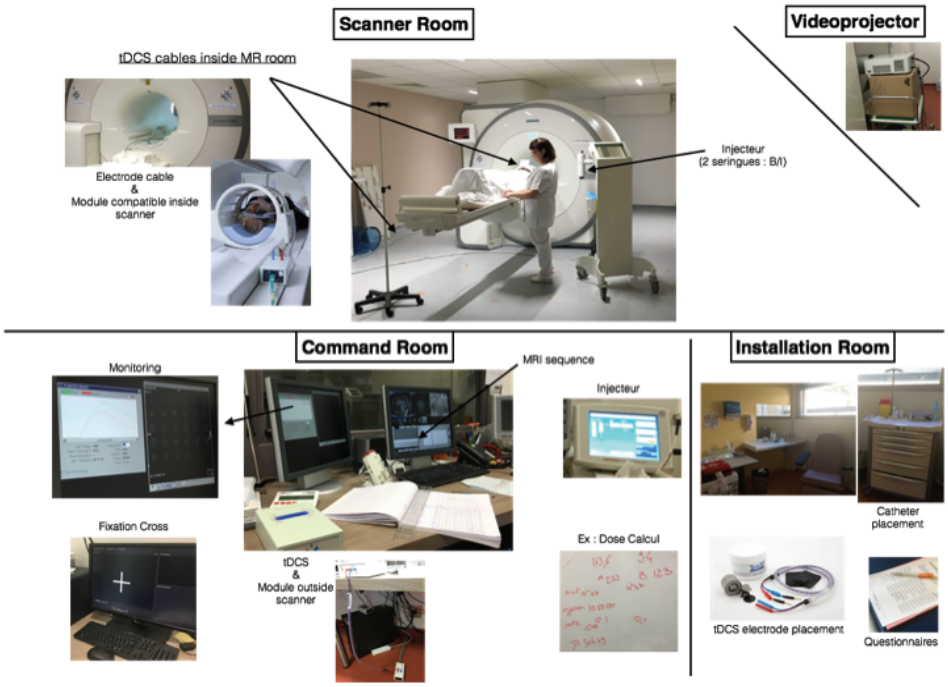
Overall, the pioneering aspects of the project are to use an innovative simultaneous multimodal imaging system, adopt a tDCS montage used in a validated therapeutic context and apply tDCS online. We expect that our unique approach will provide an imaging biomarker essential to improve our understanding of the:
1) Neurobiological effects of fronto-temporal tDCS in order to:

- Bring key element of the proof of concept of tDCS
- Optimize tDCS in a therapeutic context
- Suggest a possible marker of the therapeutic response

2) “Normal brain” and potential further deficient mechanisms underlying schizophrenia

With this study, we plan on publishing 2 articles.
The first one would combine PET, ASL and rs-fMRI data. This study would also include DTI tractography and electric field modeling in order to better understand the structural connections and the current distribution between the impacted areas.
The second one would be the DTI TBSS results. This would be of interest to the scientific community because, to date, no studies in the literature have examined the impact of a single session on structural connectivity.

6.4. Annexes



Supplementary Figure 1: Example of the organization needed for one experiment - PET-MR facility in the CERMEP, LILI department.

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Part III - General discussion

7. Chapter 7 - Results synthesis

The work presented here fits into a broad research axis intended to clarify brain mechanisms underlying frontal tDCS. As a first step, the specific aim of this thesis was to investigate this question in healthy subjects, specifically in relation to the dopaminergic system, with the goal to move on to patients in subsequent studies. In this context, two studies were performed using different electrode montages, which are being increasingly used in the literature (bifrontal tDCS and frontotemporal tDCS). We used on online study design and combined the stimulation with several imaging techniques, with the subject at rest. The online implementation of the stimulation allowed for deciphering changes induced not only after but also during the stimulation.

7.1. Frontal tDCS widespread action reaching the dopaminergic system

7.1.1. Underneath the electrodes

Our results report no significant effect in perfusion changes in regions located underneath the electrodes, i.e left DLPFC (anode) and left TPJ (cathode). These results are in line with Stagg and colleagues (2013) reporting similar results with a single session of bifrontal tDCS. Indeed, they did not observe any time dependent modification of perfusion during and after tDCS. However, these result contrast with other studies reporting variations in regional cerebral blood flow (rCBF) underneath the stimulation electrodes after tDCS (Lang et al., 2005; Homan et al., 2011; Zheng et al., 2011; Antal et al., 2014b). For example, Antal and colleagues (2014) reported only an effect (increased rCBF) under the anode compared to the sham group, as no effect under the cathode was found, whereas Homan and colleagues (2011) reported a reduced rCBF underneath the cathode after repeated tDCS session (no information is given regarding the anode). Nevertheless, another study reported an increased rCBF regardless of the polarity after the end of the stimulation, compared to sham tDCS (Lang et al, 2005). However, these effects may vary depending on the time window investigated (i.e. during the stimulation or after the end of the stimulation). Indeed, Zheng and colleagues (2011) reported offline polarity-specific after-effects of the stimulation, with the anode increasing rCBF, whereas the cathode decreased it compared to baseline. However, this same study reported an increased rCBF regardless of the polarity during the stimulation (Zheng et al, 2011). This increase in perfusion after both anodal and cathodal stimulation could underlie a similar increase in regional synaptic activity that may be excitatory or inhibitory, which would then lead to differential excitability changes depending on the subpopulation of neuronal or non-neuronal cells recruited.

Furthermore, other imaging modalities such as magnetic resonance spectroscopy (MRS) have investigated the effects underneath the electrodes. Some reported reduced GABA (Stagg et al., 2009; Stagg et al., 2011) and increased glutamine/glutamate after the end of anodal tDCS over M1 (Clark et al., 2011). However, a recent study investigating the effects of a single bifrontal tDCS session (1mA, 30min) reported online elevated N-acetylaspartate (NAA) in the prefrontal cortex (region under the anode), and no after-effect changes. No effects on glutamine/glutamate or GABA were reported (Hone-Blanchet et al, 2016).

These discrepancies in the literature could be due to the variability between subjects, which could hide potential small changes in perfusion. In sum, the effects of frontal tDCS under the stimulating electrodes

are not so clear and further studies are needed to clarify this point. The use of electric field modeling seems warranted to improve the understanding and interpretation in a subject specific manner. Additionally, the use of frontal dopamine receptor visualization using PET with [^{11}C]FLB457 tracer could allow investigating further the impact of tDCS in cortical regions, such as the ones under the electrodes.

7.1.2. Network effect

Going further, we reported that frontal tDCS has an impact on brain perfusion in regions interconnected with the stimulation sites. Indeed, we reported a decreased perfusion in the bilateral precuneus compared to the sham group. In line with our result, Stagg and colleagues (2013) also reported widespread perfusion decrease induced by bifrontal tDCS after the stimulation compared to baseline and during the stimulation, notably in the precuneus. In contrast with our study, they also showed an increased perfusion in regions closely connected to the stimulating electrode during anodal stimulation, whereas they reported widespread decreases during cathodal stimulation. However, it is important to note that they were less statistically conservative than in our study. These caveats were reported in a commentary published by Nord and colleagues (2013) and could potentially explain the discrepancies between our online effects. Moreover, other studies reported a polarity-specific effect on perfusion, i.e increased perfusion after anodal tDCS and decreased perfusion after cathodal tDCS (Lang et al., 2005; Wachter et al., 2011). These changes in rCBF were reported to remain stable throughout the 50-min period of PET scanning (Lang et al, 2005).

Interestingly, the precuneus responds to a wide range of cognitive processes (Cavanna and Trimble, 2006) and is notably an important node in a resting-state network called the default mode network (DMN) (Raichle et al., 2001). This network is involved in self-referential thinking, recollection, prospection and mind wandering, while goal-directed cognitive tasks suspend the activity of this network (Gusnard et al., 2001; Raichle et al., 2001; Spreng and Grady, 2010; Fox et al., 2015). Furthermore, the DMN is anti-correlated with networks involved in attention and cognitive engagement, such as the frontal-parietal network (FPN) or cingular-opercular/salience network (CON). The decreased perfusion in the precuneus induced by tDCS suggests a disturbance of the integrity of the DMN. More specifically, diminished DMN perfusion could induce an activation of anti-correlated networks and thus facilitate reallocation of cerebral resources for various cognitive tasks. At the functional level, the impact on spontaneous network organization is in line with other tDCS studies showing that bifrontal tDCS increased connectivity in the FPN (Keeser et al, 2011, Pena-Gomez et al, 2012). The effects regarding the DMN are not so clear. Keeser and colleagues (2011) reported an increased co-activation in frontal parts of the DMN, whereas Pena-Gomez and colleagues (2012) reported a diminished spatial robustness of the DMN. Furthermore, Park and colleagues (2013) found that tDCS increased inter-hemispheric connectivity. Thus, tDCS seems to be able to modulate resting-state connectivity in the human brain, supporting the fact that tDCS is not focal and acts in a network manner.

With this in mind, these effects of fronto-temporal tDCS beg the question of possible mechanisms leading to its impact on the dopaminergic network. The literature supports two possible mechanisms: a direct

pathway, via voltage dependent calcium channels in the striatum (Christie et al., 2011; Das et al., 2016) or an indirect pathway, involving polysynaptic glutamatergic and GABAergic cortical projections (Stagg et al, 2011; Worsching et al, 2016).

Concerning the direct pathway, dependent on current flow distribution, several mechanisms are possible to explain these changes, notably regarding the implication of intracellular calcium concentration. Indeed, several studies have reported an increase in calcium levels after anodal tDCS in animal studies (Islam et al., 1995; Bikson et al., 2004). The exact mechanism underlying these variations remains unclear, however some point to a possible effect of tDCS on voltage dependent calcium channels (for review see: Das et al, 2016). Thus, acting on sub-threshold somatic depolarization sufficient to activate axonal voltage dependent calcium channels, tDCS potentializes neurons via changes in calcium levels (Christie et al, 2011). Furthermore, low calcium level was shown to reduced neuronal activity, whereas high calcium results in enhanced activity. However even larger calcium levels reduced neuronal activity, possibly via a counter-regulative activation of potassium channels (Lisman, 2001; Misonou and Trimmer, 2004). This process mediates neuroplastic after-effect of tDCS acting via N-methyl-D-aspartate (NMDA) receptors (Liebetanz et al, 2002; Nitsche et al, 2003; Nitsche et al, 2004). Thus, increasing intracellular calcium levels could be one possible mechanism underlying the impact on interconnected regions after tDCS.

Concerning the indirect pathway, animal experiments using brain slices suggest that pyramidal cells in layer V are the most sensitive to tDCS effects (Radman et al, 2009). Thus, tDCS will impact first and foremost the glutamatergic system as well as its projection to GABAergic interneurons. The effects would be dependent on the position of both the anode and cathode as the current will impact several functionally and anatomically interconnected regions of the brain in a polysynaptic manner and not only the regions directly under the electrodes. When looking at our results, the region showing variation of perfusion (i.e. the precuneus) after a single-session of fronto-temporal tDCS is connected to both stimulation sites.

Furthermore, the functional connectivity within these networks could depend on dopamine levels. Notably, studies using pharmacological challenges in healthy volunteers reported that, compared to placebo, a dopamine agonist increased functional connectivity between the subcortical dopaminergic areas and resting-state networks, while a dopamine antagonist decreased the functional connectivity between those regions (Kelly et al., 2009; Cole et al., 2013; Handley et al., 2013). More specifically relating to our results, Cole and colleagues (2013) reported dopamine-dependent connectivity between the DMN and dopaminergic midbrain (ATV and SN). Thus, tDCS effects on the precuneus could be in part related to dopamine activity in the dopaminergic neurons in the midbrain.

Additionally, in order to directly investigate the impact of tDCS on dopamine levels in cortical regions, PET studies could be conducted using the [^{11}C]FLB457 tracer. Indeed, a PET study using this tracer reported that another NIBS approach, such as TMS applied over the left frontal cortex, released dopamine in the anterior cingulate and medial orbitofrontal cortex (Cho and Strafella, 2009). This suggests that frontal TMS released dopamine in other cortical regions than the ones stimulated. Thus, it is important to keep in mind the functional and structural connections in regards to the impact of the stimulation.

7.1.3. Subcortical effect in the striatum

We were able to confirm that frontal tDCS has an impact in specific subcortical structures. More specifically, a single session of frontal tDCS increased dopaminergic transmission in regions of the striatum connected to the stimulation sites, i.e. more ventral parts of the striatum, involved in the cognitive and executive processes related to DLPFC activity (Haber, 2016). These modulations were significant only after the end of the stimulation. Interestingly, both bifrontal and fronto-temporal tDCS, with anode over the left DLPFC as a common montage design, report similar effects concerning the location of the release of dopamine. Moreover, this effect is in line with other studies using animal models (Tanaka et al., 2013; Leffa et al., 2016) or other NIBS, such as TMS (Strafella et al, 2001; for review see Ko & Strafella, 2012; Cirillo et al, 2017), showing release of dopamine in the striatum after the stimulation.

Based on the possible mechanisms explaining the network effect of tDCS, the literature supports two possible mechanisms leading to dopamine release in the striatum after a single session of fronto-temporal tDCS: a direct pathway, via voltage dependent calcium channels in the striatum (Christie et al, 2011; Das et al, 2016) or an indirect pathway, involving polysynaptic glutamatergic and GABAergic cortical projections involved in the cortico-striatal network (Peanlikhit et al, 2017; Taber and Fibiger, 1995; Stagg et al, 2011; Hone-Blanchet et al, 2016, Grace et al, 2016). Thus, concerning the indirect pathway, two mechanisms are possible: cortical projections on mesostriatal dopamine neurons in the midbrain projecting back to the striatum and/or cortical projections on the striatum via relays such as the hippocampus or the amygdala (**Figure 35**). Both mechanisms could be involved in tDCS effects, according to animal studies showing that stimulation of the PFC could promote activation in both striatal and ventral tegmental regions (Peanlikhit et al. 2017; Taber and Fibiger 1995). With the notion that tDCS modulates glutamatergic and GABAergic activity under the electrodes (Stagg et al. 2009), frontal tDCS may impact the glutamatergic projections from the DLPFC, and consequently modify subcortical activity, as shown by a recent MR-spectroscopy study reporting that bifrontal tDCS had fast excitatory effects in the left striatum (Hone-Blanchet et al. 2016).

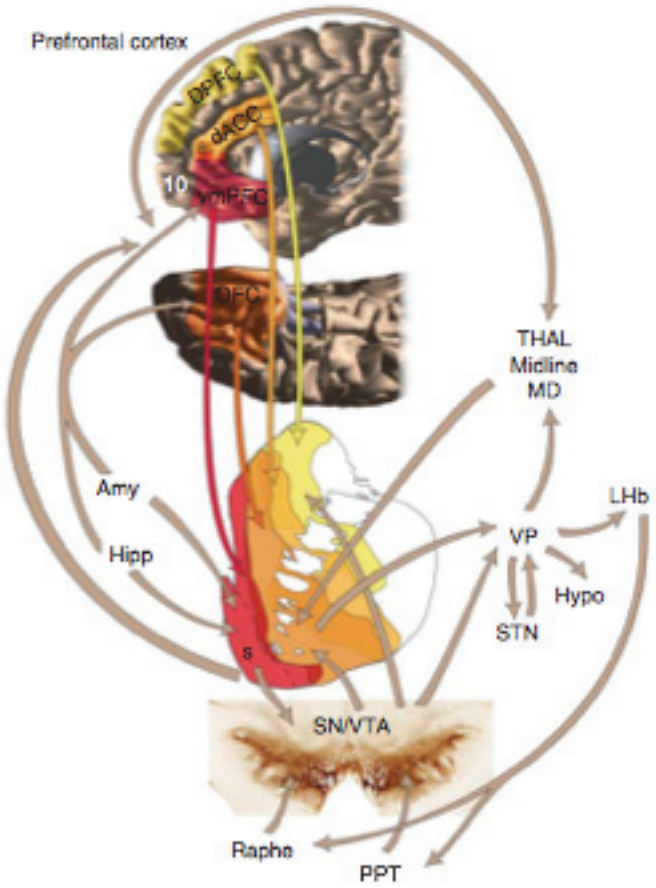

Figure 35: **Cortico-striatal network involved in the regulation of dopamine release in the striatum** - Pathways between the cortex, relays (hippocampus, amygdala), striatum and midbrain neurons. Adapted from Haber and Hudson, 2010 - *Abbreviations: DPFC: dorsolateral prefrontal cortex; dACC: dorsal anterior cingulate cortex; cmPFC: ventromedial prefrontal cortex; OFC: orbitofrontal cortex; Amy: amygdala; Hipp: Hippocampus; THAL: thalamus; VP: ventral pallidum; STN: subthalamic nucleus; Hypo: Hypothalamus; SN: substantia nigra; VTA: ventral tegmental area.*

7.1.4. Effects online vs offline

Even though clear evidence is in favor of effects of tDCS in regions interconnected with the stimulation sites and seemingly organized in a network manner, these effects seem to differ with respect to time, i.e during and/or after stimulation effects. According to the studies, behavioral and biological effects can be investigated online (during the stimulation) and/or offline (after the stimulation period). To date, few studies have investigated the combination of both online and offline effects of stimulation in one experiment.

In both studies presented in this thesis, we were able to investigate the tDCS effects both online and offline due to the design used. tDCS effects on the dopaminergic network were reported significant after the end of the stimulation. The absence of significant effects during the stimulation could be considered as contrasting with some previous online stimulation studies applied over the frontal cortex (Stagg et al, 2013; Hone-Blanchet et al, 2016). Indeed, elevated N-acetylaspartate in the left DLPFC and elevated glutamate/glutamine in the left striatum have been observed during a single bifrontal tDCS session (1mA, 30min) using online MRS, while no changes were observed under the anode (i.e. left DLPFC) after the end

of the stimulation. However, no measurements were conducted in the left striatum after the stimulation (Hone-Blanchet et al, 2016). The discrepancy with our results could be explained by several factors. First, regarding technical features, MRS measures a mixture of compounds involved in neurotransmission and metabolism in every cellular compartment of the voxel. Here, with [^{11}C]raclopride PET, we addressed dopamine neurotransmission in terms of extracellular dopamine. Second, regarding physiological features, it cannot be ruled out that the time-scale of glutamate and dopamine variations is different. With this hypothesis, changes in the macro- and microenvironment induced during tDCS could trigger dopamine release only when the stimulation ends. Another explanation could be a matter of statistically significant threshold. Indeed, the curves extracted over time in both studies show a continuous decrease starting at the beginning of the stimulation, only for the active tDCS group. However, this decrease reaches significance only after the end of the stimulation compared to the sham group.

7.1.5. Laterality

Another point to notice was a potential statistically significant laterality effect reported in both our studies. Indeed, the first study using a bifrontal montage reported more robust effects in the right striatum, whereas the second study using a fronto-temporal montage reported significant effects in the left striatum. In the literature, contradictory results exist but a recent review and meta-analysis reported no laterality of the stimulation (i.e. left vs right DLPFC) effect on cognitive task performance (Dedoncker et al, 2016). However, asymmetries within the dopamine system have been reported in the healthy human brain. Indeed, a striatal dopamine D2 receptors mapping study in healthy human reported a left-right asymmetry, with higher D2 binding ratio in the right hemisphere (Larisch et al., 1998). Furthermore, studies suggested that individual differences in striatal and cortical dopamine asymmetries could contribute to the personality dimensions and impact cognition (Tomer et al., 2008, 2013, 2014). In this line, a loss of physiological asymmetry in striatal dopamine transmission is reported during aging and might be involved in age-related declines of cognitive performance (Vernaleken et al., 2007). However, very few studies to date have studied the relevance of asymmetric dopamine activity on behavioral effects. Thus, the laterality effect seen in our studies could be related to specific state induced by tDCS and further studies are needed to clarify this point.

7.2. Cognitive and clinical implication

As variations in the dopamine system are reported when applying tDCS over the frontal cortex, this suggests a possible behavioral and clinical relevance. Here, our results are put into perspectives depending on their possible implication in 1) neuro-cognitive effects, 2) anhedonia, 3) positive symptoms of schizophrenia, 4) interaction with medication, and 5) recreational use of tDCS. These perspectives should be taken with caution as our results are obtained in healthy subjects after a single session of tDCS, whereas clinical applications are based on the administration of repeated tDCS sessions in patients possibly exhibiting alterations in dopamine transmission and still under medication (interaction with tDCS effects).

7.2.1. Neuro-cognitive effects

Our findings of an increase in extracellular dopamine spatially located in the ventral striatum, obtained after a single session of frontal tDCS, are in line with the possible pro-cognitive effects seen after frontal tDCS in healthy subjects and in pathological conditions (Kuo and Nitsche, 2015; Lefaucheur et al., 2017). Indeed, connectivity studies have traced the structural and functional coupling between individual striatal and cortical regions and have shown that the DLPFC projects extensively to the ventral striatum, an overlap between regions involved in affective and cognitive processes, corresponding to the reward/motivation network. According to these studies, our clusters are located in the cognitive and affective regions of the striatum, regions anatomically linked to the DLPFC targeted by the tDCS montage. Moreover, multiple studies focus on the reciprocal influence of cognitive functions and variations in dopamine. Imaging studies have detected increases in ventral striatal extracellular dopamine concentrations during task components such as motor learning and execution, reward-related processes, stress and cognitive performance (Egerton et al., 2009). Moreover, predictions about anticipated future rewarding have been shown encoded in dopamine concentration in the ventral striatum, and that the amount of dopamine itself encodes the distance from the reward (Howes et al., 2013). In this line, pharmacological manipulations that enhance dopamine transmission such as addictive drugs and dopamine agonists, often act as neuroenhancers (Wise, 2004; Nutt et al., 2015). For example, a recent study supports this idea reporting that administration of dopamine's precursor L-Tyrosine (increasing dopamine level) can enhance working memory performance assesses with an N-back task (Jongkees et al, 2017).

However, it is important to acknowledge that different neurotransmitter systems, other than dopamine, interact to organize integrated behavior (Higgins and Fletcher, 2003; Kenny et al., 2009; Vlachou and Markou, 2010). In this line, tDCS studies have also reported an impact on other neurotransmitter systems such as serotonin, glutamate and GABA (Stagg et al, 2011; McLaren et al, 2017).

Furthermore, decreases in regions, such as the precuneus, part of the default mode network (DMN) were reported to enhance cognitive processes (Raichle, 2015). Indeed, during the performance of a novel or attention-demanding task, the DMN connectivity decreases while connectivity in its anti correlated network, the task positive network (TPN), increases.

Thus, our result seem to support the neuro-enhancement theory following frontal tDCS, even though the studies were not associated with behavioral findings. However, one thing to also keep in mind is that there is a mental cost to cognitive enhancement. Indeed, an interesting study suggested that enhancing one function could impair another. For example, stimulation of the parietal cortex facilitated numerical learning while impaired automaticity for the learned material. Conversely, stimulation to the DLPFC impaired the learning process and enhanced the automaticity (Iuculano and Cohen Kadosh, 2013).

7.2.2. Anhedonia

Our result with bifrontal tDCS suggest great promise in extending tDCS applications aiming to decrease symptoms and improve cognition in psychiatric and neurologic conditions characterized by dopamine transmission abnormalities within the reward/motivation network. Indeed, encouraging beneficial effects have already been reported after repeated bifrontal tDCS sessions in patients with depression (Brunoni et al, 2016; Brunoni et al, 2017; Loo et al, 2018; Sampaio-Junior et al, 2018; Pavlova et al, 2018), substance-related and addictive disorder (Jansen et al, 2013; Coles et al, 2018), schizophrenia with predominant negative symptoms (Palm et al, 2013; Pontillo et al, 2018; Osoegawa et al, 2018; Kennedy et al, 2018) and the cognitive alterations in Parkinson's disease (Leite et al, 2014). The potential efficacy of tDCS, possibly acting via increased dopamine after the stimulation, is in line with the current medication provided from these patients, which target the dopaminergic system.

Furthermore, the regions of the ventral striatum reported in our study are linked to several cognitive processes and are involved in the pathophysiology of several psychiatric disorders (Price and Drevets, 2012; Belujon & Grace, 2017; Brunelin et al, 2013; Zahodne et al., 2012; Hanganu et al, 2015; Nutt et al, 2015; Maia and Frank, 2017). Notably, anhedonia, the absolute or relative inability to experience pleasure, considered as a transnosographic criteria (RDoc, Cuthbert and Insel, 2013; Bedwell et al., 2014; Insel, 2014), is linked to alterations, such as decreased activation in ventral basal ganglia areas (Berton and Nestler, 2006; Gorwood, 2008; Bewernick et al., 2010; Zhang et al., 2016). For example, fMRI studies show that activation response of the ventral striatum to rewarding stimuli is decreased in depressive versus control groups and is correlated with the level of anhedonia (for review see Treadway and Zald, 2011; Price and Drevets, 2012). The involvement of the ventral striatum in depression is also sustained by the beneficial therapeutic effect obtained by deep brain stimulation of the nucleus accumbens (Schlaepfer et al., 2014). In this line, a recent meta-analysis supported the notion that anhedonia is characterized by alterations in reward processing and relies on frontal-striatal brain circuitry, using a transdiagnostic approach (Zhang et al, 2016).

Thus, we can hypothesize that targeting the frontal cortex with tDCS could restore the fronto-striatal dysregulation driving the anhedonia on a transnosographic basis. Similarly to drug treatment, frontal tDCS would not typically have an acute affect by rather show effects after repeated exposure (Remue et al., 2016). Thus, the dopamine release induced by a single tDCS session may not be directly responsible for these beneficial effects. Multiple sessions could involve adaptive downstream mechanisms leading to changes in dopamine receptor expression (Gershon et al., 2007).

Going further, future studies could investigate if the basal dopamine activity level or the change in extracellular dopamine evoked by a first bifrontal tDCS session could be a predictive marker of the therapeutic response obtained after applying multiple tDCS sessions over several days, protocol used in studies developing tDCS as a treatment for psychiatric disorders.

7.2.3. Positive symptoms of schizophrenia

Our results with fronto-temporal tDCS suggest great promise in extending tDCS applications aiming to decrease symptoms and improve cognition in psychiatric conditions characterized by dopamine transmission abnormalities. Indeed, encouraging beneficial effects have already been reported after repeated tDCS sessions in patients with schizophrenia with predominant positive symptoms (Brunelin et al., 2012; Bose et al., 2017). However, the potential clinical efficacy of tDCS, possibly acting via increased dopamine after the stimulation could seem contradictory to the pathophysiology of schizophrenia, reported to have a hyper-responsive dopaminergic system (Grace et al, 2016; Weinstein et al, 2017). Moreover, to date, current medications block the dopaminergic system, specifically via D2 receptors. Nevertheless, several hypotheses could be put forth explaining our result.

The first thing to keep in mind is that both antipsychotics drugs and fronto-temporal tDCS do not have an immediate clinical effect but need several weeks of exposure in order to obtain a clinical improvement. Here, we only investigated an immediate effect of a single session of fronto-temporal tDCS in healthy subjects without any ongoing medication. Thus, our acute effect could be thought of as contradictory with the pathophysiology of schizophrenia but maybe repeated tDCS would have a different impact notably involving adaptive downstream mechanisms (Fritsch et al., 2010). Another hypothesis, concerning the increase of dopamine reported after tDCS possibly reflecting a decrease in symptoms, could be that those changes in basal extracellular level induce changes in tonic versus phasic dopamine signaling mode, notably perhaps by the recruitment of dopamine D2 autoreceptors (Grace et al, 1991; Suaud-Chagny et al, 2004; Brunelin et al, 2013). In turn, this could perhaps decrease the hyper-reactive state seen in patients with schizophrenia. Furthermore, clinical studies deliver fronto-temporal tDCS concurrently to antipsychotic medication. Thus, perhaps this combination helps lead to a decreased dopamine reactivity and improvement of positive symptoms. In this line, a recent study reported that the affinity of the antipsychotic drug could predict tDCS effects. More specifically, patients with a high affinity antipsychotic showed less clinical improvement after add-on tDCS compared to patients with medication with lower affinity (Agarwal et al., 2016). Another possibility could be that the dopamine release observed here may be linked to the improvement of cognitive symptoms of schizophrenia rather that the positive symptoms also reported after fronto-temporal tDCS (Brunelin et al, 2012). Thus, the decrease of positive symptoms could be due to modifications of other brain areas.

Interestingly, alterations in resting-state networks are reported notably in the DMN (Nekovarova et al, 2014) could underlie an involvement in self-monitoring. Indeed, rs-fMRI studies have put forward an aberrant functional connectivity between the DMN and the TPN as a substrate to AVH severity (Jardri et al., 2012; Manoliu et al., 2014). More specifically, the DMN suppression usually reported in healthy subjects during a cognitive task is reduced in patients with schizophrenia (Nekovarova et al., 2014). Thus, our finding of a decreased perfusion in a hub of the DMN after fronto-temporal tDCS could support a potential clinical improvement.

Thus, one hypothesis would be that fronto-temporal tDCS could in part counterbalance the dysfunctions seen in the reward-motivation dopaminergic network and induce clinical efficacy after repeated sessions.

7.2.4. Interaction with medication

One important potential factor influencing the previous results could be tDCS interaction with medication given to patients, notably regarding dopamine. Indeed, different reviews have investigated the effect of medication on tDCS efficacy. The various types of pharmacological agents, influencing multiple neurotransmitter systems (GABA, dopamine, serotonin..) were reported to all impact tDCS effects (Brunoni et al, 2012; McLaren et al, 2017). More specifically, regarding the dopamine system, these studies have shown that modulating dopaminergic activity has a differential non-linear influence on the underlying cortical excitability (for details see intro Chapter 4). Based on these studies, a recent study evaluated the influence of antipsychotic drugs' affinity with dopamine D2 on the impact of tDCS in schizophrenia. Indeed, even though all antipsychotics bind to the D2 receptor, they vary with respect to their interaction with the receptor (Kapur and Seeman, 2000). That is, high affinity antipsychotic will allow for less availability of these receptors in comparison to low affinity antipsychotics. With this in mind, a study reported that patients with high affinity antipsychotic treatment showed less clinical improvement after add-on tDCS compared to patients on medication with lower affinity (Agarwal et al, 2016). Thus, these studies put forth the importance of taking into account medication when stimulation the brain.

7.2.5. Recreational use

tDCS devices are being increasingly used in a recreational manner with little or no warning to interaction with medication or psycho-stimulant, in particular those interacting with dopamine transmission. However, tDCS studies have found that recreational drugs, such as ketamine, phencyclidine, stimulant medications (amphetamines), and nicotine may affect the effects of tDCS (Nitsche et al, 2003, Nitsche et al, 2004, Nitsche et al, 2009, Grundey et al., 2012). To date, very few studies have investigated those interactions in repeated tDCS sessions.

Thus, our work reinforces the ongoing importance of controlled studies when using tDCS and should boost the research in this field to prevent the potentially unsafe use of tDCS in uninformed people.

7.3. Variability

Contradictory studies exist leading to significant doubts about the efficacy of tDCS, despite its promising cognitive and clinical potential. Evidence of high variability of tDCS responses between and within participants (Chew et al., 2015; Lopez-Alonso et al., 2014; Lopez-Alonso et al., 2015) lead to the formation of subgroups of participants depending on their response to the parameter investigated, may it be a clinical assessment or a cognitive task. With this in mind, the study designs need to take into account the individual variability and potential placebo effects in the results obtained (Horvath et al., 2014; Krause and Cohen Kadosh, 2014; Li et al., 2015; Wörsching et al., 2016; Bikson et al., 2017). In this context of the ongoing debate surrounding tDCS in the literature and based on the results of this thesis, the levels of dopamine activity and reactivity should be a new element adding to other parameters such as genetic variability, brain state dependency, placebo effect as well as individual head anatomy and electrode position for a general

hypothesis of brain modulation by bifrontal tDCS (Kraus & Cohen Kadosh, 2014; Opitz et al, 2015; Wörsching et al, 2016).

7.3.1. Brain state dependency

One potential confounding factor concerning the variability of tDCS effects is brain-state dependency. Indeed, as tDCS creates a small change in membrane voltage of cortical neurons, it cannot directly induce action potential firing but rather induces changes that can lead to action potential firing with sufficient endogenous activity. Studies have put forth the fact that the network needs to be active in order to induce visible effects in the brain. Moreover, the activated state or brain state has received recent and increasing interest in the entire NIBS community. Indeed, TMS studies have suggested that the effects of the stimulation are not only determined by the stimulus itself but are also affected by the underlying endogenous brain state (Silvanto and Pascual-Leone, 2008; Thut et al., 2011). In this line, tDCS studies suggest that the effects of tDCS are associated with the brain state of the subjects before and during the stimulation (Marshall, 2004; Antal et al., 2007; Dockery et al., 2009; Bortoletto et al., 2015). Furthermore, the timing of the stimulation might have a differential impact on performance or clinical efficacy depending if the stimulation is applied during or before/after the task (Nitsche et al, 2008). One possible explanation is related to the priming effect. This phenomenon occurs when a stimulus, deemed priming stimulus, activate neurons that remain active when a second stimulus, the test stimulus, is presented (Maljkovic and Nakayama, 1994, 1996). This increased level of activity is thought to facilitate target detection if the neurons activated are linked to the process encoding the test stimulus. Thus, tDCS could gate or modulate task-related plasticity (Ziemann and Siebner, 2008).

With a focus on the dopamine system, the level of brain activity is in part dependent on baseline dopaminergic activity. Notably, the brain-derived neurotrophic factor (BDNF) Val66Met and the catechol-O-methyltransferase (COMT) Val(108/158)Met polymorphisms are of relevant interest as they play a role in regulating dopamine levels and are involved in cognitive processes. Indeed, BDNF influences the release of dopamine in the ventral striatum (Berton et al., 2006), while COMT impacts dopamine availability in the frontal cortex (Meyer-Lindenberg et al., 2005). More specifically, in the general population two common variants of the COMT gene exist. Val-allele carriers were reported to have relatively high COMT activity leading to low baseline dopamine levels, whereas Met-allele carriers have relatively low COMT activity leading to high baseline dopamine levels. Furthermore, BDNF Val66Met and COMTVal158Met have been shown to interact on the fronto-striatal functional connectivity, depending on their polymorphism (**Figure 36**, Wang et al., 2015). Thus, it is thought that Met-allele carriers have better cognitive performance than those with the Val-allele (Cools and D'Esposito, 2011; Frank and Fossella, 2011).

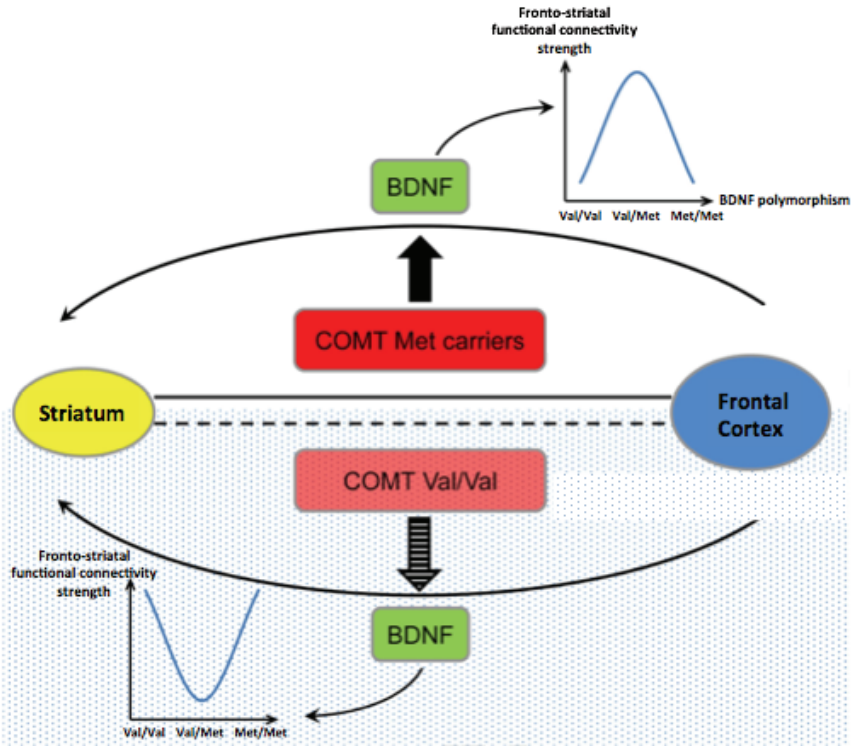

Figure 36 - Schematic representation of the interaction between BDNF Val66Met and COMT Val158Met. COMT Val-carrier would lead to a U-shape relationship depending on BDNF polymorphism concerning the strength of fronto-striatal functional connectivity. COMT Met-carrier would lead to an inverted U-shape relationship depending on BDNF polymorphism concerning the strength of fronto-striatal functional connectivity. Adapted from Wang et al, 2015

With this in mind, tDCS studies have investigated the impact of different allele carriers on the effect of tDCS. To date, no evidence is yet available for an association between BDNF polymorphisms and tDCS on cognitive functions. Indeed, Brunoni and colleagues (2013) found no impact of this genetic variant on therapeutic tDCS effects. However, concerning the COMT polymorphism, studies reported a differential effect on tDCS effects, i.e. anodal tDCS over the left DLPFC was associated with a reduction of executive functioning in COMT Met/Met carriers, whereas cathodal tDCS induced a deterioration of the inhibitory response in COMT Val/Val carriers only (Plewnia et al., 2013; Nieratschker et al., 2015; Wiegand et al., 2016). One hypothesis concerning the differential effects of tDCS depending on the COMT polymorphism is the inverted-U shape theory of frontal dopamine levels. Indeed, too high or too low dopamine levels are associated with impairment (Cools and D'Esposito, 2011; Schacht, 2016). **Figure 37** illustrates this hypothesis.

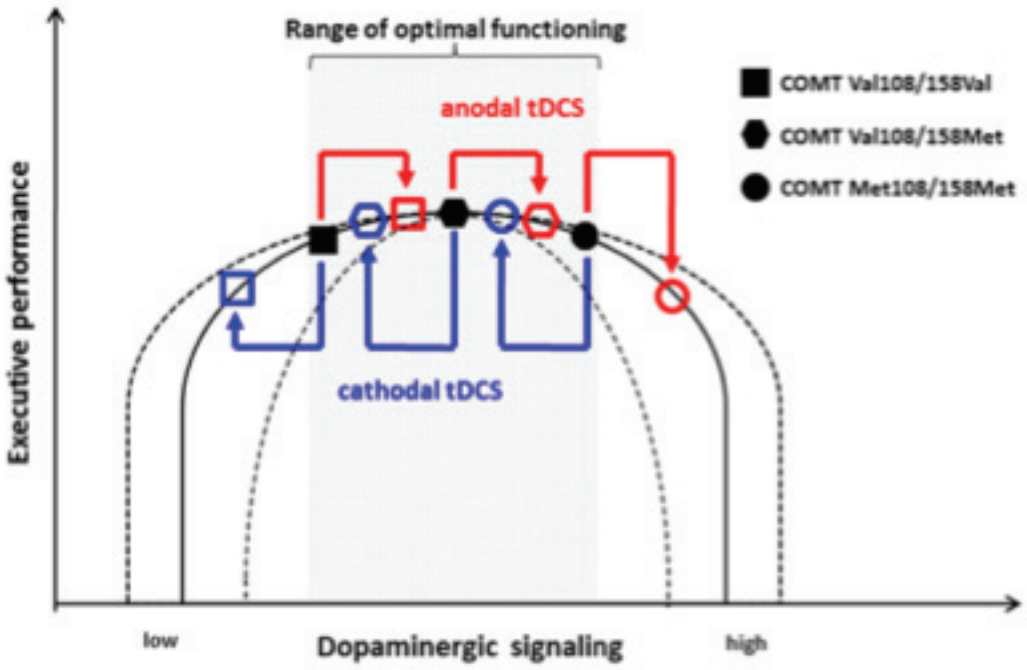

Figure 37 - Role of COMT polymorphism on tDCS effects - Inverted U-shape hypothesis - Based on the U-shape hypothesis, anodal and cathodal tDCS could exert a differential impact of executive performance depending on the individual dopaminergic signal level in the frontal cortex, which is related to individual genetic profile. COMT Val/Val would have a lower dopaminergic signal compared to COMT Val/Met, whereas COMT Met/Met would have a higher dopaminergic signal. Adapted from Wiegand et al, 2016.

Notably, subjects with a high dopamine baseline level in the frontal cortex would have reduced effects of anodal tDCS, whereas subjects with a low dopamine baseline level in the frontal cortex would have reduced effects of cathodal tDCS (Wiegand et al, 2016). Thus, depending on a participant's baseline, tDCS could shift their brain activity in or out of the optimum range of executive performances and possibly account for some of the unexpected effects reported (Li et al, 2015). This non-linear effect of tDCS is reported both in pharmacological studies (Monte-Silva et al, 2009; Monte-Silva et al, 2010; Fresnova et al, 2014a; Fresnova et al, 2014b) and in cognitive performances studies (Berryhill and Jones, 2012; Tseng et al., 2012; Hsu et al., 2014; Benwell et al., 2015; Learmonth et al., 2015; Heinen et al., 2016; Hsu et al., 2016; Looi et al., 2016). Thus, the impact of dopamine baseline seems to be an important factor to keep in mind and investigate further; especially regarding clinical population where altered dopaminergic activity is reported.

Thus, these studies point towards a complex interaction between genetic factors, variations in neuronal activity and the cognitive effects of tDCS. Therefore, we should be aware of these potential confounding factors when designing tDCS studies and analyzing our results.

7.3.2. Placebo, tDCS sham and dopamine

Dopamine has also been linked to the placebo effect, a powerful process improving several medical conditions by creating a response to an inert treatment intervention.

Molecular neuroimaging studies have contributed to our understanding of the neurobiological systems underlying the placebo effect, such as the top-down modulation of sensory and motor systems and the influence of cognition on the efficacy of a therapeutic intervention. However, these studies have shown that they are multiple placebo effects (Benedetti, 2014; Peciña et al., 2014; Peciña and Zubieta, 2015). On the one hand, concerning the antidepressant placebo effect, its mechanisms overlap with regions involved in the medication effects, suggesting that patients receiving placebo have a facilitation of brain changes needed for depression remission (Mayberg et al., 2002). For example, the baseline resting state functional connectivity within the salience network is linked to clinical placebo responses in major depression, i.e. an increased baseline resting state functional connectivity was associated with greater response to a placebo (Sikora et al., 2016). On the other hand, a study involving patients with Parkinson's disease reported a release of endogenous dopamine in the striatum in response to a placebo, this release being greater when patients perceived placebo benefit (De la Fuente-Fernández et al., 2001). With these observations, the placebo effect seems to involve more generally the dopamine system. Furthermore, this notion usually involved in clinical settings could be broadened to healthy control subjects to tDCS administration, perhaps via the process of expectation. This hypothesis seems comforted by a study reporting a dopamine release in the nucleus accumbens during a placebo administration (Scott et al., 2007).
However, not all subjects exhibit a placebo effect. Some studies have focussed on identifying reliable personality predictors of the placebo response. Thus, the responsiveness might be underlain by different traits such as optimism, empathy, motivation and extraversion (Geers et al., 2007; Darragh et al., 2014). The placebo-controlled study design developed in our studies overcame in part this problem. Moreover, the psychological assessment conducted did not reveal differences between active and sham groups regarding personality traits, motivation and anxiety.

Furthermore, in tDCS studies, a sham arm is needed to account for this potential physiological placebo effect. It is thought to keep participants and experimenters unaware of the intervention (active or sham) administered by mimicking typical initial sensations of active tDCS underneath the electrode sites (e.g., tingling, itching). However, what about sham tDCS effect in itself? Can it induce a biological effect independent of the placebo effect? Could this parameter contribute to the variability seen in clinical trials? This issue has not been thoroughly investigated in the literature so far and multiple sham protocols are being used with varying intensity (0.034mA to 2mA) and duration (5s to 30min) of sham stimulation. **See Annex 5; Fonteneau et al, Letter to Editor in preparation.**

7.3.3. Individual head anatomy and Electrode placement

When applying tDCS, two main challenges arise in order to be reproducible within and across studies: 1) The electrode positioning on skull landmarks do not always correspond to the same underlying brain targets (Seibt et al, 2015); 2) The current flow distribution is dependent on the underlying individual head anatomy and structural/functional connections (Datta et al, 2011).
The first thing to keep in mind is that tDCS electrodes are never inactive and always include at least two electrodes, an anode and a cathode. Thus, the use of terms such as "active" and "reference" or "return"

electrodes could be misleading. When investigating the biological effect of the stimulation, we should always keep in mind the current flow distribution that passes from an electrode to the other(s).
In the majority of studies (including ours), the electrodes are positioned on the scalp according to the International 10-20 system. For example, when targeting the DLPFC, the electrodes are positioned over F3/FP1 and F4/FP2 landmarks. Studies have shown that these landmarks were the closest to the regions targeted (Herwig et al., 2003; Okamoto et al., 2004; Jurcak et al., 2005). However, this method faces problems regarding the reliability of placement (manual measurements) and is time consuming. Recently, a new study indicated that current flow patterns could be optimized by positioning tDCS electrodes according to the individual anatomy (Seibt et al, 2015). This method, called the Omni-Lateral-Electrode-System (OLE-system; **Figure 38**), has been proven to precise the location of the target region and increase electrical field intensity in the targeted region. Interestingly, it is easy to use (i.e does not require MRI or neuronaviguation) and could help standardize procedure for electrode positioning.

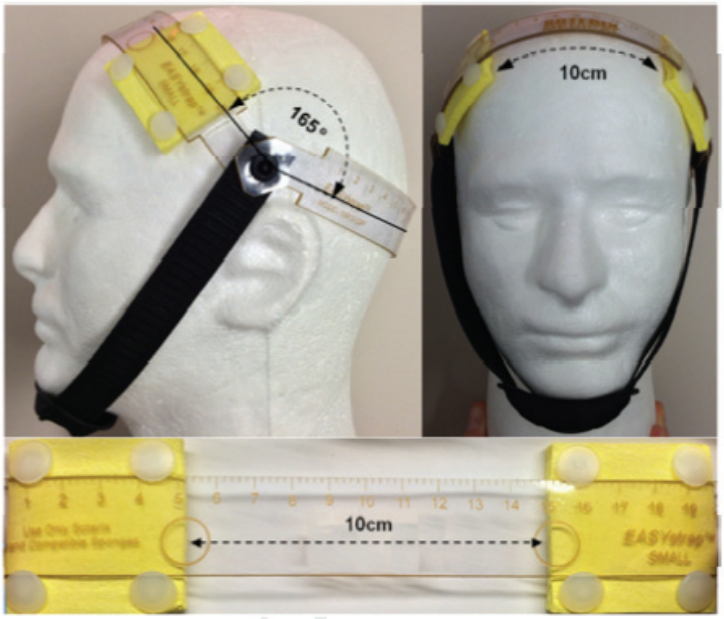

Figure 38 - OLE-system - The placement procedure is done in four steps. 1) EasyStrap size selection (small, medium, large); 2) Place the midpoint of occipital strap over inion; 3) Position the hinges that link occipital-, electrode- and chin strap over the most dorsal point on the ear; 4) Adjust the angle between occipital and electrode strap to 165 and the distance across the stars between the dorsal electrode edges to 10cm. Adapted from Seibt et al, 2015.

Furthermore, using electric field modeling, displacement or drift of the electrode about 1cm was reported to either have no significant impact on current flow (Bai et al, 2014) or change significantly the distribution of the electric field (Woods et al., 2015). Thus, the position of the electrode should be considered as a potential factor of variability.

7.4. A potential solution: Individualizing neuromodulation?

Even though the field of neurostimulation is booming for both clinical and recreational uses, caveats, such as those described above, need to be addressed. It is though that by better understanding the variability in

tDCS studies, we could increase its efficacy. Notably, novel approaches have been developed in order to reduce inter-individual variability, such as personalized stimulation using modeling to optimize targeting of a specific region or network (Cancelli et al., 2015). Furthermore, combining functional imaging and neurostimulation could help to improve our understanding of the underlying neuromodulation effects and find biomarkers of responsiveness (Bergmann et al., 2016). This approach is supported by the current NIMH directives in order to "achieve rigorous, reproducible and informative findings that support impactful device-based interventions" (Bikson et al, 2017).

7.4.1. Computational neurostimulation

In order to better understand the biological effects of tDCS and optimize its use, a novel field has emerged called computational neurostimulation, where biologically constrained computational models are used to provide mechanistic explanations of previous behavioral studies. They can also be used to test novel hypotheses for how the cellular and network effects of tDCS determine the behavioral outcome (Bestmann, 2015; Bikson et al, 2015; Rahman et al, 2015; Bonaiuto and Bestmann, 2015).

Modeling the effects of tDCS can be done on multiple levels depending on the question asked: (1) modeling the electric field distribution; (2) modeling cell polarization; (3) modeling the complex network; (4) modeling correlates of behavior and test novel hypotheses. For example, a recent study by Hammerer and colleagues (2016) introduced computational neurostimulation as a mechanistic tool to study neurostimulation effects on cognition. Indeed, using a biophysical attractor model and electric field modeling, they compared the behavioral predictions generated from a simulated neurostimulation (altered membrane polarization) to an actual human tDCS experiment.

Furthermore, concerning the individual head anatomy, studies have reported that the current flow reaching the brain deeply depends on individual anatomy as well as on structural and functional connections (Dmochowski et al., 2011; Datta et al., 2012; Dmochowski et al., 2013; Shahid et al., 2013, 2014; Opitz et al., 2015; Huang et al., 2017b), making it a possible parameter explaining inter-individual variability.

Going further, computational models can be used to design and simulate new electrode montages that could later be tested in healthy controls or clinical populations.

As of today, different tools are available for clinicians and researchers to implement individualized tDCS modeling such as workflow packages (ROAST, Huang et al., 2017a), stand-alone workflows (SCIRun, Dannhauer et al., 2012, http://www.sci.utah.edu/cibc-software/scirun.html), GUI-based simulation (BONSAI and SPHRERES, Truong et al., 2014; HD Explore, Soterix Medical, https://soterixmedical.com/research/software/hd-explore). A new promising open software: SimNIBS (Thielscher et al., 2015, Nielsen et al, in revision, http://simnibs.de/), has been developed in which one can implement individual anatomical MRI and DTI data as well as the tDCS montage. Adding DTI data is an especially interesting feature in order to estimate the conductivity tensor individually and thus create a better prediction of current flow.

This new avenue in computational neuromodulation seems promising, pending further validation, in order to guide and optimize clinical trials and neurocognitive studies.

7.4.2. Computational psychiatry

Nowadays, the psychiatric field faces challenges regarding disease classification. Indeed, the traditional approach is based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) delineating different diseases (e.g., depression, schizophrenia), which show some limitation due to substantial biological and clinical heterogeneity. Thus, to try and grasp this complexity, a recent field has emerged - computational psychiatry - which is based on mathematical modeling of psychiatric diseases. This approach is promising in order to understand the underlying mechanisms in psychiatric disease processes, such as the role of different neurotransmitters or the resting state networks disturbances, as well as to establish clinically useful single-subject results (Anticevic et al., 2012; Iglesias et al., 2016; Yang et al., 2016; Krystal et al., 2017)(Anticevic et al, 2012; Iglesias et al, 2016; Yang et al, 2016; Krystal et al, 2017). Interestingly, this fits in a novel research framework investigating mental disorders, i.e the Research Domain Criteria (RDoC), based on a transnosographic approach, leading to the clustering of disease in different subgroups. In addition, the interpretation of computational models could additionally guide the development of novel pharmacological or cognitive treatment strategies. The main objectives of this field have been summarized in **Figure 39**.

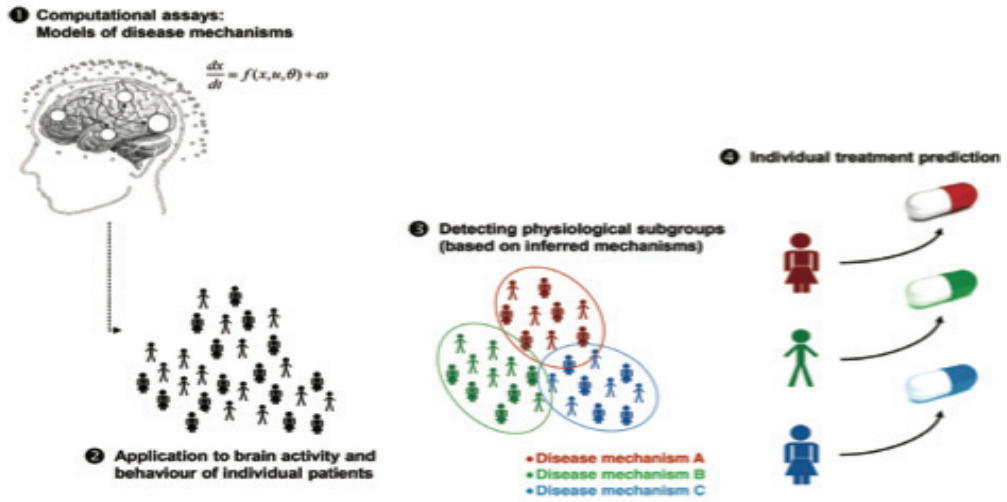

Figure 39: Computation Psychiatry - Main objectives of computational psychiatry in order to improve individual treatment. Adapted from Iglesias et al, 2016

Conclusion

The work presented here fits into a broad research axis intended to clarify brain mechanisms underlying frontal tDCS. As a first step, the specific aim of this thesis was to investigate this question in healthy subjects, specifically in relation to the dopaminergic system, with the goal to move on to patients in subsequent studies. In this context, two studies were performed using different electrode montages, which are being increasingly used in the literature (bifrontal tDCS and frontotemporal tDCS). We used an online study design and combined the stimulation with several imaging techniques, with the subject at rest. The online implementation of the stimulation allowed for deciphering potential changes induced not only after but also during the stimulation.

The first study reported the effect of a single-session of bifrontal tDCS with the anode over the left DLPFC and the cathode over the right DLPFC on the subcortical dopaminergic transmission during and immediately after the stimulation. Using a double blind sham-controlled design, 32 healthy subjects randomly received a single-session of either active (20min, 2mA; n=14) or sham (n=18) bifrontal tDCS during a dynamic positron emission tomography (PET) scan using [^{11}C]Raclopride binding. During the stimulation period, no significant effect of tDCS was observed. After the stimulation period, compared with sham tDCS, active tDCS induced a significant decrease in [^{11}C]Raclopride binding-potential ratio (BP_R) in the a specific part of the striatum, the right ventral striatum. This [^{11}C]Raclopride BP_R decrease suggests an increase in extracellular dopamine in a part of the striatum involved in the reward-motivation network. The present study provides the first evidence that bifrontal tDCS induces neurotransmitter release in polysynaptic connected subcortical areas.

The second study investigated the combined neurobiological impact of a fronto-temporal tDCS using a simultaneous multimodal imaging approach (PET-MR), in order to create a coherent ensemble to better understand the mechanisms of action of tDCS. In a double blind sham-controlled study, 30 healthy subjects randomly received an online single-session of either active (30min, 1mA; n=15) or sham (n=15) fronto-temporal tDCS during a simultaneous PET-MR scan.

The simultaneous acquisition of PET and MRI data offers new possibilities for understanding numerous aspects of brain function, such as integrating spatial and temporal components of neurotransmission, connectivity and brain activity. In our study, we explored the distributed changes in the dopaminergic system at rest through 1) Specific and localized dopaminergic transmission evaluated by PET using dopaminergic D2 subtype receptor availability via [^{11}C]Raclopride binding. 2) Spontaneous functional connectivity assessed by functional magnetic resonance imagery (fMRI). 3) Brain activity assessed by cerebral blood flow quantitatively and directly measured by arterial spin labeling (ASL). 4) Connectivity assessed by diffusion tensor imaging (DTI). However, using a novel scanner and combining these different modalities is not without its challenges and part of this thesis work was also to address them. Before analyzing the PET data, we optimized the attenuation correction (MaxProb, Merida et al, 2017) and movement correction (EBER; Reilhac et al, 2017) in order to have good quantification of our results. PET analysis showed a decrease in [^{11}C]Raclopride BP_{ND}, using the Logan Plot approach, reflecting an increase in extracellular dopamine induced by a dopamine release evoked by a single tDCS session. The impact on

dopamine transmission reaches statistical significance during the 15 to 30min period following the end of stimulation, in the associative/executive part of the left striatum. Interestingly, we report similar effects in dopaminergic subcortical structures with both the bifrontal and fronto-temporal montage. Furthermore, ASL analysis suggested a significant impact on brain perfusion in a region interconnected with the stimulation sites. Indeed, we showed a decreased perfusion in the bilateral superior parietal gyrus, notably the precuneus region, compared to the sham group. However, no significant effect on brain perfusion was reported in regions located underneath the electrodes, i.e. left DLPFC (anode) and left TPJ (cathode). As of today, the rs-MRI and DTI analyses are still ongoing. All the preprocessing has been performed. We expect to that they will bring a new piece to the puzzle in order to better understand the neurophysiological effects of fronto-temporal tDCS specifically on structural and functional networks related to the dopaminergic system. This analysis in an independent modality manner is however just the tip of the iceberg. Using a simultaneous PET-MR imaging, the goal will be to combine these modalities in order to create a coherent ensemble of the underlying neurobiological effects of a single-session of fronto-temporal tDCS.

Interestingly, when looking at both studies, both bifrontal and fronto-temporal tDCS, with anode over the left DLPFC as a common montage design, report similar effects concerning the location of the release of dopamine in a subregion of the striatum involved in the reward/motivation network. Thus, in the context of the ongoing debate surrounding tDCS in the literature and beyond the simple ‘excitatory-inhibitory’ model, levels of dopamine activity and reactivity should be a new element of the mosaic, adding to other parameters such as individual head anatomy variability, electrode position and brain state dependency for a general hypothesis of brain modulation by frontal tDCS.

With these notions in mind, further projects, notably expanding the protocol to neuropsychiatric patients, are of interest to translate our results to the clinical field. For example, a multi-centric clinical research program (PHRC-STIMZO-2015, 138 patients) granted our team to extend tDCS application to patient with schizophrenia using the same fronto-temporal montage and several imaging sequences such as resting-state fMRI and DTI.

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ANNEXES

1. Review - Does prefrontal non invasive brain stimulation alleviating symptoms in depression and schizophrenia impact emotion processing?

Revue - Santé mentale au Quebec

Titre

Neurostimulation du cortex préfrontal dorsolatéral : quels effets sur la symptomatologie et les émotions dans la dépression et la schizophrénie ?

Title

Does prefrontal non invasive brain stimulation alleviating symptoms in depression and schizophrenia impact emotion processing?

Auteurs

Marion PSOMIADES [a,*] ; Clara FONTENEAU [a,*] ; Marie-Françoise SUAUD-CHAGNY [a] ; Frédéric HAESEBAERT [a] ; Jérôme BRUNELIN [a, b, µ]

[*] MP et CF ont contribué de manière égale à ce travail

Affiliation

[a] : INSERM, U1028, Lyon Neuroscience Research Center, ΨR^2 Team, Lyon, F-69000, France ; CNRS, UMR5292, Lyon Neuroscience Research Center, ΨR^2 Team, Lyon, F-69000, France ; University Lyon, F-69000, France ; University Lyon 1, Villeurbanne, F-69000, France ; Centre Hospitalier Le Vinatier.

[b] : Centre interdisciplinaire de recherche en réadaptation et en intégration sociale (CIRRIS) de l'Université Laval, Québec (QC), Canada

[μ] Correspondance : Unité de Recherche UCB Lyon 1/Centre Hospitalier Le Vinatier Pole Est - Bat. 416 - 1er étage BP 300 39- 95 bd Pinel - 69678 BRON Cedex.

courriel : jerome.brunelin@ch-le-vinatier.fr

Tel : +33-4 37 91 55 65 ; Fax : +33-4 37 91 51 02

Titre

Neurostimulation du cortex préfrontal dorsolatéral : quels effets sur la symptomatologie et les émotions dans la dépression et la schizophrénie ?

Résumé

La stimulation magnétique transcrânienne répétée (rTMS) et la stimulation transcrânienne par courant continu (tDCS) sont des techniques de stimulation cérébrale non invasive actuellement utilisées comme solutions thérapeutiques dans plusieurs pathologies psychiatriques. Appliquées au niveau du cortex préfrontal dorsolatéral (CPFDL), elles ont montré leur efficacité pour diminuer les symptômes pharmaco-résistants chez les patients déprimés et chez les patients schizophrènes avec symptômes négatifs prédominants (SN). Le CPFDL est une structure cérébrale impliquée dans l'expression de ces symptômes et dans d'autres processus dysfonctionnels de ces deux pathologies comme les processus émotionnels. Le but de cette revue est d'établir s'il existe ou non un lien entre l'amélioration clinique et la modulation des processus émotionnels suite à la stimulation du CPFDL dans ces deux pathologies. Les données collectées montrent que l'amélioration des processus émotionnels n'est pas en lien avec l'amélioration clinique ni chez les patients déprimés ni chez les patients SN. Nos résultats suggèrent que bien que partageant des structures cérébrales communes, les réseaux cérébraux impliqués dans les processus émotionnels d'une part et les symptômes dépressifs ou les SN d'autre part seraient distincts.

Mots clés : Cortex prefrontal dorsolateral ; stimulation transcranienne ; émotion ; dépression ; schizophrénie

Title

Does prefrontal non invasive brain stimulation alleviating symptoms in depression and schizophrenia impact emotion processing?

Abstract

Repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are noninvasive brain stimulation techniques currently used as therapeutic tools in various psychiatric conditions. Applied over the dorsolateral prefrontal cortex (DLPFC), they showed their efficacy in reducing drug-resistant symptoms in patients with major depression and in patients with schizophrenia with predominantly negative symptoms. The DLPFC is a brain structure involved in the expression of these symptoms as well as in other dysfunctional processes observed in theses conditions such as emotional processes. The goal of this review is to establish whether or not a link exists between clinical improvements and modulation of emotional processes following the stimulation of the DLPFC in both conditions. The data collected show that improved emotional processes is not linked to a clinical improvement neither in patients with depressions nor in patients with negative schizophrenia. Our results suggest that although sharing common brain structures, the brain networks involved in both symptoms and in emotional processes would be separate.

Key words: Schizophrenia; Depression; Transcranial stimulation; Emotion; Dorsolateral Prefrontal Cortex

Introduction

Les techniques de stimulation transcrânienne non invasive comme la stimulation magnétique transcrânienne répétée (rTMS) et la stimulation transcrânienne par courant continu (tDCS) permettent de moduler la connectivité et l'activité cérébrale entrainant des modifications comportementales et cognitives subséquentes. Les études de neuroimagerie ont montré que les patients souffrant d'un épisode dépressif majeur et les patients schizophrènes avec symptômes négatifs prédominants (SN) présentent des anomalies structurales et fonctionnelles au niveau du cortex préfrontal dorsolatéral (CPFDL). Chez les patients présentant des symptômes pharmaco-résistants, il a été proposé d'appliquer ces techniques de neurostimulation comme des solutions thérapeutiques en les appliquant en regard de ces aires cérébrales dysfonctionnelles (Tortella *et al.*, 2015). Depuis la première étude menée en 1993 (Höflich *et al.*, 1993) chez les patients déprimés, plusieurs études contrôlées randomisées et plusieurs meta-analyses ont confirmé l'efficacité clinique de la rTMS appliquée au niveau du CPFDL pour diminuer les symptômes chez les sujets déprimés et chez les patients SN (Lefaucheur *et al.*, 2014). Des résultats prometteurs montrant l'efficacité clinique de la tDCS appliquée au niveau du CPFDL ont également été rapportés récemment chez les patients déprimés (Tortella *et al.*, 2015) et chez les patients SN (Mondino *et al.*, 2015).

Bien que l'efficacité clinique des techniques de neurostimulation soit bien établie dans ces deux indications, les mécanismes de l'effet thérapeutique restent incertains. Si le CPFDL est impliqué dans les symptômes dépressifs et dans les symptômes négatifs de la schizophrénie, il est également impliqué dans de nombreuses fonctions cognitives et notamment dans les processus émotionnels (Herrington *et al.*, 2005). Les processus émotionnels sont connus comme étant dysfonctionnels chez les patients déprimés (Beevers, 2005), et chez les patients schizophrènes (O'Driscoll *et al.*, 2014). Nous proposons ici de discuter les études qui ont mesuré les effets des techniques de neurostimulation appliquées en regard du CPFDL sur les symptômes et sur les processus émotionnels chez les patients déprimés et chez les patients SN afin d'établir s'il existe un lien ou non entre l'amélioration clinique et la modulation des processus émotionnels dans ces deux pathologies.

1. Stimulation du CPFDL chez les patients déprimés

L'épisode dépressif majeur (EDM) est un trouble invalidant avec une forte prévalence dans le monde occidental. Malgré les avancées des approches pharmacologiques, environ 30% des patients déprimés demeurent symptomatiques et sont considérés comme résistants aux traitements (Berlim et Turecki, 2007), justifiant le développement de nouvelles approches thérapeutiques. Plusieurs études (De Raedt *et al.*, 2014; Tortella *et al.*, 2015) ont rapporté l'efficacité des techniques de stimulation pour diminuer les symptômes dépressifs résistants. Dans cette indication, les zones cibles de traitement sont les CPFDL gauche et droit. La sélection de ces cibles est basée sur les études de neuroimagerie qui ont montré une asymétrie préfrontale chez les patients déprimés avec une hypoactivité du CPFDL gauche et une hyperactivité du CPFDL droit (Grimm *et al.*, 2008). Les protocoles de rTMS proposés utilisent soit une stimulation à haute fréquence sur le CPFDL gauche soit une stimulation à basse fréquence sur le CPFDL droit (Lefaucheur *et al.*, 2014). La tDCS a montré son efficacité avec le montage positionnant l'anode au niveau du CPFDL gauche et la cathode sur le CPFDL droit, la région supra-orbitaire droite ou dans une position extra-céphalique (Tortella *et al.*, 2015).

Par ailleurs, des études ont montré qu'il existait un déficit des processus émotionnels chez les patients déprimés avec notamment un biais attentionnel vers les stimuli négatifs ou un déficit de reconnaissance des émotions faciales (Gotlib et Joormann, 2010; Dalili *et al.*, 2015). Plusieurs études cliniques se sont ainsi intéressées au lien entre l'amélioration clinique de la symptomatologie dépressive dans sa globalité, évaluée par des échelles d'évaluations psychométriques standardisées (e.g., MADRS, HDRS) et les effets des techniques de neurostimulation sur l'« humeur » évaluée par des échelles visuelles analogiques (EVA), par des échelles d'évaluation psychométriques standardisées telle que la Profile of Mood States (POMS), la Positive and Negative Affect Schedule (PANAS) ou encore par des tests cognitifs comme le Go/NoGo affectif.

1.1 Effet sur l'humeur

Dans une première étude contrôlée randomisée, Dang et collaborateurs (Dang *et al.*, 2007) ont montré que bien que la rTMS active diminuait significativement les symptômes de la dépression (Avery et al., 2006), elle n'entrainait pas d'effet sur l'humeur des patients déprimés. Dans cette étude, 68 patients (35 actifs, 33 placebo) devaient coter leur humeur sur une EVA à 5 items mesurant tristesse, anxiété, joie, fatigue et douleur. Les patients recevaient 15 sessions de rTMS à haute fréquence (10Hz) sur le CPFDL gauche. Dans une autre étude évaluant l'effet de 10 et 15 sessions de rTMS haute fréquence (10 Hz) en regard du CPFDL gauche, Anderson et collaborateurs (2009) ont montré que chez 20 patients non cliniquement répondeurs à la rTMS, la rTMS n'avait pas non plus d'effet sur l'humeur mesurée à l'aide d'une EVA à 15 items (joie, irritabilité, colère, excitation, confusion, calme, tristesse, anxieux, nerveux, ennuyeux, relaxé, fatigué, distrait, douleur, inconfort).

Ces résultats sont en contradiction avec l'étude contrôlée de Szuba et collaborateurs (2001) réalisée chez 14 patients (9 actifs, 5 placebo) qui recevaient 10 séances de rTMS haute fréquence (10 Hz) sur le CPFDL gauche. Cette étude, incluant des patients déprimés sans traitements médicamenteux a montré une amélioration immédiate des items dépression, anxiété et colère mesurés par la Profile of Mood States (POMS) suite à la rTMS active comparée à la stimulation placebo. Cette amélioration n'était néanmoins pas significativement liée à une amélioration clinique. Finalement, dans une étude contrôlée en cross-over versus placebo utilisant l'échelle PANAS (Positive and Negative Affect Schedule) qui mesure l'humeur et les sensations, Palm et collaborateurs (2012) ont montré que 10 séances de tDCS (1 ou 2 mA, 20 min) avec l'anode appliquée sur le CPFDL gauche et couplée à la cathode sur l'aire supra-orbitale droite entrainaient une augmentation significative des émotions positives et une tendance à la diminution pour les émotions négatives chez 22 patients déprimés. Dans cette étude, la tDCS active appliquée au niveau du CPFDL n'entrainait cependant pas de modification des symptômes dépressifs. Ces études suggèrent qu'il n'y a pas de lien entre amélioration clinique et changement de l'humeur chez les patients déprimés.

1.2 Effets sur les processus attentionnels émotionnels

Bermpohl et collaborateurs (2006) ont mis en évidence que la réponse clinique à la rTMS et l'impact sur les émotions variaient selon le site de stimulation et la sévérité de la dépression. Les auteurs ont stimulé 18 patients déprimés à différents stades de la maladie, 10 en phase aigüe et 8 en rémission partielle ou totale. Les stimulations étaient délivrées à basse fréquence (1 Hz) sur trois zones cibles (CPFDL gauche, CPFDL droit ou Cortex Occipital). Chaque sujet recevait chacune des séances de stimulation de manière randomisée et réalisait une tâche de Go-No-Go affective (AGN). Dans cette tâche, les sujets devaient répondre à des stimuli d'une valence émotionnelle spécifique tout en inhibant la réponse aux stimuli de valences opposées. La rTMS appliquée sur le CPFDL droit améliorait les performances pour les patients déprimés en phase aigue comparativement aux patients en rémission partielle ou totale. La stimulation du CPFDL gauche entraînait une altérations des performances chez les patients en rémission partielle ou totale mais pas chez les patients en phase aigue. Ces résultats sont en adéquation avec la théorie d'un mauvais équilibre entre le CPFDL gauche hypoactif et le CPFDL droit hyperactif observé dans la physiopathologie de la dépression. A noter que dans cette étude, les effets de la rTMS ne sont pas différents pour les réponses aux stimuli positifs ou négatifs. Aucun effet clinique n'a été recherché dans cette étude.

En utilisant la même tâche AGN, Boggio et collaborateurs (2007) ont montré que 10 séances de tDCS active (2mA, 20min) avec l'anode appliquée sur le CPFDL gauche et la cathode sur l'aire supra-orbitale droite amélioraient les performances pour les images avec un contenu émotionnel positif chez des patients non traités par traitement antidépresseurs pharmacologiques. Dans cette étude, aucune amélioration clinique n'a été rapportée chez ces 12 patients recevant la tDCS active suggérant qu'il n'y a pas de lien direct entre amélioration des processus émotionnels et amélioration des symptômes.

Dans une dernière étude, Leymann et collaborateurs (Leyman *et al.*, 2011) ont observé une corrélation entre l'amélioration clinique et l'amélioration de performances des sujets pour inhiber leur réponse aux stimuli tristes. Les processus inhibiteurs des informations émotionnelles étaient mesurés grâce à la tâche « Negative Affective Priming » (NAP) avec des visages émotionnels neutres, exprimant la joie ou la tristesse. La rTMS

était appliquée à haute fréquence (10Hz) en regard du CPFDL gauche chez 14 patients déprimés ne recevant pas de traitement antidépresseur pharmacologique. Les auteurs ont rapporté que 9 des 14 patients répondaient cliniquement à la stimulation (10 sessions) avec une diminution d'au moins 50% de leur score de dépression mesuré par des échelles cliniques psychométriques standardisées (HDRS) tandis qu'aucun effet de la rTMS n'a été observée sur l'humeur cotér avec une EVA à 5 items mesurant dépression, colère, tension, fatigue, vigueur. Cette étude suggère qu'il n'y a pas de lien entre amélioration des symptômes et amélioration de l'humeur mais qu'il existe un lien entre amélioration des symptômes et amélioration des performances d'inhibition des processus attentionnels émotionnels dans une tâche avec des émotions faciales.

2. Stimulation du CPFDL chez les patients schizophrènes

La schizophrénie est une pathologie psychiatrique sévère et invalidante avec une expression clinique hétérogène (Buckley *et al.*, 2009). Les symptômes de la schizophrénie sont traités en première intention par antipsychotiques mais environ 30% des symptômes sont résistants à la pharmacologie. Ces dernières années, les techniques de neurostimulations ont montré leur intérêt dans les traitements des symptômes résistants de la schizophrénie et notamment les symptômes négatifs (SN). Dans cette indication, des stimulations rTMS à haute fréquence (Lefaucher *et al.*, 2014) ou par tDCS anodique sont appliquées en regard du CPFDL gauche (Mondino *et al.,* 2015 ; Tortella *et al.*, 2015).

A ce jour, bien que de nombreuses études incriminent un déficit de processus émotionnel comme la reconnaissance des émotions faciales chez les patients schizophrènes (e.g., Kohler *et al.*, 2010), peu d'études se sont intéressés aux corrélats entre amélioration clinique et modulation des processus émotionnels ou de l'humeur suite à des stimulations du CPFDL.

Dans une étude récente, Wolwer et collaborateurs (2014) ont montré que 10 sessions de rTMS haute fréquence (10 Hz) appliquées en regard du CPFDL gauche chez 18 sujets atteints de schizophrénie amélioraient leurs performances dans une tâche de reconnaissances des émotions faciales (joie, peur, colère,

surprise, dégout et tristesse) comparé au groupe de 14 patients ayant reçu la rTMS placebo. Le pourcentage de réponses correctes pour l'identification des émotions (joie, peur, colère, surprise, dégout et tristesse) était augmenté par la rTMS active (+8.9%) comparativement à la rTMS placebo (+1.6%). Dans cet échantillon de patients, Cordes et collaborateurs (2010) n'ont pas montré d'amélioration clinique après rTMS active comparée à la rTMS placebo. Cependant, une analyse en sous-groupe montrait une amélioration significative des scores PANSS (Positive And Negative Syndrome Scale) après rTMS active dans le sous-groupe de patients présentant des symptômes négatifs sévères comparé au placebo. Dans ce sous-groupe de patients, il y avait également une amélioration significative des performances en tâche de reconnaissance des émotions faciales après rTMS active comparée à placebo. Cependant aucune corrélation n'a été trouvée entre ces deux améliorations.

Dans une étude plus récente, Rassovsky et collaborateurs (2015) ont montré que la tDCS avec l'anode en regard du CPFDL gauche et la cathode en regard du CPFDL droit améliorait l'identification des émotions faciales chez les patients atteints de schizophrénie. Dans cette étude, 36 patients atteints de schizophrénie complétaient la tâche de Facial Emotion Identification Test (FEIT). Les 24 patients qui recevaient la séance de tDCS active (20 minutes, 2 mA) amélioraient spécifiquement leur performance de reconnaissance des émotions indépendamment d'autres processus cognitifs (perception sociale et interférence sociale) comparativement aux 12 sujets qui recevaient la tDCS placebo. Aucun lien avec les symptômes n'a été recherché dans cette étude.

Discussion

Au travers de cette revue des travaux portant sur les effets des techniques de stimulation du CPFDL sur les processus émotionnels dans les pathologies dépressives et schizophréniques, nous avons mis en exergue des impacts cliniques et émotionnels spécifiques (humeur, processus attentionnel émotionnel, reconnaissance des émotions faciales).

Dans la dépression, les études n'ont pas montré de modification de l'humeur chez les patients traités par techniques de neurostimulation (Dang *et al.*, 2007 ; Anderson *et al.*, 2009 ; Leyman *et al.*, 2011). Les études ayant mesuré les capacités attentionnelles émotionnelles à l'aide du Go-NoGo affectif n'ont pas montré de relation entre amélioration clinique et amélioration des processus émotionnels après stimulation chez des patients traités par antidépresseurs pharmacologiques (Bermpohl *et al.*, 2006). Au total, la stimulation du CPFDL peut modifier les processus émotionnels chez les patients déprimés non traités par antidépresseurs pharmacologiques en induisant une modification de l'humeur (mesuré par une EVA Szuba *et al.*, 2001) ou une modificaiton des processus attentionnels émotionnels (Leyman *et al.,* 2011 ; Boggio *et al.*, 2009). Seuls, Leyman et collaborateurs (2011) ont rapporté une corrélation entre amélioration clinique et amélioration des processus émotionnles sans toutefois montrer un effet sur l'humeur. De nouvelles études permettraient d'identifier les liens entre symptômes depressifs, humeur et processus attentionnles émotionnels.

Concernant la schizophrénie, les résultats suggèrent que les techniques de neurostimulation sont capables de modifier la reconnaissance des émotions faciales chez les patients (Rassovsky *et al*., 2015 ; Wölwer *et al.,* 2014) mais aucun effet sur l'humeur n'a été rapporté. Cependant, aucune corrélation n'a été retrouvée entre l'amélioration de la symptomatologie négative et l'amélioration des performances de reconnaissance des émotions faciales chez les patients atteints de schizophrénie après stimulation du CPFDL (Wölwer *et al.,* 2014).

Au final, ces données montrent que l'amélioration de l'humeur n'est pas en lien avec l'amélioration clinique observée chez les patients déprimés. Il semble ne pas y avoir de lien entre l'amélioration des processus émotionnels et l'amélioration clinique ni chez les patients déprimés recevant un traitement pharmacologique ni chez les patients schizophrènes. Nos résultats suggèrent que les réseaux neuronaux sous-tendant la symptomatologie des patients et les réseaux neuronaux impliqués dans les processus émotionnels et l'humeur sont des réseaux partiellement distincts qui partagent au moins une structure commune : le CPFDL. Cependant, l'application des techniques de neurostimulation en regard du CPFDL ne suffirait pas à

moduler simultanément les symptômes, l'humeur et les processus émotionnels. Cependant, cet effet plafond des traitements pharmacologiques n'empêche l'effet effet antidépresseur de la stimulation, qui dans ce cas ne serait pas lié à l'effet sur les émotions. Cette dissociation pose à lors la question d'un mécanisme distinct des stimulations, des antidépresseurs et de la combinaison de ces deux approches sur les symptômes et sur les émotions. Les données actuelles ne permettent pas d'expliquer ce phénomène.

Dans la dépression, cette absence de lien entre symptomatologie et processus émotionnel est en accord avec certains résultats observés dans des études cognitives qui n'ont pas montré de corrélation entre l'intensité des symptômes (mesurés par la BDI) et la sévérité des déficits dans les processus émotionnels (Bylsma *et al.*, 2008). Néanmoins, de nombreuses autres études ont montré des associations entre les déficits des processus émotionnels et la symptomatologie des patients atteints de dépression (Dalili *et al.*, 2015; Rottenberg *et al.,* 2002). A côté de ces travaux sur la dépression, chez les patients souffrant de schizophrénie, aucun consensus n'existe sur l'association entre les déficits des processus émotionnels et les symptômes. Certaines études ont montré qu'il existait un lien entre les déficits de processus émotionnels et la symptomatologie négative (Balogh *et al.*, 2014) alors que d'autres ont montré qu'il existait un lien entre les déficits de processus émotionnels et la symptomatologie positive et les déficits cognitifs (Laroi *et al.,* 2010). Ces travaux restent toutefois limités.

Il est important de noter qu'il est difficile de comparer les études entre elles de part la multiplication des échelles de mesures des symptômes (e.g., HDRS, MADRS, PANSS, SANS) et des façons de mesurer les processus émotionnels (e.g., EVA de l'humeur, PANAS, POMS, test cognitifs émotionnel, test de reconnaissance des émotions faciales). Par ailleurs, dans la plupart des études, les patients inclus bénéficiaient de traitements pharmacologiques (antidépresseurs et antipsychotiques). Hors, les traitements pharmacologiques peuvent modifier les processus émotionnels. En effet, s'agissant des traitements antidépresseurs, des études réalisées chez les sujets sains et les patients déprimés ont montré un effet bénéfique de ces traitements sur la capacité de reconnaissance des émotions (Harmer *et al.,* 2013; Shiroma

et al., 2014; Tranter *et al.*, 2009). Il est intéressant de noter que les seules études qui ont montré un effet des neurostimulations sur les processus émotionnels ont inclus des sujets atteints de dépression ne bénéficiant pas de traitement antidépresseur pharmacologique (Szuba *et al*., 2001 ; Leyman *et al.*, 2011 ; Boggio *et al.*, 2007). Ces données suggèrent qu'un traitement antidépresseur pharmacologique ou par neurostimulation peut améliorer les processus émotionnels chez les sujets déprimés. Les résultats négatifs des études de neurostimulation ne montrant pas d'amélioration des processus émotionnels chez des sujets déprimés avec traitement pharmacologique pourraient s'expliquer par un effet plafond des traitements antidépresseurs sur les processus émotionnels. Cependant, cet effet plafond des traitements antidépresseurs pharmacologiques n'empêche pas l'effet antidépresseur de techniques de neurostimulation mais uniquement son effet sur les processus émotionnels. Cette dissociation pose la question d'un mécanisme distinct des traitements antidépresseurs pharmacologiques, des techniques de neurostimulation et de l'association de ces deux approches sur les symptômes dépressifs, l'humeur et les processus attentionnels émotionnels.

Dans la schizophrénie, les résultats concernant les effets des traitements antipsychotiques sur les processus émotionnels sont controversés. Bien qu'une revue récente de la littérature rapporte un manque de preuve quant à l'efficacité des traitements antipsychotiques sur les processus émotionnels (Hempel *et al.*, 2010), Fakra et collaborateurs (2009) ont montré que les traitements antipsychotiques pouvaient améliorer les processus de discrimination des expressions faciales émotionnelles. Nos résultats suggèrent que, contrairement à ce que l'on observe dans la dépression, les neurostimulations peuvent moduler les processus émotionnels chez les sujets schizophrènes qui reçoivent des traitements pharmacologiques. Les relations entre techniques de neurostimulation et traitement pharmacologique restent à être explorer dans des futures études chez les patients déprimés et schizophrènes.

L'effet des techniques de neurostimulation ne dépend pas seulement des paramètres de stimulation (e.g., cible, fréquence, intensité) et des traitements associés, mais également de l'état d'activation des réseaux neuronaux pendant la stimulation (Silvanto & Pascual-Leone, 2008). En ce sens, Isserles et collaborateurs

(2011) ont montré que la modulation de l'état émotionnel des patients atteints de dépression pendant les séances de rTMS permettait d'améliorer l'efficacité clinique de la stimulation. Ainsi appliquer la rTMS lorsque les patients ressentent des émotions positives est plus efficace que lorsque les sujets ressentent des émotions négatives. Cette étude suggère qu'il existe une interaction entre les processus émotionnels et l'effet thérapeutique des techniques de neurostimulation.

Enfin, si le lien entre amélioration clinique et amélioration des déficits des processus émotionnels et de l'humeur est loin d'être linéaire après stimulation du CPFDL, l'effet sur les émotions pourrait cependant être utilisé comme marqueur prédictif de la réponse chez les patients déprimés recevant de la stimulation sur d'autres zones cérébrales dysfonctionnelles ou à l'aide d'autres techniques. En ce sens, Downar et collaborateurs (2014) ont montré que les sujets déprimés qui répondaient à la stimulation par rTMS à haute fréquence du cortex préfrontal dorsomédian (CPFDM), présentaient des scores d'anhédonie plus bas que les sujets non répondeurs avant les séances de stimulation. Cette étude suggère que l'intensité de l'anhédonie qui est liée aux processus émotionnels pourrait être un marqueur prédictif de la réponse thérapeutique à la stimulation. Dans une autre étude, Levkovitz et collaborateurs (2011) ont montré que moins les sujets étaient apathiques, plus ils avaient des chances de répondre à des séances de « deep TMS » appliquée à haute fréquence sur le CPFDL.

Conclusion

Au total, il semblerait qu'il n'y ait pas de lien direct entre l'amélioration des processus émotionnels (humeur, processus attentionnels émotionnels et reconnaissance des émotions faciales) et l'amélioration clinique chez les patients déprimés et schizophrènes recevant des stimulations en regard du CPFDL. L'effet des traitements pharmacologiques et notamment des antidépresseurs pourrait être un facteur confondant, dont l'impact reste à préciser. L'étude des processus émotionnels (anhédonie et apathie) pourrait cependant s'avérer intéressante dans le but de dégager des marqueurs prédictifs de réponse aux techniques de neurostimulations.

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2. Book Chapter - Schizophrenia - tDCS in Neuropsychiatric Disorders: Clinical Principles and Management

Chapter Schizophrenia In “Transcranial Direct Current Stimulation in Neuropsychiatric Disorders: Clinical Principles and Management », by Brunoni AR, Nitsche MA, Loo CK. Springer NY, USA

Title

Schizophrenia

Authors

Marine MONDINO [a,b], Clara FONTENEAU [c], Jérôme BRUNELIN [a,c]

Affiliation

a Centre interdisciplinaire de recherche en réadaptation et en intégration sociale, Université Laval, Québec, CANADA

b Centre de Recherche de l’Institut Universitaire en Santé Mentale de Québec, Université Laval, Québec, CANADA

c INSERM, U1028, CNRS, UMR5292, Lyon Neuroscience Research Center, ΨR2 Team, Lyon, F-69000, France ; University Lyon, F-69000, France ; University Lyon 1, Villeurbanne, F-69000, France ; Centre Hospitalier Le Vinatier.

ABSTRACT

This chapter proposes an overview of current evidence and future directions for using tDCS in schizophrenia. To date, the effects of tDCS have been investigated in three main outcomes: 1) to alleviate auditory verbal hallucinations using a fronto-temporal tDCS montage (the anode placed over the dorsolateral left prefrontal cortex coupled with the cathode placed over the left temporoparietal junction); 2) to alleviate negative symptoms using a frontal montage (the anode placed over the left dorsolateral prefrontal cortex coupled with the cathode placed over the right dorsolateral prefrontal cortex, the right supraorbital region or extra-cephalically) and 3) to enhance cognitive functions, using different tDCS montages. Promising results have been reported for these 3 outcomes. tDCS can decrease the severity of symptoms such as auditory verbal hallucinations and negative symptoms by about 30% and enhance a wide range of cognitive functions (e.g., working memory, self-monitoring, facial emotion recognition). However, most studies to date are case-reports and open labeled studies with small samples. Thus, large randomized controlled studies are needed to confirm the usefulness of tDCS in schizophrenia.

KEYWORDS

Schizophrenia, auditory verbal hallucinations, negative symptoms, cognition, tDCS.

1. INTRODUCTION

Schizophrenia is a frequent and debilitating psychiatric condition occurring in about 1% of the general population. The clinical expression of schizophrenia is heterogeneous and symptoms are usually classified into five main dimensions: positive (e.g., hallucinations, delusions), negative (e.g., flat expression, avolition), depression, disorganization and grandiosity/excitement. Symptoms of schizophrenia are usually alleviated by psychopharmacological medications. However, up to 30% of treated patients still report disabling symptoms such as auditory verbal hallucinations, negative symptoms and cognitive deficits (Shergill et al. 1998; Murphy et al. 2006). These treatment-resistant symptoms are associated with a higher risk of relapse and worse prognosis, justifying the need for developing novel alternative approaches.

Over the last decade, various non-pharmacological approaches such as non-invasive brain stimulation (NIBS) techniques have been developed in order to alleviate treatment-resistant symptoms in patients with schizophrenia. NIBS techniques are safe tools to modulate brain activity and connectivity in living humans. These approaches were based on neuroimaging studies that have highlighted some brain correlates of schizophrenia symptoms: auditory verbal hallucinations were associated with hyperactivity in the left temporoparietal region (Jardri et al. 2011) and fronto-temporal dysconnectivity (Lawrie et al. 2002); negative symptoms and cognitive deficits were associated with structural and functional abnormalities in the prefrontal cortices (Sanfilipo et al. 2000). According to their neuromodulatory effects, NIBS techniques were thus proposed to reduce treatment-resistant symptoms in patients with schizophrenia by targeting the brain regions that showed abnormal activities. One of the NIBS techniques recently used in these patients is transcranial direct current stimulation (tDCS).

The first studies investigating the use of tDCS to improve symptoms of schizophrenia have been published in 2011. Since then, a rapid increase in the number of published articles in the field was observed (Figure 1) – in fact, 20 studies investigating the clinical interest of tDCS in schizophrenia were indicated as

'ongoing' on clinicaltrials.gov database in September 2015 (10 in North America, 4 in Europe, 2 in Middle East, 1 in Australia, 1 in South America, 1 in Africa and 1 in East Asia) suggesting the international growing interest of tDCS for schizophrenia.

INSERT FIGURE 1 ABOVE HERE

Two tDCS montages for schizophrenia have been mostly used. The first one, a fronto-temporal electrode montage, is proposed to reduce treatment-resistant auditory verbal hallucinations. In this montage, the anode (presumably excitatory) was placed over the left prefrontal cortex and the cathode (presumably inhibitory) was placed over the left temporoparietal junction (Brunelin et al. 2012a,b). The second one is proposed to reduce treatment-resistant negative symptoms and to improve cognitive functions by targeting the left prefrontal region. In this montage, the anode was placed over the left dorsolateral prefrontal cortex (DLFPC) and the cathode over the right supraorbital region, the right DLPFC or extra-cephalically (Palm et al. 2013a, 2014).

The aim of this chapter is to investigate whether tDCS can alleviate symptoms and improve cognitive functions in patients with schizophrenia. Hence, we reviewed studies investigating the clinical effects of tDCS on auditory verbal hallucinations, negative symptoms and other symptoms of schizophrenia. We also reviewed studies focusing on the effects of tDCS on cognitive functions in patients with schizophrenia. After a description of current evidence regarding the interest of using tDCS in patients with schizophrenia and the brain correlates of clinical and cognitive improvements, we also discuss the safety of this approach and how tDCS parameters can be optimized to improve efficacy.

2. EFFECTS OF FRONTO-TEMPORAL TDCS ON AUDITORY VERBAL HALLUCINATIONS

Twenty-one studies investigated whether tDCS targeting the fronto-temporal network can improve the symptoms of treatment-resistant auditory verbal hallucinations in patients with schizophrenia (see table I). Among them, 3 randomized sham-controlled studies have reported a significant effect of active tDCS on auditory verbal hallucinations as compared to sham (Brunelin et al. 2012b; Mondino et al. 2015a,b). In the first one (Brunelin et al. 2012b), 30 patients with schizophrenia received 10 sessions of 20 minutes of either active (2mA) or sham tDCS delivered twice daily on five consecutive days. Electrodes were placed on the scalp based on the 10/20 international EEG system, with the center of the anode placed between F3 and FP1 (assuming to correspond to the left prefrontal cortex) and the center of the cathode placed between T3 and P3 (assuming to correspond to the left temporoparietal junction). Auditory verbal hallucinations were assessed using the Auditory Hallucination Rating Scale (AHRS). Patients receiving active tDCS reported a significant 31% decrease of their treatment-resistant auditory verbal hallucinations whereas patients receiving sham tDCS reported a non-significant 8% decrease (Brunelin et al. 2012b). Remarkably, the effect of tDCS on auditory verbal hallucinations was still significant at 1 and 3-month follow-up (Brunelin et al. 2012b).

Similar results were reported using the same tDCS protocol in 2 randomized controlled studies published in 2015 (Mondino et al. 2015a, b). It is important to stress that samples enrolled in these studies partially overlapped with the study sample of Brunelin et al (2012b). In the first study, Mondino et al. (2015a) reported a significant 46% reduction in the frequency of auditory verbal hallucinations assessed by the first item of the AHRS after 10 sessions of active tDCS whereas a non-significant 10% decrease was reported in the sham group. In the second one, a significant 28% decrease in auditory verbal hallucinations measured by the AHRS was reported after the 10 sessions of active tDCS whereas a non-significant 10% decrease was reported in patients receiving sham tDCS (Mondino et al. 2015b).

Using the same electrodes montage, promising effects of tDCS for reducing auditory verbal hallucinations were also reported in 4 open labeled studies including 23 (Shivakumar et al. 2015), 21 (Bose

et al. 2014), 16 (Brunelin et al. 2015) and 6 (Ferrucci et al. 2014) patients with schizophrenia. All studies included patients with schizophrenia receiving 10 sessions of 20 minutes of active 2mA tDCS delivered twice daily on five consecutive days. In the first one, Shivakumar et al. (2015) recruited 23 patients and assessed their auditory verbal hallucinations using the 'auditory hallucination' subscale of the Psychotic Symptom Rating Scale (PSYRATS). Patients showed a nearly 30% significant decrease of their treatment-resistant auditory verbal hallucinations after tDCS. Bose et al. (2014) recruited 21 patients and assessed the auditory verbal hallucinations, also using the 'auditory hallucination' subscale of the PSYRATS. After tDCS, patients showed a significant decrease (32.7%) in auditory verbal hallucinations. Brunelin et al. (2015) recruited 16 patients and assessed their auditory verbal hallucinations using the AHRS. After tDCS, patients showed a significant 20% decrease in auditory hallucinations. In Ferrucci et al. (2014), 6 patients were included and assessed using the Cardiff Anomalous Perceptions Scale (CAPS). After tDCS, patients showed a 33% decrease in frequency and a 40% decrease in distress of auditory verbal hallucinations.

Thirteen case-reports also investigated the effects of fronto-temporal tDCS on auditory verbal hallucinations in patients with schizophrenia. Of note, three of them observed a complete remission of auditory verbal hallucinations after tDCS (Rakesh et al. 2013; Shivakumar et al. 2013; 2014). Indeed, Rakesh et al. (2013) and Shivakumar et al. (2013) assessing auditory verbal hallucinations with AHRS, reported that 10 sessions of 20 minutes of active 2mA tDCS delivered twice daily on five consecutive days allowed complete remission of auditory verbal hallucinations. Shivakumar et al. (2014), assessing auditory verbal hallucinations with the 'auditory hallucinations' subscale of the PSYRATS, reported a complete remission of auditory verbal hallucinations for at least 3 months after 10 sessions of tDCS delivered twice daily for 20 minutes at 2mA. Two case studies also highlighted the efficacy and safety of maintenance tDCS sessions for 1 and 3 years (Shivakumar et al. 2014; Andrade, 2013). Shivakumar et al. (2014) reported a complete remission of auditory verbal hallucinations assessed with the PSYRATS 'auditory hallucinations' subscale during 1 year after 10 sessions of tDCS delivered twice daily for 20 minutes at 2mA. In fact, the

patient presented 3 relapses within one year, which were successfully managed with only 2 sessions of tDCS (in one day). Andrade (2013) reported a decrease in auditory verbal hallucinations assessed with clinical scales during 3 years of tDCS delivered domiciliary once then twice daily, for 20 then 30 minutes at 1 to 3 mA intensity. Within 2 months, the patient self reported a 90% improvement.

Finally, a randomized sham controlled study failed to replicate the beneficial clinical effect of tDCS on auditory verbal hallucinations assessed by a single item on the Positive and Negative Syndrome Scale (PANSS) measuring hallucinations severity (Fitzgerald et al. 2014). In this study, 15 sessions of tDCS (2mA, 20 minutes) were delivered once a day during 3 consecutive weeks using either a left fronto-temporal montage (with the anode over F3 and the cathode over the T3-P3) in 11 patients with schizophrenia or an original bilateral montage with 4 electrodes (two anodes over F3 and F4 and two cathodes over T3-P3 and T4-P4) in 13 patients with schizophrenia. In a recent case-report study, Bose et al. (2015) reported that 18 sessions of left fronto-temporal tDCS (with the anode placed midway between F3 and FP1 and the cathode over the T3-P3) had no effect on auditory verbal hallucinations as assessed by the 'auditory hallucination' subscale of the PSYRATS. However, when switching the electrode montage to the right side of the brain with the anode placed over the right DLPFC (between F4 and FP2) coupled with the cathode over the right TPJ (between T4 and P4), 20 sessions of tDCS induced a 31.4% reduction of auditory verbal hallucinations.

INSERT TABLE I ABOUT HERE

In sum, among the studies investigating the effects of fronto-temporal tDCS on auditory verbal hallucinations, the intensity of stimulation varied from 1 to 3 mA for a 15 to 30-minute duration. The size of the electrodes was mostly 35 cm² (7 x 5 cm), but some studies used 25 cm² electrodes (5 x 5 cm; Praharaj et al. 2015; Andrade, 2013). tDCS regimen consisted in 5 to 20 sessions of tDCS delivered either once or twice daily. Auditory verbal hallucinations were assessed using various standardized multidimensional

scales such as the PSYRATS or the AHRS, but also using single item assessments such as the 'auditory hallucinations' item of the PANSS (Fitzgerald et al. 2014) or the 'frequency' item of the AHRS (Mondino et al. 2015a). These assessments and outcomes may not have the same sensitivity to capture changes in auditory verbal hallucinations. Further studies are needed to confirm promising effects observed on auditory verbal hallucinations following fronto-temporal tDCS in patients with schizophrenia.

Effects of fronto-temporal tDCS on other symptoms.

Remarkably, among studies reporting a reduction of auditory verbal hallucinations in patients with schizophrenia following tDCS, some also observed a decrease in general symptoms of schizophrenia (Homan et al. 2011; Brunelin et al. 2012a,b; Andrade, 2013), positive symptoms (Shiozawa et al. 2013a), negative symptoms (Ferrucci et al. 2014; Shiozawa et al. 2013a; Narayanaswamy et al. 2014; Mondino et al. 2015b) and insight into the illness (Rakesh et al. 2013; Shivakumar et al. 2013; Bose et al. 2014). In addition, Shiozawa et al. (2013a) investigated the effect of 10 sessions of tDCS with the anode over F3 and the cathode over the occipital region (Oz) followed by 10 sessions with the anode over F3 and the cathode over the temporoparietal cortex (T3-P3) on visual and auditory verbal hallucinations in a patient with schizophrenia. They reported that 10 sessions of each electrode montage lead to a reduction of hallucinations in both visual and auditory modalities.

Predictive markers of response to fronto-temporal tDCS on auditory verbal hallucinations

Two open labeled studies investigated potential predictive markers of response to tDCS (Shivakumar et al. 2015; Brunelin et al.; 2015). Shivakumar et al. (2015) investigated the effects of fronto-temporal tDCS in 23 patients with treatment-resistant auditory verbal hallucinations divided into 2 groups depending on their COMT Val158Met polymorphism. A significant reduction of auditory verbal hallucinations was observed in both groups. However, patients with the val/val COMT polymorphism (n=11) showed a greater reduction in

auditory verbal hallucinations than met-allele carriers (val/met or met/met polymorphism; n= 12). The COMT Val158Met polymorphism may thus modulate response to tDCS. An excessive dopamine transmission has been implicated in the clinical expression of positive symptoms. The Val variant catabolizes frontal dopamine at up to four times the rate of its methionine counterpart, suggesting that lower extracellular dopamine rates in the frontal region predicts beneficial clinical outcome in patients with AVH. Brunelin et al. (2015) reported a mean 20% decrease of auditory verbal hallucinations following 10 sessions of fronto-temporal tDCS in 16 patients with treatment-resistant auditory verbal hallucinations. In this sample, patients with a comorbid tobacco use disorder showed a non-significant 6% reduction in auditory verbal hallucinations whereas non-smokers displayed a significant 46% reduction in auditory verbal hallucinations. Thus, smoking may prevent the effect of repeated sessions of fronto-temporal tDCS in patients with treatment-resistant auditory verbal hallucinations. It has been hypothesized that interactions between antipsychotic medication and nicotine may influence dopamine transmission and in turn modulate tDCS effects on neural plasticity.

Furthermore, one case study suggested that some clinical characteristics such as attentional salience of auditory verbal hallucinations could influence site-specific response to tDCS. Namely, Bose et al. (2015) described the case of a patient with high attentional salience auditory verbal hallucinations that failed to respond to left-sided fronto-temporal tDCS but that decreased after right-sided fronto-temporal tDCS.

Brain correlates of the effects of fronto-temporal tDCS on auditory verbal hallucinations.

Several studies used fMRI and EEG to investigate how tDCS modulates the brain when reducing auditory verbal hallucinations in patients with schizophrenia.

In a first single case study, Homan et al. (2011), reported that tDCS decreased the regional cerebral blood flow in Wernicke's area (BA22), left Heschl's gyrus (BA41/42) and Broca's area (BA44/45), as well as auditory verbal hallucinations. This work supports the hypothesis that tDCS applied over the left

temporoparietal junction reduces auditory hallucinations by normalizing brain activity, specifically by suppressing the hyperactivity observed in the language-related network during auditory verbal hallucinations (Jardri et al. 2011).

In a randomized sham controlled study including 23 patients with schizophrenia, Mondino et al. (2015b) reported that active tDCS decreased resting state functional connectivity of the left TPJ with the left anterior insula and the right inferior frontal gyrus and increased resting state functional connectivity of the left TPJ with the left angular gyrus, the left dorsolateral prefrontal cortex and the precuneus as compared to sham tDCS. These changes in functional connectivity were accompanied by a reduction of auditory verbal hallucinations. Moreover, there was a correlation between the reduction of auditory verbal hallucinations and the reduction of the resting state functional connectivity between the left TPJ and the left anterior insula. These results also suggest that the reduction of auditory verbal hallucinations induced by tDCS was associated with a modulation of the brain activity within an auditory verbal hallucinations -related brain network, including brain areas involved in inner speech production and monitoring.

Using EEG, Nawani al. (2014b) investigated the effects of 10 sessions of left fronto-temporal tDCS on auditory verbal hallucinations and on the amplitude of the auditory evoked potential N100 in 5 patients with schizophrenia. The N100 amplitude was measured when patients were listening to speech stimuli and when they were asked to produce speech. The authors reported that patients with schizophrenia showed no difference at baseline between N100 amplitudes generated in talk and listen conditions. This absence of N100 modulation during talking as compared to listening is suggested to reflect abnormalities in the corollary discharge. After tDCS, the amplitude of N100 was significantly smaller during talking than listening. Thus, tDCS seems to restore the N100 amplitude modulation when reducing auditory verbal hallucinations.

In a case study, Nawani et al. (2014a) tested whether the same protocol of left fronto-temporal tDCS had an effect on cortical plasticity measured by EEG. Namely, they measured the N100 amplitude evoked by an

auditory oddball task before and after a tetanic block before and after tDCS. The authors reported that 10 sessions of fronto-temporal tDCS reduced auditory hallucinations and increased the modulation of the N100 amplitude induced by the tetanic block. This effect was measured in the frontal region only. Since a change in N100 amplitude after tetanic block is considered as an indicator of neuroplasticity, these results suggested that tDCS modulates cortical neuroplasticity in patients with schizophrenia.

3. EFFECTS OF FRONTAL TDCS ON NEGATIVE SYMPTOMS AND OTHER SYMPTOMS OF SCHIZOPHRENIA

Five studies investigated the clinical effect of tDCS on treatment-resistant negative symptoms of schizophrenia. In these studies, the targeted brain region was the DLPFC, mainly its left part. This brain region was targeted with tDCS by placing the anode over the left DLPFC (F3) and the cathode either over the supra orbital region (FP2), the right DLPFC (F4) or the right deltoid. In the first study, Palm et al. (2013a) reported that 10 sessions of tDCS delivered once a day with the anode placed over the left DLPFC (F3) and the cathode electrode placed over the right supra orbital region (FP2) reduced treatment-resistant negative and positive symptoms in a patient with schizophrenia. In a further randomized sham controlled trial with 20 patients with negative symptoms, Palm et al. (2014) reported that 10 daily sessions of active tDCS as compared to sham tDCS decreased negative symptoms as measured by the SANS and general symptoms as assessed by the PANSS. These beneficial clinical effects were maintained at the 2-week follow-up assessment.

These beneficial effects of tDCS on negative symptoms were also reported more recently in an open-label study including 9 patients with schizophrenia (Kurimori et al. 2015) and in a randomized sham-controlled study including 15 patients with schizophrenia (Gomes et al. 2015). In the first study, patients received 10 daily sessions of tDCS with the anode placed over the left DLPFC (F3) and the cathode placed over the

right deltoid muscle (Kurimori et al. 2015). After tDCS, patients showed a significant 24% reduction in negative symptoms assessed by the PANSS negative subscale as compared to baseline. In the second study, patients received 10 daily sessions of either active or tDCS with the anode placed over the left DLPFC (F3) and the cathode placed over the right DLPFC (F4) (Gomes et al. 2015). After tDCS, patients receiving active tDCS showed a significant 20% reduction in negative symptoms as measured by the PANSS negative subscale whereas patients receiving sham tDCS showed no significant difference. Patients receiving active tDCS also reported a significant 15% reduction in PANSS general symptoms as compared to patients receiving sham tDCS.

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Brain correlates of the effects of frontal tDCS on negative symptoms

Only one case study and one randomized controlled study investigated how tDCS modulates the brain when reducing negative symptoms in patients with schizophrenia. In the case study, Palm et al. (2013a) used fMRI to measure the effects of 10 sessions of tDCS with the anode placed over the left DLPFC and the cathode placed over the right supraorbital region (FP2) on resting-state functional connectivity. Following tDCS, the patient showed a reduction in positive and negative symptoms and a reduced functional connectivity in the anterior part of the default mode network including the subgenual cortex, the anterior cingulate, the medial frontal gyrus and superior frontal gyrus. In a larger sample including 20 patients with schizophrenia, the same group of authors reported that the clinical improvement in negative symptoms observed after patients received tDCS was accompanied by a significant reduced functional connectivity within the nucleus accumbens, the subgenual cortex and the striatum (Palm et al. 2014).

Effects of frontal tDCS on other symptoms

In a case study, Shiozawa et al. (2013b) reported a reduction in severity of catatonic symptoms in a patient suffering from treatment- and electroconvulsive therapy-resistant catatonic schizophrenia following 10 sessions of tDCS delivered once a day with the anode over F3 and the cathode over F4. After one month, the remission of symptoms was complete and lasted for at least 4 months.

4. EFFECTS OF TDCS ON COGNITIVE FUNCTIONS

Cognitive deficits are a key feature in patients with schizophrenia. Several studies explored whether tDCS could improve cognitive functions in patients with schizophrenia.

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In the first study, Vercammen et al. (2011) reported that a single session of active tDCS had a facilitating effect on probabilistic association learning measured by the weather prediction test in patients who displayed the best learning abilities before stimulation. In this study the anode was placed over the left DLPFC (F3) and the cathode over the right supraorbital region (FP2). In another study, Hoy et al. (2014) observed beneficial effects of the same electrode montage on working memory performances measured using the n-back task. These beneficial effects lasted up to 40 minutes after the end of the stimulation period and were associated with an increase in frontal gamma event related synchronization (Hoy et al. 2015). Ribolsi et al. (2013) reported a reduction of visuospatial attention deficit in patients with schizophrenia after a single session of tDCS where the anode electrode was placed over the right parietal (P4) and cathode over the left shoulder.

Several studies investigated the effects of anodal tDCS applied over the left DLPFC on cognitive functioning of patients with schizophrenia using a standardized battery of cognitive tests. In one of them,

Rassovsky et al. (2015) tested the effect of a single session of either anodal or cathodal tDCS applied over FP1 or FP2 (with the reference electrode placed over the upper right arm) on social cognition and cognitive functions in 36 patients with schizophrenia. Social cognition was measured using the Mayer–Salovey–Caruso Emotional Intelligence Test (MSCEIT) that assesses four components of emotional processing, the Facial Emotion Identification Test (FEIT) that assesses the identification of facial emotion, the Profile of Nonverbal Sensitivity that assesses social perception, and the Awareness of Social Inference Test that assesses theory of mind. Cognitive functions were assessed using the MATRICS Consensus Cognitive Battery (MCCB) composite score. Following anodal tDCS, patients showed a significant improvement in the FEIT only, indicating that a single session of anodal tDCS over the prefrontal cortex might enhance identification of facial emotion in patients with schizophrenia.

In another study, Schretlen et al. (2015) compared the effects of two 30-minute sessions of tDCS, applied either with the anode over the left and cathode over the right DLPFC or with the reverse montage, on working memory and on a brief battery of cognitive measures in five outpatients with schizophrenia and six first-degree relatives of patients with schizophrenia. No differences were reported between tDCS conditions on motor speed assessed by the Grooved Pegboard Test and the Finger Tapping Test and on processing speed assessed by the Perceptual Comparison Test. No effects of tDCS condition were observed on attention assessed by the Wechsler Adult Intelligence Scale, 3rd Ed. Digit Span and Wechsler Memory Scale, 3rd Ed. Spatial Span. Working memory performances assessed by backward digit and spatial span were shown to be improved during anodal stimulation of the left DLPFC relative to cathodal stimulation. In addition, patients showed an increase in novel design production without alteration of overall productivity at the calibrated ideational fluency assessment during anodal versus cathodal tDCS.

Finally, only few studies investigated the effects of repeated sessions of tDCS on cognition in patients with schizophrenia. For instance, in a randomized double-blind, sham-controlled study, Smith et al. (2015) investigated the effects of 5 sessions of either active or sham tDCS on cognition assessed by the

MCCB composite score, psychiatric symptoms assessed by the PANSS, and smoking and cigarette craving in 37 patients with schizophrenia or schizoaffective disorder who were current smokers. tDCS was delivered with the anode placed over F3 and the cathode electrode placed over the right supra orbital region (FP2). Patients receiving active tDCS, as compared to sham, showed a significant improvement in the MCCB composite score, in the MCCB working memory score and in attention-vigilance domain scores. However, no significant effects were observed on clinical symptoms assessed by the PANSS, hallucinations, cigarette craving, and cigarettes smoked.

In a double-blind sham controlled study, Mondino et al. (2015a) tested the effects of 10 sessions of left fronto-temporal tDCS on source monitoring performance and treatment-resistant auditory verbal hallucinations in 28 patients with schizophrenia. Source monitoring was defined as the ability to discriminate between internally generated words and externally produced words. After 10 sessions of active tDCS, patients performed better at recognizing internally generated words as compared to sham tDCS. In addition, there was a negative correlation between the reduction in the frequency of treatment-resistant auditory verbal hallucinations and the increased recognition of internally generated words.

5. SAFETY OF USING TDCS FOR TREATING SCHIZOPHRENIA

The reviewed articles also investigated the impact of at least one tDCS session on more than 300 patients with schizophrenia. The duration of the tDCS session lasted from 10 to 30 minutes, with the intensity of stimulation ranging from 1 to 3 mA. Among expected adverse events following a session of tDCS (Brunoni et al. 2011), patients with schizophrenia more commonly reported tingling or itching sensations under the electrodes as well as sleepiness. No study reported any serious adverse event. In addition, 10 sessions of tDCS delivered once or twice daily were well tolerated by specific populations such as patients with childhood-onset schizophrenia (mean age 15 years old; range 10-17) (Mattai et al. 2011), female patients during pregnancy (Shenoy et al. 2015), and patients with comorbid skin condition (Shiozawa et al. 2013c).

Importantly, these studies did not observe any worsening of symptoms. An important improvement for patients with severe handicaps would be to have the possibility of tDCS to be delivered at home. Indeed, this was suggested for one patient with schizophrenia (Andrade, 2013). However, to allow this practice, the national authorities should establish recommendations (Fregni et al. 2014, also discussed in Chapter 26 of this book).

6. OPTIMIZING TDCS EFFICACY ON SYMPTOMS OF SCHIZOPHRENIA

Optimizing tDCS parameters

The use of tDCS in schizophrenia is just at its beginning. Thus, there are still numerous unanswered questions including optimal stimulation parameters such as intensity, duration, and the number of sessions. Concerning stimulation intensity, tDCS has been mostly delivered at 1, 1.5 and 2mA. Some studies comparing 1 to 2 mA stimulation suggested that 2mA is the cut off for an optimal efficiency in reducing clinical symptoms and improving cognitive functions in schizophrenia (Andrade, 2013, Hoy et al. 2014). In that line, an interesting case study reported the safety of a 3 mA stimulation (Andrade, 2013). Concerning the duration of a session, most studies used sessions of a 20-minute duration each. However, few studies reported beneficial effects of different session durations. For instance, Homan et al. (2011) reported reduced auditory verbal hallucinations following 10 sessions of tDCS delivered once daily at 1 mA during 15 minutes in a patient with schizophrenia. In another single case study, Andrade (2013) enhanced tDCS duration from 20 to 30 minutes without adverse effects. In a randomized controlled study, Gomes et al. (2015) reported the effects of 10 sessions of tDCS delivered once daily at 2 mA during 10 minutes on negative symptoms and general symptomatology in 15 patients with schizophrenia. Concerning the number of sessions to deliver, patients with schizophrenia showed improvement after 10 sessions delivered once or twice per day. One study, delivering 15 sessions of tDCS once per day, did not show any significant effect on auditory hallucination (Fitzgerald et al. 2014). In one case study, delivering 5 sessions of tDCS once per

day induced a substantial reduction of auditory hallucinations that lasted at least 6 days (Praharaj et al. 2015). To sum up, even if there is still much to learn about the tDCS optimal parameters, gathered evidence suggests that 10 sessions of tDCS of 20-minute duration and at a 2mA intensity delivered once or twice per day produce a positive outcome such as reducing symptoms and improving cognition in patients with schizophrenia.

Other modalities of transcranial electric stimulation in schizophrenia

Other forms of transcranial electric stimulation besides tDCS have been tested in schizophrenia, for instance, high frequency oscillatory unidirectional *transcranial random noise stimulation* (tRNS) (Terney et al. 2008). To date, two studies investigated the effects of unidirectional tRNS with high frequencies ranging from 100 to 640Hz, in patients with schizophrenia. Palm et al. (2013b) reported an improvement in negative symptoms after 20 sessions of tRNS with the anode applied over the left dorsolateral prefrontal cortex and the cathode over the right supraorbital cortex. Haesebaert et al. (2014), using the left fronto-temporal montage during 10 sessions of tRNS, observed a reduced severity of auditory hallucinations and an improved insight into the illness. Moreover, one study investigated the effects of transcranial slow oscillatory direct stimulation applied at a frequency of 0.75Hz during phase 2 of sleep in 14 patients with schizophrenia (Göder et al. 2013). In this study, slow oscillatory tDCS was applied at an intensity of 0.3 mA through two spherical 8 mm diameter electrodes placed bilaterally over F3 and F4 and at the mastoids. Stimulation was delivered for five blocks of 5 minutes separated by 1-min intervals free of stimulation. The authors reported that patients displayed greater performances to retain verbal information following active as compared to sham stimulation. A significant elevated mood was also observed in the morning after stimulation as compared to the morning after sham stimulation.

Combining tDCS with other approaches

tDCS studies most often include patients with schizophrenia suffering from treatment-resistant symptoms, and thus, treated with several medication classes including typical, atypical antipsychotics and selective serotonin reuptake inhibitors. These treatments should be taken into account when studying the impact of tDCS sessions. Indeed, in studies involving healthy subjects, dopaminergic, serotonergic and GABAergic agents/drugs have been shown to have an impact on motor cortex excitability after tDCS sessions (Nitsche et al. 2006; Monte-Silva et al. 2009). For example, tDCS after-effects in healthy subjects is considerably reduced with sulpiride (Nitsche et al. 2006). With this in mind, it seems important that the studies investigating the effect of tDCS in patients with schizophrenia should determine the optimal association between pharmacology and the tDCS protocol. For example, a major depression study showed that bifrontal tDCS efficacy was reduced with concomitant use of benzodiazepine drugs (Brunoni et al. 2013). Such interactions might also occur in patients with schizophrenia. Future work is therefore needed to study the association between tDCS effects, medication and even nicotine intake (Brunelin et al. 2015) with tDCS efficacy in schizophrenia.

Another interesting approach, with the aim to improve tDCS effects on symptoms, could involve combination with neurocognitive strategies such as cognitive remediation therapy (Thorsen et al. 2014; d'Amato et al. 2011). For example, tDCS has been shown to improve working memory (Brunoni and Vanderhasselt 2014), therefore it could work with cognitive training as to enhance both cognitive and clinical efficacy. Further studies are needed to determine the optimal associations with the aim of improving clinical outcomes.

7. CONCLUSION

In this chapter, we reviewed and discussed studies investigating the usefulness of tDCS to reduce symptoms and improve cognitive functions of patients with schizophrenia. To date, two electrode montages seem to stand out: one fronto-temporal montage with the anode placed over the left prefrontal cortex and the cathode

placed over the left temporoparietal junction, which may reduce auditory verbal hallucinations; and one frontal montage with the anode placed over the left DLPFC and the cathode placed over the right DLPFC or the right supra orbital region which may also have beneficial clinical outcomes, mainly on negative symptoms. However, as the use of tDCS is quite recent and since most studies reviewed here were case-reports and open labeled studies with small samples, further randomized controlled trials with large samples are needed to confirm the efficacy of tDCS in schizophrenia. Moreover, further investigations have to be conducted to determine biological correlates and the optimal stimulation parameters to use to better impact on the symptoms of schizophrenia.

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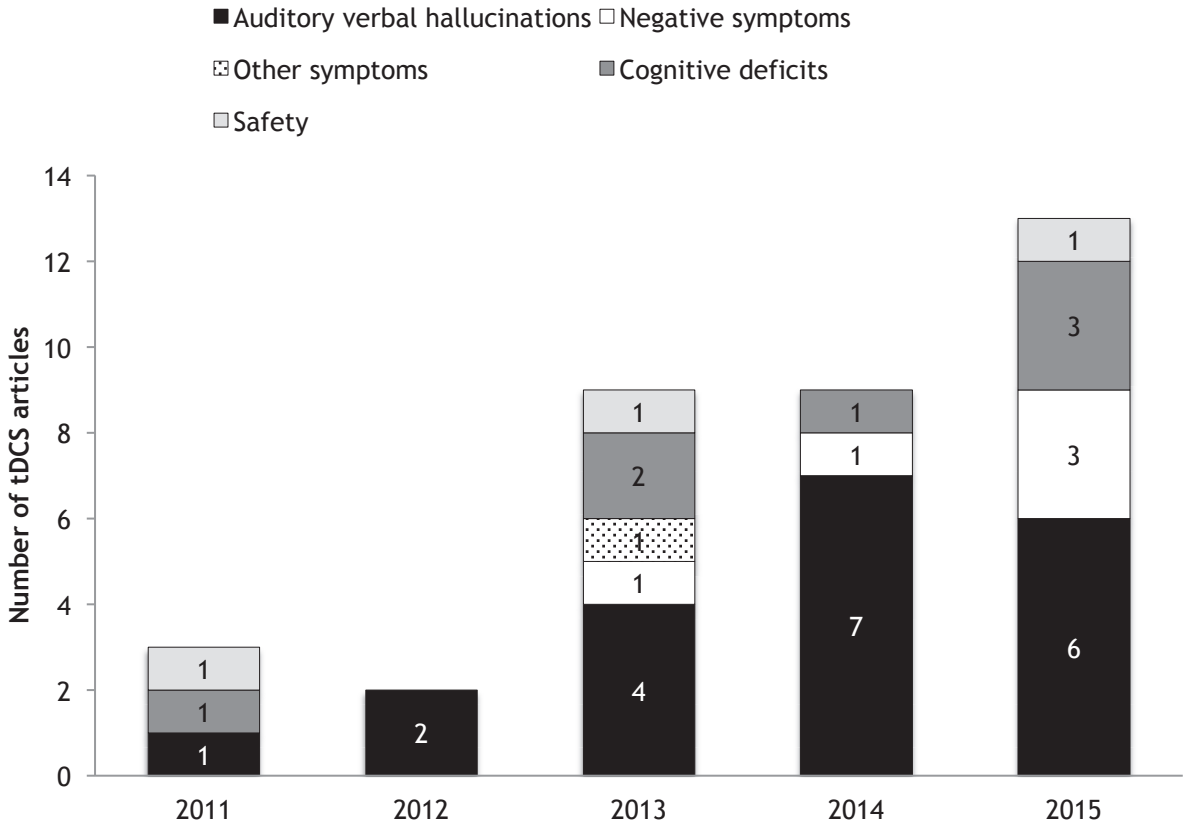
Figure 1: Number of published articles per year examining the effects of transcranial Direct Current Stimulation (tDCS) in patients with schizophrenia. Articles investigating the effects on auditory verbal hallucinations, negative symptoms, other symptoms, cognitive deficits and safety were listed. (Source: PubMed/Medline).



3. Article - Integrity of the arcuate fasciculus in patients with schizophrenia with auditory verbal hallucinations: A DTI-tractography study

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Integrity of the arcuate fasciculus in patients with schizophrenia with auditory verbal hallucinations: A DTI-tractography study

Marion Psomiades[a], Clara Fonteneau[a], Marine Mondino[b], David Luck[c,d], Frederic Haesebaert[a], Marie-Françoise Suaud-Chagny[a], Jerome Brunelin[a,b,*]

[a] INSERM U1028, CNRS UMR5292, PSYR2 Team, Lyon Neuroscience Research Center, Centre Hospitalier Le Vinatier, Université Claude Bernard Lyon 1, Bron, France
[b] Centre Interdisciplinaire de Recherche en Réadaptation et en Intégration Sociale (CIRRIS), Centre de Recherche de l'Institut Universitaire en Santé Mentale (CRIUSM), Université Laval, Québec, QC, Canada
[c] Institut Universitaire en Santé Mentale de Montréal Research Center, Québec, QC, Canada
[d] Department of Psychiatry, University of Montreal, QC, Canada

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ABSTRACT

Auditory verbal hallucinations (AVH) of schizophrenia are associated with a disrupted connectivity between frontal and temporoparietal language areas. We hypothesized that this dysconnectivity is underpinned by white matter abnormalities in the left arcuate fasciculus, the main fiber bundle connecting speech production and perception areas. We therefore investigated the relationship between AVH severity and the integrity of the arcuate fasciculus measured by diffusion tensor imaging (DTI) tractography in patients with schizophrenia. Thirty-eight patients with treatment-resistant schizophrenia were included: 26 presented with daily severe treatment-resistant AVH, 12 reported prominent negative symptoms and no AVH. Fractional anisotropy (FA) was measured along the length of the left and right anterior arcuate fasciculi and severity of AVH was assessed using P3 PANSS item.

FA values were significantly higher in the left arcuate fasciculus in patients with AVH than in no AVH patients ($F_{(1,35)} = 3.86$; $p = 0.05$). No difference was observed in the right arcuate fasciculus. There was a significant positive correlation between FA value in the left arcuate fasciculus and the severity of AVH ($r = 0.36$; $p = 0.02$). No correlation was observed between FA values and PANSS total score suggesting a specific relationship between AVH severity and the left arcuate fasciculus integrity.

These results support the hypothesis of a relationship between left frontotemporal connectivity and AVH in patients with schizophrenia and suggest that whilst a disruption of frontotemporal connectivity might be present to ensure the emergence of AVH, more severe anatomical alterations may prevent the occurrence of AVH in patients with schizophrenia.

1. Introduction

Auditory verbal hallucinations (AVH) are key symptoms of schizophrenia. The brain networks underlying such experiences are still unclear. Several neuroimaging studies have associated AVH with impaired functional connectivity within large-scale networks (Hoffman and Hampson, 2012). More specifically, it has been reported that patients with AVH displayed an abnormal resting functional connectivity between frontal and temporal areas including brain networks involved in the perception and production of speech (Lawrie et al., 2002; for a review see Alderson-Day et al., 2015). Post mortem and genetic studies suggest that such abnormalities in functional connectivity between frontal and temporal regions may be underpinned at the structural level by white matter (WM) microstructural abnormalities within bundles connecting these two regions (for review see Takahashi et al., 2011). However, the direct link between AVH symptoms and WM anatomy remains unclear. Thanks to recent advances in brain imaging, it is possible to noninvasively explore and quantify WM microstructure in the living human using magnetic resonance diffusion tensor imaging (DTI). Fractional anisotropy (FA) is the most frequently used measure of anisotropic diffusion and is assumed to reflect WM fiber organization, or axonal and myelin integrity (Beaulieu, 2002). DTI studies investigating WM integrity in schizophrenia reported fiber tracts abnormalities in the left frontal lobe and the left temporal lobe in various populations: individuals at risk to develop schizophrenia (Samartzis et al., 2014), medicated and unmedicated patients with schizophrenia (Ellison-Wright and Bullmore, 2009; Mandl et al., 2013), patients at the early stage of disorder and patients with first-

* Corresponding author at: CH Le Vinatier, Batiment 416, 95 boulevard Pinel, BP 300 39, 69 678, BRON cedex, France.
E-mail address: jerome.brunelin@ch-le-vinatier.fr (J. Brunelin).

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episode schizophrenia (Kyriakopoulos and Frangou, 2009; Luck et al., 2011).

For example, as compared with healthy controls, some studies reported a decreased FA within several fiber tracts including the uncinate fasciculus, the inferior longitudinal fasciculi, the cingulum bundle and the arcuate fasciculus in patients with schizophrenia (Minami et al., 2003; Phillips et al., 2009; for a review see Kubicki et al., 2007). By contrast, other studies reported an increased FA in bundles such as the arcuate fasciculus (Knöchel et al., 2012; for a review see Kubicki et al., 2007). To date, the discrepancy in findings is not explained. The clinical heterogeneity of schizophrenia patients included in published studies could be a confounding factor. The stratification of schizophrenia patients according to their prominent symptoms (such as AVH or negative symptoms) could help to clarify contradictory results.

Several studies investigated the relationship between FA and AVH and most of them focused on fiber tracts belonging to the networks involved in language such as the arcuate fasciculus and the superior longitudinal fasciculus (SLF). These bundles connect frontal areas with the posterior part of the temporoparietal junction. In a recent metaanalysis including 5 DTI studies that compared patients with schizophrenia suffering from AVH (AVH+; n = 106) to matched healthy controls (n = 150), Geoffroy et al. (2014) reported a significant mean reduced FA in the left, but not in the right, arcuate fasciculus in AVH+ patients.

Despite the fact that some studies compared WM integrity of the arcuate fasciculus between AVH+ patients with schizophrenia and patients with schizophrenia without AVH (no-AVH) in order to distinguish AVH-specific from schizophrenia-specific effects, the relationship between severity of AVH and integrity of the left and/or right arcuate fasciculus remains unclear. For instance, McCarthy-Jones et al. (2015) and de Weijer et al. (2011) reported that FA was significantly reduced in the left arcuate fasciculus in AVH+ patients as compared with no-AVH patients. A reduced FA was also observed in right arcuate fasciculus of AVH+ as compared with no-AVH patients (Catani et al., 2011). Conversely, other studies showed an increased FA in the superior temporal gyrus (Lee et al., 2009) and in the arcuate fasciculus of AVH+ patients as compared to no-AVH patients (Hubl et al., 2004). Moreover, Seok et al. (2007) observed an increased FA in the left SLF of AVH+ patients as compared to no-AVH patients. Remarkably, few studies investigated the relationship between AVH severity and WM integrity of the arcuate fasciculus in patients with schizophrenia. Rotarska-Jagiela et al. (2009) observed a positive correlation between FA in the arcuate fasciculus and AVH whereas Ćurčić-Blake et al. (2015) reported the inverse relationship. Two studies reported a positive correlation between AVH severity and FA in the SLF either in the left part (Seok et al., 2007) or bilaterally (Shergill et al., 2007). Finally, a recent study in unmedicated subjects at risk to develop psychosis reported larger abnormalities in WM integrity and functional connectivity within the left perisylvian language network in subjects who had not developed AVH as compared with subjects who had developed AVH (Benetti et al., 2015).

Several considerations can explain these discrepancies between studies ranging from clinical characteristics of included patients, age and medication to DTI methodology and anatomical definition of the bundle. In the present study, we aimed to investigate the relationship between AVH and the integrity of the arcuate fasciculus in patients with treatment-resistant schizophrenia according to the presence or absence of AVH. The resistance was defined as the presence of symptoms after two well-conducted antipsychotic treatments, in sufficient dose and duration. Patients included in this study were clinically characterized either by the presence of daily resistant AVH or absence of daily resistant AVH. Hence, we undertook a DTI-tractography study comparing FA values in both left and right arcuate fasciculi between these two samples of patients. We also investigated the relationship between the integrity of the arcuate fasciculus and AVH severity. We hypothesized that severity of AVH was associated with abnormal FA values especially in the left arcuate fasciculus.

2. Material and methods

2.1. Subjects

Thirty-eight patients with DSM IV-TR criteria for schizophrenia were enrolled in the study (mean PANSS score 76.3 ± standard deviation 11.8). Patients were free from any other DSM IV disorders as assessed by a structural interview using the Mini-International Neuropsychiatric Interview (MINI, Sheehan et al., 1998). The study was approved by a local ethic committee (CPP Sud EST 6, France) and all patients gave their written informed consent.

The sample was separated into two groups based on the presence of daily AVH (AVH+ and no-AVH patients). The severity of AVH was assessed by the Positive and Negative Syndrome Scale (PANSS) P3 item (i.e., hallucinations). Since all the recruited patients experienced mainly AVH, the P3 item was used to measure exclusively AVH severity in our study. Twenty-six patients presented with daily severe treatment-resistant AVH (PANSS P3 item > 3), 12 patients had no current or past history of AVH (PANSS P3 item ≤ 3) and presented with disabling treatment-resistant negative symptoms.

Since brain lateralization and thickness of WM fibers may vary with handedness (Parker et al., 2005; Knecht et al., 2000), only right-handed subjects were included in this study. All included patients were treated with antipsychotic medication for at least three months without changes in dose (713 equivalent chlorpromazine mg/day ± 584). Patients (11 women and 27 men) were aged between 22 and 57 years old (mean: 38.3 ± standard deviation: 9.4) with an educational level of 11.5 ± 2.6 years and an illness duration of 11.2 ± 8.2 years. Characteristics of groups are given in Table 1.

2.2. Image acquisition

DTI and T1-weighted MRI images were acquired on a 1.5-T Siemens Magnetom Sonata Maestro Class system equipped with a standard headcoil, at the "CERMEP-Imagerie du vivant" research imaging center of Lyon, France. Head motion was restricted using foam padding. Structural images were obtained with a standard T1-weighted pulse sequence: TR = 1.97 ms, TE = 3.93 ms, flip angle of 15°, FOV = 256 × 256 mm, voxel size: 1.0 × 1.0 × 1.0 mm. Whole brain DTI images were then acquired using a spin-echo EPI sequence (TR = 3800 ms, TE = 96 ms) with 128 × 128 phase-encoding over a FOV of 320 × 320 mm^2 and 51 axial slices of 2.5 mm thickness. DTI images were acquired in 24 directions with b values of 0 and 1000 s/mm^2. Six acquisitions were made and averaged at b = 0 and 3 acquisitions in other directions. The scan duration for the DTI sequence was about 10 min.

2.3. Data analyses

2.3.1. Image processing

DTI data of each subject were processed using MedINRIA software. Each run was linearly registered to the first diffusion series using the

Table 1
Socio-demographic and clinical characteristics of patients with schizophrenia.

	AVH	no-AVH	p
n	26	12	
Female/Male	10/26	1/12	0.12
Age (years)	36.4 ± 8.3	42.4 ± 10.7	0.06
Educational level	11.4 ± 2.7	11.5 ± 2.5	0.93
Illness duration (years)	9.9 ± 6.8	13.7 ± 10.1	0.19
Medication (eq cpz mg/day)	745 ± 561	650 ± 648	0.65
PANSS	73.5 ± 12.3	82.4 ± 8.3	0.02
P3 item (hallucinations)	5.8 ± 0.9	1.7 ± 0.9	<0.001

b = 0 image, while assuming that no motion occurred within a set (visual check). No eddy current correction was performed. Color FA map was obtained and tractography (DTI track) for the arcuate fasciculus was performed by seeding the voxels with a FA > 0.2 and a constraining angle lower than 60°.

2.3.2. Region of interest definition

Here, with a 2-ROI approach based on FA color maps, only green fibers were selected on an axial and coronal plan for each subject. These fibers corresponded to fibers with anterior-posterior orientation (arcuate fasciculus-anterior). In each hemisphere, two regions of interest (ROI) were used to extract the arcuate fasciculus fibers. All ROIs were independently drawn on the FA weighted colored maps by two investigators blinded to the hallucination status. The ROIs were placed referring to Oishi et al.'s MRI atlas of human white matter (Oishi et al., 2011) as follows: the first ROI was placed on an axial slice corresponding to Talairach 5/6, MNI z = 27.5 to 32.5 and the second ROI was placed on a coronal slice corresponding to Talairach E2/E3, MNI y = −12.5 to −17.5 as described on the MRI atlas of human white matter, second edition (Oishi et al., 2011) pages 95–99 and 155–159 respectively (Fig. 1). The obtained fiber tract throughout these 2 ROIs was then analyzed using bundle manager tool in MedINRIA. The value used in the analysis was the mean of the FA values obtained by the 2 independent experimenters (MP & CF), with an inter-rater correlation of 0.93. The fiber was tracked separately on each of the hemisphere using the same methodology.

2.4. Statistical analyses

Clinical characteristics of patients were compared using 2-tailed Student t-tests and using Fischer's exact test for gender. As a trend toward a significant difference in age was observed between groups (p = 0.06) and since age is known to influence FA values (Voineskos et al., 2010; Bijanki et al., 2015), age was added as covariate in the analysis. For each hemisphere, FA values of the arcuate fasciculus were compared between AVH+ and no-AVH patients using an ANCOVA with age as covariate. Intra group comparisons (right versus left FA) were analyzed using Student t-test and Effect size (Hedges' g) were calculated.

Relationships between FA values (left and right) and severity of symptoms (AVH measured by P3 item PANSS score and symptoms of schizophrenia measured by PANSS total scores) were analyzed using Pearson correlation tests. Significance was set at $p < 0.05$.

3. Results

3.1. Comparison between AVH + and no-AVH patients

The ANCOVA with age as a covariate revealed a significant difference ($F_{(1,35)} = 3.86$; $p = 0.05$) in FA values of the left arcuate fasciculus between AVH+ (0.434 ± 0.019) and no-AVH patients (0.417 ± 0.019; Fig. 2, part A). No difference was observed for FA values in the right arcuate fasciculus ($F_{(1,35)} = 1.09$; $p = 0.30$) between AVH+ (0.412 ± 0.027) and no-AVH patients (0.399 ± 0.027; Fig. 2, part B).

3.2. Comparison between right and left arcuate fasciculus

In AVH+ patients, the FA value was significantly greater in the left arcuate fasciculus as compared to the right arcuate fasciculus (Hedges g = 0.93; 95% CI = 0.36–1.51; p = 0.001) whereas only a trend toward a significant difference between left and right arcuate fasciculi was observed in no-AVH patients (Hedges' g = 0.73; 95% CI = −0.09–1.56; p = 0.08).

3.3. Relationship analysis between FA and AVH severity

A significant positive correlation was observed between the FA value in the left arcuate fasciculus and the severity of AVH measured by P3 PANSS item ($r = 0.36$; $p = 0.02$). No significant correlation was observed between the FA value in the left arcuate fasciculus and the severity of general symptoms of schizophrenia measured by PANSS total score ($r = -0.04$; $p = 0.80$).

No correlations were found between the FA value in the right arcuate fasciculus and the severity of AVH measured by P3 PANSS item ($r = 0.165$; $p = 0.32$) as well as between the FA value in the right

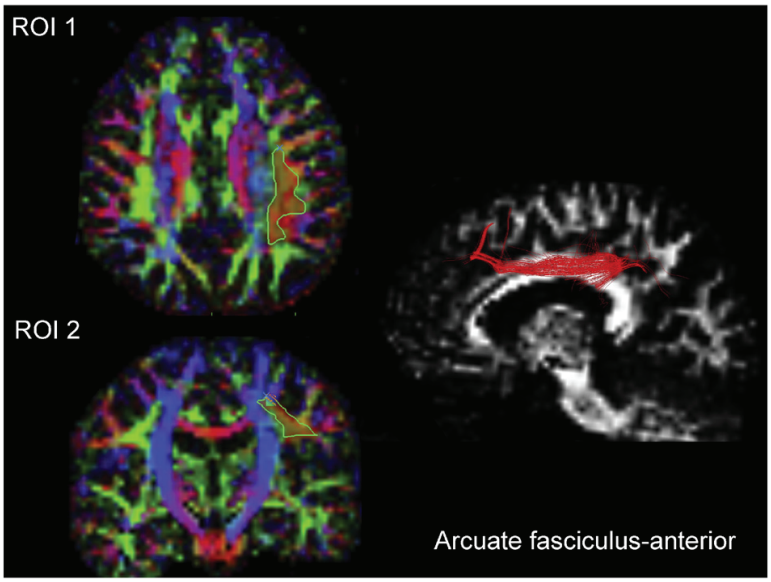

Fig. 1. Left panel (ROI 1/ROI 2) shows FA-weighted colored maps with ROI positions (in red and delineated in green). Right panel shows representative 3D illustrations of the arcuate fasciculus-anterior in a patient with schizophrenia (in red).

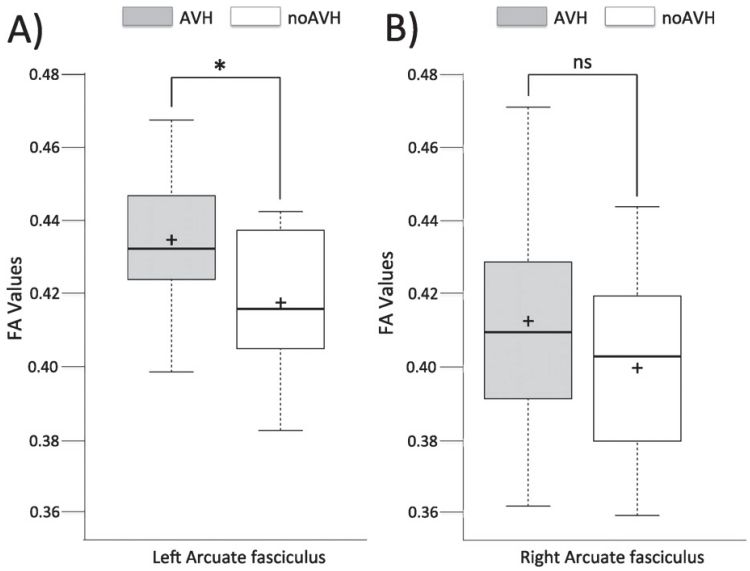

Fig. 2. FA value in the left (part A) and right (part B) arcuate fasciculus in patients with schizophrenia with and without daily auditory hallucinations. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means. n = 26, 12, 26, 12 sample points.

arcuate fasciculus and the severity of symptoms measured by PANSS total score ($r = 0.023$; $p = 0.89$).

4. Discussion

In this study, we used DTI-tractography to investigate WM abnormalities in both left and right arcuate fasciculi in AVH+ patients as compared to no-AVH patients with schizophrenia. We reported that FA was greater in the left arcuate fasciculus of AVH+ patients as compared with no-AVH patients whereas no difference was observed for the right arcuate fasciculus between the 2 groups. Moreover, the FA value was significantly greater in the left arcuate fasciculus as compared to the right arcuate fasciculus in AVH+ patients, whereas only a trend toward a significant difference between left and right arcuate fasciculi was observed in no-AVH patients.

We also found a significant positive correlation between FA values in the left arcuate fasciculus and the severity of AVH measured with 'P3' item of the PANSS. This correlation seems to be specifically lateralized since no correlation was found between FA in the right arcuate fasciculus and severity of AVH. The left-side specificity of the correlation between AVH and WM integrity of the arcuate fasciculus may be related to the fact that speech-relevant areas are predominantly located in the left hemisphere in >90% of right-handed subjects (Catani et al., 2007; Vernooij et al., 2007). The observed increase in FA within the left arcuate fasciculus seems to be specifically associated with AVH severity since no significant relationship was observed between FA (right and left) and total PANSS score. We ensured that our results were not influenced by individual characteristics that are known to influence FA values in patients with schizophrenia by introducing age (Mori et al., 2007; Voineskos et al., 2010; Bijanki et al., 2015) as a covariate in our analysis. Moreover, we ensured that AVH+ and no-AVH groups were not different for illness duration, gender and medication (Minami et al., 2003; Okugawa et al., 2004; Takase et al., 2004).

Our results are in line with previous findings investigating WM abnormalities in patients with schizophrenia and reporting increased FA value in the left arcuate fasciculus in AVH+ patients with schizophrenia as compared with no-AVH patients (Hubl et al., 2004; Seok et al., 2007). However, these results are in contradiction with other studies reporting a reduced FA value in the left arcuate fasciculus in AVH+ patients as compared to no-AVH patients and healthy controls (de Weijer et al., 2011; Catani et al., 2011; Geoffroy et al., 2014; Ćurčić-Blake et al., 2015; McCarthy-Jones et al., 2015). These discrepancies between findings might be explained by difference in DTI methodology. Indeed, most previous studies have used a VBM approach that assesses WM alterations on large regions without an a priori hypothesis by calculating a mean FA value on a ROI on a FA map with WM templates. Thus VBM approach does not take advantage of DTI's ability to depict WM systems through fiber tracking. Here, we used DTI-tractography that assembles the local diffusion tensor data to infer the paths of fiber tracts (Mori et al., 1999), based on a priori hypothesis, where scalar metrics, such as FA, along these tracts allow for the precise localization of WM abnormalities in the arcuate fasciculus.

The heterogeneity in the definition of the tract between studies could also be taken into account (e.g., parts of the arcuate fasciculus, the perisylvian language network, parts of the SLF). Indeed, tractography studies have revealed that anatomy of the arcuate fasciculus is complex (Catani et al., 2005) and delimitation between the SLF and the arcuate fasciculus is not yet clearly established (Dick and Tremblay, 2012). Here, we used a tract-specific measurements method that allowed us to quantify the microstructural integrity of the arcuate fasciculus. However, tractography approach has limitations such as sensitivity to ROI placement and partial volume effect (Lifshits et al., 2009). In this way, with the same objective and using a tractography method, the portion of the arcuate fasciculus considered differs from one study to the other according to the parameters used to specify anatomical boundaries. Here, with a 2-ROIs approach based on FA color maps, only green fibers were selected on an axial and coronal plan for each subject. These fibers corresponded to fibers with anterior-posterior orientation (arcuate fasciculus anterior, van Beek et al., 2014). In contrast, de Weijer et al. and McCarthy-Jones et al. developed measurement designs giving access to U-shape fibers of the arcuate fasciculus (de Weijer et al., 2011; McCarthy-Jones et al., 2015).

The heterogeneity in clinical characteristics of included patients in the different studies could also in part explain the observed differences.

The characteristics of AVH and the characteristic of the no-AVH groups can have influenced FA values. The main discrepancies concern the clinical characteristics of no-AVH populations who are either patients with no lifetime AVH (this study and Seok et al., 2007) or patients with no AVH in the last month (Ćurčić-Blake et al., 2015). The AVH+ populations included patients with remitted AVH (McCarthy-Jones et al., 2015), or treatment-resistant AVH (Seok et al., 2007). In the current study, we included patients with treatment-resistant daily chronic AVH and patients with treatment-resistant negative symptoms and no current or past history of AVH. Interestingly, the clinical characteristics of our populations and our results are similar to those of Seok and collaborators suggesting that the increase of FA in left arcuate fasciculus in AVH+ patients compared with no-AVH patients is specific to treatment-resistant AVH (Seok et al., 2007).

The findings of a reduced FA value in the left arcuate fasciculus of patients with prominent treatment resistant negative symptoms as compared with AVH+ patients are consistent with studies reporting lower FA in patients with prominent negative symptoms as compared with healthy controls (Hovington et al., 2015). Furthermore, in healthy subjects, studies show that most subjects have leftward lateralization of the arcuate fasciculus (Catani et al., 2007). In our results, this leftward lateralization of the arcuate fasciculus is found in AVH+ patients but not in no-AVH patients. One can hypothesize that whilst disruption of connectivity might be present to ensure the emergence of AVH as compared with healthy controls (Geoffroy et al., 2014), more severe WM alterations may prevent the occurrence of AVH in patients with predominantly negative symptoms as suggested by Benetti et al. (2015). This hypothesis is consistent with McCarthy-Jones and collaborators findings showing that patients with larger FA decreases in the arcuate were less likely to have current AVH (McCarthy-Jones et al., 2015). Our findings could also be interpreted as meaning that AVH+ patients may have normal FA and no-AVH patients abnormal low FA. The lack of a healthy subject group doesn't allow us to solve this point. However, the positive correlation found between FA of the left arcuate fasciculus and AVH severity suggests that the FA in AVH+ patients is abnormal.

Our results are in line with functional imaging studies reporting a hypercoupling between frontal and temporal regions in non-psychotic individuals with hallucinations (Diederen et al., 2013) and in AVH+ patients with schizophrenia (Lavigne et al., 2015). The WM alterations observed in patients with predominantly negative symptoms could be linked to a high susceptibility of WM tracts to inflammatory mediators in this population (Alba-Ferrara and de Erausquin, 2013). Indeed, inflammatory mediators, such as C-reactive protein (CRP), were associated with reduced FA (Prasad et al., 2015) and higher CRP levels in schizophrenia in patients with more severe negative symptoms (Garcia-Rizo et al., 2012; Joseph et al., 2015). Regarding to patient with prominent AVH, to our knowledge, no data linking cytokine levels and AVH severity in patients with schizophrenia are available to date.

Differences in the types of fibers altered could also explain the differences in FA value between patients with prominent negative symptoms and patients with treatment-resistant daily chronic AVH. In white mater regions, higher FA is typically associated with favorable neurobiological factors, such as increased tissue density and fiber organization and conversely. Thus, a reduced FA is commonly associated with clinical populations and interpreted as reflecting axonal disorganization or alterations of myelin. However, evidence for increased FA has been reported in some clinical populations, such as anorexia nervosa (Travis et al., 2015) and individuals born preterm (Groeschel et al., 2014). Interestingly, in these two populations increased FA was found in the left superior fasciculus and supposed to reflect increased fiber coherence from a reduction in the number or density of crossing fibers (Groeschel et al., 2014). Thus, a reduction in crossing fibers within the arcuate fasciculus could increase FA in AVH+ patients and offset the FA decrease due to alterations specific to the arcuate fasciculus fibers. Investigating the integrity of other bundles might also be of interest to better understand the relationship between AVH and WM abnormalities. For instance, a significantly greater FA in the fiber connecting homotopic auditory areas via the corpus callosum was observed in AVH+ as compared to no-AVH patients, a trendwise correlation between FA values and severity of AVH symptoms was also reported (Mulert et al., 2012). Moreover, a recent study performing cluster analysis on 18 fiber tracts showed two patterns of WM abnormalities in patients with schizophrenia as compared to healthy subjects. These patterns of WM alteration allowed discriminating two subgroups of patients: patients with widespread WM abnormalities showing more severe negative symptoms than patients with circumscribed regional WM abnormalities, mostly in the left superior longitudinal fasciculus (Sun et al., 2015). Combined with our findings, these results suggest the existence of two neurobiologically distinct subgroups of patients with schizophrenia according to their prominent symptomatology. Moreover, it suggests that WM abnormalities can provide a biomarker for subtyping patients.

Finally, abnormalities in the left arcuate fasciculus integrity, the most important fiber bundle between Broca's area and Wernicke's area could in part explain the emergence of AVH in patients with frequent AVH. More severe WM abnormalities in patients with prominent negative symptoms may prevent the occurrence of AVH. The correlation between FA and AVH symptom severity suggest that integrity of the left arcuate fasciculus could be a biological marker of AVH. Our results outline the importance of stratifying patients with schizophrenia according to their prominent symptoms in further imaging studies.

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Contributors

JB & MM wrote the first draft of manuscript. MM assisted with the data collection. JB & DL assisted with data analyses; MP & CF have analyzed imaging data. FH assessed clinical characteristics of patients. MFSC supervised analyses. All authors contributed to and have approved the final manuscript.

Conflict of interest

None of the authors have any conflicts of interest related to this work.

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4. Article - N-Acetyl-Aspartate in the dorsolateral prefrontal cortex in men with schizophrenia and auditory verbal hallucinations: A 1.5T Magnetic Resonance Spectroscopy Study

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NAA and AVH

Title

N-Acetyl-Aspartate in the dorsolateral prefrontal cortex in men with schizophrenia and auditory verbal hallucinations: A 1.5T Magnetic Resonance Spectroscopy Study.

Authors

Marion PSOMIADES [1,2,3], Marine MONDINO [1,2,3], Clara FONTENEAU [1,2,3], Rémy BATION [1,2,3], Frederic HAESEBAERT [1,2,3], Marie-Françoise SUAUD-CHAGNY[1,2,3], Jérome BRUNELIN [1,2,3,*]

Affiliation

1. INSERM, U1028; CNRS, UMR5292; Lyon Neuroscience Research Center, Psychiatric Disorders: from Resistance to Response Team, Lyon, F-69000, France;

2. University Lyon 1, Villeurbanne, F-69000, France;

3. Centre Hospitalier Le Vinatier, Bron, France;

Correspondence

J Brunelin, Centre Hospitalier le Vinatier - Batiment 416, 95 boulevard Pinel, BP 300 39, 69678 Bron cedex, France, jerome.brunelin@ch-le-vinatier.fr

Abstract

Auditory verbal hallucinations (AVH) in patients with schizophrenia are linked to abnormalities within a large cerebral network including frontal and temporal regions. Whilst abnormalities of frontal speech production and temporal speech perception regions have been extensively studied, alterations of the dorsolateral prefrontal cortex (DLPFC), a region critically involved in the pathophysiology of schizophrenia, have rarely been studied in relation to AVH. Using 1.5T proton magnetic resonance spectroscopy, this study examined the relationship between right and left DLPFCs N-AcetylAspartate (NAA) levels and the severity of AVH in patients with schizophrenia.

Twenty-seven male patients with schizophrenia were enrolled in this study, 15 presented daily treatment-resistant AVH (AVH+) and 12 reported no AVH (no-AVH).

AVH+ patients displayed higher NAA levels in the right DLPFC than no-AVH patients ($p=0.033$). In AVH+ patients, NAA levels were higher in the right DLPFC than in the left ($p=0.024$). No difference between the right and left DLPFC was observed in no-AVH patients. There was a positive correlation between NAA levels in the right DLPFC and the severity of AVH ($r=0.404$, $p=0.037$).

Despite limited by magnetic field strength, these results suggest that AVH may be associated with increased NAA levels in the right DLPFC in schizophrenia.

Keywords

Dorsolateral prefrontal cortex, auditory verbal hallucination, schizophrenia, Magnetic resonance Spectroscopy, N-Acetylaspartate.

Introduction

Auditory verbal hallucinations (AVH) are a disabling and frequent symptom in patients with schizophrenia. Classically, AVH have been linked to abnormal activities within bilateral inferior frontal and temporal regions associated with speech generation and speech perception [1,2]. Also, patients with AVH have been reported to display abnormal structural [3,4] and functional [5] fronto-temporal connectivity and abnormal inter-hemispheric connections between auditory cortices [6]. However, language-related areas are not the only brain structures involved in the generation of AVH. Numerous functional magnetic resonance imaging (fMRI) studies highlighted the involvement of the left [5,7,8,9,10] and the right [1,7,8,11] dorsolateral prefrontal cortex (DLPFC) in AVH. However, only few studies have explored the functional connectivity of the right DLPFC by comparing patients with AVH (AVH+ patients) and patients with no AVH (no-AVH patients). In this line, measuring local neuronal activity by regional homogeneity analysis of BOLD, Cui et al (2016)[8] put forward an increased contribution of the right DLPFC in AVH+ patients compared to no-AVH patients. At the neurochemical level, only few studies have investigated the contribution of the DLPFC in AVH in patients with schizophrenia. In a recent study using proton magnetic resonance spectroscopy (^{1}H-MRS), Ćurčić-Blake et al (2017)[12] reported higher Glx levels in the left DLPFC in AVH+ patients compared with no-AVH patients whereas the right of the DLPFC was not considered. When measured by *in vivo* ^{1}H-MRS, Glx is an index of glutamate and glutamine levels. This result suggested a relationship between high glutamatergic neurotransmission in the left DLPFC and AVH in schizophrenia. Another important brain metabolite that is often investigated using ^{1}H-MRS is N-AcetylAspartate (NAA). NAA is the second most abundant amino acid in the brain after glutamate and is exclusively synthetized in neuronal mitochondria. It is described as a reliable marker of neuronal viability and functioning[13,14]. Evidence suggests that NAA levels are decreased in the DLPFC of patients with schizophrenia [15]. However, more studies are needed to directly investigate the relationship between NAA levels in each hemispheric part of the DLPFC and AVH in schizophrenia.

Here, our objective was to study the link between AVH and the DLPFC viability and function as measured by NAA levels. We proposed to compare NAA levels in the left and right DLPFC of AVH+

and no-AVH patients. Based on Cui et al (2016)[8] showing an increased contribution of the right DLPFC in AVH+ patients as compared with no-AVH patients, we hypothesized that AVH+ patients would display higher NAA levels in the right DLPFC as compared to no-AVH patients.
Moreover, as an exploratory outcome, we conducted correlation analyses to investigate the relationship between AVH severity and left and right DLPFC NAA levels.

Results

Sociodemographic and clinical characteristics of patients

Twenty-seven male patients with schizophrenia presenting with treatment-resistant symptoms were recruited in this study. The sample was separated into two groups based on the presence and the absence of daily AVH (AVH+ and no-AVH patients, respectively). The final analyzed samples consisted of 15 AVH+ patients with daily AVH (Positive and Negative Syndrome Scale (PANSS) P3 item > 3), and 12 no-AVH patients with no current or past history of AVH (PANSS P3 item ≤ 3). There was no difference between groups regarding age, medication, duration and severity of the illness (PANSS score). The characteristics of patients are given in Table 1.

** INSERT TABLE 1 ABOUT HERE **

Levels of NAA in the left and right DLPFC in AVH+ and no-AVH male patients

A mixed model ANOVA with group (AVH+, no-AVH) as between factor and laterality (left DLPFC, right DLPFC) as within factor revealed a significant interaction between group and laterality ($F_{(1,25)}$ = 5.808; partial η^2 = 0.189; p = 0.024; figure 1). No main effects of laterality ($F_{(1,25)}$ = 0.644; partial η^2 = 0.025; p = 0.43) or group ($F_{(1,25)}$ = 0.552; partial η^2 < 0.022; p = 0.47) were reported.
Post hoc analyses revealed a significant difference between groups for NAA levels in the right DLPFC (p = 0.033). NAA levels in the right DLPFC were higher in AVH+ patients (mean = 7.54 ± standard deviation = 0.97) than in no-AVH patients (6.67 ± 1.02). No difference between groups was reported for NAA levels in the left DLPFC (p = 0.219).

In AVH+ patients, NAA levels were significantly higher in the right DLPFC (7.54 ± 0.97) than in the left DLPFC (6.63 ± 0.91; p = 0.024). In no-AVH patients, there was no difference between NAA levels in the left DLPFC (7.12 ± 1.08) and in the right DLPFC (6.67 ± 1.02; p = 0.29).

** INSERT FIGURE 1 ABOUT HERE **

Relationship between NAA levels and severity of AVH

A significant correlation was observed between NAA levels in the right DLPFC and the severity of AVH measured by P3 PANSS item (r = 0.404; p = 0.037; figure 2).

No significant correlation was reported between NAA levels in the left DLPFC and AVH severity (r = -0.325; p = 0.098).

** INSERT FIGURE 2 ABOUT HERE **

Discussion

In this study we used ^{1}H MRS to assess NAA levels in the left and right DLPFC in AVH+ and no-AVH male patients with schizophrenia. We reported that NAA levels were higher in the right DLPFC as compared with the left DLPFC in AVH+ patients. No difference in NAA levels was observed between left and right DLPFC in no-AVH patients. NAA levels in the right DLPFC were higher in AVH+ than in no-AVH patients and no between-group differences were reported in the left DLPFC. To the best of our knowledge, this study is the first to report a significant difference in NAA levels measured in the right DLPFC between two sub-groups of patients with schizophrenia based on the presence of AVH. Indeed, in the literature, the majority of studies that investigated prefrontal NAA levels in schizophrenia has compared patients with schizophrenia with healthy subjects and has measured NAA levels within the DLPFC regardless of the laterality or only in the left DLPFC. These studies showed conflicting results: some reported a decrease in NAA levels measured in the left DLPFC in patients with schizophrenia compared to healthy subjects and some failed to find significant differences[15]. These discrepancies could be explained by the clinical heterogeneity of patients'

symptomatology. Indeed, only two studies have investigated prefrontal NAA levels in relation with schizophrenia symptomatology. The first study by Callicott et al (2000) [16] reported a negative correlation between the NAA/Creatine levels in the DLPFCs and the severity of negative symptoms. However, the authors have not investigated separately the relationship between the left and the right DLPFC and the severity of symptoms and used a mean ratio of NAA/Creatine in the both DLPFCs. The second study by Sigmundsson et al (2003) [17] found a negative correlation between the severity of positive symptoms and NAA levels in the right DLPFC but not between the severity of positive symptoms and NAA levels in the left DLPFC. Moreover, the severity of general symptoms negatively correlated with NAA levels in the left and in the right DLPFC. However, these studies did not permit to isolate DLPFC hemispheric abnormalities linked to AVH. This is one of the reason we chose to directly compare AVH+ to no-AVH-patients.

The current findings of increased NAA levels in the right DLPFC in AVH+ patients as compared to no-AVH patients are consistent with some recent neuroimaging studies showing an increased contribution of right DLPFC in AVH+ patients [1,8,11]. For instance, Cui et al. (2016) [8] recently reported higher regional brain activation in the right DLPFC in AVH+ patients as compared with no-AVH patients. Using resting-state functional connectivity fMRI, two studies also found an increased involvement of the right DLPFC within widespread functional brain networks in AVH+ patients [1,11]. However, one study reported a reduced functional connectivity between the right DLPFC and inferior frontal gyrus in AVH+ patients as compared to healthy controls [7]. Finally, current results could appear in contradiction with results from Ćurčić-Blake et al (2017) [12] showing higher Glx levels in the left DLFPC in AVH+ patients than in no-AVH patients since numerous studies have reported a functional coupling between NAA and Glx in healthy subjects [18]. However, a decoupling between NAA and Glx levels was observed in the DLPFC in patients with schizophrenia [19]. In the current study, we also found a positive correlation between NAA levels in the right DLPFC and the severity of AVH, reinforcing the link between the right DLPFC and AVH.

NAA and AVH

It is important to note that the findings of a significant difference in NAA levels between left and right DLPFCs in AVH+ patients but not in no-AVH patients are difficult to interpret in the absence of a group of healthy subjects. Interestingly, Brambilla et al (2004) [20] observed no difference in NAA levels between the right and left DLPFCs in healthy subjects, suggesting that NAA levels in the DLPFC showed no hemispheric lateralization in healthy subjects. Taken together, these findings suggested that a prefrontal imbalance in NAA levels might be linked to AVH presence. However, further studies including a group of healthy subjects are needed to test this imbalance hypothesis.

Finally, our findings highlight the role of the DLPFC in AVH and are in accordance with cognitive models of AVH. In these models, the DLPFC is described as a key structure involved in "top-down" and monitoring processes suggesting that AVH generation results from a failure in speech monitoring. [21] More specifically, cognitive models suggest that AVH generation results from a functional breakdown in appropriate monitoring of inner speech generation leading to misattribution of inner speech to externally perceived event. This misattribution may be supported by an abnormal "corollary discharge", a mechanism by which the frontal regions can modulate the temporal area activity by sending inhibitory information. [22]

Some limitations may have influenced our results. First, our sample size was limited and only male patients were included. Although NAA levels in the prefrontal region seem not to be influenced by gender [23], these results need to be confirmed by further studies including larger sample sizes and including female patients. Second, it has been reported that antipsychotic medication may modulate metabolic levels measured by MRS. Some studies reported that antipsychotic medication increased NAA levels in the prefrontal cortex [24] and others reported that some antipsychotic such as risperidone can decrease NAA in the medial prefrontal cortex [25]. This discrepancy can be explained by clinical heterogeneity of patients with schizophrenia included in studies, level of treatment-resistance or by the diversity of antipsychotic treatments used in studies. Moreover, NAA alterations were also found in drug free subjects at-risk for developing schizophrenia[23]. In our study, AVH+ and no-AVH patients were all under pharmacological treatments and experienced treatment-resistant symptoms. There was

no difference between groups regarding antipsychotic treatment in term of dosage (expressed as equivalent chlorpromazine per day), molecule (first and second generation of antipsychotic) and duration of illness. Thus, the between-group difference in NAA levels might not be related to a between-group difference in antipsychotic medication.

Third, methodological issues need to be considered. The strength of the magnetic field used in our study (1.5T) can be considered as a limitation. However, NAA is one of the most abundant amino acid in the brain and 1.5T is sufficient to quantify NAA levels with accuracy, as has been done in other studies, [26] even recently [27]. Similar NAA abnormalities in patients with schizophrenia were described by studies using a 1.5T scan as well as by studies using higher magnetic field strength [26]. Moreover, although creatine has been commonly used as an internal reference to quantify metabolites levels in 1H-MRS studies, in this study we chose not to express NAA levels as a ratio to creatine. Indeed, some recent data suggested that the use of creatine as an internal reference may not be appropriate for psychiatric research [28] since creatine levels were decreased in patients with schizophrenia compared to healthy subjects [28,29] especially in the left but not right DLPFC [30]. Finally, the interpretation of NAA levels remains difficult to date. Whereas it is accepted that NAA can be used as a marker of mitochondrial activity and osmotic balance[14], NAA levels can also reflect neuronal activity and energy metabolism [13,14], leading some authors to conclude that NAA function remains unknown[31].

In summary, the current study highlighted a relationship between AVH severity and DLPFC NAA levels in patients with schizophrenia. Whereas no-AVH patients showed no difference in NAA levels between left and right DLPFC, AVH+ patients showed higher NAA levels in the right DLPFC as compared to the left. Moreover, our findings suggested a close relationship between NAA levels in the right DLPFC and the severity of AVH in patients with schizophrenia. The hemispherical imbalance between DLPFCs should thus be taken into account in studies investigating pathophysiology of schizophrenic symptoms.

Methods

The study was approved by a local ethics committee (CPP Sud Est 6, France) and all patients gave their written informed consent. All experiments were performed in accordance with relevant guidelines and regulations. Patients were referred to our research unit and have participated in clinical studies registered on clinicaltrials.gov database (NCT00870909 and NCT00875498). The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Participants. Twenty-seven male patients who met DSM IV-TR criteria for schizophrenia were enrolled in this study between February 2009 and January 2016. The severity of symptoms was assessed using the Positive and Negative Syndrome Scale (PANSS). The severity of AVH was assessed by the PANSS P3 item (i.e., hallucinations). Since all the AVH+ patients experienced mainly AVH, the P3 item was used to measure exclusively AVH severity in our study. Patients were divided into two subgroups based on the presence of daily treatment-resistant AVH (PANSS P3 item > 3, AVH+ patients) or the absence of AVH (PANSS P3 item ≤ 3, no-AVH patients). The cut off P3 ≤ 3 has been fixed according to remission definition proposed by Andreasen et al (2005)[32]. The subgroup of AVH+ patients consisted of 15 patients and the subgroup of no-AVH patients consisted of 12 patients. Patients presented with treatment-resistant symptoms defined as the persistence of symptoms despite two well conducted antipsychotic treatments from different pharmacological classes, in sufficient dose and duration [33]. All included patients were treated with antipsychotic medication for at least three months without change in dose and still presenting with disabling treatment-resistant symptoms.

At inclusion, patients were treated with several antipsychotic agents from different class. In the AVH+ group, 3 patients received first generation antipsychotic, 8 patients received second-generation antipsychotic and 4 received a combination of both. In the no-AVH group, 1 patient received first generation antipsychotic, 10 patients received second-generation antipsychotic and 1 received a combination of both. No difference between groups was observed regarding the class of antipsychotic received (Fisher Exact Probability Test = 0.37).

Image acquisition. Images were acquired on a 1.5-T Siemens Magnetom Sonata Maestro Class system equipped with a standard headcoil. A structural 3D image was obtained with a standard T1-weighted pulse sequence: 176 transverse slides, TR = 1970 ms, TE = 3.93 ms, flip angle of 15°, FOV = 256 x 256 mm, voxel size: 1.0 x 1.0 x 1.0 mm. In vivo ^{1}H MRS measurements were acquired with PRESS sequence (TR = 1500 ms; TE = 30 ms; Spectral Bandwidth = 1000 Hz; Averages = 16; 1024 samples) with and without suppression of water signal (CHESS sequence). A 2x2x3 voxel of interest (12 cm^3) was positioned on the 3D anatomical MRI to acquire signal in the left and the right DLPFC (Figure 3).

** INSERT FIGURE 3 ABOUT HERE **

Data analysis. In vivo ^{1}H MRS analyses were processed using Tarquin 4.3.8 software. Total NAA absolute concentration was determined and corresponded to the sum of NAA and N-Acetyl-Aspartyl-Glutamate (NAAG). NAA levels were calculated relative to the unsuppressed water signal from the same voxel.

Quantification reliability of results was assessed using Cramer-Rao lower bound (CRLB), a common metric for the goodness of spectral fit. The threshold for CRLB standard deviation values was set at 20 %. Thus, a CRLB standard deviation value greater than or equal to 20% was considered unreliable. Data quality was verified using the mean and standard deviation for CRLB, Signal to Noise Ratio (SNR), and Full width at half maximum (FWHM) and water peak in each group. No significant difference between groups was observed regarding CRLB, SNR, FWHM and water peak in the both DLPFCs (please see Supplementary Material 1).

Tissue segmentation was performed in both ROIs. No significant difference between groups was observed regarding CSF, gray matter and white matter composition per ROI in the both DLPFCs (please see Supplementary Material 2).

Statistical analyses. Statistical analyses were performed with SPSS-22 (Statistical Package for the Social Sciences, version 22). Statistical significance was set at $p<0.05$, two-tailed for all analyses.

Sociodemographic and clinical characteristics of patients were compared using 2-tailed student t-tests for continuous variables and Fischer's exact test for gender and antipsychotic medication class. MRS data quality and tissue segmentation were compared between groups (AVH+ and no-AVH) using 2-tailed student t-tests. Bonferroni correction for multiple comparisons was applied.

To investigate difference in NAA levels in the DLPFC between groups, NAA levels were entered in a mixed model ANOVA with group (AVH+, no-AVH) as between factor and laterality (left DLPFC, right DLPFC) as within factors. In case of significance, post hoc tests were performed with Bonferroni correction for multiple comparisons.

Relationships between AVH severity (measured by P3 item PANSS score) and NAA levels in the left and right DLPFC were investigated using Pearson correlation tests.

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Author Contributions

MP & MM have acquired MRS data. RB & FH have assessed clinical data.

MP, JB & MFSC have analysed and interpreted data. MM has undertaken statistical analyses. CF has helped in analyzing and interpreting MRS data.

MP & JB have written the first draft of the manuscript, MM & MFCS have provided critical revisions of the manuscript. All the authors have approved the final version of the manuscript.

Competing interests

The author(s) declare no competing interests.

Table 1: Sociodemographic and clinical characteristics of patients with schizophrenia with (AVH+) and without (no-AVH) auditory verbal hallucinations (AVH).

The results are given as the mean (standard deviation).

PANSS: Positive And Negative Syndrome Scale. eq cpz: chlorpromazine equivalent

	AVH+	no-AVH	p
n	15	12	
Age (years)	33.9 (4.9)	37.3 (7.3)	0.16
Educational level	10.8 (2.6)	11 (2.7)	0.83
Illness duration (years)	10.8 (5.2)	13.5 (7.3)	0.27
Medication (eq cpz mg/day)	759.5 (483.8)	546.6 (641.8)	0.36
PANSS total	73.9 (13)	75.3 (11.9)	0.77
P3 item (AVH)	5.7 (0.7)	1.5 (0.9)	< 0.001

Figure 1. N-AcetylAspartate (NAA) levels measured by ^{1}H Magnetic Resonance Spectroscopy in the left and right Dorsolateral Prefrontal Cortex (DLPFC) in patients with schizophrenia with (AVH+, n = 15) and without (no-AVH, n = 12) auditory verbal hallucinations (AVH). NAA levels were higher in the right DLPFC in AVH+ patients than in non-AVH patients and in the left DLPFC ($F_{(1,25)} = 5.808$; partial $\eta^2 = 0.189$; $p = 0.024$).

Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots.

Figure 2. Relationship between N-AcetylAspartate (NAA) levels in the right Dorsolateral Prefrontal Cortex (DLPFC) and severity of auditory verbal hallucinations (AVH) measured by Positive And Negative Syndrome Scale - PANSS P3 item in patients with schizophrenia. ($r = 0.404$; $p = 0.037$)

Figure 3. A: Example of voxel placement on subject's 3D anatomical MRI and B: example of MRS spectra obtained with Tarquin 4.3.8 software.

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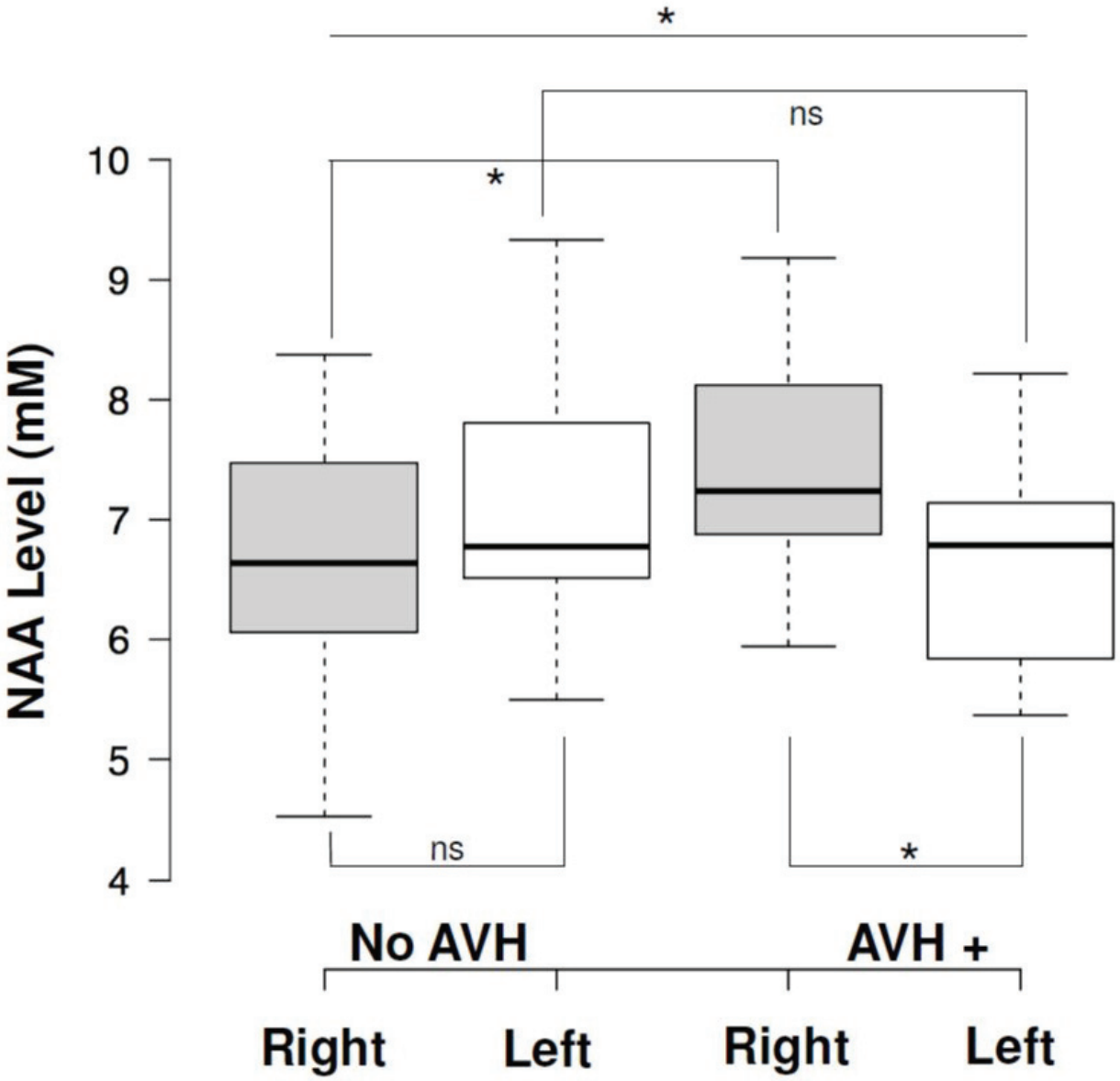
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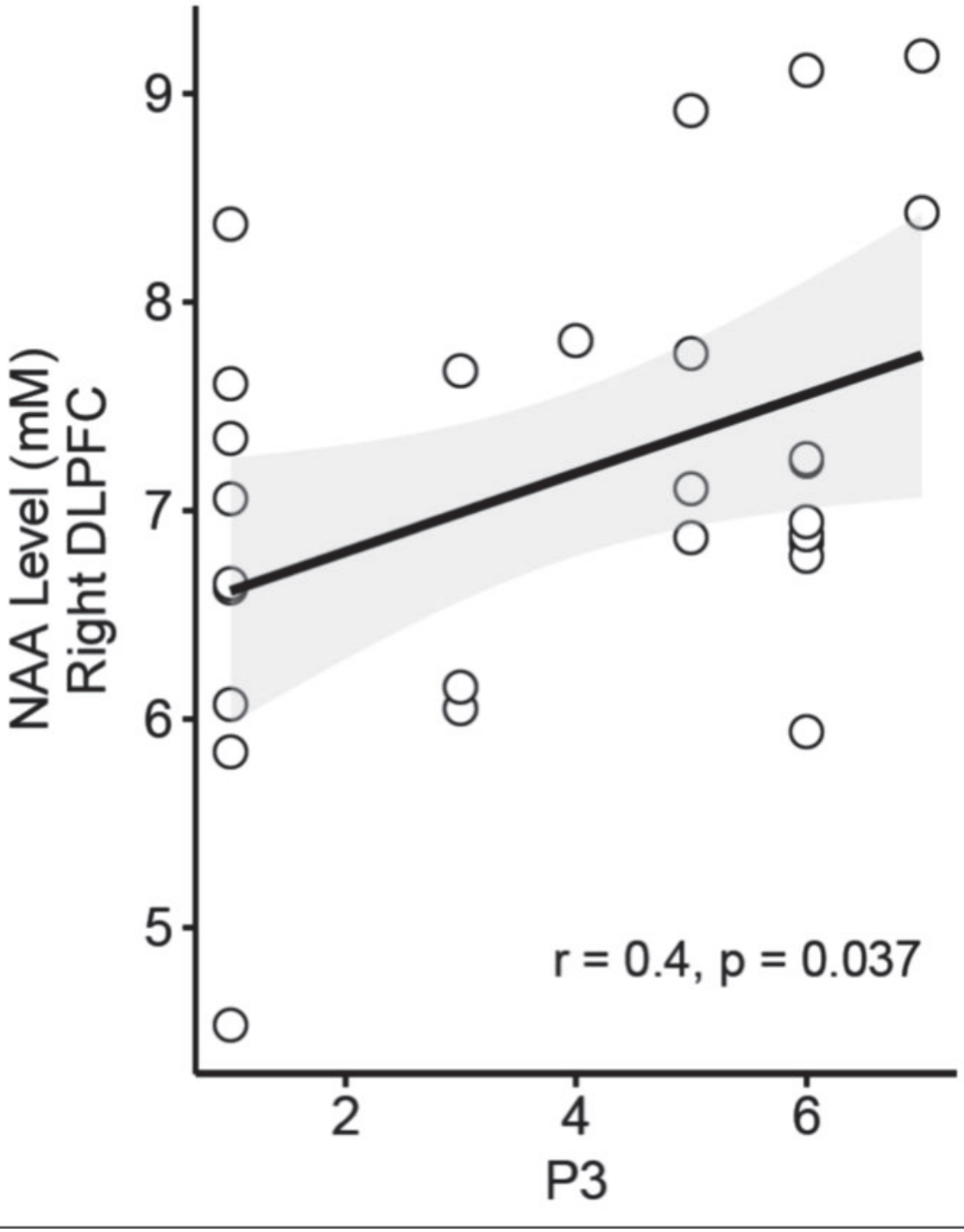
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r = 0.4, p = 0.037

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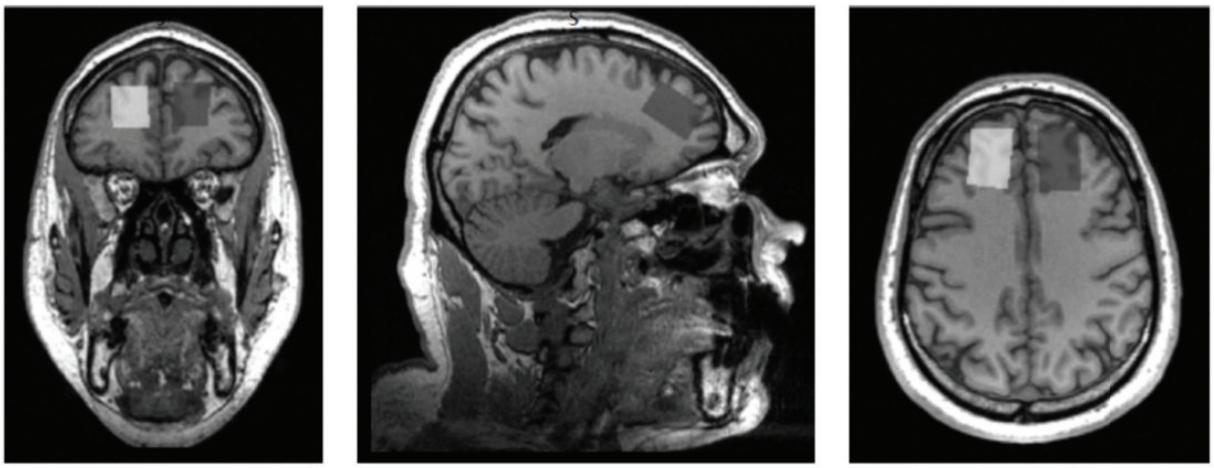
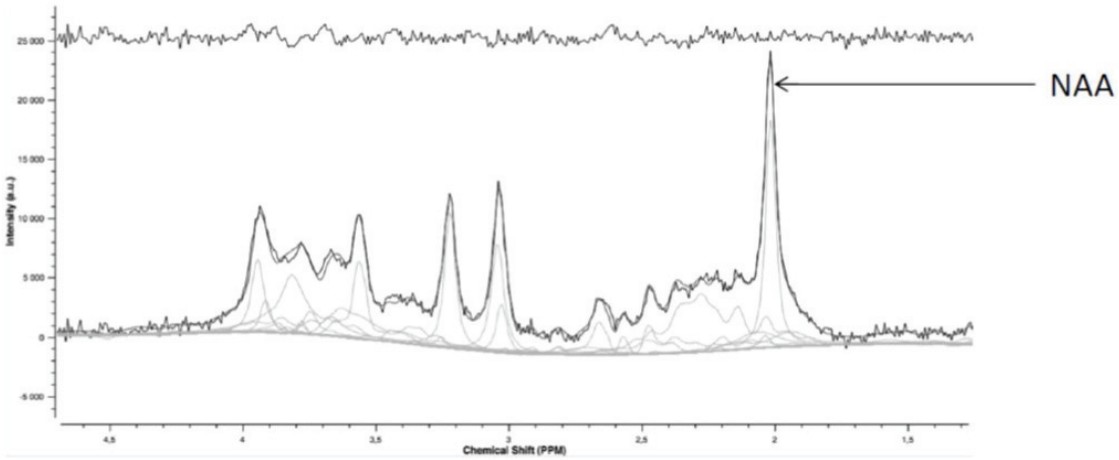


B


NAA
Intensity (a.u.)
25 000
20 000
15 000
10 000
5 000
0
-5 000
4,5
4
3,5
3
2,5
2
1,5
Chemical Shift (PPM)

N-Acetyl-Aspartate in the dorsolateral prefrontal cortex in men with schizophrenia and auditory verbal hallucinations: A 1.5T Magnetic Resonance Spectroscopy Study.

Marion PSOMIADES, Marine MONDINO, Clara FONTENEAU, Rémy BATION, Frederic HAESEBAERT, Marie-Françoise SUAUD-CHAGNY, Jérome BRUNELIN.

Supplementary Material 1. MRS data quality

	AVH+	no-AVH	P
CRLB NAA			
Right DLPFC	4.5 (1.4)	6.6 (2.8)	0.14
Left DLPFC	7.9 (3.5)	6.5 (4.2)	1
SNR			
Right DLPFC	26.4 (5.7)	25.2 (6.3)	1
Left DLPFC	24.6 (4.6)	27.1 (4.7)	1
FWHM NAA (ppm)			
Right DLPFC	0.053 (0.013)	0.065 (0.020)	0.79
Left DLPFC	0.068 (0.015)	0.061 (0.016)	1
Water width (ppm)			
Right DLPFC	0.087 (0.007)	0.095 (0.013)	0.49
Left DLPFC	0.105 (0.015)	0.092 (0.015)	0.36

Data quality was verified using the mean and standard deviation for CRLB, Signal to Noise Ratio (SNR), and Full width at half maximum (FWHM) and water peak in each group. No significant difference between groups was observed regarding CRLB, SNR, FWHM and water peak in the both DLPFCs.

MRS data quality was compared between groups (AVH+ and no-AVH) using 2-tailed student t-tests. Bonferroni correction for multiple comparisons was applied.

Supplementary Material 2. Tissue segmentation

	AVH+	no-AVH	P
Grey matter (%)			
Right DLPFC	41.7 (3.4)	37.6 (6.6)	0.30
Left DLPFC	39.4 (4.3)	36 (6.6)	0.73
White matter (%)			
Right DLPFC	52.2 (4.6)	53.7 (9.2)	1
Left DLPFC	54.1 (5.0)	55.1 (9.5)	1
CSF (%)			
Right DLPFC	5.9 (3.7)	8.5 (5.3)	0.87
Left DLPFC	6.3 (3.6)	8.8 (4.8)	0.84

Tissue segmentation was performed in both ROIs (right and left DLPFC) in patients with AVH and in patients with no AVH.

No significant difference between groups was observed regarding CSF, gray matter and white matter composition per ROI in the both DLPFCs.

Tissue segmentation was compared between groups (AVH+ and no-AVH) using 2-tailed student t-tests. Bonferroni correction for multiple comparisons was applied.

5. Letter to editor: Blinding or real biological effect? Thoughts around sham transcranial direct current stimulation

in preparation

Blinding or real biological effect? Thoughts around sham transcranial direct current stimulation

Clara FONTENEAU [1,2,3], Marine MONDINO [1,2,3], Marie-Françoise SUAUD-CHAGNY [1,2,3], Jérome BRUNELIN [1,2,3]

1) INSERM U1028; CNRS UMR5292; Lyon Neuroscience Research Center, Psychiatric Disorders: from Resistance to Response Team, F-69000, France

2) University Lyon 1, Villeurbanne, F-69000, France

3) Centre Hospitalier Le Vinatier, Lyon, F-69000, France

Corresponding author:

Clara Fonteneau

Centre Hospitalier Le Vinatier

Equipe de Recherche PSYR2

Pôle Est - Bâtiment 416 – 1er étage

BP 300 39 – 95 boulevard Pinel

69678 BRON Cedex – FRANCE

Phone Number : +33(0)437915565

e-mail: fonteneau.clara@gmail.com

Highlights:

- inconsistent sham tDCS protocols
- biological effect of sham tDCS
- reproducibility aspects of stimulation parameters

LETTER TO THE EDITOR

Blinding or real biological effect?

Thoughts around sham transcranial direct current stimulation

Dear editor,

In light of the increasing interest surrounding reproducible transcranial direct current stimulation (tDCS) studies, guidelines emerge notably pointing to the importance of masking (Brunoni et al., 2011; Woods et al., 2016; Bikson et al., 2017). This process, also called blinding, is an essential part of tDCS designs, developed to keep participants and experimenters unaware of the intervention administered (active or sham) and thus avoid bias and unrelated observable effects in order to establish study validity and prevent false positive conclusions regarding the efficacy of tDCS.

Although this is of major relevance for creating reproducible tDCS experiments, we want to emphasize here a related point that is under-addressed in the literature and also critical for reproducibility, which are sham tDCS effects. Indeed, the main constant across placebo controlled tDCS studies is that the sham stimulation would exert similar responses as a placebo pill administration. This sham stimulation is thought to mimic sensory effects and thus blind the subjects to the intervention provided, especially when participants receive both treatments (active and sham stimulation). In most of cases, sham tDCS consisted in delivering a short period of active stimulation at the beginning of the stimulation session (from 10s at 0.1 mA to 120s at 2 mA) followed by no stimulation. Participants feel typical initial sensations of active tDCS underneath the electrode sites (e.g., tingling, itching).

To date, this sham mode is thought only as a way to minimize any potential neuromodulatory effects unrelated to the stimulation itself. Moreover, based on studies using tDCS and transcranial magnetic stimulation (TMS) over the motor cortex, sham stimulation is thought unlikely to produce lasting changes in cortical excitability (Nitsche et al., 2008). However, these tDCS and TMS studies have also revealed opposite effects of the stimulation on cortical excitability which can be linked to different intensity and duration of the stimulation (Monte-Silva et al., 2013). On the one hand several studies have investigated tDCS effects with parameters similar to those of sham parameters (i.e. short stimulation duration) and some report an effect (Priori et al., 1998; Kuo et al., 2006; Furubayashi et al., 2008; Antal et al., 2011; Javadi et al., 2012), whereas others report no effect (Nitsche et al, 2000). On the other hand, some placebo-controlled studies report no effect of the sham group (Peña-Gómez et al., 2012; Stagg et al., 2013; 30s stimulation) and a recent study on motor cortex excitability reported a moderate level of reliability

concerning the null effect of sham stimulation (Dyke et al., 2016; 30s stimulation). Nevertheless, to our knowledge no tDCS study investigated the neurobiological effects of parameters used in sham conditions as the primary objective. Thus, sham stimulation could possibly by itself exert neuromodulatory effects independent of the placebo effect of the stimulation and possibly with either similar or different action than the active stimulation.

Furthermore, numerous sham modes are being used in the community and these differences across protocols could lead to sham stimulation that might induce multiple physiological/biological effects. Indeed, in the literature, a variety of sham protocols are reported (**Figure 1**). Based on the recent review from Bikson and colleagues (2017), a majority of tDCS studies report using the approach suggested by Gandiga and colleagues (2006). This sham protocol consists in a 10s ramp-up followed by 30s of sham stimulation before turning off the stimulator. However, this protocol has been adapted with various degrees of changes concerning the intensity and duration of the active current being delivered (from “no current” to 2 minute stimulation), the duration of ramp-in and ramp-out phases (e.g. 5-30s) and as well as the number of ramps carried out throughout the stimulation. Indeed, new sham protocols include 2 periods of sham stimulation, including ramps up-down with 10-30s of stimulation in between, over the first and last seconds of the stimulation (Palm et al., 2013). Furthermore, in order to help practitioners deliver adequate sham treatment interventions, several commercial stimulators provide a study mode, which delivers a built-in-sham mode. However, it should be noted that this sham mode varies across stimulator brands, which could be a confounding factor when comparing studies and in multi-centric studies using various devices across centers. We urge scientists and clinicians to be aware of the built-in-sham mode of their respective stimulator (i.e. NeuroConn device (GmbH, Germany): 20min active=40s sham stimulation / 30min active=1min sham with ramps; Soterix Medical Inc (New York, NY) and NeuroElectrics StarStim (Barcelona, Spain): only 2 ramps beginning and end).

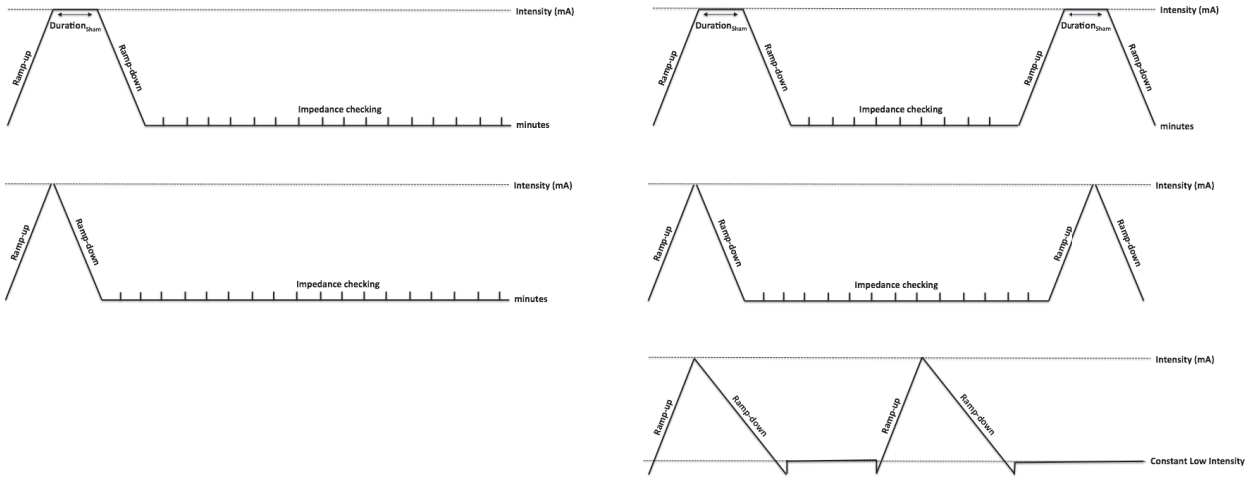


Figure 1 - Different types of sham - Different Duration of sham (5s, 8s, 10s, 15s, 20s, 30s, 40s, 60s, 2min). Different duration of ramps (5s, 8s, 10s, 15s, 30s). Either same or reduced intensity as active stimulation. Some studies exert a constant low intensity sham stimulation (0.034mA). Either 1 or 2 ramps per session (beginning, middle or/and end).

With this in mind, these different sham stimulations could be a possible confounding factor in clinical and cognitive studies but also when investigating the neurophysiological effects of tDCS. Indeed, functional neuroimaging, at different spatial and temporal levels (biological (PET; MRS), functional (BOLD fMRI, ASL, EEG, MEG) and structural (VBM, DTI)) is used to gain new useful information for inferring tDCS's mechanisms of action. However, these studies draw conclusions based on comparison between active and sham groups. Thus, could sham tDCS itself have a neurobiological impact? Or does it only reflect a placebo effect? Are all sham stimulations equal regarding their biological effects? What about repeated sham tDCS? Could sham tDCS contribute to the blurry vision surrounding the mechanisms of action of tDCS?

Indeed, to date the field of tDCS is clouded with mixed results both in cognitive and clinical studies (Lefaucheur et al., 2017) and when looking at the sham parameters, they differ greatly between studies. Thus, sham could be an important parameter among others (session duration, number of session, current intensity) to keep in mind when designing tDCS studies, not only for blinding but also to investigate potential specific neuromodulatory effects linked to the sham stimulation itself.

Ultimately, more research seems needed to make sure sham protocols are reliable and do not induce unexpected neurobiological effects. In addition, accurately reporting sham interventions is crucial to help increase reproducibility in the tDCS research field. Moreover, this interrogation fits in the current interest of

the NIMH to ‘validate that sham interventions are biologically inactive’ (Bikson et al., 2017). Our hope is that a better understanding of these neurobiological processes can improve clinical efficacy.

Conflict of interest

None of the authors have any conflict of interests related to this letter.

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Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Built in sham mode							
Hoy et al (2014)	Schizophrenia	18	Y	F3/FP2	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	Built it sham mode, Sham stimulation began with a fade in of 120 s to a peak current of 2 mA, followed by 30 s of stimulation at constant current before being immediately faded out over 15 s
Hoy et al (2015)	Schizophrenia	16	Y	F3/FP2	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	Built it sham mode, Sham stimulation began with a fade in of 120 s to a peak current of 2 mA, followed by 30 s of stimulation at constant current before being immediately faded out over 15 s
Bose et al (2017)	Schizophrenia	25	Y	F3FP1/T3P3	20	Neuroconn DC Stimulator Plus	current was delivered at 2 mA strength for the first 40 s, After 40 s of stimulation, only a small current pulse was delivered every 550 ms (110-μA over 15 ms with peak current lasting for 3 ms).
Brunelin et al (2012a)	Schizophrenia	15	Y (15)	F3FP1/T3P3	20	Eldith DC stimulator	40 seconds of real stimulation (2 mA), only a small current pulse occurred every 550 msec (110 mA over 15 msec) through the remainder of the 20-minute period.
Frohlich et al, (2016)	Schizophrenia	23	Y	F3FP1/T3P3 /Cz	20	Eldith Stimulator Plus manufactured by neuroConn,	sham dual mode tDCS (40s stim)
Keeser et al (2011)	Healthy	13	Y	F3/FP2	20	Eldith DC stimulator (neuroConn)	built-in placebo mode , ramps at the beginning and end of sham stimulation
Balconi & Vitaloni (2012)	Healthy	34	Y	FP2/F3	15	custom-built placebo stimulator	custom-built placebo stimulator was used
Brunoni et al (2017)	Depression	91 + 94	Y (60)	F3/F4	30	Soterix Medical	current was turned off automatically after 30 seconds (build in sham mode)
Mondino et al (2015)	Schizophrenia	23	Y	F3FP1/T3P3	20	Eldith DC stimulator Plus	first 30 sec of the 20-min period; code (Gandiga)
Orlov et al,(2016)	Schizophrenia	24	Y (25)	F3/FP2	30	Eldith Stimulator Plus manufactured by neuroConn,	stimulation was applied for 30 s with the same ramping parameters (30s) built in sham mode
Bennabi et al (2015)	Depression	24	Y	F3/Fp2	30	'Eldith'' stimulator, Ilmenau, Germany	current was gradually ramped down to zero, (built in sham mode)
Mulquiney et al (2011)	Healthy	10	Y	F3/FP2	10	Eldith Stimulator Plus manufactured by neuroConn,	constant current faded in for 20 s before being immediately faded out for 20 s. (coding stimulator)
Teo et al, (2011)	Healthy	12	Y	F3/FP2	20	Eldith Stimulator Plus manufactured by neuroConn,	15s fade-in and fade-out before turning off (code stimulator)

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	**Population**	**n**	**sham**	**Anode/Cathode placement**	**Duration of stim**	**Stimulator**	**Sham parameters**
Sampaio-Junior et al, (2018)	Depression	59	Y	F3/F4	30	Soterix Medical	ramp-up 30s/ramp-down 15s, 30s sham stimulation (code)
Hoy et al, (2013)	Healthy	18	Y	F3/FP2	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	120s ramp in; 30s stim; 15s ramp down (same ramps than active; only 30s stim)
Plewnia et al, (2013)	Healthy	46	Y	F3/FP2	20	NeuroConn GmbH, Ilmenau, Germany	current was applied only for 40 sec (ramps 5s, id active). Predefined codes.
Shiozawa et al (2016)	Schizophrenia	10	Y	F3/F4	20	NA	study mode (turned off after 60s)
Turned off after 5s							
Fregni et al (2005)	Healthy	15	Y	F3/F4	10	Schneider Electronic	stimulator was turned off after 5 s (Siebner and colleagues 2004)
Fregni et al (2006)	Depression	18	Y	F3/FP2	20	NA	stimulator was turned off after 5 s (Siebner and colleagues 2004)
Mylius et al (2012)	Healthy	12	Y	F3/F4 or F4/F3	20	Schneider Electronic, Gleichen, Germany	as usual, by turning off the stimulator after the subjects felt the initial tingling sensation for 5 s (Nitsche et al., 2003)
Turned off after 8s							
Hammer et al, (2011)	Healthy	36	Y	F3/FP2	30	Eldith Stimulator Plus manufactured by neuroConn,	current was applied for 8 s and was then turned off
Turned off after 10s with very low current							
Huey et al (2007)	Dementia	10	Y	F3/FP2	40	Phoresor® II Auto Model PM850 iontophoresis	10 seconds of very low current (0.1 mA) and was then shut off.
Turned off after 10s							
Iyer et al (2005)	Healthy	103	Y	F3/FP2	20	Exp1:Grass CCU1 constant current unit, controlled by a S11 stimulator Exp2-3:Phoresor, Iomed Inc., Salt Lake City, UT.	current was delivered for 10 seconds,
Ohn et al (2008)	Healthy	15	Y	F3/FP2	30	Phoresor PM850 (IOMED, Salt Lake City, Utah, USA)	current was applied for 5 s, and was then tapered off over 5s
Dockery et al (2009)	Healthy	24	Y	F3/FP2	15	Rolf Schneider Electronic	current was applied for 5 s, and was then tapered off over 5s
Plazier et al (2012)	Healthy	17	Y	F3/F4 F4/F3	20	Mind Alive Inc.,Edmonton, Alberta, Canada	current was delivered for 10 seconds, with the same ramp up as during real stimulation. Afterward the device was turned off

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	**Population**	**n**	**sham**	**Anode/Cathode placement**	**Duration of stim**	**Stimulator**	**Sham parameters**
Priori et al (2008)	Healthy	15	Y	F3 or F4 / right deltoid	10	NA	stimulator was turned off after 10 s
Jo et al, (2009)	Stroke	10	Y	F3/FP2	30	Phoresor II PM850 (Iomed Inc., Salt Lake City, UT)	stimulator was turned on for only 10 secs during which the current intensity was gradually increased and then decreased to di- minish the perception of the sham.
Knechtel et al (2014)	Healthy	16	Y	F3/FP2	20	Transcranial Direct Current Stimulator PLUS, Magstim	10 s of gradually increasing to 2 mA, followed by maintaining 2 mA for 10 s before gradually decreasing the current over 10 s from 2 mA to 0 mA
Knechtel et al (2014)	Schizophrenia	14	Y	F3/FP2	20	Transcranial Direct Current Stimulator PLUS, Magstim	10 s of gradually increasing to 2 mA, followed by maintaining 2 mA for 10 s before gradually decreasing the current over 10 s from 2 mA to 0 mA
Turned off after 15s							
Weber et al (2014)	Healthy	11	Y (11)	F4 / F3	15	Magstim Eldith stimulator (Carmarthenshire, UK)	stimulator was turned off after 15 s of stimulation
Filmer et al (2014)	Healthy	18	Y	F3/FP2	8	Eldith Stimulator Plus manufactured by neuroConn,	30s ramps and total current lasted for 1min15s (i.e 15s stim)
Leite et al (2013)	Healthy	16	Y	F3/F4	duration of experiment (30min max)	battery-driven Eldith Stimulator DCþ (Neuroconn, Germany)	15 s ramp up, 15 s of plateau and then 15 s down
Turned off after 20s							
Boggio et al (2007)	Depression	26	Y (7)	F3/FP2	20	sboggio@colband.com.br	stimulator was turned-on for 20 s only
Conson et al (2015)	Healthy	16	Y	F3/F4 F4/F3	15	BrainStim	stimulator was turned-on for 20 s only
Nitsche et al (2012)	Healthy	14-17	Y	F3/FP2	10 or 20	Schneider Electronic, Gleichen, Germany	current flow was terminated after 20 s
Vanderhasselt et al, (2013)	Healthy	25	Y	F3/FP2	20	NA	current was ramped down after 20 seconds
Jeon et al, (2012)	Healthy	32	Y	F3/F4 F4/F3	20	Phoresor II Auto Model PM850 (IOMED, Salt Lake City, USA)	intensity of the current was gradually decreased after 10 seconds, then being turned off after 20 seconds
Turned off after 30s							

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Loo et al (2010)	Depression	20	Y (20)	F3/FP2	20	DC stimulator made by J. Lagopoulos + Eldith DC-stimulator (NeuroConn GmbH, Germany)	the current was gradually ramped down (30s) to zero after 30s of stimulation
Vercammen et al (2011)	Schizophrenia	20	Y	F3/FP2	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	stimulator was turned off after 30 s of stimulation(id Loo 2010)
Boggio et al (2008)	SUD (alcohol)	13	Y	F3/F4 F4/F3	20	sboggio@colband.com.br.	stimulator was turned off after 30 s of stimulation (custom button)
Boggio et al (2009)	SUD (nicotine)	27	Y	F3/F4 F4/F3	20	sboggio@colband.com.br.	stimulator was turned off after 30 s of stimulation (custom button)
Boggio et al (2010)	SUD (cannabis)	25	Y	F3/F4 F4/F3	20	sboggio@colband.com.br.	stimulator was turned off after 30 s of stimulation (custom button)
Fregni et al (2008)	SUD (food)	23	Y	F3/F4 F4/F3	20	sboggio@colband.com.br.	stimulator was turned off after 30 s of stimulation (custom button)
Fregni et al (2008)	SUD (nicotine)	24	Y	F3/F4 F4/F3	20	sboggio@colband.com.br.	stimulator was turned off after 30 s of stimulation (custom button)
Fitzgerald et al (2014)	Schizophrenia	24	Y	F3/T3P3	20	Eldith DC stimulator Plus	ramp up of stimulation and 30 s of stimulation prior to stimulation off set
Mondino et al (2014)	Schizophrenia	28	Y	F3FP1/T3P3	20	Eldith DC stimulator Plus	first 30 sec of the 20-min period
Lattari et al (2017)	Parkinson	17	Y	F3/Fp2	20	TCT, China	stimulator was turned off after 30 s of stimulation (Gandiga et Boggio)
Hortensius et al (2012)	Healthy	60	Y	F3/F4 F4/F3	15	Magstim Eldith DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany)	initial 5 s ramp-up period, real stimulation lasted for 30 s followed by a ramp-down period of 5s (Gandiga)
Orlov et al, (2017)	Schizophrenia	28	Y	F3/FP2	30	Eldith Stimulator Plus manufactured by neuroConn,	30s stim
Monte-Silva et al (2009)	Healthy	12	Y	left M1/FP2	anode:13 cathode: 9	Schneider Electronic	ramp 10s then stimulator was turned on only for 30 s (Gandiga et al., 2006).
Fecteau et al (2007)	Healthy	35 12	Y	F3/F4 or F4/F3 F3/FP2 or F4/FP1	15-20	sboggio@colband.com.br.	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Knoch et al (2007)	Healthy	64	Y	FP1/F4	14	sboggio@colband.com.br.	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Rigonatti et al (2008)	Depression	42	Y	F3/FP2	20	sboggio@colband.com.br.	stimulator was turned on only for 30 s (Gandiga et al., 2006). (Boggio 2008)
Park et al (2013)	Healthy	25	Y (14)	F3/FP2	20	DC-STIMULATOR MR (neuroConn GmbH, Ilmenau, Germany)	stimulator was turned off after 30 s of stimulation

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Pena-Gomez et al (2012)	Healthy	10	Y	F3/FP2 F4/FP1	20	Phoresor PM850 (IOMED, Salt Lake City, UT)	stimulator was turned off after 30 s of stimulation and ramp off (id Fregni 2005)
Blumberger et al (2012)	Depression	13	Y (11)	F3/F4	20	CX-6650; Rolf Schneider Electronics, Germany	stimulator was turned off after 30 s of stimulation (Gandiga + Ambrus 2010)
Boggio et al (2008)	Depression	40	Y	F3/FP2	20	sboggio@colband.com.br.	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Gorini et al (2014)	SUD (cocaine)	18	Y	F3/F4 or F4/F3	20	HDC-stim, Newronika, Milan, italy	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Metuki et al (2012)	Healthy	21	Y	F3/FP2	11	battery-driven, constant-current stimulator (Rolf Schneider Electronics, Germany)	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Nelson et al (2014)	Healthy	19	Y	F3/F4	10	MagStim DC stimulator (Magstim Company Limited; Whitland, UK)	30s stim + ramps 15s
Nozari et al (2013)	Healthy	24	Y	F3/F4	20	Magstim Eldith 1 Channel DC Stimulator Plus, Magstim Company Ltd., Whitland, Wales	30s stim + ramps 30s
Iuculano and Cohen Kadosh et al (2013)	Healthy		Y	F3/F4	20	NeuroConn Eldith DC-Stimulator Plus	stimulator was turned on only for 30 s (Gandiga et al., 2006).
Salehinejad et al (2017)	Depression	24	Y	F3/F4	20	“ActivaDose Iontophoresis” manufac- tured by Activa Tek	ramped up for 30s and then turned off (Palm, 2013)
Pavlova et al (2018)	Depression	69	Y	F3/FP2	20 or 30	Reamed-polaris (Vozrojdenie, Russia)	stimulator was turned on only for 30 s
Turned off after 40s							
Smith et al (2015)	Schizophrenia	30	Y	F3/FP2	20	Chattanooga Ionto Device, Chattanooga Group, Chattanooga, TN	stimulation with 2 mA lasting only 40 seconds
Nilsson et al, (2015)	Aging	30	Y	F3/FP2	25	DC-STIMULATOR PLUS (neuroConn GmbH, Ilmenau, Germany)	current was terminated after 40 s
10s ramp-in; 30s active stimulation; 10s ramp-out							
Loo et al (2012)	Depression	33	Y (31)	F3/F8	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	1 mA current was applied for 30s, ramps 10s
Stagg et al (2013)	Healthy	24	Y	F3/FP2 F4/FP1	20	MR-compatible system (DC-Stimulator MR, Magstim)	stimulator was turned off after 30 s of stimulation (fade in/out 10s)

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Boggio et al (2006)	Parkinsons	18	Y	F3/Fp2	20	Schneider Electronic, Gleichen, Germany	current intensity was gradually decreased after 20 s, being then turned off after 30 s (ramp down of 10 s)
Maeoka et al (2012)	Healthy	15	Y	F3/FP2	20	DC Stimulator Plus; NeuroConn, Ilmenau, Germany	stimulator was turned off after 30 s of stimulation; ramps 10s
Motohashi et al (2013)	Healthy	11	Y	F3/FP2	20	NeuroConn, Ilmenau, Germany	the current was applied for 30 s, with ramp up and ramp down over 15 s.
Javadi et al, (2013)	Healthy	30	Y	F3/FP2	20	NeuroConn DC brain stimulator plus unit *rogue resolutions, wales UK)	30s sham stim (ramps 10s)
10s ramp-in; 30s active stimulation; 10s ramp-out + reduced intensity							
Powell et al (2014)	Depression	18	Y	F3/F8	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	1 mA (instead of 2mA) current was applied for 30 s with ramp up/down over 10 s
30s ramp-in; 30s active stimulation; 15s ramp-out							
Brunoni et al (2014a)	Depression	20	Y(17)	F3/F4	30	Chattanooga Ionto Device, Chattanooga Group, Chattanooga, TN	sham procedure consisted of an initial 30-s ramp-in phase, 30 s of active stimulation and a ramp-out phase of 15 s
120s ramp-in; 30s active stimulation; 15s ramp-out							
Andrews et al (2011)	Healthy	10	Y	F3/FP2	10	Eldith DC-stimulator (NeuroConn GmbH, Germany)	120s ramp in; 30s stim; 15s ramp down (same ramps than active; only 30s stim)
Turned off after 60s							
Brunoni et al (2012)	Depression	28	Y	F3/F4	30	NA	electric current was turned off for 60 s after stimulation onset
Brunoni et al (2013b)	Depression	60	Y (60)	F3/F4	30	Chattanooga Ionto Device, Chattanooga Group, Chattanooga, TN	electric current was turned off for 60 s after stimulation onset (Gandiga)
Brunoni et al (2014b)	Depression	12	Y(12)	F3/F4	30	Chattanooga Ionto Device, Chattanooga Group, Chattanooga, TN	electric current was turned off for 60 s after stimulation onset (id Brunoni et al, 2012)
Oliveira et al (2013)	Depression	28	Y	F3/F4	30	NA	electric current was turned off 60 s after stimulation
Gladwin et al (2012)	Healthy	20	Y	F3/FP2	10	NA	automatically switched off after 1 min
Gladwin et al (2012)	Healthy	14	Y	F3/FP2	10	NA	automatically switched off after 1 min (ramps 8s not included)
Kang et al, (2009)	Stroke	10	Y	F3/FP2	20	Phoresor II PM850; IOMED Inc., Salt Lake City, Utah	1min and then slowly tapered down to 0.

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Kang et al, (2012)	TBI	9	Y	F3/FP2	20	Phoresor II PM850; IOMED Inc., Salt Lake City, Utah	1min and then slowly tapered down to 0.
2min stimulation							
Axelrod et al (2015)	Healthy	45	Y	F3/FP2	10	Magstim	ramps of 30s (same as active) but 2min of stimulation
2min fade-in then cessation of stimulation							
Segrave et al (2014)	Depression	27	Y	F3/F8	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	active 2 min 2 mA fade-in period was delivered, followed by cessation of stimulation.
10s ramp-in; ramped down during 1min; 2nd ramp 0.5mA over 1min during the stimulation; constant current of 0.034mA							
Loo et al (2018)	Depression	130	Y	F3/F8	30	NA	current was rapidly ramped up to 1 mA over the first 10 s and slowly ramped down over the next minute. . A second ramp up and down to 0.5 mA over 1 min was delivered at either 10 min or 20 min, again to aid blinding.The tDCS device also emitted a constant current of 0.034 mA throughout sham stimulation.
Palm Protocol - 2 ramps 15s up and down, beginning and end of stimulation							
Palm et al (2012)	Depression	11	Y (11)	F3/FP2	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	specific program: 20-minute off interval between ramp in and out periods of 15 seconds
Palm et al (2016)	Schizophrenia	20	Y	F3/F4	20	Eldith DC-stimulator (NeuroConn GmbH, Germany)	novel sham mode (Palm et al, 2013)
Dickler et al (2017)	SUD (gambling)	16	Y	F4/F3	30	MR compatible DC Stimulation NeuroConn	was delivered for 30 minutes with active current applied only during the first and last 30 seconds of the 89 session following standard procedure (gandiga 2006)
Wörsching et al (2017)	Healthy	20	Y	F3/F4	20	Eldith stimulator MR (neuroConn)	current was ramped up at the beginning and end of the stimulation period to mimic the somatosensory sensation of real tDCS, but turned off in between alternated with low-threshold direct-current impulses (Palm et al., 2013b).
Manenti et al (2013)	Healthy	32	Y	F3/FP2	6	BrainStim, EMS, Bologna, Italy	current was turned off 10 s after the start of the stimulation and was turned on for the last 10 s of the stimulation period (plus the duration of the fade-in and fade-out periods = 10 s)
only ramps no stimulation							
Doruk et al (2014)	Parkinson	18	Y	F3/Fp2	20	Soterix Medical Inc., New York, NY) Chattanooga Ionto device	current was applied only for the initial 30-s ramp up and 30-s ramp down

Supplementary Table - Sham parameters - Studies using bifrontal (F3/F4 - F3/FP2) and fronto-temporal Montage

Study				tDCS parameters			
Author Date	Population	n	sham	Anode/Cathode placement	Duration of stim	Stimulator	Sham parameters
Cerruti et al (2009)	Healthy	30	Y	F3/FP2	20	(Phoresor; Iomed Inc., Salt Lake City, UT)	stimulation ramped up 20s then 10s ramped down
Leite et al (2011)	Healthy	30	Y	F3/FP2	15	Eldith Stimulator Plus manufactured by neuroConn,	only 15 sec ramp up and down
Keshvari et al, (2013)	Healthy	60	Y	F3/F4 F4/F3	20	Activa Dose Iontophoresis manufactured by Acti- vaTek	ramps of 30s
Sela et al, (2012)	Healthy	22	Y	F3/F4 or F4/F3	15	Magstim LtD, Wales	ramps of 30s
Electrodes placed and ramps but stimulator with no active current							
Hone-Blanchet et al (2016)	Healthy	15	Y	F3/F4	30	MRI–compatible DC-STIMULATOR (neuroConn GmbH, Ilme- nau, Germany)	Sham stimulation was delivered for 30 minutes following standard procedure with a ramp up and a ramp down of 30 seconds with the remaining time with no active current
Electrodes placed but stimulator turned off (no stimulation)							
Marshall et al (2004)	Healthy	30	Y	F3/F4 + Mastoids	15s on/off over 30min	NA	electrodes were applied as in the stimulation sessions, but the stimulator remained off.
Marshall et al (2005)	Healthy	12	Y	F3/F4 + Mastoids	15s on/off over 30min	NA	electrodes were applied as in the stimulation sessions, but the stimulator remained off.
NA							
Palm et al (2014)	Schizophrenia	10	Y (10)	F3/FP2	20	NA	NA
Gomes et al (2015)	Schizophrenia	7	Y (8)	F3/F4	20	NA	NA
Ironside et al (2015)	Healthy	60	Y	F3/F4	20	DC Stimulator Plus, Neuroconn, Germany	NA
Nienow et al (2016)	Schizophrenia	10	Y	F3/FP2	20	Soterix Medical	NA

DÉVELOPPEMENTS D'UNE METHODE DE CORRECTION DE MOUVEMENT EN IRM -TEP CEREBRALE

Anthonin Reilhac (1), Rania Berrada (1), Zacharie Irace (1), Inés Mérida (1), Clara Fonteneau (2), Marie-Françoise Suaud-Chagny (2), Nicolas Costes (1)

1. CERMEP – Imagerie du vivant, 59 bd Pinel, 69003 Lyon, France;

2. Centre de Recherche en Neurosciences de Lyon, Equipe PSYR2, Lyon, France

Introduction

L'exploitation optimale de données acquises sur la nouvelle génération d'IRM-TEP combinées nécessite des développements méthodologiques tirant parti de la combinaison des enregistrements de données des deux modalités. Les mouvements du patient dégradent la qualité des images TEP, et réduisent la précision des indices biologiques calculés à partir de ces images de concentration radioactive. Le but de ce travail est de mettre au point une méthode de correction de mouvement de tête du sujet dans les données TEP dynamiques, basée sur l'utilisation de séquences IRM rapides acquises simultanément.

Matériel et Méthode

La connaissance des mouvements de tête est basée sur une estimation de la matrice rigide de mouvement calculé grâce aux données TEP reconstruites sans correction d'atténuation [1], ou sur les images IRM acquises simultanément.

Principe : L'application des matrices de mouvement s'effectue directement sur le mode liste des coïncidences enregistrées en TEP : pour chaque événement le rebinner réattribue une nouvelle position spatiale de la ligne de réponse, en fonction de la connaissance de la matrice de mouvement connue à l'instant de la détection. Le framing TEP n'est donc pas nécessairement coïncident avec le découpage temporel des matrices de mouvements. Le rebinner prend en considération les corrections de sensibilité et d'efficacité des lignes de réponse avant et après application de la transposition de la LOR, ainsi que les zone de gap existant entre couronnes de détection.

Données : la mise au point et l'évaluation de la méthode sont effectuées sur des données simulées par le simulateur de Monte-Carlo SORTEO [1], récemment adapté à la géométrie du détecteur de l'IRM-TEP Siemens mMR [2]. Un ensemble de données expérimentales d'acquisition dynamiques au [^{11}C]Raclopride permet également de comparer les résultats de correction de mouvement basée sur l'image par rapport à la méthode mise au point. L'évaluation du mouvement à partir de la TEP reconstruite sans atténuation, ou à partir des séquences IRM acquises simultanément (EPI BOLD, ASL ou DTI), mais de façon non continue, sont également comparés.

Résultats

L'analyse des acquisitions simulées montre que le rebinner produit des sinogrammes corrigés permettant de récupérer l'ensemble du signal recueilli initialement sans perte de sensibilité ni artéfact lié à au rebinning des lignes de réponse. Concernant les données réelles, la figure 1 illustre une image statique de 90 minutes d'enregistrement TEP au [^{11}C]Raclopride.

Notre méthode de correction élimine totalement l'artéfact de mouvement visible sur le scalp, ainsi que le flou et l'erreur de quantification crée sur les noyaux gris centraux.

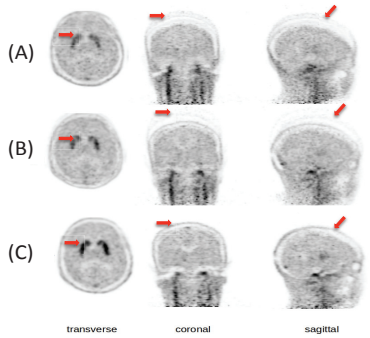

Fig. 1. Image TEP (A) sans correction, (B) avec une correction basée sur du recalage d'image, (C) avec la nouvelle méthode basée sur un rebinning par intervalles de 100s.

Discussion

L'étude quantitative des cinétiques régionales montrent que seule la correction directe sur le mode liste permet de récupérer une quantification juste. L'analyse des corrections de mouvement basées sur les séquences IRM ou sur les acquisitions TEP seulement doit finir d'être analysées pour une nombre de cas important, car les premiers résultats indiquent que la qualité de la correction est dépendante de la quantité de mouvements du sujets, et de la continuité des séquences IRM disponible simultanément.

Conclusion

La méthode développée permet de disposer d'un outil fiable de correction de mouvement rigide d'acquisitions dynamiques TEP. Il peut se décliner par l'enregistrement de champs de mouvement par IRM ou par dispositifs externes.

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7. Poster Presentation - Attenuation correction with a multi-atlas method for brain PET-MR imaging: assessment with realistic simulated [^{11}C]raclopride bolus-infusion PET data

BrainPET 2017

Attenuation correction with a multi-atlas method for brain PET-MR imaging: assessment with realistic simulated [^{11}C]raclopride bolus-infusion PET data

Inés Mérida[1,2,3], Alexander Hammers[4,5], Jérôme Redouté[3], Colm McGinnity[5], Clara Fonteneau[6,7], Marie-Françoise Suaud-Chagny[6,7], Anthonin Reilhac[3], Nicolas Costes[3]

[1]Université de Lyon 1, INSERM, CNRS, Lyon Neuroscience Research Center, France,
[2]Siemens Healthcare France SAS, Saint-Denis, France,
[3]CERMEP-Imagerie du vivant, Lyon, France,
[4]Neurodis Foundation, Lyon, France,
[5]King's College London & Guy's and St Thomas' PET Centre, Division of Imaging Sciences and Biomedical Engineering, Kings' College London, UK,
[6]Centre de Recherche en Neurosciences de Lyon, Equipe PSYR2 (INSERM U1028, CNRS UMR5292, UCBL, Université de Lyon), Lyon, France
[7]Centre Hospitalier Le Vinatier, Lyon, France

Objectives
We have recently shown that inaccurate MR-based attenuation maps used in PET-MR systems can induce an error on dynamic PET data that depends on tracer distribution and varies over time [1]. Here we assess the impact of different MR-based attenuation correction (AC) methods on PET quantification and kinetic modelling. We compare our multi-atlas technique *(MaxProb)* [2] and the segmented-UTE [3] to ground-truth CT.

Methods
Brain PET data was simulated for twelve subjects with PET-SORTEO [4] to reproduce a bolus-infusion [^{11}C]raclopride protocol. For the simulations, emission phantoms were defined with 15 regions of interest on MRI [5], and the input 90-minute time-activity-curves were derived from real PET/CT data. Simulated PET data was reconstructed using *MaxProb*, segmented-UTE and ground-truth CT AC. Simple tissue-to-reference ratios were used to estimate the BP_{ND} in caudate, accumbens and putamen, at equilibrium, with cerebellum as the reference region.

Results
For the cerebellum, mean bias on time-activity-curves varied over time from -7.7 to -13.1% with segmented-UTE and from -2.5 to -5.2% with *MaxProb*. Mean bias in caudate, accumbens and putamen varied from -2.8 to -7.1% for segmented-UTE and from -2.2 to -4.6% for *MaxProb*. The bias tended to increase at later time-points. Mean error on tissue-to-reference ratios reached +5.4% for UTE but remained below +1% for *MaxProb*.

Conclusions
Compared with segmented-UTE, *MaxProb* produces less bias for time-activity-curves and hardly any bias for tissue-to-reference ratios. Multi-atlas AC may enhance sensitivity to detect physiological variations between groups of subjects or experimental conditions. Further work will focus on the sensitivity of the AC method to detect tracer displacement induced by endogenous dopamine.

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PSMR 2017

Motion correction and multi-atlas attenuation correction applied to a simultaneous bolus/infusion PET-MR brain study

Inés Mérida[1,2,3], Clara Fonteneau[4,5], Anthonin Reilhac[3,6], Jérôme Redouté[3], Zacharie Irace[3], Marie-Françoise Suaud-Chagny[4,5], Alexander Hammers[7,8], Nicolas Costes[3]

[1] Université de Lyon 1, INSERM, CNRS, Lyon Neuroscience Research Center, France,
[2] Siemens Healthcare France SAS, Saint-Denis, France,
[3] CERMEP-Imagerie du vivant, Lyon, France,
[4] Centre de Recherche en Neurosciences de Lyon, Equipe PSYR2 (INSERM U1028, CNRS UMR5292, UCBL, Université de Lyon), Lyon, France,
[5] Centre Hospitalier Le Vinatier, Lyon, France,
[6]CIRC, NUS-ASTAR, Singapore,
[7] Neurodis Foundation, Lyon, France,
[8] King's College London & Guy's and St Thomas' PET Centre, Division of Imaging Sciences and Biomedical Engineering, Kings' College London, UK

Abstract—With the aim to improve PET quantification acquired during a simultaneous PET-MR brain imaging, we compared attenuation and motion correction techniques in a bolus/infusion PET protocol studying variations of endogenous dopamine concentration across time. The target parameter was the [^{11}C]raclopride striatum/cerebellum binding ratio. Acquired data were corrected for both motion, with the list-mode rebinner *Eber*, and for photon attenuation, with standard UTE solution and the multi-atlas-based *MaxProb* approach. Combination of *Eber* and *MaxProb* improved the signal quality significantly by reducing the variance of binding ratio calculated on each region of interest, and at each time-point.

Keywords—PET-MR; attenuation correction; motion correction; kinetic modelling; neuroimaging; dopaminergic neurotransmission.

Introduction

In simultaneous PET-MR systems, accurate attenuation maps need to be derived from the MR (or PET) data to ensure correct PET quantification. Inaccurate attenuation correction (AC) affects PET quantification [1]. We have recently shown [2], [3] that dynamic PET data and physiological parameters derived from kinetic modelling, such as Binding Ratios (BR), can also be affected by inaccurate AC, especially in the late frames of the acquisition. In addition, when dealing with real data, subject motion during long acquisitions can also increase the quantification error, in particular in small brain structures. In this work we apply a novel approach for motion correction (*Eber*, Reilhac et al., abstract submitted to PSMR 2017) combined with our multi-atlas AC method [2] on PET data acquired with a simultaneous PET-MR system and investigate the influence of those techniques on data quality.

Materials and Methods

Protocol and data

PET-MR imaging was used to explore dopaminergic neurotransmission in healthy subjects in subcortical areas, such as the striata. Eighteen subjects (8 male, 10 female) [mean age ± SD, 25.6 ± 2.9 y; range, 22-34 y] had a simultaneous PET-MR exam with a 110 minute [^{11}C]raclopride bolus-infusion protocol to measure variations of D2 receptor occupancy [4]. T1 and UTE MRI sequences were acquired (as well as other functional sequences

- BOLD, ASL and DTI - not analysed in this preliminary work).

PET motion and attenuation corrections

PET data were corrected for head motion with the *Eber* algorithm, which corrects the listmode data directly by rebinning the detected events according to the estimated inter-frame motion. For motion estimation, dynamic PET data were first reconstructed without AC in 63 frames of 100s each. Then the 63 motion correction matrices were applied to the listmode data, rebinned in sinograms of 21 regular 5-minute frames. Images were reconstructed from the corrected sinograms with AC, and the OP-OSEM3D algorithm incorporating PSF, using 12 iterations and 21 subsets. For AC, *MaxProb* [2] which is a recently proposed multi-atlas method and the standard UTE [5] method were used.

Data analysis

Time-activity curves (TACs) were extracted from the striatal regions (caudate and putamen) and from the cerebellum, considered here as the reference region. Tissue-to-reference BR were deduced from these TACs for both striatal regions. Parametric BR images were generated from the 21-frame dynamic series, for the following time-points after the injection of the tracer: T1 (30-40 min), T2 (45-70 min), T3 (75-90 min) and T4 (90-105 min)). Means and standard deviations of BRs were extracted from the ratio images. Results were compared with or without applying *Eber* motion correction, incorporating *UTE* or *MaxProb* AC. An analysis of variance was performed with a Tukey honest significant difference (HSD) test for each time-point.

Results

Example

Figure 1 shows the BR curves across time for one subject that showed important motion artefacts. In the absence of motion correction (top), regional BR decreased after 30 minutes, whereas the BR was recovered after applying *Eber* motion correction (bottom).

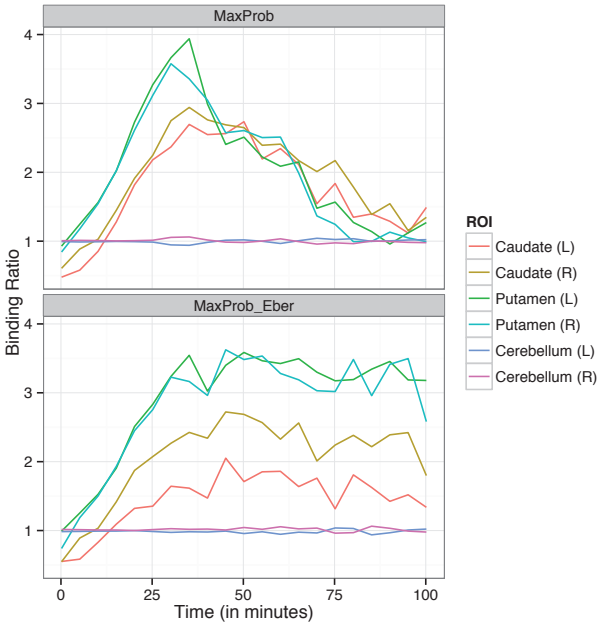

Fig. 1. Example of BR TACs per ROI for one subject with important motion before motion correction (top) and after *Eber* motion correction (bottom). *MaxProb* AC was applied in the two cases.

Quantitative results

Overall, both *MaxProb* AC and *Eber* motion correction contributed individually to decrease the intra-regional variance of regional BR.

At T1, the mean standard deviation over subjects and striatal regions decreased from 1.23 to 1.09 for *UTE* and from 1.16 to 1.03 for *MaxProb* when applying *Eber* motion correction. Significant improvement produced by motion correction was seen for T1 and T2. At T1, no significant differences were found between *UTE* and *MaxProb*, with or without *Eber* motion correction. At T2, T3, T4, the BR standard deviation was significantly reduced with *MaxProb*, compared to *UTE* AC. For example, at T4, the BR standard deviation was 1.65 for *UTE_Eber*, and 1.52 for *MaxProb_Eber*.

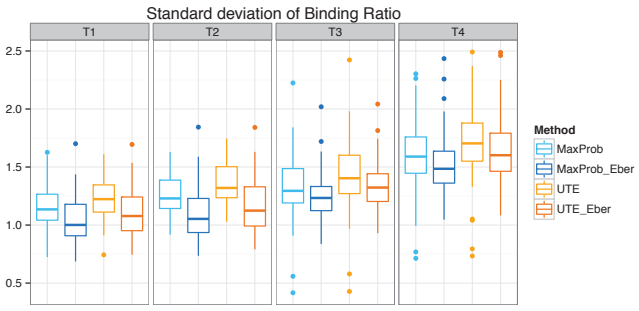

Fig. 2. Boxplot of standard deviation of intra-regional BR, measured on the striata, per time-point, and per AC method applied. *: $p < 0.05$ with the Tukey HDS test. Significant statistical tests for motion correction comparisons are indicated by brackets on the top of the graph, and significant statistical tests for AC comparisons at the bottom of the graph.

Conclusion

Used singly and together, *Eber* motion correction and multi-atlas *MaxProb* AC contributed to reduce the intra-regional variance of BR. Further work will investigate the sensitivity gain generated by the proposed methods in stimulation conditions aiming to evoked extracellular dopamine concentration variation by contrasting groups receiving an active or placebo stimulation.

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9. Questionnaires

9.1. Questionnaires sur les effets secondaires aigus de la tDCS

Cotation de la sévérité: 1- absent, 2- léger, 3- modéré, 4- sévère

Cotation du lien: 1- aucun, 2- éloigné, 3- possible, 4- probable, 5- certain

Symptômes	**Sévérité**	**Lien**	**Notes**
Mal de tête			
Douleur au cou			
Convulsions			
Brûlures au scalp			
Audition altérée			
Cognition altérée			
Difficultés de concentration			
Changement aigu de l'humeur			
Autre (spécifier)			

9.2. Echelle de motivation globale (French version)

Indique dans quelle mesure chacun des énoncés suivants correspond aux raisons pour lesquelles tu fais différentes choses en general.

Ne correspond pas du tout 1	Correspond très peu 2	Correspond un peu 3	Correspond moyennement 4	Correspond assez 5	Correspond beaucoup 6	Correspond exactement 7

EN GÉNÉRAL, JE FAIS DES CHOSES . . .

1. ... pour ressentir des émotions que j'aime. 1 2 3 4 5 6 7
2. ... parce que je ne veux pas décevoir certaines personnes. 1 2 3 4 5 6 7
3. ... pour m'aider à devenir ce que je veux être plus tard. 1 2 3 4 5 6 7
4. ... parce que j'aime faire des découvertes intéressantes. 1 2 3 4 5 6 7
5. ... parce que je m'en voudrais de ne pas les faire. 1 2 3 4 5 6 7
6. ... parce que j'éprouve du plaisir à me sentir de plus en plus habile. 1 2 3 4 5
7. ... bien que je ne vois pas ce que cela me donne. 1 2 3 4 5 6 7
8. ... parce que je vis une sensation de bien-être pendant que je les fais. 1 2 3 4 5
9. ... parce que je veux être mieux considéré-e par certaines personnes. 1 2 3 4 5
10. ... parce que je les choisies comme moyens pour réaliser mes projets. 1 2 3 4 5
11. ... pour le plaisir d'acquérir des connaissances. 1 2 3 4 5 6 7
12. ... parce que je me sentirais coupable de ne pas les faire. 1 2 3 4 5 6 7
13. ... parce que je ressens du plaisir à maîtriser ce que je fais. 1 2 3 4 5 6 7
14. ... bien que cela ne fasse pas de différence que je les fasse ou non. 1 2 3 4 5
15. ... parce que j'éprouve des sensations plaisantes en les faisant. 1 2 3 4 5 6
16. ... pour montrer aux autres ce que je vaux. 1 2 3 4 5 6 7
17. ... parce que je les choisis pour obtenir ce que je désire. 1 2 3 4 5 6 7
18. ... parce que j'y trouve de nouveaux éléments intéressants à apprendre. 1 2 3 4

19. ... parce que je m'oblige à les faire. 1 2 3 4 5 6 7

20. ... parce que j'éprouve de la satisfaction à essayer d'exceller dans ce que je fais. 1 2 3 4 5 6 7

21. ... même si je n'ai pas de bonnes raisons de les faire. 1 2 3 4 5 6 7

22. ... pour les sentiments agréables que je ressens. 1 2 3 4 5 6 7

23. ... parce que je souhaite obtenir du prestige. 1 2 3 4 5 6 7

24. ... parce que je choisis de m'investir dans ce qui est important pour moi. 1 2 3 4

25. ... parce que j'ai du plaisir en apprenant sur différents faits intéressants. 1 2 3 4

26. ... parce que je me sentirais mal de ne pas les faire. 1 2 3 4 5 6 7

27. ... parce que je ressens du plaisir à me surpasser. 1 2 3 4 5 6 7

28. ... même si je ne crois pas que cela en vaille la peine. 1 2 3 4 5 6 7

9.3.Life Orientation Test-Revisited (LOT-R) (French version)

Consigne :

Répondez aux questions ci-dessous en les appliquant à vous-même à l'aide de l'échelle suivante :

[0] = Totalement en désaccord
[1] = Plutôt en désaccord
[2] = Neutre
[3] = Plutôt d'accord
[4] = Totalement d'accord

Soyez le plus honnête possible en répondant au questionnaire, sans laisser votre réponse à une question influencer vos réponses à d'autres questions. Il n'y a pas de bonnes ou de mauvaises réponses.

_____ 1. Dans les moments d'incertitude, je m'attends habituellement au mieux.

_____ 2. J'ai de la facilité à relaxer.

_____ 3. S'il y a des chances que ça aille mal pour moi, ça ira mal.

_____ 4. Je suis toujours optimiste face à mon avenir.

_____ 5. J'apprécie beaucoup mes amis(es).

_____ 6. C'est important pour moi de me tenir occupé.

_____ 7. Je ne m'attends presque jamais à ce que les choses aillent comme je le voudrais.

_____ 8. Je ne me fâche pas très facilement.

_____ 9. Je m'attends rarement a` ce que de bonnes choses m'arrivent.

_____10. Dans l'ensemble, je m'attends a` ce que plus de bonnes choses m'arrivent que de mauvaises.

9.4. Big Five Inventory (BFI) (French version)

Instructions:

Vous allez trouver un certain nombre de qualificatifs qui peuvent ou non s'appliquer à vous. Par exemple, acceptez-vous d'être quelqu'un qui aime passer du temps avec les autres? Ecrivez devant chaque affirmation le chiffre indiquant combien vous approuvez ou désapprouvez l'affirmation :

1 désapprouve fortement
2 désapprouve un peu
3 n'approuve ni ne désapprouve
4 approuve un peu
5 approuve fortement

Je me vois comme quelqu'un qui ….

1.___ est bavard
2.___ a tendance à critiquer les autres
3.___ travaille consciencieusement
4.___ est déprimé, cafardeux
5.___est créatif, plein d'idées originales
6.___ est réservé
7.___ est serviable et n'est pas égoïste avec les autres
8.___ peut être parfois négligent
9.___ est "relaxe", détendu, gère bien les stress
10.___ s'intéresse à de nombreux sujets
11.___ est plein d'énergie
12.___ commence facilement à se disputer avec les autres
13.___ est fiable dans son travail
14.___ peut être angoissé
15.___ est ingénieux, une grosse tête
16.___ communique beaucoup d'enthousiasme
17.___ est indulgent de nature
18.___ a tendance à être désorganisé
19.___ se tourmente beaucoup
20.___ a une grande imagination
21.___ a tendance à être silencieux
22.___.fait généralement confiance aux autres
23.___.a tendance à être paresseux
24.___ est quelqu'un de tempéré, pas facilement troublé
25.___ est inventif
26.___ a une forte personnalité, s'exprime avec assurance
27.___ est parfois dédaigneux, méprisant
28.___ persévère jusqu'à ce que sa tâche soit finie
29.___ peut être lunatique d'humeur changeante
30.___ apprécie les activités artistiques et esthétiques
31.___ est quelquefois timide, inhibé
32.___ est prévenant et gentil avec presque tout le monde
33.___ est efficace dans son travail
34.___ reste calme dans les situations angoissantes
35.___ préfère un travail simple et routinier
36.___ est sociable, extraverti
37.___ est parfois impoli avec les autres
38.___ fait des projets et les poursuit
39.___ est facilement anxieux

40._____ aime réfléchir et jouer avec des idées
41._____ est peu intéressé par tout ce qui est artistique
42._____ aime coopérer avec les autres
43._____ est facilement distrait
44._____ a de bonnes connaissances en art, musique ou en littérature
45._____ cherche des histoires aux autres

Vérifier que vous avez bien répondu à toutes les questions. Merci

Score= moyenne des items dans la dimension
E (Extraversion, Energie, Enthousiasme)
8 items: 1, 6R, 11, 16, 21R, 26, 31R, 36;
A (Agréabilité, Altruisme, Affection)
10 items: 2R, 7, 12R, 17, 22, 27R, 32, 37R, 42, 45R;
C (Conscience, Contrôle, Contrainte)
9 items: 3, 8R, 13, 18R, 23R, 28, 33, 38, 43R;
N (émotions Négatives, Névrosisme, Nervosité)
8 items: 4, 9R, 14, 19, 24R, 29, 34R, 39;
O (Ouverture, Originalité, Ouverture d'esprit)
10 items: 5, 10, 15, 20, 25, 30, 35R, 40, 41R, 44;

Chaque facteur correspond à la moyenne de la somme des items en inversant les items marqués d'un R « reverse » (5 devenant 1 ; 4 : 2 ; 3 : 3, 2 : 4 et 1 : 5).

9.5. Echelle de cotation de l'anxiété-Etat de Spielberger (French version)

Consigne :

Un certain nombre de phrases que l'on utilise pour se décrire sont données ci-dessous.
Lisez chaque phrase, puis marquez d'une croix, parmi les quatre points à droite, celui qui correspond le mieux à **ce que vous ressentez A L'INSTANT, JUSTE EN CE MOMENT**.
Il n'y a pas de bonnes ni de mauvaises réponses. Ne passez pas trop de temps sur l'une ou l'autre de ces propositions, et indiquez la réponse qui décrit le mieux **vos sentiments actuels.**

En ce moment	**non**	**Plutôt non**	**Plutôt oui**	**oui**	**En ce moment**	**non**	**Plutôt non**	**Plutôt oui**	**oui**
1 .Je me sens calme.	☐	☐	☐	☐	11. Je sens que j'ai confiance en moi.	☐	☐	☐	☐
2. Je me sens en sécurité, sans inquiétude, en sûreté.	☐	☐	☐	☐	12. Je me sens nerveux(se), irritable.	☐	☐	☐	☐
3. Je suis tendu(e), crispé(e).	☐	☐	☐	☐	13. J'ai la frousse, la trouille (j'ai peur).	☐	☐	☐	☐
4. Je me sens surmené(e).	☐	☐	☐	☐	14. Je me sens indécis(e).	☐	☐	☐	☐
5. Je me sens tranquille, bien dans ma peau.	☐	☐	☐	☐	15. Je suis décontracté(e), détendu(e).	☐	☐	☐	☐
6. Je me sens ému(e), bouleversé(e), contrarié(e).	☐	☐	☐	☐	16. Je suis satisfait(e).	☐	☐	☐	☐
7. L'idée de malheurs éventuels me tracasse en ce moment.	☐	☐	☐	☐	17. Je suis inquiet(e), soucieux(se).	☐	☐	☐	☐
8. Je me sens content(e).	☐	☐	☐	☐	18. Je ne sais plus où j'en suis, je me sens déconcerté(e), dérouté(e).	☐	☐	☐	☐
9. Je me sens effrayé(e).	☐	☐	☐	☐	19. Je me sens solide, posé(e), pondéré(e), réfléchi(e).	☐	☐	☐	☐
10. Je me sens à mon aise.	☐	☐	☐	☐	20. Je me sens de bonne humeur, aimable.	☐	☐	☐	☐

9.6. Echelle visuelle analogique sur l'humeur (French version)

En ce moment, je me sens

Eveillé(e)	———	Somnolent(e)
Calme	———	Excité(e)
Fort(e)	———	Faible
Vaseux(se)	———	Les idées claires
Adroit(e)	———	Maladroit(e)
Mou(Molle)	———	Energique
Content(e)	———	Mécontent(e)
Inquiet(e)	———	Tranquille
L'esprit lent	———	L'esprit vif
Tendu(e)	———	Relax
Attentif(ve)	———	Dans les nuages
Incapable	———	Capable
Heureux(se)	———	Triste
Hostile	———	Amical(e)
Intéressé(e)	———	Ennuyé(e)
Renfermé(e)	———	Sociable

9.7. Questionnaire d'aptitude pour une expérience incluant la TMS ou tDCS

QUESTIONNAIRE D'APTITUDE POUR UNE EXPERIENCE INCLUANT la TMS ou tDCS

ID participant:_____ Numéro SIRUL:_____
Date: ___/___/___ Heure : _____ Nom du chercheur: _____

Avez-vous déjà participé à une expérience TMS ou tDCS?	☐ oui	☐ non
Avez-vous déjà eu des effets secondaires à la suite d'une expérience TMS ou tDCS?	☐ oui	☐ non
Avez-vous déjà fait une crise d'épilepsie?	☐ oui	☐ non
Avez-vous déjà eu un épisode inexpliqué de perte de conscience?	☐ oui	☐ non
Avez-vous déjà eu un accident vasculaire cérébral (AVC)?	☐ oui	☐ non
Avez-vous déjà eu un traumatisme sévère à la tête?	☐ oui	☐ non
Avez-vous déjà eu une maladie du cerveau ou une maladie neurologique?	☐ oui	☐ non
Souffrez-vous de maux de têtes fréquents ou sévères?	☐ oui	☐ non
Avez-vous des pièces métalliques à l'intérieur de la tête (sauf dans la bouche), tels des pièces chirurgicales ou des fragments métalliques?	☐ oui	☐ non
Prenez-vous des médicaments?	☐ oui	☐ non
Pensez-vous être enceinte ou susceptible de l'être?	☐ oui	☐ non
Avez-vous des implants médicaux, tels un pacemaker, un implant cochléaire ou des pompes médicales?	☐ oui	☐ non
Quelqu'un de votre famille souffre-t-il d'épilepsie?	☐ oui	☐ non
Avez-vous besoin d'explications supplémentaires concernant la TMS ou la tDCS et les risques associés?	☐ oui	☐ non

Veuillez nous fournir de l'information détaillée si vous répondez «OUI» à l'une des questions

9.8. Questionnaire d'aptitude pour l'IRM

Afin d'assurer la sécurité de toute personne accédant aux locaux de l'imageur IRM, il est très important que ce questionnaire soit complété correctement.

DÉPISTAGE PRÉLIMINAIRE POUR ÉTUDE D'IMAGERIE PAR IRM

Veuillez écrire en caractère d'imprimerie

Chercheur / Projet :	Numéro d'identification :
Nom :	Prénom :
Genre : ☐F ☐M Naissance (jj/mm/aaaa): __ / ___ / _____	Poids : _____ kg _____ lbs Grandeur : _____ m _____ pi

1. Avez-vous déjà subi une opération ?

Oui	Non		Si oui, veuillez préciser le type d'opération et la date :
☐	☐	Tête	
☐	☐	Thorax ou cœur	
☐	☐	Abdomen	
☐	☐	Bras, mains	
☐	☐	Jambes, pieds	
☐	☐	Colonne vertébrale	
☐	☐	Yeux	
☐	☐	Autres :	

2. Portez-vous :

Oui	Non	
☐	☐	Stimulateur cardiaque (Pace-maker) ?
☐	☐	Électrodes épicardiques ?
☐	☐	Clip pour anévrisme cérébral ?
☐	☐	Prothèse cochléaire ? Prothèse auditive ?
☐	☐	Filtre ou cathéter dans un vaisseau sanguin ?
☐	☐	Neurostimulateur ?
☐	☐	Stimulateur électronique pour les os ?
☐	☐	Prothèse valvulaire cardiaque ?
☐	☐	Corps étrangers métalliques ? (ex: balles, fragments d'obus, éclats métalliques)
☐	☐	Pompe à insuline implantée ?
☐	☐	Prothèse orthopédique ? (ex: clou, vis, plaque)
☐	☐	Membre (s) artificiel (s) ?
☐	☐	Maquillage permanent ? Tatouage(s) ?
☐	☐	Perçage(s) ?
☐	☐	Implant(s) magnétique(s) ou non magnétique(s) ?
☐	☐	Diaphragme, stérilet ?
☐	☐	Dentier (Appareil orthodontie) ?
☐	☐	Implant(s) ou prothèse(s) oculaire(s) ?
☐	☐	Système de distribution transdermique (ex: timbre de nitroglycérine)
☐	☐	Autres :

Exemple de Formulaire de Dépistage IRM, page 1/2

Afin d'assurer la sécurité de toute personne accédant aux locaux de l'imageur IRM, il est très important que ce questionnaire soit complété correctement.

3. Êtes-vous enceinte ou croyez-vous l'être ? ☐ **oui** ☐ **non**

4. En cas de doute, accepteriez-vous de passer un test de grossesse ? ☐ **oui** ☐ **non**

5. Êtes-vous claustrophobe ? ☐ **oui** ☐ **non**

6. Avez-vous déjà été blessé(e) par un morceau de métal ? ☐ **oui** ☐ **non**
(ex: accident de voiture ou de travail, blessure de guerre)
Si oui, veuillez préciser:_____

7. Avez-vous subi un examen par résonance magnétique ? ☐ **oui** ☐ **non**

8. Avez-vous déjà été:

	oui	**non**
Machiniste	☐	☐
Soudeur	☐	☐
Opérateur de machinerie lourde	☐	☐
Travailleur de métal	☐	☐

9. Souffrez-vous de problème respiratoire ou moteur ? ☐ **oui** ☐ **non**

On m'a expliqué les procédures à suivre lors d'une session d'IRM. On m'a informé des mesures de sécurité à appliquer et on a répondu à toutes mes questions. Je certifie que les renseignements ci-dessus sont exacts au meilleur de mes connaissances et consens à participer à une étude d'IRM.

_____ _____
Signature participant/parent/tuteur légal Date

========== **Espace réservé** ==========

	oui	**non**
Participation autorisée	☐	☐
Investigation	☐	☐

_____ _____
Signature médecin/chercheur Date

Exemple de Formulaire de Dépistage IRM, page 2/2

9.9. Echelle d’évaluation mise en insu

Quel traitement le sujet pense-t-il avoir reçu ?

ACTIF ——————————————————— PLACEBO

Commentaires :