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Le GABA comme marqueur de récupération suite à une commotion cérébrale dans le sport ?

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Résumé

L'association démontrée récemment entre les commotions cérébrales dans le sport et le développement possible de maladies neurodégénératives a suggéré la possibilité que des altérations persistantes soient présentes dans le cerveau de l'athlète commotionné. En fait, des altérations neurophysiologiques ont récemment été révélées au sein du cortex moteur primaire (M1) d'athlètes ayant un historique de commotions via la stimulation magnétique transcrânienne (SMT). Plus précisément, la période silencieuse corticale (PSC), une mesure d'inhibition liée aux récepteurs $GABA_B$, était anormalement élevée, et cette hyper-inhibition était présente jusqu'à 30 ans post-commotion. La PSC, et possiblement le GABA, pourraient donc s'avérer des marqueurs objectifs des effets persistants de la commotion cérébrale. Toutefois, aucune étude à ce jour n'a directement évalué les niveaux de GABA chez l'athlète commotionné.

Ainsi, les études cliniques et méthodologiques composant le présent ouvrage comportent deux objectifs principaux: (1) déterminer si l'inhibition excessive (GABA et PSC) est un marqueur des effets persistants de la commotion cérébrale; (2) déterminer s'il est possible de moduler l'inhibition intracorticale de façon non-invasive dans l'optique de développer de futurs avenues de traitements.

L'article 1 révèle une préservation des systèmes sensorimoteurs, somatosensoriels et de l'inhibition liée au $GABA_A$ chez un groupe d'athlètes universitaires asymptomatiques ayant subi de multiples commotions cérébrales en comparaison avec des athlètes sans historique connu de commotion cérébrale. Cependant, une atteinte spécifique des mesures liées au système inhibiteur associé aux récepteurs $GABA_B$ est révélée chez les athlètes commotionnés en moyenne 24 mois post-commotion.

Dans l'article 2, aucune atteinte des mesures SMT liées au système inhibiteur n'est révélée en moyenne 41 mois après la dernière commotion cérébrale chez un groupe d'athlètes asymptomatiques ayant subi 1 à 5 commotions cérébrales. Bien qu'aucune différence entre les groupes n'est obtenue quant aux concentrations de GABA et de glutamate dans M1 via la

spectroscopie par résonance magnétique (SRM), des corrélations différentielles suggèrent la présence d'un déséquilibre métabolique entre le GABA et le glutamate chez les athlètes commotionnés.

L'article 3 a démontré, chez des individus en bonne santé, un lien entre la PSC et la transmission glutamatergique, ainsi que le GABA et le glutamate. Ces résultats suggèrent que la PSC ne reflète pas directement les concentrations du GABA mesurées par la SRM, mais qu'un lien étroit entre la GABA et le glutamate est présent.

L'article 4 a démontré la possibilité de moduler la PSC avec la stimulation électrique transcrânienne à courant direct (SÉTcd) anodale chez des individus en santé, suggérant l'existence d'un potentiel thérapeutique lié à l'utilisation de cette technique.

L'article 5 a illustré un protocole d'évaluation des effets métaboliques de la SÉTcd bilatérale. Dans l'article 6, aucune modulation des systèmes GABAergiques révélées par la SMT et la SRM n'est obtenue suite à l'utilisation de ce protocole auprès d'individus en santé. Cet article révèle également que la SÉTcd anodale n'engendre pas de modulation significative du GABA et du glutamate.

En somme, les études incluent dans le présent ouvrage ont permis d'approfondir les connaissances sur les effets neurophysiologiques et métaboliques des commotions cérébrales, mais également sur le mécanisme d'action des diverses méthodologies utilisées.

Mots-clés : commotions cérébrales, cortex moteur, GABA, glutamate, neuromodulation, spectroscopie par résonance magnétique, stimulation électrique transcrânienne à courant direct, stimulation magnétique transcrânienne, traumatisme craniocérébral

Abstract

The recent demonstration of a link between sport concussions and the possible development of neurodegenerative disorders suggests that these injuries could induce long-term alterations in the brain of athletes. In fact, neurophysiological abnormalities have recently been shown via transcranial magnetic stimulation (TMS) in primary motor cortex (M1) of asymptomatic concussed athletes. Specifically, the cortical silent period (CSP), a measure of $GABA_B$-related inhibition, was prolonged and this hyper-inhibition was observed up to 30 years post-concussion. Therefore, the CSP, and possibility abnormal GABA transmission, may become objective markers of lingering effects of sport concussions. However, no study to date has directly assessed GABA levels in concussed athletes.

Therefore, the clinical and methodological studies included in the present thesis comprise two main objectives: (1) to determine whether excessive inhibition (GABA and CSP) is a marker of the persistent effects of concussion; (2) to assess the possibility of non-invasively modulating intracortical inhibition in order to develop future treatments aiming to normalize aberrant inhibition.

Study 1 reveals normal sensorimotor interactions, somatosensory processing and $GABA_A$-related intracortical inhibition in M1 of asymptomatic athletes who sustained multiple concussions in comparison with athletes who never sustained a concussion. However, a specific enhancement of $GABA_B$-related intracortical inhibition is observed in athletes on average 24 months after the last concussion.

In study 2, no alteration of $GABA_B$-related intracortical inhibition is revealed in a group of athletes who sustained 1 to 5 sport concussions on average 41 months after the last concussion, in comparison with control athletes. In addition, while no alterations were present for GABA and glutamate levels in M1 using magnetic resonance spectroscopy (MRS), both groups displayed differential correlations between GABA and glutamate, which suggests the presence of a slight metabolic imbalance between the two metabolites in the concussed brain.

Study 3 highlighted, in healthy individuals, a relationship between the CSP and MRS-derived glutamatergic transmission, as well as GABA and glutamate levels. These results reveal a link between excitatory and inhibitory transmission in M1 and suggest that the CSP does not directly reflect GABA concentrations measured with MRS.

Results from study 4 showed that anodal transcranial direct current stimulation (tDCS) can reduce the length of the CSP in healthy individuals, suggesting the existence of a therapeutic potential associated with the use of this technique.

Study 5 thoroughly describes a protocol that aims at assessing the effects of bilateral tDCS on M1 metabolism using MRS. Using this protocol, study 6 reveals, in healthy individuals, no significant modulation of GABAergic inhibition as assessed with MRS. The study also shows, in an additional experiment, that anodal tDCS does not modulate MRS-derived GABA and glutamate levels.

In summary, the six studies included in the present thesis have helped increase our understanding of the neurophysiological and metabolic long-term effects of sport concussions. In addition, these experiments have shed light into the mechanism of action of several methods, including TMS, tDCS and MRS.

Keywords : GABA, glutamate, magnetic resonance spectroscopy, motor cortex, neuromodulation, sport concussion, transcranial magnetic stimulation, transcranial direct currect stimulation, traumatic brain injury

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Liste des sigles

CDC : Centers for Disease Control and Prevention

LNH : Ligue Nationale de Hockey

NFL : National Football League

Liste des abréviations

ATP : Adénosine triphosphate

ÉCG : Échelle de coma de Glasgow

ÉCT : Écenphalopathie traumatique chronique

GABA : Acide gamma-aminobutyrique

Glx : Glutamine + glutamate

IICld : Inhibition intracorticale de longue durée

IICcd : Inhibition intracorticale de courte durée

M1 : Cortex moteur primaire

mIns : Myo-inositol

NAA : N-acetyl-aspartate

NMDA : N-methyl-D-aspartate

PÉM : Potentiels évoqués moteurs

PLT : Potentialisation à long terme

SÉTcd : Stimulation électrique transcrânienne à courtant direct

SM : Seuil moteur

SMT : Stimulation magnétique transcrânienne

SRM : Spectroscopie par résonance magnétique

TCC : Traumatisme craniocérébral

TCCL : Traumatisme craniocérébralléger

tCr : créatine + phosphocréatine

« Negative results are just what I want.
They are just as valuable to me as positive results. »

Thomas A. Edison

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Chapitre 1

Introduction

1.1 Introduction générale

Depuis l'an 776 avant J.C., on rapporte que l'humain participe à des sports de contact le mettant à risque de recevoir des chocs au niveau de la tête (McCrory, Ariens et Berkovic, 2000). Des symptômes de commotions cérébrales sont rapportés depuis au moins l'ère d'Hippocrate. Cependant, l'hypothèse selon laquelle les commotions cérébrales pourraient avoir des effets délétères importants est très récente (McCrory et al., 2000). Au début du XX^{e} siècle, certains scientifiques se sont particulièrement intéressés aux effets cliniques multiples des traumatismes cérébraux subis par les soldats lors de la première Guerre Mondiale (Bogousslavsky et Tatu, 2013). Quelques années plus tard, Derek Denny-Brown a soulevé l'idée que les effets pathophysiologiques des traumas crâniens pourraient être potentiellement dommageables pour le cerveau, et ce à une époque où l'existence même de la pathologie était remise en question (Kutcher et Giza, 2014). En effet, il y a de cela tout juste 30 ans, les commotions cérébrales étaient toujours reconnues comme étant peu dangereuses et donc très peu étudiées dans le milieu scientifique. Étant donné l'exposition marquée à des contacts fréquents et le haut risque de coups à la tête, les cliniciens et chercheurs ont commencé à s'intéresser aux commotions cérébrales subies dans le sport au milieu des années 90 (Kutcher et Giza, 2014). En 1997, le premier document visant à fournir des lignes directrices basées sur des données scientifiques probantes face au diagnostic et à la prise en charge des commotions cérébrales a été publié par les *Centers for Disease Control and Prevention* aux États-Unis (CDC, 1997). Depuis, le nombre d'études sur le sujet a vu une augmentation exponentielle avec près de 2000 articles scientifiques publiés sur la thématique des commotions cérébrales dans le sport depuis 1997. L'intérêt de la population générale pour le phénomène a également récemment pris de l'importance suite à la découverte d'un lien possible entre les commotions cérébrales multiples subies par des athlètes et le développement de maladies neurodégénératives (Kutcher et Giza, 2014). Le récent recours collectif contre la *National Football League* (NFL) en lien avec les impacts à long terme des commotions cérébrales impliquant le tiers des athlètes retraités a également contribué à mettre de l'avant le phénomène dans le milieu scientifique, mais également dans les médias (Kutcher et Giza, 2014). Le terme « épidémie silencieuse », mentionné pour la première fois dans le *Wall*

Street Journal en 1982, est maintenant largement utilisé afin de décrire le phénomène des commotions cérébrales.

Malgré le grand nombres d'études publiées sur le sujet ayant permis une meilleure compréhension de la pathophysiologie des commotions cérébrales, l'application des plus récentes techniques de neuroimagerie cérébrale au phénomène et la mise en place de nombreuses lignes directrices sur la gestion des commotions cérébrales et le retour au jeu des athlètes par les spécialistes du domaine, plusieurs questions demeurent sans réponse. En effet, on en connait encore très peu sur les mécanismes cellulaires pouvant mener au développement précoce de maladies dégénératives chez d'anciens athlètes. Par ailleurs, il n'existe toujours pas de marqueur objectif permettant de diagnostiquer une commotion cérébrale, d'orienter le pronostic, et par conséquent, de guider le retour au jeu de l'athlète.

Le récent développement de méthodes de stimulation cérébrale non-invasive, telle que la stimulation magnetique transcrânienne (SMT), offre la possibilité d'évaluer la présence d'altérations neurophysiologiques infracliniques chez les athlètes ayant un historique de commotions cérébrales. Cette méthode comporte plusieurs avantages en comparaison avec les méthodes de neuroimagerie actuelles. Par exemple, la SMT est portative, peu coûteuse et permet d'étudier *in vivo* la neurophysiologie du cerveau. Avec cette méthode, une augmentation anormale des mécanismes d'inhibition au sein du cortex moteur a été révélée chez de jeunes athlètes asymptomatiques ayant subi de multiples commotions cérébrales (De Beaumont, Lassonde, Leclerc et Théoret, 2007; De Beaumont et al., 2011a), ainsi que chez d'ex-athlètes asymptomatiques 30 ans après la dernière commotion cérébrale (De Beaumont et al., 2009). Des études pharmacologiques suggèrent que les mesures neurophysiologiques altérées chez les athlètes reflètent l'activité du GABA (McDonnell, Orekhov et Ziemann, 2006; Werhahn, Kunesch, Noachtar, Benecke et Classen, 1999), qui est le neurotransmetteur inhibiteur le plus important du cerveau. Toutefois, le lien entre le GABA et cette mesure demeure indirect; aucune étude n'a investigué directement la présence d'altérations dans la concentration du GABA dans le cerveau. Par conséquent, les résultats de ces études ont soulevé trois questions importantes: (a) est-ce que la SMT pourrait être utilisée comme marqueur objectif de la présence d'altérations persistantes engendrées par la commotion cérébrale; (b) est-ce que le GABA pourrait s'avérer

être un marqueur fiable, et (c) si le GABA est altéré de façon persistante, est-il possible de développer des traitements pour rétablir l'équilibre d'inhibition/excitation au sein du cerveau ?

Ainsi, le premier objectif de cette thèse est d'évaluer la pertinence de l'utilisation de la SMT comme marqueur des effets à long terme sur le GABA suite aux commotions cérébrales. Pour ce faire, les objectifs secondaires sont d'établir la spécificité des altérations neurophysiologiques avec la SMT, d'évaluer directement la présence d'altérations GABAergiques chez des athlètes commotionnés asymptomatiques avec la spectroscopie par résonance magnétique (SRM) et finalement, d'établir la correspondance entre ces mesures et des mesures directes du GABA par la SRM. Le deuxième objectif de cette thèse est d'évaluer la possibilité de moduler la transmission GABAergique, mesurée par la SMT et par la SRM, via une méthode de stimulation cérébrale non-invasive, la stimulation électrique transcrânienne à courant direct (SÉTcd).

En guise d'introduction aux études composant cette thèse, un survol de la littérature actuelle sur les commotions cérébrales dans le sport sera d'abord présenté. Plus précisément, la définition actuelle de la problématique, sa prévalence et sa symptomatologie seront exposées, ainsi que la littérature scientifique récente en lien avec la pathophysiologie et les conséquences à long terme des commotions cérébrales. Par la suite, les principes de fonctionnement de la SMT et les différentes études utilisant la méthode pour mieux comprendre le phénomène des commotions cérébrales seront examinés. Les études ayant utilisé la spectroscopie par résonance magnétique pour investiguer l'état du métabolisme cérébral suite à une commotion seront ensuite présentées. Enfin, les connaissances actuelles sur la SÉTcd et la possibilité de moduler l'excitabilité corticale seront abordées.

1.2 Le phénomène des commotions cérébrales dans le sport

1.2.1 Définition, prévalence et symptomatologie

En 2009 seulement, plus de 3.5 millions d'individus ont été diagnostiqués avec un traumatisme craniocérébral (TCC) aux États-Unis, ce qui en fait un problématique de santé

publique de grande importance (Coronado et al., 2012). Au moins 5,3 millions d'Américains vivent à ce jour avec des handicaps permanents conséquents à un TCC, ce qui engendre des dépenses annuelles moyennes de 56 milliards en coûts directs et indirects reliés à la prise en charge de ces patients (Binder, Corrigan et Langlois, 2005). De plus, les TCC représentent une des atteintes neurologiques affichant la plus haute incidence chez les jeunes adultes occidentaux (Hirtz et al., 2007). Ils sont la première cause de déficits cognitifs sévères dans cette population, ainsi que la première cause de décès.

Un TCC est causé par un choc direct ou indirect à la tête qui provoque une dysfonction du système nerveux central par une atteinte du tissu neuronal et qui engendre généralement un changement soudain de l'état de conscience et une perturbation des fonctions cognitives d'une durée et d'une sévérité variables (CDC, 1997). La sévérité d'un TCC s'étend sur un continuum allant d'une altération légère et brève de l'état de conscience à un coma profond et prolongé, voire au décès de l'individu. L'Échelle de Coma de Glasgow (ÉCG) classifie la sévérité de l'atteinte neurologique d'un patient suite à un TCC, de niveaux léger à sévère, et ce à partir d'une échelle allant de 15 (aucune altération de la conscience) à 3 (coma profond ou mort; Teasdale et Jennett, 1974). Il est estimé qu'environ 70 à 90% des TCC annuels traités sont des traumatismes crâniens légers (TCCL). L'Organisation Mondiale de la Santé en collaboration avec le *Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury* définit le TCCL comme étant une atteinte cérébrale aiguë qui résulte d'une énergie mécanique appliquée à la tête via des forces physiques externes (Carroll et al., 2004). Les critères d'identification cliniques incluent : i) un ou plus des suivants : confusion et/ou désorientation, perte de conscience de 30 minutes ou moins, amnésie post-traumatique de moins de 24 heures, et/ou d'autres anomalies neurologiques transitoires tels que signes focaux, convulsions, et lésion intracrânienne ne nécessitant pas de chirurgie; ii) un score à l'ÉCG de 13 à 15 évalué trente minutes suivant l'incident (Carroll et al., 2004).

Lorsqu'il survient dans un contexte sportif, un TCC léger est qualifié de commotion cérébrale. Malgré que dans la littérature scientifique les termes « commotion cérébrale » et « TCC léger » soient parfois utilisés de façon interchangeable (Dimou et Lagopoulos, 2014), plusieurs auteurs les considèrent comme étant deux phénomènes distincts (McCrory et al.,

2013). Ainsi, dans un souci de clarté et de cohérence, le terme « TCC léger » sera utilisé dans le présent manuscrit lorsqu'il s'agit d'un contexte non-sportif et le terme « commotion cérébrale » sera utilisé pour qualifier les TCC léger survenus dans un contexte sportif.

Plusieurs efforts ont été déployés dans la dernière décennie afin de parvenir à un consensus quant à la définition de la commotion cérébrale. Malgré cela, il n'existe toujours pas de définition unique du phénomène. Lors de la 4^{ième} conférence internationale sur les commotions cérébrales dans le sport s'étant déroulée à Zurich en 2012, les experts du domaine en sont venus à la définition suivante : la commotion cérébrale est un processus pathophysiologique complexe affectant le cerveau, induit par des forces biomécaniques (McCrory et al., 2013). La commotion cérébrale peut résulter d'un coup direct à la tête, mais également de forces externes transmises à la tête ou au corps (Dimou et Lagopoulos, 2014). Elle résulte en une altération brève de l'état de conscience et des fonctions neurologiques. Les commotions cérébrales sont aujourd'hui qualifiées comme un phénomène fonctionnel plutôt que structurel (Johnston, Ptito, Chankowsky et Chen, 2001) compte tenu de l'absence habituelle d'anomalies cérébrales structurelles visualisées aux examens d'imagerie cérébrale de routine (tomodensitométrie cérébrale ou imagerie par résonance magnétique standard). Elles sont associées à une large constellation de symptômes affectant les sphères cognitive, physique et comportementale, et qui sont habituellement transitoires. Les plus fréquents sont la présence de maux de tête, de fatigue, d'un ralentissement psychomoteur, d'irritabilité, de troubles de l'équilibre, de difficultés de concentration et de déficits mnésiques (Cantu, 1996). Cependant, les symptômes qui caractérisent le plus la commotion cérébrale sont la présence de confusion, ainsi que d'une amnésie pour l'incident et/ou pour les évènements qui le précèdent ou le suivent (McCrory et al., 2013). Normalement, ces symptômes post-commotionnels s'estompent progressivement suite à un repos physique et cognitif plus ou moins prolongé. En effet, la majorité des études rapportent une disparition complète des symptômes entre 7 et 10 jours après l'incident (McCrory et al., 2013). Ainsi, dans la littérature scientifique, on parle généralement de phase aiguë en lien avec la fenêtre normale de présence de symptômes soit à l'intérieur de deux semaines post-commotion (McCrory et al., 2013). La phase sub-aiguë est habituellement associée à un à trois mois post-commotion et on parle généralement de phase chronique à partir

de trois à six mois post-commotion, bien qu'aucun consensus ne soit établit quant à la période de temps qui caractérisent ces deux phases.

Bien que la commotion cérébrale soit auparavant associée à une perte de conscience et que celle-ci ait été longtemps nécessaire au diagnostic, les études actuelles suggèrent que seulement 10% des individus souffrant d'une commotion cérébrale vont avoir une période plus ou moins prolongée de perte de conscience (Ellemberg, Henry, Macciocchi, Guskiewicz et Broglio, 2009). En lien avec ceci, les spécialistes se sont concertés lors des dernières années dans l'optique de définir un système de gradation des commotions cérébrales étant donné l'hétérogénéité de la symptomatologie post-commotionnelle. Il existe présentement au-delà de huit systèmes de gradation des commotions cérébrales (voir Cantu, 2001 pour une revue de la littérature). Le système de gradation de l'*American Academy of Neurology* (Kelly et Rosenberg, 1997) est celui qui est le plus couramment utilisé. Selon ce système, la sévérité d'une commotion cérébrale se distribue sur une échelle de 1 à 3, selon la durée de la confusion, la durée des symptômes post-commotionnels et la présence ou non d'une perte de conscience.

Toutefois, pour évaluer la sévérité de l'incident, les systèmes de gradation sont de plus en plus laissés de côté au profit d'une analyse de la chronicité des symptômes (Echemendia, Giza et Kutcher, 2015). En fait, chez environ 10 à 15% des athlètes, les symptômes post-commotionnels vont perdurer au-delà de la fenêtre normale d'environ 7 à 10 jours (Herring et al., 2011). Chez un faible pourcentage de ces athlètes, les symptômes peuvent persister au-delà de 3 mois, ce qu'on associe à un syndrome post-commotionnel (SPC; Ryan et Warden, 2003; Bigler, 2008). Finalement, chez approximativement 10 à 20% des athlètes ayant un diagnostic de SPC, les symptômes vont persister pour plusieurs mois, voire un an après la commotion (Broshek, De Marco et Freeman, 2015; Lovell, 2008). On parle alors de syndrome post-commotionnel persistant qui suggère la présence possible d'altérations fonctionnelles permanentes (Bigler, 2008). Dans certains cas, les symptômes peuvent persister indéfiniment, laissant entrevoir la possibilité que la commotion cérébrale amorce un processus neuropathologique irréversible (Henry, Tremblay, Boulanger, Ellemberg et Lassonde, 2010). La communauté scientifique ignore toujours l'étiologie des symptômes post-commotionnels, ainsi que la nature des mécanismes pathologiques menant à leur persistance.

1.2.2 Pathophysiologie de la commotion cérébrale

1.2.2.1 Biomécanique

Une commotion cérébrale survient lorsqu'une énergie cinétique est transférée aux éléments se trouvant à l'intérieur de la boîte crânienne (Dimou et Lagopoulos, 2014). Ainsi, le cerveau se trouve alors en mouvement à l'intérieur du crâne. Plusieurs forces biomécaniques peuvent engendrer ce déplacement. Cantu (1996) fait mention de trois différents types de mécanismes pouvant causer une commotion cérébrale : 1) force de compression; 2) force de tension; 3) force de rotation. Ainsi, la tête peut entrer en contact direct avec un objet ou une personne, soit un impact direct impliquant une accélération et une décélération. L'impact peut également être indirect, par exemple lors d'une accélération ou décélération rapide de l'individu ou une onde de mouvement à la tête créée par un impact au corps (Prins, Greco, Alexander et Giza, 2013). Dans d'autres cas, le coup peut être fait de façon parallèle à la tête impliquant alors un mouvement rotationnel. De par l'évolution récente des sports de contact, incluant le perfectionnement des méthodes d'entraînement et des équipements protecteurs, les athlètes d'aujourd'hui ont une masse corporelle plus élevée que leurs prédécesseurs, mais ils sont également assujettis à de forces cinétiques plus importantes (Dashnaw, Petraglia et Bailes, 2012).

De récentes études en neuroimagerie ont permis d'élucider les déformations qui surviennent dans le cerveau suite à des accélérations rapides de la tête (Bayly et al., 2005). De par l'anatomie du crâne et l'emplacement des diverses régions cérébrales, certaines structures semblent plus susceptibles à des déformations et à entrer en contact avec des structures osseuses lorsqu'une force est appliquée à la tête. Par exemple, les régions fronto-temporales, le corps calleux et le fornix ont été identifiées comme particulièrement sensibles suite à un impact (Bayly et al., 2005). L'utilisation de modèles animaux, de reconstruction de l'impact par ordinateur et l'implantation récente de casques protecteurs munis d'accéléromètres a également permis de préciser les aspects biomécaniques de la commotion cérébrale (Dimou et Lagopoulos, 2014). Malgré qu'il soit toujours impossible de prédire la sévérité d'une commotion par de telles mesures, ces études ont permis de démontrer que les accélérations angulaires (rotationnelles) et linéaires, ainsi que certaines localisations précises au niveau du casque (p.ex. impact frontal ou

supérieur) sont liées à une probabilité plus importante de subir une commotion cérébrale (Broglio, Surma et Ashton-Miller, 2012; Pellman, Viano, Tucker, Casson et Committee on Mild Traumatic Brain Injury, 2003).

1.2.2.2 Cascade neurométabolique

Il semblerait que ces forces externes appliquées au cerveau entraînent une cascade complexe de changements biochimiques, métaboliques et d'expression génétique qui met en péril la survie des neurones. Le récent développement de modèles animaux des traumatismes crânio-cérébraux a permis de suggérer de possibles processus pathophysiologiques engendrant la commotion cérébrale. Cette chaîne d'évènements survenant dans le cerveau suite à un choc à la tête, qualifiée de cascade neurométabolique, a été d'abord suggérée par Giza et Hodva (2001). Selon leur modèle, ces évènements impliquent une dépolarisation neuronale massive, une libération excessive de neurotransmetteurs, dont le glutamate, et une production diminuée d'adénosine triphosphate (ATP), qui mènent à des processus cellulaires pathologiques tels que de l'inflammation, un stress oxydant, des dysfonctions de la mitochondrie, une excitotoxicité, de l'oedeme et une hypoxie (Barkhoudarian, Hovda et Giza, 2011; Giza et Hovda, 2001).

Plus précisément, la force appliquée au cerveau provoque la libération massive de neurotransmetteurs, surtout le glutamate, ainsi que la sortie incontrôlée d'ions dans l'espace extracellulaire. La liaison du glutamate avec les récepteurs N-methyl-D-aspartate (NMDA) accroît la dépolarisation incontrôlée des neurones, ce qui provoque la libération accrue des ions potassium et l'entrée des ions calcium. Ce déséquilibre ionique actionne donc de façon effrénée les pompes ioniques ATP-dépendantes dans le but de retrouver une homéostasie métabolique. Puisque ces pompes requièrent beaucoup d'énergie afin de rétablir le potentiel membranaire, on constate une augmentation fulgurante du métabolisme du glucose. Cette phase s'observerait normalement dans les 30 minutes suivant l'incident et correspondrait à un état d'hypermétabolisme (Yoshino, Hovda, Kawamata, Katayama et Becker, 1991). Cependant, cette augmentation du métabolisme a lieu dans un contexte de diminution du flot sanguin cérébral. Selon les auteurs, cela a pour conséquence de causer un épuisement neuronal en raison d'un manque d'oxygène pour actionner de façon optimale le processus de respiration cellulaire

géré par la mitochondrie (Barkhoudarian et al., 2011; Giza et Hovda, 2001). Ainsi, l'état d'hypermétabolisme se transformerait dans les 5 à 6 heures post-commotion vers un état d'hypométabolisme cérébral. L'étude initiale de Giza et Hodva (2001) a révélé un retour à l'équilibre métabolique environ 5 à 7 jours suivant la commotion cérébrale, ce qui correspond à la fenêtre attendue de résolution des symptômes.

Dans un contexte d'hypo- ou hyper-métabolisme, il serait très dangereux de recevoir un autre traumatisme au cerveau, malgré qu'il soit d'intensité plus faible que le premier coup subit. En effet, il semblerait que la chaîne d'événements métaboliques survenant suite à la commotion cérébrale place le cerveau dans un état de vulnérabilité importante qui pourrait ainsi précipiter des mécanismes de neuroinflammation menant à un œdème cérébral fatal. En effet, de nombreux cas de fatalité ont été documentés suite à un second impact reçu dans un court laps de temps suivant la commotion cérébrale, le processus étant nommé syndrome du second impact (Cantu, 1998). La fenêtre temporelle de vulnérabilité demeure méconnue, bien que certaines recherches animales suggèrent que celle-ci se prolongerait sur une période de 3 à 5 jours après l'incident (Longhi et al., 2005). Les impacts à court terme de ce dérèglement neurométabolique peuvent donc être catastrophiques, toutefois on en connait encore peu sur leurs effets à long terme. De surcroit, par l'étude du phénomène de la cascade neurométabolique chez les modèles animaux, Giza et Hodva (2001) en sont venus à postuler l'hypothèse d'une discordance métabolique entre les demandes énergétiques et les réserves en énergie créant une vulnérabilité cellulaire durable prédisposant ainsi à un second traumatisme crânien. Plusieurs facteurs peuvent influencer les chances de subir une deuxième commotion. Il semble toutefois que le métabolisme cérébral soit fragilisé par le premier choc subi.

Cette cascade neurométabolique serait également influencée par une force d'extension mécanique appliquée aux axones. En effet, plusieurs études portant sur la pathophysiologie des traumatismes crâniens ont été axées sur la compréhension des dommages axonaux diffus observés suite à des TCC modérés à sévères (Bayly et al., 2005). Toutefois, les études animales suggèrent que des dommages axonaux peuvent également être observés suite à des TCC légers (Spain et al., 2010; Xu et al., 2014). Dans ces cas, les mouvements rapides effectués par le cerveau, suite aux forces appliquées à la boîte crânienne, engendreraient un étirement des axones

qui est supérieur au seuil physiologique et qui provoquerait alors un déchirement des fibres de matière blanche (Bayly et al., 2005). Le dommage axonal ainsi créé aurait également un impact sur le métabolisme cellulaire en provoquant une perturbation du fonctionnement de la mitochondrie, du cytosquelette et des influx de calcium (Giza et Hovda, 2001). Enfin, les études animales suggèrent également l'implication d'autres neurotransmetteurs, tels que le GABA et l'acétylcholine, dans la cascade neurométabolique (Giza et Hovda, 2001). Par exemple, des altérations de l'inhibition GABAergique ont également été observées suite à un TCC, ce qui pourrait expliquer le développement d'épilepsies post-traumatiques dans les cas de TCC modérés à sévères (Lee, Lui, Wong, Yeh et Tzaan, 1995).

1.2.3 Commotions multiples et effets à long terme

Un champ d'études particulièrement fécond porte sur l'étude des effets cumulatifs des commotions cérébrales chez les athlètes. Ces études ont démontré que les athlètes ayant un historique de commotion cérébrale ont plus de chances de développer des symptômes post-commotionnels chroniques (Bazarian et al., 1999) et une sévérité plus importante de symptômes lors d'un impact subséquent (Collins et al., 2002). Par ailleurs, le fait de subir une première commotion cérébrale augmenterait le risque d'en subir une subséquente dans le futur (Guskiewicz et al., 2005). En effet, une étude effectuée auprès de 4251 joueurs de football a mis en lumière que les joueurs ayant un historique de 3 commotions ou plus ont trois fois plus de chances d'en subir une autre (Guskiewicz et al., 2003). Par ailleurs, une étude prospective effectuée auprès de 15 304 joueurs de football sur une durée de deux ans a démontré que les joueurs ayant un historique de commotion cérébrale ont 5.8 fois plus de chances de subir une commotion subséquente en comparaison avec les individus n'ayant pas d'historique (Zemper, 2003). Ces données prospectives concordent avec l'hypothèse de vulnérabilité métabolique soulevée par Giza et Hodva (2001).

Outre une augmentation de la susceptibilité à subir une commotion ultérieure, de récentes données suggèrent que le fait de subir plusieurs commotions cérébrales pourrait causer des effets délétères persistants sur le cerveau et même mener au développement de troubles cognitifs chroniques ou de conditions neurologiques dégénératives avec le vieillissement

(McCrea, Broshek et Barth, 2015). Cet intérêt pour l'étude des possibles effets à long terme catastrophiques des commotions multiples a notamment pris naissance suite à la mort précoce (vers l'âge de 45-50 ans) de trois anciennes vedettes du football américain probablement liée aux conséquences de l'encéphalopathie traumatique chronique (ETC) confirmée par autopsie (Cantu, 2007). Mike Webster, Terry Long et Andre Waters étaient tous trois reconnus pour leurs frappes violentes et l'utilisation très fréquente de leur tête lors des contacts. À la fin de leur vie, ils ont présenté des symptômes neurodégénératifs similaires, incluant une détérioration cognitive marquée, des pertes de mémoire et des symptômes psychiatriques, tels que la paranoïa, la dépression et le trouble panique (Cantu, 2007). Des analyses pathologiques de leur cerveau ont permis de lier ces symptômes à la présence de l'encéphalopatie traumatique chronique, ainsi identifiée pour la première fois chez des joueurs de football (Omalu et al., 2005; 2006). Depuis, des analyses post-mortem des tissus cérébraux ont révélé des signes importants de cette maladie dégénérative chez d'autres athlètes professionnels ayant subi de multiples commotions (McKee et al., 2009). Des centres de recherche, comme le *Center for the Study of Traumatic Encehalopathy* à l'Université de Boston, sont maintenant entièrement dédiés à l'étude pathologique du cerveau d'anciens athlètes. Des signes pathologiques de l'ÉTC ont même été découverts chez des athlètes universitaires de 18 et 21 ans décédés des suites d'un suicide, suggérant que le processus pathophysiologique menant à la maladie dégénérative puisse se développer très tôt chez les athlètes soumis à des contacts répétés (McKee, Daneshvar, Alvarez et Stein, 2014).

L'association entre les traumatismes crâniens et cette maladie n'est toutefois pas récente. L'ETC a d'abord été identifié chez des boxeurs professionnels, c'est pourquoi elle a longtemps été surnommée la démence du pugiliste. En 1928, Dr. Harrison Martland a dévoilé 23 cas de boxeurs professionnels qui présentaient des symptômes qualifiés de *punch-drunk* (Martland, 1928). L'article décrit un cas en particulier d'un ancien boxeur professionnel de 38 ans qui présentait des tremblements, une ataxie et une dysfonction pyramidale sans atteinte à l'intelligence. Subséquemment, Roberts a étudié 250 anciens boxeurs entre 1929 et 1955, et rapporta 37 cas de lésions du système nerveux (17% de la cohorte; Roberts, 1969). Les symptômes décrits se caractérisaient par une constellation de symptômes associés à des lésions des systèmes pyramidal, cérébelleux et extrapyramidal (McCrory, 2011). Les symptômes

évoluaient généralement d'une dysarthrie et de troubles extrapyramidaux, à un ralentissement moteur et verbal progressif, une ataxie et des troubles cognitifs importants (McCrory, 2011). Malgré un intérêt de plus en plus important envers l'étude de la prévalence de cette maladie dégénérative chez les athlètes professionnels, il n'y présentement aucun critère clinique permettant de diagnostiquer le trouble, ce dernier pouvant uniquement être réalisé par autopsie (Gardner, Iverson et McCrory, 2014). Les principaux signes pathologiques sont la présence d'enchevêtrements neurofibrillaires, l'accumulation de protéine tau et la perte cellulaire (Cantu, 2007). Gardner et collaborateurs (2014) ont récemment publié une revue de la littérature sur les études ayant investigué la présence d'ETC dans le sport professionnel. Ils ont identifié 85 cas d'autopsie ayant été effectués auprès d'athlètes au cours des 10 dernières années. De ce chiffre, 20% des athlètes présentaient des signes pathologiques concordant avec l'ETC, 52% présentaient des signes d'ETC et d'autres pathologies, 5% présentaient une neuropathologie autre et 24% ne présentaient aucune pathologie. Considérant le fait que les symptômes moteurs sont habituellement les premières manifestations cliniques de ce trouble chronique, il est plausible que le système moteur soit également affecté à un degré inférieur, chez les athlètes commotionnés asymptomatiques.

En parallèle, des études épidémiologiques effectuées auprès de joueurs de la NFL ont mis en lumière une prévalence 20% plus élevée que la population générale en ce qui concerne le développement de troubles cognitifs, de démence et de troubles affectifs incluant l'anxiété et la dépression (Amen, Wu, Taylor et Willeumier, 2011). Guskiewicz et ses collaborateurs (2005) ont découvert que les athlètes retraités qui ont souffert de commotions cérébrales multiples (3 ou plus) ont cinq fois plus de chances de développer un trouble cognitif léger que des personnes retraités n'en ayant pas subi, une condition qui est connue pour progresser vers une démence à une fréquence de 10 à 20% par année. De plus, le fait d'avoir souffert d'une ou plusieurs commotions cérébrales est le facteur de risque environnemental le plus important de la maladie d'Alzheimer (Guo et al., 2000; Heyman et al., 1984; Mortimer, French, Hutton et Schuman, 1985; Plassman et al., 2000). Finalement, de récentes études épidémiologiques suggèrent que des athlètes ayant subi de multiples commotions ont 11 fois plus de chances de développer la sclérose latérale amyotrophique, une malade neurodégénérative dévastatrice caractérisée par la

mort progressive de motoneurones supérieurs et inférieurs (Chen, Richard, Sandler, Umbach et Kamel, 2007; Piazza, Sirén et Ehrenreich, 2004).

De récentes démarches légales menées par d'anciens joueurs (plus de 4000 anciens athlètes) contre la NFL en lien avec les effets post-commotionnels persistants ont contribué à mettre de l'avant plan la problématique des effets à long terme des commotions cérébrales dans les médias. Depuis, des joueurs de la Ligue Nationale de Hockey (LNH) ont également présenté un recours collectif contre leur ligue. Cet engouement médiatique a contribué à l'augmentation du financement privé et public quant au phénomène. Par exemple, en 2012, la NFL a annoncé le versement d'un fond de 30 millions de dollars aux *National Institutes of Health for Medical Research* des États-Unis afin d'approfondir la recherche sur les impacts à long terme des commotions cérébrales. Malgré tout, le mécanisme exact par lequel les commotions cérébrales multiples contribuent au développement de conditions neurologiques sévères demeure incompris.

1.2.4 À la recherche de marqueurs objectifs des commotions cérébrales

Malgré les efforts récents pour augmenter la conscientisation de la population générale et des athlètes face aux possibles effets délétères des commotions cérébrales, il est estimé que jusqu'à 50% des commotions cérébrales seraient non-rapportées par les athlètes (Harmon et al., 2013). En lien avec ceci, un récent sondage anonyme effectué auprès de 320 joueurs de la NFL a révélé que 85% de ceux-ci seraient prêts à jouer un match de championnat malgré la présence d'une commotion cérébrale (Broshek et al., 2015). Ainsi, il est impératif de développer des marqueurs objectifs de diagnostic et pronostic, puisque malgré de récentes avancées dans les méthodes d'évaluation des commotions cérébrales, le diagnostic demeure globalement basé sur les symptômes auto-rapportés par l'athlète (Lynall et Guskiewicz, 2015).

En fait, les directives actuelles pour la prise en charge des commotions cérébrales incluent les éléments suivants (Echemendia et al., 2015; Harmon et al., 2013; McCrory et al., 2013): (a) l'athlète dont on suspecte une commotion cérébrale doit être retiré du jeu et ne peut

y retourner avant une évaluation par un spécialiste des soins de la santé; (b) le diagnostic devrait être guidé par l'utilisation d'échelles de symptômes standardisées, d'une brève évaluation cognitive et d'équilibre, ainsi que d'examens physiques supplémentaires; (c) l'utilisation d'outils permettant des évaluations comparatives de références (niveau de base) peut être envisagée (p.ex. batterie cognitive informatisée) et l'utilisation de méthodes d'imagerie cérébrale conventionnelles n'est pas recommandée à moins qu'un TCC plus sévère soit suspecté. Quant au retour au jeu, les présentes recommandations font notamment mention que (Echemendia et al., 2015; Harmon et al., 2013; McCrory et al., 2013): (a) le retour au jeu ne peut être permis avant la disparition complète des symptômes évalués par un spécialiste de la santé et celui-ci ne devrait pas être permis dans la même journée; (b) le retour à l'exercice ne devrait pas être permis avant la disparition complète des symptômes et ce dernier devrait se faire de façon graduelle avec l'aide d'outils supplémentaires, tels que des protocoles de retour au jeu progressifs; (c) une évaluation formelle neurologique ou neuropsychologique peut être utile dans l'optique d'émettre des recommandations quant au retour au jeu. Ainsi, une brève analyse de ces recommandations révèle une très grande part de subjectivité dans l'évaluation, ainsi qu'une interrogation particulière face à l'authenticité et l'exactitude des symptômes rapportés par l'athlète (Ruff et al., 2009). De surcroit, les recommandations actuelles se basent sur le principe voulant qu'une récupération des symptômes soit associée à une récupération complète de la commotion cérébrale. Or, les études liant un historique de commotions cérébrales au développement de maladies dégénératives suggèrent que des dommages subtils, mais permanents, pourraient être présents malgré une absence de symptomatologie.

Par conséquent, le défi actuel est de se distancer des évaluations diagnostiques courantes afin de mettre en place des méthodes diagnostiques objectives qui reflètent la pathophysiologie des commotions cérébrales et qui permettraient d'émettre des recommandations fiables quant au pronostic, ainsi que sur l'état du rétablissement (Dimou et Lagopoulos, 2014). Cet aspect est d'autant plus important compte tenu du potentiel de développement de séquelles à long-terme (Echemendia et al., 2015; Ellemberg et al., 2009; Randolph et Kirkwood, 2009; Reddy et Collins, 2009). Toutefois, il n'existe présentement aucun marqueur qui permet de prédire la présence de dommages cérébraux aigus ou persistants.

Le développement récent d'une multitude de nouvelles méthodes de neuroimagerie permettant de visualiser des anomalies infracliniques ou microscopiques offre le potentiel d'améliorer la compréhension de la réponse du cerveau à la commotion cérébrale (voir Yuh, Hawryluk et Manley, 2014 pour une revue de la littérature). Cependant, ces méthodes sont très coûteuses et malheureusement, très peu accessibles en clinique. Par ailleurs, les méthodes d'analyse que requièrent les nouvelles séquences d'imagerie sont souvent très complexes et nécessitent la présence de professionnels en neuroimagerie. Ainsi, d'un point de vue diagnostic, il n'est présentement pas possible d'utiliser ces méthodes de façon régulière auprès des athlètes.

Une cible particulièrement intéressante dans l'étude du développement de potentiels marqueurs de la commotion cérébrale serait la possibilité d'obtenir un indice du métabolisme cérébral et de la neurophysiologie, et ce compte tenu de la cascade neurométabolique suspectée suite à l'incident. En effet, étant donné la présence probable d'altérations au niveau du métabolisme cérébral et de production de neurotransmetteurs suggérées par les modèles animaux, les interactions excitatrices et inhibitrices au sein du cerveau semblent être impliquées dans la réponse neuronale à la commotion cérébrale (Dimou et Lagopoulos, 2014). En lien avec ceci, le récent développement des méthodes de stimulation cérébrale non-invasive offre une opportunité unique d'explorer la présence possible de marqueurs neurophysiologiques de la commotion cérébrale. La stimulation magnétique transcrânienne, qui permet d'étudier *in vivo* l'excitabilité corticale, serait un candidat potentiel à cette fin. En effet, la SMT a permis de fournir un apport considérable dans la compréhension de la pathophysiologie de multiples désordres du système nerveux au cours des dernières années, incluant la maladie de Parkinson (Udupa et Chen, 2013) et la sclérose latérale amyotrophique (Vucic, Ziemann, Eisen, Hallett et Kiernan, 2013). Cette technique comporte les avantages d'être peu coûteuse, portative, facile d'utilisation et dont les résultats ne demandent aucune analyse supplémentaire (Chen et al., 2008). Ainsi, advenant l'identification d'un marqueur de la commotion cérébrale avec cette technique, la SMT pourrait être utilisée directement auprès des joueurs, par exemple dans le vestiaire ou dans le centre d'entraînement.

1.3 La stimulation magnétique transcrânienne comme mesure du métabolisme cérébral

1.3.1 Principes de fonctionnement

Introduite par Barker et collègues en 1985, la SMT permet d'investiguer la neurophysiologie du cerveau de façon objective, non-invasive et sans risque. Cette technique repose sur le principe d'induction électromagnétique découvert en 1831 par Michael Faraday. Selon ce principe, la force électromagnétique se manifeste à la fois dans les champs magnétiques et électriques. Plus précisément, un courant électrique détient la capacité de générer un champ magnétique et à l'inverse, une variation de champ magnétique permet de produire un courant électrique. De ce fait, il est possible de produire un bref champ magnétique lorsqu'une charge électrique passe à travers un conducteur, et ce dernier peut à son tour générer un champ électrique dans un conducteur donné. Ainsi, la SMT est basée sur la génération d'une charge électrique relâchée dans une bobine de cuivre recouverte d'une gaine isolante, le stimulateur, produisant ainsi une stimulation magnétique de quelques Tesla. Lorsque le stimulateur est placé sur la tête d'un participant, le champ magnétique ainsi généré passe au travers du crâne sans aucune atténuation. Étant donné la conductivité du tissu cérébral, le champ magnétique produit un courant électrique ionique dans le cortex, et subséquemment une dépolarisation de la population neuronale qui se trouve sous cette influence. Le cortex moteur est la région cérébrale la plus étudiée puisque les effets de la SMT y sont facilement observables et quantifiables. En effet, puisque le cortex moteur primaire (M1) comporte des projections directes avec la moelle épinière, sa stimulation avec la SMT active automatiquement la voie corticospinale et provoque une contraction involontaire du muscle correspondant à la région corticale stimulée. Cette contraction musculaire peut être mesurée de manière fiable grâce à l'électromyographie et est connue sous le nom de potentiel évoqué moteur (PÉM).

Les PÉM induits par la SMT peuvent être utilisés pour quantifier l'activation motrice produite par la dépolarisation des neurones corticaux. Lorsque l'intensité de la stimulation appliquée au dessus de M1 est gardée constante, les variations d'amplitudes des PÉM reflètent le niveau d'activité intrinsèque des neurones pyramidaux de M1 (Reis et al., 2008). En variant

les différents paramètres de stimulation (p.ex. intensité, muscle au repos ou en contraction, fréquence de stimulation), il est possible d'obtenir diverses mesures des processus d'inhibition et de facilitation intra-corticaux du cortex moteur.

1.3.2 Mesures de l'excitabilité et de l'inhibition dans le cortex

D'une part, un ensemble de protocoles permettent d'évaluer les processus d'excitabilité intracorticale. Ceux-ci incluent notamment les *seuils moteurs* (SM) de repos et d'activité qui reflètent l'excitabilité globale du système corticospinal, notamment l'excitabilité de la membrane des neurones corticaux et des interneurones, ainsi que l'activité des récepteurs NMDA (Kobayashi et Pascual-Leone, 2003; Paulus et al., 2008). Par ailleurs, il est possible de mesurer l'excitabilité corticale par l'entremise de la *courbe de recrutement* (Hallett, 2007), obtenue par des stimulations simples successives d'intensité variable, et la *facilitation intracorticale*, obtenue par deux stimulations successives à un intervalle de 8 à 30 ms (Kujirai et al., 1993). Ces deux protocoles reflèteraient, respectivement, l'activité glutamatergique (Di Lazzaro et al., 2003), ainsi que l'activité des interneurones modulée par l'activité des récepteurs glutamatergiques (Liepert, Schwenkreis, Tegenthoff et Malin, 1997) et $GABA_A$ (Ziemann, 2004). Finalement, le *temps de conduction motrice centrale*, une mesure du délai entre l'activation de M1 et l'activation des neurones moteurs du tronc cérébral ou de la moelle épinière (Kobayashi et Pascual-Leone, 2003), permet de mesurer l'intégrité de l'excitabilité de la voie corticospinale.

D'autre part, un ensemble de mesures SMT permettent de mesurer les systèmes inhibiteurs au sein de M1. Une première mesure, l'*inhibition intracorticale de courte durée* (IICcd), est obtenue par une stimulation double avec un court intervalle inter-stimulus (1 à 5 ms) et reflèterait l'activité des récepteurs $GABA_A$ (Di Lazzaro et al., 2000; Ilić et al., 2002). De plus, l'activité des récepteurs $GABA_B$ peut être évaluée via l'*inhibition intracorticale de longue durée* (IICld), qui est obtenue via une stimulation pairée à un intervalle de 100-200 ms (McDonnell et al., 2006; Werhahn et al., 1999), et la *période silencieuse corticale* (PSC; Werhahn et al., 1999). La PSC est obtenue par l'application d'une pulsation unique au-dessus de M1 tandis que le participant maintient une légère contraction volontaire du muscle

controlatéral cible, provoquant une période de pause dans le signal électromyographique suite au PÉM.

Le lien entre ces diverses mesures et ces processus d'inhibition/excitation a été obtenu grâce à des études pharmacologiques lors desquelles des agonistes ou antagonistes à des neurotransmetteurs et/ou récepteurs spécifiques sont administrés. Lorsque l'agent pharmacologique est en mesure de moduler la réponse électromyographique suite à un protocole SMT particulier, on suggère alors que la molécule est impliquée dans le mécanisme neurophysiologique mesuré. Par exemple, l'administration d'un agoniste $GABA_B$ augmente la durée de la période silencieuse corticale (Werhahn et al., 1999), ce qui suggère que cette mesure reflète l'activité de ces récepteurs spécifiques.

1.3.3 La SMT et intégrité des systèmes excitateurs et inhibiteurs suite à une commotion cérébrale

La SMT a d'abord été utilisée pour étudier l'intégrité du système moteur suite à un TCC léger par Chistyakov et ses collaborateurs (1998). Les résultats de cette étude pionnière suggéraient une réduction de l'excitabilité corticale, révélée par une augmentation du seuil moteur au repos, et ce deux semaines suivant le TCC léger. Cette altération de l'excitabilité corticale était résorbée 3 mois suivant le TCC (Chistyakov et al., 1998). Dans une étude ultérieure effectuée auprès d'individus ayant subi un TCC léger deux semaines post-incident, une augmentation des seuil moteurs, du temps de conduction motrice centrale et de la durée de la période silencieuse corticale étaient observés (Chistyakov et al., 2001). Ces premières études suggéraient donc la présence d'altérations au niveau de la voie corticospinale, ainsi que des processus d'inhibition et d'excitation au sein du cortex moteur primaire. La persistance de telles altérations au niveau de M1 demeurait alors inconnue.

Les travaux de De Beaumont et collaborateurs (2007) se sont penchés sur la possible nature persistante de ces altérations neurophysiologiques chez des athlètes de football universitaire asymptomatiques ayant subi une ou plusieurs commotions cérébrales. En

comparaison avec des athlètes n'ayant jamais subi de commotion cérébrale, les athlètes commotionnés ne présentaient pas d'altération au niveau des mesures d'excitabilité corticale (SM, courbe de recrutement, facilitation intracorticale), ainsi qu'au niveau de l'inhibition intracorticale de courte et longue durée. Toutefois, le groupe d'athlètes ayant subi de multiples commotions cérébrales présentait une augmentation de la durée de la PSC en comparaison avec le groupe contrôle. De plus, leurs résultats ont démontré un lien entre la sévérité des commotions cérébrales, et l'altération de la période silencieuse corticale. Ceci suggère que les récepteurs des interneurones inhibiteurs intracorticaux $GABA_B$ du système moteur seraient particulièrement vulnérables aux effets des commotions dans les sports. Par ailleurs, aucun lien n'a été observé entre la durée de la période silencieuse et le temps passé depuis la dernière commotion. Les résultats provenant de l'étude de De Beaumont et collaborateurs (2007) indiquent donc pour la première fois que les commotions peuvent engendrer des dysfonctions infracliniques du système moteur qui sont liées à des anomalies du système inhibiteur intracortical chez de jeunes athlètes asymptomatiques.

Dans une étude ultérieure, De Beaumont et collègues (2009) ont cherché à savoir si les altérations neurophysiologiques motrices engendrées par les commotions cérébrales se résorbaient après une période de temps de l'ordre de plusieurs décennies. Dans une étude où ils ont comparé 19 anciens athlètes ayant subi leur dernière commotion il y a plus de trente ans à 21 ex-athlètes n'ayant jamais subi de commotion cérébrale, ces chercheurs ont rapporté chez les commotionnés : (a) une baisse de performance aux tests neuropsychologiques de mémoire épisodique; (b) une diminution de l'amplitude de la composante de potentiels évoqués P300 et une augmentation de sa latence; (c) une prolongation de la PSC; (d) une diminution significative de la vélocité du mouvement (bradykinésie; De Beaumont et al., 2009). Ces auteurs ont également démontré la présence d'une forte corrélation entre la longueur de la PSC et la lenteur à l'exécution de la tâche motrice, suggérant ainsi que les altérations neurophysiologiques au sein des régions motrices pourraient sous-tendre en partie la bradykinésie observée.

Dans deux études subséquentes, le groupe de De Beaumont et collaborateurs a également montré, en moyenne 9 mois post-commotion, une augmentation de l'inhibition intracorticale chez des athlètes commotionnés asymptomatiques révélée par une prolongation de la PSC (De

Beaumont et al., 2011a; 2011b) et de l'inhibition intracorticale de longue durée (De Beaumont et al., 2011a). Des altérations au niveau de la plasticité synaptique et de l'apprentissage moteur implicite ont également été révélées et celles-ci étaient corrélées avec l'inhibition intracorticale excessive GABAergique (De Beaumont et al., 2011b). Des altérations du contrôle postural ont également été mises en lumière (De Beaumont et al., 2011a). Ainsi, ces études suggèrent que les dysfonctions des réseaux inhibiteurs dans M1 pourraient avoir un impact fonctionnel significatif sur l'apprentissage moteur et sur l'état des capacités motrices de l'athlète. Ce facteur est d'autant plus important compte tenu du fait que ces athlètes étaient de retour au jeu depuis plusieurs mois.

1.3.4 État actuel des connaissances et avenues à explorer

En résumé, les données disponibles suggèrent la présence d'une altération spécifique et persistante de la transmission GABAergique au niveau de M1 (possiblement limitée aux récepteurs de type B) suite à une commotion cérébrale. Toutefois, ces études ont été effectuées avec la stimulation magnétique transcrânienne, laquelle permet une mesure *indirecte* de l'activité du GABA. En effet, bien que la SMT mesure de façon directe la neurophysiologie motrice, son association avec le métabolisme cérébral, telle que la transmission GABAergique, demeure indirecte. Il n'existe pas, à ce jour, d'évidences directes démontrant l'implication du GABA dans la réponse à la commotion cérébrale chez l'athlète.

1.4 La spectroscopie par résonance magnétique comme mesure directe du système inhibiteur

1.4.1 Intégrité du GABA : études animales et pharmacologiques

Comme il a été décrit dans le modèle de Giza et Hodva, une libération excessive de glutamate serait une des réponses neurométaboliques principales suite à un TCC (Baker, Moulton, MacMillan et Shedden, 1993; Faden, Demediuk, Panter et Vink, 1989), ce qui engendrerait une excitotoxicité glutamatergique et une neurodégénération (Rothman et Olney,

1986). En lien avec ceci, une des explications possibles à l'augmentation du niveau relatif de transmission GABAergique révélée par les mesures SMT chez les athlètes commotionnés serait liée au déploiement d'un mécanisme de protection contre l'excitotoxicité glutamatergique survenant lors de la cascade neurométabolique. En effet, le l'acide gamma-aminobutyrique (GABA) jouerait un rôle régulateur important lors d'une crise énergétique au sein du cerveau (Wu et Sun, 2014). De plus, dans le cas d'un dommage axonal, la libération de GABA aurait un rôle neuroprotecteur (Fern, Waxman et Ransom, 1995). Cet effet est aboli lorsqu'un antagoniste de $GABA_B$ est administré (Wu et Sun, 2014). Par ailleurs, des études effectuées chez l'animal ont démontré une augmentation de la transmission GABAergique suivant la provocation d'un bref épisode ischémique (Dave et al., 2005; Kuramoto et al., 2007), ce qui concorde avec l'hypothèse de neuroprotection en phase aiguë. Toutefois, les études en SMT suggèrent que cette altération dans la transmission du GABA pourrait persister au-delà de la fenêtre aiguë post-TCC. Ainsi, certains modèles animaux du TCC ont mis en lumière des niveaux supranormaux de GABA (Kobori et Dash, 2006; Pascual et al., 2007), qui se normalisaient suite à l'administration d'un antagoniste GABAergique. Les auteurs ont ainsi soulevé l'hypothèse de la présence d'une « inhibition GABAergique excessive » provoquée par le TCC (Kobori et Dash, 2006). D'autres auteurs ont aussi suggéré que cette augmentation de la transmission GABAergique surviendrait probablement dans l'optique d'un rétablissement de l'équilibre entre l'excitation et l'inhibition, afin de diminuer l'excitotoxicité causée par une trop grande transmission glutamatergique, ainsi que pour jouer un rôle neuroprotecteur contre la mort neuronale (Kuramoto et al., 2007).

Étant le neurotransmetteur inhibiteur principal, le GABA est libérée à travers l'ensemble des neurones du cerveau et interagit avec d'autres neurotransmetteurs, notamment le glutamate (Bacci, Huguenard et Prince, 2005). Ainsi, un débalancement prolongé du GABA pourrait non seulement altérer l'équilibre entre les transmissions excitatrices et inhibitrices, mais également affecter le fonctionnement de plusieurs systèmes (p.ex. système moteur) (Amin et al., 2006). D'autres auteurs ont en effet associé les altérations GABAergiques suite au TCC comme étant « maladaptives » puisqu'elles contribueraient à l'excitotoxicité et au développement des épilepsies post-traumatiques. En effet, des modèles animaux des épilepsies post-traumatiques associées aux TCC sévères ont mis en lumière une augmentation de l'inhibition GABAergique

en réponse à l'augmentation de l'excitabilité corticale (Nichols, Perez, Wu, Adelson et Anderson, 2015), tandis que d'autres auteurs ont démontré une perte progressive d'inhibition synaptique liée aux récepteurs $GABA_A$ en phase aiguë qui pourrait contribuer à l'hyperexcitabilité observée en phase aiguë post-TCC (Drexel et al., 2015).

À la lumière des évidences répertoriées ci-haut, les études animales suggèrent la présence d'altérations au niveau GABAergique en phase aiguë. Compte tenu des récentes études en SMT, il apparaît primordial de mieux comprendre les effets d'une commotion cérébrale sur la transmission GABAergique, et ce de manière directe et non-invasive chez l'humain.

1.4.2 Spectroscopie par résonance magnétique et commotions cérébrales

La spectroscopie par résonance magnétique permet la détection et la quantification *in vivo* de différents neurométabolites dans un voxel spécifique (aire à 3 dimensions) localisé dans l'IRM anatomique. Aussi appelée « biopsie virtuelle », la SRM se base sur les propriétés moléculaires uniques à chacun des neurométabolites. L'acquisition des concentrations des métabolites s'étend alors sur un spectre où chaque métabolite est associé à un pic de résonance, et ce à une fréquence précise (Puts et Edden, 2012). Cette technique permet de vérifier l'intégrité neuronale et la fonction de multiples régions cérébrales, fournissant ainsi une évaluation sensible et non invasive de possibles altérations neurochimiques (Ashwal et al., 2004; Dimou et Lagopoulos, 2014).

En plus des neurométabolites communément étudiés avec la SRM, soit la créatine/phosphocréatine (tCr : marqueur de métabolisme énergétique et de la fonction mitochondrique), le myoinositol (mIns : marqueur de l'activité des cellules gliales et d'œdème), le N-acetylaspartate (NAA : marqueur d'intégrité neuronale et de neuroprotection), et le glutamate + glutamine (Glx : marqueur de transmission excitatrice), il est désormais possible de quantifier de façon précise la concentration de GABA dans le cerveau humain. En effet, la quantification du GABA est plus complexe que celle des autres métabolites. C'est seulement avec l'avènement du scanneur à 3 Tesla qu'il a été possible de développer des méthodes d'acquisition stables du métabolite (Jissendi Tchofo et Balériaux, 2009). En effet, la résonance

du GABA est en chevauchement avec celle d'autres neurométabolites, ce qui la rend difficilement identifiable. Par ailleurs, la concentration, et par conséquent le ratio signal sur bruit, du GABA dans la matière grise est basse contrairement aux autres métabolites, ce qui complexifie son acquisition (Choi, Lee, Merkle et Shen, 2006; Jensen, Frederick, Wang, Brown et Renshaw, 2005).

Le développement et le raffinement dans les différentes séquences d'acquisition des neurométabolites par la SRM au cours des dernières années ont permis son utilisation pour évaluer l'impact de plusieurs pathologies sur le métabolisme cérébral. Dans le cas des TCC, des études ont d'abord été effectuées chez l'animal où il a été possible de démontrer l'état métabolique du cerveau après l'induction d'un TCC (Harris et al., 2012; Kobori et Dash, 2006; Viant, Lyeth, Miller et Berman, 2005; Xu et al., 2011). Dans certaines études, des niveaux supranormaux de glutamate et de GABA ont été observés dans la phase aiguë post-TCC chez l'animal (Fievisohn, Sajja, Vandevord et Hardy, 2014; Sajja et al., 2014). Chez l'humain, la spectroscopie par résonance magnétique a été d'abord été utilisée auprès de patients ayant subi des TCC de sévérités variables, et ce dans diverses régions du cerveau. En général, ces études ont montré une diminution du niveau de NAA dans la phase aiguë post-TCC (Brooks, Friedman et Gasparovic, 2001; Govindaraju et al., 2004; Macmillan et al., 2002; Marino, Ciurleo, Bramanti, Federico et De Stefano, 2011). Bien que moins systématiques à travers les études, des altérations ont également été observées pour d'autres métabolites dans la phase aiguë post-TCC, tel que des niveaux anormaux de Glx (Babikian et al., 2006; Shutter, Tong et Holshouser, 2004), et de lactate, choline et myoinositol (Brooks et al., 2001; Marino et al., 2011). Aucune de ces études n'a quantifié le GABA.

Quelques études ont également évalué l'impact spécifique des commotions cérébrales sur le métabolisme cérébral avec la SRM, sans toutefois mesurer les niveaux de GABA. À l'intérieur d'un mois post-commotion, des réductions de la concentration de NAA ont été observées (Cimatti, 2006; Henry et al., 2010; Johnson et al., 2012; Vagnozzi et al., 2008; 2010; 2012), tout comme une réduction du niveau de glutamate (Henry et al., 2010). Une seule étude a évalué les altérations métaboliques dans la phase chronique, c'est-à-dire 6 mois post-commotion (Henry et al., 2011). Une diminution de NAA a été observée dans les régions

motrices et prémotrices, ainsi qu'une augmentation du myoinsitol dans le cortex moteur primaire (Henry et al., 2011). Il semble donc que la SRM soit relativement sensible aux changements dans les concentrations de neurométabolites suite à une commotion cérébrale et puisse contribuer à une meilleure compréhension de l'impact des commotions cérébrales sur le métabolisme cérébral.

1.4.3 État actuel des connaissances et avenues à explorer

La SRM n'a pas été utilisée pour investiguer directement l'intégrité du système GABAergique chez des athlètes commotionnées ou suite à un traumatisme crânien léger. Par ailleurs peu d'études ont évalué l'impact à long terme d'une commotion cérébrale en utilisant cette méthode. Ceci est d'autant plus important compte tenu des études en SMT montrant des altérations persistantes des récepteurs $GABA_B$.

1.5 L'hypothèse GABAergique : une possibilité de traitement?

La découverte d'un marqueur probable de la commotion cérébrale, tel que l'inhibition intracorticale excessive, ouvre la voie au développement de possibles avenues de traitement. Dans cette optique, une modulation de la transmission GABAergique permettrait un rétablissement de l'équilibre entre l'excitation et l'inhibition au sein du cortex moteur. Bien que certaines interventions pharmacologiques puissent agir sur ces transmissions, le développement récent de méthodes de stimulation non-invasive, telle que la stimulation électrique transcrânienne à courant direct (SÉTcd), offrent la possibilité d'agir de façon non-invasive sur l'excitabilité corticale (Jacobson, Koslowsky et Lavidor, 2011). Ces méthodes détiennent donc un potentiel clinique unique. En effet, la SÉTcd a été récemment associée à une diminution de symptômes chez une grande variété de troubles du système nerveux central, tels que la maladie de Parkinson, la maladie d'Alzheimer et la sclérose en plaques, pour n'en nommer que quelques uns (voir Floel, 2013 pour une revue de la littérature).

La stimulation électrique transcrânienne à courant direct permet de moduler l'excitabilité corticale de façon non-invasive par l'application d'un courant électrique de faible intensité (1-2 mA) au niveau du scalp via deux électrodes en caoutchouc. Le courant voyage ainsi de l'anode, dont la polarité est positive, vers la cathode dont la polarité est négative. L'effet de la stimulation sur une région d'intérêt dépend donc de la polarité de l'électrode qui se trouve au dessus, c'est-à-dire qu'une stimulation anodale a pour effet d'augmenter l'excitabilité corticale et qu'une stimulation cathodale diminue celle-ci. Lorsque les régions motrices sont stimulées, il est possible de mesurer directement ces effets avec la SMT par les différences dans l'amplitude des potentiels évoqués moteurs générés avant et après la stimulation. Généralement, l'électrode active est positionnée au dessus de M1 et l'électrode « contrôle » est positionnée au niveau du pôle frontal.

Avec ce type de montage, de nombreuses études ont montré des effets systématiques et relativement durables de la SÉTcd sur les PÉM, c'est-à-dire la présence d'une augmentation des PÉM suite à une stimulation anodale, et d'une réduction suite à une stimulation cathodale (Lang et al., 2005). Quelques études ont investigué les effets de la stimulation sur les marqueurs d'inhibition/excitation intracorticale, et les résultats suggèrent la possibilité de moduler certains marqueurs. Suite à une stimulation anodale, certaines études ont montré une réduction de l'inhibition intracorticale de courte durée (Antal, Terney, Kühnl et Paulus, 2010; Nitsche et al., 2005) et une augmentation de la faciliation intracorticale (Nitsche et al., 2005), mais aucun effet sur l'inhibition intracorticale de longue durée (Antal et al., 2010). Quant à la stimulation cathodale, Nitsche et collaborateurs (2005) ont montré une diminution de la facilitation intracorticale et une augmentation de l'inhibition intracroticale de courte durée, tandis que d'autres auteurs n'ont rapporté aucun impact significatif sur ces mesures (Di Lazzaro et al., 2012). Seulement deux études ont investigué la possibilité de moduler la PSC chez des individus en santé. Aucun effet n'a été rapporté par Suzuki et collaborateurs (2012) suite à des stimulations anodale et cathodale, tandis qu'une augmentation de la durée de la PSC a été rapportée suite à une stimulation cathodale (Hasan et al., 2011). Une investigation plus approfondie de l'impact de la SÉTcd sur ces marqueurs neurophysiologiques permettrait non seulement de vérifier le potentiel clinique de la méthode dans l'optique de l'utiliser auprès d'une population d'athlètes

commotionnés, mais également de mieux comprendre les mécanismes par lesquels cette technique permet de moduler l'excitabilité corticale.

Les mécanismes sous-jacents aux effets de la SÉTcd ont été principalement étudiés via des modèles animaux et des études pharmacologiques. Ces études suggèrent que la SÉTcd module l'excitabilité corticale en agissant sur le potentiel de repos de la membrane (Fritsch et al., 2010; Liebetanz, Nitsche, Tergau et Paulus, 2002). En effet, contrairement à la SMT, cette technique ne permettrait pas de dépolariser la membrane cellulaire, mais modulerait à la hausse ou à la baisse le seuil de dépolarisation (Stagg et Nitsche, 2011). Par ailleurs, la SÉTcd permettrait d'agir sur les connexions synaptiques par des mécanismes de potentialisation et dépression à long terme, qui seraient modulés par les neurones GABAergiques et glutamatergiques (Froc, Chapman, Trepel et Racine, 2000; Trepel et Racine, 2000). Chez l'humain, l'effet de la SÉTcd sur les systèmes excitateurs et inhibiteurs a également été étudié avec la spectroscopie par résonance magnétique. Stagg et collaborateurs (2009) ont investigué l'effet de la SÉTcd cathodale et anodale sur le GABA et le glutamate au niveau du cortex moteur primaire. Ils ont observé une réduction du GABA suite à la stimulation anodale et une réduction du GABA et du glutamate suite à une stimulation cathodale. Dans une étude ultérieure, le même groupe de chercheurs a démontré que la réduction de GABA provoquée par la stimulation anodale était corrélée avec la performance à une tâche motrice suggérant que les mesures du neurotransmetteur reflètent des gains au niveau du fonctionnement moteur (Stagg, Bachtiar et Johansen-Berg, 2011a).

1.5.1 État actuel des connaissances et avenues à explorer

Ainsi, ces études suggèrent que la SÉTcd peut agir sur la plasticité cérébrale, ainsi que sur le GABA et le glutamate tels que mesurés par la SRM et la SMT. Toutefois, l'effet de la SÉTcd sur les mesures inhibitrices possiblement altérées suite à une commotion cérébrale demeure méconnu. Par ailleurs, l'impact de la SÉTcd sur les mesures directes du GABA et du glutamate n'a été évalué que par un groupe de chercheurs avec un protocole de stimulation spécifique. Il est donc impératif d'approfondir les connaissances actuelles des effets métaboliques de la SÉTcd avec des protocoles de stimulation pouvant être efficaces auprès de

diverses pathologies. Une meilleure compréhension des effets de cette méthode de stimulation cérébrale non-invasive est essentielle dans l'optique de développer des essais cliniques auprès d'une population d'athlètes commotionnés.

1.6 Objectifs expérimentaux et hypothèses

1.6.1 La SMT comme marqueur d'altération du GABA

L'objectif premier de cette thèse est d'évaluer la possibilité d'utiliser la présence d'altérations GABAergiques, telles que mesurés par la SMT, comme marqueur des effets persistants des commotions cérébrales chez des athlètes asymptomatiques. Ceci permettra de mieux comprendre les processus neurophysiologiques et métaboliques qui sont responsables des effets à long terme des commotions cérébrales dans le sport, mais également d'évaluer la pertinence de la SMT comme mesure du GABA et comme mesure objective de récupération. Cet objectif général forme la thématique centrale des articles 1, 2 et 3 qui forment cet ouvrage.

1.6.1.1 Article 1 : Spécificité des altérations neurophysiologiques suite à une commotion cérébrale

Dans l'article 1, la spécificité des altérations neurophysiologiques persistantes engendrées par des commotions cérébrales multiples est étudiée chez un groupe d'athlètes universitaires asymptomatiques au moins 9 mois après la dernière commotion, et ce en comparaison avec un groupe d'athlètes n'ayant jamais subi de commotion cérébrale. À cette fin, l'intégrité des systèmes inhibiteurs GABAergiques, de la vitesse de conduction de la voie corticospinale et des interactions sensorimotrices est évaluée avec la SMT. Par ailleurs, l'intégrité du système somatosensoriel est évaluée via les potentiels évoqués somatosensoriels. Considérant les études antérieures effectuées auprès de populations de TCCL et d'athlètes commotionnés, nous sommes en mesure de formuler les hypothèses suivantes :

1) la SMT peut être utilisé comme marqueur d'altérations spécifiques du système GABAergique au sein du cortex moteur chez une population d'athlètes commotionnés multiples;
2) les systèmes sensoriels et les interactions sensorimotrices seront intacts chez les athlètes comotionnés.

1.6.1.2 Article 2 : Effets persistants des commotions cérébrales sur les mesures directes et indirectes du GABA

Dans l'article 2, les effets à long terme des commotions cérébrales dans le sport sur le système GABAergique sont étudiés chez des athlètes asymptomatiques ayant un historique de commotions cérébrales via la SMT et la SRM, et comparés à un groupe d'athlètes n'ayant aucun historique de commotion cérébrale. L'intégrité des régions motrices est également évaluée par l'entremise de mesures d'épaisseur corticale. Compte tenu des résultats obtenus à l'étude 1 et des résultats d'études en SRM effectuées en phase aiguë et chronique (Henry et al., 2010; 2011), nous émettons les hypothèses suivantes :

1) chez les athlètes commotionnés, une augmentation de la transmission GABAergique (révélée par la SMT) sera observée comparativement à un groupe d'athlètes non-commotionnés ;
2) chez les athlètes commotionnés, les mesures directes de GABA et possiblement de glutamate révéleront une plus grande concentration comparativement à un groupe d'athlètes non-commotionnés.

1.6.1.3 Article 3 : Comparaison des mesures GABAergiques obtenues par la SMT et la SMR

Dans l'article 3, la correspondance entre les mesures indirectes du système GABAergiques obtenues par la STM et les mesures directes du GABA obtenues par la SRM est étudiée auprès d'une population d'individus en santé. Nous émettons les hypothèses suivantes:

1) compte tenu des résultats antérieurs suggérant l'absence de lien entre l'inhibition intracorticale de longue durée et le GABA, aucune corrélation ne sera observée entre ces mesures ;
2) la période silencieuse corticale et les mesures directes (SRM) du GABA seront corrélées positivement ;
3) aucune corrélation ne sera observée entre les mesures d'inihibition (SMT) et le glutamate.

1.6.2 SÉTcd et modulation de la transmission GABAergique

Compte tenu des possibles atteintes au niveau de l'équilibre entre l'excitation et l'inhibition au sein des régions motrices, impliquant notamment le GABA, il est impératif d'évaluer la possibilité de développer de possibles traitements pour les commotions cérébrales, mais également pour d'autres pathologies présentant des anomalies neurophysiologiques semblables. Ainsi le second objectif du présent ouvrage est d'évaluer la possibilité de moduler la transmission GABAergique telle que mesurée par la SMT et la SRM avec une méthode de stimulation corticale non-invasive, soit la SÉTcd. Ce second objectif forme la thématique centrale des articles 4, 5 et 6 qui composent cet ouvrage.

1.6.2.1 Article 4 : Modulation des marqueurs SMT du GABA par la SÉTcd

Dans l'article 4, la possibilité de moduler, avec la SÉTcd, les deux marqueurs d'altérations GABAergiques mesurés par la SMT qui étaient altérés chez les athlètes commotionnés dans l'étude 1 est étudiée. Nous émettons les hypothèses suivantes :

1) l'excitabilité corticale du cortex moteur primaire sera modulée d'une manière dépendante à la polarité de la stimulation, c'est-à-dire diminuée par la SÉTcd cathodale et augmentée par la SÉTcd anodale ;
2) la stimulation anodale engendrera une réduction du GABA et la stimulation cathodale engendrera une augmentation du GABA, telle que révélée par une modulation des marqueurs SMT.

1.6.2.2 Article 5 : Évaluer la possibilité de mesurer les concentrations de métabolites par la SRM suite à l'utilisation de la SÉTcd à l'intérieur du scanneur

Dans l'article 5, la possibilité d'obtenir des mesures fiables des concentrations de métabolites suite à l'application d'un courant électrique transcrânien est étudiée. Cet article méthodologique vise à décrire de façon précise le protocole expérimental dans l'optique de favoriser la réplication des résultats. Ainsi, aucune hypothèse spécifique n'est liée à celui-ci.

1.6.2.3 Article 6 : Modulation des marqueurs SRM du GABA et du glutamate par la SÉTcd

Dans l'article 6, la possibilité de moduler, par l'entremise de la SÉTcd anodale, les concentrations de GABA et glutamate telles que mesurées par la SRM est étudiée. Par ailleurs, cette étude a comme objectif d'évaluer l'impact de la SÉTcd bilatérale, qui est utilisée dans le traitement des atteintes motrices suite à des accidents vasculaires cérébraux, sur les concentrations de GABA et glutamate mesurées par la SRM, ainsi que l'excitabilité corticale mesurée par la SMT. Nous émettons les hypothèses suivantes :

1) la SÉTcd anodale permettra de réduire la concentration de GABA et d'augmenter la concentration de glutamate au sein du cortex moteur primaire ;
2) la SÉTcd bilatérale permettra de réduire la concentration de GABA et d'augmenter la concentration de glutamate sous l'anode, et inversement sous la cathode ;
3) la SÉTcd bilatérale permettra d'augmenter l'excitabilité corticale mesurée par la SMT sous l'anode, et inversement sous la cathodale.

Chapitre 2

Article 1: Evidence for the specificity of GABA-mediated intracortical inhibitiory dysfunction in asymptomatic concussed athletes

Evidence for the specificity of intracortical inhibitiory dysfunction in asymptomatic concussed athletes

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2.1 Abstract

Sports concussions affect thousands of individuals every year and are a major public health concern. Still, litte is known about the long-term and cumulative effects of concussions on brain neurophysiology. The principal objective of this study was to investigate the long lasting effects of multiple sports concussions on sensorimotor integration and somatosensory processing in a sample of 12 concussed athletes and 14 non-concussed athletes of similar age (mean = 23 years) and education (mean = 16 years). Right median nerve stimulation was paired with transcranial magnetic stimulation (TMS) of the left primary motor cortex to investigate sensorimotor integration with short latency afferent inhibition (SAI) and long latency afferent inhibition (LAI) at five interstimulus intervals (18, 20, 22, 100, 200 ms). Somatosensory evoked potentials (SEP) were recorded from the left centro-parietal region. We also investigated primary motor cortex inhibitory mechanisms with three TMS protocols : cortical silent period, long interval intracortical inhibition and short interval intracortical inhibition. Motor evoked potentials were recorded from the right abductor pollicis brevis muscle. No differences were observed between groups for SAI, LAI and SEP. However, cortical silent period duration was prolonged and long interval intracortical inhibition was enhanced in the concussed group. These findings suggest that multiple sports concussions lead to specific, long-term neurophysiological dysfunctions of intracortical inhibitory mechanisms in primary motor cortex while somatosensory processing and sensorimotor integration are spared. This study provides additional evidence for the presence of specific and stable alterations of $GABA_B$ receptor activity in primary motor cortex that may be of clinical value for prognosis and diagnosis.

Keywords: afferent inhibition; intracortical inhibition; somatosensory evoked potentials; sports concussions; TMS

2.2 Introduction

The Centers for Disease Control and Prevention (CDC; 1997) estimate that every 21 seconds someone sustains a traumatic brain injury (TBI) in the United States, which represents about 1.2 million Americans per year. The CDC also estimate that of all traumatic brain injuries occurring in a year, 75% of them are mild TBI (mTBI). When mTBI takes place in a sports context, it is called a sports concussion, which is defined as a complex pathophysiological process induced by a near instant transfer of kinetic energy that affects the brain (McCrory et al., 2005). In the United States only, between 50 000 and 300 000 contact sports athletes will sustain a concussion within the course of a single season. This high prevalence makes sports related concussion a major public health concern (CDC, 1997).

Post-concussion symptoms usually consist of headache, dizziness, visual difficulties, memory disturbance and concentration problems (Cantu, 1996). It is generally agreed that post-concussive symptoms typically disappear between 2 to 10 days after the incident (McCrory et al., 2005), a time window that closely coincides with the resolution of the neurometabolic cascade of concussion (Giza and Hovda, 2001). In addition to the transient effects on cognition, motor function alterations in the form of gait stability and balance control have been documented in the acute post-concussion phase (Cavanaugh et al., 2005; Guskiewicz et al., 2001; Parker 2005). In the 48 hours following head injury, athletes display changes in postural control that appear to be linked to deficits in sensory interactions between the visual, somatosensory and vestibular systems (Guskiewicz et al., 2001). Furthermore, Catena and collaborators (2007a,b) have shown that concussed individuals show increased medial/lateral motion in gait stability tasks.

Despite the apparent ephemeral nature of post-concussive symptomatology and the uncommon presence of tissue damage using routine imaging techniques such as CT-scan (Kibby and Long, 1996), recent studies suggest detrimental long-term effects of concussions, which might eventually evolve into devastating neurological conditions with aging. Neuropathological analysis of brain tissue has revealed chronic traumatic encephalopathy, a progressive taupathy, in athletes that suffered multiple concussive injuries (McKee et al., 2009) and diffusion tensor

imaging (DTI) has shown numerous white matter alterations after mild TBI (Maller et al., 2010). Studies have also shown that approximatively 17% of retired professional boxers will eventually develop *dementia pugilistica*, a disorder characterized by motor and cognitive symptoms resembling those of Parkinson's disease (Rabadi and Jordan, 2001). The exact mechanism by which concussions contribute to the development of severe neurological conditions remains largely unknown, and only a handful of studies have investigated the neurophysiological impact of brain injury in otherwise healthy individuals beyond the acute phase.

Despite the fact that concussed athletes typically stop complaining about motor symptoms 10 days following concussion, a recent study suggests long lasting gait stability abnormalities whereby athletes showed altered postural stability in a dual-task more than 28 days following concussion (Parker et al., 2006). Supporting these findings, neurophysiological studies of mTBI using transcranial magnetic stimulation (TMS) have recently shown primary motor cortex (M1) dysfunctions in the acute phase (Chistyakov et al., 2001) that seem to be long-lasting (De Beaumont et al., 2007). In a series of studies, Chistyakov and collaborators (1998, 1999, 2001) used TMS to investigate the presence of M1 neurophysiological alterations after traumatic brain injuries of different severity. Chistyakov and collaborators reported altered motor cortex excitability in minor to severe TBI (Chistyakov et al., 1998, 1999, 2001) as well as prolonged cortical silent period (CSP) duration in mild to moderate TBI (Chistyakov et al., 2001), which is indicative of dysfunctional intracortical inhibitory systems. While these results documented immediate neurophysiological dysfunctions, the possible persistence of those alterations were unknown. In fact, it is only recently that researchers investigated the possible long-term effects of sport concussions on M1 neurophysiology. Using TMS, De Beaumont and collaborators (2007) have reported the presence of long lasting intracortical inhibitory system abnormalities within the primary motor cortex of university football athletes who sustained multiple concussions. In line with the results of Chistyakov (2001), they observed a lengthening of the CSP, which was linked to concussion severity but independent of the time elapsed since the last concussion. This suggests that sports concussions can produce neurophysiological alterations that persist well beyond the acute phase. Although the neurophysiological underpinnings of the CSP are still debated, the majority of pharmacological studies have attributed CSP lengthening to alterations of $GABA_B$ inhibitory receptors. Indeed, CSP is

prolonged by GABA reuptake inhibitor tiagabine (Werhahn et al., 1999) and intrathecal $GABA_B$ agonist baclofen (Siebner et al., 1998). Interestingly, M1 GABA alterations in concussed athletes appear to be limited to $GABA_B$ receptors, as reflected by preserved short latency intracortical inhibition (SICI; De Beaumont et al., 2007a; 2009), a measure of $GABA_A$-related intracortical inhibition (Ziemann et al., 1996; Ilic et al., 2002).

In addition to specific alterations of $GABA_B$ receptor activity, there is evidence for an alteration of cholinergic systems in severe traumatic brain injury resulting in diffuse axonal injury (Fujiki et al., 2006), where short latency afferent inhibition (SAI), a marker of cholinergic activity involved in sensorimotor integration, was reduced (Tokimura et al., 2000). Alterations in cholinergic activity and motor inhibitory circuits have been found in diverse pathologies such as Gilles de La Tourette syndrome (reduced SAI: Orth et al., 2005), asymptomatic *Parkin* mutation carriers (reduced SAI: Bäumer et al., 2007), Alzheimer's disease (reduced SAI : Di Lazarro et al., 2002; 2004; 2005) and Parkinson's disease (reduced long latency afferent inhibition; LAI : Sailer et al., 2003). Knowing that the aforementioned pathologies all share motor/memory dysfunctions similar to those associated with post-concussion syndrome (Jotwani and Harmon, 2010) and that SAI/LAI interacts with concussion-vulnerable inhibitory circuits of the primary motor cortex for sensorimotor integration (Chen, 2004), assessing the integrity of this system in concussed athletes is of particular clinical interest.

In contrast with the growing body of evidence for M1 inhibitory mechanism alterations (De Beaumont, 2007a; 2009), few concussion studies have investigated the presence of neurophysiological abnormalities in non-motor areas. Somatosensory evoked potential (SEP) testing represents a useful technique to assess the integrity of somatosensory cortex as well as afferent conduction and it is routinely used as a clinical tool in severe TBI to predict functional recovery (Chistyakov et al., 1999; Lew et al., 2003). Abnormalities in the SEP N20 component were found in comatose, diffuse axonal injury patients and in moderate head injury victims (Chistyakov et al., 1999) and altered N20 latency seems to be related to clinical disability in severe TBI (Rappaport et al.,1990). Abnormal N60 latencies lasting up to three months were also found following concussion in a sample of consecutive patients presenting to the emergency (Zumsteg et al., 2006). Prolonged central sensory conduction time (CSCT) has also been

reported in the acute coma phase after head injury (Chistyakov et al., 1999) and abnormalities in central motor conduction time (CMCT) were found after head injury, mostly in patients who sustained axonal damage (Chistyakov et al., 1999).

The principal objective of this study was to investigate the specificity of the previously reported long lasting inhibitory dysfunction in primary motor cortex of concussed athletes. To this end, a comprehensive neurophysiological evaluation of sensorimotor function was performed in concussed athletes to better circumscribe areas of dysfunction in the aim of developing objective markers of concussion to facilitate diagnosis, gather prognostic insights, and facilitate return-to-play decisions. This is especially relevant in the case of asymptomatic athletes, where neuropsychological testing, neurological examination, and symptom checklist often fail to reveal any lingering dysfunction. A sample of symptom-free concussed athletes who sustained their last concussion on average two years prior to testing were assessed on the following measures: short latency afferent inhibition, long latency afferent inhibition and somatosensory evoked potentials. Furthermore, the integrity of ascending and descending pathways was evaluated with sensorimotor conduction times. Finally, M1 GABA-mediated intracortical inhibition was evaluated with measures of short interval intracortical inhibition, long interval intracortical inhibition and cortical silent period.

2.3 Methods

2.3.1 Participants

Data were obtained from 26 participants who were active football players from Canadian university football and were recruited through the team physician. The following exclusion criteria were used to determine participation in the study: no history of psychiatric illness, learning disability, alcohol or drug abuse, neurological condition (i.e. seizures, brain tumor), TBI unrelated to sports or medical conditions requiring daily medication. The inclusion criteria for concussed participants were: two or more concussions, last concussion more than 12 months prior to testing, absence of symptoms, and active university-level football player. Participants were all right handed. The study was approved by the local ethics committee and all participants

provided written informed consent prior to testing. Subjects received a financial compensation of $60 CDN for their participation.

The study included two experimental groups. The first group consisted of 14 athletes with no history of sports concussion with a mean age of 23 years (mean: 22.36; SD = 1.69) and a mean level of education of 16 years (mean: 15.93; SD = 1.33). The second group consisted of 12 athletes with a history of two or more sports concussions (mean: 3.25; SD = 0.97) that occurred more than one year prior to testing (mean: 23.17 months; SD = 5.92). Concussion history was based on medical records for accidents that occurred throughout the athletes' university years while previous concussion history was self-reported. At the time of testing, concussed athletes were asymptomatic, reporting very few, if any, symptoms on the Post-Concussion Symptoms Scale (mean: 2.15; SD = 2.08; Maroon et al., 2000). Concussion severity ratings were provided by the team physician and were graded according to the American Academy of Neurology parameters (1997), from grade 1 (confusion for less than 15 minutes without amnesia or loss of consciousness) to grade 3 (loss of consciousness, duration either brief (seconds) or prolonged (minutes), with a mean grade of severity of 2 (mean: 2.00; SD = 0.67). All concussions were rated as mild (score of 13 to 15) on the Glasgow Coma Scale.

2.3.2 Procedure

The experiment consisted of a single 90-minute testing session during the football off-season. This session included the administration of a concussion history questionnaire, a general health questionnaire, the Post-Concussion Symptoms scale (PCS) (refer to De Beaumont et al., 2007b) to obtain more details on these questionnaires), and the acquisition of TMS and SEP recordings.

2.3.3 TMS recordings

TMS was delivered through an 8-cm figure-of-eight coil connected to a MagPro transcranial magnetic stimulator (Medtronic, Minneapolis, MN). The stimulating coil was placed flat on the skull with the handle pointing backwards and 45° away from the midline. The induced current flow was biphasic with a posterior-anterior direction. Pulses were delivered

over the optimal position to elicit a maximal electromyographic (EMG) response of the controlateral abductor pollicis brevis (APB) muscle to conform to afferent inhibition paradigms (Kessler et al., 2005). The EMG signal was amplified using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA), filtered with a band pass 20-1000Hz and digitized at a sampling rate of 4 KHz. Motor evoked potentials (MEPs) were recorded using Scope v4.0 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. A Brainsight frameless stereotaxic system (Rogue Research Inc., Montréal, Canada) was used to ensure stable coil positioning over the stimulation site.

Intracortical inhibition

The resting motor threshold (rMT) was first established as the minimum stimulation intensity necessary to evoke MEPs of 50μV in 50% of 10 consecutive trials while the targeted hand was at rest. According to the method described by Kujirai and collaborators (1993), short interval intracortical inhibition (SICI) was elicited by applying a subthreshold conditioning stimulus (80% of the resting motor threshold) 2 ms before a suprathreshold test stimulus (TS) adjusted to reliably induce MEPs of approximatively 1 mV peak-to-peak amplitude. Ten MEPs were recorded for this SICI paradigm. A single pulse TS condition of 15 consecutive trials was used as baseline. To evoke long interval intracortical inhibition (LICI), two pulses set at an intensity that produced a TS MEP between 0.20 and 1.50 mV were administered with an interstimulus interval of 100 ms. Ten pairs of such MEPs were collected. To induce a cortical silent period (CSP), single-pulse stimulations set at TS intensity (1 mV peak-to-peak amplitude) were applied over the left primary motor cortex while the participant maintained a voluntary isometric contraction of the right APB muscle at approximately 10% of maximal strength. Ten MEPs were recorded for this condition. TMS paradigms were delivered in a pseudo-randomized order.

LICI was expressed as the ratio of the test stimulus relative to the conditioning stimulus, whereas SICI was measured by comparing MEP amplitude evoked by the TS when preceded by the conditioning stimulus with that elicited in the unconditioned condition (TS alone). The

length of the CSP was assessed manually and was defined as the period from the onset of EMG suppression until the resumption of sustained post-stimulus EMG activity.

Spinal and central motor conduction times

To obtain spinal conduction time, TMS stimulations were applied directly on the surface of the C7-C8 dorsal root at an intensity of 60% of maximal stimulator output. Spinal conduction time was defined as the period between stimulation and onset of EMG response. Corticospinal conduction time was the mean latency of the MEPs recorded after TMS stimulation over M1. To obtain central motor conduction time, the mean latency of the spinal stimulation was subtracted from the mean corticospinal stimulation latency. Ten MEPs were recorded from the APB muscle for each condition.

Afferent inhibition by somatosensory input from the hand

Afferent inhibition was elicited by applying a median nerve electrical conditioning stimulus followed by a TMS test stimulus at different time intervals. To elicit short latency and long latency afferent inhibition, a Grass S88 stimulator (Grass, Co., Quincy, Mass., USA) was used to stimulate the median nerve at the level of the right wrist. Standard bipolar electrodes were used with the cathode positioned proximally. The electrical stimulation consisted of a square wave pulse of 0.2 ms duration. Conditioning electrical stimulation (CS) intensity was adjusted slightly over the threshold to evoke a small muscle twitch at the thumb. The intensity of the test stimulus (TS) applied over the left motor cortex was adjusted to evoke a MEP in the resting APB of approximately 1 mV peak-to-peak amplitude. In keeping with previous studies, interstimulus intervals (ISI) of 18, 20 and 22 ms between the CS and TS were used to produce short latency afferent inhibition (Tokimura et al., 2000) while ISIs of 100 and 200 ms were used to elicit long latency afferent inhibition (Nakamura et al., 1997; Chen et al., 1999). A TS-alone control condition was also performed to subsequently compute ratios of the mean amplitude of the MEPs recorded for each ISI with respect to the mean amplitude of the control, unconditioned response (SAI and LAI conditions / TS-alone). Due to technical difficulties, data for SAI and LAI were not collected in four participants (two in each group).

2.3.4 Somatosensory evoked potentials recordings and data analysis

In a subset of 21 participants (9 concussed, 12 controls), somatosensory evoked potentials were recorded. Electrical stimulation was applied with a Grass S88 stimulator (Grass, Co., Quincy, Mass.,USA) to stimulate the median nerve at the level of the right wrist through standard bipolar electrodes, with the cathode positioned proximally. A square wave pulse of 0.2ms duration was used. Stimulation intensity was adjusted to evoke a small muscle twitch at the thumb. For SEP recordings, a 32-channel acquisition system (Neuroscan Labs; El Paso; Texas; USA) was used. Continuous EEG signals were recorded from the 7th cervical vertebra (Cv7) and four stainless steel electrodes placed on the following sites according to the 10-20 International system: C3', C4', Oz and Fpz (Cooper et al., 1980). The left and right mastoids were used as references and the electrode Fpz as the ground. Skin-electrode impedance was kept under 5000 ohms. The session consisted of 500 electrical stimulations at 3Hz. The timing was controlled by PsyScope X software running on a MacBook Pro computer (Apple, Cupertino, USA).

BrainVision Analyser software (Brain products, Inc., Germany) was used for data analysis. The duration of a single epoch was 100 ms with a pre-stimulus period of 30 ms. A semi-automatic artifact exclusion was performed where epochs including blinking or ocular movements and cardiac artifacts exceeding 150uV in peak-to-peak amplitude were excluded. Averaged N20 component amplitude recorded at C3' electrode was measured from peak-to-peak amplitude with P27, which is the first positive component following the N20 peak. N20 and N13 latencies were also measured. The central sensory conduction time (CSCT) was defined as the interpeak latency of the cervical N13 and cortical N20.

2.3.5 Statistical analysis

All values are expressed as means plus/minus standard deviations (SD). Intracortical inhibition measures, SAI/LAI, SEP data and central conduction time data were subjected to standard descriptive statistics and ANOVAs. Independent sample T-tests were performed to assess differences between the two groups.

2.4 Results

2.4.1 Intracortical inhibition of M1

In agreement with previous findings (De Beaumont et al., 2007a; 2009) increased intracortical inhibition was found at baseline in athletes with a history of multiple concussions. Compared to their unconcussed counterparts, LICI was significantly enhanced ($t_{24} = 2.11$; $p = .05$) and CSP duration was significantly prolonged ($t_{24} = 2.35$; $p = .03$). Consistent with recent findings, SICI did not differ between groups ($t_{24} = 1.67$; $p = .11$; Table 1).

2.4.2 Short and long latency afferent inhibition

SAI. A one-way ANOVA was first performed on raw MEP amplitude to verify that the CS modulated TS response. There was a main effect of ISI ($F_{3, 23} = 13.11$; $p = .0001$), which was caused by inhibition of the conditioned TS compared to TS alone at all intervals. Using the ratio of the conditioned stimulus over the test stimulus alone, a mixed ANOVA (Group X ISI) revealed no main effect of ISI ($F_{2, 23} = 2.88$; $p = .08$), no main effect of Group ($F_{2, 23} = 0.02$; $p = .88$) and no interaction ($F_{2, 23} = 0.15$; $p = .80$; Fig. 1A).

LAI. A one-way ANOVA was first performed on raw MEP amplitude to verify that the CS modulated TS response. There was a main effect of ISI ($F_{2, 23} = 16.78$; $p = .0001$), which was caused by inhibition of the conditioned TS compared to TS alone at all intervals. Using the ratio of the conditioned stimulus over the test stimulus, mixed ANOVA (Group X ISI) revealed a significant main effect of ISI ($F_{2, 23} = 9.80$; $p = .005$), no main effect of Group ($F_{2, 23} = 0.02$; $p = .88$) and no interaction ($F_{2, 23} = 0.15$; $p = .80$). The main effect of ISI was explained by increased inhibition at the 100ms interval (Fig. 1B).

2.4.3 Somatosensory evoked potentials

Between group comparisons for each SEP component are shown in Table 2. Both N20 and N60 components were found in each participant for electrode C3'. Student's t-tests revealed no significant difference between the two groups for N20 latency ($t_{19} = 1.77$; $p = .10$) and N20

amplitude (t_{19} = .60 ; p = .56). Student's t-tests also revealed no significant difference between the two groups for N60 latency (t_{19} = 1.16; p = .26) and N60 amplitude (t_{19} = .66 ; p = .53). A N13 component was found in every participant for electrode Cv7. Student's t-tests revealed no significant difference between groups for N13 amplitude (t_{19} = .50; p = .63) and N13 latency (t_{19} = .04; p = .97). One participant in the concussed group displayed a large N20 component that considerably increased variability. Removing this participant from analysis did not lead to a different between-group outcome and N20 amplitudes were below 3SD.

2.4.4 Conduction time

Mean conduction times and standard deviations for both groups are shown in Table 3. For central sensory conduction time (CSCT), student's t test revealed no statistical difference between groups (t_{17} = 1.19 ; p = .25). Similarly, TMS assessment of central motor conduction time (CMCT) (t_{23} = 0.26 ; p = .80), spinal conduction time (t_{23} = 0.53 ; p = .58) and corticospinal conduction time (t_{23} = 0.55 ; p = .21) did not reveal any significant difference between groups.

2.5 Discussion

The major finding of the present study is the specificity of long-term intracortical inhibitory dysfunction in primary motor cortex of concussed athletes that is presumably $GABA_B$-mediated. Compared to unconcussed athletes, CSP and LICI measures were abnormal, suggesting increased $GABA_B$ inhibition. By contrast, $GABA_A$-mediated inhibition (SICI), sensorimotor integration assessed with cholinergic-dependent SAI, and basic somatosensory processing (SEPs) were similar in the concussed and unconcussed groups. Finally, both afferent and efferent sensorimotor conduction times were of similar duration in the two groups.

Cholinergic abnormalities have been reported in patients with severe TBI associated with diffuse axonal injury, where short latency afferent inhibition was significantly reduced (Fujiki et al., 2006). Administration of a single dose of an acetylcholinesterase inhibitor was sufficient to restore normal SAI in this population, in line with previous studies showing similar effects in Alzheimer's disease (e.g. Di Lazzaro et al., 2002, 2004). The present findings suggest that

cholinergic circuits in sensorimotor areas are unaffected by sports concussions two years after the last concussive event. The absence of significant differences between the two groups suggests that sensory signals originating from the median nerve interact normally with primary motor cortex circuits in the concussed brain. SAI appears to be partly mediated by $GABA_A$ receptors at the motor cortex level (Di Lazzaro et al., 2007). Data from the present and other (De Beaumont et al., 2007, 2009) studies have shown spared $GABA_A$-mediated SICI in athletes tested 1-2 years after their last concussion. Although SAI and SICI appear to involve different subtypes of $GABA_A$ receptors (Di Lazzaro et al., 2007), it has been shown that they are reciprocally connected (Alle et al., 2009). Normal LAI was also found in the present population of formerly concussed athletes, and it is believed that LAI and SAI are mediated through different sensory-motor circuits. Although M1-S1 cortico-cortical interactions appear to underlie LAI, the exact nature of this inhibitory phenomenon is unclear (Pirio et al., 2009). Nevertheless, the present data point to the absence of long-term effects of sports concussions on the interaction between sensory input and corticospinal excitability. Whether this represents a state of recovery from short-term alterations is an open issue that warrants further investigation.

It is important to note that in contrast with previous studies from our group (De Beaumont et al., 2007b; 2009), MEPs were recorded from the APB rather than the first dorsal interosseus muscle (FDI). It has been shown that excitatory and inhibitory patterns induced with TMS differ between muscle groups. For example, MEP area for muscles at rest is greater and onset latency longer for small hand muscles compared to forearm muscles, while CSP durations are longer for small hand muscles (Wu et al., 2002). Differential effects have also been reported between proximal and distal muscles, where increased intracortical inhibition and decreased intracortical facilitation is present in proximal compared to distal muscles (Abbruzzese et al., 1999). More relevant to the present study, it has been suggested that function may explain differences in inhibition between muscles, with intrinsic hand muscles playing an active role in fine motor acts requiring enhanced inhibitory control (Abbruzzese et al., 1999). As such, it is unlikely that the selection of one hand muscle over the other significantly affected the present data, but it is an open question whether similar group effects would be observed in proximal muscle groups.

The absence of sensorimotor integration dysfunction was matched by a lack of short/middle latency SEP abnormalities in concussed athletes. Concussions have been shown to increase middle-latency SEPs (N60) in the acute phase, an effect which tended to normalize to pre-injury levels three months after the concussive event (Zumsteg et al., 2006). The same study reported no significant latency differences in the early SEP components between concussed individuals and healthy controls (Zumsteg et al., 2006). The present data are in agreement with these findings, revealing no differences between concussed and control athletes on measures of SEP amplitude and latency. As such, if an increase in N60 latency in the acute and post acute (3 months) phases was present in our group of concussed athletes, a post-injury period of more than one year is sufficient to restore basic somatosensory processing to normal levels. It should be noted, however, that participants in the Zumsteg et al. (2006) study had lost consciousness following concussion, were older (35.4 years), spanned a much wider range of ages (22 to 62 years), and included patients with varied mTBI causes. This contrasts with the current sample, which was younger and much more homogeneous. As such, it is difficult to ascertain whether the lack of SEP abnormalities in the present sample of concussed athletes reflects recuperation from a dysfunctional state or merely the absence of SEP effects in the acute phase. This is an important issue in light of the fact that short-latency SEPs have been repeatedly shown to be good predictors of outcome in patients with severe TBI, and are one of the best predictors of coma outcome (Carter and Butt, 2005). If SEP abnormalities are present acutely following sports concussions, it would be of great clinical interest to determine whether they can predict the severity of long lasting impairments that have recently been discovered in the cognitive and motor domains.

In keeping with the lack of SEP abnormalities and sensorimotor integration dysfunction, the present data suggest that ascending and descending peripheral and central pathways are not affected by the presence of multiple sports concussions. In addition to normal SEP latencies, central somatosensory and motor conduction times were similar between groups. Numerous studies have used diffusion tensor imaging (DTI) to investigate the integrity of white matter fibers in mTBI (for review, see Maller et al., 2010). Of particular relevance to the present findings, reduced fractional anisotropy (FA) has been reported in the corticospinal tract and internal capsule of mTBI individuals (e.g. Bendlin et al., 2008). Indeed, reduced FA in the

internal capusle of mTBI patients has been shown numerous times (Bendin et al., 2008; Lipton et al., 2008; Miles et al., 2008) and has been observed up to 6 years post injury (Inglese et al., 2005). Similarly, reduced white matter integrity of the corticospinal tract has been reported both in the short- (2 months post-injury; Bendin et al., 2008) and long-term (107 months post-injury; Kraus et al., 2007). Our physiological data show that if structural damage is present in white matter fibers of the corticospinal tract, it is not sufficient to modify ascending and descending conduction times. It should be noted that DTI studies of mTBI sometimes included patients with visible structural brain damage, prolonged unconsciousness, variable age groups, and presence of cognitive impairments, which contrasts with the homogeneous nature of the present sample. Indeed, every participant in the current study reported no overt symptom, was young and active, and had identical educational profiles. In this specific population, integrity of the corticospinal tract may be spared altogether.

The lack of significant somatosensory, sensorimotor, and conduction abnormalities found in the present study thus points to the specificity of long-term intracortical inhibitory dysfunction in concussed athletes. Previous findings of increased CSP duration in concussd athletes were replicated and additional evidence for the involvement of $GABA_B$ receptor activity was provided in the form of increased LICI. In healthy subjects, administration of selective $GABA_B$ agonist Baclofen increases LICI, possibly through facilitation of inhibitory post-synaptic potentials (McDonnell et al., 2006). As such, increased CSP and LICI measures suggest that both magnitude and duration of $GABA_B$-mediated intracortical inhibition (McDonnell et al., 2006) is affected in sports concussion. It is important, however, to consider that TMS is an indirect measure of GABAergic activity. Evidence for an association between activity of specific receptor subtypes and TMS-measures of intracortical inhibition comes primarily from pharmacological studies where $GABA_A$ or $GABA_B$ agonists or antagonists modulate M1 responses to TMS (Ziemann, 2004). Direct confirmation of the involvement of specific GABA receptors in the pathophysiology of sports concussions are needed to establish this fact with certainty.

A possible mechanism explaining presumed increased GABA transmission in concussion may be related to protective effects against glutamate excitotoxicty. Excessive

glutamate stimulation is a core feature of brain response to TBI (Faden et al., 1989; Baker et al., 1993) leading to 'glutamatergic excitotoxicity' and neurodegeneration (Rothman and Olney, 1986). Increased levels of glutamate are associated with NMDA receptors and beneficial effects of NMDA receptor antagonists in TBI patients have been reported (e.g. Yurkewicz et al., 2005). In addition to modulation of glutamate levels, there is evidence for the involvement of GABA in the response to TBI, which could be secondary to the increase in glutamate and represent an attempt at minimizing glutamatergic excitotoxicity. Although the effects of TBI on GABA transmission in man have been poorly studied, it has been reported that GABA concentration in ventricular CSF is greatly elevated in patients with severe head injury (Palmer et al., 1994). There is significant data suggesting increased GABA levels following TBI in rat models of injury. For example, impact injury of rats has been shown to induce long lasting working memory (WM) deficits that are associated with increased GABA levels for as long as 1 month post-TBI (Kobori and Dash, 2006). Reduction of GABA levels in those rats by administration of GABA antagonists restored memory function, suggesting that TBI is associated with "excess GABA-mediated inhibition" (Kobori and Dash, 2006). Magnetic resonance spectroscopy has shown a similar pattern of response following TBI in rats, where GABA concentration is elevated after injury (Pascual et al., 2007). The idea that long-term GABA increases in concusssed athletes is a response to glutamatergic excitotoxicity is obviously highly speculative. Indeed, it has recently been shown with MR spetroscopy that glutamate levels are *decreased* in the motor cortex of concussed athletes 3-4 days post-injury (Henry et al., 2010). However, since animal models have shown an immediate release of glutamate following mTBI (Katayama et al., 1990), complex intractions between excitatory and inhibitory neurotransmitters may significantly modulate the initial response in the days following concussive injury.

It is important to take into account the fact that the neurophysiological profile that is reported here comes from a relatively sample and homogeneous small made up of young, otherwise healthy high-level athletes. This has some important advantages, such as the presence of a comparable control group and reduction in the prevalence of comorbid conditions. It should therefore be emphasized that the present results may not easily generalize to a broader population of individuals with TBI, where etiology, age, general health and comorbidity are important variables. For example, it remains to be seen whether individuals with more severe

TBI also display abnormal M1 intracortical inhibition. With respect to concussed athletes, however, we can safely assume that our results are generalizable despite the small sample size since similar dysfunction of inhibition in M1 have been found in older former athletes (DeBeaumont et al., 2009) and in another sample of young, active athletes (DeBeaumont et al., 2007).

In conclusion, the current data show that intracortical GABA dysfunction in the motor cortex of concussed athletes shows a surprising degree of specificity. The reported alteration appears to be long-lasting and stable, as abnormal CSP durations have also been found more than 30 years after the last injury (De Beaumont et al., 2009). The specificity and duration of M1 dysfunctions make it plausible that TMS measures of cortical excitability may in the long run provide diagnostic and prognostic cues in TBI. In light of the fact that depression and post traumatic stress disorder (PTSD) are often present in TBI patients (Kim et al., 2007; Rogers et al., 2007), objective measures of M1 neurophysiology may find useful clinical utility. This is particularly relevant since both depression and PTSD have specific cortical excitability dysfunction profiles that are different from that of TBI. In PTSD, hemispheric-specific reductions in SICI and SAI (Rossi et al., 2009) have been reported, whereas depressive patients show a consistent pattern of right-left hemisphere motor threshold differences (Maeda et al., 2000) and reduced CSP durations (Bajbouj et al., 2006). Further studies will be necessary to determine the value of TMS measures in the differential diagnosis of these pathologies. Additionally, at present, return-to-play decisions following sport concussion are higly dependent on the athlete being physically and cognitively asymptomatic, as assessed by medical and neuropsychological evaluations (Reddy and Collins, 2009). The present data show that when these symtoms subside, highly-specific dysfunctions in primary motor cortex may linger for years in some athletes. The development of objective, neurophysiological measures of brain dysfunction following concussion may therefore provide valuable return-to-play information in the future. However, whether the presence of intracortical inhibitory dysfunction can be a predictor of future concussive events, for example, is an open issue that will need to be adressed directly in future studies.

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2.7 Author disclosure statement

No competing financial interests exist.

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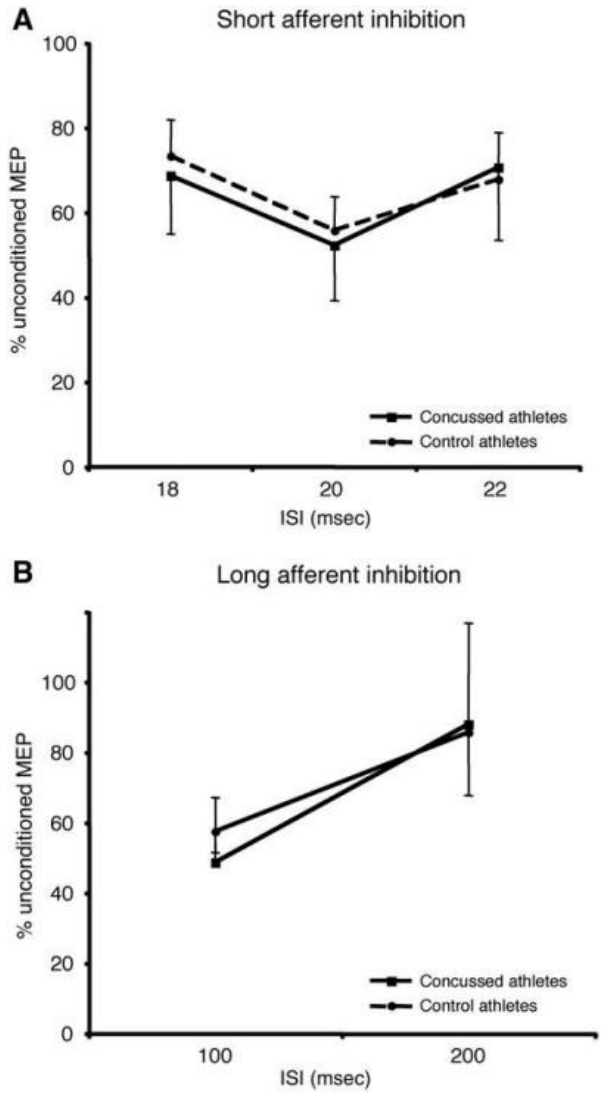
2.9 Figures

Figure 1. Short afferent inhibition and long afferent inhibition

Legend : A) SAI ratios for every time condition. There is no significant difference between groups. Error bars represent standard error of the mean. B) LAI ratios for every time condition. There is a significant difference between the two time conditions, where LAI 100ms produces stronger inhibition than LAI 200ms. Error bars represent standard error of the mean.



2.10 Tables

Table 1. Between group comparisons of M1 intracortical inhibition parameters

Parameters	Groups	Means (SD)	*t* test	*P* value
Long interval intracortical inhibition (ratio)	Control	.42 (0.23)	2.11	.05
	Concussed	.23 (0.21)		
Short interval intracortical inhibition (ratio)	Control	.40 (0.17)	1.67	.11
	Concussed	.31 (.17)		
Cortical silent period duration (ms)	Control	137.38 (27.95)	2.35	.03
	Concussed	166.78 (31.08)		

Table 2. Between group comparisons of conduction time parameters

Parameters	Groups	Means (SD)	*t* test	*P* value
Central sensorimotor condution time (ms)	Control	7.70 (4.03)	1.19	.25
	Concussed	10.67 (6.63)		
Central motor conduction time (ms)	Control	7.22 (1.59)	.003	.99
	Concussed	7.22 (.94)		
Spinal motor conduction time (ms)	Control	15.40 (.79)	.68	.50
	Concussed	15.19 (.70)		
Corticospinal conduction time (ms)	Control	22.32 (1.79)	.75	.21
	Concussed	22.41 (1.33)		

Table 3. Between group comparisons of somatosensory evoked potentials

Parameters	Groups	Means (SD)	*t* test	*P* value
N20 component amplitude (mV)	Control	.80 (.72)	1.77	.10
	Concussed	1.66 (1.31)		
N20 component latency (ms)	Control	23.41 (2.78)	.60	.56
	Concussed	22.67 (2.91)		
N60 component amplitude (mV)	Control	3.47 (1.13)	1.16	.26
	Concussed	2.87 (1.22)		
N60 component latency (ms)	Control	66.91 (2.25)	.66	.53
	Concussed	65.55 (5.90)		
N13 component amplitude (mV)	Control	- .97 (1.25)	.50	.63
	Concussed	- .71 (1.13)		
N13 component latency (ms)	Control	15.17 (3.71)	.04	.97
	Concussed	15.22 (3.19)		

Chapitre 3

Article 2 : Multimodal assessment of primary motor cortex integrity following sport concussion in asymptomatic athletes

Multimodal assessment of the integrity of the primary motor cortex following sport concussion in asymptomatic athletes

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3.1 Abstract

Objective. Recent studies have shown, in asymptomatic concussed athletes, metabolic disruption in the primary motor cortex (M1) and abnormal intracortical inhibition lasting for more than six months. The present study aims to assess if these neurochemical and neurophysiological alterations are persistent and linked to M1 cortical thickness.

Methods. Sixteen active football players who sustained their last concussion, on average, three years prior to testing and 14 active football players who never sustained a concussion were recruited for a single session of proton magnetic resonance spectroscopy (^{1}H-MRS) and transcranial magnetic stimulation (TMS). Measures of M1 and whole brain cortical thickness were acquired, and ^{1}H-MRS data were acquired from left M1 using a MEGA-PRESS sequence. Cortical silent period (CSP) and long-interval intracortical inhibition (LICI) were measured with TMS applied over left M1.

Results. No significant group differences were observed for metabolic concentrations, TMS measures, and cortical thickness. However, whereas GABA and glutamate levels, and GABA levels and M1 mean thickness were positively correlated in control athletes, these relationships were absent in concussed athletes.

Conclusion. These data suggest the absence of persistent neurophysiologic or metabolic disruptions in concussed athletes. However, further correlational studies suggest the presence of a slight persistent metabolic imbalance in the primary motor cortex of concussed athletes.

Significance. The present study highlights the importance of evaluating the role of slight metabolic or neurophysiologic dysfunction in sport concussions.

Keywords: sport concussion, traumatic brain injury, magnetic resonance spectroscopy, transcranial magnetic stimulation, GABA, glutamate

Highlights:

- Absence of M1 neurophysiologic disruptions following concussion, as assessed by the magnitude of TMS and anatomic measures.
- Normal concentration of GABA, glutamate and NAA in M1 of concussed athletes.
- Abnormal correlation between GABA and glutamate in concussed athletes suggesting a slight metabolic imbalance in M1.

3.2 Introduction

Over the past decades, interest in sport concussion research has increased considerably as the phenomenon evolved from being considered a minor injury to being considered a public health priority (Wiebe et al., 2011). In the United States of America, the Center for Disease Control and Prevention estimates that sport concussions affect about 1.6-3.8 million athletes annually (Rutland-Brown et al., 2006), most commonly in contact sports such as boxing and American football (Guskiewicz et al., 2003). However, this could be vastly underestimated because as many as 50% of sport concussions may go unreported (Harmon et al., 2013). This "silent epidemic" has recently gained general public and media attention following reported cases of chronic traumatic encephalopathy (CTE) in former athletes, a neurodegenerative disorder resembling tau-related dementias, parkinsonism, and amyotrophic lateral sclerosis (Chin et al., 2013).

In a recent position statement by the American Society of Sports Medicine, concussion has been defined as a traumatically induced transient disturbance of brain function involving a complex pathophysiological process (Harmon et al., 2013). Clinical symptoms of concussion include cognitive impairments such as memory and attention deficits, headaches, confusion, and behavioural changes (Barkhoudarian et al., 2011), which typically resolve completely within 2-3 weeks post-concussion (Lovell et al., 2003; McCrea et al., 2003). However, repeated concussions have been associated with greater symptom severity (Collins et al., 2002), longer recovery time (Guskiewicz et al., 2003), higher susceptibility to sustain a subsequent concussive event in both humans (Guskiewicz et al., 2003) and animals (Barkhoudarian et al., 2011), and a higher risk of developing dementia (Guskiewicz et al., 2005). Although standard imaging

techniques such as CT-scan or magnetic resonance imaging (MRI) usually fail to show any gross structural damage following a concussive event, the consequences of multiple concussions suggest that the injury may induce "silent" pathophysiological or molecular changes to the brain. A particularly interesting mechanism explaining the susceptibility of the brain following a concussion is derived from animal models of traumatic brain injury (Blennow et al., 2012). Giza and Hovda (2001) first described the complex metabolic cascade of neurochemical and neurometabolic changes initiated by acceleration and deceleration forces induced by the concussive event. These events include massive depolarization, excessive release of glutamate (Glu), and decreased ATP production (Giza & Hovda, 2001; Barkhoudarian et al., 2011) leading to pathological cellular processes such as inflammation, oxidative stress, mitochondrial dysfunction, excitotoxicity, oedema and hypoxia (Harris et al., 2012).

Although there is currently no biomarker of these cellular dysfunctions, recent studies suggest that proton magnetic resonance spectroscopy (^{1}H-MRS) could be a powerful approach to assess metabolic disruption following concussion (Harris et al., 2012). This technique allows sensitive *in vivo* detection and quantification of brain metabolites (Ashwal et al., 2004; Holshouser et al., 2006) including creatine/phoschocretaine (tCr), a general energy marker; phosphocholine (PCho), a marker of glial proliferation and membrane turnover; *N*-acetylaspartate + *N*-acetylaspartylglutamate (tNAA), a marker of neuronal integrity, bioenergetics and neuroprotection; Glu + glutamine (Gln) (Glx), a marker of excitatory neurotransmission; *myo*-inositol (mIns), a glial and oedema marker. Recent technological advances have allowed detection and quantification in humans of gamma-aminobutyric acid (GABA), a marker of inhibitory neurotransmission (Mescher et al., 1998). The assessment of metabolic disruption using ^{1}H-MRS has been mostly studied in patients who sustained different severities of traumatic brain injuries (TBI). In these populations, studies have shown consistent decreases in NAA within a month following injury, which is usually considered the acute phase (Brooks et al., 2001; Macmillan et al., 2002; Govindaraju et al., 2004; Marino et al., 2011). Although less consistent (Xu et al., 2011), results for other brain metabolites showed altered Glx (Shutter et al., 2004; Babikian et al., 2006) and elevated lactate (lac), total choline (tCho), and mIns (Brooks et al., 2001; Marino et al., 2011) in the acute phase. Studies conducted with a population of concussed athletes showed a similar pattern of reduction in NAA (Cimatti, 2006;

Vagnozzi et al., 2008; Vagnozzi et al., 2010; Henry et al., 2010; Johnson et al., 2012) and an increase in Glu (Henry et al., 2010) within a month post-concussion. Henry and collaborators (2011) also found chronic metabolic disruptions 6 months post injury in motor areas, where a decrease of NAA in premotor and primary motor (M1) cortices, and an increase of mIns in M1 were observed. Although motor function deficits are not included in the definition of a concussion, these results highlight the possibility of a specific vulnerability of motor areas following brain injury.

This hypothesis is consistent with recent literature showing persistent motor dysfunctions following concussion (De Beaumont et al., 2012). For example, postural stability is now increasingly used as part of post-concussion return-to-play protocols (Harmon et al., 2013) as a growing body of evidence suggests the presence of balance deficits following injury (Guskiewicz, 2001a,b; Cavanaugh et al., 2005; Parker et al., 2006). Neurophysiological motor alterations have also been reported in concussed athletes using transcranial magnetic stimulation (TMS). Long term abnormal intracortical inhibition was observed in young asymptomatic athletes who sustained multiple concussions, as revealed by increased duration of the cortical silent period (CSP: De Beaumont et al., 2007; Tremblay et al., 2011; De Beaumont et al., 2011a,b) and increased long interval intracortical inhibition (LICI; Tremblay et al., 2011; De Beaumont et al., 2011b). Increased CSP duration was also found in former athletes more than 3 decades after their last concussion, along with a significant slowness of movement resembling bradykinesia (De Beaumont et al., 2009). The physiological mechanisms underlying CSP and LICI have been suggested by pharmacological studies where both parameters have been found to be mediated by $GABA_B$ receptors (Ziemann, 2004; McDonnell et al., 2006), thus suggesting long lasting alterations in GABAergic transmission following concussion. Furthermore, De Beaumont and collaborators (2011a) have shown abnormal M1 long term potentiation (LTP)-like synaptic plasticity in asymptomatic athletes, as revealed by suppressed paired-associative stimulation (PAS), and reduced implicit motor learning. This result is consistent with the hypothesis of altered inhibitory mechanisms in M1 following concussion, as GABAergic transmission, more specifically $GABA_B$ receptors, is involved in LTP-like mechanisms (McDonnell et al., 2007). Although recent animal ^{1}H-MRS studies have shown altered GABA concentrations in the hours and days following induced traumatic brain injury (Xu et al., 2011;

Harris et al., 2012) no study has directly assessed the long-term effects of sport concussions on GABA levels in M1.

The main objective of the present study was to investigate, by combining multiple neuroimaging methods, the possible long-term effects of sport concussion on the primary motor cortex in asymptomatic, active university-level athletes. First, the integrity of M1 metabolism was assessed by ^{1}H-MRS. Second, transcranial magnetic stimulation was used to assess $GABA_B$ transmission in M1 by CSP and LICI measurements. Finally, possible effects of concussions on whole brain and M1 neuronal integrity was assessed by standard cortical thickness analyses and anatomical connectivity analyses using the Mapping Anatomical Correlations Across Cerebral Cortex (MACCAC) method (Lerch et al., 2006).

3.3 Methods

3.3.1 Participants

All participants in the present study were active male football players from Canadian universities recruited with the help of team physicians and physiotherapists. Athletes were excluded if they had a history of psychiatric illness; alcohol and/or substance abuse; learning disability; neurological condition (i.e., seizures, brain tumor); TBI unrelated to sport; and medical conditions requiring daily medication. Concussed athletes were included in the study if they sustained their last concussion at least 10 months prior to the experimentation and were asymptomatic at the time of testing. The study was approved by the local ethics committee and all participants provided written informed consent prior to testing. Participants received a financial compensation of Can $80 for their participation in the study.

Participants were divided into two groups. The control group consisted of 14 university-level football athletes who never sustained a concussion and the experimental group consisted of 16 university-level football players who sustained their last sport concussion at least 10 months prior to testing. Both groups did not differ in age ($t_{(28)} = .25$, $p = .80$) and level of education ($t_{(28)} = .35$, $p = .73$; Table 1). All athletes were right-handed in the concussed group

(right: 16, left: 0) whereas two athletes out of the control group were left-handed (right: 12; left: 2). Athletes in the concussed group sustained 1 to 4 sport-related concussions (M = 1.88) and the time since the last concussion ranged from 10 to 96 months (M = 41.25 +/- 29.71). Information regarding concussions that occurred during university years was acquired from team medical records, whereas past concussions were self-reported. In order to obtain detailed information for any head injury that could have occurred prior to testing, a standardized concussion history questionnaire was administered to all participants in an interview setting. The questionnaire aimed to collect detailed information on the number of previous concussions (if any), approximate date(s) of each concussion(s), the description of the incident(s), the nature and duration of relevant post-concussion symptoms (i.e., loss of consciousness, confusion, retrograde and/or anterograde amnesia, disorientation). Concussion grade was assessed according to the American Academy of Neurology (1997) from grade 1 (confusion for less than 15 min without amnesia or loss of consciousness) to grade 3 (loss of consciousness, from few seconds to prolonged), with a mean of severity of grade 2 (SD = 0.89). All concussions were rated as mild (score of 13 to 15) on the Glasgow Coma Scale. Retrospective reports of past concussions by athletes may introduce a bias in the evaluation of the number of concussive events sustained by participants. This methodological caveat was compensated by a standardized evaluation of past concussive events.

3.3.2 Procedure

The experimental setting consisted of a single session of MRS of 1 h duration preceded by the administration of the concussion questionnaire. The TMS session was administered either immediately prior to the MRS testing or within 2 months after the MRS session (experimental group: 6 athletes post-MRS, 10 athletes pre-MRS; control group: 7 athletes pre-MRS, 7 athletes post-MRS). These settings were established due to the availability of the material and of the athletes.

3.3.3 MR acquisition

MR acquisitions were performed using the 3T whole-body system (MAGNETOM Trio, a TIM systems, Siemens, Erlangen, Germany) at the *Unité de Neuroimagerie Fonctionnelle, Centre de recherche de l'Institut universitaire de gériatrie de Montréal*. Radiofrequency transmission was performed with the built-in body coil, and signal was received with at 12-channel receive-only head coil. The prescription of M1 voxel and detection of potential structural abnormalities were performed using anatomical images of the brain obtained with a T_1-weighted MPRAGE sequence (T_R = 2300 ms; T_E = 2.91 ms; FA: 9°; FOV = 256 x 256 mm^2; 256 x 256 matrix; 160 axial slices of 1 mm; acquisition time: 9 min 50 s). The voxel of interest (27 x 24 x 32 mm^3) was positioned over the left hand area of the primary motor cortex using two accepted anatomical landmarks (Yousry et al., 1997) (Figure 1a). These authors evaluated the location of the motor hand area within the precentral gyrus and described the region as a knob-like structure that can be identified using the two following landmarks: an omega shape in the axial plane and a hook-like shape in the sagittal plane (Yousry et al., 1997). MRS data were acquired using a MEGA-PRESS sequence (Mescher et al., 1998) with double-banded pulses used to simultaneously suppress water signal and edit the γ–CH_2 resonance of GABA at 3 ppm. Additional water suppression, using variable power with optimized relaxation delays (VAPOR), and outer volume suppression (OVS) techniques (Tkác et al., 1999) were optimized for the human 3T system and incorporated prior to MEGA-PRESS. The final spectra were obtained by subtracting the signals from alternate scans with the selective double-banded pulse applied at 4.7 ppm and 7.5 ppm ('EDIT OFF') and at 1.9 ppm and 4.7 ppm ('EDIT ON') (Figure 2). MEGA-PRESS data were acquired in four interleaved blocks of 32 ('EDIT OFF', 'EDIT ON') scans each with frequency drift correction between blocks. FIDs were stored separately in memory for individual frequency and phase correction using the tCr signal at 3.03 ppm, as well as correction for residual eddy-current using unsuppressed water signal obtained from the same voxel.

3.3.4 Analysis of MRS data.

Both 'EDIT OFF' and difference spectra were analyzed using LCModel 6.2-1A (Provencher, 1993; 2001) which calculated the best fit of the experimental spectrum as a linear combination of model spectra. The basis set for 'EDIT OFF' spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and metabolite spectra simulated with home-written software based on density matrix formalism (Henry et al., 2006) in MATLAB, using known chemical shifts and *J* couplings (Govindaraju et al., 2000). The simulated spectra of the following 20 brain metabolites were included in the basis set: acetyl moiety of NAA (sNAA), alanine (Ala), ascorbate (Asc), aspartate (Asp), aspartate moiety of NAA (mNAA), CH_2 group of Cr (Cr-CH_2), CH_3 group of Cr (Cr-CH_3), CH_2 group of PCr (PCr-CH_2), CH_3 group of PCr (PCr-CH_3), GABA, glucose (Glc), Glu, Gln, glycerophosphorylcholine (GPC), glycine (Gly), glutathione (GSH), lactate (Lac), mIns, *N*-acetylaspartylglutamate (NAAG), phosphorylcholine (PCho), phosphorylethanolamine (PE), scyllo-inositol (sIns), and taurine . From LCModel's default simulations of lipid and macromolecular resonances, only 'Lip13a' (modeling a broad peak at 1.28 ppm) was allowed during the LCModel fitting that was performed over the spectral range from 0.2 to 4.0 ppm, and modeling of the baseline was restricted to 6 spline knots (the minimum allowed by the program). The basis set for difference spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and the experimentally measured spectra from 100 mM phantoms of NAA, GABA, Glu and Gln at 37°C and with pH adjusted to 7.2. No LCModel's default simulations of lipid and macromolecular resonances were allowed during the LCModel fitting that was performed over the spectral range from 0.5 to 4.0 ppm, and modeling of the baseline was restricted to 9 spline knots. No baseline correction, zero-filling, or apodization functions were applied to the in vivo data prior to LCModel analysis. Visual inspection of the spectra led to exclusion of nine subjects (4 in the control group, 5 in the experimental group) because of contamination from subscapular lipid signal for a final cohort of 12 concussed athletes and 10 controls. Cramér-Rao lower bounds (CRLB) were < 40% for Glx, tNAA, mIns, and tCr (Cr-CH_3 + PCr-CH_3) and taurine. For six participants, Cramér-Rao lower bounds (CRLB) were > 40% for GABA. The six participants were therefore excluded from all analysis involving GABA, leading to a group

of 8 control athletes and 8 concussed athletes. Because of the reduction in sample size, analysis was also performed with participants showing GABA concentrations with a CRLB < 60%, which eliminated one participant. Linewidth of water spectra were all < 10 Hz. A scaling factor between the simulated and measured basis sets was calculated using the group average of tNAA measured from 'EDIT OFF' spectra and the group average tNAA from difference spectra. tCr, mIns, taurine, and tNAA concentrations were obtained from 'EDIT OFF' spectra, and GABA and Glx concentrations were obtained from difference spectra. The concentration of metabolites was expressed as ratios to tCr.

3.3.5 Cortical thickness analysis

Cortical thickness was extracted from the T_1-weighted images using the CIVET pipeline of the Brain-Imaging Centre of the Montreal Neurological Institute (McGill University, Montreal, Canada; Lyttelton et al., 2007). Mean cortical thickness of the whole brain was calculated and the hand representation over left M1 was calculated following the anatomical guidelines from Yousry and collaborators (1997; figure 1b). Statistical analyses were performed on the cortical thickness data using the SurfStat toolbox for Matlab© (http://www.math.mcgill.ca/keith/surfstat/), corrected for multiple comparisons across space using False discovery rate (FDR; Storey, 2002). Anatomical correlations between thickness of left M1 and all cortical vertices were computed using the MACACC method (Lerch et al., 2006), which allows the investigation of correlated changes in cortical thickness across and within diverse cortical networks.

3.3.6 Transcranial magnetic stimulation protocol.

TMS was delivered through an 8 cm figure-of-eight coil connected to a MagPro stimulator (MagVenture, Farum, Denmark). The coil was positioned flat on the head of participants with an angle of 45° from the midline, with the handle pointing backwards. A biphasic current was induced with an anterior-posterior direction. The optimal site of stimulation was defined as the coil position from which TMS produced motor evoked potentials (MEPs) of

maximum amplitude in the first dorsal interosseus (FDI) muscle of the contralateral hand. The optimal site was then marked down on a cap placed over the head of the participant prior to TMS. In order to measure muscle contractions, two self-adhesive electrodes were placed on the FDI muscle of the right hand and a ground electrode was positioned over the wrist. The EMG signal was filtered with a bandwidth of 20-1000 Hz and digitized at a sampling rate of 4 kHz using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using Scope v4.0 software (ADInstruments, Colorado Springs, USA) and stored for offline analysis. TMS pulses were delivered at a frequency of 0.1 to 0.2 Hz for all TMS protocols to avoid long lasting modulation of M1 excitability (Chen et al. 1997).

The resting motor threshold (RMT) was initially determined for each participant and defined as the minimum intensity used to elicit MEPs of 50 μV in 6 of 10 trials. For cortical silent period measurement, subjects were asked to maintain a voluntary isometric muscle contraction of the right FDI at approximately 20% of maximal strength while single pulse TMS was administered at intensities of 120% and 130% of RMT. To induce LICI, two pulses were applied at an intensity to produce test (TS) and conditioning (CS) stimulus amplitudes of approximately 1mV at an interstimulus interval of 100 ms. Ten MEPs were collected for each condition.

3.3.7 TMS analysis

The length of the CSP was manually evaluated and defined as the beginning of EMG activity suppression until the resumption of sustained EMG activity. For LICI, ratios of the conditioning stimulus over the TS were collected. Percentage of inhibition of the CS over the TS was then calculated.

3.3.8 Statistical analysis

All values are expressed as means (SDs). A p value of < 0.05 was considered significant. Group differences on MRS-derived metabolite concentrations and TMS-derived CSP and LICI were tested with independent samples Student's t-tests. Pearson correlations were computed

for each group to assess the relationship between TMS and MRS measures of intracortical inhibition/excitation. To assess the difference between both groups in correlation coefficients, a Fisher's exact test was applied. The impact of the number of concussions, the severity of concussions and the time elapsed since the last injury on TMS and MRS measures were also assessed with Pearson correlations. A Bonferonni correction for multiple comparisons was applied to multiple correlations.

Statistical analyses were performed on the cortical thickness data using the SurfStat toolbox for Matlab© (http://www.math.mcgill.ca/keith/surfstat/), corrected for multiple comparisons across space using False discovery rate (FDR; Storey, 2002) Anatomical correlations between thickness of left M1 and all cortical vertices were computed using the MACACC method (Lerch et al., 2006). Pearson correlations were also computed to assess the relationship between M1 mean thickness and MRS and TMS derived measures of intracortical inhibition. A Fisher's exact test was then computed to assess differences between both groups.

3.4 Results

MRS. Demographic data are shown in Table 1. Independent Student's t-tests showed no significant differences between groups for all metabolites of interest (see Table 2). For the control group, two-tailed Pearson correlations showed a significant correlation between GABA and Glx (r = .82, p = .01). However, concussed athletes showed no correlation between metabolites (r = -.04, p = .92). To assess the difference between coefficients, a Fisher's exact test was computed and showed a trend towards a significant difference between groups for the relationship between Glx and GABA (z = 1.87, p = .06). When participants with higher CRBL (< 60%) were included to enhance statistical power, the correlations obtained for both groups were similar (control athletes: r = .75, p = .01; concussed athletes: r = -.16, p = .63) and the difference between both coefficients was significant (z = 2.21, p = .03; Figure 3a).

TMS. Because both CSP conditions (120-130%) were highly correlated (r = 86, p = .0001), they were used as a compound to reduce the number of comparisons. Independent

Student's t-tests showed no significant differences between groups for LICI ($t_{(26)}$ = 1.00, p = .32; figure 4a) and CSP duration ($t_{(28)}$ = .29, p = .77; Figure 4b). Two-tailed Pearson correlations were computed to verify the relationship between TMS and MRS-derived measures of intracortical inhibition/excitation for both groups individually. Controls participants showed no significant relationship between GABA and LICI (r = -.12, p = .71) or CSP (r = .253, p = .18). They also showed no significant relationship between Glx and LICI (r = .12, p = .74) or CSP (r = .34, p = .34). Concussed athletes also showed no significant correlation between GABA and LICI (r = .42, p = .30) and CSP (r = .35, p = .39). No significant correlation was observed between Glx and LICI (r = -.12, p = .71) or CSP (r = .30, p = .35). Further exploratory analyses were computed including GABA concentrations with CRBL < 60%. With this larger group, concussed athletes showed a significant correlation between LICI and GABA (r = .92, p = .0001), which was absent in control athletes (r = -.12, p = .72). To assess the difference between both coefficients, a Fisher's exact test was computed and showed a significant difference between groups for the relationship between LICI and GABA (z = 3.25, p = .001; Figure 3b).

Cortical thickness. No significant differences were observed for whole brain cortical thickness and left M1 thickness between groups. MACCAC anatomical correlations between M1 thickness and whole brain vertices also showed no significant differences between groups. Two-tailed Pearson correlations were also computed in order to assess the correlation between MRS/TMS-derived measures of intracortical inhibition/excitation and M1 mean thickness. No significant correlations were observed for both controls (r = .40, p = .33) and concussed athletes (r = .003, p = .99). Further exploratory analyses were computed including GABA concentrations with CRBL < 60%. In this case, although correlations were not significant, both groups showed opposite relationships between GABA and M1 thickness. Control athletes showed a positive correlation between both measures (r = .50, p = .14), whereas concussed athletes showed a negative correlation (r = -.34, p = .30). Fisher's exact test showed a trend towards a significant difference between both coefficients (z = 1.76, p = .08; Figure 3c).

3.5 Discussion

In the present study, the long-term impact of sport concussions on M1 metabolism, anatomy and physiology was investigated in a sample of asymptomatic athletes who sustained their last concussion on average 3 years prior to testing. The study revealed four main findings: 1) no significant alteration in intracortical inhibition, as measured by CSP and LICI, was observed in the concussed group; 2) no significant metabolic alteration was observed in the M1 of concussed athletes; 3) concussed athletes showed no significant cortical thickness abnormalities in M1 or the whole brain, as well as no abnormalities in M1-whole brain connectivity; and 4) group differences were observed in the relationship between GABA and glutamate and GABA and LICI, suggesting the presence of subtle alterations in M1 inhibition/excitability balance in concussed athletes.

TMS data revealed no significant alterations of the magnitude of M1 $GABA_B$-related intracortical inhibition in concussed athletes, as shown by CSP and LICI measurements. In contrast, previous studies have shown long term alterations in M1 intracortical inhibition in samples of both young asymptomatic and retired athletes. These studies revealed 1) increased CSP duration in asymptomatic university-level football players who sustained multiple concussions from an average of 13 months (De Beaumont et al., 2011a) to 31 months prior to testing (De Beaumont et al., 2007); 2) increased LICI and CSP duration in asymptomatic active university-level football players who sustained multiple concussions from an average of 19 months (De Beaumont et al., 2011b) to an average of 24 months prior to testing (Tremblay et al., 2011b); and 3) increased CSP duration in former athletes who sustained multiple concussions more than 30 years prior to testing.(De Beaumont et al., 2009) Several factors could account for these divergent results. First, the number of concussions suffered in our sample ranged from 1 to 4 with an average of less than two concussions; in contrast, all previous studies consisted of samples of athletes who sustained at least two concussions. The lower number of concussions suffered in our sample did not allow us to look at differences in TMS measures between single and multiple concussions. However, a negative relationship (non-significant) was found between the number of concussions and both TMS measures suggesting the absence of an impact of this factor on our results. Second, the time elapsed since the last concussive

event in our study differed considerably with previous studies conducted with young asymptomatic athletes. Athletes from our study sustained their last concussion an average 3 years prior to testing, suggesting a possible recovery of inhibitory dysfunction. Finally, alterations in M1 intracortical inhibition may not be a widespread and stable feature of the neurophysiological response to concussion.

Results from the present study also suggest the absence of long-term disruptions in the concentration of metabolites in primary motor cortex after sport concussion. Recent studies have shown effects of sport concussions on brain metabolism in the acute and chronic phases (Vagnozzi et al., 2008; 2010; Henry et al., 2011). However, no study has investigated metabolic alterations in athletes beyond the establishment of chronicity phase (that is, more than 6 months post-concussion). Moreover, there is currently no consensus on the acute or chronic metabolic effects of sport concussions; results from Henry and collaborators have shown the presence of chronic NAA/Cr and M-I disruption 6 months post-injury in M1 (Henry et al., 2011), whereas other studies have shown complete recovery of NAA/Cr levels within 45 days in the frontal lobe (Vagnozzi et al., 2008; 2010). Given the present data, we can hypothesize that alterations seen in M1 during the chronic phase eventually recover 3 years after the concussive event. Additionally, results from most ^{1}H-MRS studies do not include the measurement of GABA and consequently, use a smaller voxel of interest. In the present study, the VOI included some contamination from somatosensory regions, which could also explain the discrepancy between our data and previous studies. Finally, we cannot conclude on metabolic disruptions that could be seen in other brain regions such as the corpus callosum or the hippocampus, which have been shown to display some vulnerability to concussion in moderate to severe TBI (Babikian et al., 2010; Harris et al., 2012). As a result, variability in the regions of interest used to assess neurometabolic alterations in concussed athletes could contribute to the lack of consensus in the literature.

Since no alterations were found in the concussed groups using highly sensitive measures such TMS and ^{1}H-MRS, it is not surprising that group differences were not found in the cortical thickness analysis, a less direct measure of neuronal function. Furthermore, results using the MACCAC method (Lerch et al., 2006) also suggest that sport concussion does not affect

anatomical connectivity, as cortical thickness correlations between M1 and multiple cortical regions were not different between groups. In the current TBI literature, there are very few studies that have assessed cortical thickness integrity in adults as most studies have assessed populations of children and adolescents, or animals models of pediatric TBI (Fineman et al., 2000; Merkley et al., 2008; Turken et al., 2009; Hanten et al., 2011; Palacios et al., 2012). One recent study reported measures of cortical thickness following concussion in healthy aging adults and found no difference in cortical thickness between groups, but a link between regions of cortical thinning and episodic memory deficits (Tremblay et al., 2012). However, no study has looked at the effect of cortical thinning in relation to concussion in younger athletes. Results from the present study suggest that acute and chronic metabolic or neurophysiological dysfunctions in M1, as revealed in more recent studies (De Beaumont et al., 2012), have no long-term impact on cortical thickness.

Since multiple studies have shown long term alterations in M1 intracortical inhibition mediated by GABAergic transmission after sport concussion (De Beaumont et al., 2012), we hypothesized altered GABA concentrations in the M1 of concussed athletes. Although no alterations were seen in measures of the GABAergic system by TMS and ^{1}H-MRS, further correlational analysis suggest the presence of subtle changes in inhibitory M1 mechanisms in concussed athletes. Control athletes showed a significant positive correlation between GABA and Glx, whereas concussed athletes displayed no correlation between both metabolites. Data in control athletes are in line with recent studies showing a positive correlation between these inhibitory and excitatory neurometabolites in healthy individuals using ^{1}H-MRS (Stagg et al., 2011; Tremblay et al., 2012; Prescot et al., 2013). Therefore, the non-existent relationship between GABA and Glx in concussed athletes suggests that sport concussions could cause an imbalance between excitability and inhibition in M1. A trend towards a differential correlation between GABA and M1 cortical thickness was observed as controls showed a positive non-significant correlation between the two variables whereas concussed athletes showed a negative non-significant relationship. Although these results are exploratory, they indicate that subtle alterations in GABA transmission and organization in M1 could be present in the brain of concussed athletes even though the absolute metabolite concentrations do not differ from non-concussed athletes.

The exact mechanism underlying this possible slight metabolic imbalance is unknown. Based on recent studies, it could be hypothesized that increases in intracortical inhibition revealed by increased CSP duration and LICI (De Beaumont et al., 2012) and the abnormally high glutamate concentration in M1 in the chronic phase (Henry et al., 2011) could trigger a long-lasting disruption in the interaction between GABA and glutamate. In the even longer term, this subtle imbalance could make the brain more susceptible to a subsequent concussion and partly explain recent findings suggesting a link between sport concussion and abnormal aging (Broglio et al., 2012). Normal aging is typically associated with structural and chemical changes together with a functional impairment of neurons. However, the decline in cognitive function associated with these brain changes could be related to the amount of "cognitive reserve" available (Broglio et al., 2012), which may be influenced by concussive and sub-concussive hits. Therefore, we can hypothesize that, if the differential relationships seen in the present study are related to alterations in metabolic interactions, these dysfunctions in brain metabolism could accelerate or accentuate the neurodegenerative process of aging and increase the odds of developing an abnormal aging trajectory.

Moreover, concussed athletes also showed a differential relationship between GABA and LICI, where concussed athletes showed a positive significant correlation while no correlation was observed in the control group. The absence of a relationship between GABA levels as measured by ^{1}H-MRS and TMS GABA-mediated inhibitory measures in the control group is surprising. However, this result is in line with two recent studies that showed no correlation between MRS and multiple TMS-derived GABA measures in healthy controls (Stagg et al., 2011; Tremblay et al., 2011). This finding suggests that H-MRS GABA does not precisely reflect GABAergic synaptic activity (Stagg et al., 2011), and more specifically that involving $GABA_B$ receptors. Surprisingly, however, LICI and GABA levels were correlated solely in concussed athletes. Although difficult to interpret, this result could reflect the presence of subtle inhibitory dysfunctions in M1. Moreover, the present data suggest that LICI and CSP, both measures of the GABAergic system, are likely to tap into different mechanisms underlying $GABA_B$-related inhibition in the primary motor cortex as they did not correlate in the control group. Physiological studies support this hypothesis as CSP was found to be linked to spinal

inhibition (Inghilleri et al., 1993), whereas LICI was found to rely on cortical inhibition (Werhahn et al., 1999).

It should be noted that several factors can limit the generalization of the present results. First, sub-concussive blows to the head throughout an athlete's career may have resulted in subtle brain alterations. This is coumpounded by the fact that many real concussions go undiagnosed. A recent study conducted in a sample of high-school football players revealed a very high average of impacts to the head, and of markedly high rotational and linear acceleration forces (Broglio et al., 2011). Indeed, Chamard and collaborators (2012) reported the presence of metabolic disruptions in non-concussed hockey players throughout a season that was hypothesized to be caused by cumulative effects of sub-concussive events. As a result, metabolic disruptions in primary motor cortex of concussed athletes may have been underestimated. To control for this, further studies should include an additional control group comprising high-level athletes who do not participate in contact sports, such as track and field. Nevertheless, direct comparisons between athletes that play the same contact sport can reveal important information. Most notably, since it can be assumed that the prevalence of sub-concussive blows is similar between athletes of both groups (concussed and non-concussed), something specific about diagnosed concussions may emerge. This appears to be the case indeed, since most concussion studies using a similar recruiting approach to the one sued in the present study have revealed wide-ranging brain abnormalities. Second, there is increasing evidence suggesting cumulative and deleterious effects of repeated concussions (Blennow et al., 2012). For instance, Guskiewicz and collaborators (2005) showed that football players with a history of multiple concussions have a strikingly increased risk of developing long lasting cognitive impairments. Unfortunately, the size of the present sample did not allow a direct comparison between athletes that had suffered one or many concussive events throughout their career. It should also be noted that there exists a possibility that the TMS measures, which were taken before MR acquisition in a portion of the sample of athletes, may have altered glutamate and GABA concentration in M1. However, this appears unlikely since a low frequency of stimulation was used (between 0.1 and 0.2 Hz), which has been shown not to modify cortical excitability (Chen et al. 1997) and only a limited number of pulses were applied for the measurement of LICI and CSP.

In conclusion, the present data suggest the general absence of neurophysiologic, neurometabolic and neuroanatomical disruptions in M1, three years after the last concussion in a sample of active university-level football players. However, correlational analyses suggest the presence of a slight metabolic imbalance between GABA and glutamate concentrations in the primary motor cortex of concussed athletes. Even though this abnormality appears relatively modest, it highlights the need for multimodal evaluations of the impact of concussions that may not be seen using neuropsychological or standard functional evaluations. The present data also stress the importance of assessing the long-term impact of sport concussions on brain function and evaluating the role of subtle disruptions in metabolic balance that may contribute to abnormal aging (Tremblay et al. 2012).

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3.8 Figures

Figure 1. Voxel of interest and M1 region of interest

Legend : A) Position of the voxel of interest (27 x 24 x 32 mm^3) over the left hand area of the primary motor cortex in (A) sagittal, (B) axial (C) and coronal slices. B) M1 region used for cortical thickness analysis.

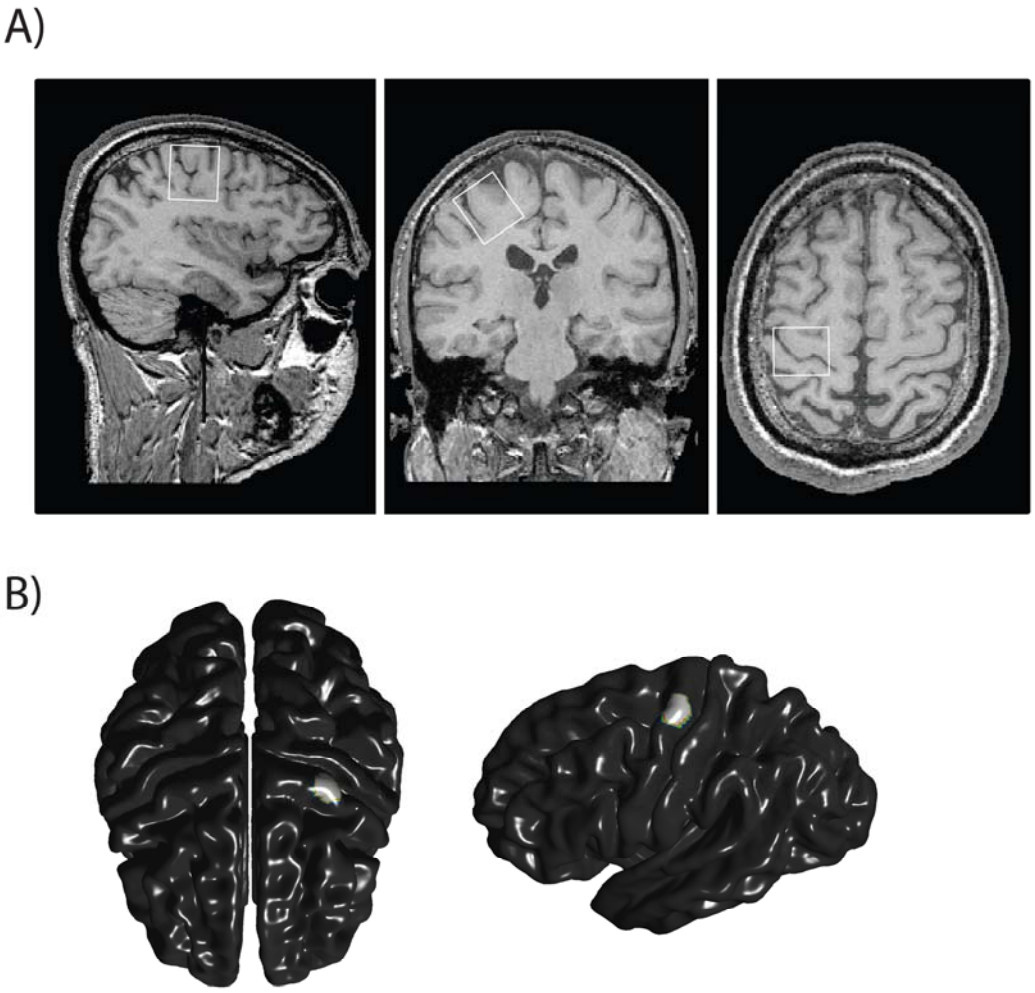


Figure 2. Representative MEGA-PRESS spectrum

Legend : Representative 'EDIT OFF', 'EDIT ON', and difference ('DIFF') spectra. tCr was obtained from 'EDIT OFF' spectrum, Glx, and GABA from difference spectrum, and tNAA from both. 'EDIT OFF' and 'EDIT ON' spectra are the average of 128 scans each.

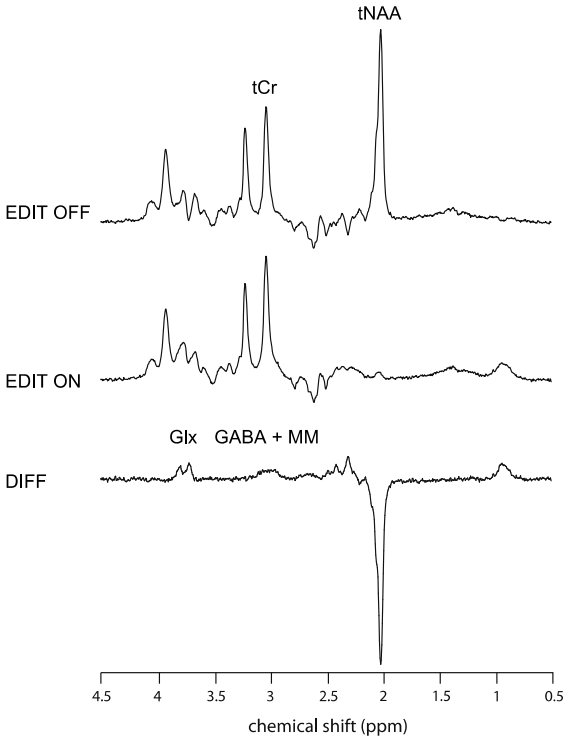


Figure 3. Between-group comparisons of correlations

Legend : (A) Pearson correlations for Glx/tCr and GABA/tCr levels for both groups. Concussed group: r = -.16, p = .63; Control group r = .75, p = .01. (B) Pearson correlations for GABA/tCr levels and LICI percentage of inhibition for both groups. Concussed group r = .92, p = .0001; Control group: r = -.12, p = .72 (C) Pearson correlations for M1 mean thickness and GABA/tCr levels for both groups. Concussed group: r = -.34, p = .30; Control group: r = .50, p = .14

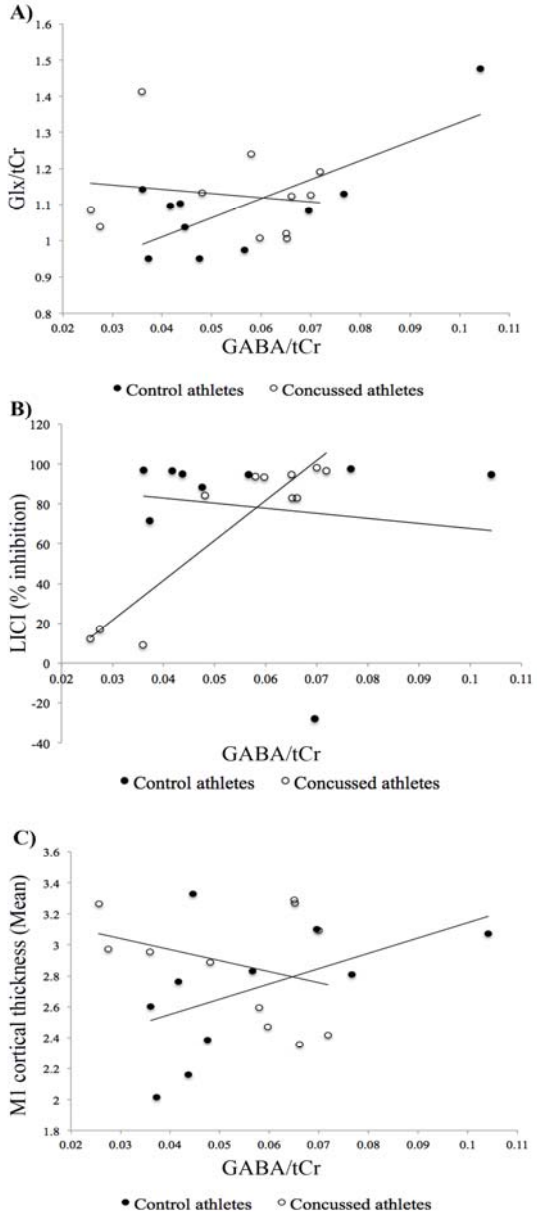
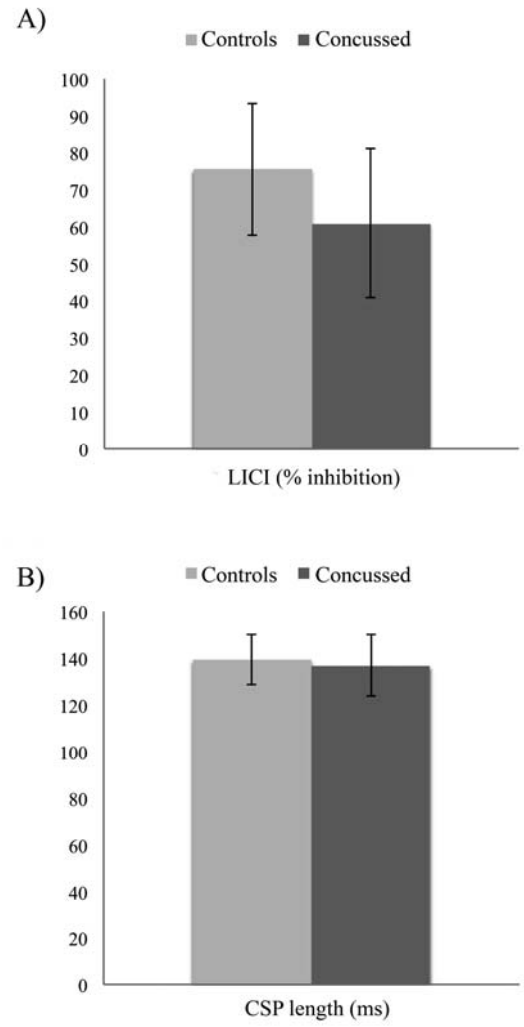


Figure 4. Group comparisons for for LICI and CSP

Legend : (A) Mean LICI percentage inhibition for both groups. (B) Mean CSP duration for both groups



3.9 Tables

Table 1. Between group comparisons of demographic and concussion history information

Variables	Group	Mean (SD)	t test	p value
Age (years, months)	Control	22.03 (1.08)	0.25	0.80
	Concussed	22.00 (1.09)		
Education (years, months)	Control	15.11 (1.09)	0.35	0.73
	Concussed	15.09 (1.08)		
Number of concussion	Control		N/A	N/A
	Concussed	1.88 (0.89)		
Maximum severity (grade)	Control		N/A	N/A
	Concussed	2.00 (.89)		
Time since the last concussion (years, months)	Control		N/A	N/A
	Concussed	3.05 (2.06)		

Table 2. Between group comparisons of metabolites concentrations

Metabolite	Group	Mean (SD)	t test	p value
GABA/tCr	Control	0.056 (0.022)	0.222	0.827
	Concussed	0.054 (0.017)		
Glx/tCr	Control	0.855 (0.101))	0.783	0.443
	Concussed	0.825 (0.079)		
tNAA/tCr	Control	1.314 (0.110)	0.753	0.446
	Concussed	1.264 (0.184)		
Ins/tCr	Control	0.611 (0.082)	0.777	0.446
	Concussed	0.637 (0.071)		
tau/tCr	Control	0.186 (0.027)	1.413	0.173
	Concussed	0.204 (0.031)		
Asp/tCr	Control	0.282 (0.040)	1.041	0.310
	Concussed	0.308 (0.070)		
Lac/tCr	Control	0.034 (0.013)	0.988	0.335
	Concussed	0.039 (0.011)		

Chapitre 4

Article 3 : Relationship between transcranial magnetic stimulation measures of intracortical inhibition and spectroscopy measures of GABA and glutamate+glutamine

The relationship between transcranial magnetic stimulation measures of intracortical inhibition and spectroscopy measures of GABA and glutamate+glutamine

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4.1 Abstract

Transcranial magnetic stimulation (TMS) can provide an index of intracortical excitability/inhibition balance. However, the neurochemical substrate of these measures remains unclear. Pharmacological studies suggest the involvement of $GABA_A$ and $GABA_B$ receptors in TMS protocols aimed at measuring intracortical inhibition, but this link remains inferential. Proton magnetic resonance spectroscopy (^{1}H-MRS) permits measurement of GABA and glutamate + glutamine (Glx) concentrations in the human brain, and might help in the direct empirical assessment of the relationship between TMS inhibitory measures and neurotransmitter concentrations. In the present study, MRS-derived relative concentrations of GABA and Glx measured in the left M1 of healthy participants were correlated with TMS measures of intracortical inhibition. Glx levels were found to correlate positively with TMS-induced silent period duration whereas no correlation was found between GABA concentration and TMS measures. The present data demonstrate that specific TMS measures of intracortical inhibition are linked to shifts in cortical Glx, rather than GABA neurotransmitter levels. Glutamate might specificaly interact with $GABA_B$ receptors, where higher levels of MRS-derived Glx concentrations seem to be linked to higher levels of receptor activity.

Keywords: Magnetic resonance spectroscopy; Motor cortex; Cortical silent period; MEGA-PRESS

4.2 Introduction

Transcranial magnetic stimulation (TMS) is contributing significantly to our understanding of the pathophysiology of many neurological and psychiatric disorders (Chen et al. 2008). By using single and paired-pulse TMS over primary motor cortex (M1) it is possible to investigate physiological interactions between excitatory and inhibitory circuits (Hallett 2007). Furthermore, the combination of TMS protocols with the administration of central nervous system drugs permits indirect evaluation of the mechanism underlying these circuits (Teo et al. 2009) and potentially implicated receptors (Ziemann 2004). It has been suggested that short interval intracortical inhibition (SICI; (Kujirai et al. 1993) induced by paired-pulse TMS protocols is mediated by gamma-aminobutyric acid A receptors ($GABA_A$). Indeed, the administration of benzodiazepine, a positive modulator of $GABA_A$, was found to enhance SICI (Ziemann et al. 1996a; Di Lazzaro et al. 2005). In parallel, pharmacological studies suggest that long interval intracortical inhibition (LICI) and the cortical silent period (CSP), which are TMS measures of long lasting intracortical inhibition, are increased by the administration of $GABA_B$ receptor agonists tiagabine (LICI: McDonnell et al. 2006) and baclofen (CSP: Werhahn et al. 1999).

A better understanding of the effects of pharmacological agents on TMS measures of cortical excitability has also contributed to a better definition of the pathophysiology of numerous motor system disorders (Chen et al. 2008). For example, TMS studies have shown that both short- and long-interval intracortical inhibition was affected (Ziemann et al. 1997b; Mills, 2003) in patients with amyotrophic lateral sclerosis, a neurodegenerative disease selectively affecting motoneurons. In addition, abnormal intracortical inhibition was found in patients with Parkinson's disease, where a shorter CSP (Cantello et al. 1991) and reduced SICI (Ridding et al. 1995) were observed. Other studies have suggested the presence of reduced intracortical inhibition in dystonia (Di Lazzaro et al. 2009) and Tourette syndrome (Ziemann et al. 1997a). Recent studies have also demonstrated the presence of altered $GABA_B$ function in motor cortex inhibition in asymptomatic, concussed athletes (De Beaumont et al. 2007, 2012).

These studies suggest that TMS may present diagnostic utility in a variety of pathologies affecting primary motor cortex as well as providing a safe and rapid way of evaluating treatment

response. However, TMS and pharmacological studies only allow an indirect measure of excitatory/inhibitory mechanisms and their implicated neurotransmitter systems. It is possible to directly and non-invasively evaluate the presence of alterations in brain neurochemistry by using proton magnetic resonance spectroscopy (1H-MRS). This technique allows *in vivo* detection and quantification of different neurometabolites, providing a sensitive and reliable assessment of neurochemical alterations (Ashwal et al. 2004; Holshouser et al. 2006). In addition to common neurometabolites (creatine (Cr) and phosphocreatine (PCr) (tCr = Cr + PCr), *myo*-inositol (mI), *N*-acetylaspartate + *N*-acetylaspartylglutamate (tNAA), glutamate (Glu) and glutamine (Gln) (Glx = Glu + Gln)), recent technological advances have allowed the detection and quantification of GABA neurotransmitter in the human brain (Mescher et al. 1998).

Similarly to TMS, MRS has provided a better understanding of the underlying biochemistry of different neuropathologies (Jissendi Tchofo and Balériaux 2009). For example, abnormal Glu concentration ratios characterize several brain pathologies, where a reduction of Glu/tCr was found in Parkinson's disease (Griffith et al. 2008), while abnormally elevated Glx concentrations were implicated in amyotrophic lateral sclerosis symptoms (Han and Ma 2010). Such neurometabolic alterations over regions of interest has also been shown in the acute concussion phase, where injured athletes exhibit reduced NAA and Glu concentrations within the primary motor cortex (Henry et al. 2010).

Despite the parallel development of the TMS and MRS techniques, it remains unclear how the *direct* assessment of GABA and Glu concentrations corresponds to synaptic GABAergic and glutamatergic activity *indirectly* assessed by TMS. The nature of this link could help further understand what both techniques are specifically measuring. Stagg and collaborators (2011a) recently addressed this issue and reported no correlation between MRS-derived measures of GABA neurotransmitter levels and TMS measures of synaptic $GABA_A$ (SICI; 2.5 ms) and $GABA_B$ (LICI) receptor activity in M1. By contrast, a significant correlation between overall cortical excitability (input/output curve) and Glu levels was reported. Surprisingly, MRS-GABA levels were found to correlate positively with the slope of the input/output curve, whereby individuals with the greatest levels of M1 excitability (TMS) also

showed the highest GABA concentration (MRS). These data suggest that MRS-derived GABA levels may not reflect specific synaptic activity, whereas MRS-derived Glu levels may relate to synaptic glutamatergic activity indirectly measured by the TMS input/output curve (Stagg and Nitsche 2011). The present study was conducted to provide further empirical insights into the presumed association between GABA concentration and TMS measures of intracortical inhibition, and to assess the link between GABA and the cortical silent period, a TMS inhibitory measure used in clinical and experimental settings.

4.3 Methods

4.3.1 Participants

The study group consisted of 24 right-handed participants (12 men and 12 women), from 20 to 38 (M = 24.7, SD = 4.1) years of age. The following exclusion criteria were used: psychiatric or neurological history, traumatic brain injury or concussion, presence of a pacemaker, use of central nervous system-active medication, metal implanted in the skull, history of fainting, history of seizures or history of substance abuse. The study was approved by the local ethics committee, and all participants provided written informed consent prior to testing. Subjects received a financial compensation of $85 CAN for their participation. The experiment consisted of a single session of approximately 90 minutes, comprising 30 minutes of TMS immediately followed by a 50-minute session of MRS.

4.3.2 TMS

TMS was delivered through an 8 cm figure-of-eight coil connected to a MagPro stimulator (MagVenture, Farum, Denmark). The coil was positioned flat on the head of participants with an angle of 45° from the midline and with the handle pointing backwards. The induced current was biphasic with an anterior-posterior direction. The optimal site of stimulation was defined as the coil position from which TMS produced MEPs of maximum amplitude in the target muscle of the contralateral hand. The optimal site was then marked down on a cap placed over the head of the participant prior to TMS. Two self-adhesive electrodes were placed on the first dorsal interosseus (FDI) muscle to measure motor contraction. A ground electrode

was positioned over the wrist. The EMG signal was filtered with a bandwidth of 20-1000 Hz and digitized at a sampling rate of 4 KHz using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using Scope v4.0 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. TMS pulses were delivered at a frequency of 0.1 to 0.2 Hz for all TMS protocols to avoid long lasting modulation of M1 excitability (Chen et al. 1997).

Resting motor threshold: The resting motor threshold (RMT) was initially determined for each subject. The RMT was defined as the minimum intensity used to elicit MEPs of 50 μV in 6 of 10 trials.

Paired pulse paradigms: The intensity of stimulation was first adjusted to produce MEPs of approximately 1 mV in amplitude. The protocol for short interval intracortical inhibition (SICI) was conducted in accordance with the method of Kujirai and colleagues (1993). A conditioning stimulus (CS) with an intensity of 70% of the MT was paired with a test stimulus (TS) of 1 mV using an interstimulus interval (ISI) of 3 ms. Ten MEPs were collected in addition to the TS alone. The protocol for long-term intracortical inhibition (LICI) was then performed by applying two pulses at an intensity adjusted to produce CS and TS amplitudes of approximately 1 mV peak-to-peak at an ISI of 100 ms.

CSP: To induce a CSP, single pulse TMS with an intensity of 120% and 130% of RMT was performed while participants maintained a voluntary isometric muscle contraction of the right FDI at approximately 20% of maximal strength. Ten MEPs were collected for both intensities.

4.3.3 Analysis of TMS data

For SICI, ratios of CS-TS on TS alone were computed. For LICI, ratios of the CS on the TS were computed. The length of the CSP was manually evaluated by an investigator blind to MRS data and defined as the beginning of EMG activity suppression until the resumption of sustained electromyographic activity. The two different intensities of stimulation for CSP

(120% and 130%) were computed as a single variable (csp average) for analysis. Incomplete acquisition of TMS data led to the exclusion of one participant.

4.3.4 MR acquisition

Magnetic resonance (MR) acquisitions were performed using the 3T whole-body system (MAGNETOM Trio, a TIM systems, Siemens, Erlangen, Germany) at the "Unité de Neuroimagerie Fonctionnelle, Centre de recherche de l'Institut universitaire de gériatrie de Montréal". Radiofrequency transmission was performed with the built-in body coil, and signal was received with at 12-channel receive-only head coil. The prescription of M1 voxel and detection of potential structural abnormalities were performed using anatomical images of the brain obtained with a T_1-weighted MPRAGE sequence (T_R = 2300 ms; T_E = 2.91 ms; FA: 9°; FOV = 256 x 256 mm^2; 256 x 256 matrix; 160 axial slices of 1 mm; acquisition time: 9 min 50 s). The voxel of interest (27 x 24 x 32 mm^3) was positioned over the left hand area of the primary motor cortex using two accepted anatomical landmarks (Yousry et al. 1997; Figure 1). MRS data were acquired using a MEGA-PRESS sequence (Mescher et al. 1996, 1998) with double-banded pulses used to simultaneously suppress water signal and edit the γ–CH_2 resonance of GABA at 3 ppm. Additional water suppression using variable power with optimized relaxation delays (VAPOR) and outer volume suppression (OVS) techniques (Tkac et al. 1999) was optimized for the human 3T system and incorporated prior to MEGA-PRESS. The final spectra were obtained by subtracting the signals from alternate scans with the selective double-banded pulse applied at 4.7 ppm and 7.5 ppm ('EDIT OFF') and the selective double-banded pulse applied at 1.9 ppm and 4.7 ppm ('EDIT ON') (Figure 2). MEGA-PRESS data were acquired in four interleaved blocks of 32 ('EDIT OFF', 'EDIT ON') scans each with frequency drift correction between blocks. FIDs were stored separately in memory for individual frequency and phase correction using the tCr signal at 3.03 ppm, as well as correction for residual eddy-current using unsuppressed water signal obtained from the same voxel.

4.3.5 Analysis of MRS data

Both 'EDIT OFF' and difference spectra were analyzed using LCModel 6.2-1A (Provencher 1993, 2001), which calculated the best fit of the experimental spectrum as a linear combination of model spectra. The basis set for 'EDIT OFF' spectra was simulated using home-written software based on density matrix formalism (Henry et al. 2010) in MATLAB, using known chemical shifts and *J* couplings (Govindaraju et al. 2000). The simulated spectra of the following 20 brain metabolites were included in the basis set: alanine (Ala), ascorbate (Asc), aspartate (Asp), Cr, GABA, glucose (Glc), Glu, Gln, glycerophosphorylcholine (GPC), glycine (Gly), glutathione (GSH), lactate (Lac), myo inositol (mI), NAA, N-acetylaspartylglutamate (NAAG), PCr, phosphorylcholine (PCho), phosphorylethanolamine (PE), scyllo-inositol (sI), and taurine (Tau). Default simulations of lipids and macromolecular resonance were allowed during the LCModel fitting that was performed over the spectral range from 0.2 to 4.0 ppm. The basis set for difference spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and the experimentally measured spectra from 100 mM phantoms of NAA, GABA, Glu and Gln at 37°C and with pH adjusted to 7.2. The LCModel fitting was performed over the spectral range from 0.5 to 4.0 ppm, restricting modeling of the baseline by the use of the minimal number of spline knots allowed by the program. No baseline correction, zero-filling, or apodization functions were applied to the in vivo data prior to LCModel analysis. Visual inspection of the spectra led to exclusion of two subjects because of contamination from subscapular lipid signal. All remaining Cramér-Rao lower bounds (CRLB) were > 40% for GABA, Glx, tNAA and tCr. Linewidth of water spectra were all < 10 Hz, but two were larger than 2*SD over the mean and were excluded from further analysis. The scaling factor for the simulated and measured basis sets was calculated using the group average of tNAA measured from 'EDIT OFF' spectra and the group average tNAA from difference spectra. This scaling factor allowed for the fitted values to be on the same scale. Measures of GABA, Glx, and tNAA were extracted from difference spectra, whereas tNAA and tCr were extracted from 'EDIT OFF' spectra. The metabolites of interest, GABA and Glx, were expressed as ratios to tCr.

4.3.6 Statistical analysis

T-tests were computed to verify the efficacy of TMS inhibitory protocols. Pearson correlations were also computed to look at the relationship between intracortical inhibition/facilitation protocols and metabolite concentration ratios. A p value of < 0.05 was considered significant.

4.4 Results

Average GABA/tCr and Glx/tCr values across participants were 0.06 (±0.01) and 1.05 (± 0.11), respectively. Cramér-Rao lower bound from LCModel analysis was 24.05 (±4.48) for GABA and 3.37 (±0.50) for Glx. Paired-sample t-tests were first conducted to verify the inhibitory effects of the TMS protocols. SICI ($t_{(18)} = 6.56$, $p = 0.0001$) and LICI ($t_{(18)} = 2.88$, $p = 0.01$) induced a significant inhibition of the TS. Correlations between MRS and TMS variables were then computed. Two-tailed Pearson correlations between TMS parameters and metabolite ratios are shown in Figure 3 and 4. Because both CSP conditions (120-130%) were highly correlated ($r = 0.88$, $p < 0.0001$), they were calculated as a compound measure to reduce the number of comparisons. No significant correlation was found between GABA/tCr ratio and SICI ($r = 0.26$, $p = 0.30$; Figure 3a), LICI ($r = 0.31$, $p = 0.20$; Figure 3b), or CSP ($r = 0.20$, $p = 0.41$; Figure 3c). There was no significant correlation between SICI and Glx/tCr ($r = 0.35$, $p = 0.14$; Figure 4a) or LICI and Glx/tCr ($r = 0.12$, $p = 0.62$; Figure 4b). However, a significant positive correlation was found between Glx/tCr ratio and CSP duration ($r = 0.57$, $p = 0.03$, Bonferroni-corrected; Figure 4c), which remained significant when corrected for GABA ($r = 0.57$; $p = 0.04$, Bonferroni-corrected). Multiple regression analysis was performed to evaluate the contribution of TMS inhibitory measures (SICI, LICI, CSP) to Glx/tCr concentration values. The regression model was significant ($r^2 = 0.44$, $p = 0.03$) with CSP duration being the only significant predictor ($\beta = 0.54$, $p = 0.14$). Multiple regression analysis with GABA/tCr and the TMS inhibitory measures was not significant ($r^2 = 0.21$; $p = 0.31$). The correlation between GABA/tCr and Glx/tCr ratios was also computed and revealed a significant positive correlation ($r = 0.58$, $p =$

0.01; Figure 5). Finally, none of the TMS measures were correlated with one another (SICI vs LICI: $r = -0.13$, $p = 0.61$; SICI vs CSP: $r = 0.06$, $p = 0.80$; LICI vs CSP: $r = 0.07$, $p = 0.77$).

4.5 Discussion

This study was conducted to investigate the relationship between TMS measures of intracortical inhibition and levels of GABA and Glx in human primary motor cortex. We report two major findings: 1) MRS-derived GABA did not reflect $GABA_A$ or $GABA_B$ synaptic activity measured by TMS; 2) A positive correlation was found between $GABA_B$ synaptic activity (CSP) and MRS-derived Glx.

The lack of correlation between GABA synaptic activity and MRS-derived GABA levels replicates previous results reported by Stagg and collaborators (2011a), where no relationship between TMS-derived $GABA_A$ (SICI) and $GABA_B$ (LICI) synaptic activity and MRS-GABA concentration was found. We can hypothesize that a major difference in the specificity of the two methods can be responsible for this result. Indeed, studies have shown that TMS protocols reflect specific activity of $GABA_A$ or $GABA_B$ receptors (Reis et al. 2008), whereas MRS mostly reflects extracellular and intracellular GABA concentrations (Maddock and Buonocore 2012). GABA is found in two major pools in the human brain (Stagg et al. 2011b; Maddock and Buonocore 2012), a large cytoplasmic pool (primarily produced by glutamate) and a small vesicular one (primarily found in pre-synaptic boutons). The ability of MRS to detect vesicular GABA, which plays an important role in inhibitory synaptic neurotransmission, remains unknown (Maddock and Buonocore 2012).

Unlike GABA levels that do not seem to correspond to synaptic inhibitory activity, a counterintuitive relationship between the CSP, thought to provide a measure of $GABA_B$ synaptic activity (Ziemann 2004), and Glx/tCr was found in M1. Glx (Glu+Gln) signal mostly comes form Glu, which like GABA, is present in multiple pools. Glu is present in all cell types with the largest pool in glutamatergic neurons and smaller pools in GABAergic neurons and astroglia (Danbolt 2001). It plays a central role in Glu-Gln neurotransmitter cycle. Gln is synthesized from Glu by Gln synthethase in the astroglia and it is broken down to Glu by phosphate-activated

glutaminase in neurons (Danbolt 2001). The exact mechanism underlying the relationship between $GABA_B$ synaptic activity and Glx remain unknown. However, animal studies suggest a close relationship between pre-synaptic $GABA_B$ and glutamatergic neurons (Chalifoux and Carter 2011), where $GABA_B$ agonist Baclofen has a significant effect on excitatory rather than inhibitory transmission in the visual system (Luo et al. 2011).

A similar phenomenon was reported previously, where MRS-GABA levels were found to correlate positively with the slope of the TMS input/output curve (Stagg et al. 2011a), which indexes global corticospinal excitability. Moreover, Stagg and collaborators (2011a) also found a relationship between MRS-glutamate levels and TMS input/output curve. Authors suggest that this relationship could reflect the fact that greater pre-synaptic glutamate stores is linked to higher levels of glutamate (Stagg et al. 2011a). Moreover, pharmacological studies suggest that TMS measures of intracortical facilitation indirectly involve several neurotransmitters including glutamate (Reis et al. 2006) and GABA (Ziemann et al. 1996b), which could explain why the input/output curve is linked to both MRS-levels of GABA and glutamate in their study. Although intracortical facilitation was not measured in the present study, combining results from both studies gives a better picture of the relationship between GABA, glutamate and TMS measures of inhibiton/excitation. Indeed, both results suggest the existence of a close relationship between GABA and glutamate within primary motor cortex, a notion that is compounded by the fact that GABA and Glx/tCr levels measured by spectroscopy correlate strongly. As such, an increase in the concentration of glutamate was associated with parallel increases in GABA concentration levels and $GABA_B$ synaptic activity. A different measure of $GABA_B$ activity (LICI) and a measure of $GABA_A$ activity failed to correlate with Glx/tCr levels in the same region. This confirms data from a previous report (Stagg et al. 2011a) and is not surprising in light of the fact that the three TMS inhibitory measures failed to correlate between them.

Our data thus show that $GABA_A$- and $GABA_B$-related synaptic activity measured with TMS interact differently with glutamate as measured with MRS. Physiological studies suggest that GABAergic neurons exerts rapid synaptic inhibition via anion permeable $GABA_A$ receptors (Isaacson and Scanziani 2011), while $GABA_B$ receptors are responsible for slow inhibition via

the opening of K+ channels (Lüscher et al. 1997). Although we should be cautious in translating these results to our findings, it could be hypothesized that, knowing this physiological discrepancy in their mechanism of action, $GABA_A$ activity would rapidly decrease in response to an increase of glutamate, while $GABA_B$ activity would exert a fine tuning on the balance between excitatory and inhibitory mechanisms by slowly increasing its activity in response to enhanced excitability of the neuron.

At the same time, the present data highlight the fact that LICI and CSP are likely to tap into different mechanisms underlying $GABA_B$-related inhibition in motor cortex. Indeed, it has been shown that the early part of CSP relies on spinal inhibition (Inghilleri et al. 1993), whereas LICI appears to be linked exclusively to cortical inhibition (Werhahn et al. 1999). Moreover, Ziemann and collaborators (1996a) have shown that $GABA_B$-agonist Baclofen can enhance LICI, but has no impact on the CSP duration. Finally, as mentioned earlier, the CSP and LICI measures of inhibition did not correlate in the present study. It should also be noted that there exists a possibility that the TMS measures, which were taken before MR acquisition, may have altered glutamate and GABA concentration in M1. This appears unlikely since a low frequency of stimulation was used (between 0.1 and 0.2 Hz), which has been shown not to modify cortical excitability (Chen et al. 1997). Furthermore, a limited number of TMS pulses were applied to M1, as only MT, LICI and SICI were evaluated, with 10 pulses for each condition. Finally, between the end of TMS and the start of MRS acquisition, approximately 30 minutes elapsed due to participant preparation and anatomical MRI acquisition.

4.5.1 Conclusion

Our data show that the amount of intracortical inhibition assessed by TMS does not reflect global levels of GABA neurotransmitters in the primary motor cortex. Instead, the cortical silent period, a TMS-measure of intracortical inhibition, appears to be linked to cortical glutamate levels. Further research is needed to fully understand the mechanisms of action underlying these complex interactions. In addition, these results suggest that cautious, complementary interpretations should be given to research data assessing the GABAergic system with MRS or TMS. Greater emphasis should be given to the fact that both techniques

can only provide reliable information about specific aspects of GABAergic inhibiton. This is particularly relevant in the study of patient populations when a mechanistic explanation of disease is needed.

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4.8 Conflict of interest disclosures

Dr. Pascual-Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Allied Mind, Neosync, and Novavision, and is an inventor on patents and patent applications related to noninvasive brain stimulation and real-time integration of TMS with EEG and fMRI.

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4.10 Figures

Figure 1. Voxel of interest

Legend : Position of the voxel of interest (27 x 24 x 32 mm^3) over the left hand area of the primary motor cortex in (A) sagittal, (B) axial (C) and coronal slices.

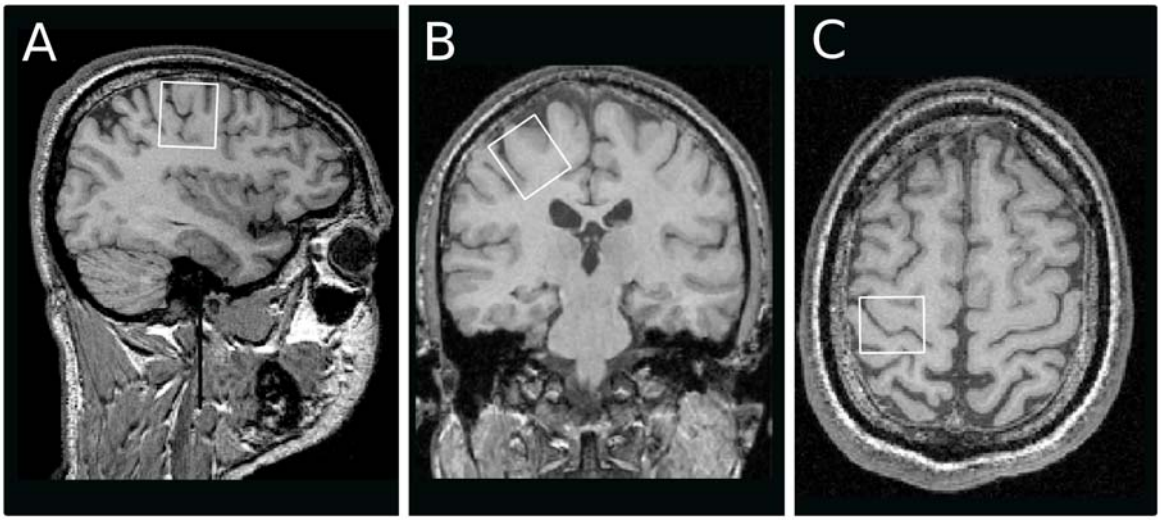


Figure 2. Representative 'EDIT OFF', 'EDIT ON', and difference ('DIFF') spectra.

Legend : tCr was obtained from 'EDIT OFF' spectrum, Glx, and GABA from difference spectrum, and tNAA from both. 'EDIT OFF' and 'EDIT ON' spectra are the average of 128 scans each.

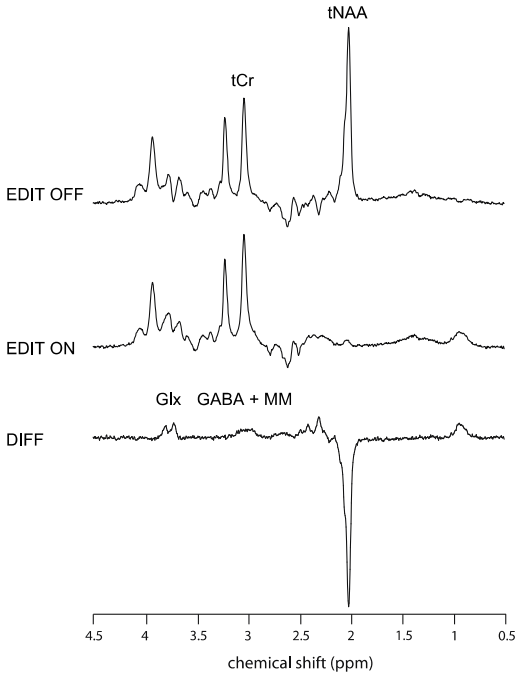


Figure 3. Correlation between TMS and MRS-GABA/tCr measures.

Legend : Relationship between GABA-MRS levels and (A) SICI, (B) LICI, (C) CSP.

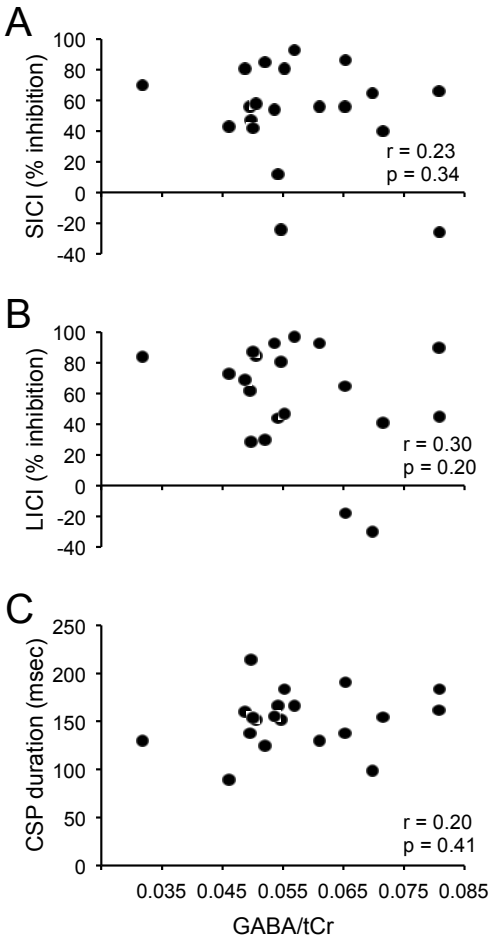


Figure 4. Correlation between TMS and MRS-Glx/tCr measures.

Legend : Relationship between Glx-MRS levels and (A) SICI, (B) LICI, (C) CSP.

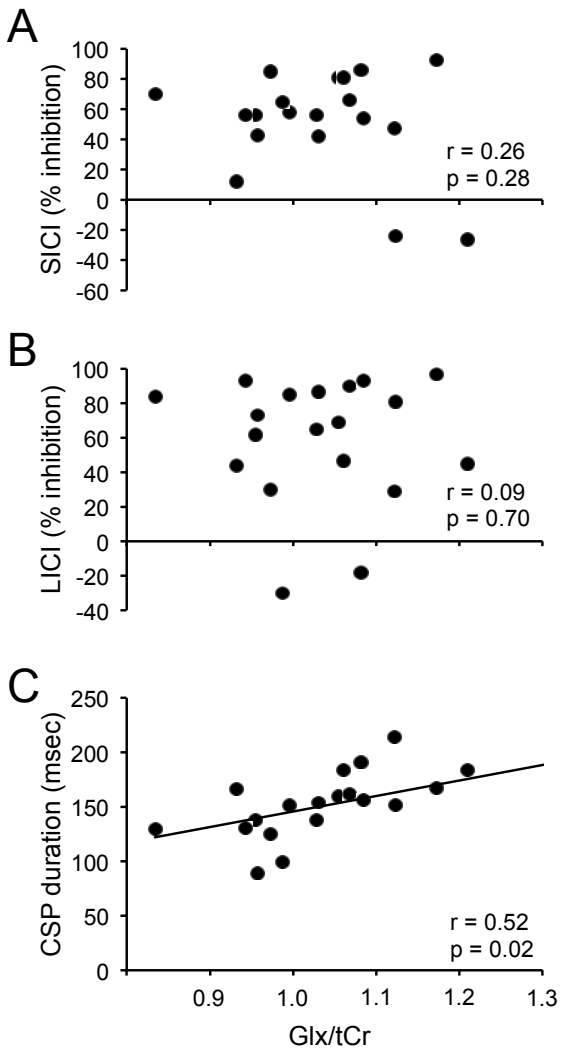
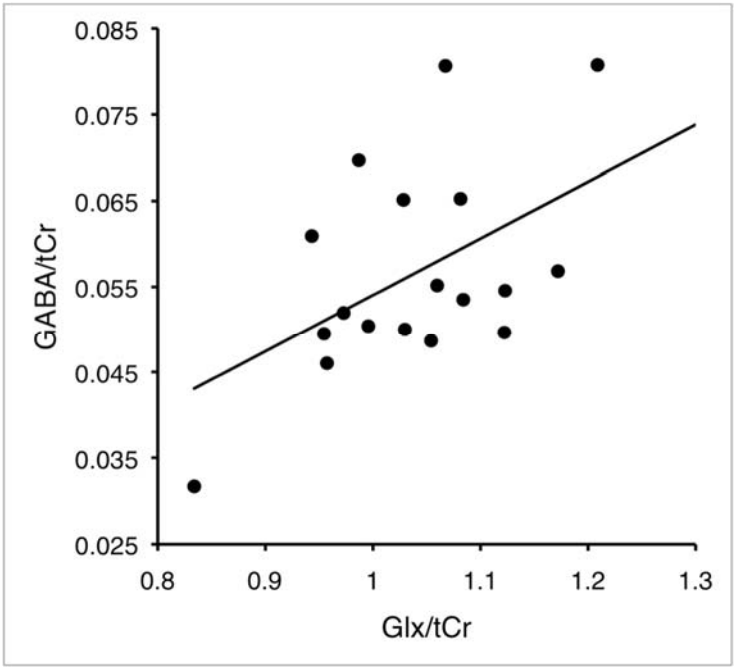


Figure 5. Correlation between Glx/tCr and GABA/tCr MRS levels.



Chapitre 5

Article 4 : Anodal transcranial direct current stimulation modulates $GABA_B$-related intracortical inhibition in M1 of healthy individuals

Anodal transcranial direct current stimulation modulates $GABA_B$-related intracortical inhibition in M1 of healthy individuals

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5.1 Abstract

It is known that transcranial direct current stimulation (tDCS) can induce polarity-specific shifts in brain excitability of the primary motor cortex (M1) with anodal tDCS enhancing and cathodal tDCS reducing cortical excitability. However, less is known about its impact on specific intracortical inhibitory mechanisms, such as $GABA_B$ mediated inhibition. Consequently, the aim of the present study was to assess the impact of anodal and cathodal tDCS on M1 intracortical inhibition in healthy human participants. Long interval intracortical inhibition (LICI) and cortical silent period (CSP) duration, both presumably mediated by $GABA_B$ receptors, were assessed with transcranial magnetic stimulation immediately before and immediately after a 20-minute session of tDCS over the left M1. Anodal tDCS significantly enhanced motor evoked potential (MEP) size and reduced CSP duration, whereas it had no effect on LICI. Cathodal stimulation did not significantly modulate MEP size, CSP duration or LICI. This study provides evidence that anodal tDCS, presumably by synaptic plasticity mechanisms, has a direct effect on $GABA_B$-meditated inhibition assessed by the CSP, but not by LICI. Our results further suggest that CSP and LICI probe distinct intracortical inhibitory mechanisms as they are differentially modulated by anodal tDCS. Finally, these data may have clinical value in cases where a pathological increase in CSP duration is present, such as schizophrenia.

Keywords: cortical silent period, gamma-aminobutyric aci, transcranial direct current stimulation, transcranial magnetic stimulation

5.2 Introduction

Transcranial direct current stimulation (tDCS) allows the modulation of cortical excitability by polarity-specific weak electric current stimulation. Anodal stimulation enhances cortical excitability, whereas cathodal stimulation reduces it. These modulations in cortical excitability are reflected in changes in transcranial magnetic stimulation (TMS) induced motor evoked potential (MEP) size, which are enhanced in anodal tDCS and reduced in cathodal tDCS [1]. Pharmacological studies suggest that these inhibitory and facilitatory effects are influenced by drugs modifying both membrane excitability and synaptic plasticity [1; 2], mediated by N-Methyl-D-Aspartate receptors [3]. This is in agreement with recent animal studies confirming the involvement of activity-dependent synaptic plasticity mechanisms in tDCS polarity-specific excitability shifts [4]. Therefore, it is thought that anodal stimulation results in long-term potentiation (LTP; [4]) a long lasting enhancement in signal transmission implicated in learning and memory.

To investigate the impact of tDCS on brain excitability, most studies use variation in MEP size to quantify changes in cortical excitability [5]. Only a handful of TMS studies have examined the effect of anodal [6-8] or cathodal stimulation [5;7;9] on intracortical inhibitory mechanisms. Results from these studies suggest that anodal stimulation decreases short interval intracortical inhibition (SICI; [6; 8]), presumably mediated by $GABA_A$ receptors [10], but does not affect long interval intracortical inhibition (LICI; [6]), presumably mediated by $GABA_B$ receptors [11]. However, the little evidence available on the effects of tDCS on the cortical silent period (CSP), presumably also mediated by $GABA_B$ receptors [12], is contradictory; one study reported an increase of CSP duration in controls after cathodal stimulation [9], whereas other studies reported no effect of anodal [6;7] or cathodal tDCS [7] on CSP.

Recently, Stagg *et al.* [13] showed that excitatory anodal tDCS reduces GABA levels as revealed by magnetic resonance spectroscopy (MRS), suggesting that the facilitatory effects of tDCS could be partly explained by a decrease in GABAergic inhibition. However, this study does not permit to pinpoint the effect of tDCS on specific $GABA_B$ processes. The present study

aimed to investigate the specific effect of cathodal and anodal tDCS on intracortical inhibitory mechanisms mediated by $GABA_B$ receptors.

5.3 Methods

5.3.1 Subjects

A total of 10 participants (5 men and 5 women), aged 19 to 28 years (M= 23.3), were recruited through advertisements. The following exclusion criteria were used: psychiatric or neurological history, traumatic brain injury, presence of a pacemaker, piece of metal implanted in the skull, history of fainting, history of seizures or history of substance abuse. Participants were all right handed. The study was approved by the local ethics committee and all participants provided written informed consent prior to testing. Subjects received a financial compensation of $60 CAN for their participation.

5.3.2 Procedure

The study consisted of two 90-minute sessions separated by at least 48 hours. One session consisted of anodal tDCS and the other session consisted of cathodal tDCS. Session order was pseudorandomly determined across participants. TMS stimulation of the left primary motor cortex (M1) was performed before and after tDCS.

5.3.3 Transcranial magnetic stimulation.

TMS was delivered through an 8cm figure-of-eight coil connected to a MagPro stimulator (Medtronic, Minneapolis, MN). The coil was positioned flat on the head of participant with an angle of 45° from the midline and with the handle pointing backwards. The induced current was biphasic with an anterior-posterior direction. The optimal site of stimulation was defined as the coil position from which TMS produced MEPs of maximum amplitude in the target muscle of the controlateral hand. Two self-adhesive electrodes were placed on the first dorsal interosseus (FDI) muscle to measure motor contraction. A ground electrode was positioned over the wrist. The EMG signal was amplified with a bandwidth of 20-1000 Hz using

a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using Scope v4.0 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. A Brainsight frameless stereotaxic system (Rogue Research Inc., Montréal, Canada) was used to ensure stable coil positioning over the stimulation site and to properly position tDCS electrodes over M1.

Two identical TMS sessions were conducted before and after tDCS. At first, the intensity of stimulation was adjusted to elicit MEPs of averaged amplitude of about 1mV peak-to-peak. To induce CSP, single pulse TMS with an intensity of 1mV peak-to-peak was performed while participants maintained a voluntary isometric muscle contraction of the right FDI at approximately 20% of maximal strength (see [14]). A long-interval intracortical inhibition protocol was then performed by applying two pulses at an intensity adjusted to produce test stimulus (TS) amplitudes between 0.20 and 1.50 mV at an ISI of 100ms. The intensity of the conditioning stimulus and the TS were identical. Ten MEPs were collected for CSP and LICI. Twenty MEPs were also collected at 1mV peak-to-peak intensity. All TMS pulses were applied at a time interval of 7 to 10 seconds. For LICI and CSP measurements, TMS intensity was adjusted again after tDCS to produce peak-to-peak amplitudes of 1mV to ensure that modulation of LICI and CSP was not linked to modifications in threshold, but rather to changes in intracortical inhibition.

5.3.4 Transcranial direct current stimulation

Electrical current was delivered by a Magstim DC Stimulator (Magstim Ltd, Wales, U.K.) via a pair of conductive rubber electrodes inserted into saline-soaked sponges. A small squared electrode (25cm^2) was positioned over the left FDI vertex, previously determined using TMS as the site inducing maximal FDI muscle contractions. A second rectangular electrode (35cm^2) was positioned above the right supraorbital area. The electrodes were oriented parallel to the central sulcus and eyebrows. It has been shown that this site provides optimal modulation of corticospinal excitability in M1 [1]. The polarity of the electrical stimulation (anodal or cathodal) was dependent on the polarity of the electrode positioned over M1. A constant electric

current of 1.5mA was applied for 20 minutes for both conditions. Current was gradually increased and decreased during the first and last 15 seconds to avoid peripheral sensations.

5.3.5 Data analysis

For LICI, ratios of the conditioning stimulus on the test stimulus were collected. The length of the CSP was manually evaluated and defined as the beginning of EMG activity suppression until the resumption of sustained electromyographic activity. Paired-sample t-tests were computed to evaluate the potential effect of tDCS on cortical excitability and inhibitory mechanisms. A p value of $< .05$ was considered significant. All values were expressed as means +/- SEM. Participants were removed form further analysis of a specific variable if the TMS data was +/- 2 SD from the mean. With this criterion, a single subject was removed from LICI analysis.

5.4 Results

Anodal stimulation. Results of anodal stimulation are presented in Figure 1 and Table 1. Anodal stimulation resulted in a 68% average increase in MEP size (pre: 1.14 mV; post: 1.92 mV), where 8 of 10 participants showed the effect. A paired-sample t-test revealed that this difference was significant ($t_{(9)} = 2.67$, $p = .026$). Duration of the CSP was shortened by an average of 12% (pre: 110 ms; post: 96 ms), where 9 of 10 participants showed the effect. A paired-sample t-test revealed that this difference was significant ($t_{(9)} = 2.88$, $p = .018$). Finally, anodal stimulation did not modulate the strength of LICI (pre: 0.59; post: 0.50) as shown by a paired t-test ($t_{(8)} = .75$, $p = .47$)

Cathodal stimulation. Results of cathodal stimulation are presented in Figure 2 and Table 2. Cathodal stimulation did not modulate MEP size ($t_{(9)} = .12$, $p = .90$), CSP duration ($t_{(9)} = 1.39$, $p = .20$) or LICI strength ($t_{(9)} = .42$, $p = .69$).

5.5 Discussion

The main finding of the present study is that 20 minutes of anodal tDCS can significantly shorten the duration of the CSP. Conversely, despite an increase in corticospinal excitablity reflected in greater MEP size, anodal tDCS failed to modulate LICI, another presumed measure of $GABA_B$ inhibition.

The present study is, to our knowledge, the first to show that anodal tDCS can reduce CSP duration. This is in contrast with previous studies where anodal tDCS failed to modulate CSP duration [6; 7]. This discrepancy could be due in part to differences in stimulation parameters and protocol. Indeed, in previous studies, stimulation intensity, duration and electrode sizes varied considerably from the ones used here (1.5mA; 20 minutes, 25 cm^2 for M1 stimulation and 35 cm^2 for supraorbital stimulation). Moreover, participants in one of the studies suffered from chronic pain [6], hindering the generalizability of the results. The effect of cathodal stimulation on CSP is also unclear. Hasan and collaborators [9] reported that 9 minutes of cathodal tDCS could increase duration of the CSP in a sample of healthy participant, whereas another study [7] recently reported no impact of 10 minutes of cathodal tDCS on CSP duration in healthy participants. It should also be mentioned that the strength of the isometric muscle contraction was not adjusted post-tDCS in the present study. As such, tDCS may have modified absolute strength exerted by the participants and led to the increase in CSP duration.

Theoretically, an increase in excitability could be explained by either enhanced excitatory transmission, or a reduction of inhibitory transmission [15]. A recent magnetic resonance spectroscopy study reported a decrease of GABA levels after tDCS, but no impact on the excitatory glutamate [13], suggesting that intracortical inhibition rather than excitatory transmission could be specifically affected by tDCS and partially responsible for its changes on cortical excitability. This is concordant with the reduction of $GABA_B$ synaptic activity observed here. The mechanism underlying this reduction of inhibitory transmission is thought to result from the modification of NMDA receptor activity and associated LTP mechanisms [2; 15; 16]. Studies on motor learning point towards the implication of intracortical inhibition in synaptic plasticity, as it has been shown that learning a motor sequence reduces GABA levels in M1,

presumably through LTP [17]. A recent TMS study also supports the close link between $GABA_B$ receptors and LTP as it was found that abnormal prolongation of the CSP in concussed athletes was linked to suppression of LTP plasticity [18].

Although both CSP and LICI have been linked to $GABA_B$ activity [12], anodal tDCS failed to modulate the strength of LICI. It has been shown previously that LICI and CSP measurements correlate, with increased CSP durations being associated with deeper LICI [19,20]. In addition, parallel dysfunctions of CSP and LICI have been reported in individuals with succinic semialdehyde dehydrogenase deficiency [21]. In this case, however, although LICI was reduced and CSP shortened, the two measures failed to correlate [21]. Interestingly, correlations between LICI and CSP have also been reported in concussed athletes, but only at certain TMS intensities [20]. Adding to the discrepancies, the $GABA_B$ agonist Baclofen has been shown to increase LICI without modulating CSP duration [11], and CSP and LICI values did not correlate in the present study. Anodal stimulation has been shown not to impact LICI in patients with chronic pain [6] although, as mentioned previously, study parameters differed significantly with those of our study. The present data thus suggest that anodal tDCS has a differential impact on LICI and CSP measures of $GABA_B$- related activity, which could be due to different underlying mechanisms subtending both forms of inhibition. For example, the early part of the CSP is believed to rely on spinal inhibition [22], whereas LICI appears to be exclusively cortical [23]. Intrasubject variability in MEP amplitude because of a relatively low number of pulses [19] may also be different for LICI and CSP, and increasing the number of MEPs could help stabilize the response and permit a better evaluation of the differential effects of anodal tDCS on LICI and CSP. Finally, the intersubject coefficients of variation were much higher for LICI compared with CSP, suggesting that LICI is more susceptible to differences between participants, which may partly explain the differential effects of tDCS on LICI and CSP.

The results of cathodal stimulation on intracortical inhibition are much more difficult to interpret. In contrast to most previous studies [24], cathodal tDCS failed to reduce MEP size.

The absence of inhibitory effects following cathodal stimulation has been observed in several cognitive studies but more rarely in published studies of motor cortex excitability [24]. Although the lack of significant results could in part be attributable to the limited statistical power of the small sample size, significant inhibitory cathodal effects on MEP size have been reported in studies with similar sample sizes [1]. The absence of cathodal tDCS inhibition on corticospinal excitability suggests that the null CSP/LICI effect should be taken with caution, and further studies are needed to determine with certainty whether cathodal tDCS can reliably modulate $GABA_B$-related intracortical inhibition

5.5.1 Conclusion

Anodal tDCS can reduce CSP duration, suggesting that reduced $GABA_B$-related inhibition may be implicated in the excitatory effect of anodal tDCS on the primary motor cortex. Cathodal stimulation, on the other hand, did not modulate MEP size or CSP duration

5.6 Acknowledgements

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5.7 Conflicts of interest

There are no conflicts of interest.

5.8 References

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5.9 Figures

Figure 1. Anodal tDCS effect on intracortical inhibition.

Legend : A) Anodal tDCS significantly increased MEP size B) Anodal tDCS significantly reduced CSP duration. C) Anodal tDCS had no effect on strength of LICI. CSP, cortical silent period; LICI, long interval intracortical inhibition; MEP, motor evoked potential; tDCS, transcranial direct current stimulation. * : $p < .05$

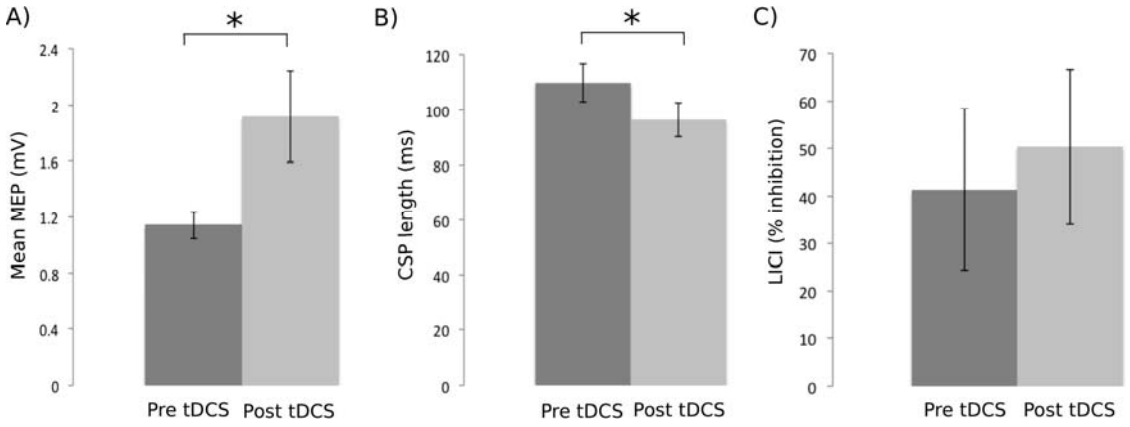
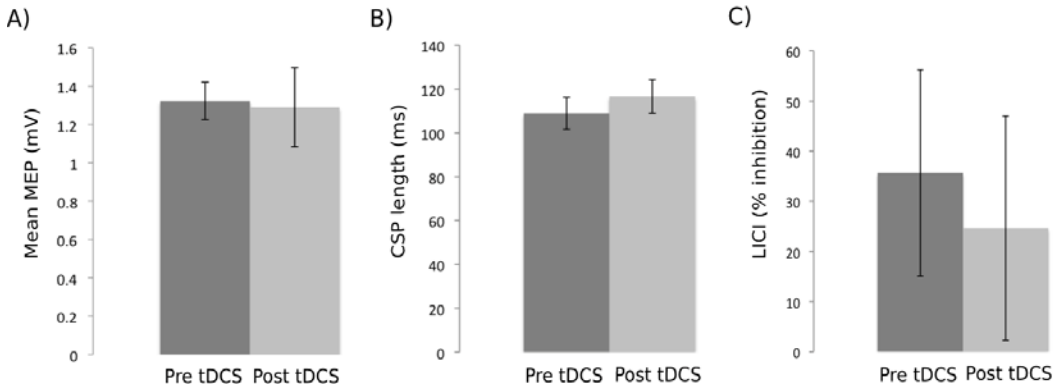


Figure 2. Cathodal tDCS effect on intracortical inhibition.

Legend : No significant effect of cathodal stimulation was found for A) mean MEPs size; B) duration of the CSP; C) strength of LICI. CSP, cortical silent period; LICI, long interval intracortical inhibition; MEP, motor evoked potential; tDCS, transcranial direct current stimulation.



5.10 Tables

Table 1. Anodal tDCS

Participants	MEP pre tDCS (mV)	MEP post tDCS (mV)	CSP pre tDCS (ms)	CSP post tDCS (ms)	LICI pre tDCS (ratio)	LICI post tDCS (ratio)
1	0.90	3.16	67.68	60.40	0.63	0.10
2	0.90	1.79	119.55	81.40	0.04	0.03
3	0.68	1.14	127.40	125.93	0.11	0.33
4	1.13	1.00	119.48	93.83	0.07	0.11
5	0.94	1.08	81.10	76.75	0.99	1.50
6	1.54	3.21	140.88	105.93	0.46	0.14
7	1.65	3.38	108.63	97.73	-	-
8	1.24	0.73	125.20	111.98	1.66	1.02
9	1.26	1.28	106.33	113.93	0.36	0.35
10	1.16	2.42	103.05	96.43	0.96	0.89

Table 2. Cathodal tDCS

Participants	MEP pre tDCS (mV)	MEP post tDCS (mV)	CSP pre tDCS (ms)	CSP post tDCS (ms)	LICI pre tDCS (ratio)	LICI post tDCS (ratio)
1	0.80	1.36	81.18	75.28	0.71	0.33
2	1.26	2.20	111.38	118.70	0.04	0.04
3	1.16	1.78	118.23	141.56	0.21	0.22
4	1.28	1.61	148.60	134.85	0.05	0.03
5	1.01	1.10	97.53	105.48	0.24	0.26
6	1.70	0.35	133.83	138.95	0.43	1.34
7	1.58	0.11	81.88	129.40	2.13	0.14
8	1.40	1.81	127.75	135.35	1.79	1.58
9	1.25	1.37	99.80	106.88	0.72	1.23
10	1.79	1.20	89.18	80.13	1.61	1.75

Chapitre 6

Article 5 : The use of magnetic resonance spectroscopy as a tool for the measurement of bi-hemispheric transcranial electric stimulation effects on primary motor cortex metabolism

The use of magnetic resonance spectroscopy as a tool for the measurement of bi-hemispheric transcranial electric stimulation effects on primary motor cortex metabolism

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6.1 Abstract

KEYWORDS:

proton magnetic resonance spectroscopy, transcranial direct current stimulation, primary motor cortex, GABA, glutamate, stroke

SHORT ABSTRACT:

This article aims to describe a basic protocol for combining transcranial direct current stimulation (tDCS) with proton magnetic resonance spectroscopy (^{1}H-MRS) measurements to investigate the effects of bilateral stimulation on primary motor cortex metabolism.

LONG ABSTRACT:

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that has been increasingly used over the past decade in the treatment of neurological and psychiatric disorders such as stroke and depression. Yet, the mechanisms underlying its ability to modulate brain excitability to improve clinical symptoms remains poorly understood [33]. To help improve this understanding, proton magnetic resonance spectroscopy (^{1}H-MRS) can be used as it allows the *in vivo* quantification of brain metabolites such as γ-aminobutyric acid (GABA) and glutamate in a region-specific manner [41]. In fact, a recent study demonstrated that ^{1}H-MRS is indeed a powerful means to better understand the effects of tDCS on neurotransmitter concentration [34]. This article aims to describe the complete protocol for combining tDCS (NeuroConn MR compatible stimulator) with ^{1}H-MRS at 3 T using a MEGA-PRESS sequence. We will describe the impact of a protocol that has shown great promise for the treatment of motor dysfunctions after stroke, which consists of bilateral stimulation of primary motor cortices [27,30,31]. Methodological factors to consider and possible modifications to the protocol are also discussed.

6.2 Introduction

The idea of applying electricity to the human brain to modulate its activity has been studied since ancient times. In fact, writings from as early as the 11th century have been found that describe the use of the torpedo electric fish in the treatment of epileptic seizures[1]. Yet, it is not until recently that non-invasive brain stimulation has received widespread interest in the scientific community as it was shown to produce modulatory effects on cognitive function and motor response[2]. While transcranial magnetic stimulation (TMS) has been extensively studied since the early 1980's[3], recent interest in transcranial direct current stimulation (tDCS) has increased as it is now considered a viable treatment option for a wide range of neuropathologies, such as stroke[4], alcohol addiction[5], and chronic pain[6]. tDCS has many advantages over neurostimulation techniques like TMS, for example, since it is relatively inexpensive, painless, well tolerated by patients, and portable, thus making it possible to administer at bedside[7]. In fact, only a small percentage of patients experience a mild tingling sensation during stimulation[8]. However, this sensation usually disappears after a few seconds[9]. Consequently, tDCS allows robust double-blind, sham-controlled studies since a majority of participants cannot differentiate sham stimulation from real stimulation[9,10].

tDCS involves the induction of a constant low-amperage electric current (1-2 mA) applied to the cortex via surface electrodes positioned on the scalp of the subject. The electrodes are usually placed into saline-soaked sponges or directly on the scalp with an EEG-type paste. To conduct a tDCS study, four main parameters need to be controlled by the experimenter: 1) the duration of stimulation; 2) the intensity of stimulation; 3) the electrode size; and 4) the electrode montage. In standard protocols, the "active" electrode is positioned over the region of interest while the reference electrode is usually placed over the supraorbital region. The current flows from the positively charged anode towards the negatively charged cathode. The effect of tDCS on primary motor cortex (M1) is determined by the polarity of the stimulation where anodal stimulation enhances the excitability of a population of neurons and cathodal stimulation reduces it [11]. Unlike TMS, the induced current is insufficient to produce action potentials in cortical neurons. The changes in cortical excitability are believed to be due to the modulation of the membrane neuronal threshold leading to either the hyperpolarization of membrane potentials

or a facilitation of depolarization of neurons depending on the direction of the current flow [8,11]. The duration of the excitability changes can persist for up to 90 min after the offset of stimulation, depending on stimulation duration [11,12].

6.2.1 tDCS and motor rehabilitation

The M1 has been extensively used as a target of stimulation since excitability changes elicited by tDCS can be quantified through motor evoked potentials (MEPs) induced by single pulse TMS [3]. Early studies showing the possibility of measuring polarity-specific excitability changes induced by tDCS have used M1 as a target of stimulation [11,12]. Since then, M1 has remained one of the primary targets of tDCS in studies involving both clinical populations and healthy subjects because of its importance in motor function, memory formation, and consolidation of motor skills [13].

The brain relies on a complex interaction between motor regions of both hemispheres to perform a movement [14]. When one area is damaged, after suffering a stroke for example, inter-hemispheric interactions are altered. Studies on brain plasticity have shown that the motor areas of the brain adapt to this modification in different ways [15]. First, the intact, surrounding regions of the damaged area can become overactived, leading to inhibition of the damaged area - a process called intra-hemispheric inhibition. Second, the homologous region of the damaged area can become overactivated and exert inhibition on the injured hemisphere - a process called inter-hemispheric inhibition. The affected M1 can therefore be twice penalized: first by the lesion and second by the inhibition coming from both the unaffected M1 and the surrounding region of the affected M1 [16]. A recent study has shown that increased excitability in the unaffected hemisphere is linked to slower rehabilitation [17], which has been described as maladaptive inter-hemispheric competition [18].

Understanding the plasticity occurring after a stroke may lead to the development of neuromodulation protocols that can restore interhemispheric interactions [19]. Three main tDCS treatments have been proposed in patients with motor deficits following stroke [20,21]. The first treatment aims to reactivate the injured motor cortex by unilateral *anodal* stimulation (a-tDCS).

In this case, stimulation aims at *directly* increasing activity in perilesional areas, which are believed to be essential for recovery. In fact, studies have shown improvement of the paretic upper or lower limb following this treatment [22-26]. The second treatment was developed with the aim of reducing the over-activation of the contralesional hemisphere by applying unilateral *cathodal* tDCS (c-tDCS) over the intact M1. Here, stimulation aims at *indirectly* increasing activity in perilesional areas through interhemispehric interactions. Results from these studies have shown improvement of motor function after c-tDCS [4,27-29]. Finally, the third treatment aims at combining the excitatory effects of a-tDCS over the injured M1 with the inhibitory effects of c-tDCS over the unaffected M1 using *bilateral* tDCS. Results have shown improvements in motor function after bilateral tDCS [27,30,31]. Moreover, one study demonstrated greater improvements following bilateral tDCS compared to both unilateral methods [32].

6.2.2 Physiological mechanisms of tDCS

Despite the increasing use of tDCS in the treatment of stroke, the physiological mechanism underlying its effects remains unknown [33]. A better understanding of the physiological effects could help develop better treatment options and could lead to standardized protocols. As mentioned earlier, the effects of tDCS can last for up to 90 min after the offset of stimulation [11,12]. Therefore, hyperpolarization/depolarization processes cannot completely explain long lasting effects [33,34]. Different hypotheses have been suggested regarding the physiological mechanism underlying tDCS after-effects on M1 including changes in neurotransmitter release, protein synthesis, ion channel function, or receptor activity [34,35]. Insights into this matter were first acquired through pharmacological studies showing a suppression of the after effects of anodal and cathodal stimulation on M1 excitability by the glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist dextromethorphan [36,37] whereas the opposite effect was shown using a NMDA receptor agonist [38]. NMDA receptors are thought to be involved in learning and memory function through long term potentiation (LTP) and long term depression (LTD), both mediated by glutamatergic and GABAergic neurons [39,40]. Animal studies are in line with this hypothesis as they have shown that a-tDCS induces LTP [13].

Despite the important progress made in our understanding of the mechanisms of action underlying tDCS effects, pharmacological protocols present important limitations. Indeed, drug action cannot be as spatially specific as tDCS, especially in the context of human experimentation, and the mechanism of action of their effects is mostly due to post-synaptic receptors [34]. Therefore, there is a need to investigate more directly the effects of tDCS on the human brain. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a good candidate as it allows non-invasive *in vivo* detection of neurotransmitter concentrations in a specific region of interest. This method is based on the principle that every proton-containing neurochemical in the brain has a specific molecular structure and consequently, produces chemically specific resonances that can be detected by ^{1}H-MRS [41]. The acquired signal from the brain's volume of interest is generated from all protons that resonate between 1 and 5 ppm. The acquired neurochemicals are represented on a spectrum and plotted as a function of their chemical shift with some clearly distinguishable peaks, but where many resonances from the different neurochemicals overlap. The signal intensity of each peak is proportional to the concentration of the neurometabolite [41]. The amount of neurochemicals that can be quantified depends on the strength of the magnetic field [42,43]. However, low-concentration metabolites, which are obscured by very strong resonances, are hard to quantify at lower field strength such as 3 T. One way to obtain information about such overlapping signals is to remove the strong resonances via spectral editing. One of such techniques is a MEGA-PRESS sequence, which allows detection of γ-aminobutyric acid (GABA) signals [44,45].

Only a few studies have investigated the effect of tDCS on the brain metabolism using ^{1}H-MRS in motor [34,46] and non-motor regions [47.] Stagg and collaborators [34] assessed the effects of a-tDCS, c-tDCS, and sham stimulation on M1 metabolism. They found a significant reduction in GABA concentration following a-tDCS, and a significant reduction of glutamate+glutamine (Glx) and GABA following c-tDCS. In another study, it was reported that the amount of changes in GABA concentration induced by a-tDCS over M1 was related to motor learning [46].

These studies highlight the potential of combining ^{1}H-MRS with tDCS to increase our understanding of the physiological mechanism underlying the effect of tDCS on motor function. In addition, the use of clinical protocols such as a-tDCS and c-tDCS over M1 is useful because

their behavioral effects are well studied and can be directly related to physiological results. Therefore, a standard protocol for combining bilateral tDCS and ^{1}H-MRS is demonstrated in healthy participants using a 3 T MRI system. Bihemispheric tDCS is presented to contrast data with a previous MRS study where unilateral cathodal or unilateral anodal tDCS were applied over motor cortex [34]. The protocol is described specifically for stimulation with a NeuroConn stimulator in a Siemens 3 T scanner performing MEGA-PRESS ^{1}H-MRS.

6.3 Protocol

The study was approved by the Research and Community Ethics Boards of Unité de Neuroimagerie Fonctionnelle and University of Montréal and was done in compliance with the code of ethics as stated in the Declaration of Helsinki. All subjects gave written informed consent following careful screening for MRI compatibility and were financially compensated for their participation.

6.3.1 tDCS material

1) Make sure all necessary materials are available before starting the experiment (see figure 1 for list).

Note: Different electrode sizes are available for tDCS. For this study, two 5x7 cm rubber electrodes will be used. Other sizes can be chosen depending on the area of stimulation and the desired focality of the stimulation [48].

2) Make sure to check that the batteries of the DC-stimulator are charged and to periodically charge them since the device cannot be charged or plugged-in during stimulation for safety reasons.

6.3.2. Planning of the conditions for stimulation

1) Turn on the tDCS device according to the instructions included with the device. Pre-set the tDCS device for two different stimulation modes (active and sham).

2) As some devices do not have a pre-set mode, select the appropriate sham parameters before starting stimulation.

2.1) Pre-define a set of parameters by loading a setting. Press the button 2 or 4 to select from the main menu the "system" option (see Figure 2).

2.2) Move the cursor to line 2 on the display by pressing the button 3.

2.3) Press the button 2 or 4 until the "load setting" shows on the display. Press the button 3.

2.4) Select the letter of the setting (A, B, C or D) by pressing the button 2 or 4.

2.5) Move the cursor upwards with the button 1. The display will automatically show the "parameters" option.

2.6) Set the tDCS device to a current of 1 mA. To do so, press the button 1 to select the line 3 of the "parameters" menu of the display. Select the "current" option by pressing the button 2 or 4. Press the button 3 to reach the line 4 and modify the intensity to 1000 μA by pressing the button 2 or 4.

2.7) Press the button 1 to go back to the line 3. Select the "fade in" option from the screen menu of the device by pressing the button 2 or 4. Press the button 3 to go to the line 4 and press the buttons 2 and 4 to adjust the duration to 15 s.

Note: Fade in durations can be modified.

2.8) Press the button 1 to go back to the line 3. Select the "fade out" option from the screen menu of the device by pressing the buttons 2 or 4. Press the button 3 to go to the line 4 and press the buttons 2 and 4 to adjust the duration to 15 s.

Note: Fade in durations can be modified.

2.9) Press the button 1 to go back to the line 3. Press the button 2 or 4 until the "duration" option shows on the display menu. Press the button 3 to go to the line 4 and press the button 2 and 4 to adjust the duration to the minimum duration available on the device (15 s for the present device; see Figure 3b).

Note: This will induce a tingling sensation similar to the active stimulation.

2.10) Press the buttons 1 and 3 simultaneously to save the changes of the setting.

3) Pre-program the active stimulation parameters. To do so, follow the same instructions as for the setting of the sham stimulation, but program the duration to 1200 s (20 min; see figure 3a).

4) Pre-program the test stimulation parameters. To do so, follow the same instructions as for the setting of the sham stimulation but program the duration to 45 s.
Note: The test stimulation will be used for the measurement of the impedance prior to the experimentation.

5) Pseudo-randomly assign the conditions of stimulation to participants.

6) Assign a number to each of the three conditions for a blind experimentation: 1) bilateral: anodal right, cathodal left; 2) bilateral: anodal left, cathodal right; 3) sham: anodal right, cathodal left.

6.3.3. Consenting the participants

1) Inform the participant of the procedure and sign consent form.

1.1) Verify that participants do not have any contraindication to tDCS: a psychiatric or neurological history, the presence of a pacemaker, metal implanted in skull, a history of fainting, a history of seizures, a history of substance abuse, a family history of seizure, a history of febrile fits, a lack of sleep in the preceding night, a history of skin sensitivity, and any alcohol consumption the previous day.

1.2) Inform the participant of the most reported side-effects of tDCS: mild tingling; moderate fatigue; light sensation of itching under the electrodes; slight burning sensation.

2) Inform the participant of the usual MR contraindications and side effects.

6.3.4 Measurements for electrodes placement

1) Use the 10/20 international system to find the following landmarks on the participant head: nasion and inion (Figure 4a), preauricular points, and the two targeted areas: C3 and C4 (Figure 4b).

1.1) Locate the nasion as the distinct depressed area located on the bridge of the nose at he level between both eyes. Locate the inion as the most prominent projection of the occipital bone located at the lower part of the skull. Locate the preauricular point near each ear; it is the indentation above the zygomatic notch. Locate the C3 and C4 based on measurements as described below.

2) Use a measuring tape to measure the distance between the nasion and inion along the midline of the head and make a mark at 50% of the distance with a non-permanent hydro marker.

3) Use a measuring tape to measure the distance between the two preauricular points and make a mark with a non-permanent hydro marker at 50% of the distance in line with the previous mark. This point corresponds to Cz (vertex).

4) From the Cz, along the line created between the preauricular points, mark two points, one on each side, with a non-permanent hydro marker that correspond to 20% of the total distance. These marks correspond to the target areas (C3 and C4, figure 4b).

Note: Other methods such as TMS or neuronavigation can also be used to localize M1.

6.3.5 Placement of electrodes

1) Move as much hair as possible away from the targeted areas that will be stimulated. Apply an EEG-type exfoliating gel with a cotton-swab to clean the targeted areas.

2) Clean the targeted areas with a 70% isopropyl alcohol and pumice prepping pad to enhance electrode contact.

3) Generously cover the entire electrode with an EEG-type conductive paste. Ensure that the paste is approximately 5 mm thick across the entire surface. Make sure the entire rubber area is covered with paste. Lightly wet the target areas and the conductive paste on the electrodes with a saline solution.

4) Position the electrodes as shown in Figure 4b and press the electrodes firmly onto the targeted areas. Place a rubber band around the head of the participant to ensure optimal stability of the electrodes. Adjust it in such a way that the participant will experience no pain or discomfort during the scanning session.

5) Make sure that the leads do not come in contact with the skin to avoid potential burns.

6.3.6 tDCS test outside the scanner room

1) Use a multimeter to verify the proper functioning of the electrode cable and resistance.

2) Turn on the tDCS device and load the test stimulation settings.

2.1) Press the button 2 or 4 to select from the main menu the "system" option. Move the cursor to line 2 on the display by pressing the button 3. Press the button 2 or 4 until the "load setting" shows on the display. Press the button 3. Select the letter of the pre-programmed test setting (A, B, C or D) by pressing the button 2 or 4.

2.2) Move the cursor upwards with the button 1. The display will automatically show "parameters" option. On the first line, press the button 2. The display will show "stimulation?" with the different pre-programmed parameters.

3) Press the button 1 to start the stimulation. The display will show the impedance level and automatically stop if it reaches more than 20 kΩ. If the impedance level is over 20 kΩ, unplug the electrode wires from the inner box and exit the scanning room to verify the positioning of the electrodes.

4) Redo the test stimulation. When a good level of impedance is reached and when the test stimulation is over, unplug the electrodes from the inner box.

6.3.7 tDCS setup

1) As shown in figure 5, place the tDCS device and the outer box in the scanner control room.

Note: The tDCS device and the outer box are not MR compatible and should not be taken into the magnet environment.

2) Plug the outer box wires into the tDCS device and then plug the long box cable into the outer box.

3) Run the tDCS box cable from the scanner control room into the MRI room. Make sure to run this cable as straight as possible, avoiding any kinks or loops, along the wall of the MRI room towards the back of the MRI scanner. Put multiple MR compatible sandbags on the cable to ensure its stability, as shown in figure 5.
4) Bring the inner box into the MRI room and plug the long box cable into it (Figure 5).

6.3.8 MRI scan preparation

1) Ask the participant to enter the MRI room, if not already in there from the tDCS test, and to put in earplugs.

2) Put a thin cushion under the coil area of the MRI table. Ask the participant to lie down on the table. Put a cushion under the legs of the participant for comfort and a blanket if needed. Give the participant the alarm button for security purposes.

3) Put separate headphones over both ears to allow transmission of information from the scanner control room to the participant in the MRI room.

4) Position the participant's head as high as possible under the area where the head coil will be positioned (top of the head as close as possible to the top of the table where the coil will be placed). Put the electrode wires along the right side of the head of the participant, as recommended by the tDCS device company.

5) Place the 32-channel receive-only coil around the head of the participant. Run the electrode cables through the right side of the coil. Position the head of the participant as straight as possible using a red positioning laser (built-in feature of the scanner).

6) Ask the participant to move arms and legs into a comfortable position, while making sure that hands do not touch. Make sure to remind the participant to stay as still as possible during the entire session. When the participant is ready, move the table past the middle line to reach the electrode wires at the back of the scanner.

7) Use medical tape to stabilize the electrode cable on the right side of the back of the coil. Plug the electrode wires located inside the scanner into the tDCS inner box. Put the inner box on the right side of the scanner with a sandbag on it for maximal stability.
8) Move the table back into its final position. Keep the tDCS turned on and the electrodes plugged into the outer box for the entire MRI session.

6.3.9 Pre-tDCS ^{1}H-MRS session

1) Run a localizer sequence to acquire images needed to verify the proper positioning of the head and to compare to a second localizer which will be acquired at the end of the session to check for overall movement.

2) Acquire anatomical T_1-weighted MPRAGE images for the positioning of the M1 voxel and detection of possible structural abnormalities (T_R = 2300 msec; T_E = 2.91 msec; FA: 9°; FOV = 256 x 256 mm; 256 x 256 matrix; T_I : 900 msec; 176 slices; orientation: sagittal; acquisition time: 4 min 12 s).

3) Perform a multi-planner reconstruction of the images in planes that are more appropriate for visualization of the spectroscopy volume-of-interest (VOI).

3.1) In the 3D card, browse the MPRAGE raw images (sagittal orientation). From the "creating parallel ranges" window select "axial 2X2". Adjust the position of the parallel lines and click on save to create the axial orthogonal view.

3.2) From the "creating parallel ranges" window select "coronal 2X2". Adjust the position of the parallel lines and click on "save" to create the coronal orthogonal view.

4) Locate the left M1 based on Yousry and collaborators' [49] anatomical landmarks on the three orientation slices. Then, position the VOI (30 x 30 x 30 mm^3) on the area without any angulation relative to the scanner axis (figure 6).

5) Acquire a line-width scan (21 s).

5.1) Select the spectroscopy card to measure water line-width on the real part of the signal from this line-width scan. Load the line-width raw data from the browser. Load the line-width measurement protocol (protocols menu: select the protocol).

5.2) Adjust the phase using the scanner software interactive post-processing tools. Select the phase correction section and adjust the phase for the baseline with the cursor.

5.3) In order to reduce the line-width, run the FAST(EST)MAP [50] sequence three times. Repeat the line-width scan and the line-width measurement (step 9.5). Note the final water line-width.

6) Start 4 blocks of 64 metabolite scans (32 "EDIT OFF" and 32 "EDIT ON", interleaved) with a MEGA-PRESS sequence [44,45], where VAPOR [51], OVS [51] and individual storage of FIDs are enabled (T_R = 3 s, T_E = 68 msec, total acquisition time: 12 min)

7) Acquire a water reference using MEGA-PRESS sequence without MEGA water suppression, with VAPOR suppression ("only RF off") and with a delta measurement at 0 ppm. Acquire a single block of 4 metabolite scans instead of 64 (acquisition time: 42 s).

6.3.10 tDCS procedure

1) Inform the participant that the tDCS stimulation will start and that the scanner will be silent for the entire stimulation.

2) Select one of the two previously programmed parameters according to the condition and start the stimulation. Keep track of the impedance and voltage during the 20 min of stimulation. When the stimulation is over, notify the participant that the post-tDCS MRS session will begin. Do not turn off the tDCS device.

6.3.11 Post-tDCS ^{1}H-MRS session

1) Run the same metabolite scans with MEGA-PRESS sequence as the pre-tDCS scan but double the blocks of acquisition (8 blocks of 64 scans (32 "EDIT OFF" and 32 "EDIT ON", interleaved)) to acquire the metabolites at two different time points post-tDCS.

2) As with the pre-tDCS session, acquire a water reference scan using the same parameters. Finish the session with a localizer sequence.

3) Visually compare the localizer images acquired at the beginning and end of the scanning session as an index of head motion.

4) Access the viewing card and go to the browser menu. Select the first and second localizer raw images. Load the images in the viewing card and compare both images. Export data in the dicom format through the server.

6.3.12 Analysis of the ^{1}H-MRS data

1) Import data using a programming and processing software, and adjust frequency and phase of individually stored FIDs using tCr and tCho signal between 2.85 and 3.40 ppm. To do so, use the software's lsqnonlin function to fit frequency and phase of each individual Fourier-transformed FIDs (spectra) to the average spectra of the session.
Note: this is a site-specific approach and other methods for importing and analyzing data will not necessarily affect data quality.

2) To obtain the final spectra, subtract the signals from alternate scans with the selective double-banded pulses that were applied at 4.7 ppm and 7.5 ppm ("EDIT OFF") and at 1.9 ppm and 4.7 ppm ("EDIT ON") (figure 7).

3) Use the LCModel [52] for the analysis of both difference and "EDIT OFF" spectra. Deactivate default simulations and baseline modeling.
4) Perform a visual inspection of the spectra to exclude sessions with contamination from subscapular lipid signal (see figure 9).

5) As part of quality control, exclude spectra with linewidth of tCr-CH_3 above 10 Hz. Only include in the analysis metabolites (GABA, Glx, tCr, tNAA) which were quantified with Cramer-Rao lower bounds (CRLB) lower than 35%.
Note: CRLB provide estimated error of the metabolite quantification. CRLB > 50% is not reliable and is a recommended cut-off by LCModel manual. Many in the field have used a CRLB lower than 35% as a standard. [53-55] Additionally, the CRLB should be kept in mind when interpreting the results.

6) Obtain GABA and Glx quantifications from the "DIFF" spectra, tCr from the "EDIT OFF" spectra, and tNAA from both "EDIT OFF" and "DIFF". Express concentrations of the different metabolites of interest as ratios over tCr. For GABA and Glx, multiply the ratio by the following group-averaged correction factor to account for the different basis set used for the numerator and denominator (tNAA from "EDIT OFF" spectra / tNAA from "DIFF" spectra).
Note: GABA and Glx concentrations can also be quantified using water or NAA signal.

6.4 Representative results

Figure 6 shows the position of the VOI located on the representation of hand in M1 where all MRS measures were taken. In figure 6D, a 3D visualization shows a clear representation of the tDCS electrodes positioned on the scalp over the putative primary motor cortex. Figure 7 shows representative "EDIT OFF" and difference ("DIFF") spectra acquired in M1. Peaks corresponding to Glx, GABA+MM as well as NAA can be clearly seen.

Figure 8 shows the percentage of change between the MRS acquisition pre-tDCS and post-tDCS for the three different conditions in a single participant. Results from the post-tDCS session are separated into two time points to illustrate the evolution of change over time. Figure 8a shows the percentage of change for Glx. For sham stimulation, Glx concentration displays no notable modulation. For bilateral stimulation 1 (left anodal, right cathodal), again no notable modulation of Glx is observed; however, modulation of the concentration over time is opposite to what is observed in the sham stimulation. Finally, regarding bilateral stimulation 2 (left cathodal, right anodal), a similar pattern is observed to the sham stimulation but with a slight reduction of the Glx concentration in the second time-point following stimulation.

Figure 8b shows the percentage of change in the concentration of GABA in relation to the condition of stimulation. For the sham stimulation, GABA concentration displays no notable modulation. However, a slight reduction is observed at both time points. The modulation of GABA following the sham stimulation is more important than for the Glx. In contrast, a notable increase of GABA concentration is seen in the second-time point after bilateral stimulation 1 (left anode, right cathode). Finally, a similar pattern of change to the sham stimulation is observed for bilateral stimulation 2 (left cathode, right anode).

Figure 9 shows the obtained spectra from two different participants. Figure 9a shows a spectrum of good quality with an acceptable lipids signal. Figure 9b shows a spectrum with large lipids signals, which was excluded after visual inspection. Finally, figure 10 shows displacement of the location of the voxel of interest following 5 mm participant movement.

6.5 Discussion

The present paper aimed to describe a standard protocol for combining tDCS and ^{1}H-MRS using a 3 T scanner. In the next section, methodological factors will be discussed.

6.5.1 Critical steps

6.5.1.1 Contraindications screening

Previous to the experiment, it is crucial to screen participants for any contraindication regarding the use of tDCS and ^{1}H-MRS. The use of the following exclusion criteria is recommended for tDCS: a psychiatric or neurological history, the presence of a pacemaker, a piece of metal implanted in the skull, a history of fainting, a history of seizures or a history of substance abuse. Because only metabolites from the left M1 will be acquired, the exclusion of left handed participants from the study is recommended. In fact, a recent study has shown differential interhemispheric inhibition between the dominant and non-dominant hemispheres depending on the hand preference, which could modulate the effect of stimulation [15]. Moreover, before starting the experiment, check for any lesion on the scalp and ask for any skin disease [56]. If there is a lesion present, try to avoid stimulating directly the affected area. It is also recommended to inspect the skin after stimulation [57]. Also, screen for the presence of allergies to any of the products used for electrode montage. For ^{1}H-MRS, the exclusion criteria should be the same as for any magnetic resonance imaging study including a careful screening of any prior surgeries for the presence of metal in the body.

It is also important to determine if the participant felt any discomfort during tDCS stimulation. Again, after the experiment, participant should be asked about any side effects. It is possible to use a record-form including the most reported side effects to quantify their presence in relation to the protocol (see [58] for an example). The most reported side effects are mild tingling (70.6%), moderate fatigue (35.3%), a light sensation of itching under the electrodes (30.4%), and slight burning sensation (21.6%) [58].

6.5.1.2 Movement artefacts reduction

Movement of the participant in the scanner is a major issue during ^{1}H-MRS as this is one of the main factors affecting the quality of the data [59]. As shown in figure 10, a movement of the subject (from 1 mm to 5 mm) can lead to large lipids signal in the spectrum thus altering the quality of the data and consequently, to the exclusion of this acquisition from the data. Therefore, it is crucial to carefully explain to the participant the importance of head stability

during the entire scan. During the positioning of the participant in the scanner, it is important to ask the subject to find the most comfortable position to avoid any further movement. During positioning of the VOI, it is also important to notify the participant that even though the scan is silent, it is essential to remain still.

In addition, the duration of the experiment is an important factor to help minimize total amount of movement. First, it is important to use an optimal length for the anatomical sequence, as short as possible, but long enough to obtain good quality images for placement of the VOI. Second, the use of a short sequence of metabolite acquisition is recommended before tDCS. Third, in order to capture the temporal course of stimulation effects, the use of a longer sequence of acquisition after stimulation is advised. Fourth, compare pre- and post-experiment localizer images to estimate participant movement.

6.5.1.3 Analysis

The MEGA-PRESS sequence is used to acquire localized, water suppressed, and edited spectra. A spatial localization in PRESS is performed using a 90° Hamming-filtered sync pulse (bandwidth time product = 8.75, duration = 2.12 msec, bandwidth (FWHM) = 4.2 kHz) and two 180° mao pulses (duration = 5.25 msec, bandwidth = 1.2 kHz). All localization pulses are executed at 3 ppm. A selective double-banded 180° Shinnar-Le Roux pulse is applied at 1.9, the resonance frequency of β–CH_2 of GABA, and 4.7 ppm alternating with 7.5 and 4.7 ppm. Additional water suppression using variable power with optimized relaxation delays (VAPOR) and outer volume suppression, OVS[50] were adapted for the human 3 T system and incorporated prior to MEGA-PRESS and are used to suppress water and to improve the localization of the VOI. When the selective pulse is applied at 1.9 ppm, the resonance at 1.9 ppm and the resonances within the bandwidth of the pulse are inverted causing refocusing of γ–CH_2 resonance of GABA ("EDIT ON"). When the selective pulse is applied at 7.5 ppm, the usual spectrum at T_E of 68 msec is obtained ("EDIT OFF") with the γ–CH_2 resonance of GABA phase modulated. The subtraction of signals from alternate scans results in selective observation of outer lines of GABA triplet and cancelation of the total creatine (creatine + phosphocreatine) resonance ("DIFF"). Due to the bandwidth of the inversion pulse, additional resonances of

NAA, Glu + Gln, and macromolecules are also observed. The whole protocol is divided into four interleaved acquisitions and the frequency is updated before each individual scan to minimize the frequency drifts due to the hardware. The interleaved acquisition and single FID storage allows the correction of frequency and phase in post-processing.

The analysis method described in the protocol allows the calculation of the best fit of the experimental spectrum as a linear combination of model spectra. Model spectra in the basis set for "EDIT OFF" spectra were simulated based on density matrix formalism [59] and known chemical shifts and *J* couplings [60], and included the following: acetyl moiety of *N*-acetylaspartate (sNAA), alanine (Ala), ascorbate (Asc), aspartate (Asp), aspartate moiety of NAA (mNAA), CH_2 group of Cr (Cr-CH_2), CH_3 group of Cr (Cr-CH_2), CH_2 group of PCr (PCr-CH_2), CH_3 group of PCr (PCr- CH_2), GABA, glucose (Glc), Glu, Gln, glycerophosphorylcholine (GPC), glycine (Gly), glutathione (GSH), lactate (Lac), *myo*-inositol (mI), *N*- acetylaspartylglutamate (NAAG), phosphorylcholine (PCho), phosphorylethanolamine (PE), *scyllo*-inositol (sI), and taurine.

The basis set for "DIFF" spectra was generated from experimentally measured spectra of four 100 mM solutions of NAA, GABA, Glu, and Gln (600 ml spherical glass flasks) using the same parameters and scanner as for *in vivo* experiments. Each solution additionally contained K_2HPO_4 (72 mM), KH_2PO_4 (28 mM), sodium azide (0.1 mM), 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (TSP; 2 mM), formate (200 mM; optional), and distilled water. The basis set spectra were acquired at the physiological temperature of 37°C and every effort was made to minimize cooling (~1°C within the 15 of acquisition) by preheating the phantoms in a large water tank before placing each one in a smaller water-filled isolated plastic container, which was placed in the coil. Temperature and pH are particularly important in spectroscopy because they affect the chemical shift of the metabolites. Additionally, for both "EDIT OFF" and "DIFF" spectra, basis sets included a metabolite-nulled macromolecular spectrum experimentally measured from 10 subjects from the occipital cortex using the inversion-

recovery (inversion time, T_I = 760 msec) technique using the same parameters as the regular MEGA-PRESS acquisition (except for T_R = 2.7 s) [61].

6.5.1.4 Phantom testing

Testing the procedure on a 100 mM GABA phantom with and without the tDCS stimulator that will be used on participants with the exact scanner and sequence parameters is strongly advised prior to the first participant being studied. The procedure should include a localizer sequence, an anatomical sequence (i.e. MPRAGE), a line-width scan and 16 "EDIT ON" and "EDIT OFF" scans. This should be repeated if stimulator, stimulation parameters or scanners are changed. In order to investigate the presence of artefacts on the signal, one should review spectra for changes in SNR with and without the tDCS simulator, presence of spikes and noise at certain frequencies, and the SNR values and any important artefact on the anatomical images.

6.5.2 Possible modifications to the protocol

6.5.2.1 ^{1}H-MRS parameters

To acquire metabolite concentrations using ^{1}H-MRS, it is necessary to localize a specific region and excite signals in this volume [35]. In the present paper, the procedure for the placement of a single VOI over left M1 was described. However, many different modifications to this protocol can be applied. Successful measurement of metabolite concentrations have been demonstrated in various cortical and subcortical regions, such as the prefrontal cortex [62], hippocampus [63], cerebellum striatum and pons [64], visual cortex [66], and auditory cortex [67]. The size of the VOI can also differ as a function of the region of interest, but the volume typically ranges between 3 and 27 cm^3 [68]. However, it is hard to obtain concentration of low-concentration metabolites such as GABA from voxels smaller than 20 cm^3. An important issue is to make sure to avoid any contact of the VOI with the cranial bones, meninges, and extra-cerebral cerebrospinal fluid. In smaller brains, the VOI might include part of the left lateral ventricle. In this case, the inclusion of the ventricle is preferable over the inclusion of cranial bones.

Additionally, depending on the selected acquisition sequence, different metabolites can be quantified [69]. Previous methods, such as the Point-RE-Solved spectroscopy (PRESS) sequence [70] and stimulated echo acquisition mode (STEAM) [71], did not allow quantification of GABA at 1.5 T. However, because of the polarity-specific effect of tDCS on cortical excitability, the quantification of both excitatory (glutamate) and inhibitory (GABA) neurotransmitters is essential. In the present protocol, the use of the MEGA-PRESS spectral editing sequence [44,45] was shown, which allows the quantification of the major neurochemicals, including GABA (see figure 6). Other sequences allowing GABA quantification, such as ultra-short TE MRS and *J*-resolved MRS, have been developed over the last few years (see [41] for a review).

Finally, since metabolite concentrations are usually expressed as a ratio in relation to another metabolite (relative concentration), the choice of the reference metabolite is highly important, and particularly so in studies employing clinical populations [69]. The most commonly used reference metabolites are tCr and NAA, as their concentrations are found to be relatively stable in the human brain. It should be noted it is also possible to use an absolute quantification of metabolites which requires referencing to either an external (e.g., phantom) or internal signal (e.g., water signal) [68]. The use of an internal water reference requires an additional step of tissue correction since the water concentration and relaxation properties differ between grey matter, white matter and cerebrospinal fluid (CSF) [72]. The tissue correction can be performed either using the estimated tissue composition in the VOI of all participants or using subject specific tissue composition from segmentation [73]. Additionally, it should be noted that tDCS carries the theoretical risk of inducing oedema, which could have a minor impact on water concentrations. However, Nitsche and collaborators [74] directly assessed this specific concern and showed no evidence of oedema following tDCS on the frontal cortex. Consequently, the use of a water reference is considered a viable option.

6.5.2.2 tDCS parameters

Different electrodes sizes can be used [9] depending on the region of stimulation and the desired focality of stimulation [75,76]. Da Silva and collaborators [56] provide a comprehensive

description of the different types of electrodes that are currently available for tDCS. Furthermore, as described in the present paper, ^{1}H-MRS is a useful technique that can be used to verify the underlying mechanisms of action of specific tDCS protocols that have been shown to improve symptoms in different clinical populations. Electrode positioning and duration of stimulation can be modified to investigate the effects of these specific tDCS protocols, such as those one used in the treatment of pain, depression, tinnitus, Parkinson's, migraine, and alcohol abuse (see [77] for a description of the protocols). It should also be noted that if the impedance level is above 20 kΩ, the device will not stimulate and display an impedance error message on the screen. Different factors that can cause a high impedance include: 1) insufficient amount of conductive paste on the electrodes; 2) insufficient pressure on the electrodes; 3) bad contact with the scalp (caused by hair); 4) thickening of the scalp due to baldness; 5) problems with connections; 6) problems with wiring; (7) problems with stimulator; and 8) problems with electrodes.

It should also be noted that localization of primary motor cortex for tDCS could be made more precise. In the present protocol, the 10/20 EEG system is used, which may introduce slight misalignment between maximum electrical field projection and actual representation of M1 within precentral gyrus. One possible way to circumvent this issue is to use transcranial magnetic stimulation to precisely localize the hand representation in M1 through the TMS-induced muscular response. Availability of a TMS unit in the vicinity of the MR scanner may limit this possibility.

6.5.3 Safety of tDCS and ^{1}H-MRS

6.5.3.1 Safety of tDCS

Multiple studies have shown that tDCS is a safe neuromodulation technique producing only minor adverse effects in both non-clinical and clinical populations [10]. In fact, no case of epileptic seizure has ever been reported following tDCS [10]. However, the safety of tDCS has yet to be investigated in children and pregnant women [78].

6.5.3.2 MR compatible materials

Caution should be taken when stimulating inside a MR scanner. All materials brought into the MR room must be MR compatible (see figure 1). Because of the possible interaction between the electric current produced by the tDCS and the MR scanner, tDCS should always be turned on, and the electrodes should remain connected, during the MR sequences described in the present protocol. Coiling of the wires under the head coil can produce artefacts and distortions in the signal. Moreover, improper connection of the wires could potentially produce a current strong enough to burn the participant [79]. Finally, it is important to never disconnect the electrodes while the current is flowing as this might cause an unwanted high-voltage stimulation.

6.5.4 tDCS-MRS technique

Using tDCS in conjunction with MRS offers the possibility to better understand the mechanism underlying modulation of brain activity with this relatively new neuromodulation technique. However, some limitations of the technique should be addressed. First, the electrodes used in tDCS are usually rather large and the effects of stimulation are believed to cover a wide spatial extent of brain tissue. Coupled with the fact that MRS acquisition is limited to a small voxel of interest, tDCS-MRS only allows for the assessment of spatially circumscribed effects despite presumed widespread modulation of brain excitability. One possible way to circumvent this problem is to use multiple voxels of interest distributed throughout the brain. However, this will significantly increase duration of the experimental session, which is already a major limitation of the present technique. Indeed, when considering participant preparation, pre-tDCS MRS, tDCS intervention and post-tDCS MRS, a full session may easily last up to two hours. Duration can also increase if one wishes to map the time course of tDCS effects on metabolite concentration.

An important issue related to the duration of the experiment is the possibility that electrode impedance will increase after the participant is in the scanner. Since tDCS can easily begin more that 45 minutes after electrode placement, there is a risk that the stimulating

electrodes will gradually lose adherence to the participant's scalp if paste application is not optimal and electrodes are not held tightly enough. If impedance reaches more than 20 kΩ, stimulation will not be possible and the participant will need to be removed from the scanner to solve the problem. Since the described procedure involves multiple scanning of the same area pre- and post-tDCS, removing the participant from the scanner may create important displacement of the voxel of interest. It is therefore very important to test impedance immediately prior to scanning and to take great care when installing electrodes.

Theoretically, the current flow of the tDCS could produce artefacts in the MR signal. Antal and collaborators [80] investigated this specific concern by measuring the impact of different tDCS conditions (with and without electrodes, with and without stimulation, etc.) on the quality of functional magnetic resonance images. However, to our knowledge, the presence of artefacts in the spectroscopy signal due to the presence of the tDCS device in the scanner has yet to be assessed.

Finally, care should be taken with regards to the resistors in the electrode cables. The MR field may damage resistors, thus preventing stimulation. As a precautionary measure, resistance should be tested outside the scanner environment prior to every MRS session. In addition, an impedance of more than 20 kΩ can lead to skin reactions and high impedance may reflect an incipient or actual problem with the stimulator. Therefore, the stimulator should be checked carefully before every participant and impedance levels checked outside the scanner room prior to every MRS session.

Combined tDCS and ^{1}H-MRS is a powerful tool that provides a quantitative measure of the effect of clinically used treatments on brain metabolism. As the physiological mechanism of tDCS effects remains poorly understood, there is a need for multimodal approaches that can shed light on these processes. With the recent surge in interest in tDCS as a clinical tool for pathologies such as stroke [27,30,31] and depression [81], it is clear that combination of tDCS with MRS may be an important tool to better understand the therapeutic effects of tDCS. Furthermore, tDCS-MRS may serve as an early tool to determine which patients have a better

chance to respond clinically to tDCS. If such a marker is found, tDCS-MRS may be used as a screening test prior to enrolling patients in a tDCS intervention.

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6.7 Disclosures

The authors have nothing to disclose.

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6.9 Figure

Figure 1: Materials

Legend : 1) Saline solution; 2) Conductive paste; 3) Electrode gel; 4) Alcohol prepping pad; 5) Measuring tape; 6) EEG pencil; 7) Rubber bands; 8) Inner box; 9) tDCS device; 10) Outer box; 11) Inner box cable; 12) Outer box cable; 13) Electrodes; 14) Long box cable

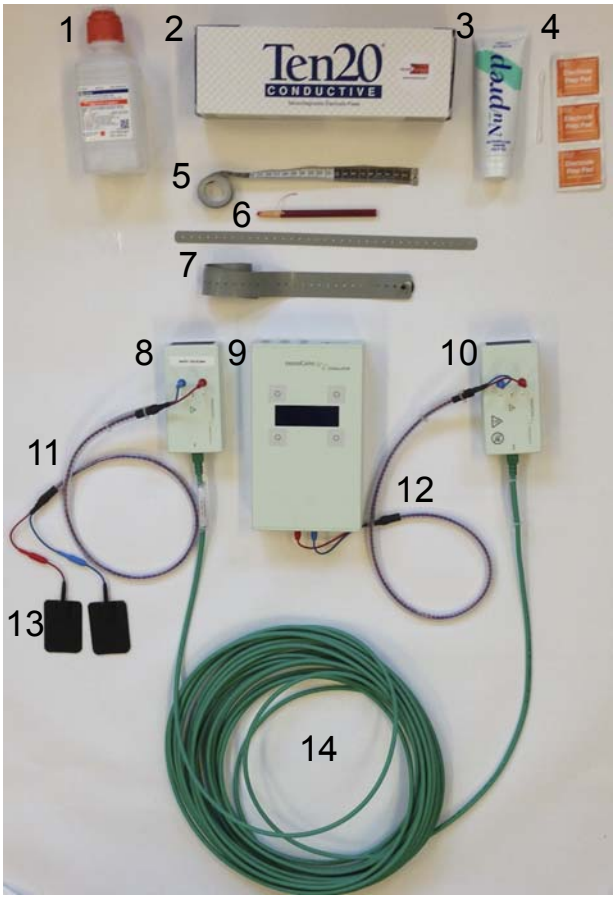


Figure 2: tDCS device

Legend : Image of the positioning of the buttons on the specific tDCS device used in the present protocol. These buttons are used to pre-set the different settings.

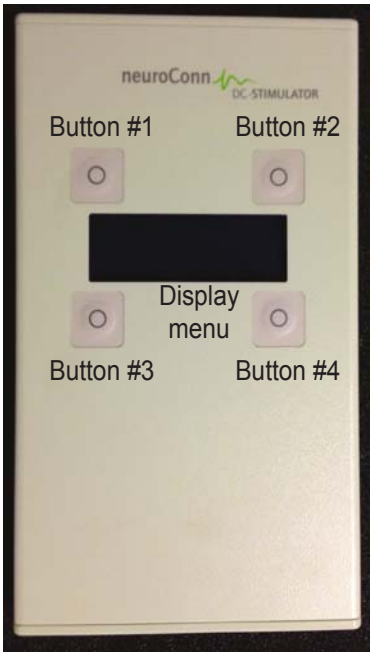


Figure 3: Time course of tDCS conditions

Legend : A) Time course of the active tDCS condition. After the pre-tDCS metabolite acquisition, turn on the tDCS device and ramp-up the current for 15 s until an intensity of 1 mA is reached. Stimulate for 20 min and ramp-down the current for 15 s until an intensity of 0 mA is reached. Do not turn off the tDCS device and proceed to the post-stimulation metabolite acquisition. B) Time course of the sham tDCS condition. After the pre-tDCS metabolite acquisition, turn on the tDCS device and ramp-up the current for 15 s until an intensity of 1 mA is obtained. Stimulate for 15 s (the minimum time available on the current device) and ramp-down the current for 15 s until an intensity of 0 mA is reached. Wait for 20 min. Do not turn off the tDCS device and proceed to the post-stimulation metabolite acquisition.

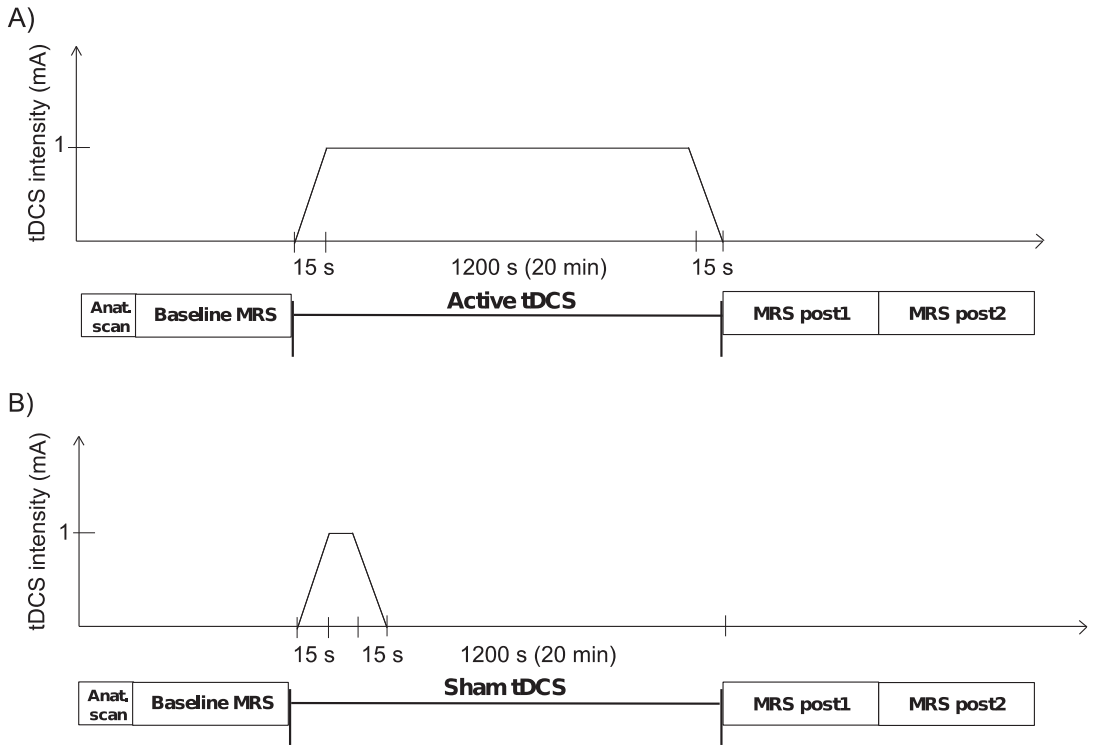
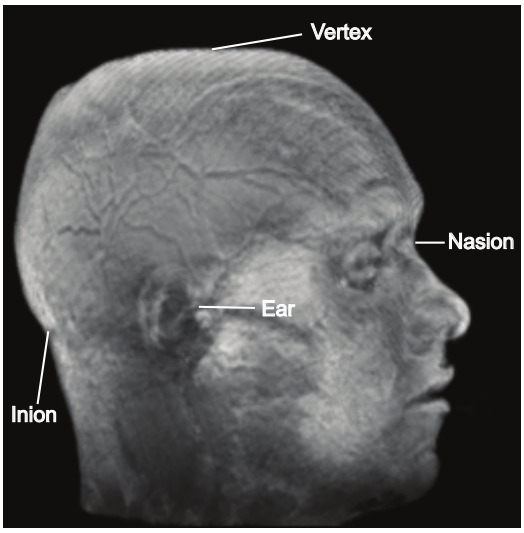


Figure 4: Electrode positioning

Legend : A) 10/20 international system landmarks used for the identification of C3 and C4. The vertex (Cz) corresponds to 50% of the distance between the nasion and the inion, and 50% of the distance between the two preauricular points. B) C3 and C4 correspond to 20% of the total distance between the preauricular points, measured from the vertex point. Make sure to leave at least 8 cm of distance between both electrodes.

A)



B)

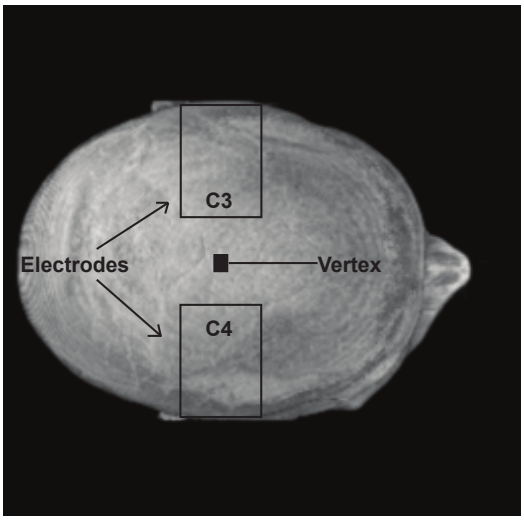


Figure 5: Schematic view of the MR room

Legend : Placement of the materials in the MR scanning and console rooms. It is essential to follow the protocol for the positioning of the different parts of the device in order to obtain a MR signal of good quality and for safety purposes.

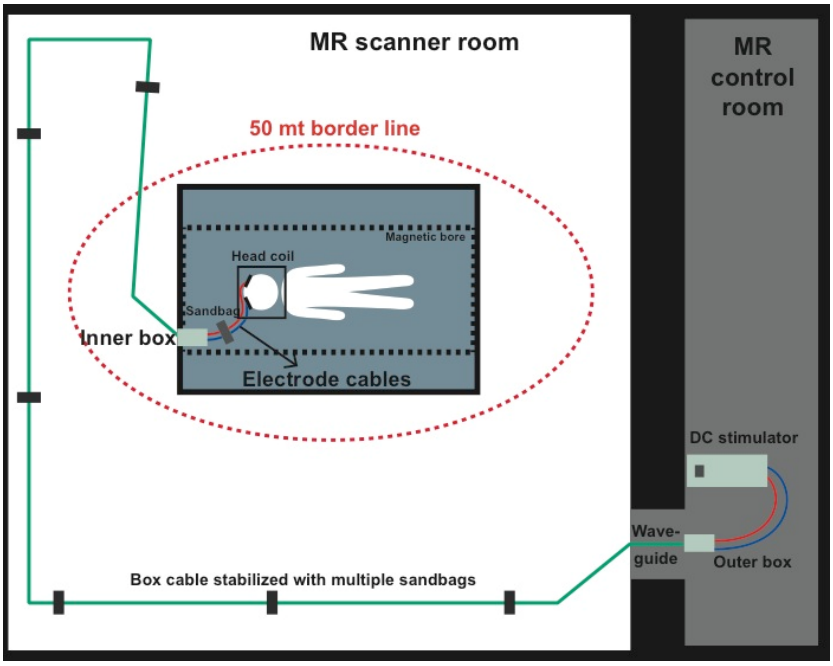


Figure 6: VOI placement

Legend : Position of the VOI (30 x 30 x 30 mm^3) over the left hand area of M1 in (A) sagittal, (B) coronal, and (C) axial slices. 3D visualization of the positioning of the electrodes is shown in (D).

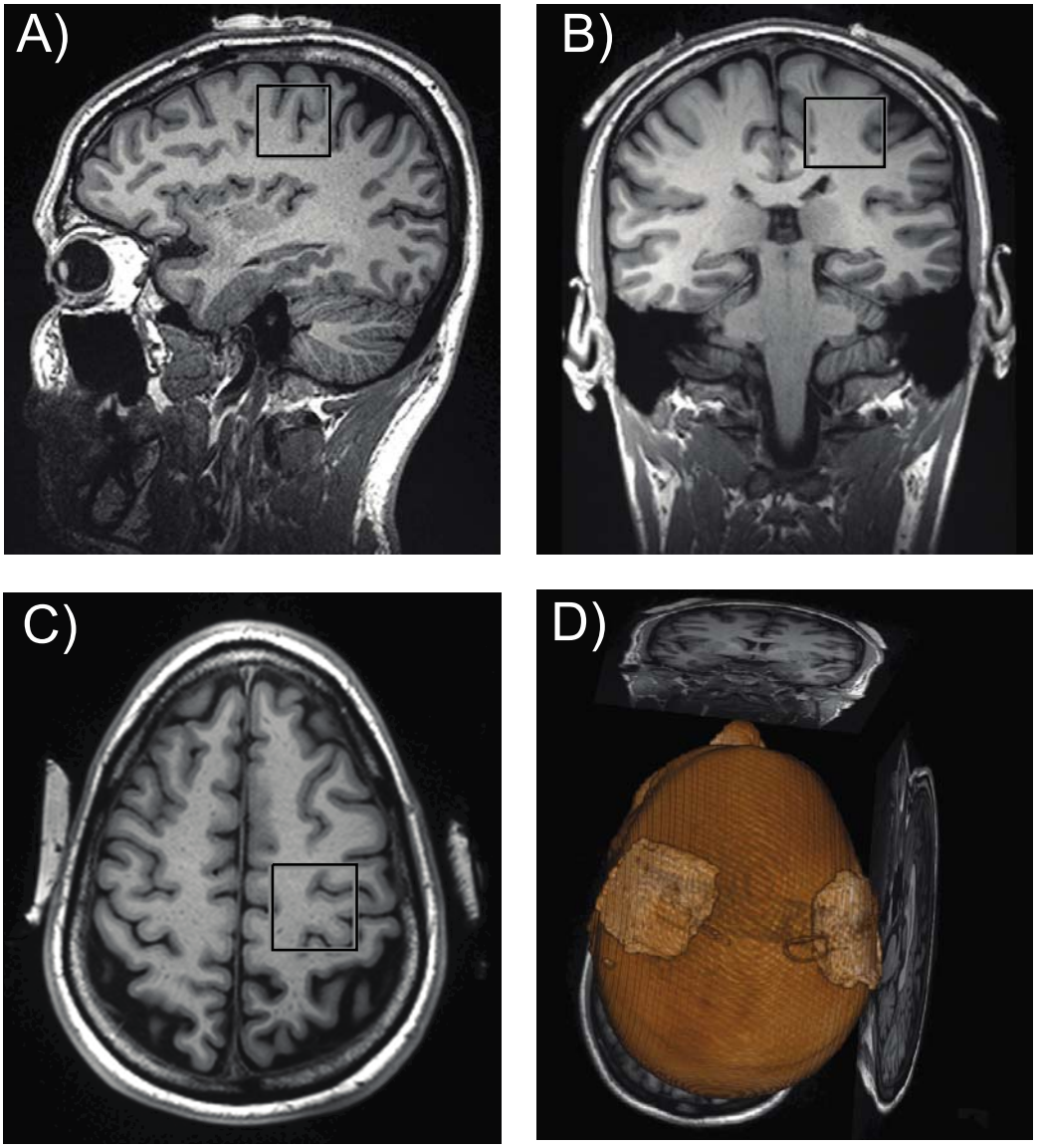


Figure 7: ^{1}H-MRS metabolite spectrum

Legend : Representative (A) "EDIT OFF" and (B) difference ("DIFF") spectra acquired with the MEGA-PRESS sequence [44,45] including the raw data, the fit from LCModel and the residuals.

Cr: total creatine (creatine + phosphocreatine (Cr-CH_3 + PCr-CH_3)); NAA: *N*-acetyl-aspartate + NAAG (sNAA + NAAG); Glx : glutamate + glutamine (Glu + Gln); GABA + MM: γ-aminobutyric acid + macromolecules

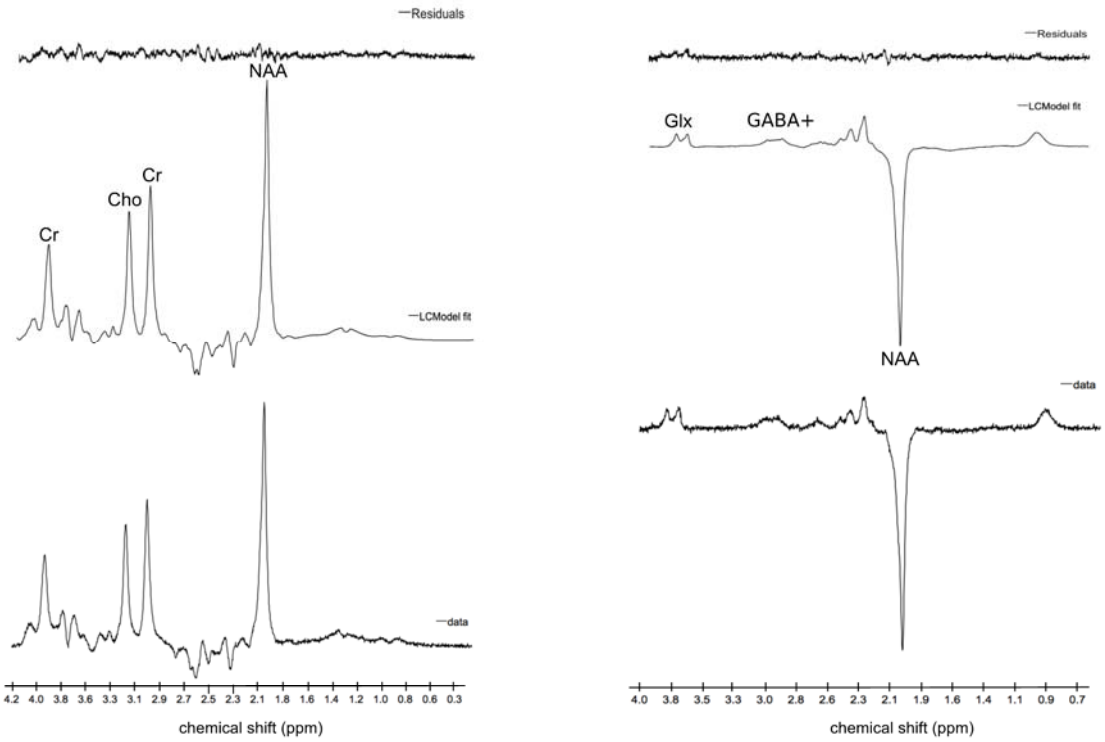


Figure 8: Effects of bilateral tDCS on Glx and GABA for a single subject

Legend : A) tDCS effects on Glx concentration are shown for the three conditions. Results are expressed as percentage of change between the pre-tDCS acquisition and the two post stimulation acquisitions. B) tDCS effects on GABA concentration are shown for the three conditions. Results are expressed as percentage of change between the pre-tDCS acquisition and the two post stimulation acquisitions.

Sham: Bilateral, Bilateral 1: left anode, right cathode; Bilateral 2: left cathode, right anode

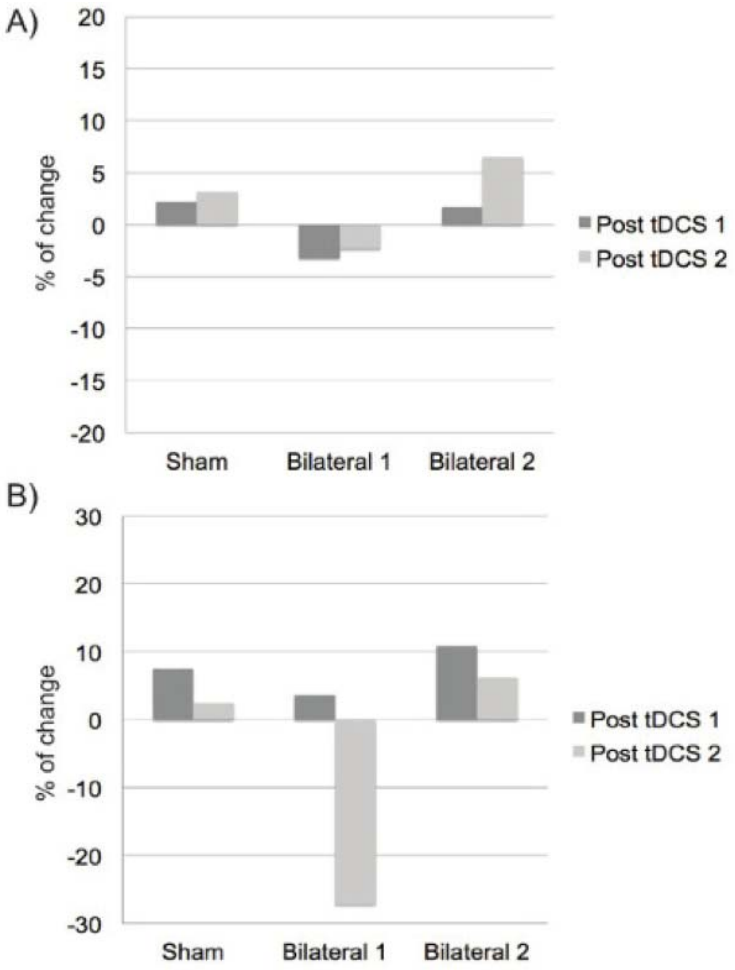


Figure 9: Visual inspection of the spectra

Legend :

A) Example of a good quality data. The figure shows the ”EDIT OFF” and ”DIFF” spectra with an acceptable amount of lipids.

SNR from analysis of ”DIFF” spectra: 56

CRLB of the GABA signal: 14%

Lw of tCr-CH_3 at 3 ppm: 5.6 Hz.

B) Example of a poor quality data caused by excessive movement of the participant. The figure shows the ”EDIT OFF” and ”DIFF” spectra.

SNR from analysis of ”DIFF” spectra: 39

CRLB of the GABA signal: 47%

Lw of tCr-CH_3 at 3 ppm: 4.4 Hz

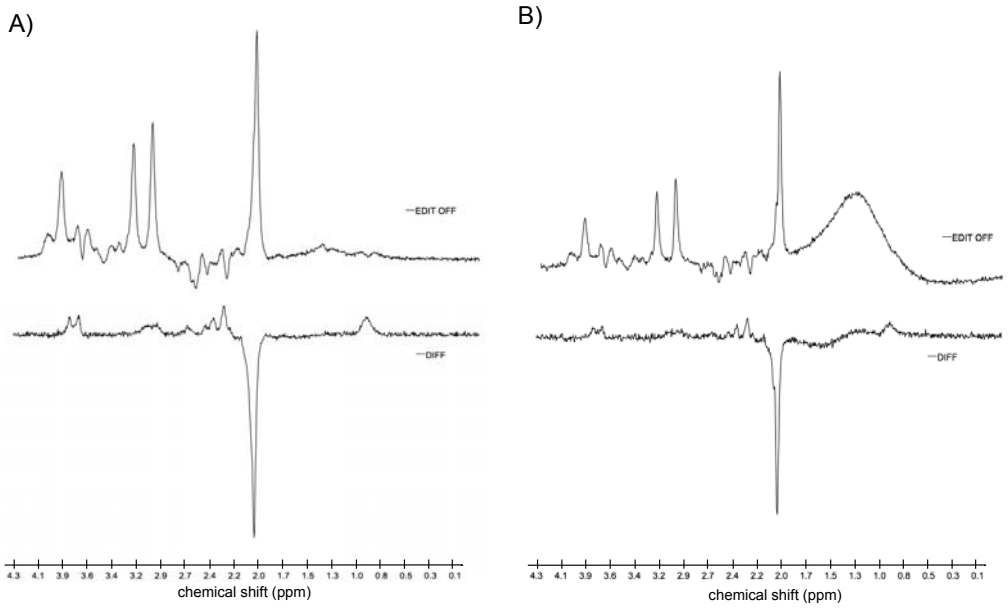
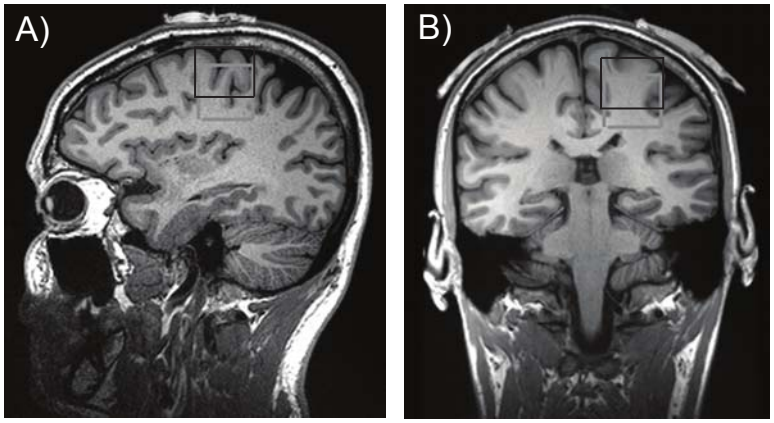


Figure 10: VOI location after movement

Legend : Position of the VOI (30 x 30 x 30 mm^3) over the left hand area of M1 in (A) sagittal and (B) coronal slices after a movement of 5 mm. Inclusion of the cranial bones and the meninges in the box would lead to inclusion of lipids and elimination of the scan. The light grey square shows the initial position of the VOI.



Chapitre 7

Article 6 : The neurophysiological and metabolic effects of bi-hemispheric transcranial direct current stimulation over primary motor cortex

Soumis à Neuroimage

The neurophysiological and metabolic effects of bi-hemispheric transcranial direct current stimulation over primary motor cortex

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Running title: Metabolic effects of bilateral and unilateral tDCS

Keywords: magnetic resonance spectroscopy, transcranial direct current stimulation, motor cortex, GABA, glutamate, MEGA-PRESS

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Article en revision dans *Neuroimage*

7.1 Abstract

Proton magnetic resonance spectroscopy (^{1}H-MRS) studies suggest that γ-aminobutyric acid (GABA) and glutamate are involved in corticospinal excitability changes induced by unilateral transcranial direct current stimulation (tDCS). However, results are inconsistent and little is known about the effects of bilateral tDCS, a technique that has been shown to improve motor function following stroke. The aim of the present study was to assess, in healthy individuals, the impact of bilateral tDCS on primary motor cortex (M1) excitability using transcranial magnetic stimulation (TMS) and sensorimotor metabolism using ^{1}H-MRS. Additionally, the effects of unilateral anodal tDCS were tested using ^{1}H-MRS. No effect of bilateral tDCS on corticospinal excitability was found. Similarly, bilateral tDCS did not significantly modulate ^{1}H-MRS-derived metabolite concentrations. Unilateral anodal tDCS, on the other hand, was associated with significantly higher NAA concentrations compared to sham stimulation. The present results show limited neurophysiological and metabolic effects of bilateral tDCS applied over sensorimotor cortex. Although high inter-subject variability in the response to tDCS is likely involved in the absence of group effects, TMS and ^{1}H-MRS may lack sensitivity to reliably detect the neural substrates of tDCS-induced behavioral changes and clinical improvement.

Abbreviations. A-tDCS, anodal transcranial direct current stimulation; C-tDCS, cathodal transcranial direct current stimulation; Cr, creatine; CRBL, Cramér-Rao lower bounds; CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; Glu, glutamate; Gln, glutamine; Glx: glutamate + glutamine; GM, grey matter; H_2O, water; ^{1}H-MRS, proton magnetic resonance spectroscopy; LA/RC, left anodal/right cathodal; LC/RA, left cathodal/right anodal; LTP, long-term potentiation; LTD long-term depression; M1, primary motor cortex; mIns, *myo*-inositol; MEP, motor evoked potential; MM, macromolecules; MR, magnetic resonance; MRI, magnetic resonance imaging; NAA: *N*-acetylasparte; PCr, phosphocreatine; tCr, Cr + PCr; tDCS, transcranial direct current stimulation; tNAA, acetyl moiety of NAA+ *N*-acetylaspartylglutamate; T_E, echo time; TMS, transcranial magnetic stimulation; T_R, repetition time; WM, white matter.

7.2 Introduction

Over the past twenty years, there has been growing interest in the use of non-invasive brain stimulation to assess plasticity and promote recovery of function following brain damage, such as in the case of stroke (Liew *et al.*, 2014; Simonetta-Moreau, 2014). Early research on the topic primarily focused on the use of repetitive transcranial magnetic stimulation (TMS) to modulate brain activity, but recent years have seen the resurgence of transcranial direct current stimulation (tDCS), a technique that was used in animal models over half a century ago (Dayan *et al.*, 2013). In 2000, Nitsche and Paulus reported that tDCS could be used to safely modulate corticospinal excitability in the human motor cortex. They applied low amplitude currents (1 mA) through a battery-powered direct current device connected to a pair of rubber electrodes (anode and cathode) positioned over the scalp, and demonstrated polarity-dependent effects. A general consensus followed suggesting that when the anode is placed over the primary motor cortex and the cathode is positioned over the contralateral frontal pole (anodal tDCS, A-tDCS), corticospinal excitability is generally enhanced (as assessed via TMS-induced motor evoked potentials; MEP) but decreased when the current flow is reversed (cathodal tDCS, C-tDCS) (Lang *et al*., 2005).

Since this demonstration, tDCS has gained recognition as a promising clinical tool because of its ability to modulate a large array of behaviours and cognitive functions in both healthy individuals (Jacobson *et al*., 2011) and clinical conditions including depression, schizophrenia, Parkinson's disease, stroke and addiction (Sandrini & Cohen, 2013; Schulz *et al*., 2013). Despite a wealth of encouraging results, the apparent therapeutic potential of tDCS has led to its use in clinical settings without a comprehensive understanding of optimal parameters for treatment, intra- and inter-individual variability of response, individual factors modulating response, and most importantly mechanisms of action underlying its physiological effects (Gomez Palacio Schjetnan *et al.*, 2013; Horvath *et al.*, 2014). As such, recent studies have highlighted the current challenges facing the use of tDCS. For instance, two prospective studies have shown the presence of highly variable effects in the motor cortex of healthy individuals following tDCS (López-Alonso *et al.*, 2014; Wiethoff *et al.*, 2014). Similarly, a recent quantitative review found very little evidence supporting the use of single-session tDCS

to modulate cognitive function (Horvath *et al.*, 2015a), a conclusion that was similar for prefrontal cortex stimulation (Tremblay *et al.*, 2014b).

The current understanding of the technique's underlying mechanism of action mainly comes from pharmacological studies and animal models. tDCS is thought to modulate corticospinal excitability through its effects on resting membrane potentials (Liebetanz *et al.*, 2002; Fritsch *et al.*, 2010; Stagg & Nitsche, 2011), and synaptic connections via long-term potentiation (LTP) and long-term depression (LTD) (Stagg & Nitsche, 2011). In the neocortex, these mechanisms are believed to be partially mediated by GABAergic and glutamatergic neurons (Froc *et al.*, 2000; Trepel & Racine, 2000). Using TMS, recent studies have assessed the impact of tDCS on several indirect measures of GABA and glutamate receptor activity. Although some studies report significant modulation of these measures, such as cortical silent period duration (Tremblay *et al.*, 2013) and intracortical facilitation (Di Lazzaro *et al.*, 2012), a recent meta-analysis found no consistent impact of tDCS on neurophysiological measures of intracortical inhibition and facilitation (Horvath *et al.*, 2015b). In fact, out of 30 neurophysiological TMS measures, only MEP amplitude was shown to produce significant and reliable effects following unilateral tDCS over motor cortex (Horvath *et al.*, 2015b).

Although the effects of unilateral anodal and cathodal tDCS on motor cortex excitability have been widely studied using TMS, only few studies have assessed the impact of bilateral tDCS on M1 excitability. One recent study reported no significant effect of bi-hemispheric tDCS (left anode/right cathode) over bilateral M1 (O'Shea *et al.*, 2013), while other studies have shown the expected polarity-sensitive effects on M1 excitability (Mordillo-Mateos *et al.*, 2012; Tazoe *et al.*, 2014). Although little information is available regarding the neurophysiological mechanism underlying the cortical effects of bilateral tDCS, the technique has been used in several clinical studies (e.g., Vines *et al*., 2008; Di Lazzaro *et al*., 2014). More specifically, in stroke patients, a number of recent clinical studies have shown that bilateral stimulation, through the combination of anodal tDCS over contralesional M1 and cathodal tDCS over ipsilesional M1, induces greater and longer-lasting effects on motor recovery than unilateral stimulation (Vines *et al.*, 2008; Lindenberg *et al.*, 2013; Sehm *et al.*, 2013).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a powerful tool to directly assess the underlying effects of tDCS on GABA and glutamate transmission. Using this method, Stagg and collaborators (2009) showed a significant reduction of GABA levels in sensorimotor cortex following unilateral A-tDCS and significant reductions of both GABA and glutamate + glutamine (Glx) levels following unilateral cathodal stimulation. However, other ^{1}H-MRS studies have yielded mixed results. One study reported reductions in M1 GABA concentration following A-tDCS, but no effect of C-tDCS (Kim *et al.*, 2014), while an increase in Glx (Clark *et al.*, 2011; Hunter *et al.*, 2015) and *myo*-inositol (mIns) was reported following stimulation of non-motor areas (Rango *et al.*, 2008). To our knowledge, the effect of bilateral tDCS on motor cortex metabolism has yet to be investigated.

The objective of the present set of experiments was to: 1) assess the neurophysiological effects of bilateral tDCS on M1 corticospinal excitability using TMS; 2) assess the impact of bilateral tDCS on sensorimotor metabolism using ^{1}H-MRS; and 3) further investigate the effects of unilateral anodal tDCS on sensorimotor metabolism using ^{1}H-MRS.

7.3 Material and methods

7.3.1 Ethical approval

The experiments described in the current manuscript conformed to the standards set by the *Declaration of Helsinki*, and all of the procedures were approved by the research ethics board of the Comité Mixte d'Éthique de la Recherche du Réseau de Neuroimagerie du Québec (CMER-RNQ). All participants provided written informed consent prior to testing.

7.3.2 Experiment 1: neurophysiological effects of bilateral tDCS

7.3.2.1 Participants and procedure

Ten right-handed healthy volunteers (6 women; mean age: 25 ± 6 years; age range: 20-40 years) were recruited for this part of the study. The following exclusion criteria were used: psychiatric or neurological history, presence of a pacemaker, metal implanted in skull, history of fainting, history of seizures, history of substance abuse, family history of seizure or febrile

fits, history of skin sensitivity, and any alcohol consumption the previous day. The experiment consisted of three sessions of approximately 40 min, separated by at least 72 h. Each participant received in pseudo-randomized order each of the three following interventions: 1) active stimulation left anodal/right cathodal (LA/RC); 2) active stimulation left cathodal/right anodal (LC/RA); 3) sham stimulation LA/RC.

7.3.2.2 Experimental procedures

Trancranial magnetic stimulation

TMS was delivered through an 8 cm figure-of-eight coil connected to a Magstim 200^2 stimulator (Magstim Company Ltd, Spring Gardens, UK). The coil was positioned flat on the head of participants at a 45° angle from the midline, with the handle pointing backwards. A biphasic current was induced with an anterior-posterior direction. The optimal site of stimulation was defined as the coil position from which TMS produced motor evoked potentials of maximum amplitude in the first dorsal interosseus (FDI) muscle of the contralateral hand. The optimal site was marked directly on the scalp of participants using a non-permanent hydromarker, which was then used as reference for the placement of the tDCS electrodes. This procedure was repeated for both hemispheres. Stimulation was delivered over left M1. To ensure stable coil positioning, a stereotaxic neuronavigation system (Brainsight; NeuroConn GmbH, Ilmenau, Germany) was used. To quantify muscle contractions, two self-adhesive electrodes were positioned over the FDI muscle of the right hand and a ground electrode was positioned over the right wrist. The EMG signal was filtered with a bandwith of 20-1000 Hz and digitized at a sampling rate of 4 kHz using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using LabChart7 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis. The intensity of stimulation was adjusted to elicit MEPs of average amplitude of about 1 mV peak-to-peak. Twenty MEPs were collected immediately before tDCS (pre). Twenty MEPs were also collected, at the same intensity of stimulation used for baseline assessment, immediately after tDCS (post0) and 12 min post-tDCS (post12).

Transcranial direct current stimulation

Electrical current was delivered by a Magstim DC Stimulator (Magstim Company Ltd, Spring Gardens, UK) through a pair of rectangular conductive rubber electrodes (35 cm^2)

inserted into saline-soaked sponges. The electrodes were positioned over the left and right first-dorsal interosseous muscle M1 representation, previously determined using TMS. The electrodes were oriented parallel to the central sulcus and eyebrows. For both active conditions, a constant electric current of 1 mA was applied for 20 min, and the current was gradually increased and decreased during the first and last 15 s of stimulation. For the sham condition, current was ramped up for 15 s and then no current was delivered for 20 min.

7.3.3 Experiment 2: neurometabolic effects of bilateral tDCS

7.3.3.1 Participants and procedure

Eight right-handed healthy volunteers (4 women; mean age: 29 ± 6 years; age range: 24-40 years) participated in this part of the study. The same exclusion criteria as in Experiment 1 were used, in addition to standard magnetic resonance (MR) contraindications. The experimental protocol consisted of three sessions of 2 h duration, separated by at least 72 h. Each session consisted of a first ^{1}H-MRS acquisition, 20 min of bilateral tDCS that was administered inside the scanner, and two consecutive ^{1}H-MRS acquisitions post-stimulation. Each participant received in pseudo-randomized order each of the three following interventions: 1) active stimulation LA/RC; 2) active stimulation LC/RA; 3) sham stimulation LA/RC.

7.3.3.2 Experimental procedures

Transcranial direct current stimulation

Electrical current was delivered using a MR-compatible NeuroConn DC-stimulator plus (NeuroConn GmbH, Ilmenau, Germany) through a pair of rectangular conductive rubber electrodes (35 cm^2). Electrodes were entirely covered with an EEG-type conductive paste. They were positioned over C3 and C4, according to the 10/20 international system, which corresponds to the left and right primary motor regions, respectively. The electrodes were oriented parallel to the central sulcus and eyebrows. Once the electrodes were properly positioned, the impedance level was tested outside the scanning room prior to testing. If an adequate impedance level was present (< 20 kΩ), participants were positioned comfortably in the scanner and were instructed to lie at rest for the entire scanning session. The electrodes were plugged into the MR-compatible tDCS box, which was positioned inside the scanner. For both active conditions, current was

ramped up for 15 s, remained constant at 1 mA intensity for 20 min, and then ramped down for 15 s. For the sham condition, current was ramped up for 15 s and then no current was delivered for 20 min. No MR data was acquired during tDCS. See Tremblay *et al*. (2014a) for a comprehensive description of the protocol.

Proton magnetic resonance spectroscopy

MR acquisitions were performed using a 3 T whole-body system (MAGNETOM Trio, Siemens, Erlangen, Germany) at the *Unité de Neuroimagerie Fonctionnelle, Centre de recherche de l'Institut Universitaire de Gériatrie de Montréal*. Radiofrequency transmission was performed with the built-in body coil, and signal was received with at 32-channel receive-only head coil. The prescription of sensorimotor voxel and detection of potential structural abnormalities were performed using anatomical images of the brain obtained with a T_1-weighted MPRAGE sequence (T_R = 2300 ms; T_E = 2.91 ms; FA: 9°; FOV = 256 x 256 mm; 256 x 256 matrix; T_I: 900 ms; 176 slices; orientation: sagittal; acquisition time: 4 min 12 s). The voxel of interest (30 x 30 x 30 mm^3) was manually positioned over the left precentral knob without any angulation relative to the scanner reference space and using two accepted anatomical landmarks (Figure 1; Yousry *et al*., 1997). First and second order shims were adjusted using FAST(EST)MAP (Gruetter & Tkác, 2000). ^{1}H-MRS data were then acquired using a MEGA-PRESS sequence (Mescher *et al.*, 1998) as previously described (Tremblay *et al.* 2014a). The MEGA-PRESS acquisition consisted of four blocks of 64 metabolite scans (32 editOff and 32 editOn, interleaved) each with frequency update between blocks (T_R = 3 s, T_E = 68 ms, total acquisition time: 12 min), as well as single blocks of unsuppressed-water reference scans (4 editOff and 4 editOn, interleaved; acquisition time: 42 s; same parameters as for metabolite scans, but MEGA and VAPOR water suppression off). Free induction decays were stored separately in memory for individual frequency and phase correction using tCr and choline signals between 2.85 and 3.40 ppm. The final spectra were obtained by subtracting (for metabolite scans) or averaging (for unsuppressed-water reference scans) the signal from editOff and editOn scans as described previously (Tremblay *et al.* 2014a) (Figure 1). The ^{1}H-MRS metabolite acquisition was performed prior to tDCS (^{1}H-MRS pre) and repeated twice following tDCS (^{1}H-MRS post1, ^{1}H-MRS post2), and the ^{1}H-MRS unsuppressed-water reference acquisition followed the acquisition of the pre and post 2 metabolite spectra. A localizer scan

was performed prior to the ^{1}H-MRS session and following the last metabolite acquisition to visually compare both scans for head movement.

7.3.3.3 Analysis of ^{1}H-MRS data

Both editOff and difference spectra were analyzed using LCModel 6.3-1 (Provencher, 1993, 2001) which estimated the best fit of the experimental spectrum as a linear combination of model spectra. The basis set for editOff spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and metabolite spectra simulated with home-written software based on density matrix formalism (Henry *et al.*, 2006) in MATLAB, using known chemical shifts and *J* couplings (Govindaraju *et al.*, 2000) as described previously (Tremblay *et al*. 2014a). From LCModel's default simulations of lipid and macromolecular resonances, only "Lip13a" (modeling a broad peak at 1.28 ppm) was allowed during the LCModel fitting that was performed over the spectral range from 0.2 to 4.0 ppm, and LCModel spline model of the baseline was deactivated using the NOBASE = T input parameter. The basis set for difference spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and the experimentally measured spectra from 100 mM phantoms of NAA, GABA, Glu and Gln at 37°C and with pH adjusted to 7.2. No LCModel default simulations of lipid and macromolecular resonances were allowed during the LCModel fitting that was performed over the spectral range from 0.5 to 4.0 ppm, and LCModel spline model of the baseline was also deactivated using the NOBASE = T input parameter. No baseline correction, zero-filling, or apodization functions were applied to the *in vivo* data prior to LCModel analysis. tCr (Cr-CH_3 + PCr-CH_3), mIns, and tNAA (sNAA+NAAG) concentrations were obtained from editOff spectra, and GABA and Glx concentrations were obtained from difference spectra. A scaling factor between the simulated and measured basis sets was calculated using the group average of tNAA measured from editOff spectra and the group average tNAA from difference spectra. The spectra were visually inspected for contamination from subscapular lipid signals and Cramér-Rao lower bounds (CRLB) and linewidth of water spectra were examined for outliers. This led to rescanning of 8 ^{1}H-MRS sessions. For each time point, the final CRLB were < 30% for Glx, tNAA, and tCr. For each GABA time point, CRLB were generally < 40%. However, the CRLB of the "post2" time point of the cathodal condition was 46% for participant 1, and the three time

points were > 65% for participant 4 (sham condition). After a visual inspection of the ^{1}H-MRS data and localizer images for head motion, data from participant 1 were not excluded, but GABA concentrations from participant 4 (sham condition) were excluded from further analyses. The linewidth of each water spectra was < 10 Hz. Metabolite concentrations were quantified using the water reference and in secondary analysis using tCr.

7.3.3.4 Quantification using water reference

Quantification was performed using an unsuppressed water signal obtained from the same voxel after eddy current correction (Kolse, 1990) and after averaging editOff and editOn scans. The pre-tDCS water reference scan was used for the quantification of the pre-tDCS metabolite scan, and the post-tDCS water reference scan was used for the quantification of both post-tDCS metabolite scans. Concentrations were corrected for cerebrospinal fluid (CSF) content. The tissue composition was obtained from the high-resolution anatomical MR images of the sham session of each subject, which were segmented to gray matter (GM), white matter (WM), and CSF content using the automated *FreeSurfer* pipeline (V 5.3.0, http://freesurfer.net). The fractional volumes of GM, WM, and CSF were obtained for the ^{1}H MRS voxels. The relative densities of MR-visible water for GM, WM, and CSF were assumed to be 0.78, 0.65, and 0.97 (Gasparovic *et al.* 2006), respectively. The T_1 and T_2 relaxation times of water used in the calculation of attenuation factors were taken from published reports [T_1(GM) = 1.29 s, T_1(WM) = 0.87 s, T_1(CSF) = 4 s, T_2(GM) = 110 ms, T_2(WM) = 80 ms, and T_2(CSF) = 400 ms] (Wansapura *et al.*, 1999; Rooney *et al.*, 2007). The water attenuation was computed using the fractional volume of each compartment (Gasparovic *et al.* 2006).

7.3.3.5 Distance between M1 and the scalp

Given that previous studies have shown an influence of the scalp-to-cortex distance on TMS measures such as motor threshold, scalp-to-cortex distances were assessed in all participants taking part in the ^{1}H-MRS study to determine their impact on individual responses to stimulation. From the sagittal view of the MPRAGE scan (sham condition) of each individual participant, the distance between M1 and the scalp was measured using previously developed method (McConnell et al. 2001) (Figure 2).

7.3.4 Experiment 3: neurometabolic effects of anodal tDCS

7.3.4.1 Participants and procedure

Six right-handed healthy volunteers (3 women; mean age: 28 ± 5 years; age range: 23-34 years) participated in this part of the study. The same exclusion criteria as in Experiments 1 and 2 were used. The experimental protocol consisted of two sessions of 2 h duration, separated by at least 72 h. Each session consisted of a first ^{1}H-MRS acquisition, 20 min of unilateral A-tDCS that was administered inside the scanner, and two consecutive ^{1}H-MRS acquisitions post-stimulation. Each participant received in pseudo-randomized order each of the two following interventions: 1) active left A-tDCS; 2) sham left A-tDCS.

7.3.4.2 Experimental procedures

The tDCS protocol was identical to Experiment 1, with the exception of the positioning of electrodes. For both sham and active conditions, the anode was positioned over C3 (left M1) and the cathode was positioned over the right supraorbital region. The electrodes were oriented parallel to the central sulcus and eyebrows. ^{1}H-MRS was performed and analyzed in a manner identical to that described in Experiment 2. For each time point, the final CRLB values were < 30% for all metabolites of interest and linewidth of the water spectra were < 10 Hz. Metabolite concentrations were quantified using the water reference and in secondary analysis using tCr.

7.3.5 Statistics

Data were analysed separately using a standard statistical software package (version 21.0, SPSS inc, Chicago, IL, USA). For Experiment 1, mean MEP amplitudes were determined for each time point. A specific time point MEP measure was removed form analysis if the data was +/- 2 SD from the mean. Normalized (ratios of change from baseline) MEP data were compared using a general linear model repeated-measure analysis of variance (ANOVA), with factors of Polarity (LA/RC, LC/RA, sham) and Time (post0, post12). For Experiment 2, metabolite concentrations quantified using tCr and water (normalized as ratios of change from baseline) were compared using a general linear model repeated-measure ANOVA, with factors of Polarity (LA/RC, LC/RA, sham) and Time (post1, post2). For Experiment 3, both metabolite concentrations quantified using tCr and water (normalized as ratios of change from baseline)

were compared using a general linear model repeated-measure ANOVA, with factors of Polarity (A-tDCS, sham) and Time (post1, post2). When necessary, non-sphericity was adjusted using Greenhouse-Weisser correction. Two-tailed Pearson correlation coefficients were computed between scalp-to-cortex measures and metabolite concentrations. A p value of $< .05$ was considered as statistically significant. When significant effects were observed, *post-hoc* analysis were computed and the p value was adjusted for multiple comparisons (Bonferonni correction). When judged necessary, power analyses were computed to determine the required sample size to observe significant difference between factors using G*power software (version 3.1.9.2, Heinrich Heine Universität Düsseldorf; Faul *et al*., 2009). When significant effects or statistical trends were observed, effect size was calculated and expressed as eta-squared (η^2).

7.4 Results

7.4.1 Experiment 1

Mean MEP amplitudes for the three tDCS conditions at the three time points are shown in Figure 3. To assess the effects of tDCS on MEP size, a 2 X 3 repeated measures ANOVA with *time* and *polarity* as factors was computed on change ratios (post0/pre; post12/pre). No significant main effect of time ($F_{(2,9)} = 1.72$; $p = .22$) or polarity ($F_{(2,9)} = .79$; $p = .41$) was observed. The interaction was also not significant ($F_{(2,9)} = .15$; $p = .86$). Power analyses were computed to determine the required sample size needed to observe a significant difference between the sham and active conditions. When both post-tDCS time points were averaged, a sample size of 158 participants would have been required to obtain a significant difference between LC/RA and sham tDCS, while a sample size of 62 participants would have been required to obtain a significant difference between LA/RC and sham tDCS.

7.4.2 Experiment 2

7.4.2.1 Water-quantified metabolite concentrations

Average percent changes in concentrations of metabolites following bilateral tDCS are shown in Table 1. Change ratios between pre-tDCS and post-tDCS measures (Post1/Pre; Post2/Pre) were calculated for each metabolite of interest and were used for analysis. To assess the effects of tDCS on metabolite concentration, a 2 X 3 repeated measures ANOVA with *time*

and *polarity* as factors was computed for each metabolite of interest. Figure 4 shows changes in concentration ratios. For GABA, no significant main effect of time ($F_{(2,6)} = .16$; $p = .70$) or polarity ($F_{(2,6)} = .03$; $p = .97$) was found. The interaction was also not significant ($F_{(2,6)} = 3.51$; $p = .063$; $\eta^2 = .13$). Because the interaction was close to significance, post-hoc exploratory contrasts were calculated with paired-sample *t*-tests between the three polarities at both points. No significant effect was found. Power analysis revealed that a sample size of at least 25 participants would be required to reach a p value of $< .05$ for individual contrasts. Examination of individual data showed high variability in the direction of GABA changes over time (Figure 5).

For Glx, no significant main effect of time ($F_{(2,7)} = .10$; $p = .78$) or polarity ($F_{(2,7)} = .50$; $p = .61$) was found. The interaction was also not significant ($F_{(2,7)} = .30$; $p = .75$). For mIns, no significant main effect of time ($F_{(2,7)} = 3.31$; $p = .11$) or polarity ($F_{(2,7)} = 1.32$; $p = .30$) was found. The interaction was also not significant ($F_{(2,7)} = .38$; $p = .69$). For tNAA, no main effect of time ($F_{(2,7)} = 3.63$; $p = .10$) or polarity ($F_{(2,7)} = 3.08$; $p = .08$; $\eta^2 = .27$) was found. The interaction was also not significant ($F_{(2,7)} = 2.26$; $p = .14$). Because the interaction was close to significance, post-hoc exploratory contrasts were calculated with paired-sample *t*-tests between the three polarities at both points. No significant effect was found. Power analyses revealed that a total sample size of 23 and 24 participants, respectively, would be required to reach a p value of $< .05$. Ratios of Glx over GABA were also computed to measure the interaction between the two metabolites (Figure 6). Change ratios were computed as for previous metabolite measurements. No significant main effect of time ($F_{(2,7)} = 2.84$; $p = .14$) or polarity ($F_{(2,7)} = .03$; $p = .96$) was found. The interaction was also not significant ($F_{(2,7)} = 2.35$; $p = .13$).

7.4.2.2 tCr quantified metabolite concentrations

Prior to tCr scaling, a 3 x 3 repeated measures ANOVA with *time* and *polarity* as factors was computed on raw tCr concentrations to confirm the stability of the reference metabolite. No significant main effect of time ($F_{(2,7)} = .49$; $p = .63$) or polarity ($F_{(2,7)} = .74$; $p = .49$) was found. The interaction was also not significant ($F_{(2,7)} = .90$; $p = .48$). Metabolite concentrations were

then computed as ratios over tCr for secondary analyses. Results were highly similar to those of water-quantified metabolites. Statistical analyses are presented in Table 2.

7.4.2.3 Scalp-to-cortex measures

The average scalp-to-cortex distance was 17.36 mm (SD = 2.73 mm), which is comparable to previous studies using the same protocol (McConnell *et al.*, 2001; Lepage *et al.*, 2011). Bivariate Pearson's correlations were performed between scalp-to-M1 distance and absolute percent change (water-scaled) following both active conditions. Bonferonni corrections were applied for multiple comparisons. No significant correlation was found for any metabolite of interest. Given the exploratory nature of this analysis, absolute percent change for all metabolites and all conditions was also computed in a single correlation (N = 128) to increase statistical power. The correlation was not significant ($r = .10$, $p = .25$).

7.4.3 Experiment 3

7.4.3.1 Water-quantified metabolite concentrations

Average percent change in metabolite concentrations following unilateral tDCS are shown in Table 3. As for experiment 2, change ratios between pre-tDCS and both post-tDCS measures (Post1/Pre; Post2/Pre) were calculated for each metabolite and were used for analysis. Figure 7 shows average concentration ratios. To assess changes in concentration levels, a 2 X 2 repeated measures ANOVA with *time* and *polarity* as factors was computed for each metabolite of interest. For GABA, no significant main effect of time ($F_{(1,5)} = .39$; $p = .56$) or polarity ($F_{(1,5)} = 1.00$; $p = .36$) was found. The interaction was also not significant ($F_{(1,5)} = .15$; $p = .71$). For Glx, no significant main effects of time ($F_{(1,5)} = .15$; $p = .71$) or polarity ($F_{(1,5)} = 2.90$; $p = .15$) was found. The interaction was also not significant ($F_{(1,5)} = .43$; $p = .54$). For mIns, no significant main effect of time ($F_{(2,7)} = .05$; $p = .83$) or polarity ($F_{(2,7)} = .02$; $p = .90$) was observed. The interaction was also not significant ($F_{(2,7)} = .74$; $p = .43$). For tNAA, a significant interaction between factors ($F_{(1,5)} = 17.39$; $p = .001$) was found. Post-hoc contrasts revealed a significant difference between sham and A-tDCS at time post1 ($t_{(5)} = 2.84$; $p = .036$; Cohen's $d = 1.16$). Analysis of raw data showed that this effect was mainly driven by the difference between pre and post1 time points in the sham condition ($p = .052$). For ratios of Glx over GABA (Figure 8)

no significant main effect of time ($F_{(1,5)} = .28$; $p = .62$) or polarity ($F_{(1,5)} = .75$; $p = .43$) was found. The interaction was also not significant ($F_{(1,5)} = 01$; $p = .94$).

7.4.3.2 tCr quantified metabolite concentrations

Prior to tCr scaling, a 3 X 3 repeated measures ANOVA with *time* and *polarity* as factors was computed on raw tCr concentrations to confirm the stability of the reference metabolite. No significant main effect of time ($F_{(2,5)} = 1.25$; $p = .31$) or polarity ($F_{(2,5)} = .10$; $p = .91$) was found. The interaction was also not significant ($F_{(2,5)} = .16$; $p = .85$). Metabolite concentrations were computed as ratios over tCr for secondary analyses. As for Experiment 2, results were highly similar to those of water-scaled metabolites. Statistical analyses are presented in Table 2.

7.4.3.3 Scalp-to-cortex measures

The average scalp-to-cortex distance was 16.03 mm (SD = 2.46 mm). Bivariate Pearson's correlations were performed between scalp-to-M1 measures and absolute percent change following both active conditions. Bonferonni corrections were applied for multiple comparisons. No significant correlation was observed for all metabolites of interest. Absolute percent change for all metabolites were also computed in a single correlation (N = 48). The correlation was not significant ($r = -.01$, $p = .96$).

7.5 Discussion

The present set of experiments investigated the effects of bilateral tDCS on M1 corticospinal excitability, as well as the effects of bilateral tDCS and unilateral A-tDCS on sensorimotor cortex metabolism. Bilateral tDCS (LA/RC and LC/RA) did not significantly modulate corticospinal excitability compared to sham stimulation. Similar results were obtained with ^{1}H-MRS, where bilateral tDCS also failed to modulate GABA, Glx, tNAA or mIns concentrations. A significant difference between unilateral anodal stimulation and sham tDCS was found, however, for tNAA concentrations. Subsequent analysis on raw data suggests that this effect is mainly driven by the sham condition, and therefore, variability within this condition likely accounts for this effect.

7.5.1 Neurophysiological effects of bilateral tDCS

The failure of bilateral tDCS to modulate corticospinal excitability in the present study is in line with a previous report where identical stimulation parameters were used (O'Shea *et al.*, 2013). In that study, bilateral tDCS (1 mA, 20 min, 35 cm^2 electrode size; 13 participants) resulted in no MEP size difference between LA/RC stimulation and sham tDCS. In contrast, two studies have reported polarity-dependent M1 effects following tDCS, with a reduction of corticospinal excitability under the cathode and an increase under the anode (Mordillo-Mateos *et al.*, 2012; Tazoe *et al.*, 2014). Another study found increased excitability under the anode following LC/RA stimulation (Kidgell *et al.*, 2013). The discrepancy between reported results could be partly explained by significant differences in stimulation parameters. For example, stimulation duration and intensity varied considerably between studies (1 mA for 13 min; 1 mA for 20 min; 1.5 mA for 15 min; 2 mA for 5 min). Results from a recent study suggest a reversal of polarity-dependent effects using unilateral C-tDCS when the intensity of stimulation is doubled from 1 mA to 2 mA (Batsikadze *et al.*, 2013). Other studies suggest that longer durations of stimulation can reduce the modulatory response to tDCS (Fricke *et al.*, 2011). These studies suggest that the effects of unilateral tDCS vary widely and non-linearly with slight modifications of the stimulation protocol. A systematic evaluation of bilateral stimulation parameter efficiency is needed and could be clinically relevant considering its use in clinical trials with stroke patients (e.g. Vines *et al.* 2008; Lindenberg *et al.* 2013; Sehm *et al.* 2013).

Due to strong interactions between primary motor cortices, the effects of bilateral tDCS on corticospinal excitability may be more complex than those of unilateral tDCS. In fact, by stimulating both M1 simultaneously, it is likely that the effects of tDCS not only occur in each M1 separately, but also in the balance of inhibitory/excitatory interactions between both areas. The modulation of interhemispheric interactions could occur without significant increases or decreases of corticospinal excitability in either M1. Consequently, an absence of MEP modulation following bilateral tDCS does not necessarily imply a lack of stimulation effects. Interestingly, O'Shea and collaborators (2013) reported that although bilateral tDCS failed to significantly modulate corticospinal excitability, its response could be predicted by the effects of unilateral anodal or unilateral cathodal stimulation. This suggests the presence of weak,

polarity-dependent effects of bilateral stimulation despite the absence of significant group effects.

7.5.2 tDCS effects on sensorimotor metabolism

In line with neurophysiological results, bilateral tDCS did not significantly modulate metabolite concentrations in sensorimotor cortex. Although this is the first study to report ^{1}H-MRS-derived effects on metabolite levels following bilateral tDCS, four previous studies investigated the effects of unilateral tDCS with ^{1}H-MRS and all reported changes in at least one metabolite concentration following stimulation. However, these studies reported divergent results that limit generalizability of the data. Rango and colleagues (2008) evaluated the effect of anodal tDCS (right M1/left shoulder) compared to sham stimulation on right M1 metabolism using ^{1}H-MRS at 1.5 T (1.5 mA for 30 min outside the scanner; 5 participants). Anodal tDCS was shown to significantly increase mIns levels but failed to modulate tNAA and Glx concentrations (GABA was not measured). Following this first study, Stagg *et al.* (2009) investigated the effects of cathodal and anodal unilateral tDCS (left M1 / right supraorbital region) compared to sham tDCS using ^{1}H-MRS at 3 T (1 mA for 10 min inside the scanner; 11 participants). It was found that anodal tDCS significantly reduced GABA/NAA levels whereas cathodal tDCS reduced both GABA/NAA and Glx/NAA levels. In an additional experiment (same protocol; 7 participants), glutamate, glutamine, and creatine levels were measured at 7 T following cathodal tDCS. A significant reduction of Glu/Cr levels was found whereas glutamine and creatine levels were not significantly altered (Stagg et al., 2009). Clark *et al.* (2011) assessed the effects of anodal tDCS on water-scaled metabolite concentrations in the parietal cortex (left and right) using ^{1}H-MRS at 3T (2 mA for 30 min outside the scanner; 7 participants). A significant increase of tNAA and Glx levels in right parietal cortex was found, but not in left (GABA was not measured). Importantly, however, active tDCS was not compared to sham stimulation. Finally, Kim and collaborators (2014) reported the effects of anodal, cathodal, and sham tDCS (left M1 / right supraorbital) on right and left M1 metabolism using ^{1}H -MRS at 7 T (1.5 mA for 15 min outside the scanner; 35 participants). A reduction of GABA/tNAA levels in left M1 (but not right M1) was found following anodal tDCS whereas cathodal stimulation failed to modulate GABA levels. Additionally, glutamate, glutamine and NAA levels were not altered

following either anodal or cathodal stimulation. It should be noted, however, that the effect of anodal tDCS on GABA/tNAA levels was only marginally significant (p = .051) and although the sample size was larger than that of previous studies (n = 35), stimulation conditions were a between-subjects factor.

The lack of specificity and inconsistency in the modulatory effects of tDCS measured with ^{1}H-MRS could be linked to highly inconsistent tDCS parameters (stimulation duration and intensity, among others) as well as significant differences in ^{1}H-MRS experimental protocols. For example, 3 of the 4 previous ^{1}H-MRS studies performed tDCS outside the scanner (Rango *et al*., 2008; Clark *et al*., 2011; Kim *et al*., 2014). Furthermore, metabolite concentration was scaled to water in two studies (Rango *et al*., 2008; Clark et al., 2011) and scaled to tNAA in two others (Stagg *et al*., 2009; Kim *et al*., 2014). Finally, all studies used relatively small samples or between-subjects designs. As highlighted by power analyses in the present study, high sample sizes would be required to produce significant tDCS-induced group effects in M1 neurophysiology and neurochemistry.

Despite the possibility that sample size may be a contributing factor to negative findings associated with tDCS, recent studies suggest that inter-subject variability is the main reason why physiological and metabolic studies of tDCS effects have produced conflicting results. For example, Wiethoff and collaborators (2014) reported that in a large sample of healthy participants (n = 53), approximately half of the subjects displayed no significant facilitation or inhibition of corticospinal excitability following unilateral anodal or cathodal stimulation (2 mA, 10 min). It was also determined that using this protocol to compare two groups of participants, 87 subjects per group would be needed to reach statistical significance. In a similar study with 56 healthy participants, López-Alonso *et al*. (2014) found no significant effect of unilateral anodal tDCS (1 mA, 13 min), and cluster analysis revealed that only 45% of subjects showed the expected increase in corticospinal excitability following stimulation. It is important to note that different strategies could be used to circumvent the inherent physiological variability associated with tDCS, especially in clinical settings. For example, it has been shown that MEP latencies, when TMS is applied in the antero-posterior orientation (an index of I-wave recruitment), are correlated with increases in MEP size following anodal tDCS (Wiethoff et al.,

2014). It is therefore possible that pre-treatment screening with TMS may predict which patients will respond in the expected manner to a tDCS intervention. In the case of bilateral tDCS, the presence of response predictors could guide patient selection in stroke rehabilitation procedures. It was not possible to determine whether specific MRS measures can predict bilateral tDCS effects in the present study due to the small sample size, but further studies may pinpoint key metabolic factors, which can be measured with ^{1}H-MRS, that can identify probable responders.

Taking all of this into account, it is not completely surprising that TMS and MRS failed to detect significant differences in MEP size or metabolite concentration following bilateral stimulation. Indeed, for both TMS and MRS measures, a high degree of inter-individual variation in response to stimulation was observed. It is unlikely, however, that variability in ^{1}H-MRS measures *per se* can explain this result. Indeed, the within-session reproducibility of four MEGA-PRESS acquisitions over the dorso-lateral prefrontal region at 3 T was recently reported (O'Gorman *et al.*, 2011). High reproducibility was found, with low coefficients of variation between the four acquisitions: 0.07 for GABA, 0.06 for Glx, and 0.04 for NAA. Similar coefficients of variation were observed in the present study for sham tDCS (pre, post1, post2) in the bilateral (GABA = 0.09; Glx = 0.03; NAA = 0.01) and unilateral (GABA = 0.11; Glx = 0.02; NAA = 0.01) conditions. Coefficients of variation, however, were much more elevated for TMS measures: in the sham condition (pre, post1, post2) the coefficient of variation was 0.25. Intra-individual variation of TMS-induced MEP amplitudes is a well-documented phenomenon (e.g. Kiers *et al*., 1993; Pitcher *et al*., 2003; Darling *et al*., 2006), with MEP trial-to-trial coefficients of variation reaching upwards of 0.5 depending on TMS intensity (Pitcher *et al*., 2003; Darling *et al*., 2006). Ngomo and collaborators (2012) investigated the short-term reliability (4 days between 2 sessions) of TMS-induced MEP amplitude measures. They found intraclass correlations of 0.70 and 0.87 and coefficients of variation of 0.36 and 0.43 depending on TMS intensity (110% and 120% motor threshold, respectively). Results from the present study are therefore in general agreement with previous studies with regards to normal variations of MEP amplitudes and metabolite concentrations in the primary motor cortex of healthy subjects.

7.5.3 Conclusion

The present study suggests that both TMS and ^{1}H-MRS lack sensitivity to reliably quantify the neurophysiological and metabolic effects of bilateral tDCS in small sample sizes. This contrasts with the behavioral literature, where numerous studies have reported bilateral tDCS-induced changes in a variety of cognitive and motor tasks (see Reis & Fritsch, 2011; Tremblay *et al*., 2014b). Similarly, bilateral tDCS has been shown to improve motor function following stroke (Ludemann-Podubecka *et al.*, 2014). It remains to be determined whether some behavioral outcomes are more sensitive to the effects of bilateral tDCS than TMS and ^{1}H-MRS. Multimodal tDCS studies that combine behavioral outcome with neurophysiological and metabolic measures, that systematically evaluate stimulation parameters effects, and that identify factors predicting outcome are greatly needed to support its use in clinical settings.

7.6 References

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7.8 Figures

Figure 1. Representative ^{1}H-MRS spectrum

Shows placement of the ^{1}H-MRS voxel over the left sensorimotor region in a single subject, with a representative spectrum obtained with the MEGA-PRESS sequence. tCr = total creatine; tNAA: *N*-acetyl-aspartate + NAAG; Glx: glutamate + glutamine; GABA + MM: γ-aminobutyric acid + macromolecules; mIns: *myo*-inositol.

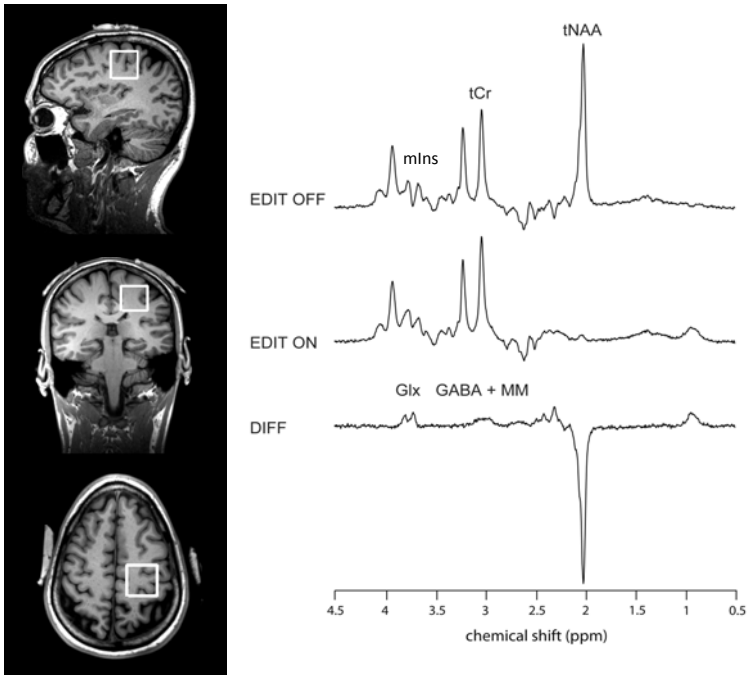

Figure 2. Scalp-to-M1 measurements

Example of a measurement of the scalp-to-M1 distance for a single participant.

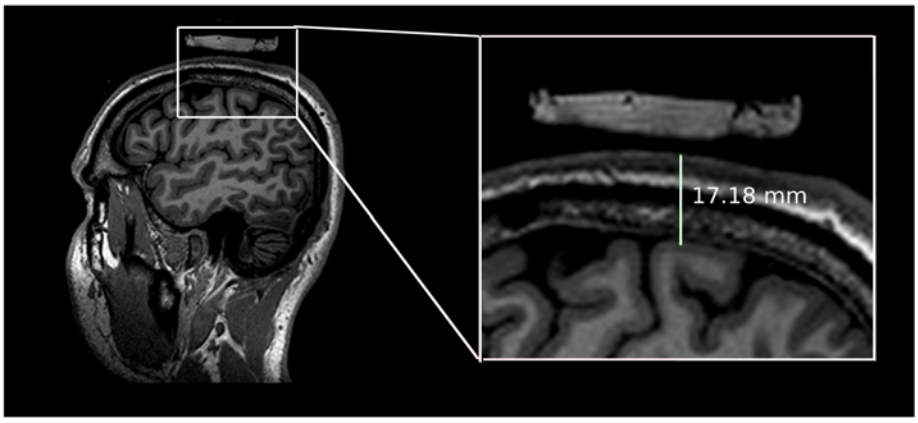

Figure 3. Effects of bilateral tDCS on MEPs

Mean MEP amplitude (± SD) before and at the two time-points following the three bilateral tDCS conditions. No significant modulation is observed for sham tDCS and for both active bilateral conditions.

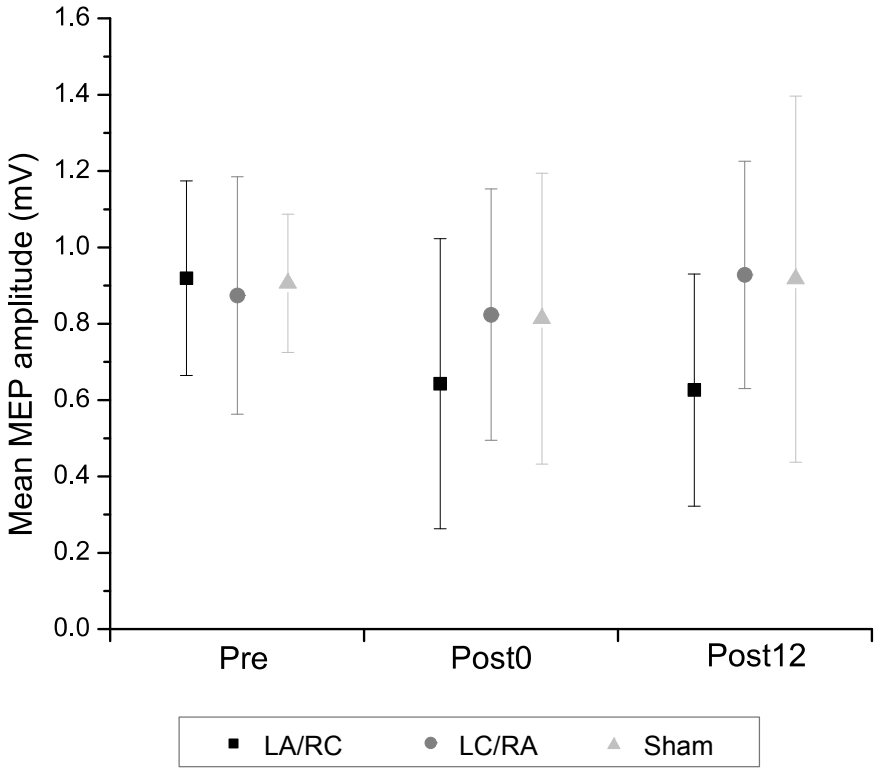

Figure 4. Effects of bilateral tDCS on water-quantified concentrations of metabolites

Average change ratios (± SD) of water-quantified metabolite concentrations between pre-tDCS and post-tDCS measures. No significant modulation is observed for any metabolite concentration ratios at all time points.

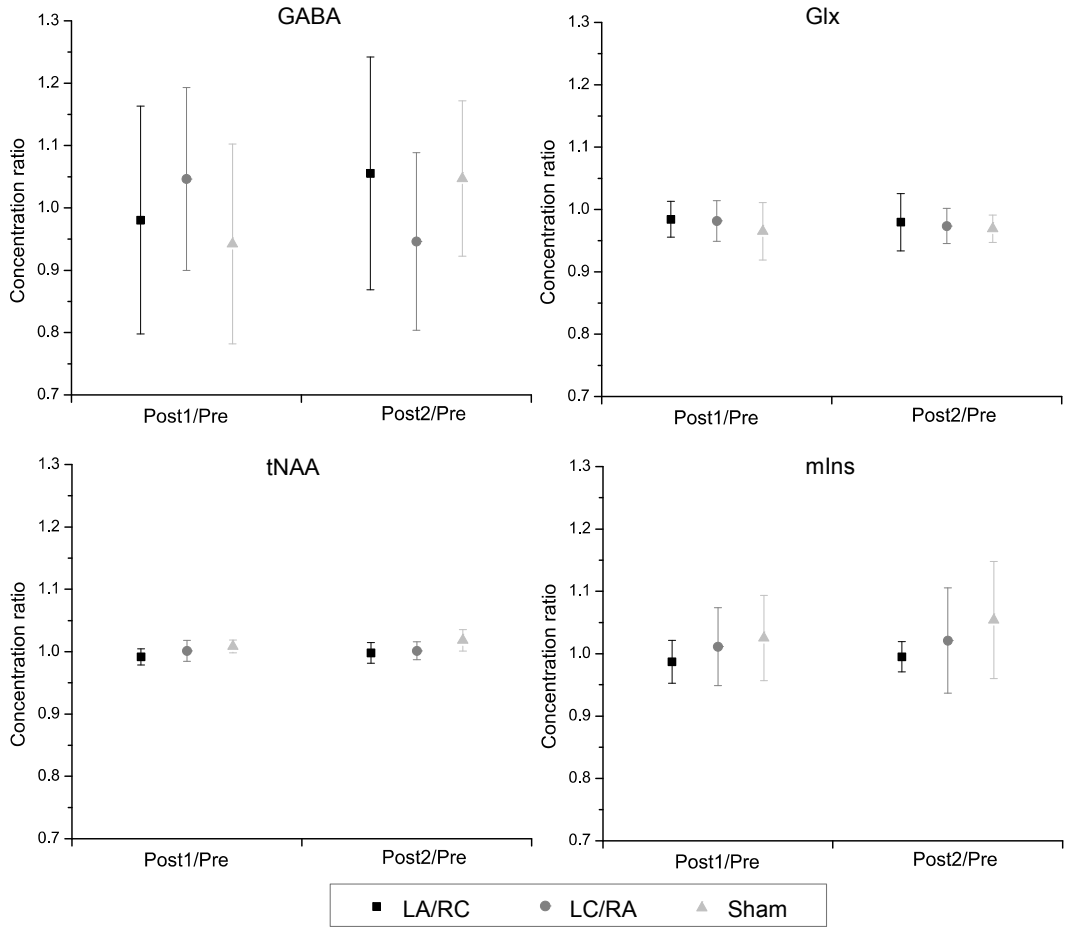

Figure 5. GABA individual change ratios

Individual change ratios of water-quantified GABA concentrations following both active *bilateral* tDCS conditions. The dotted lines represent the mean of the change ratios.

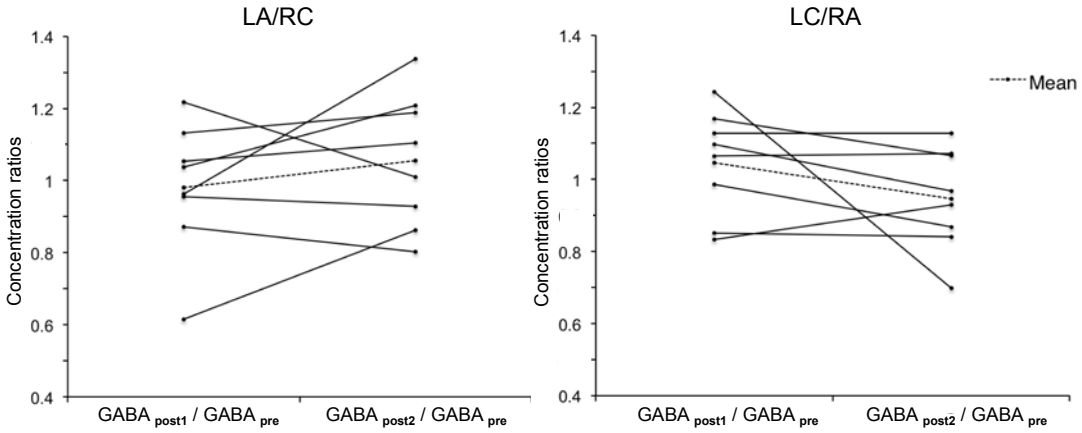

Figure 6. Effects of bilateral tDCS on Glx/GABA ratios

Average change ratios (± SD) following *bilateral* tDCS for the ratio of water-quantified Glx over GABA levels. No significant change is observed.

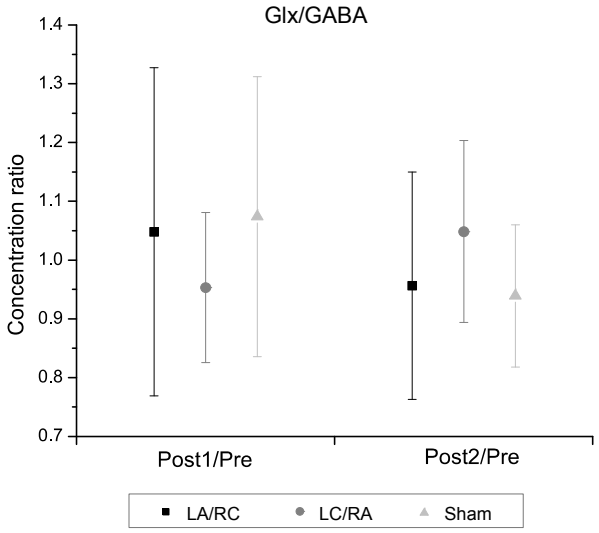

Figure 7. Effects of unilateral A-tDCS on water-quantified concentrations of metabolites
Average change ratios (± SD) of metabolite concentrations between pre-tDCS and post-tDCS measures. No changes in metabolite concentration following anodal tDCS in comparison with sham are observed for GABA, Glx and mIns. For tNAA, a significant difference is noted between the change ratios of sham and anodal tDCS at the first time point post-tDCS. * $p < .05$

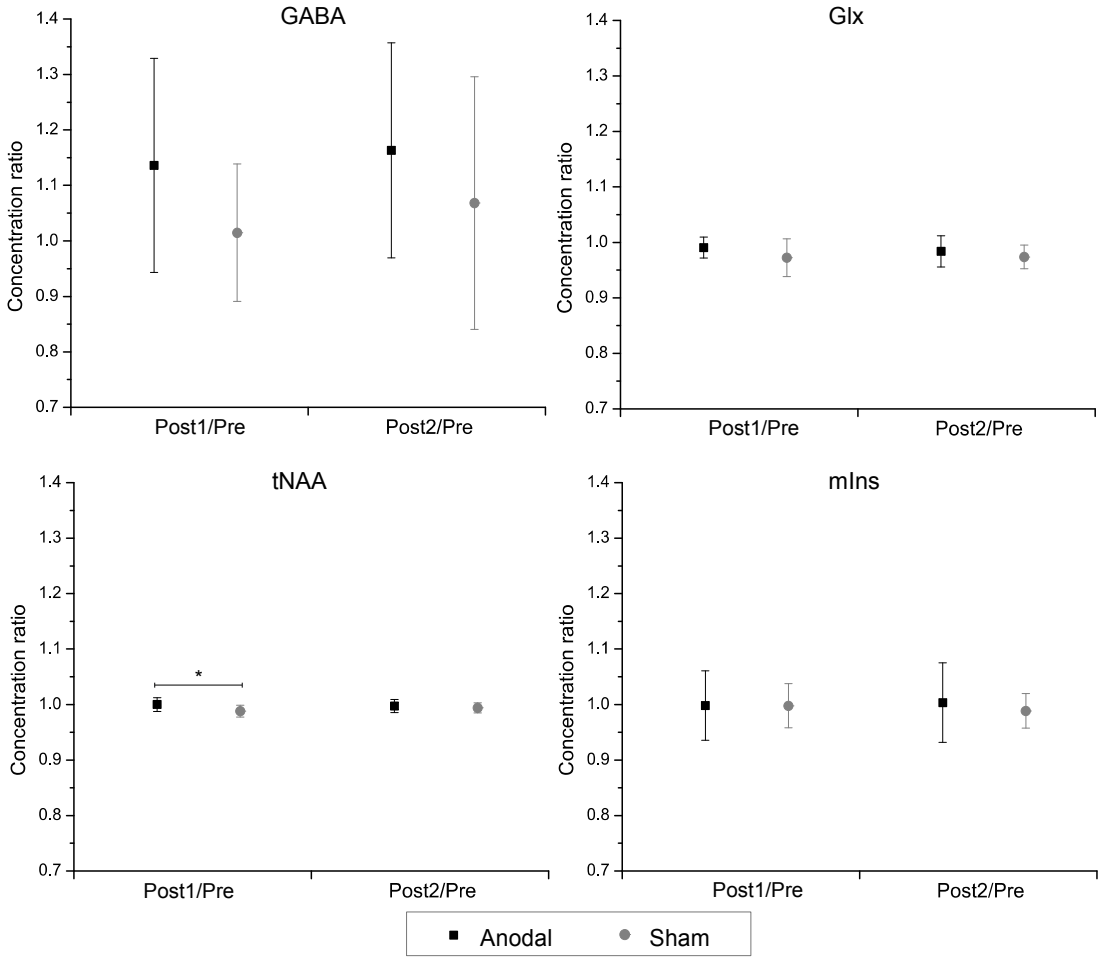

Figure 8. Effects of unilateral A-tDCS on Glx/GABA ratios

Average change ratios (± SD) following *unilateral* tDCS for the ratio of water-quantified Glx over GABA levels. No significant change is observed.

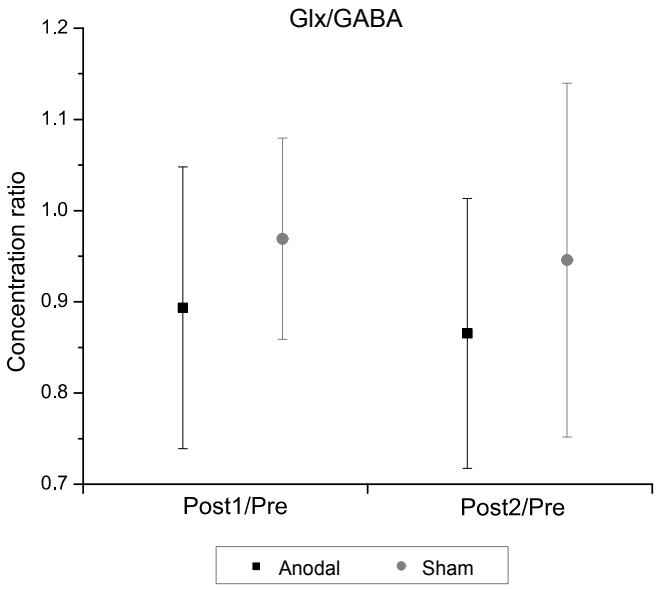

Table 1. Percent change in water-quantified concentrations of metabolites following bilateral tDCS in 8 participants

	Sham tDCS Percent change (M ± SD)		**LA/RC tDCS** Percent change (M ± SD)		**LC/RA tDCS** Percent change (M ± SD)	
Metabolite	Post1	Post2	Post1	Post2	Post1	Post2
GABA	-5.78 ± 16.04	4.71 ± 12.44	-1.96 ± 18.27	5.53 ± 18.67	4.65 ± 14.65	-5.36 ± 14.25
Glx	-1.53 ± 8.32	-3.07 ± 2.17	-1.56 ± 2.85	-2.03 ± 4.58	-1.84 ± 3.26	-2.64 ± 2.81
tNAA	0.85 ± 1.01	1.82 ± 1.73	-0.82 ± 1.28	-0.19 ± 1.65	0.14 ± 1.68	0.12 ± 1.44
mIns	2.51 ± 6.84	5.40 ± 9.39	-1.30± 3.44	-0.50 ± 2.42	1.12 ± 6.24	2.11 ± 8.44

Table 2. Repeated measures ANOVA for concentration of metabolites quantified using tCr

tDCS montage	Metabolite	Main effect: Time		Main effect: Polarity		Interaction	
		F value	*p* value	F value	*p* value	F value	*p* value
Bilateral	GABA	.16	.70	.02	.98	2.89	.10
	Glx	.57	.47	.67	.53	.07	.93
	tNAA	.20	.67	2.89	.09	.05	.95
	mIns	1.58	.25	1.16	.34	.31	.74
	GABA/Glx	2.85	.14	.04	.96	2.35	.14
Unilateral	GABA	.33	.59	.86	.40	.07	.80
	Glx	.96	.37	.95	.38	.17	.70
	tNAA	.93	.38	.17	.70	.43	.54
	mIns	.13	.73	.00	.98	1.23	.32
	GABA/Glx	.32	.60	.73	.43	.01	.19

Table 3. Percent change in water-quantified concentrations of metabolites following unilateral tDCS in 6 participants

	Sham tDCS Percent change (M ± SD)		**Anodal tDCS** Percent change (M ± SD)	
Metabolite	Post1	Post2	Post1	Post2
GABA	1.47 ± 12.38	6.83 ± 22.78	13.61± 19.30	16.31 ± 19.37
Glx	-2.76 ± 3.40	-2.61 ± 2.15	-0.92 ± 1.88	-1.63 ± 2.80
tNAA	-1.18 ± 1.09	-0.58 ± 0.89	0.01 ± 1.21	-0.24 ± 1.16
mIns	-0.23 ± 3.97	-1.13 ± 3.11	-0.18 ± 6.25	0.36 ± 7.15

Chapitre 8

Discussion

8.1 Discussion générale

L'objectif premier de cette thèse consistait en l'évaluation de la possibilité d'utiliser la stimulation magnétique transcrânienne comme marqueur d'altérations du GABA suite à une commotion cérébrale, et ce dans l'optique d'obtenir une mesure objective des effets pathophysiologiques des commotions cérébrales. Dans un premier temps, le but était de vérifier la spécificité des atteintes au niveau du GABA suite à la commotion cérébrale par l'entremise de méthodes permettant de mesurer l'intégrité neurophysiologique et neurométabolique du cortex moteur primaire. Cet objectif est central aux expérimentations et résultats des articles 1 et 2 présentés dans cet ouvrage. Dans un deuxième temps, le but était de vérifier la correspondance entre les deux méthodologies qui ont été utilisées pour mesurer l'intégrité du système GABAergique suite aux commotions cérébrales, et ce chez des individus non-athlètes et sans historique de commotion cérébrale. Cet objectif est central à l'expérimentation et aux résultats présentés dans l'article 3.

Le second objectif du présent ouvrage était de déterminer la possibilité de rétablir, de façon non-invasive, un niveau normal d'inhibition au sein du cortex moteur dans l'optique à long terme d'offrir une avenue de traitement pour les individus montrant des altérations de l'excitabilité corticale suite à une commotion cérébrale. Dans un premier temps, le but était de vérifier, auprès d'une population normale, si la SÉTcd permet de moduler les mesures du GABA qui ont été montrées comme étant de possibles marqueurs d'une récupération incomplète suite à une commotion cérébrale. Cet objectif est central aux expérimentations et résultats présentés dans l'article 4. Dans un deuxième temps, l'objectif était d'évaluer au plan méthodologique l'impact de la SÉTcd sur le métabolisme cérébral, incluant le GABA et le glutamate, de façon directe via la spectroscopie par résonance magnétique. Cet objectif est central aux expérimentations et résultats présentés dans les articles 5 et 6.

Ainsi, la présente discussion vise à mettre à jour les différentes hypothèses soulevées dans l'introduction à la lumière des résultats obtenus dans les études présentées. Les objectifs principaux et secondaires seront d'abord discutés et une conclusion générale, incluant des perspectives futures, sera présentée.

8.2.1 Aspect clinique : commotions et altérations GABAergiques

8.2.1.1 La SMT comme mesure de l'inhibition intracorticale

Tel que révélé par la première étude (article 1), les atteintes neurophysiologiques présentes chez un groupe de joueurs de football de niveau universitaire ayant subi de multiples commotions cérébrales semblent circonscrites à la période silencieuse corticale et l'IIC de longue durée, reflétant l'activité des récepteurs $GABA_B$. En effet, les athlètes commotionnés n'ont présenté aucune anomalie liée à la transmission somatosensorielle ascendante et descendante (temps de conduction motrice et potentiels évoqués somatosensoriels) et aux interactions sensorimotrices modulées par le système cholinergique (inhibition afférente M1-cortex somatosensoriel primaire). Par ailleurs, l'IIC de courte durée s'est avérée normale chez les athlètes commotionnés en comparaison à un groupe d'athlètes sans historique de commotion cérébrale, ce qui suggère une préservation du fonctionnement des récepteurs $GABA_A$.

Tout d'abord, ces résultats permettent de constater la spécificité des atteintes neurophysiologiques présentes suite à des commotions multiples, ainsi que la persistance de celles-ci puisque l'échantillon était constitué d'athlètes asymptomatiques étant de retour au jeu depuis au moins un an. Par ailleurs, cette première étude réplique les résultats obtenus par les précédentes études effectuées par De Beaumont et collaborateurs (2007; 2009; 2011a; 2011b), ce qui supporte l'hypothèse que la présence d'une prolongation de la période silencieuse corticale et d'une augmentation de l'inhibition intracorticale de longue durée pourraient s'avérer de bons marqueurs des atteintes neurophysiologiques persistantes suite à des commotions cérébrales multiples.

Cependant, contrairement à cette première étude, l'étude 2 a révélé l'absence de modifications significatives de l'inhibition intracorticale, telle que mesurée par la PSC et l'IICld, chez un groupe de joueurs de football universitaire asymptomatiques ayant un historique d'au moins une commotion cérébrale. Ces résultats vont donc à l'encontre des études précédentes, ainsi que des hypothèses préalablement soulevées.

Ainsi, comment expliquer les divergences entre ces deux études ? Premièrement, le nombre de commotions cérébrales subies dans cet échantillon s'étendait de 1 à 4 avec une moyenne de près de deux commotions cérébrales; en revanche, la majorité des études précédentes ont observé des différences significatives chez des athlètes ayant subi au moins 2 commotions cérébrales. En effet, dans la première étude réalisée par De Beaumont et collaborateurs (2007), l'augmentation de la durée de la PSC était exclusivement observée dans le groupe de commotionnés multiples. Qui plus est, dans le premier article inclus dans le présent ouvrage, l'échantillon était entièrement composé d'athlètes ayant subi de multiples commotions. Le faible nombre de participants formant notre groupe expérimental ne nous a pas permis d'évaluer les différences dans les mesures d'inhibition intracorticale entre les athlètes ayant subi une ou plusieurs commotions cérébrales. Cependant, une relation négative (non significative) a été trouvée entre le nombre de commotions cérébrales et les deux mesures SMT, ce qui laisse supposer l'absence d'impact de ce facteur sur nos résultats. Il est toutefois à noter que bien que la plupart des études ont montré des altérations de la PSC et de l'IICld chez des individus ayant un historique de commotions multiples, d'autres études ont également suggéré la présence de telles altérations au sein d'échantillons hétérogènes incluant des individus ayant subi de 1 à 5 commotions cérébrales (De Beaumont et al., 2009; 2011a).

Deuxièmement, le temps écoulé depuis le dernier incident dans notre deuxième étude se différencie considérablement de la majorité des études antérieures menées avec de jeunes athlètes asymptomatiques. En effet, les études précédentes ont été réalisées en moyenne 13 mois (De Beaumont et al., 2011b), 19 mois (De Beaumont et al., 2011a), 24 mois (article 1) et 31 mois (De Beaumont et al., 2007) après la dernière commotion cérébrale. Les athlètes inclus dans notre étude ont subi leur dernière commotion en moyenne 41 mois avant l'expérimentation. Ainsi, il est possible de soulever l'hypothèse qu'après un tel délai post-commotion, il y ait présence d'un rétablissement de la dysfonction inhibitrice observée plus tôt (étude 1). Cependant, si ce facteur peut expliquer l'absence d'altérations neurophysiologiques observées dans notre deuxième étude, il est difficile d'expliquer la présence d'anomalies de la PSC persistant en moyenne 30 ans après la dernière commotion cérébrale (De Beaumont et al., 2009).

Enfin, ces résultats divergents pourraient également refléter le fait que les modifications dans l'inhibition intracorticale de M1 ne sont peut-être pas une caractéristique généralisée et stable de la réponse neurophysiologique à la commotion cérébrale. En lien avec ceci, nous avons récemment effectué une revue de la littérature portant sur l'utilisation de la SMT comme marqueur d'altérations neurophysiologiques suite à une commotion cérébrale ou un TCCL (voir Annexe 1). À ce jour, 17 articles ont été publiés sur le sujet, incluant les deux études présentées dans cet ouvrage. Il est intéressant de constater que des 12 études ayant obtenu une mesure de PSC en phase aiguë ou chronique, 9 études ont rapporté une augmentation de sa durée, deux études n'ont révélé aucune altération (incluant l'article 2) et une étude a suggéré une réduction de sa durée. En ce qui concerne l'IIC de longue durée, des 7 études ayant obtenu une mesure en phase aiguë ou chronique, trois études ont rapporté une augmentation de l'inhibition, trois études ont révélé une inhibition normale et une étude a rapporté une réduction de l'inhibition. Finalement, des huit études ayant évalué l'IIC de courte durée, seulement une étude a rapporté une anomalie. Une vue d'ensemble des études recensées sur le sujet suggère que la PSC est la mesure qui a été le plus souvent rapportée comme étant altérée, bien que des résultats divergents en lien avec la mesure soient présents. Ainsi, les effets à court et à long terme des commotions cérébrales sur le cortex moteur primaire semblent se refléter par une tendance générale à une hypoexcitabilité corticale diffuse (ou hyperinhibition) telle que mesurée par la SMT.

Par ailleurs, l'analyse des 17 études recensées suggère que l'effet immédiat d'une commotion cérébrale se traduirait par une excitabilité corticale réduite, qui évoluerait ensuite vers une augmentation de la transmission GABAergique (probablement liée à l'activité des récepteurs $GABA_B$) en phase chronique (Powers, Cinelli et Kalmar, 2014). Ainsi, cela soulève la question suivante : est-ce que l'augmentation de l'inhibition corticale est une adaptation bénéfique aux changements pathophysiologiques survenant suite à une commotion cérébrale ou un marqueur de récupération incomplète? Un indice possible à cet égard réside dans le fait qu'un grand nombre des altérations de l'excitabilité corticale révélées par la SMT ont été corrélées avec des anomalies fonctionnelles liées au fonctionnement moteur. Par exemple, l'augmentation de la durée de la PSC a été associée à une vitesse d'exécution motrice réduite (De Beaumont et al., 2009), à une réduction de l'apprentissage moteur (De Beaumont et al., 2011b), ainsi qu'à des altérations au niveau des temps de réponse moteurs, de la durée des mouvements et de la

performance attentionnelle (Pearce et al., 2014a). Par ailleurs, une étude récente a démontré une corrélation entre la durée de la PSC et les temps de réaction visuo-moteurs, ainsi que le contrôle moteur fin (Pearce et al., 2014b) dans un groupe d'anciens athlètes ayant un historique de commotions cérébrales (21 années après le dernier incident). Ces données suggèrent que les données neurophysiologiques fournies par la SMT peuvent présenter une valeur clinique significative, et ce malgré les divergences entre les études.

8.2.1.2 La SRM comme mesure de l'inhibition intracorticale

La SMT étant une mesure indirecte de la concentration GABAergique corticale, l'article 2 visait à évaluer directement l'intégrité des concentrations de ce neurotransmetteur au sein des régions sensorimotrices chez le même échantillon d'athlètes ayant un historique de 1 à 4 commotions cérébrales. En plus de l'absence d'altérations neurophysiologiques, aucune anomalie du métabolisme cérébral n'a été observée dans le cortex moteur primaire des athlètes commotionnés, et ce tant au niveau du GABA, du glutamate, du myo-inositol, que du NAA. Qui plus est, aucune anomalie structurelle au niveau de M1, telle que révélée par des mesures de l'épaisseur corticale de M1 et de l'ensemble du cerveau, ainsi qu'aucune altération de la connectivité structurelle entre M1 et l'ensemble du cortex n'ont été révélées par l'étude.

Les résultats de l'article 2 suggèrent donc l'absence de perturbations à long terme de la concentration de métabolites dans le cortex moteur primaire après une commotion cérébrale dans un contexte sportif. Cette étude est la première à évaluer cet aspect de la pathophysiologie de la commotion cérébrale au-delà du début de la phase chronique, et elle est la première étude à obtenir une mesure du GABA chez des athlètes commotionnés. En effet, jusqu'à maintenant, les effets des commotions cérébrales sur le métabolisme cérébral ont été observés en deçà de six-mois post-commotion cérébrale (Henry et al., 2010; 2011; Vagnozzi et al., 2008; 2010; 2012). De plus, il n'existe aucun consensus quant aux effets métaboliques des commotions cérébrales à l'intérieur de cette fenêtre temporelle. Par exemple, Henry et collaborateurs (2011) ont montré la présence de perturbations du niveau de NAA et du myo-inositol 6 mois post-commotion au niveau de M1, tandis que d'autres études ont rapporté une normalisation complète des niveaux de NAA 45 jours post-commotion au niveau du lobe frontal (Vagnozzi et al., 2008;

2010; 2012). Une récente revue de la littérature portant sur l'utilisation de la SRM dans l'évaluation du TCCL et des commotions cérébrales (11 études recensées) arrive à la conclusion suivante : les différences entre les paramètres d'acquisition, les régions d'intérêts et l'intervalle post-commotion ne permettent pas de mettre en lumière un résultat systématique à travers les études (Gardner, Iverson et Stanwell, 2013). Le résultat observé le plus fréquemment (9 études) est une réduction du niveau de NAA, et le deuxième plus fréquent est une modification des niveaux glutamatergiques (2 études). Ainsi, compte tenu des données actuelles, nous pouvons émettre l'hypothèse que tout comme les dysfonctions neurophysiologiques, les modifications métaboliques rapportées dans les études antérieures allant de la phase aiguë au début de la phase chronique pourraient éventuellement se résorber en moyenne trois années après l'incident.

Malgré qu'aucune altération des mesures du système GABAergique obtenues par la SMT et la SRM n'ait été observée chez les athlètes ayant un historique de commotion cérébrale dans l'étude 2, une analyse plus approfondie des corrélations entre les mesures GABAergique et glutamatergique nous a permis de démontrer la présence de changements subtils dans les mécanismes inhibiteurs du cortex moteur primaire. En effet, les athlètes sans historique de commotion cérébrale ont montré une corrélation positive significative entre le GABA et le Glx, alors qu'aucune corrélation entre les deux métabolites n'a été observée chez les athlètes avec un historique de commotion cérébrale. Il est intéressant de constater que les données chez les athlètes contrôles concordent avec les résultats de l'étude 3 effectuée chez des individus en santé, chez qui une corrélation similaire a été observée (voir prochaine section). Par conséquent, l'absence de lien entre la concentration de GABA et de Glx chez les athlètes commotionnés suggère que les commotions cérébrales pourraient provoquer un déséquilibre entre l'excitabilité et l'inhibition au sein du cortex moteur primaire. De plus, une tendance à une corrélation différentielle entre le GABA et l'épaisseur corticale du cortex moteur primaire a aussi été observée; chez les athlètes contrôles, une corrélation non significative positive entre les deux variables a été observée alors que les athlètes commotionnés ont montré une relation négative non significative. Bien que ces résultats soient exploratoires, ils indiquent que de subtiles altérations dans la transmission du GABA et l'organisation de M1 pourraient être présentes dans le cerveau d'athlètes commotionnés, même si les concentrations absolues de métabolites ne diffèrent pas de celles observées chez les athlètes non-commotionnés. Enfin, une relation

différentielle entre le GABA et l'IICld a été obtenue entre les deux groupes, où les athlètes avec un historique de commotions cérébrales ont montré une corrélation positive significative entre les deux mesures, alors qu'aucune corrélation n'a été observée dans le groupe d'athlètes contrôles. Étonnamment, les niveaux de GABA et l'IICld étaient corrélés uniquement chez les athlètes avec un historique de commotion cérébrale. Bien que difficile à interpréter, ce résultat pourrait également refléter la présence de dysfonctionnements subtils de l'inhibition au niveau de M1.

Le mécanisme exact sous-tendant ce déséquilibre métabolique nous est inconnu. En se basant sur les études récentes suggérant une hypoexcitabilité corticale persistante suite à la commotion cérébrale, nous soulevons l'hypothèse que les niveaux élevés d'inhibition intracorticale révélés par les mesures de PSC et d'IICld (De Beaumont, Henry et Gosselin, 2012) et la concentration anormale du glutamate rapportée dans la phase aiguë (Henry et al., 2011), puissent déclencher un déséquilibre persistant dans l'interaction entre le glutamate et le GABA dans le cerveau commotionné qui ne se reflèterait pas directement dans les concentrations respectives de ces métabolites.

8.2.1.3 Articles 1 et 2 : impact des contacts répétés à la tête

Il est important de souligner qu'un facteur en particulier peut limiter la généralisation des résultats des deux études effectuées auprès d'athlètes ayant un historique de commotions cérébrales inclues dans le présent ouvrage. Premièrement, il est possible que les perturbations métaboliques et neurophysiologiques dans le cortex moteur primaire des athlètes commotionnés aient été sous-estimées étant donné la nature du groupe contrôle. En effet, malgré une entrevue semi-structurée avec chaque athlète afin de s'assurer de l'absence d'historique de commotions cérébrales, il demeure impossible de complètement éliminer la possibilité que les athlètes aient pu subir une commotion cérébrale dans le passé sans la rapporter à l'entrevue. Ceci est d'autant plus probable compte tenu du haut pourcentage de commotions cérébrales qui demeurent non-diagnostiquées et non-rapportées (Harmon et al., 2013).

Par ailleurs, le football étant un sport impliquant des contacts très fréquents, les athlètes ont été exposés à de nombreux chocs à la tête depuis un jeune âge. Avec le développement récent d'accéléromètres implantés dans les casques des athlètes, il est possible de mesurer le nombre et l'intensité des coups reçus à la tête à l'entraînement et lors de matchs. En utilisant cette technologie, une étude récente menée dans un échantillon de joueurs de football a révélé une moyenne très élevée d'impacts reçus à la tête, plus spécifiquement liés à des impacts rotationnels et linéaires (Broglio, Eckner, Paulson et Kutcher, 2012). Ceci suggère que les joueurs de football subissent des coups répétitifs qui pourraient avoir un effet délétère cumulatif sur le fonctionnement du cerveau. Autrement dit, le fait d'avoir accumulé de nombreux coups à la tête ne causant pas de symptômes cliniques suffisants pour établir un diagnostic formel de commotion cérébrale peut avoir entrainé des altérations cérébrales subtiles. Ceci suggère donc que les deux groupes expérimentaux incluent dans nos études seraient « équivalents » en terme du nombre d'impacts sous-commotionnels et qu'ils se différencieraient uniquement par la présence ou non de diagnostics formels de commotion cérébrale. Cette question est d'ailleurs grandement alimentée par le rôle potentiel des coups répétés à la tête dans le développement de l'encéphalopathie traumatique chronique (Concannon, Kaufman et Herring, 2014).

En lien avec ceci, une récente étude a rapporté la présence de perturbations métaboliques chez des joueurs de hockey sans historique de commotion cérébrale. Ces résultats ont été interprétés comme reflétant les effets cumulatifs d'évènements « sous-commotionnels » (Chamard et al., 2012). Des résultats similaires en SRM ont été rapportés par un autre groupe ayant effectué un suivi longitudinal d'athlètes sans historique de commotion cérébrale en comparaison avec des non-athlètes (Poole et al., 2014). Bien que très peu d'études aient investigué l'impact des coups répétés à la tête sur le métabolisme cérébral et qu'aucune étude à ce jour ne se soit penché sur cette problématique en utilisant la SMT, les perturbations métaboliques et neurophysiologiques dans le cortex moteur primaire d'athlètes commotionnés peuvent avoir été sous-estimées par la présence d'altérations chez le groupe contrôle. Pour contrôler ce facteur, les études ultérieures devraient inclure un groupe contrôle supplémentaire comprenant des athlètes de haut niveau qui ne participent pas à des sports de contact.

Néanmoins, les comparaisons directes entre des athlètes pratiquant le même sport de contact peuvent révéler des informations importantes. Plus spécifiquement, puisque l'on peut supposer que la prévalence des coups « sous-commotionnels » est similaire entre les athlètes des deux groupes, on peut imaginer qu'un effet spécifique aux commotions cérébrales diagnostiquées émerge de ce type de comparaison. Ceci semble être effectivement le cas, puisque la plupart des études sur le sujet, en utilisant une approche de recrutement similaire à celle des études 1 et 2, ont révélé des anomalies dans plusieurs régions cérébrales par l'entremise de plusieurs techniques de neuroimagerie.

8.2.1.4 Articles 1 et 2 : conclusions

En somme, les articles 1 et 2 de la présente thèse suggèrent que la SMT et la SRM peuvent être utilisées pour évaluer les changements neurophysiologiques et neurométaboliques associés à une commotion cérébrale. Bien que les résultats entre les deux études ne concordent pas tout à fait, l'analyse de la littérature actuelle suggère la présence d'une altération persistante de l'excitabilité corticale suivant la commotion cérébrale. La SMT semble particulièrement sensible à ces changements à la fois dans les phases aiguës et chroniques (jusqu'à 30 ans après le dernier incident). Plus spécifiquement, la présence d'altérations de l'inhibition intracorticale semble être un marqueur potentiel d'une récupération incomplète. L'analyse de la littérature en SRM et les résultats de l'article 2 suggèrent qu'un déséquilibre métabolique, impliquant possiblement le GABA et le glutamate, puisse être présent chez les athlètes commotionnés.

Les mécanismes sous-tendant le possible excès d'inhibition révélé par les mesures SMT demeure inconnu et l'hypothèse voulant que cette hyper-inhibition survienne en réponse à un état d'excitotoxicité glutamatergique demeure hautement spéculative. En effet, les études en SMT et SRM suggèrent plutôt la présence d'une réduction de l'excitabilité corticale en phase aiguë post-TCCL ou post-commotion, telle que révélée par une réduction du niveau de glutamate (Henry et al., 2010), une augmentation des seuils moteurs (Chistyakov et al., 1998; 2001) et une réduction de la facilitation intracorticale (Powers et al., 2014) se normalisant en phase chronique. Il semble donc y avoir des discordances entre les résultats obtenus auprès d'individus ayant subi un TCCL et les modèles animaux de la pathophysiologie des TCC. En

lien avec ceci, certains auteurs ont suggéré la présence d'une hypoexcitabilité corticale liée à une diminution du glutamate et une augmentation du GABA suite à une commotion cérébrale (Powers et al., 2014). Nous sommes d'avis qu'il est probable que la commotion cérébrale crée une augmentation du glutamate dans les quelques heures suivants l'incident, mais que celle-ci serait suivie par la suite par un hypo-métabolisme glutamatergique et une hyper-inhibition persistante.

Finalement et tel que soulevé précédemment, ces études soulèvent plusieurs questions importantes en lien avec la nature de l'impact fonctionnel d'une altération de l'excitabilité corticale sur le système moteur, mais également de leur possible implication dans le développement d'une trajectoire de vieillissement anormal. Bien que hautement spéculatif, nous avons soulevé quelques hypothèses en lien avec ces problématiques dans les articles 1 et 2. Par exemple, compte tenu des études pathophysiologiques suggérant la présence d'une vulnérabilité cellulaire prolongée suite au TCC, il est probable que le déséquilibre persistant au niveau de l'excitabilité corticale, bien que subtil, puisse rendre le cerveau plus enclin à subir une commotion cérébrale subséquente. Par ailleurs ces résultats pourraient expliquer en partie les conclusions récentes suggérant un lien entre les commotions cérébrales et le vieillissement pathologique (Broglio et al., 2012). En effet, le vieillissement normal est généralement associé à des changements structurels et chimiques, ainsi qu'à une dégradation fonctionnelle des neurones. Toutefois, certains auteurs ont suggéré que l'ampleur du déclin cognitif associé à ces changements survenant dans le cerveau pourrait être lié à la quantité de « réserve cognitive » disponible (Broglio et al., 2012), qui peut être influencée par les commotions cérébrales et l'accumulation de coups répétés à la tête. Par conséquent, nous pouvons émettre l'hypothèse que, si les corrélations différentielles observées dans l'étude 2 sont liées à des modifications des interactions métaboliques corticales, ces dysfonctionnements dans le métabolisme du cerveau pourraient accélérer ou augmenter l'ampleur du processus neurodégénératif associé au vieillissement normal et ainsi augmenter la probabilité d'une trajectoire de vieillissement anormal. Par exemple, une étude récente en SRM suggère la présence, chez d'anciens athlètes ayant un historique de commotions cérébrales, de signes de vieillissement anormal (p.ex. ventricules élargis) et d'altérations métaboliques liées à un déclin de la mémoire épisodique (Tremblay et al., 2012). Dans le même ordre d'idée, les altérations du fonctionnement de

récepteurs spécifiques, tels que les récepteurs $GABA_B$, pourraient déclencher des processus pathophysiologiques délétères qui, associés au vieillissement normal, pourraient déclencher des processus de vieillissement anormal. Cette hypothèse est également supportée par de récentes études montrant des liens entre les altérations neurophysiologiques chez d'anciens athlètes et un déclin des fonctions motrices et cognitives (De Beaumont et al., 2009; Pearce et al., 2014b).

8.2.2 Aspect méthodologique: le GABA tel que mesuré par la SMT et la SRM

Compte tenu des études en SMT suggérant la présence d'une hypoexcitabilité persistante au niveau du cortex, il est important de mieux comprendre ce que représentent les mesures indirectes du GABA révélées par la SMT en termes de métabolisme cérébral. Cette question formait l'essentiel de l'article 3 présenté dans cette thèse.

D'une part, l'étude a révélé une absence de corrélation entre les mesures du GABA obtenues par la SMT, à savoir la PSC, l'IIC de longue durée et l'IIC de courte durée, et les concentrations GABAergiques obtenues par la SRM. Ce résultat a priori surprenant concorde en partie avec les hypothèses soulevées dans l'introduction, et est en accord avec une récente étude effectuée par Stagg et collaborateurs (2011b) où une absence de corrélation entre les mesures SMT reflétant l'activité spécifique des récepteurs $GABA_A$-$GABA_B$, et le GABA tel mesuré par la SRM a été révélée. Il semblerait donc que les deux méthodes mesurent des aspects physiologiques GABAergiques distincts. En effet, les connaissances actuelles sur les deux méthodes d'évaluation du système GABAergique suggèrent que la SMT reflèterait l'activité de récepteurs des interneurones du cortex (Reis et al., 2008), tandis que la SRM reflèterait les concentrations GABAergiques extracellulaire et intracellulaire (Maddock et Buonocore, 2012). De plus, la possibilité de détecter le GABA contenu dans les vésicules pré-synaptique via la SRM est inconnue (Maddock et Buonocore, 2012). Ces différences inhérentes à la méthodologie peuvent donc créer des divergences dans la sensibilité de détection du GABA par l'entremise de la SMT et la SRM.

D'autre part, les résultats ont mis en lumière une relation étroite entre les mesures SMT d'inhibition et les mesures SRM d'excitabilité. En effet, une relation contre-intuitive entre la PSC et la concentration de Glx a été obtenue dans M1. Par ailleurs, un lien entre la concentration de GABA et de Glx a été observé, suggérant une interaction étroite entre l'excitation et l'inhibition dans le cortex moteur primaire. Au plan physiologique, ce lien peut être expliqué par différents facteurs. En effet, de récentes études animales ont suggéré un lien étroit entre le $GABA_B$ pré-synaptique et les neurones glutamatergiques (Chalifoux et Carter, 2011; Raiteri, 2008). Par exemple, suite à l'administration d'un agoniste $GABA_B$, le Baclofen, des effets sur le GABA, mais également sur le glutamate ont été rapportés dans le système visuel (Luo, Wang, Su, Wu et Chen, 2011). De plus, la facilitation intracorticale, une mesure SMT qui est principalement modulée par l'administration d'un agoniste glutamatergique, est modulée par l'administration d'agonistes $GABA_B$ chez l'humain en santé (Ziemann et al., 2014).

La mise en commun des résultats de l'étude de Stagg et al. (2011b) et de l'étude 3 permet d'obtenir une meilleure compréhension du lien entre les mesures SMT et SRM du GABA et du glutamate. En effet, les deux études suggèrent l'existence d'un lien étroit entre le GABA et le glutamate dans le cortex moteur primaire, une notion qui est corroborée par la corrélation élevée entre les concentrations de GABA et de Glx obtenues par la SRM. Ainsi, dans notre étude, une augmentation du glutamate était associée à une augmentation parallèle du GABA et de l'activité du $GABA_B$ liée à la période silencieuse corticale.

Par ailleurs, nos données montrent que les mesures SMT de $GABA_A$ et $GABA_B$ interagissent différemment avec le glutamate mesuré avec la SRM, puisque seule la PSC était corrélée avec ce métabolite. Ce résultat n'est pas surprenant compte tenu des études chez les athlètes commotionnés montrant, dans une majorité des cas, des altérations spécifiques du $GABA_B$ sans anomalie des mesures du $GABA_A$ (Annexe 1). Il semble donc qu'au niveau physiologique, la commotion cérébrale puisse agir différemment sur chacun des sous-types de récepteurs GABAergiques. En fait, des études physiologiques suggèrent que les neurones GABAergiques exercent une inhibition synaptique rapide par les récepteurs ionotropiques $GABA_A$ (Isaacson et Scanziani, 2011), tandis que les récepteurs métabotropiques $GABA_B$ sont responsables de l'inhibition lente via l'ouverture des canaux de potassium et l'implication de

seconds messagers (Lüscher, Jan, Stoffel, Malenka et Nicoll, 1997). Bien que cette hypothèse demeure spéculative, étant donné les différences liées au mécanisme d'action physiologique entre les deux types de récepteurs, il est possible que l'activité du $GABA_A$ augmente rapidement en réponse à une augmentation de glutamate, tandis que l'activité du $GABA_B$ exerce un réglage fin de l'équilibre entre les mécanismes excitateurs et inhibiteurs en augmentant lentement son activité en réponse à une plus grande excitabilité du neurone. Ceci pourrait expliquer pourquoi les commotions cérébrales semblent ne pas avoir le même impact sur les deux types de récepteurs, et pourquoi ces derniers ne corrèlent pas de la même façon avec le glutamate.

8.2.2.1 Article 3 : conclusions

En somme, notre troisième étude suggère plusieurs éléments nouveaux concernant le lien entre les mesures SMT et SRM. D'une part, le niveau d'inhibition intracorticale évalué par la SMT ne reflète pas les concentrations GABAergiques dans le cortex moteur primaire. D'autre part, la période silencieuse corticale, une mesure d'inhibition intracorticale, semble plutôt liée à la transmission glutamatergique. D'autres études sont nécessaires pour mieux comprendre les mécanismes d'action sous-tendant ces interactions complexes. En outre, ces résultats suggèrent que la prudence est nécessaire dans l'interprétation des données visant à évaluer le système GABAergique avec la SMT et la SRM. Une plus grande attention devrait être accordée au fait que les deux techniques fournissent des informations spécifiques et complémentaires quant au fonctionnement des systèmes GABAergique et glutamatergique.

8.3 Objectif 2 : modulation de la transmission GABAergique par la SÉTcd

8.3.1 Aspect clinique : modulation des marqueurs SMT de l'inhibition GABAergique

Compte tenu du grand nombre d'études suggérant la présence d'altérations de l'excitabilité corticale suite à une commotion cérébrale, l'article 4 visait à étudier la possibilité

de moduler, via la SÉTcd, l'inhibition intracorticale telle que mesurée par la SMT chez des individus en santé.

Tout d'abord, l'étude a mis en lumière la possibilité de moduler de façon significative l'excitabilité corticale du cortex moteur primaire qui s'est reflétée par une augmentation de l'amplitude moyenne des PÉM en comparaison avec les mesures pré-stimulation. Ce résultat va de pair avec la tendance générale observée dans la littérature quant aux effets excitateurs associés à la SÉTcd anodale (Lang et al., 2005). Lorsque la polarité des électrodes était inversée (stimulation cathodale), aucune modulation significative de l'excitabilité corticale n'a été obtenue. Ceci contraste avec les résultats de l'étude initiale publiée par Nitsche et collègues (Nitsche et Paulus, 2000), ainsi qu'avec une méta-analyse récente (Jacobson et al., 2011), montrant qu'une réduction de l'amplitude des PÉM est généralement présente suite à la SÉTcd cathodale. Contrairement aux études neurophysiologiques, l'absence d'un effet inhibiteur suivant la stimulation cathodale a été observée dans plusieurs études cognitives (voir Annexe 3). Bien que l'absence de résultats significatifs pourrait être en partie attribuable à la puissance statistique limitée liée à la taille de l'échantillon, des effets significatifs ont été rapportés dans des études avec des tailles d'échantillon similaires (Nitsche et Paulus, 2000). Compte tenu des résultats de deux récentes études prospectives menées dans de grands échantillons suggérant la présence d'une variabilité inter-sujet importante dans la réponse à la SÉTcd (López-Alonso, Cheeran, Río-Rodríguez et Fernández-del-Olmo, 2014; Wiethoff, Hamada et Rothwell, 2014), nous croyons que ce résultat pourrait être attribuable à des différences individuelles dans la réponse à la stimulation cathodale dans notre échantillon.

Quant à l'objectif principal d'évaluer la possibilité de moduler l'inhibition intracorticale liée aux récepteurs $GABA_B$, les résultats ont montré qu'il est possible de réduire la durée de la PSC suite à 20 minutes de stimulation anodale du cortex moteur, ce qui concorde avec l'hypothèse initiale. Ceci contraste avec une étude précédente, dans laquelle la SÉTcd anodale n'a pas produit de modification significative de la durée de la PSC (Suzuki et al., 2012). Par ailleurs, la stimulation anodale a échoué à moduler l'IICld, une autre mesure de l'inhibition intracorticale liée au $GABA_B$. Les données actuelles suggèrent que la SÉTcd anodale a un impact différentiel sur les mesures de PSC et l'IICld, ce qui concorde avec une hypothèse

récemment soulevée stipulant que les deux mesures du système GABAergique représenteraient différents mécanismes inhibiteurs (Ziemann et al., 2014).

Quant à la SÉTcd cathodale, aucune modulation des deux index d'inhibition intracorticale n'a été induite par la stimulation. Ce résultat concorde avec les résultats d'une étude précédente où aucune modulation de la PSC n'a été observée chez des individus en bonne santé suite à 10 minutes de stimulation cathodale (Suzuki et al., 2012). Toutefois, Hasan et collaborateurs ont montré une augmentation de la durée de la PSC suite à 9 minutes de SÉTcd cathodale (Hasan et al., 2012).

Le manque de consensus quant aux effets des deux types de stimulation sur les mesures d'inhibition intracorticale de M1 pourrait être attribuable en partie à des différences quant au choix des paramètres de stimulation. En effet, dans les études précédentes, on observe des divergences quant à l'intensité et la durée de la stimulation, ainsi que la taille des électrodes, en comparaison avec les paramètres utilisés dans l'article 4 (intensité : 1.5 mA; durée : 20 min; électrodes : 25 cm^2 pour la stimulation M1 et 35 cm^2 pour la stimulation supra-orbitale).

Néanmoins, la modulation significative de la PSC suggère que la SÉTcd anodale pourrait réduire l'hypoexcitabilité corticale présente suite aux commotions cérébrales. Toutefois, les mécanismes par lesquels la SÉTcd engendre une réduction de l'inhibition demeurent méconnus. En théorie, une augmentation de l'excitabilité corticale (ou réduction du niveau d'inhibition) pourrait s'expliquer par une transmission excitatrice accrue (p.ex. augmentation du glutamate) ou une réduction de la transmission inhibitrice (p.ex. diminution de l'activité des récepteurs $GABA_B$; Reis et Fritsch, 2011). De ce fait, les études animales et pharmacologiques suggèrent qu'une réduction de la transmission inhibitrice pourrait impliquer des modifications de l'activité des récepteurs NMDA (Liebetanz et al., 2002; Reis et Fritsch, 2011; Rossini et al., 1994) et le déclenchement de mécanismes de plasticité cérébrale similaires aux mécanismes de plasticité à long terme (PLT; Stagg et Nitsche, 2011). Quant aux études effectuées chez l'humain, une récente étude en SRM suggère qu'une réduction du GABA, et non une augmentation glutamate, pourrait sous-tendre les effets excitateurs engendrés par la stimulation anodale (Stagg et al., 2009). De plus, une association entre la diminution des concentrations de GABAergiques dans

M1 et l'apprentissage d'une séquence motrice a récemment été révélée (Floyer-Lea, Wylezinska, Kincses et Matthews, 2006). Les auteurs ont soulevé l'hypothèse que ces changements pourraient être associés aux mécanismes de PLT (Floyer-Lea et al., 2006).

Ces études suggèrent deux points importants en lien avec la possible utilisation de la technique auprès d'athlètes commotionnés. D'abord, elles suggèrent qu'il est possible d'agir sur le GABA avec la SÉTcd anodale et donc que l'inhibition intracorticale plutôt que la transmission excitatrice pourrait être spécifiquement affectée par la SÉTcd anodale et partiellement sous-tendre les changements dans l'excitabilité corticale généralement engendrés par la stimulation. Ensuite, elles mettent en lumière la possibilité d'agir sur la fonction motrice et la plasticité cérébrale. Ceci est d'autant plus pertinent compte tenu des études rapportant une altération de la plasticité cérébrale chez des athlètes commotionnés (De Beaumont et al., 2011b) et des individus ayant subi un TCCL (Annexe 2).

8.3.1.1 Article 4 : conclusions

En somme, la présente étude démontre la possibilité de moduler la PSC suite à une stimulation anodale chez individus en santé. Ceci suggère l'existence d'un potentiel thérapeutique lié à l'utilisation de la SÉTcd. Toutefois, la littérature actuelle comporte plusieurs résultats divergents quant aux effets neurophysiologiques de la SMT, possiblement associés à la grande diversité de paramètres de stimulation employés et la grande variabilité inter-individuelle de la réponse à la stimulation.

8.3.2 Aspect méthodologique : modulation du métabolisme cérébral suite à la SÉTcd

Les résultats de l'étude 4 montrant la possibilité de moduler la PSC avec la SÉTcd anodale, ainsi que les résultats d'une étude récente suggérant la possibilité de moduler le GABA avec le même protocole de stimulation (Stagg et al., 2009) suggèrent un potentiel clinique important pour la SÉTcd. Toutefois, d'autres protocoles de stimulation, comme la stimulation

bilaterale, ont également montré des effets bénéfiques sur la cognition lorsqu'appliqués au niveau préfrontal (Annexe 3), ainsi que sur l'excitabilité corticale et les symptômes moteurs suite à un accident vasculaire cérébral (AVC; Reis et Fritsch, 2011). Étant donné le peu de connaissances des mécanismes d'action sous-tendant l'effet de ce protocole et de la SÉTcd en général, l'article 5 avait pour but de développer un protocole expérimental visant à tester l'effet de la SÉTcd bilatérale appliquée simultanément aux deux cortex moteurs primaires sur le métabolisme cérébral. Suite à la démonstration et la mise sur pied du protocole, nous avons effectué les trois expérimentations incluses dans l'article 6. La première visait à évaluer les effets de la SÉTcd bilatérale sur l'excitabilité corticale de M1. La deuxième avait pour but d'utiliser le protocole développé dans l'article 5 auprès d'un échantillon de huit individus en bonne santé. Enfin, la troisième avait pour objectif de répliquer les effets obtenus par Stagg et ses collègues (2009) sur le métabolisme cérébral suite à la SÉTcd anodale.

Dans la première étude de l'article 6, nous avons révélé l'absence d'une modulation significative de l'excitabilité corticale par la SÉTcd bilatérale, à la fois lorsque l'anode se trouvait au-dessus du M1 gauche et la cathode au-dessus du M1 droit (anode/cathode), que lorsque les électrodes étaient inversées (cathode/anode). Malgré le fait que ces résultats contredisent l'hypothèse émise dans l'introduction, ils répliquent néammoins ceux récemment obtenus par O'Shea et collaborateurs (2013), où aucune modulation significative des PÉM n'a été observée suite à une stimulation bilatérale anode/cathode avec les mêmes paramètres de stimulation que ceux utilisés dans l'étude 6 (1 mA; électrodes de 35 cm^2; 13 participants). À l'inverse, deux études récentes ont montré les effets théoriquement attendus suite à une stimulation bilatérale, à savoir une augmentation des PÉM sous l'anode et une réduction sous la cathode (Mordillo-Mateos et al., 2012; Tazoe, Endoh, Kitamura et Ogata, 2014). Une autre étude a également montré un effet attendu, soit une augmentation de l'excitabilité corticale sous l'anode dans un protocole bilatéral anode/cathode (Kidgell, Goodwill, Frazer et Daly, 2013).

Tout comme pour l'article 4 où une absence d'effet a été observée pour la SÉTcd cathodale, les présentes divergences entre les études peuvent être en partie attribuées aux paramètres de stimulation. Par exemple, il a récemment été démontré que si l'on double l'intensité d'une stimulation cathodale de 1 mA à 2 mA, l'effet obtenu est excitateur et non

inhibiteur (Batsikadze, Moliadze, Paulus, Kuo et Nitsche, 2013). Quant à la durée de la stimulation, de récentes études suggèrent que les effets modulateurs sont réduits lorsque celle-ci est augmentée (Fricke et al., 2011). Il semble donc y avoir des effets non-linéaires associés à la modification des paramètres de stimulation. Il est à noter que ces études ont généralement évalué les effets de la SÉTcd unilatérale (M1 / région supra-orbitale). Une évaluation systématique de l'efficacité de divers paramètres de stimulation bilatérale est nécessaire afin de développer des protocoles de traitement pour diverses populations, tels que les athlètes commotionnés et les personnes ayant subi un AVC.

En lien avec ces résultats, les expérimentations 2 et 3 en SRM n'ont révélé aucune modulation significative des principaux neurotransmetteurs (GABA, Glx, NAA, mIns) comparativement à une stimulation placebo, et ce suite à 20 minutes de stimulation anodale et suite aux deux conditions de SÉTcd bilatérale (anode/cathode et cathode/anode). Bien que ce soit la première étude à évaluer l'effet d'un protocole bilatéral sur le métabolisme cérébral, quatre études récentes ont étudié les effets de la SÉTcd unilatérale anodale et/ou cathodale. Bien que chacune de ces études ait rapporté une modulation d'au moins un métabolite suite à une stimulation unilatérale, aucun consensus n'émerge des résultats. L'étude présentant une méthodologie la plus similaire à la notre a montré une réduction des concentrations GABAergiques suite à une stimulation anodale des régions motrices et une réduction du GABA et du glutamate suite à une stimulation cathodale (Stagg et al., 2009). Par ailleurs, une étude a montré une réduction du GABA suite à une stimulation anodale, mais aucun effet de la stimulation cathodale (Kim, Stephenson, Morris et Jackson, 2014), tandis que des études mesurant une région non-motrice ont montré une augmentation du glutamate et du NAA (Clark, Coffman, Trumbo et Gasparovic, 2011), ainsi que du myo-inositol (Rango et al., 2008) suite à une stimulation anodale.

Plusieurs facteurs peuvent limiter la généralisation des résultats des études antérieures. Tout d'abord, trois des quatre études ont effectué la SÉTcd à l'extérieur du scanneur, ce qui engendre l'introduction de plusieurs facteurs confondants (Clark et al., 2011; Kim et al., 2014; Rango et al., 2008). Notre protocole et celui de Stagg et collaborateurs (2009) incluaient une stimulation à l'intérieur du scanneur et donc aucun mouvement du participant entre les deux

mesures du métabolisme cérébral. De plus, une étude a discuté d'un effet sur le GABA non-significatif ($p = 0.051$) et utilisé un protocole d'expérimentation inter-sujets quant aux différentes conditions de stimulation (Kim et al., 2014), tandis qu'une étude n'a pas utilisé de condition placébo (Clark et al., 2011). Par ailleurs, tout comme dans notre étude, de faibles tailles d'échantillon ont été utilisées. Des analyses de puissance statistique ont suggéré que notre échantillon devait être triplé pour d'obtenir des résultats significatifs.

Ainsi, comment expliquer le peu de consensus présent dans la littérature en lien avec les effets neurophysiologiques et métaboliques de la SÉTcd ? Premièrement, ceci pourrait s'expliquer par l'utilisation de paramètres de stimulation divergents (p.ex. durée et intensité) et par la présence d'une grande variabilité inter-individuelle dans la réponse à la stimulation, tel que discuté en lien avec l'article 4. Bien que le facteur de variabilité ait été étudié suite à la stimulation unilatérale (López-Alonso et al., 2014; Wiethoff et al., 2014), il n'a pas été évalué à ce jour suite à une stimulation bilatérale. Malgré une taille d'échantillon ne permettant pas l'évaluation de ce facteur, l'analyse des coefficients de variation de nos mesures métaboliques et neurophysiologiques sont en accord avec les études précédentes menées en SRM (O'Gorman, Michels, Edden, Murdoch et Martin, 2011) ou SMT (Darling, Wolf et Butler, 2006; Kiers, Cros, Chiappa et Fang, 1993; Pitcher, Ogston et Miles, 2003). Ainsi, nous suggérons que la variabilité observée n'est pas expliquée par une faille méthodologique liée à la mesure, mais bien par la réponse du participant à la stimulation. De surcroit, l'analyse des coefficients de variation associés à chacune des méthodes met en lumière des coefficients beaucoup plus petits pour la SRM (autour de .06) que pour la SMT (autour de .25). Ceci suggère que malgré l'absence d'un effet significatif de la SÉTcd sur le métabolisme et la neurophysiologie des régions motrices, la SRM pourrait être une mesure plus stable, et donc plus sensible aux changements produits par la stimulation.

8.3.2.1 Articles 5 et 6 : conclusions

La présente étude suggère que la SRM et la SMT sont peu sensibles pour quantifier de manière fiable les effets neurophysiologiques et métaboliques de la SÉTcd bilatérale avec de petits échantillons. Ceci contraste avec la littérature comportementale, où de nombreuses études

ont révélé des changements associés à la stimulation bilatérale dans une variété de tâches cognitives et motrices (voir Reis et Fritsch, 2011; Annexe 2). De plus, de récentes études ont montré une amélioration des fonctions motrices suite à ce type de stimulation (Lüdemann-Podubecká, Bösl, Rothhardt, Verheyden et Nowak, 2014). Il reste à déterminer si les mesures comportementales sont plus sensibles aux effets de la SÉTcd bilatérale que la SRM et la SMT. Des études combinant des mesures comportementales, neurophysiologiques et métaboliques, qui évaluent systématiquement les effets des paramètres de stimulation, et qui identifient les facteurs prédictifs d'une réponse à la stimulation sont nécessaires pour soutenir son utilisation auprès de populations cliniques.

8.4 Conclusion générale et perspectives futures

Les deux études effectuées auprès d'une population d'athlètes ayant subi une ou plusieurs commotions cérébrales, ainsi que la littérature actuelle portant sur cette problématique, nous laissent croire que l'hypoexcitabilité corticale, probablement sous-tendue par un dysfonctionnement des récepteurs $GABA_B$ et un déséquilibre inhibiteur/excitateur, pourrait être un marqueur des effets à long terme de la commotion cérébrale et d'une récupération incomplète. Toutefois, les études méthodologiques présentées dans cet ouvrage suggèrent que le lien entre les index d'excitabilité corticale révélés par la SMT et les mesures directes de GABA et de glutamate obtenues par la SRM sont plus complexes qu'il n'avait d'abord été suggéré. Ces études ont plutôt révélé un lien entre l'activité des récepteurs $GABA_B$ et le glutamate. Par conséquent, est-ce que le GABA pourrait être un marqueur d'une récupération incomplète suite à une commotion cérébrale dans le sport ? Les études présentées dans le présent ouvrage ne nous permettent pas de répondre par l'affirmative. Il semble toutefois que la période silencieuse corticale, qu'elle soit liée aux récepteurs $GABA_B$ et/ou au glutamate, soit un marqueur potentiel.

Il reste encore plusieurs étapes à franchir avant d'inclure l'utilisation de la SMT dans les futures lignes directrices liées à la gestion et l'évaluation des commotions cérébrales dans le sport. Premièrement, il est essentiel de développer des études longitudinales auprès de grandes cohortes d'athlètes afin d'élucider la progression des changements de l'excitabilité corticale

suite à une commotion cérébrale, par la SMT, mais également par la SRM. Deuxièmement, il est impératif d'obtenir plus d'information concernant l'impact de ce dysfonctionnement des mécanismes d'inhibition sur la motricité des athlètes. Étant donné la récente inclusion de mesures d'équilibre au sein des protocoles d'évaluation des commotions cérébrales (p.ex. au sein du SCAT-3; Echemendia et al., 2015), il serait facile d'inclure de telles mesures en complément aux mesures neurophysiologiques. Troisièmement, il serait pertinent d'évaluer les effets des coups multiples à la tête dans les sports de contact en incluant un second groupe contrôle composé d'athlètes ne participant pas à des sports de contacts. Finalement, considérant l'anatomie du cerveau et les connaissances actuelles sur la pathophysiologie des commotions cérébrales, il est peu probable que seul le cortex moteur primaire soit affecté suite à une commotion cérébrale. Toutefois, ce dernier est une cible quasi-systématique dans les études en SMT puisqu'il est possible d'obtenir une mesure objective de l'excitabilité corticale. Le récent développement de méthodologies combinant la SMT à l'électroencéphalographie offre désormais la possibilité d'obtenir des mesures relativement fiables de l'inhibition intracorticale, incluant l'IIC de longue durée, dans des régions non-motrices, telles que les régions frontales et préfrontales (Farzan et al., 2010; Fitzgerald et al., 2008; Fitzgerald, Maller, Hoy, Farzan et Daskalakis, 2009). Il serait donc intéressant d'étudier la possible présence d'altérations de l'excitabilité corticale dans ces régions chez l'athlète commotionné, et les comparer à des mesures neurophysiologiques au niveau des régions motrices.

Par ailleurs, il semble que la SÉTcd anodale puisse moduler à la baisse la durée de la PSC et ainsi offrir une avenue de traitement intéressante en lien avec les atteintes neurophysiologiques persistantes. La prochaine étape serait donc de tester cette méthode de stimulation corticale non-invasive auprès d'une population d'athlètes commotionnés présentant des anomalies de la PSC. Dans l'éventualité de résultats positifs, des études cliniques randomisées pourraient être mises sur pied pour vérifier l'impact clinique de cette méthode dans le traitement des commotions cérébrales. À court terme, ceci pourrait favoriser la récupération suite à une commotion cérébrale et diminuer les atteintes motrices fonctionnelles. À long terme, il est possible d'imaginer qu'une telle approche pourrait freiner le développement d'atteintes persistantes, et possiblement réduire la « vulnérabilité cellulaire » présente dans le cerveau d'athlètes commotionnés.

En parallèle, des études méthodologiques sont essentielles au développement de la SÉTcd comme traitement auprès de populations cliniques incluant les commotions cérébrales. En effet, les futures études devraient, à notre avis, être orientées vers une meilleure compréhension de la variabilité de la réponse à la stimulation, l'évaluation systématique des paramètres de stimulation optimaux auprès de larges échantillons et l'utilisation de multiples méthodes d'investigation pour élucider les mécanismes sous-tendant les effets de la SÉTcd. Une meilleure compréhension de ces facteurs, ainsi que des facteurs prédictifs d'une réponse positive au traitement, permettrait le développement de traitements individualisés auprès de plusieurs populations cliniques.

En somme, bien que plusieurs avenues demeurent à explorer, les études incluent dans le présent ouvrage ont permis d'approfondir les connaissances sur les effets neurophysiologiques et métaboliques des commotions cérébrales, mais également sur le mécanisme d'action des diverses méthodologies utilisées.

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Annexe 1

Probing the effects of mild traumatic brain injury with transcranial magnetic stimulation of the primary motor cortex

Probing the effects of mild traumatic brain injury with transcranial magnetic stimulation of the primary motor cortex

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Abstract

Primary objective: The present paper systematically reviews studies using transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) to assess cortical excitability, intracortical inhibtion/facilitation and synaptic plasticity following mild traumatic brain injury (mTBI).

Methods: Articles using TMS over M1 in patients with mTBI or sport-related concussion indexed in PubMed and published between 1998 and September 2014 were included in the present review.

Main outcomes and results: From the 17 articles that matched search criteria, results from various TMS paradigms were summarized and divided in three main areas of interest: motor cortical excitability/facilitation, motor cortical inhibition, and cortical plasticity. Although studies suggest a trend of abnormal intracortical inhibition following mTBI, no clear and specific pattern emerges from the surveyed data.

Conclusions: At this time and with the possible exception of intracortical inhibitory measures, TMS cannot reliably detect changes in M1 excitability in individuals with mTBI or a concussion at both the acute and chronic stages of injury. This may be explained by the small number of studies and large variety of stimulation parameters. Additional longitudinal and multimodal studies are needed to better understand the nature of the excitability changes that may occur within M1 following mTBI.

Introduction

In 2009 only, over 3.5 million individuals in the USA were diagnosed with a traumatic brain injury (TBI), which represents a serious public health problem [1]. The most frequent causes of TBI being falls, motor-vehicle collisions, and violence, TBI affect all age groups, and more importantly adolescents and young adults, children and the elderly [1,2]. It is estimated that amongst treated brain injuries, about 70 to 90% are considered mild traumatic brain injuries (mTBI) [2]. Considering the large number of mild traumatic brain injuries that are not treated, the incidence of mTBI is estimated at 600 per 100,000 [2].

The World Health Organization (WHO) Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury proposes a definition of mild traumatic brain injury (mTBI) as an 'acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include: (i) 1 or more of the following: confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Scale score of 13–15 after 30 minutes post-injury or later upon presentation for healthcare' [3]. When a mTBI occurs in a sport context, the brain injury caused by a direct or indirect blow to the head is called a concussion. The terms concussion and mTBI, however, have often been used interchangeably in the literature [4]. A wide range of physical, cognitive, behavioural and affective symptoms follow mild traumatic brain injury or concussion, and are used to determine recovery. Among the most frequently reported symptoms are headaches, fatigue, slowness, irritability, balance problems, attention, and concentration and memory deficits [5,6].

Mild traumatic brain injury has long been considered a minor and completely reversible injury. This belief was supported by the typical spontaneous resolution of symptoms within 2-3 weeks post-injury and the failure to observe any gross brain damage as assessed by standard neuroimaging techniques [4]. However, the recent association of sport concussions with neurodegenerative disorders, such as dementia [7] and chronic traumatic encephalopathy [8] suggests that there may be 'silent' pathological changes occurring in the brain following mTBI

that could cause long-lasting alterations. Models of TBI derived from animal studies described by Giza and Hodva [9] suggest that the initial impact to the head provokes a pathological cascade of cellular and metabolic disruptions, including inflammation, excitotoxicity, oedema, and mithocondrial dysfunction. This neurometabolic cascade is thought to involve a sudden imbalance in glutamatergic, cholinergic and GABAergic levels, as well as ion channels and N-methyl-D-aspartate (NMDA) receptor malfunctions [9,10].

The majority of patients with mTBI recover within days to weeks, but some experience symptoms that last for months or even become essentially permanent. Following a concussion, the potential for long-lasting neurobiological consequences remains unresolved, and the question of when recovery is complete and thus normal activities can be resumed is uncertain as we lack reliable biomarkers [11-14]. Traditional, purely clinical measures of the consequence of a concussion are unreliable indicators of brain injury, poor predictors of full recovery, return to work, sport activity or duty decisions. Therefore, there is a need to develop objective markers to facilitate diagnosis and gather prognostic insights, test treatment efficacy, and establish objective criteria on which to base activity decisions [14]. Neurophysiologic [15-17] and neuroimaging [18,19] methods, while promising, are still insufficiently reliable, perhaps because they fail to directly assess the pathophysiology of post-concussion symptoms and consequences. Indeed, mechanistic insights into the etiology of (lasting) deficits after a concussion are sparse. The recent development of powerful tools of noninvasive brain stimulation opens a new opportunity to explore diagnostic and prognostic markers of mTBI. More specifically, numerous recent studies have used transcranial magnetic stimulation (TMS) of the primary motor cortex to identify areas of abnormality at both the acute and chronic stages of mTBI, and there is hope that such indices may serve as diagnostic and prognostic predictors for subjects after a concussion.

Introduced by Barker and collaborators in 1985 [20], TMS allows the assessment of motor neurophysiology via non-invasive, safe and painless stimulation of the human brain [21]. TMS is based on the principle that when a magnetic pulse is delivered over a brain region, the pulse induces a secondary ionic current in the brain, which produces a depolarization of a population of neurons [21]. When applied over the primary motor cortex (M1), the

depolarization activates the corticospinal pathway and induces a twitch in the corresponding muscle. This muscular activity can be quantified using electromyography, i.e. the motor evoked potential (MEP). Using specific paradigms involving single and paired pulses, it is possible to assess the integrity of excitatory and inhibitory mechanisms within M1, as well as synaptic plasticity, sensorimotor interactions and motor pathway integrity [22]. The present paper reports a systematic review of TMS studies in patients with mTBI to reveal patterns of primary motor cortex abnormalities and guide future efforts aiming at developing reliable markers of incomplete recovery.

Material and methods

A systematic review of the literature was performed using the following databases: PubMed (1998 to sept 2014) and Medline (1998 to sept 2014). The following search keywords were used: 'TMS', 'transcranial magnetic stimulation', 'traumatic brain injury', 'TBI', 'concussion', 'motor cortex', and 'M1'. We initially identified 106 articles corresponding to our search criteria. After carefully reviewing the abstract of all papers, we identified 15 articles investigating mild traumatic brain injury in humans. We also looked through the references of the selected papers for additional relevant articles, which led to the inclusion of two additional papers. Subsequently, we read through the full texts of the final sample of articles to gather the following information: population (athletes or non-athletes), number of participants, age of participants, number of concussions, time post injury, TMS parameters, and results. Studies were only included if they were published in English and described thoroughly their methodology.

Results

Note that results are restricted to M1 stimulation, even though some studies investigated other regions or other methods were used (i.e. EEG, neuropsychological testing). When different levels of severity of traumatic brain injury were included in the studies, only results related to mTBI and sport-related concussions are reported. In order to be succinct, only the main results

of the different studies are reported (see table 1 for full results). For a clearer understanding of the impact of mTBI on the neurophysiology of the primary motor cortex, the results are divided into each TMS paradigm used to measure three main areas of interest: motor cortical excitability and facilitation, motor cortical inhibition and cortical plasticity (table 2). Note that for a clear identification of the populations, the term concussion refers to sport-related mTBI and the term mTBI refers to non-athlete populations.

Motor cortical excitability

Resting motor threshold (rMT)*:* The resting motor threshold is usually obtained by a single stimulation of the primary motor cortex (M1) at the lowest intensity of stimulation producing a 50μV peak-to-peak amplitude muscle contraction (MEP) of the resting targeted contralateral muscle in a minimum of five out of ten consecutive trials. The rMT is believed to reflect global excitability of the corticospinal system, including membrane excitability of corticospinal neurons and interneurons, and synaptic connections at the cortical and spinal levels [21]. Pharmacological studies suggest that motor thresholds are mediated by NMDA glutamate receptor activity [23]. The resting motor threshold was found to be normal in mTBI groups, i.e. equivalent to controls, in the majority of reviewed studies, in both acute and chronic phases. However, two studies found an elevated rMT 2 weeks after mTBI that returned to normal levels 3 months post-injury [24,25]. An elevated rMT was also observed, in both symptomatic and asymptomatic non-athletes, 5 years after injury [26,27].

Active motor threshold (aMT): The active motor threshold is usually obtained by a single stimulation of the primary motor cortex (M1) at the lowest intensity of stimulation producing a 100 μV peak-to-peak amplitude activation (MEP) of the contracted targeted contralateral muscle in a minimum of 50% of ten consecutive trials [21]. Compared to the rMT, the active motor threshold is believed to depend more directly on axon thresholds [23]. Two studies reported results for the aMT. Pearce and colleagues [28] reported normal aMT values at 2, 4 and 10 days post-injury in amateur Australian football players while Chistyakov, et al. [25] reported a higher active threshold 2 weeks post-mTBI.

Motor evoked potential amplitude: To assess MEP amplitude, single pulse stimulation at a typical intensity of 120% rMT is delivered over M1 and mean peak-to-peak amplitudes are measured. MEP amplitudes reflect global excitability of the corticospinal tract, including the motor cortex, and can be used to assess the physiologic integrity of motor pathways [21]. Pharmacological studies suggest that MEP amplitude is mediated by the activity of many receptors, including $GABA_A$ and dopamine [23]. MEP amplitude was shown to be normal in the acute [28,29] and chronic phases post-injury [30,31].

MEP/M amplitude ratio: The MEP/M amplitude ratio is calculated by dividing the average peak-to-peak MEP amplitude by the MEP amplitude obtained through supramaximal peripheral electrical stimulation, which is related to activation of the M-wave [25]. A significant increase in the MEP/M amplitude ratio was observed between day 3 and day 5 following a sport concussion [32,33]. Two weeks post-injury, MEP/M wave amplitude ratios were shown to be reduced in another study [25].

MEP latency: MEP latency is defined as the onset latency of the peripheral motor response and is thought to reflect the integrity of fast-conducting fibres along the corticospinal pathway [34]. Livingston and colleagues [32,33] reported a significant prolongation of MEP latency between day 1 and day 10 following injury. However, other studies found normal latencies in the acute phase [28,29].

Input-output curve: Also called recruitment curve, this measure is obtained by successive single stimulations of varying intensities, where increased intensities lead to higher MEP amplitudes [22]. The slope of the MEP input-output curve is believed to reflect corticospinal excitability, with steeper slopes indicating increased excitability [35]. Insights from pharmacological studies suggest a relationship with glutamatergic activity [36]. The input-output curve was shown to be normal in the chronic phase (approximately 5 years and 30 years after the last sport concussion) [37,38]. Conversely, Pearce and collaborators [30] reported reduced inpout-output curves in retired athletes 21 years post-concussion.

Central motor conduction time (CMCT): CMCT represents the delay between motor cortex activation and motor neuron activation in the brainstem or in the spinal cord [22]. To assess CMCT, the latency of the peripheral conduction time, which is usually obtained by the stimulation of the spine root over the intravertebral foramina central, is subtracted from the central MEP latency [22]. Chistyakov, et al. [25] found prolonged CMCT two weeks after mTBI while other studies found normal conduction time in non-athletes and concussed athletes [24,32,33,39].

Intracortical facilitation (ICF): Based on a protocol first described by Kujirai, et al. [40], this paradigm consists of pairing of a sub-threshold stimulation (70 to 90% of rMT) to a supra-threshold (usually 120% of rMT) stimulation with a 8ms to 30ms inter-stimulation interval (ISI) [41], which produces a facilitation of the motor response. This facilitation is believed to reflect interneuron activity and is thought to be modulated by glutamatergic [42] and GABAergic activity ($GABA_A$ receptors) [43]. ICF was found to be reduced in concussed individuals between week 1 and week 4 post-injury [44]. In contrast, Bashir, et al. [45] have shown greater ICF at weeks 2 and 6 in a concussed individual. In the chronic phase, ICF was shown to be normal [37,38].

Motor cortical inhibition

Cortical silent period (CSP) duration: In this paradigm, a supra-threshold single pulse stimulation is applied over M1 while the participant maintains a slight voluntary contraction of the targeted muscle, creating a pause in the EMG signal occurring after the MEP [21,22]. The duration of this silent period, more precisely the later part, is thought to be modulated by intracortical inhibition [22]. Pharmacological studies suggest that the CSP reflects $GABA_B$ receptor activity [46]. In the acute phase of mTBI, the CSP tends to be prolonged on the first days [28,29], weeks [25,29] and months [29] following a concussive event. This prolongation of the CSP seems to be maintained over time as demonstrated in studies where athletes were tested more than 9-12 months post-injury [31,38,39,47]. Furthermore, prolongation of the CSP observed in athletes tested more than 9 months post-concussion was shown to further increase when retested 6-15 months after a new injury[38]. De Beaumont, et al. [37] have also shown

that abnormally high CSP duration values can be observed 30 years after the last concussion in retired athletes with a history of multiple concussions. In contrast, a study found a reduction of CSP duration in Australian football players tested on average 21 years post injury [30]. Also, the presence of CSP durations within normal limits was reported in the first 4 weeks [44] and again more than 10 months post injury [48].

Short intracortical inhibition (SICI): Based on a protocol first described by Kujirai, et al. [40], this paradigm consists of pairing of a sub-threshold stimulation (70 to 90% of rMT) to a supra-threshold (usually 120% of rMT) stimulation with a 1ms to 5ms inter-stimulation interval (ISI) [41], which produces an inhibition of the motor response. This protocol is believed to reflect intracortical inhibition, more specifically $GABA_A$ receptor activity [49,50]. In the acute phase, SICI was shown to be normal [28,44,45]. In the chronic phase, SICI was also shown to be normal in most studies [37-39]. However, one study reported a reduction of SICI in athletes tested at 21 years post-injury [30].

Long intracortical inhibition (LICI): Using a paired-pulse paradigm, suppression of the motor response can also be induced by two supra-threshold pulses with a longer inter-stimulus interval (50-200ms) [41]. This inhibitory measure is believed to be modulated by $GABA_B$ receptor activity [46,51]. In the acute phase, Powers, et al. [44] found normal LICI in the first 4 weeks post-injury in football athletes. However, Bashir, et al. [45] observed an absence of inhibition in a patient at 2weeks post-injury. This altered inhibition had returned to normal levels when retested at week 6 post-injury. In the chronic phase, LICI was shown to be normal in concussed athletes more than 10 months post-injury in one study [48]. In contrast, LICI was found to be enhanced more than 9-12 months post-concussion in football athletes [31,39,47]. When tested on average 21 years post-concussion, LICI was also found to be altered, but inhibition was reduced [30].

Short latency afferent inhibition (SAI): This paired-pulse protocol involves a peripheral stimulation followed by a cortical stimulation to examine sensorimotor interactions. Afferent inhibition of motor cortex excitability is elicited by an electrical stimulation of the median nerve. This stimulation is paired with a magnetic stimulation of the contralateral M1 with inter-

stimulation intervals of 19 to 21ms [52]. SAI is thought to be a marker of sensorimotor integration and involves cholinergic [53] and $GABA_A$ [54] receptor activity. One study investigated SAI and reported normal inhibition in concussed athletes more than a year after the last concussive event [39].

Long latency afferent inhibition (LAI): Inhibition of the motor excitability is also possible using the same protocol as SAI, but with an ISI of 100 to 200ms [22]. This measure is thought to reflect M1-primary sensory cortex (S1) interactions [55]. Tremblay, et al. [39] reported no significant LAI differences between concussed and control athletes tested one year after the last concussion.

Cortical plasticity

Potential for cortical plasticity can be evaluated by numerous TMS protocols. Depending on specific parameters, it is possible to modulate motor cortex excitability by increasing or inhibiting the potential for motor response. Two plasticity mechanisms are thought to be modulated by TMS: long-term potentiation (LTP), when synaptic strength is increased, and long-term depression (LTD), when synaptic strength is decreased [22].

Repetitive transcranial magnetic stimulation (rTMS): With rTMS, successive pulses of the same intensity are applied at low (1Hz or less) or high (usually 5 Hz to 20Hz) frequencies. Generally, inhibition of motor excitability can be achieved by low-rate rTMS, whereas high-rate rTMS increases excitability [21]. Only one study has reported findings from rTMS in patients with a mTBI. A low frequency (1Hz) paradigm was used and showed irregular shape alternations of the MEP waveform. These abnormalities were observed 2 weeks after injury and normalized in 9 out of 15 patients when retested 3 months after injury [24].

Theta burst stimulation (TBS): In theta burst stimulation (TBS), bursts of stimulation are used to induce plasticity related to LTP and LTD. The effects of TBS depend upon the temporal pattern of stimulation: continuous (cTBS) trains of stimulation (3 stimuli at 50Hz every 200ms, for 20-40 sec) induce a reduction in excitability and intermittent (iTBS) trains of stimulation (2

sec bursts every 10 sec for a total duration of 190 sec) induce an increase of excitability [56]. Bashir, et al. [45] used cTBS and measured its effect on excitability at several time-points following stimulation (0, 5, 10, 20 minutes post-cTBS). In contrast with the expected inhibition and with the results obtained in control subjects, two weeks after the injury, a single patient presented MEP facilitation at every time point. However, normal inhibition was found in the patient 6 weeks post-injury.

Paired associative stimulation (PAS): This paradigm involves peripheral electric stimulation of the median nerve (afferent somatosensory pathway) paired with magnetic stimulation of M1. If the afferent peripheral signal arrives in motor cortex synchronously with a TMS pulse applied to M1, and paired stimulation is applied repetitively, it induces plasticity-like changes in excitability [57]. This synchronicity is usually obtained with a 25ms ISI (PAS_{25}) and is thought to reflect LTP-like plasticity [22]. This technique has been shown to induce MEP amplitude and CSP duration increases [57] in healthy subjects. However, if a 10ms ISI is used (PAS_{10}), the response is inhibited and is thought to reflect LTD-like plasticity [22]. A single study has reported the effects of PAS in individuals with a concussion during the chronic phase [31]. As expected, controls showed the usual increase in MEP amplitude and prolongation of the CSP following PAS_{25} whereas athletes with a history of multiple concussions did not. For PAS_{10}, a similar pattern was observed: controls showed the usual decrease in cortical excitability whereas concussed athletes did not.

Discussion

An increasing number of studies have explored the physiological integrity of primary motor cortex using TMS after mTBI or sport-related concussion. The present review highlights the wide variety of paradigms and parameters that have been used to assess cortical reactivity and plasticity following mTBI. Due to a significant disparity in procedures and results, there is a lack of consensus regarding the short- and long-term physiological response to mTBI or concussion. Whereas altered inhibitory function is generally observed, no clear pattern of

changes in excitatory systems emerges from the literature. Additionally, very few studies have addressed the impact of mTBI on M1 plasticity.

The most frequently reported alteration in M1 function following mTBI is an abnormal level of intracortical inhibition. In both the acute and chronic phases, most studies have reported significantly altered values in inhibitory TMS protocols (CSP, SICI and LICI). Nine studies found increased CSP durations in concussed individuals, two studies found no difference with healthy controls and one reported a reduction in CSP duration (see table 1). As CSP duration is thought to reflect $GABA_B$ receptor activity [46], similar results should be expected with LICI, which also appears to be modulated by $GABA_B$ receptor activity [51]. Here again, alterations are commonly reported, but no clear pattern emerges. In the acute phase, two studies reported both normal and abnormal levels of LICI, although impaired LICI was found in a single patient and the abnormal values returned to baseline 6 weeks post injury [45]. In the chronic phase, LICI was reported to be enhanced in three studies, normal in one study and reduced in one study (see table 1). It has been hypothesized that altered intracortical inhibition at both the acute and chronic stages of mTBI are related to the complex neurometabolic events that accompany trauma to the head [28]. More specifically, the reviewed data point to significantly altered GABA transmission [30,37] in the motor cortex of concussed individuals.

It should be noted, however, that TMS is essentially an indirect measure of neurotransmitter function [43]. Neurotransmitter concentrations can be more directly assessed with proton magnetic resonance spectroscopy (^{1}H-MRS), which allows sensitive *in vivo* detection and quantification of brain metabolites [58,59,60] such as *N*-acetylaspartate, glutamate and GABA [61]. It has been shown that athletes with a concussion suffer an initial increase in glutamate concentration (1-6 days post-injury) that resolves at 6 months post-injury [2]. MRS data have also shown that concussed athletes tested on average 3 years after their last concussion display normal MRS-GABA levels within M1 [48]. There is an apparent discrepancy, therefore, between the numerous TMS studies reporting altered GABAergic inhibition in M1 and the one study assessing GABA directly with MRS. However, it appears that MRS measures of GABA correlate weakly and imperfectly with TMS measures of inhibition, such as LICI and CSP [63,64]. This suggests that TMS and MRS may reflect distinct GABAergic mechanisms and

thus be affected differently by mTBI, arguing in favour of multimodal approaches that could provide complementary information regarding neurotransmitter disruption and validate TMS measures in this population.

In addition to evaluating inhibitory function within M1, TMS provides a simple way to assess numerous other specific characteristics of M1 reactivity and plasticity in response to mTBI. Except for motor threshold, it is difficult at this point to detect any specific pattern of abnormalities that accompany mTBI due to the limited number of studies that have used each of these protocols. Four studies have reported increased resting motor threshold and one study found increased active motor threshold in both the acute and chronic phases following mTBI. Studies reporting higher MTs in brain-injured patient, combined with a single study showing reduced intracortical facilitation [44], suggest a general tendency towards reduced cortical excitability of the primary motor cortex. This would fall in line with the multiple reports of increased GABA-related intractortical inhibition in mTBI (See table 1).

Thus, when taken together, TMS studies of the short- and long-term effects of mTBI on primary motor cortex excitability suggest a general trend towards diffuse cortical hypoexcitability. Powers and collaborators [44] have suggested that the immediate effect of a concussion would be that of reduced cortical excitability, which would then move towards increased GABAergic tone (presumably related to $GABA_B$ receptor activity) in the chronic phase. This raises the important issue of determining whether increased cortical inhibition/reduced cortical excitability is a beneficial adaptation to the cellular trauma that occurs following concussion or a marker of incomplete recovery. A possible clue in that regard is the fact that many of the reported M1 abnormalities have been found to correlate with specific TMS measures of cortical excitability/inhibition. For example, cortical silent period lengthening in concussed athletes has been associated with reduced motor execution velocity [37], reduced motor sequence learning [31] and reduced motor response time, movement time and attention performance [28]. Interestingly, correlations were also reported between CSP duration and visuomotor reaction time and fine motor control [30] in a group of athletes with a history of concussions (21 years after last concussive event) that displayed *reduced* CSP durations. These data suggest that TMS assessment of M1 function may have clinical value.

In conclusion, this systematic review shows that TMS, with the possible exception of intracortical inhibitory measures, cannot reliably detect changes in M1 excitability in individuals with mTBI or a concussion at both the acute and chronic stages of injury. As it stands, the clinical and predictive utility of this technique remains to be determined, as no clear pattern of excitability/inhibition impairments emerges from the literature. As the time post-trauma at which participants are tested varies significantly between studies, it is necessary to conduct longitudinal assessments of M1 reactivity and plasticity to get a better picture of the timeline of excitability changes that accompany mTBI. Additionally, these studies would benefit from larger sample sizes as the number of participants in all the reviewed studies is relative low. Finally, multimodal evaluations that include TMS measures would also greatly enhance the value of future studies. For example, MRS, magnetic resonance imaging (MRI; diffusion imaging, resting-state imaging, susceptibility weighted imaging) and electroencephalography could provide important complementary information that would allow evaluation of the relevance of TMS results in a greater context and validation of some of the specific findings. Finally, because TMS is a non-invasive, portable, and relatively easy to use technique, it could be used as a means to get very early physiological data that could help predict individual responses to mTBI, contribute to return to activity/play decisions, objectively evaluate recovery and test treatment efficacy.

Declaration of interest

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Tables

Table 1. Main parameters and results

Authors	Population	Groups : N	Age range (Mean ; +/- SD)	TBI history	Time post injury	Target cortical region hand muscle	TMS Parameters			Results
								rMT		mTBI group: higher rMT
							Single pulse	CMCT		No difference
							rTMS slow rate (1Hz)	Relative Amplitude variability		MTBI group: decrease of amplitude during session, irregularity in amplitude and waveform
		mTBI : 39 controls: 21	19-59 (33,1) 18-54 (36,2)		2 weeks	M1 / APB		Latency variability		No difference, stable
							Single pulse	rMT		9 out of 15 mTBI: return to normal
1998 Chistyakov et al. [24]	Non-athletes	mTBI: 15			3 months	M1/ APB	rTMS slow rate (1Hz)	Relative Amplitude Variability		9 out of 15 mTBI: return to normal, did not decrease during session
	Non-Athletes with minor, mild, moderate TBI Scan results: no structural									Significantly increased in mild and moderate groups. Increased in diffuse and combined injury
2001 Chistyakov et al. [25]		mTBI: 38 controls: 20	18-60 (31,9) 20-54 (33,2)		2 weeks	M1 / APB	Single pulse	rMT		Significantly increased in mild and moderate groups
								aMT	MEP/M amplitude	Reduction in mild and moderate groups

	injury, diffuse focal, combined dammage							ratio CMCT	Slightly prolonged in all concussed group, but significant only in mild injury group Significantly prolonged in the diffuse and combined injury groups
								CSP Duration stimulation at 125%,150%, 175% of aMT, at 130% of rMT	At 130%, CSP duration significantly prolonged in mild and moderate TBI. At 125% of aMT, significantly prolonged in moderate group only. Other intensities: no effect. Minor TBI did not differ from controls Prolonged in focal and combined injury at 130%
								Interthreshold differences - MEP/SP	Significant increase in mild/moderate TBI group: Abnormal in 54% of patients with mild TBI and 50% of moderate TBI.
2007 De Beaumont et al. [38]	Asympotmatic American football athletes	Multiple concussions (2+) : 15 Single concussion (1): 15 Controls: 15	23,38±2,63 22,94±2,84 22,5±2,53	Multiple: 2 or more (2,75±1,29)	more than 9months (59,12±69, 52 months for single, 31.00±22, 08 months for multiple)	Left M1 / FDI	Single pulse	rMT	No difference
								Input-output curve Stimulation at 80%, 90%, 100%, 110%, 115%, 120%, 130%, 140% of rMT	No difference, normal curves
								CSP Duration Stimulation at 120% of rMT	CSP significantly prolonged when multiple concussions group was compared to normal controls. No other group interactions reached significance.
								SICI, ISI: 1,2,3 ms	No difference

							Paired pulse	ICF, ISI: 6,9,12,15 ms	No difference
					retest 6-15 months after new concussion	Left M1 /FDI			All measures were equivalent to time 1, but CSP which was prolonged at time 2 (after a new concussion) in patient group.
2009 De Beaumont et al. [37]	Retired hockey and American football athletes	Concussion: 19 Controls: 21	50-65 61±5,16 59±9,07	1 to 5. (Last concussion in young adulthood)	30 years (27 to 41, M= 34,74±9,21)	Left M1 /FDI	Single pulse	rMT	No difference
								Input-output curve 90%, 100%, 110%, 120%, 130%, 140% of rMT	No difference
								CSP Duration 110%,120%, 130% of rMT	Significantly longer in concussed group in all levels of stimulation
							Paired pulse	SICI, ISI: 2,3 ms	No difference
								ICF, ISI: 9,12,15 ms	No difference
2011 Tremblay et al. [39]	Asymptomatic American football athletes	Concussion: 12 controls: 14	22,36±1,69	2 or more (3,25±0,97)	12 months or more (23,17±5,92)	Right Median nerve + Left M1 /APB		SAI, ISI: 18, 20, 22 ms	No difference

								LAI, ISI: 100, 200ms	Effect of time, 100ms interval increases inhibition compared to 200ms. No group effect
						M1 / APB		CMCT	No difference
							Single pulse	CSP duration	Significantly prolonged in concussed group
							Paired pulse	SICI, ISI: 2ms	No difference
								LICI, ISI: 100ms	Significantly enhanced in concussed group
2011 De Beaumont et al. [47]	Asymptomatic American football athletes	Concussion: 21 Controls: 15	19-26 22,3±3,45	more than 2 (2,65±1,45)	more than 9 months (19,03±13,77)	Left M1 /FDI	Single pulse	CSP Duration 110%,120%,130% of rMT	Longer CSP duration in all levels of stimulation
							Paired pulse	LICI, ISI: 100ms	Lower LICI ratios LICI correlated with CSP duration when stimulation 120 and 130%
2012 De Beaumont et al. [31]	Asymptomatic American football athletes	Concussion: 13 controls: 19	19-27 23,4±3,11	more than 2 : 2,87±1,41	more than 9 months 13,74±6,26	Left M1 /APB	Single pulse	rMT	No difference
								MEP amplitude	No difference
								CSP duration 120%, 130% of rMT	Significantly longer in concussed group
							Paired pulse	LICI, ISI: 100ms	Significantly enhanced in concussed group
						Right Median nerve + Left M1 /APB	PAS_{25}	MEP amplitude	Significant increase in MEP size in control group. Inhibition of response in concussed but not significant

								CSP duration	CSP prolongation after PAS in control group. Not prolonged after PAS in concussed group
								MEP amplitude	Significant difference in control group. No effect in concussed group after PAS
							PAS_{10}	CSP duration	No difference
2012-10 Livingston et al. [32,33]	Athletes	Concussion: 9 controls: 9	20,4±1,3	not in last 6 m	1,3,5,10 days	Left M1 / APB and ADM	Single pulse	rMT	No difference
								MEP latency	Significant difference in APB latencies between day 1 and 5, and day 1 and 10. Linear increase of latency over days ADM: no difference
								MEP/M amplitude ratio	ADM amplitude significantly smaller on day 3 than day 5. APB: no difference
								CMCT	No difference
2012 Bashir et al. [45]	Non-athlete	mTBI: 1 controls: 12	44 30±14	1	2 and 6 weeks	Left M1	Paired pulse	ICF, ISI: 12ms	Significantly greater in patient at both time points
								SICI, ISI: 3ms	No difference
								LICI, ISI: 100ms	Absent at week 2, but returned to normal at week 6
							cTBS	MEP amplitude	Significant MEP facilitation at week 2 at 0,5,10,20 minutes after cTBS in patients compared to controls. Normal inhibition at week 6.
2012-13 Tallus et al. [26,27]	Non-athletes	Symptomatic: 11 Recovered: 8 controls: 9	43,7±11,63 35,9±15,9 33,6±13,2		5 years (6,1±5,4 /3,8±1,1)	Left M1 / APB	Single pulse	rMT	Both mTBI groups had a MT significantly higher than controls. More variance in results for MTBI groups

2014 Miller et al. [29]	Non-athletes	mTBI: 15 controls: 15	20,8±1,2 21,1±1,3	1 or 2	3 days, 1 and 2 weeks, 1 and 2 months	M1 / FDI	Single pulse	rMT	No effect of group or time over the 2 months. No difference
								MEP amplitude	No effect of group or time over the 2 months. No difference
								MEP latency	No effect of group or time over the 2 months. No difference
								CSP duration 120%of rMT	Significantly longer in concussed group at 72hrs and over the 2 months. No effect of time, duration stayed the same over the 2 months.
2014 Pearce et al. [28]	Amateur Australian football (AF) players	Concussion: 8 controls: 15	25,1±4,5	excluded if 1 in last 1 2months	2, 4, 10 days	M1 / FDI	Single pulse	rMT	No difference
								aMT	No difference
								MEP Amplitude	No difference
								MEP latency	No difference
								CSP duration 125% of aMT	Significantly longer in concussed group at 48h and 4days.
							Paired pulse	SICI, ISI : 2ms	No difference
2014 Pearce et al. [30]	Retired elite and amateur Australian football (AF) players	AF: 40 (20 elite, 20 amateur) controls: 20	49,74±5,67 48,46±6,86 47,56±6,85	multiple (M = 3.2)	21,9 years	M1 / FDI	Single pulse	MEP amplitude	No difference
								Input-output curve 90%, 110%, 130%, 150% of aMT	No difference in amplitude and CSP. But, difference in curve top which is significantly higher in controls
								CSP duration 125% of aMT	Significantly reduced in AF groups, no difference between amateur and elite
							Paired pulse	SICI, ISI : 2ms	Significantly reduced in AF groups, no difference between amateur and elite
								LICI, ISI: 100ms	Significantly reduced in AF groups, no difference between amateur and elite
2014	American football	Concussion:	20,16±1,1	0 to 2	1-4 weeks	Left M1 /	Single	rMT	No difference

Powers et al. [44]	athletes	8 Controls: 8	9 20,28±1,47			FDI	pulse	Voluntary activation	Lower maximal voluntary activation in concussed group
								CSP duration	No difference
							Paired pulse	ICF, ISI: 10 ms	Reduced in concussed group
								SICI, ISI: 2ms	No difference
								LICI, ISI: 100ms	No difference
2014 Tremblay et al. [48]	Asymptomatic American football athletes	Concussion: 16 Controls: 14	22,00±1,09 22,03±1,08	1 to 4 (M:1,88)	more than 10 months	Left M1 /FDI	Single pulse	rMT	
								CSP duration 120%, 130% of rMT	No difference
							Paired pulse	LICI, ISI: 100ms	No difference

Legend: ADM= abductor digiti minimi; aMT = active motor threshold; APB = abductor pollicis brevis ; CMCT = central motor conduction time; CSP = cortical silent period; cTBS= continuous theta burst stimulation; FDI = first dorsal interosseus; ICF= intracortical facilitation; ISI = inter-stimulus interval; LAI = long latency afferent inhibition; LICI = long intracortical inhibition; M = Mean; M1 = primary motor cortex; MEP = motor evoked potentials; mTBI= mild traumatic brain injury; N= sample size; PAS= paired associative stimulation; rMT= resting motor threshold; rTMS= repetitive transcranial magnetic stimulation; SAI = short latency afferent inhibition; SD = standard deviation; SICI = short interval intracortical inhibition; TMS= transcranial magnetic stimulation. * : indicates studies that used the same cohorts.

Table 2. Results of mTBI/concussed groups compared to controls.

	Time post injury	Ref.	TMS PARADIGMS																			
			Cortical excitability/facilitation								Cortical inhibition					Plasticity						
			rMT	aMT	MEP amplitude	MEP/M amplitude ratio	MEP latency	Input output curve	CMC T	ICF	CSP	SICI	LICI	SAI	LAI	cTBS	PAS_{25} MEP amplitude	PAS_{25} CSP	PAS_{10} MEP amplitude	PAS_{10} CSP	slow rate rTMS MEP amplitude
Recovery phase	1,3,5,10 days	[32,33]	normal			Lower day 5 vs 3	Longer day 10 vs 1		normal												
	2,4,10 days	[28]	normal	Normal	normal		normal				longer day 2 and 4	normal									
	3 days, 1 and 2 weeks	[29]	normal		normal		normal				longer										
	1 to 4 weeks	[44]	normal							reduced	normal	normal	normal								
	2 weeks	[24]	hieher						normal												lower
	2 weeks	[25]	hieher	hieher		reduced			longer		longer										
	2 weeks	[45]								enhanced		normal	absent inhibition			MEP facilitation					
	6 weeks	[45]								enhanced		normal	normal			normal					
	1 and 2 months	[29]	normal		normal		normal				longer										
	3 months	[24]	normal																		normal
Chronic phase	more than 9 months		normal					normal		normal	longer	normal									
	6-15months after new concussion	[38]	normal					normal		normal	longer than time 1	normal									

more than 9 months	[47]										longer		enhanced								
more than 9 months	[31]	normal		normal							longer		enhanced				slighthly inhibited	not prolonged	not reduced	normal	
more than 10 months	[48]										normal		normal								
more than 12 months	[39]								normal		longer	normal	enhanced	normal	normal						
5 years	[26,27]	higher																			
21 years	[30]			normal			lower				reduced	reduced	reduced								
30 years	[37]	normal					normal		normal		longer	normal									

Legend: aMT = active motor threshold; CMCT = central motor conduction time; CSP = cortical silent period; cTBS= continuous theta burst stimulation; ICF= intracortical facilitation; LAI = long latency afferent inhibition; LICI = long intracortical inhibition; MEP = motor evoked potentials; PAS= paired associative stimulation; rMT= resting motor threshold; rTMS= repetitive transcranial magnetic stimulation; SAI = short latency afferent inhibition; SICI = short interval intracortical inhibition; TMS= transcranial magnetic stimulation. * : indicates studies that used the same cohorts.

Annexe 2

Theta burst stimulation to characterize changes in brain plasticity following mild traumatic brain injury : a proof-of-principle study

Theta burst stimulation to characterize changes in brain plasticity following mild traumatic brain injury: a proof-of-principle study

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Running head: cTBS and mild traumatic brain injury

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Abstract

Purpose. Recent studies investigating the effects of mild traumatic brain injury (mTBI) suggest the presence of unbalanced excitatory and inhibitory mechanisms within primary motor cortex (M1). Whether these abnormalities are associated with impaired synaptic plasticity remains unknown. *Methods.* The effects of continuous theta burst stimulation (cTBS) on transcranial magnetic stimulation-induced motor evoked potentials (MEPs) were assessed on average two weeks and six weeks following mTBI in five individuals. *Results.* The procedure was well-tolerated by all participants. Continuous TBS failed to induce a significant reduction of MEP amplitudes two weeks after the injury, but response to cTBS normalized six weeks following injury, as a majority of patients became asymptomatic. *Conclusions.* These preliminary results suggest that cTBS can be used to assess M1 synaptic plasticity in subacute phase following mTBI and may provide insights into neurobiological substrates of symptoms and consequences of mTBI.

Introduction

The Centers for Disease Control and Prevention estimate that between 1.4 and 3.8 millions of mild traumatic brain injuries (mTBI) occur annually in the USA (Rutland-Brown et al., 2006). Although mTBI has been long considered a short-lasting "minor" injury, current literature suggests that it may involve a clinically silent pathological process that is related to subclinical neurophysiologic and neurometabolic changes. An increasing number of studies have revealed the long term impact of mTBI or concussion since the discovery of a possible link between multiple mTBIs and the development of neurodegenerative diseases (Bazarian et al., 2009), such as Alzheimer`s disease (Guskiewicz et al., 2005; McCrory, 2011; Mortimer et al., 1985; Plassman et al., 2000), chronic traumatic encephalopathy (Cantu, 2007; McCrory et al., 2007) and amyotrophic lateral sclerosis (Piazza et al., 2004). Therefore, there is a need to better understand both the subacute and chronic impacts of mTBI on brain physiology to fully appreciate the timeline of the changes occurring in the brain following injury.

Insight from animal studies suggest that a complex neurometabolic cascade of events occurs in the brain in the acute/subacute phase following mTBI that involves NDMA receptors, ion channels and glutamate release (Giza & Hovda, 2001). In healthy humans, transcranial magnetic stimulation (TMS) has been used to non-invasively assess the neurophysiological impact of mTBI as it allows precise quantification of inhibitory and excitatory systems within primary motor cortex (M1; Hallet, 2007). Using this method in TBI, previous studies have shown sometimes long-lasting disruptions in M1 inhibitory/excitatory balance, usually taking the form of increased intracortical inhibition/reduced intracortical facilitation (Chistyakov et al., 2001; De Beaumont et al., 2007, 2009; Miller et al., 2014; Pearce et al., 2014a; Powers et al. 2014; but see Tremblay et al., 2014; Pearce et al., 2014b) Moreover, chronic alterations in synaptic plasticity, possibly reflecting faulty long term potentiation (LTP) – and long term depression (LTD) - like mechanisms were found after multiple concussions, and were associated with intracortical inhibition abnormalities (De Beaumont et al., 2012). Taken together, these studies suggest the presence of impaired balance between primary motor cortex excitatory and inhibitory mechanisms following mTBI both in the subacute and chronic phases, which may be related to abnormal M1 plasticity.

To our knowledge, the integrity of synaptic plasticity mechanisms following human mTBI in the subacute phase has yet to be investigated. This is of major importance since impairments in M1 plasticity could prevent adaptive plastic changes to occur following injury and may themselves be the cause of pathological processes and functional disability. Continuous theta-burst stimulation (cTBS) is a repeated TMS protocol that induces long lasting reduction of corticospinal excitability and allows non-invasive and rapid assessment of motor cortex plasticity (Huang et al., 2005). Continuous TBS is thought to involve several neural mechanisms including long-term depression (LTD), and inhibitory mechanisms modulated by GABAergic transmission (Cárdenas-Morales et al., 2010). Continuous TBS can therefore provide important insight into the synaptic plasticity changes that may occur shortly after mild head trauma. The objective of the present proof-of-principle, case-series study was to provide preliminary evidence that cTBS can be safely and efficiently applied in the subacute phase of mTBI to assess the integrity of synaptic plasticity mechanisms in primary motor cortex.

Methods

Participants

Case 1. This 44 year-old right-handed man was playing soccer when he sustained a head to head collision with another player and then hit the ground with his head. There was loss of consciousness (LOC) for about 90 seconds, followed by confusion, blurred vision, agitation and about 1–2 minutes of retrograde and 3–4 minutes of anterograde post-traumatic amnesia (PTA). The symptoms resolved approximately 20 minutes after the event at which point his physical and neurological exams were normal, and remained normal 10 days later. He was diagnosed with a Grade 3 concussion according to the American Academy of Neurology classification (1997). For two weeks following the accident, he complained of fatigue and poor concentration, memory problems, mild headaches and some difficulty sleeping. These symptoms had markedly improved by week 6, although he still complained of mild headaches, slight fatigue, and intermittent memory difficulties. He was not taking any drugs known to alter brain excitability, plasticity, or excitation/inhibition balance. He had a history of four prior episodes diagnosed as

concussions while an athlete in college, over 20 years prior to the present episode. In two of these incidents there was no loss of consciousness, but there was varying degrees of retrograde and anterograde amnesia, mild and transient concentration and memory difficulties, headaches, and dizziness that had completely subsided within 2 months from the episode. Past medical history, review of system and family history were otherwise negative. Note that this case was previously presented in a case report by Bashir et al. (2012).

Case 2. This 24 year-old right-handed woman suffered from a bike accident during which she hit her head while wearing a helmet. She sustained a LOC of approximately 1-5 minutes duration. A brief seizure-like twitching episode (20 sec) was observed while she was unconscious. She suffered from retrograde and anterograde PTA (few minutes). A week after the injury, she was involved in a second bike accident where she hit her head again. Following this second incident, she did not report LOC, involuntary movements, seizures or retrograde/anterograde PTA. Three or four days after the second incident, she started to experience intermittent headaches and trouble concentrating. Her neurological examination was normal. She was diagnosed with a Grade 3 concussion according to the American Academy of Neurology classification (1997). She had a past medical history of attention deficit and hyperactivity disorder (ADHD) for which she was taking psychostimulant medication. She stopped taking the medication three weeks prior to the experimentation. Her past medical history, review of system and family history were otherwise negative.

Case 3. This is a 22 year-old left-handed woman who was involved in a collision with a skateboarder while she was on her bike. Following the impact, she flew over the handle bar. The front of her helmet broke and she sustained a left pre-orbital ecchymosis. The duration of the LOC is unknown. She experienced confusion and retrograde PTA for about 30 sec and anterograde amnesia for approximately 30 min. She has a past medical history of migraine. During a few days following the accident, she experienced some word finding difficulties but she did not report any increase in the frequency of her migraine or changes in her concentration. Her physical and neurological examinations were normal. She was diagnosed with a Grade 3 concussion according to the American Academy of Neurology classification (1997). She was

not taking any drugs known to alter brain excitability, plasticity, or excitation/inhibition balance. Her past medical history, review of system and family history were negative.

Case 4. This is a 28 year-old right-handed woman who was involved in a car-pedestrian collision. The presence of LOC is unknown and there was no report of anterograde/retrograde PTA. Following the incident, she experienced increased intensity and frequency of headaches. Her neurological and physical exams were normal. She was diagnosed with a Grade 2 concussion according to the American Academy of Neurology classification (1997). A computed-tomography scan (CT-scan) of her head revealed a small right parietal subgaleal scalp hematoma along the vertex with no underlying fracture. She was not taking any drugs known to alter brain excitability, plasticity, or excitation/inhibition balance. Her past medical history, review of system and family history were negative.

Case 5. This is a 22 year-old left-handed woman who was involved in a work incident during which she was hit by a stack of plates on the left post-aural region by a co-worker. There is no report of LOC or anterograde/retrograde PTA. However, she experienced dizziness and nausea after the incident, and headaches for several days post-injury. No intracerebral anomalies were observed on the CT-scan. Her neurological and physical exams were normal. She was diagnosed with a Grade 2 concussion according to the American Academy of Neurology classification (1997). She was not taking any drugs known to alter brain excitability, plasticity, or excitation/inhibition balance. Her past medical history, review of system and family history were negative.

Procedure

All participants were seen within two weeks post-mTBI (M= 14 ± 3 days) and again approximately six weeks post-injury (separated by 61 ± 19 days). All participants were first seen by a neurologist and had to meet the concussion criteria of the American Academy of Neurology (Neurology, 1997). All participants completed the TMS safety questionnaire (Rossi et al., 2011) prior to testing to screen for possible contraindications. On visit 1, a brain magnetic resonance

imaging (MRI) exam was performed, followed by baseline measures of TMS and the cTBS procedure. On visit 2, TMS and cTBS procedures were repeated. All participants gave their written informed consent for the study, which had been approved by the Institutional Review Board of Beth Israel Deaconess Medical Center.

TMS recordings

All participants underwent an anatomical brain MRI, using a 3-Tesla GE scanner, to rule out structural lesions and to generate high-resolution images to guide magnetic stimulation. For single-pulses, a Nexstim stimulator (Nexstim Ltd, Helsinki, Finland) was used, delivering biphasic pulses with a current flowing in the brain with an antero-posterior and then a postero-anterior (AP–PA) direction. For repetitive TMS, i.e. cTBS, a MagPro stimulator (MagVenture A/S, Farum, Denmark) was used, delivering biphasic pulses with the current flowing in an AP–PA direction. In order to ensure stable coil positioning over the stimulation site during the experimentation and to ensure that the exact same cortical location was targeted within each study session as defined by each individual's brain MRI, a Nexstim eXimia Neuronavigation system was used. During stimulation, surface electromyography (EMG) was recorded and monitored continuously on-line. Active electrodes were attached to the skin overlying the first dorsal interosseus (FDI) muscle. The reference electrode was placed over the metacarpo-phalangeal joint and a ground electrode was placed over the wrist bone or the ipsilateral forearm. EMG signals were filtered (8–500 Hz), amplified, displayed and stored off-line for analysis. The TMS system delivered triggered pulses that synchronized the TMS and EMG systems. Relaxation of the measured muscle was controlled by continuous visual EMG monitoring. Participants were asked to keep their eyes open throughout the experiment and were monitored for drowsiness.

TMS measurements

Participants were seated in a comfortable chair, with a head rest, and with their elbows flexed at approximately 90° and their hands resting on their laps. The optimal scalp location for

activation of the right FDI using TMS over left primary motor cortex (M1) was determined as the location from which TMS-induced motor evoked potentials (MEPs) of maximum peak-to-peak amplitude in the right FDI. Once the optimal location was identified, a marker was placed on the MRI scan to which the individual participant was registered using the eXimia navigated brain stimulation (NBS) system. This allowed the TMS coil to be placed systematically in the same location, orientation and tilt throughout each session.

Motor threshold (MT) was determined according to the recommendations of the International Federation for Clinical Neurophysiology (Rossini et al., 1994). Single TMS pulses were delivered over the optimal scalp position at supra-threshold intensity and gradually reduced by decrements of 2% of stimulator output. Resting MT (RMT) was defined, with the Nexstim stimulator used for single-pulse TMS, as the lowest stimulus intensity capable of inducing MEPs $\geq$ 50 μV peak-to-peak amplitude in at least 5 of 10 consecutive trials. EMG monitoring was performed to assure that the target muscle was at rest. Prior to cTBS, active MT (AMT) was determined, and defined as the minimum single-pulse TMS intensity required to produce MEPs $\geq$ 200 μV in at least 5 of 10 consecutive trials while participants contracted the target muscle (contralateral FDI) at approximately 20% of maximal voluntary contraction. In order to control for prior motor contraction during the measurement of AMT, participants were asked to contract the FDI muscle approximately 2 s prior to each TMS pulse and to relax it about 1 s after each TMS pulse, for at least 3 s. The cTBS protocol was applied approximately 1 min after the end of the AMT measurement procedures; the experimenters monitored the relaxation of hand muscles continuously during and after the stimulation.

cTBS procotol

Continuous TBS was applied using parameters similar to those used by Huang et al. (Huang et al., 2005): three pulses at 50 Hz, with an interval of 200 ms between the last pulse of a triplet and the first pulse of a triplet (i.e. with an interstimulus interval of 240 ms), for a total number of 600 pulses. Thus, in the present cTBS paradigm, the triplet repetition rate was about 4.17 Hz instead of 5 Hz, both frequencies being included in the theta band. The intensity was fixed at 80% of AMT. This paradigm was recently shown to induce significant suppression of

MEPs in healthy controls (see Vernet et al., 2014). Before cTBS, two to three batches of 20 to 30 MEPs (60 in total) were acquired in response to stimulation over the optimal FDI location, at an intensity of 120% of RMT and a rate of approximately 0.1 Hz (a random jitter of ±1 s was introduced to avoid any training effects). Such measures allow verifying for stability of the pre-cTBS measure of excitability; moreover, the second batch was used as the baseline to which the post-cTBS measures of excitability were compared. Following cTBS, a single batch of MEPs was measured immediately after (T0) and then at 5, 10, 20, 30, 40, 50, 60, 75 and 90 min following cTBS to track changes in amplitude over time.

Data analysis

MEP peak-to-peak amplitude was automatically determined using the Nexstim Neurophysiologic Analysis software and then visually inspected. Mean raw MEP peak-to-peak amplitudes for each time points were used for analysis. Paired-sample t-tests were conducted to assess the reproducibility of baseline MEP amplitude. A within subject repeated measure multivariate analysis of variance (MANOVA) was used to compare the impact of cTBS on MEP amplitude over time, using session (session 1 and 2) and MEP measures (11 time points) as within-group factors. Paired-sample *t*-tests were used to identify the effect of cTBS at the different time points in comparison to the baseline MEP measure. The critical *p*-value was set to 0.05. Because of the very small sample and the exploratory purpose of the present case report, no correction for multiple comparisons was applied. One participant (case 4) did not come to the second session. For statistical analyses, the missing data were replaced by the average data from the 4 other cases. All analyses were performed on raw TMS data. All statistical tests were two-tailed and performed using the Statistical Package for the Social Sciences version 21.

Results

A questionnaire was used at the beginning and at the end of each session to evaluate the presence of pain and discomfort. Two patients reported the presence of mild discomfort during the procedure. Case 2 reported, at the beginning of session 1, mild headache, for which acetaminophen was given and, at the end of session, mild neck pain. Again, at the beginning of

session 2, Case 2 reported mild headache and trouble concentrating and, at the end of session 2, a mild neck pain in addition to those symptoms. Case 3 reported mild neck pain at the beginning and at the end of both sessions. Thus, the only side-effect associated with the procedure was a mild neck pain for Case 2.

A paired-sample t-test revealed no significant difference between baseline MEP measures from both session ($t_{(4)} =$ -.07, $p = .94$). The MEP response profiles in the two sessions were not parallel as indicated by a significant [session x time] interaction (F=2.23, df=10, p<0.035) (Figure 1). Subsequent paired-sample t-tests revealed no significant reduction in the MEPs size compared to baseline at all time points for session 1 (Table 1). A significant inhibition of the MEPs compared to baseline at T0, T5, T20, T50, T60, T75 and T90 was observed for session 2 (Table 2). Individual data are shown in Figure 2.

Discussion

The goal of this proof-of-principle study was to investigate the feasibility of using cTBS to evaluate plasticity changes in the subacute phases of mTBI. The protocol was well-tolerated by all participants but induced a mild side-effect (neck pain) in one out of 5 patients. Preliminary results suggest the presence of altered plasticity 2 weeks post-mTBI, as cTBS failed to elicit the usual suppression of MEPs post-stimulation, which could reflect altered M1 LTD-like mechanisms. Significant cTBS-related suppression of MEPs was observed 6 weeks post-mTBI suggesting a resolution of plasticity abnormalities beyond the acute phase.

A common observation following mTBI is the presence of altered M1 intracortical excitability in the acute/subacute (Chistyakov et al., 2001; Pearce et al., 2014b; Miller et al., 2014; Powers et al., 2014) and chronic (De Beaumont et al., 2007,2009; Tremblay et al., 2011; Pearce et al., 2014b) phases of injury. More specifically, increased intracortical inhibition (Chistyakov et al., 2001; Pearce et al., 2014b; Miller et al., 2014) and decreased intracortical facilitation (Powers et al., 2014) have been reported in the acute and subacute phases of mTBI. Despite strong evidence suggesting inhibitory/excitatory imbalance in the primary motor cortex of individuals with mTBI, the duration of such effects is unclear. Pearce and collaborators

(2014b) found increased GABA-related inhibition 48h and 96h after concussion that normalized 10 days post-injury, whereas Miller et al. (2014) reported similarly increased inhibition that lasted up to 2 months after the concussive event. Intracortical inhibition has also been reported to be increased 1-4 weeks (Powers et al., 2014) and 9 months after a concussion (De Beaumont et al., 2007) and within normal values 41 months post-injury (Tremblay et al., 2014).

In the present study, we show reduced synaptic plasticity in the subacute phase as indexed by the response to cTBS, and that this this abnormality disappears six weeks post-injury. An association between abnormal intracortical excitability and aberrant synaptic plasticity has been previously shown in concussed athletes on average 14 months post-injury. De Beaumont et al. (2012) reported that increased silent period durations in concussed athletes, presumably reflecting faulty $GABA_B$ transmission, were negatively correlated with the level of synaptic plasticity induced with paired associative stimulation. In the present study, the hypoexcitatory or hyperinhibitory state of M1 intracortical networks could prevent the injured brain from responding adequately to the effects of cTBS and therefore be an accurate marker of early abnormal plasticity. The inability of the injured brain to respond to cTBS appears short-lived, however, which is in contradiction with the previous study by De Beaumont and collaborators (2012) who showed persistent motor cortex LTD- and LTP-like deficits in the chronic phase following sport concussion. This discrepancy could be explained by the fact that the current sample included 4 individuals with mTBI who did not have a history of multiple concussions (3 and over) and that were not subjected to recurrent sub-concussive blows through contact sports. Additionally, the age of participants in the present study ranged from 22 to 44 years, which may be a confounding factor. Indeed, it has been shown that cTBS effects are modulated by age, where less motor cortex plasticity is observed in older individuals (Freitas et al., 2011), and older age has been associated with prolonged post-concussion symptoms (King et al., 2014). It should finally be noted that TBI is a very heterogeneous condition and as such the present sample cannot be representative of the TBI population as a whole. Nevertheless, the present data show that TBS can be used safely to assess motor cortex plasticity in individuals with TBS. Studies with larger and more homogeneous samples are needed to determine the clinical usefulness of TBS for evaluating plastic changes related to TBI.

Animal studies have shown that bursts of 3-5 pulses at 50-100 Hz (theta rhythm) induce LTP/LTD when applied to the motor cortex or hippocampus (Hess & Donoghue, 1996; Larson, et al., 1986). While the exact mechanism underlying the effects of cTBS on the human brain are still unknown, it has been suggested that MEP suppression following stimulation could be related to long-term depression (LTD)-like processes mediated by N-methyl-D-aspartate receptors (NMDA-r), as NMDA-r antogatonist memantine was shown to block the after effects of cTBS (Huang et al., 2007). Modulation of GABA receptors (Thickbroom, 2007) and glutamate receptors (Glu-r) (Huang et al., 2007) has also been proposed as a possible mechanism explaining excitability changes following TBS. TBS could therefore target both excitatory and inhibitory networks within the human motor cortex (Cárdenas-Morales et al., 2010). The present data are in line with this hypothesis since M1 alterations in glutamate (Babikian et al., 2006; Henry et al, 2010; Shutter et al., 2004) and abnormal interactions between M1 GABA and glutamate (Tremblay et al., 2014) have been shown in the acute/subacute and chronic phases of TBI and sport-related mTBI using magnetic resonance spectroscopy. Abnormal GABA and glutamate transmission could therefore partly explain the inhibitory/excitatory imbalance found in the motor cortex of individuals with mTBI and its associated effects on synaptic plasticity.

Continuous TBS has been used with various populations to non-invasively probe synaptic plasticity in the conscious human brain. This method has many advantages over other techniques such as its short application time (Huang et al., 2005), low intensity of stimulation (Huang et al., 2005) and reasonable intra-subject reproducibility over two separate sessions (Vernet et al., 2014). Recent studies, however, have reported inconsistent results with TBS, possibly due to important inter-subject variability. For example, Lopez-Alonso et al. (2014) found no significant changes in M1 corticospinal excitability following intermittent TBS (iTBS) in a sample of 56 healthy participants. A similar absence of significant modulation of excitability was reported for iTBS and cTBS in a sample of 52 healthy participants (Hamada et al., 2014). In that study, only 25% of study participants had the expected response to iTBS (increased excitability) and cTBS (reduced excitability). Interestingly, the effects of TBS were correlated with the latency of TMS-induced MEPs when the TMS current was applied in the anterior-posterior direction (Hamada et al., 2014). More specifically, only individuals in which MEP latency differences between anterior-posterior and latero-medial stimulation currents were

important showed the "traditional" pattern of excitability increases/decreases following TBS. This suggests that MEP latency may serve as a way to predict in which individuals TBS is more likely to work and thus guide its therapeutic use.

In conclusion, as recent studies have suggested that altered metabolite interactions, faulty intracortical inhibition and reduced plasticity mechanisms within M1 could be key features of mTBI pathophysiology, the goal of the present proof-of-principle study was to determine whether cTBS could be used to assess the integrity of plasticity mechanisms in the subacute phase of mTBI. Results showed that cTBS is safe and can be effectively used in mTBI individuals. Reduced LTD-like synaptic plasticity was found two weeks following injury and disappeared six weeks post-injury. Whether the altered LTD-like plasticity mechanisms seen in the subacute phase following mTBI is part of the pathophysiology of the injury or reflects a compensatory mechanism of short-duration needs to be assessed in larger prospective studies and compared to normative values. Furthermore, in light of the reported link between MEP latency and TBS effects (Hamada et al., 2014), an important next step will be to determine to what extent changes in cortical excitability associated with TBS protocols in TBI patients reflect synaptic plasticity impairments.

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RR025758). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, the Canadian Institutes of Health, the National Institutes of Health or the Sidney R. Baer Jr. Foundation.

Conflict of interest statement

Dr. Pascual- Leone serves on the scientific advisory boards for Nexstim, Neuronix, Starlab, Neuroelectrics, Axilum Robotics, Magstim, and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI).

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Figures

Figure 1. Mean MEP amplitude and standard deviations following cTBS over the different points and for session 1 and 2.

Legend: Error bars show standard deviations. No significant reductions are observed on MEP amplitudes for the first session, although a small trend is observed towards the last time points. Significant reductions of MEP amplitudes are observed for sessions two for 7 out of the 10 time points. * $p < 0.05$; ** $p < 0.01$

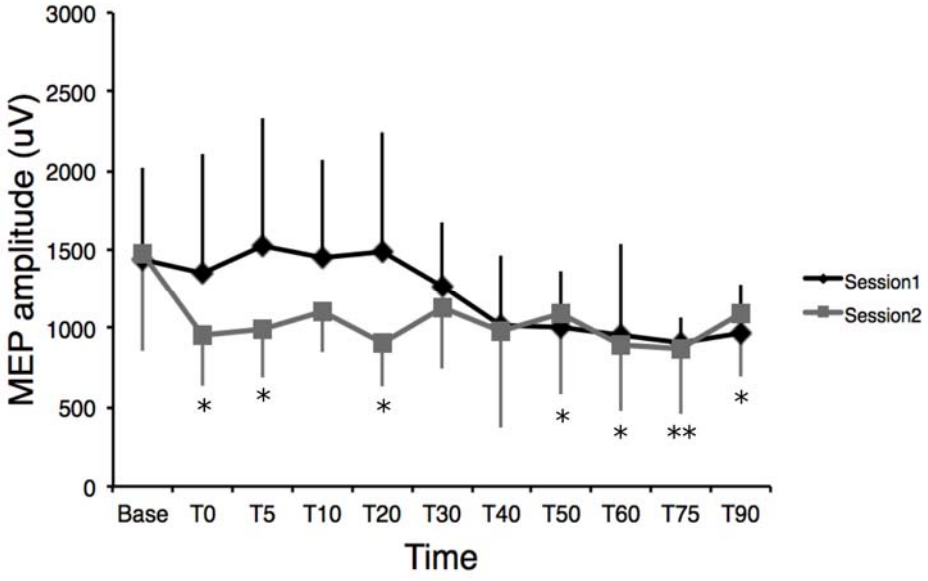
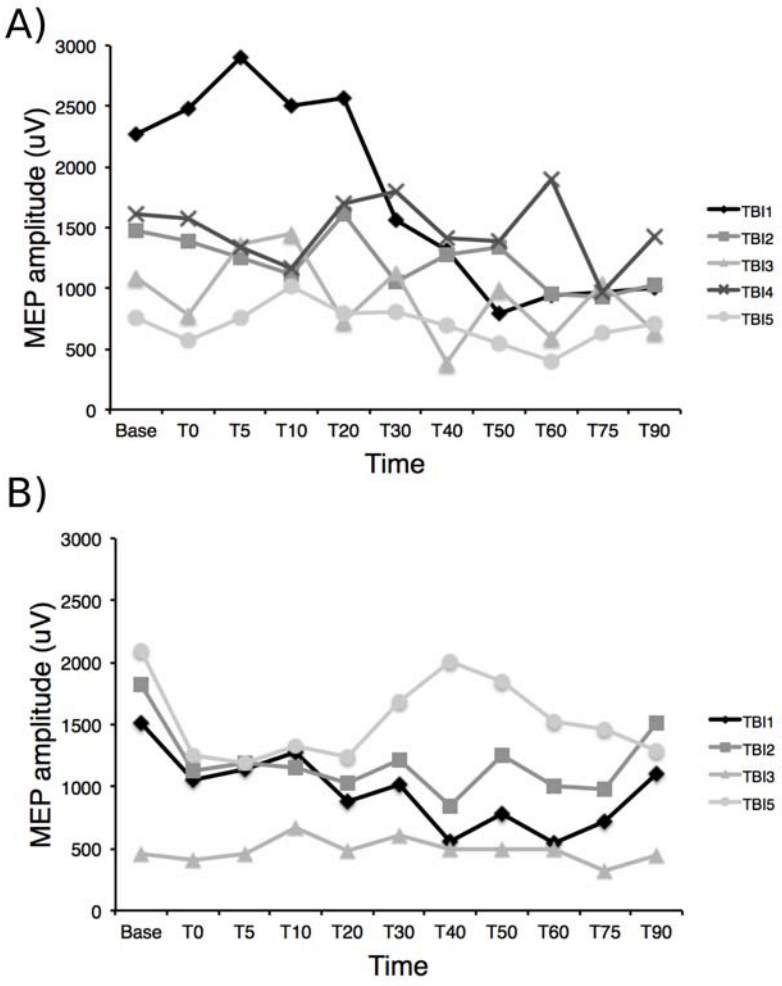


Figure 2. Individual MEP changes over time for both cTBS sessions

Legend : A) Individual mean MEP amplitudes for the first cTBS session. High variability is observed between the responses for each subject and therefore no clear inhibitory pattern can be visually observed. **B)** Individual mean MEP amplitudes for the second cTBS session. Subjects 1, 2 and 5 show a clear inhibitory response for at least the first four time points, whether only subject 3 does not seem to show an inhibitory response to the stimulation.



Annexe 3

The uncertain outcome of prefrontal tDCS

The uncertain outcome of prefrontal tDCS

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Running head: tDCS and the prefrontal cortex

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Keywords: transcranial direct current stimulation, neurostimulation, cognition, executive functions, dorsolateral prefrontal cortex

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Abstract

Background. Transcranial direct current stimulation (tDCS) is increasingly used in research and clinical settings, and the dorsolateral prefrontal cortex (DLPFC) is often chosen as a target for stimulation. While numerous studies report modulation of cognitive abilities following DLPFC stimulation, the wide array of cognitive functions that can be modulated makes it difficult to predict the precise outcome of DLPFC stimulation.

Objective: The present review aims at identifying and characterizing the various cognitive domains affected by tDCS over DLPFC.

Methods: Articles using tDCS over DLPFC indexed in PubMed and published between 2000 and January 2014 were included in the present review.

Results: tDCS over DLPFC affects a wide array of cognitive functions, with sometimes apparent conflicting results.

Conclusion: Prefrontal tDCS has the potential to modulate numerous cognitive functions simultaneously, but to properly interpret the results, a clear a priori hypothesis is necessary, careful technical consideration are mandatory, further insights into the neurobiological impact of tDCS is needed, and consideration should be given to the possibility that some behavioral effects may be partly explained by parallel modulation of related functions.

Keywords: transcranial direct current stimulation, prefrontal cortex, cognition, state-dependency, review

Introduction

In 1865, Broca introduced the idea of studying the neural basis of cognitive processes by the anatomical-correlative method [1]. While studying the effect of a brain lesion in his famous patient "Monsieur Tan", who had a neurosyphilic lesion to the left hemisphere that impaired his language production, Broca concluded that it was possible to infer a causal relationship between a specific brain region and a cognitive function [2]. This discovery ultimately sparked the emergence of neuropsychology, which aims to better understand the link between brain and behavior, and led to a wide interest in the study of patients with various brain lesions. Subsequently, remarkable progress was made using this approach, for example during World War II, where researchers were able to study the effects of focal brain lesions induced by weapons in conjunction with cognitive testing [3].

Despite the numerous and significant insights derived from the "lesion method", researchers were -and still are- confronted with methodological limitations when trying to ascertain brain-behavior relationships in patient populations. Firstly, lesions are usually large and often encompass multiple brain areas or networks, as they are most frequently acquired through stroke, ischemia, or traumatic brain injury. Secondly, and consequently, multiple functions are often altered simultaneously, inducing substantial variability in the nature and amplitude of the deficits observed in patients with relatively similar and overlapping lesions. Thirdly, patients often suffer from other medical conditions, either preexistent or consequent to injury, further contributing to the heterogeneity of the studied population. Lastly, it is difficult to conduct a study with a large sample of patients with overlapping lesions, which has led to numerous case studies and findings that have been difficult to replicate [4].

The development of non-invasive neuromodulation methods in the early 1980's offered the promise to circumvent many of the methodological caveats associated with the "lesion method", allowing causal inference in the study of brain-behavior relationship in healthy populations. While repetitive transcranial magnetic stimulation (rTMS) was increasingly used in the mid 1990's to study the influence of so-called "virtual lesions" in different regions of the brain, interest in transcranial direct current stimulation (tDCS) emerged more recently. tDCS

involves the induction of a constant low-amperage electric current (usually 1-2 mA) applied to the cortex via surface electrodes positioned on the scalp of the subject that can be used to probe and modulate cortical plasticity in the human cortex [5]. In standard protocols, the "active" electrode is positioned over the region of interest while the "reference" electrode is placed contralaterally over the homologous region or supraorbital area. The current flows from the positively charged anode towards the negatively charged cathode. The effect of tDCS on a specific region is partly determined by the polarity of the stimulation: cortical excitability is thought to be enhanced under the anode, and decreased under the cathode [6].

As with TMS protocols, initial studies using tDCS [6,7] investigated its effects on motor cortex, mainly because of the possibility to directly measure the increase or reduction of cortical excitability through TMS-induced motor evoked potentials (MEPs). Since tDCS was shown to be efficient in this regard, many studies began to report the impact of tDCS on other brain functions in healthy subjects, such as vision [8], language [9], and learning [10]. The investigation of the method's potential for the treatment of different neurological and psychiatric disorders, such as depression [11], stroke [12], and schizophrenia [13] has also recently arisen. In fact, over the past 16 years, over one thousand papers have been published on the use of tDCS on different brain functions. However, studies investigating the effect of tDCS on cognition have shown a lack of specificity and a relative inconsistency in both the modulatory effects and the choice of tDCS parameters, which has led to a large number of heterogenous results. For example, modulation of the dorsolateral prefrontal cortex (DLPFC), which is often chosen as target for tDCS because of its role in numerous high-order cognitive processes, has been associated with both an increase and a decrease in executive functions [14-16] and has been suggested to influence -among others- spatial memory [17], verbal fluency [18], risk taking [19] and craving [20].

Therefore, it remains to be determined to which extent tDCS can compensate for obvious limitations to the lesion method. For example, it is debatable whether tDCS can target specific behaviors associated with a given area when the physiologic impact of tDCS itself can vary considerably between subjects. Indeed, the effect of tDCS on a specific brain area will depend on a variety of factors including electrode montage and size, but also according to size and shape

of the participant head and fat tissue amount, among others. As a result, the amount of current induced in a given brain area may vary considerably across individuals. Furthermore, the brain region and neuronal populations that underlie a specific cognitive function may also be subject to important variations. Finally, the effects of tDCS for a given brain region are state-dependent and the state of brain activity will differ for different cognitive functions (even if the same brain area is engaged in different functions).

Another, often overlooked issue arises from the fact that stimulation of a given area produces widespread modulation of brain activity, which in turn can affect multiple cognitive functions simultaneously. This can lead to an important problem of interpretation since the observed effect of stimulation could be due to the interaction of several parallel cognitive effects, which are sometimes in opposite directions. To better understand the challenges of interpretation of results of studies using tDCS to modulate dorsolateral prefrontal cortical functions, we undertook a systematic review of the literature. Care was taken to select and compare studies that target the same area and use similar electrode montages. The international 10-20 electrode system areas F3 and F4 were chosen, as they are the most commonly used in tDCS studies of the DLPFC.

Material and methods

A systematic review of the literature was performed using the following database: PubMed (2000 to jan 2014) and Medline (2000 to jan 2014). We used the following search keywords: “tDCS”, “transcranial direct current stimulation”, “prefrontal”, “DLPFC”, “cognition”. We initially identified 202 articles corresponding to our search criteria. After carefully reviewing the abstract of the different papers, we identified 67 articles investigating only healthy subjects. Of these 67 publications, we selected the 63 articles using F3 and/or F4 as stimulation targets. Subsequently, we read through the full texts of the final sample of articles in order to gather the following information: location of stimulation; electrode montage; duration of stimulation; timing of stimulation and task; intensity; electrode size; cognitive domain; and results. We also looked through the references of the selected papers for additional relevant papers, which led to the inclusion of one additional paper. Studies were only included

if they were published in English and described thoroughly their methodology. Studies that did not directly assess the impact of prefrontal tDCS on a cognitive task were also excluded, leading to the exclusion of two additional studies and a final sample of 61 publications.

An important issue that needs to be taken into consideration when comparing tDCS studies is the electrode montage and the use of terms such as 'cathodal stimulation' and 'anodal stimulation'. It is not possible to apply anodal or cathodal stimulation, as a second electrode is always needed to deliver current to the brain. It is therefore important to emphasize that the 'site of stimulation' is not simply the location of one electrode, but rather the combination of the anode and cathode. In the present review, a distinction was made between stimulation paradigms that place one electrode (cathode or anode) over the specific target area (F3 or F4) and the other over a 'reference' site (usually the supraorbital area) and those that place both electrodes over the target area bilaterally.

Results

Using the same site of stimulation (F3 and F4, or F3/F4 and reference site), results from the 61 publications suggest that tDCS applied over the prefrontal cortex can influence the performance of a wide range of cognitive functions. The results and description of the studies are shown in Table 1. Note that these results are restricted to the effects of DLPFC stimulation on cognitive tasks, even if a study investigated other regions or if other methods were used to quantify the effects of tDCS (i.e. EEG). In order to be succinct, only the main results of the different studies are reported. Non-significant results in supplementary tasks included in the paradigms are not reported. For a clearer understanding of the effects of different types of stimulation (target regions and polarity) on cognitive function, the results are divided into the seven different types of electrode montages that were used in the included articles.

1. Cathode over left DLPFC, anode over reference site. Was shown to *decrease*: a) working memory performance [21]; b) executive function performance (mental flexibility: [22]); c) verbal and semantic performance (visual priming effect:[23]; word fluency task:[18]);

d) fear memory consolidation [17]; e) verbal memory performance [17,25-28]). Was shown to *increase*: a) working memory performance [29]; b) semantic processing performance [30-31]; c) executive functioning performance (planning: [15]). Was shown to *modulate*: a) decision making [32].

2. Cathode over right DLPFC, anode over reference site. Was shown to *decrease*: a) propensity to punish unfair behavior [33], b) executive function performance (impulsivity: [14]); c) attention control [34]. Was shown to *increase*: a) cognitive control during emotion regulation [35]; b) tolerance to heat pain [29]; c) executive functioning performance (planning: [15]).

3. Anode over left DLPFC, cathode over reference site. Was shown to *decrease*: a) working memory performance [36]; b) risk taking behaviors [37]; c) negative emotions perception [38-39]; d) categorization learning [28]); e) executive functioning performance only in a COMT Met-Met group (cognitive flexibility [40]). Was shown to *increase*: a) working memory performance [21,41-49]; b) positive emotion processing [50-52]; c) pain thresholds [53] ; d) performance on verbal tasks (verbal; word retrieval:[54]; word fluency:[18]); e) executive function performance (mental flexibility: [22]; inhibition: [46]; problem solving: [24,55-56]; planning [15]); f) control of negative emotions [39,57]; g) memory performance and learning [25,27,58-60]. Showed *no significant effect* on: a) mood [61].

4. Anode over right DLPFC, cathode over reference site. Was shown to *decrease*: a) risk taking [37]; b) propensity to punish unfair behaviors [33]. Was shown to *increase*: a) working memory performance [48]; b) visuo-spatial memory [46]; c) executive functioning performance (inhibition: [46]); d) pain thresholds [29]; e) emotion regulation [35]; f) memory performance [59]. Showed *no significant effect* on: risk taking [62].

5. Anode over left DLPFC, cathode over right DLPFC. Was shown to *decrease*: a) working memory performance [63]; b) food consumption but not craving [20]; c) executive function performance (mental flexibility: [16]). Was shown to *increase*: a) aggressive behaviours and anger [65]; b) executive function performance (mental flexibility: [16]) ; c)

language comprehension [66]; d) generation of untruthful answer [67]; e) attention and language performance [68]; f) automaticity for learned materials [66]. Was shown to *modulate*: a) responses to lies [69]; b) decision making [70].

6. Cathode over left DLPFC, anode over right DLPFC. Was shown to *increase*: a) executive function performance (mental flexibility: [16]); b) response confidence in a gambling task [71]; c) working memory performance [29]; d) generation of untruthful answers [67]; e) language comprehension [66]**.** Was shown to *decrease*: a) risk-taking behaviors [19, 62]; b) food craving and consumption [20].

7. Anode over left DLPFC, anode over right DLPFC. Was shown to *increase*: a) lie responses [72]; b) attention and vigilance [73].

To summarize, tDCS intending to modulate activity of the same target region (DLPFC) can interfere with a wide range of cognitive functions, from relatively simple and low-level attentional processes, to complex, higher-order functions such as decision-making and working memory. The results also show that the effects of tDCS are highly variable and may be dependent upon the task and stimulation parameters, as illustrated in studies probing working memory function. For instance, working memory was shown to be enhanced by cathodal tDCS over the left DLPFC [29], anodal tDCS over the left DLPFC [21,41-49]; and anodal tDCS over the right DLPFC [48];. Working memory performance was also shown to be decreased by cathodal tDCS over the left DLPFC [21], anodal tDCS over the left DLPFC [36]; and tDCS over bilateral DLPFC (left anodal/right cathodal: [63]). In general, the present review shows that 1) studies probing the same cognitive function using similar tDCS protocols can lead to opposite results; 2) a specific tDCS protocol can induce cognitive effects over a wide variety of functions.

Discussion

The present review highlights the fact that tDCS over the prefrontal cortex can modify a wide range of behaviors from various domains. Due to the presence of many important

variations in experimental protocols that have a similar aim (for example reducing excitability of the DLPFC to inhibit a specific cognitive function), it is difficult at this point in time to confidently point to a general pattern describing the effects of "prefrontal tDCS". This is further compounded by the fact that the physiological effects of tDCS themselves are highly variable and dependent upon a variety of individual characteristics.

Polarity

The highly variable effects of tDCS on cognition highlight the fact that the idea of a polarity-specific effect of tDCS, as described originally for the primary motor cortex, cannot be easily transposed to non-motor areas [74]. Theoretically, tDCS increases excitability in the area under the anode, thus facilitating performance on a specific task whereas the opposite effect would occur in the area under the cathode, inhibiting behaviour by decreasing cortical excitability. However, the reality of tDCS effects on cognition is much more complex [75]. For example, many studies report a facilitatory effect associated with stimulation of areas under the cathode [75]. It has been suggested that this effect may be due to the reduction of noise in a specific network that enables facilitation of behaviour [75]. Alternatively, it is possible that 'cathodal tDCS' inhibits a specific function, which would consequently enhance a specific behavior (e.g. faster reaction times).

In a recent study by Batsikadze and collaborators [76], 20 minutes of cathodal tDCS over the primary motor cortex (reference electrode over supraorbital area) was shown to produce an enhancement of corticospinal excitability instead of the expected inhibition when the intensity of the stimulation was doubled from 1 mA to 2 mA. This suggests that different stimulation parameters can directly affect the direction of tDCS-induced changes in cortical excitability. In the studies that were included in the present review, the intensity of stimulation ranged from 260 uA to 2 mA, stimulation duration varied from 3 min to 30 min and electrode size ranged from 8 mm diameter to 100 cm^2. This inconsistency in the choice of the parameters may contribute to the variable direction of the cognitive changes induced by prefrontal tDCS.

State-dependency

Out of the 61 articles presented in this review, 38 used a so-called “online” paradigm where the prefrontal cortex is modulated by tDCS during a specific task. Conversely, 23 studies applied tDCS before a specific task (“offline” paradigm). Both methods are thought to rely on partially distinct mechanisms, which could contribute to the apparent discrepancies among results [77]. Indeed, “offline” stimulation has been suggested to rely on modification of neuronal activity that lasts beyond the period of stimulation, whereas “online” stimulation is believed to modulate a specific network that is involved in the task [77].

Unlike TMS, tDCS does not induce a direct depolarization of neurons but rather is thought to modulate the membrane permeability of neurons leading to a change in the neuronal firing rate [78]. Therefore, theoretically, tDCS should induce a depolarization of the neurons that are the closest to firing, but that would not have necessarily fired otherwise. In an “online” paradigm, the targeted neuronal populations are already prone to discharge, given that they are presumably part of a neural network thought to be involved in the cognitive task under study [79]. Hence, the effects of prefrontal tDCS are highly dependent on the state of the underlying targeted network, a principle known as “state-dependency” [77,80,81]. In other words, any tDCS-induced activity occurs in the context of a baseline neural activity or a specific state [82]. This state-dependent effect of neuromodulation on the motor region has been taken into consideration from the very first motor studies because the level of cortical excitability is measured before and after the stimulation via MEPs. However, this is more challenging to achieve when studying cognitive functions because many factors can influence the initial state of a neuronal network, such as the level of fatigue, knowledge of the task, pre-existent network connectivity, etc. [81]. For example, a recent meta-analysis showed that “cathodal tDCS” has a very minor effect on language function, which could be explained by the strongly connected brain networks [75]. In other words, because of the high intensity of the firing rate of these strongly interconnected neurons, the current induced by tDCS might not be strong enough to significantly modulate network activity and induce behavioral changes. A further example can be drawn from a tDCS study on motor cortex where the induction of motor imagery during the application of stimulation abolished the excitatory effect of anodal tDCS [83]. In this case, the

neurons are already depolarized, which constrains the excitatory effects of the stimulation, possibly by engaging metaplasticity mechanisms.

If the effect of tDCS is dependent on the state of the networks, it must thus also be dependent on the specific task the subjects are engaged in. As a result, the targeted cognitive function has a higher probability of being modulated, and online and offline tDCS protocol would be expected to lead to different results. Similarly, the instructions given to study participants prior to the tDCS would be predicted to exert significant effects onto the results, and thus need to be scripted and controlled with care. Further investigation and leveraging of the "state-dependent" effect could benefit tDCS prefrontal studies in order to better specify the effects of stimulation of a targeted network or function. To date, very few studies have taken this important factor into consideration: within the articles included in the present review, only five mentioned the impact of state-dependency.

Inter-subject variations

Two recent large-scale prospective studies evaluated the inter-subject variation of tDCS effects on primary motor cortex excitability and showed high variability in the participants' response to stimulation [5,84]. Results from Lopez-Alonso and colleagues [5] showed that only 45% of participants respond to "anodal tDCS" over the target area. Similarly, Wiethoff and colleagues [84] showed a response ratio of 45:15 (facilitation: inhibition) after anodal stimulation of the target area and a ratio of 60:40 (facilitation: inhibition) after cathodal stimulation of the target area. As mentioned previously, there exists a large number of *stimulation* parameters that can modulate the physiologic response to tDCS. Chief among them are electrode size, stimulation duration and stimulation intensity. As can be seen from Table 1, these parameters vary widely between studies and considerably limit the generalizability and comparison of results between studies. Similarly, *participant* characteristics are also important factors that contribute to the variability observed in tDCS studies of prefrontal cortex. Participant head size and shape, as well as amount of fat tissue and fiber orientation all contribute to the physiologic effects of tDCS. When taken together, the presence of these confounding factors strongly suggest that the level of induced current in a specific brain area

can vary quite extensively. It is therefore not surprising that the behavioral response to prefrontal tDCS is also subject to large hetererogeneity. All of these factors are compounded by the fact that sample sizes are often relatively small in tDCS studies of prefrontal cortex. A study of cathodal and anodal effects on motor cortex excitability suggested that based on acquired data in healthy individuals, a minimum of 87 participants per group would be needed to achieve a sufficient level of power and confidence to detect a significant difference between patients and healthy subjects [84]. Although this seems to be an extreme case, it should be noted that the mean sample size for the studies included in the present review was only 21 participants.

Conclusion

When using tDCS over the DLPFC with a specific set of parameters, it is possible to modulate a specific cognitive function. However, as highlighted in this review, a given stimulation protocol may simultaneously modulate various other cognitive functions in similar or opposite directions (i.e. facilitation or inhibition). This implies that any effect of prefrontal tDCS on a given task is probably associated with the extensive modulation of a wide range of *multiple* cognitive functions. This, in turn, makes it hard to attribute an observed effect on a specific task to a single mechanism, at least with traditional stimulation protocols. When differing participant characteristics, stimulation parameters and state-dependency effects are also taken into consideration, it becomes clear that more neurobiologic insights of the effects of tDCS are needed to properly interpret the results of studies and appropriately conclude brain-behavior relations.

In conclusion, refined protocols that take into account the numerous caveats associated with tDCS and a better standardization of stimulation protocols are needed to improve study quality. One possible way to reduce uncertainty is to monitor the brain impact of tDCS separately and independently of behavioral and cognitive effects. Techniques such as EEG (e.g. [85]), TMS-EEG (e.g. [86]), magnetic resonance spectroscopy (e.g. [87]), functional magnetic resonance imaging (e.g. [88]) and modeling of induced currents (e.g. [89)] have all been shown to be effective in characterizing the physiologic effects of tDCS. Relating behavioral and

cognitive effects to the measured brain impact (induced current, physiologic effect) would offer a significant advance for the interpretation of tDCS data.

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Conflict of Interest Disclosure

APL serves on the scientific advisory boards for Nexstim, Neuronix, Starlab Neuroscience, Neuroelectrics, Axilum Robotics, Magstim Inc., and Neosync; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation (TMS) with electroencephalography (EEG) and magnetic resonance imaging (MRI).

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Table 1.

Authors	Target Region(s)	Montage	Timing of tDCS	Duration	Intensity	Electrode Size	Cognitive Domain	Cognitive effect
[36] Fregni *et al.* (2005)	Left DLPFC	Anodal F3 / Cathodal contra SO Cathodal F3 / Anodal contra SO Sham (F3 /SO)	Online	10 min	1 mA	35 cm^2	Working memory	1) Left anodal: increased performance (sequential letter task)
[63] Marshall *et al.* (2005)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Reference: mastoids Sham (F3/F4/mastoid)	Online	15 min (15s on and 15s off, intermittent)	260 uA	Diameter: 8 mm	Working memory	1) Bilateral (left anodal/right cathodal): decreased performance (slower RTs)
[62] Fecteau *et al.* (2007)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4) Anodal F3 / Cathodal contra SO Anodal F4 / Cahodal contra SO Sham (F3 or F4 / contra. SO)	Online	20 min	2 mA	35 cm^2	Risk taking	1) Bilateral (left cathodal/right anodal): decreased risk taking (Baloon Analogue Risk Task) 2) Right anodal: no sign. effect on risk taking
[19] Fecteau *et al.* (2007)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Online	15 min	2 mA	35 cm^2	Risk taking	1) Bilateral (left cathodal/right anodal): reduced risk taking (Risk Task)
[37] Beeli *et al.* (2008)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal ipsi. mastoid Cathodal F3 or F4 / Anodal ipsi. mastoid	Offline	15 min	1 mA	35 cm^2	Risk taking	1) Left anodal: reduced risk taking behaviours (driving stimulator) 2) Right anodal: reduced risk taking behaviours (driving stimulator)
[14] Beeli *et al.* (2008)	Right DLPFC	Anodal FC3 / Cathodal ipsi. mastoid Cathodal FC3 / Anodal ipsi. Mastoid Sham	Online	5.5 min	1.5 mA	35 cm^2	Executive function (impulsivity) Feeling of "presence"	1) Right cathodal: increases impulsiveness (Go-Nogo task)
[53] Boggio *et al* (2008)	Left DLPFC	Anodal F3 / Cathodal right SO Sham (F3 / SO)	Online	5 min	2 mA	35 cm^2	Pain perception	1) Left anodal: decreased pain perception (higher pain threshold)

[20] Fregni *et al.* (2008)	Left/right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Offline	20 min	2 mA	35 cm^2	Food craving	1) Bilateral (left anodal/right cathodal): reduced food consumption but not craving 2) Bilateral (left cathodal/right anodal): reduced food craving and consumption
[33] Knoch *et al.* (2008)	Right DLPFC	Cathodal F4 / Anodal contra. SO	Online	14 min	1.5 mA	35 cm^2 Reference: 100 cm^2	Emotions Social behaviours	1) Right cathodal: reduced propensity to punish unfair behaviour
[41] Ohn *et al.* (2008)	Left DLPFC	Anodal F3 / Cathodal right SO Sham (F3 / SO)	Online	30 min	1 mA	25 cm^2	Verbal working memory	1) Left anodal: enhanced performance (3-back)
[72] Priori *et al.* (2008)	Left/Right DLPFC	Anodal F3 and F4 / Cathodal deltoid Cathodal F3 and F4 / Anodal deltoid Sham (F3-F4 / Deltoid)	Offline	10 min	1.5 mA	Active: 32 cm^2 Reference: 64 cm^2	Decision making	1) Bilateral (left anodal/ right anodal): increased lie responses
[38] Boggio *et al.* (2009)	Left DLPFC	Anodal F3 / Cathodal contra SO Sham (F3 / SO)	Online	5 min	2 mA	35 cm^2	Emotion processing Pain	1) Left anodal: decreased negative emotions perception (unpleasantness and emotional discomfort)
[56] Cerruti *et al.* (2009)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal contra SO Cathodal F3 or F4 / Anodal contra SO Sham F3/F4	Online	20 min	1 mA	16.3 cm^2 on F3/F4 30 cm^2 on SO	Verbal problem solving	1) Left anodal: improvement (RAT task) 2) Cathodal: no sign. effect
[15] Dockery *et al.* (2009)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Anodal contra. SO Sham (F3 / SO)	Online	15 min	1 mA	35 cm^2	Executive functioning (planning)	1) Left cathodal and left anodal: enhanced performance (Tower of London)
[24] Elmer *et al.* (2009)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal mastoid Cathodal F3 or F4 / Anodal mastoid Sham (F3 or F4/ Mastoid)	Online	5 min	1.5 mA	28 cm^2 on F3/F4 100 cm^2 on SO	Verbal learning	1) Left cathodal: impaired short-term verbal learning
[54] Fertonani	Left DLPFC	Anodal F3 / Cathodal shoulder	Offline	8-10 min	2 mA	35 cm^2	Language	1) Left anodal: facilitation

et al. (2010)		Cathodal F3 / Cathodal shoulder Sham (F3/Shoulder)					(picture naming)	(faster RTs) 2) Left cathodal: no sign. effect
[70] Hecht *et al.* (2010)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Control (no stimulation)	Online	22 min	2 mA	9 cm^2	Decision making	1) Bilateral (left anodal/right cathodal): modified strategies (Probabilistic Guessing Task)
[69] Mameli *et al.* (2010)	Left/Right DLPFC	Anodal F3 / Anodal F4 / Reference right deltoid muscle Sham (F3 / F4 / Deltoid)	Offline	15 min	2 mA	Target: 32 cm^2 Reference: 64 cm^2	Decision making (lies)	1) Bilateral (left anodal/ right anodal): modulated responses to lies (decreased RTs)
[28] Ambrus *et al.* (2011)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal Cz Cathodal F4 / Anodal Cz Sham (F4/ Cz)	Online	10 min	1 mA	35 cm^2	Categorization learning	1) Left anodal and right anodal: decreased performance (accuracy of identification of prototype)
[44] Andrews *et al.* (2011)	Left DLPFC	Anodal F3/Cathodal SO	Online	10 min	1 mA	35 cm^2	Working memory	1) Left anodal: enhanced performance (digit span forward)
[26] Hammer *et al.* (2011)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Cathodal contra. SO Sham (F3 / SO)	Online	30 min	1 mA	35 cm^2	Memory	1) Left cathodal: hampered memory performance after errorful learning
[22] Leite *et al.* (2011)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Cathodal contra. SO Sham (F3 / SO)	Online	15min	1 mA	35 cm^2	Executive functions (mental/motor flexibility)	1) Left anodal: increased performance (set switching task) 2) Left cathodal: decreased performance (set switching task)
[42] Mulquiney *et al.* (2011)	Left DLPFC	Anodal F3 / Cathodal contra. SO Sham (F3 / SO)	Online	10 min	1 mA	35 cm^2	Working memory	1) Left anodal: improved speed of performance (2-back task).
[57] Peña-Gómez *et al.* (2011)	Left DLPFC	Anodal F3 / Cathodal C4 Sham (F3 / C4)	Online	20 min	1 mA	35 cm^2	Emotion processing	1) Left anodal: enhanced down-regulation of negative emotions
[43] Teo *et al.* (2011)	Left DLPFC	Anodal F3 / Cathodal right SO Sham (F3 / SO)	Online	20 min	1 mA and 2 mA	35 cm^2	Working memory	1) Left anodal: enhanced performance at 2 mA (faster RTs, no effect on accuracy)
[21] Zaehle *et al.*	Left DLPFC	Anodal F3 / Ipsi. mastoid	Offline	15 min	1 mA	35 cm^2	Working	1) Left anodal: increased

(2011)		Cathodal F3 / Ipsi. mastoid Sham (F3 / mastoid)					memory	performance 2) Left cathodal: reduced performance
[31] Balconi *et al.* (2012)	Left DLPFC	Cathodal F3 / Anodal right SO Sham (F3/ Right SO)	Offline	15 min	2 mA	35 cm^2	Semantic congruence processing	1) Left cathodal: improved performance (reduced RTs for incorrect object)
[45] Gladwin *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal right SO Sham (F3/Right SO)	Offline	10 min	1 mA	35 cm^2	Working memory	1) Left anodal: improved performance (congruent blocks of the IAT task)
[65] Hortensius *et al.* (2012)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Offline	15 min	2 mA	35 cm^2	Emotion regulation	1) Bilateral (left anodal/right cathodal): increased aggressive behaviours and anger
[58] Javadi *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Cathodal contra. SO Sham (F3 / SO)	Online	20 min	1 mA	Target: 12.2 cm^2 Reference: 30.2 cm^2	Declarative memory	1) Left anodal: enhanced memory performance (applied during encoding and recognition (trend)) 2) Left cathodal: impaired memory performance (applied during encoding and recognition)
[25] Javadi *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Cathodal contra. SO Sham (no stimulation)	Online	3 min	1.5 mA	Target: 12.2 cm^2 Reference: 30.2 cm^2	Verbal memory	1) Left anodal: enhanced memory performance (accuracy) 1) Left cathodal: impaired memory performance (accuracy)
[46] Jeon *et al.* (2012)	Left DLPFC Right DLPFC	Anodal F3 / Cathodal right SO Anodal F4 / Cathodal left SO Sham (F3 or F4/ SO)	Offline	20 min	1 mA	35 cm^2	Working memory Attention Executive functions (inhibition, mental flexibility)	1) Left anodal: enhanced performance (stroop, digit span backwards, K-BNT) 2) Right anodal: enhanced performance (stroop, visuospatial memory/attention task)
[16] Leite *et al.* (2012)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Online	<30 min	2 mA	35 cm^2	Executive functions (mental	1) Bilateral (left anodal/right cathodal): increased switching

							flexibility)	performance (letter-digit task), improved accuracy and decreased switching performance (vowel-consonant parity task) 2) Bilateral (left cathodal): increased accuracy (letter-digit task)
[39] Maeoka *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal contra. SO Sham (F3 / SO)	Offline	20 min	1 mA	35 cm^2	Emotion processing	1) Left anodal: decreased negative emotion (decreased unpleasantness subjective report)
[55] Metuki *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal Fp2 Sham (F3 / Fp2)	Online	11 min	1 mA	35 cm^2	Executive functions: Problem solving	1) Left anodal: increased performance (verbal insight task)
[71] Minati *et al.* (2012)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Online	20.5 ± 4.1min	2 mA	N.S.	Decision making	1) Bilateral (left cathodal/ right anodal): no sign. effect on risk taking (Gambling Task) but enhanced response confidence
[29] Mylius *et al.* (2012)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal contra SO Cathodal F3 or F4 / Anodal contra SO Sham (F3 or F4 / contra SO)	Online	20 min	2 mA	35 cm^2	Working memory Pain perception	1) Right anodal: increased tolerance to pain 2) Left cathodal: decreased number of outliers in 2-back task
[50] Nitsche *et al.* 2012	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F4 / Anodal contra. SO Sham (F3 / contra. SO)	Offline Online	10 min or 20 min	1 mA	35 cm^2	Emotion processing	1) Left anodal: enhanced positive emotion (positive emotion detection)
[66] Sela *et al.* (2012)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Cathodal F3 / Anodal F4 Sham (F3/F4)	Offline	15 min	1.5 mA	35 cm^2	Language comprehension (semantic processing)	1) Bilateral (left anodal/right cathodal): enhancement of performance (predictable idioms) 2) Bilateral (left cathodal/ right anodal): enhancement of performance

								(unpredictable idioms)
[18] Vannorsdall *et al.* (2012)	Left DLPFC	Anodal F3 / Cathodal Cz Cathodal F3 / Cathodal Cz Sham (F3 or F4 / Cz)	Online	30 min	1 mA	27 cm^2	Verbal fluency	1) Left anodal: increased performance (category-cued) 2) Left cathodal: decreased performance (clustered words)
[17] Asthana *et al.* (2013)	Left DLPFC	Anodal F3 / Ipsi. mastoid Cathodal F3 / Ipsi. mastoid Sham (F3 / mastoid)	Offline	12 min	1 mA	35 cm^2	Fear memory	1) Left cathodal: disrupted fear memory consolidation
[51] Cattaneo *et al.* (2013)	Left DLPFC	Anodal (between F3 and F5) / Cathodal contra. SO Sham (F3-F5 / SO)	Offline	20 min	2 mA	35 cm^2	Emotion processing	1) Left anodal: enhancement of beauty experience
[67] Fecteau *et al.* (2013)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Anodal F4 / Cahodal F3 Sham (F3 / F4)	Offline	20 min	2 mA	35 cm^2	Decision making	1) Bilateral (left cathodal/right anodal): enhanced the generation of untruthful answers 2) Bilateral (left anodal/right cathodal): enhanced the generation of untruthful answers
[35] Feeser *et al.* (2013)	Right DLPFC	Anodal F4 / Cathodal contra. SO Sham (F4 / SO)	Online	20 min	1.5 mA	Anode: 35 cm^2 Cathode: 100 cm^2	Emotion regulation	1) Right anodal: enhancement of performance (downregulation or upregulation of emotions)
[60] Foerster *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra. SO Sham (F3 / SO)	Online	13 min	2 mA	20 cm^2	Motor learning	1) Left anodal: enhanced motor learning (motor-imagery-induced learning)
[47] Hoy *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal right SO Sham (F3 / SO)	Offline	20 min	1 mA and 2 mA	35 cm^2	Working memory	1) Left anodal: enhanced performance (both 1mA and 2mA)
[64] Iuculano and Cohen Kadosh (2013)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Sham (F3/F4)	Online	20 min x 6 sessions	1 mA	3 cm^2	Learning and automaticity	1) Bilateral: impaired numerical learning but enhanced automaticity for learned materials
[27] Javadi *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra. SO Cathodal F3 / Cathodal contra. SO	Online	20 min	1.5 mA	Target: 12.2 cm^2	Long term memory	1) Left anodal: enhanced memory performance

		Sham (F3 / SO)				Reference: 30.2 cm^2		(recognition)
[23] Kongthong *et al.* (2013)	Left DLPFC	Cathodal F3 / Anodal T6 Sham (F3/T6)	Offline	20 min	1 mA	25 cm^2	Visual semantic processes	1) Left cathodal: decreased performance (elimination of priming effect)
[59] Manenti *et al.* (2013)	Left/Right DLPFC	Anodal F3 or F4 / Cathodal contra. SO Sham (F3 or F4 / SO)	Online	6 min	1.5 mA	35 cm^2	Verbal episodic memory	1) Left and right anodal: increased memory performance in young subjects 2) Left anodal: increased memory performance in older subjects
[48] Meiron *et al.* (2013)	Left DLPFC Right DLPFC	Anodal F3 or F4 / Cathodal Cz Sham (F3 / Cz)	Online	15 min	2 mA	Anode: 16 cm^2 Cathode: 35 cm^2	Working memory	1) Left anodal: improved performance (highest memory load males only) 2) Right anodal: improved performance (highest memory load females only)
[32] Mengarelli *et al.* (2013)	Left DLPFC Right DLPFC	Cathodal F3 / Anodal contra SO Cathoal F4 / Anodal contra SO Sham (F3 or F4 / SO)	Online	15 min	1 mA	35 cm^2	Decision making	1) Left cathodal: reduced behaviour-induced preference change
[61] Motohashi *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra SO Sham (F3 / SO)	Offline	20 min	1 mA	35 cm^2	Mood	1) Left anodal: no sign. effect on mood
[68] Nozari *et al.* (2013)	Left/Right DLPFC	Anodal F3 / Cathodal F4 Sham (F3 / F4)	Online	20 min	1.5 mA	25 cm^2	Attention Language	1) Bilateral (left anodal/right cathodal): increased performance (verbal task)
[40] Plewnia *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra SO Sham (F3 / SO)	Online	20 min	1 mA	35 cm^2	Executive functions	1) Left anodal: no sign. effect for whole group but decreased performance for COMT Met-Met
[34] Tanoue *et al.* (2013)	Right DLPFC	Cathodal F4 / Anodal contra. cheek Sham (F4 / contra. cheek)	Offline	10 min	1.5 mA	35 cm^2	Attention	1) Right cathodal: impairs internal attention
[52] Vanderhasse *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra SO Sham (F3 / SO)	Online	20 min	2 mA	35 cm^2	Working memory	1) Left anodal: enhanced performance (working

							Emotion processing	memory of angry faces)
[49] Vanderhasse *et al.* (2013)	Left DLPFC	Anodal F3 / Cathodal contra SO Sham (F3 / SO)	Offline	20 min	2 mA	35 cm^2	Emotion processing	1) Left anodal: enhanced cognitive control for positive stimuli
[30] Balconi *et al.* (2014)	Left DLPFC	Cathodal F3 / Anodal right SO Sham (F3/ Right SO)	Offline	15 min	2 mA	35 cm^2	Semantic processing of actions	1) Left cathodal: modulated performance (reduced RTs to incorrect object use but increased errors rates)
[73] Nelson *et al.* (2014)	Right/Left DLPFC	Anodal F3 / Cathodal F4 Cathodal F3 / Anodal F4 Sham (F3 / F4)	Online	10 min	1 mA	35 cm^2	Attention / Vigilance	1) Bilateral (anodal left/cathodal right and anodal right / cathodal left): enhanced performance (detection task)

Table Legends
*Only main results were included in the present table.
**Presented results are restricted to the DLPFC. Results from an additional method of investigation (such as EEG) or an alternative region were not included.