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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

The modulation of the cerebellar excitability with cerebellar transcranial direct current stimulation to improve bimanual coordination in the elderly

Fréderique Michiels

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Clinical Molecular Sciences

SUPERVISOR :
Prof. dr. Raf MEESEN

MENTOR :
dr. Kim VAN DUN

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1. List of abbreviations

A	Anterior
ALE	Activation Likelihood Estimate
BA	Brodmann Area
Bl	Block
BTT	Bimanual Tracking Task
CC	Corpus Callosum
CI	Confidence Interval
cbtDCS	cerebellar transcranial direct current stimulation
EEG	Electroencephalography
Ex	Externus
FC	Functional connectivity
FDR	False Discovery Rate
fMRI	Functional Magnetic Resonance Imaging
Fr	Frequency
FWHM	Full-Width Half-Maximum
GPe	Globus Pallidus externus
GPi	Globus Pallidus internus
In	Internus
M1	Primary Motor Cortex
MNI	Montreal Neurological Institute
L	Left
P	Posterior
Q	Quadrant
R	Right
SMA	Supplementary motor area
SII	Secondary somatosensory cortex
tDCS	Transcranial Direct Current Stimulation
tES	Transcranial Electrical Stimulation
V	Version
VPL	Ventral posterolateral
VPM	Ventral posteromedial

2. Foreword

When I started this internship, I never heard about bimanual coordination and the mechanisms involved. I also never performed an experiment on my own or engaged with participants. Throughout the last few months, I learned a lot about how a beginning research is established and how to do an experiment and encourage the participants to do their best when they doubted their selves. All this I learned through the excellent supervision by dr. Kim van Dun. Even when she did not even start her post doctorate, she helped me writing my proposal. She encouraged me when I was insecure and helped me where needed. So, I especially want to thank her for all the help she gave me throughout my internship.

However, she is not the only one I have to thank for this; the rest of the team, Stefanie Verstraelen and Siel Depestele, also helped me throughout the last couple months. They made me feel like I was a part of the team and not just an intern. They encouraged me to think along, even if the subject was entirely different from mine.

At first, I was nervous to ask questions or to speak up if I did not understand something. Prof. Dr. Raf Meesen quickly made it clear that there are no stupid questions by telling a story of his own experience. I kept that story in my mind for the rest of the year and reassured me when I doubted myself. Also, he taught me how to speak up, how to present and how to prepare myself. I can say for sure that he taught me a lot through the year, not only in the lab, but also as a person. He kept pushing me; at first, I thought it was quite annoying, but after a while I noticed I kind of needed it to keep giving the best of myself and he knew that.

Other people I want to mention are my parents of course. Although they had their own mountains to climb; they always made sure I kept climbing to reach the top. We often take it for granted what they do for us, but if I reflect on my university career, they made their own sacrifices to give me this chance and I always be thankful for that and try to make them proud.

Otherwise, I want to thank my friends. Some of them really helped me throughout the year, either by supporting me, or by listening to me when I was talking about my thesis, even if they did not have a clue what is was about.

I still have a lot to learn but these people already taught me a lot and helped me on my way. So, to all the people I mentioned and to all the people I did not mention like the participants, thank you very much; without you, I would not be writing this.

Fréderique Michiels

3. Summary

Introduction: Healthy aging influences the execution of bimanual coordination which constitutes a critical marker of functional independence across the lifespan. Bimanual coordination poses an important element in many daily activities and is an example where the two hemispheres have to collaborate. The cerebellum is linked to factors strongly involved in bimanual coordination and is an important if not the most important predictor of bimanual coordination performance in the elderly, little is still known about the specific role of the cerebellum in bimanual coordination and in neurological aging processes. The aim of this study is to gain more knowledge about the cerebello-cerebral activations in bimanual coordination and to which functional network the clusters, which is a collection of activations, belong and to improve performance of bimanual coordination in the elderly by modulating the cerebello-cerebral network with cerebellar transcranial direct current stimulation (cbtDCS).

Material and methods: First, a meta-analysis of functional MRI studies was performed with activation likelihood estimation method. Next, for the double-blind sham-controlled randomised study, 14 right-handed young people (μ = 21) and 13 right-handed healthy elderly (μ = 71.1) were recruited. They performed the bimanual tracking task (BTT) while being monitored with electroencephalography (EEG) and stimulated with cbtDCS (real or sham/placebo) with a wash-out period of at least one week. Three dependent variables (Average Trace Deviation, Average Target Deviation and Motion stability) were used to determine the effect of cbtDCS on the performance of both age groups.

Results: The meta-analysis identified seven clusters of which the left precentral gyrus (M1) was the largest cerebral cluster consistently activated across studies for bimanual coordination and the right anterior cerebellum was the largest cerebellar cluster. The contrast of bimanual coordination versus unimanual coordination revealed the right globus pallidus externus (GPe) as the largest cluster for bimanual coordination. The BTT revealed an improvement after stimulation both in session 1 and session 2 and between sessions showed by the interaction between time and session for Average Trace Deviation. No effect of cbtDCS was shown, except in for the Average Target Deviation. cbtDCS was not involved in an interaction, which influence cbtDCS has, remains unclear.

Discussion and Conclusion: Both cerebral and cerebellar activations were seen in motor and coordination regions and in regions related to cognition. The left M1 is located in the dominant hemisphere which mainly controls bimanual coordination. Next, since bimanual coordination may require more control than unimanual coordination, the involvement of the GPe was the largest cluster from the contrast. This structure is involved in the control of voluntary movements through the No-Go pathway. At last, the cerebellum influences muscle activity through connections with the motor areas and it represents a critical site for control, organisation and execution of bimanual movements. The interaction between time and session reveal a learning effect. Although no clear effects were observed for cbtDCS, it is speculated it may have an influence on the consolidation rather than on immediate improvement of the performance because a larger but non-significant improvement between the two sessions was seen if cbtDCS was applied in the first session.

4. Introduction

Bimanual coordination, which has a signature in many daily activities, is an example where two interconnected, yet functionally specialised hemispheres need to collaborate to achieve goal-directed behaviour (1, 2). In the beginning, the **supplementary motor area (SMA)** was proposed to be the sole controller of the integration of the two hands during bimanual coordination (3). However, since SMA lesions only caused short-term disturbances in interlimb coordination, the existence of a rather distributed neuronal network for the control of coordinated bimanual actions was proposed (3, 4). Bimanual coordination requires accurate coordination and communication between the different neurological networks integrating left and right limb movements into a functional control entity (1, 2). The interhemispheric communication is mainly covered by the **corpus callosum (CC)** which connects the two cerebral hemispheres making it an important structure in the context of bimanual coordination (1, 2, 5). The CC has both an inhibitory and an excitatory function; the excitatory function of the CC states that callosal connections are critical for interhemispheric information transfer, while the inhibitory function ensures functional specialisation and independent processing of both hemispheres (6). Studies researching the relationship between the size of CC and the microstructure on one hand and bimanual performance on the other hand found that age-related declines in callosal size and integrity were key contributors to bimanual control deficits (5, 7). All the movements, prepared by the premotor cortices and executed by the primary motor cortex (precentral gyrus or M1), are followed and monitored by the posterior parietal cortices and the cerebellum, adjusting the movements if necessary (1). Bimanual-related activity was also found in the cingulate motor area, the dorsolateral prefrontal cortex, and the basal ganglia (1, 3).

Normal aging is associated with a cognitive decline, affecting the domains of attention, memory and executive functioning which has an impact on the quality of life (8). Movements become slower and/or less accurate and are going to depend more on cognition (***Figure 1***)(5). This event is associated with age-related hyper-activations of distinct cortical areas which reflect additional neural recruitment for cognitive and sensory processing functions (***Figure 1***) (5). It is suggested that these cortical hyper-activations may be linked to the age-related hypo-activations of subcortical structures (5). The interactions among these areas are also exhibiting age-related changes, as suggested by the changed functional connectivity (FC) with aging (***Figure 1***) (5). The FC reflects the underlying architecture of anatomical connectivity and is positively correlated with indices of structural connectivity (8). Variations in white matter integrity are correlated with abilities of information processing and executive functioning, which declines during healthy aging (***Figure 1***) (8).

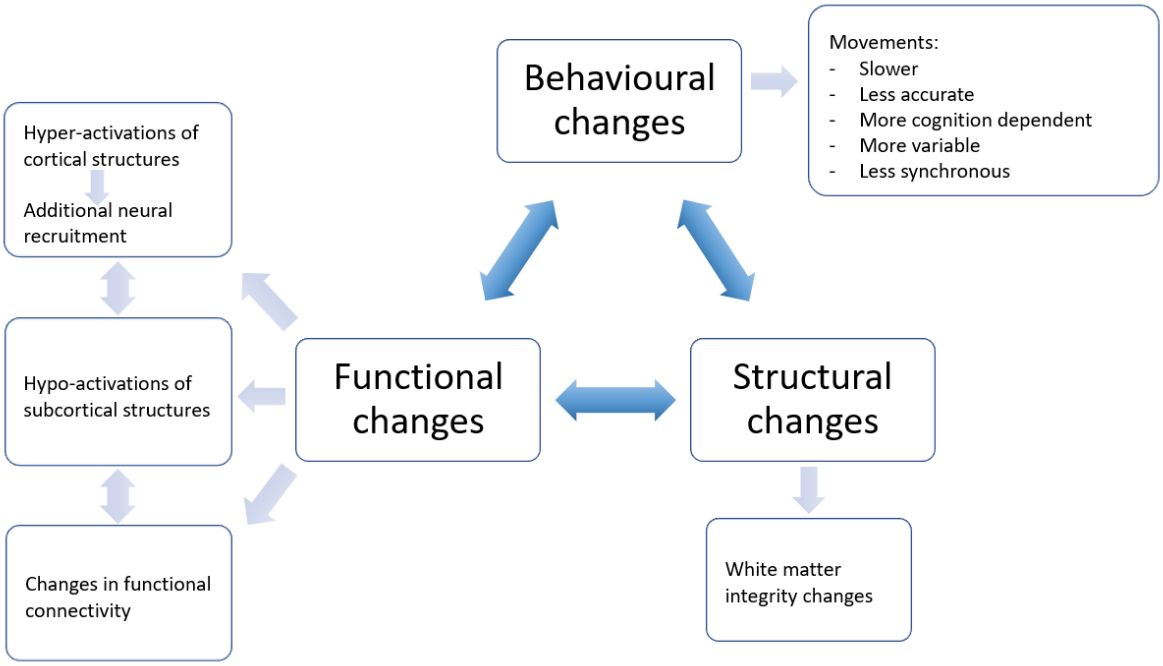

Figure 1 *– An overview of the effects of aging. The effects of aging can be divided into three categories, namely the behavioural, structural and functional changes. However, these categories are interconnected, and together, they may explain what is happening in aging. With respect to* ***behavioural changes****, movements will become slower and/or less accurate, more variable, less synchronous and more dependent on cognition. This is associated witch age-related hyper-activations of cortical structures which may be linked to hypo-activations of subcortical structures (****functional changes****). The hyper-activations demonstrate the recruitment of additional neural networks. In addition, the functional connectivity between the different networks is also changing, which might be associated with the loss of white matter integrity (****structural changes****).*

4.1. Bimanual coordination

Bimanual tasks are classified into **three categories**, namely discrete, serial or continuous actions which can be further subdivided based on the complexity and difficulty of the task (5). **Discrete bimanual actions** are tasks with a clear beginning and end and are subdivided into: (1) **nonrepetitive bimanual actions** which are movements performed in isolation such as the reaction time task; and (2) **repetitive discrete bimanual actions** involving multiple movements performed one after another until arbitrarily stopped, for example the finger tapping task (5). If multiple actions are performed in series, where the order is important, this is called **serial bimanual coordination** (5). The finger sequencing task is an example of this category. The last category, **the continuous bimanual actions**, involve the simultaneous movements or force applications that are repeated over time without a pause in between repetitions (5). The bimanual tracking task (BTT) of this study belongs to the last category.

The human body has a large number of degrees of freedom that are influenced by constraints (9). These constraints can be **concrete** (*musculoskeletal origin*: the restricted range of motion offered by the mechanical configuration of muscles and joints) or **abstract** (*neural origin*: the difficulty experienced when combining simple movements into complex rhythms) (10). However, all the constraints on coordination are mediated by the central nervous system (10).

The bimanual movements performed in daily life consist of **preferred movements** and **nonpreferred movements**. The **preferred movements** are the default coordination modes, such as **in-phase** (i.e., simultaneous timing of homologues muscle activation, or 0° phase offset) and **anti-phase** (i.e., alternated timing of activation of homologues muscles, or 180° phase offset) (***Figure 2***)(5). Accordingly, these coordination modes can be considered basic collaborations constituting the basis for the development of new and/or less preferred coordination modes (5). The **nonpreferred movements**, on the other hand, include out-of-phase coordination (e.g. 90° phase offset) (2, 5, 6). **Out-of-phase coordination** is less frequently observed during typical daily tasks (5).

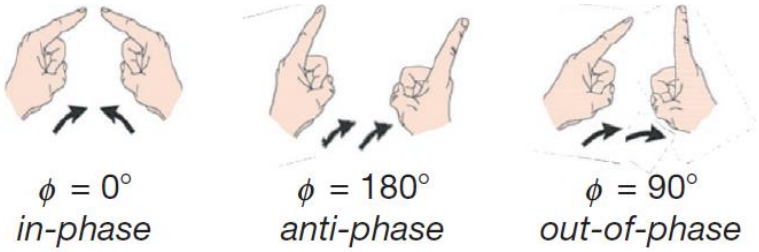

Figure 2 *– The phases of bimanual coordination with in-phase and anti-phase as the preferred movements and out-of-phase as the nonpreferred movement (2). Φ is the phase difference between limbs at continuous or discrete time points (2).*

Next to the coordination modes, there are two other parameters that determine the complexity of the bimanual tasks: the temporal parameters and the spatial parameters. As for the **temporal parameters**, the preferred movements consist of the simple rhythms (e.g., 1:1) in which the frequency of one limb motion is the same frequency of the other limp (isofrequency). The non-preferred movements (non-isofrequencies) also consist of simply rhythms, as well as polyrhythms. However, in the simple rhythm (harmonic frequency movements, e.g., 1:2 or 1:3) the frequency of one hand is an integer multiple of the other; the polyrhythms (e.g., 3:2 or 5:3) consist of non-integer ratios (2, 6). The **spatial parameters** refer to the amplitude and/or direction of the movements in which there is also a tendency to move in the same amplitude and direction (i.e., isodirectionality) (2).

Motor learning is the process by which movements are executed more quickly and accurately through repeated practice (2, 5, 11, 12). It relies on the integrity and functional interaction between the cortico-striatal and cortico-cerebellar systems (13). The process can be divided into **three stages** of which the duration is highly task specific: (1) **the initial stage** which is defined as **fast learning**, is the activation of a smaller area of activation (habituation) and is related to a slow performance; (2) **the intermediate stage** reflects sleep-dependent motor memory **consolidation** and includes skill stabilisation and improvement of a recently acquired fragile memory which is transformed into a stabile form (gradual learning); (3) and **the advanced stage** in which a larger area of activation (enhancement) was discovered which defines **slow learning** (14, 15). Maintaining the skill over time, results in **long-term retention** which strongly depends on successful **consolidation** (15). During the initial stage, the cerebellum is active. However, this activity decreases with practice and a shift from the cerebellar-cortical network to the striatal-cortical network occurs which makes the activity in the cerebellum undetectable (13, 16). Suggesting that the cerebellum set up a procedurally acquired sequence of movements which is then maintained elsewhere in the brain (16).

4.2. Aging

Aging affects the execution of bimanual movements, especially during more complex tasks (6). It is known that bimanual coordination already starts declining from **the age of 40** and a more elaborate network is recruited (17-19). This extended network is observed on the cerebral level, as well as in the cerebellum (1). The dynamic bimanual coordination network of brain areas may expand into (pre)frontal, parieto-occipital, and temporal areas and the insular cortex depending on **internal** (expertise level, age, and pathology) and **external factors** (environmental information, and task difficulty and complexity) (2).

Age-related **hyper-activations** are seen in several regions in the cortical cortex such as in the SMA, DLPFC, inferior frontal gyrus inferior parietal cortex, secondary somatosensory cortex (SII), and cingulate cortex (5). This may reflect increased demands on sensory processing and the penetration of cognition into action which is able to compensate to a certain extent but fails when task demands become higher (5). Two hypotheses of aging are proposed to explain these hyper-activations (5). The **dedifferentiation hypothesis** links the decrease in functional accuracy during task performance in elderly to the reduced neural distinctiveness, so, it is associated with unwanted activation spreading (5). While the **compensation hypothesis** states that the recruitment of additional brain areas is a compensatory mechanism for functional and/or structural deficits in these or other more scattered brain areas and is associated with a better performance and learning gain (5). However, the mechanism of aging may be a combination of the two hypotheses, in which the additional recruitment of brain areas may be a consequence of the reduced inhibitory processes (5). These hyper-activations are probably a more general indication of aging, and not specifically related to bimanual coordination (***Figure 1***) (5).

Changes in **FC** also correlate with aging (***Figure 1)***(5). To match the motor performance of the young people in bimanual coordination, the elderly need an higher FC (20). There are three potential causal factors formulated which together may be able to explain the age-related FC changes, including: (1) the **loss of white matter integrity**; (2) **dopaminergic deficits**; and (3) **amyloid deposition** (8). By manipulating the complexity (spatiotemporal) and difficulty (frequencies) of the bimanual coordination tasks, the effects of aging can be made distinguishable (5).

It is proposed that the cerebellum is more rapidly affected by aging than other cerebral structures (21). Andersen et al. (2003) have shown that especially **the anterior 'motor' cerebellum** structurally changed more with aging in comparison with the posterior cerebellum (22). A reduced or less efficient input of the cerebellum may increase the workload on the cerebrum which may cause a switch from predictive to reactive movement and therefore, a delayed motor function in the elderly (17, 18).

4.3. Transcranial direct current stimulation (tDCS) and the cerebellum

The **cerebellum** is strongly connected via polysynaptic circuits and closed parallel loops to the motor and associative regions in the cerebral cortex, and is linked to error correction, motor learning, complex movements, and attention; all of which are strongly involved in bimanual coordination (1, 23, 24). However, little is still known about the specific role of the cerebellum in bimanual coordination and in neurological aging processes (25). Yet, the cerebellar region is the strongest predictor of bimanual coordination performance in subjects with an age 60 to 80, together with the primary sensorimotor cortex (19). Because of the anatomical structure of the cerebellum, its electrical properties, and its participation in numerous closed-loop circuits involved in motor, cognitive and affective operations, the **cerebellum** poses an interesting target for non-invasive brain stimulation such as **transcranial direct current stimulation (tDCS)** (26, 27).

tDCS is a simple, robust and non-invasive brain stimulation technique that mainly acts on neurons to modulate cortical excitability and brain activity which will enhance both motor and cognitive functions (23). It causes a polarity-dependent physiological change in the neurons both intra- and extracellular which lasts for a few hours depending on intensity and duration of the stimulation. The change will induce a shift in the resting membrane potential which modifies neuronal synaptic efficiency (21, 23, 28). While the shift is not adequate to induce action potentials, it is capable to change the threshold for discharge of stimulated neurons (21, 28). **The effects of tDCS** depend on the preceding neuronal physiological state and the orientation of the structure relative to the electric field direction (23). Importantly, the direction of the current determines if the soma will hyperpolarise or depolarise. In general, anodal tDCS increases neuronal excitability which will enhance behavioural performance because of depolarisation of the membrane potential of the soma, while cathodal tDCS will decrease neuronal excitability because of hyperpolarisation of the soma (28). However, the neurons of the cerebellum follow a complex anatomical distributions over the numerous folia; some compartments will be as such hyperpolarised, while simultaneously others will be depolarised (23). One of the advantages of tDCS is that the mobility of the patient is unaffected during the stimulation (23). Modelling studies have shown that there is only a slight spread of electricity to other structures, such as the brainstem and the heart (23). Because tDCS will be applied to the cerebellum, it will be called **cerebellar transcranial direct current stimulation (cbtDCS)**.

4.4. Aim of the study

The aim of this pilot study is to achieve more clarity about the role of the different networks in the bimanual coordination in both the young and the elderly and whether modulation with cbtDCS improves bimanual coordination. It is a pilot study to eventually develop a more focussed application of non-invasive neuromodulation. The **hypothesis** states that modulating the cerebello-cerebral network with cbtDCS will improve bimanual coordination in the elderly. This study consists of two major parts.

The **meta-analysis** of functional MRI studies is the first part and aims to determine the cerebral and cerebellar activations and their functional networks that are involved in bimanual coordination and which are

specific for bimanual as compared to unimanual coordination. The Activation Likelihood Estimate (ALE) method by GingerALE will be used to determine the convergence of foci.

The second part is the experiment where the subjects perform the **BTT** while they are monitored with **EEG** and receive an **intervention**, either sham or real stimulation (cbtDCS). The aim is to determine the effect of cbtDCS on the young people and the elderly, and to identify the difference between the young people and the elderly. The experiment is divided into two sessions (session A and session B) consisting of seven blocks; in the first session, the subject either receives sham or real cbtDCS, and in the second session, the other one.

4.5. Relevance

Since the **functional independence** is related to the quality of life of the elderly, maintaining this independence poses a critical challenge for the health and well-being of the elderly (5). Bimanual skills constitute a critical marker of, and co-determines functional independence across the lifespan, so, a research effort towards improvement of our knowledge about bimanual coordination is needed (5).

In addition, bimanual coordination is a complex process since both hands have to move in an organised way in both space and time which requires more brain structures and more effort from neuronal systems to perform these movements (5). This may explain why it is more susceptible for neurodegeneration (5).

By stimulating the cerebello-cerebral networks through the cerebellum, the aim is to improve performance during the BTT and eventually, improve bimanual coordination in daily life. Thus, the information gathered in this study may enhance the quality of life of the elderly and may be used in pathological situations such as stroke, Alzheimer's disease and Parkinson's disease.

5. Materials and methods

5.1. Meta-analysis

A **meta-analysis** included functional magnetic resonance imaging (fMRI) studies with a focus on bimanual or unimanual coordination and without a focus on a learning effect. **fMRI** is a technique that measures brain activation by detecting small differences between oxygen-poor and oxygen-rich blood flow (2). By performing a meta-analysis of fMRI studies with ALE, cerebellar activations and cerebello-cerebral networks activated during the BTT can be identified when they show a consistent response across experiments (29).

It is important to know that clusters in this context refer to the assembly of activations within regions in the cerebellar and cerebral cortex and the networks refer to the functional networks the collection of activations belong to determine with the use of a specific atlas which will be discussed later.

5.1.1. Activation likelihood estimation (ALE) method

ALE is an objective, quantitative technique for coordinate-based meta-analysis of neuroimaging results (30). **GingerALE** is an automated method used for performing meta-analysis of human brain imaging studies. The software generates a whole-brain map of ALE values which is an estimate of the likelihood that at least one of the foci in a dataset was truly located at a given voxel (30). It starts with the generation of a text file with the cerebellar foci reported in each study within bimanual coordination and the number of subjects included in the study. The foci are reported in **Montreal Neurological Institute (MNI)** space, so they were converted to MNI if needed. The subject information is needed to calculate the Full-Width Half-Maximum (FWHM) of the Gaussian function used to blur the foci. The analysis yielded several images and spreadsheets of which the thresholded image was the most important as well as the cluster spreadsheet with the information about the clusters' results.

To identify the clusters that were more consistently activated in bimanual coordination, the **single contrast analysis** was used. The thresholding algorithm, the **cluster-level interference**, thresholds the data using a cluster-forming threshold. In this algorithm, GingerALE finds the clusters above the threshold and tracks the distribution of their volume. As cluster-level inference, 0.05 was used with 5000 permutations, an FDR pID of 0.001 and a minimum cluster size of 100 mm^3 analogue to the study of Stoodley (31). No minimum cluster size was set for the clusters of the cerebellum.

The thresholded images of the bimanual and unimanual data and the pooled data (the combination of the two datasets) obtained from the single study analysis are the input image for the **contrast analysis**. For the analysis, the thresholding algorithm, FDR pID, was 0.05, the p value permutations were set on 5000 and the minimum volume of the cluster was also 100 mm^3. The analysis compares and contrasts the two datasets of interest and examines them for statistically significant differences in association.

In the clusters' results, the coordinates of the peak of the maximum ALE value in the cluster were found; together with the general brain map and the Buckner atlas, they were entered in **Mango**, a viewer program, so

the functional network of the cluster could be determined. The Buckner atlas is a map in which the cerebral and cerebellar cortex is parcellated into multiple functional networks based on resting state fMRI data. The map of the brain is organised into either seven networks, which was used here, or 17 networks which is a more specific division of the seven networks (***Appendix 9.5***).

For the clusters revealed in the analysis, a **confidence interval (CI)** was computed, however, it was only available for the clusters in the cerebrum. It is a value between minus one and plus one; the closer the value is to one, the higher the confidence of the spatial location belonging to its designated network is (32).

5.2. Bimanual tracking task (BTT)

5.2.1. Study design

This study is a double-blind sham-controlled cross over design in which both the researchers as the subjects were blinded by keeping the electrodes on the head after ramping down the current to obtain the impression of comparable session lengths (23). To evaluate if the blinding was effective, the subjects were asked if they received sham or real stimulation.

The **inclusion criteria** of the study were (1) right-handedness (evaluated by *Edinburgh Handedness Inventory* (33)) and (2) an age between 18 and 30 years or an age between 65 and 77 years. The **exclusion criteria** were (1) neurologic or psychiatric conditions, (2) pregnancy, (3) a physical condition that makes it impossible to perform the BTT, and (4) contra-indications for cbtDCS. For each subject, a screening questionnaire for transcranial electrical stimulation (tES) was used to assess these contra-indications (***Appendix 10.1***) (34).

After inclusion, the following safety measurements were implemented when cbtDCS was applied: (1) the impedance was continuously monitored and documented every five minutes, and the stimulation was stopped when impedance was too high; (2) if there were skin lesions present under the electrodes or if the impedance remained too high, subjects were excluded. Before the onset of the experiment, equipment was thoroughly checked for deficiencies. In addition, the data of the EEG will be examined by a neurologist. If there are abnormalities in the recordings, the neurologist will notify the subject.

For the double-blind sham-controlled randomised study, 28 subjects were recruited, 14 young people (μ_{age} = 21y) and 14 elderly (μ_{age} = 71,1y). The names of the subjects are linked to a number in a document to which only the local researchers have access. In that document, the version of the session was documented, age, date of birth, gender, and the score of the Edinburgh Handedness inventory (***Table 12***, ***Appendix 9.1***). Originally, there were 16 young participants and 15 elderly, however, two young participants dropped out, one due to technical problems and the other one was not able to participate anymore; one of the elderly was excluded because the tES revealed a contra-indication for tDCS.

The study was approved by the ethical committee of UHasselt and an informed consent was presented to every subject before the experiment.

5.2.2. The experiment

In the **BTT**, the subjects sat on a chair watching the screen with both forearms on the table (***Figure 3***). The index finger of each hand controlled the controller (***Figure 3)***. By turning the controllers, the subject controlled the cursor on the screen. The right and left hand controlled the X- and Y-axis, respectively (***Figure 3***).

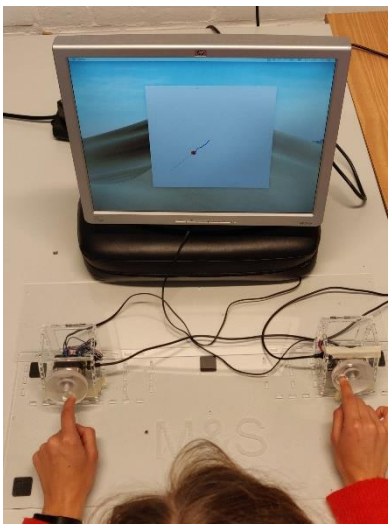

Figure 3 *– The set-up of the BTT. The index finger of each hand controlled the controller and so, moved the cursor on the screen. The right hand controlled the x-axis, while y-axis was controlled by the left hand. The forearms rested on the table*

The target line appeared on the screen, and 2s after appearing, an auditory signal indicated that the subject had to start moving the cursor as close to the target as possible, which moved at a constant pace over the target line. One trial lasted 7s in total of which the first 2s was the preparation period before the auditory signal and 5s were the time the participant had to move across the target line. The trajectory made by the cursor was drawn as a blue line (visual feedback). There were 100ms between the end of the previous line and the next one. The target line could occur in four different quadrants (Q's); quadrant 2 (Q2) and quadrant 4 (Q4) were the in-phase coordination mode, quadrant 1 (Q1) and quadrant 3 (Q3) were the anti-phase coordination mode (***Figure 4***). In the in-phase movements, the participants initially used homologous muscles; in the anti-phase movements, the participant initially used non-homologous muscles. Within each quadrant, there were three frequency ratios: 1:1, 1:3 and 3:1 (***Figure 4***).

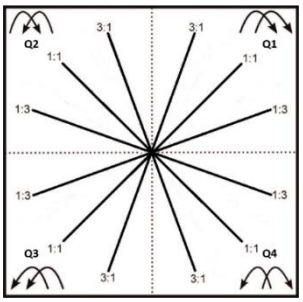

Figure 4 *– A picture of the quadrants in which Q2 and Q4 represent the in-phase coordination mode and Q1 and Q3 the anti-phase coordination mode. The 1:3 ratio, for example, means that the left hand have to turn three times faster than the right hand; this was linked to the slope of the line: so, the steeper the line, the faster the left hand should have turned.*

The experiment consisted of two sessions with a washout period of at least two weeks (***Figure 5***). Each session consisted of seven blocks; depending on the coordination mode of the block, three conditions were either performed in Q2 and Q4 or in Q1 and Q3 which gave six combinations (or six tasks, quadrant x frequency ratio) that were each repeated six times in a row, giving a total of 36 trials per block. Within each block, the sequence of the combinations was randomised. Because there were an uneven number of blocks, two versions were made; one with four blocks in-phase and one with four blocks anti-phase. The order of the two versions was counterbalanced: one week, the subject received one of the two versions, while in the other week, he received the other version.

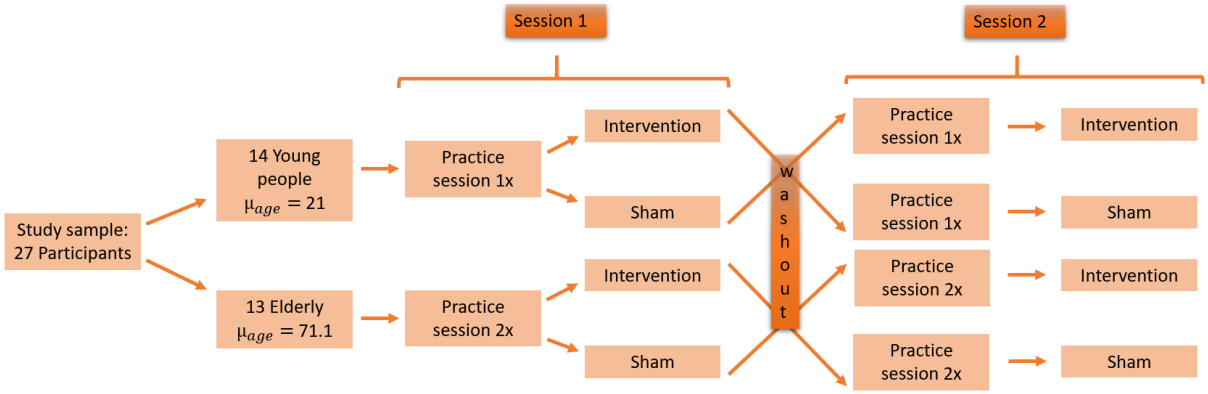

Figure 5 *– A simplified overview of the study design of the BTT. The experiment consists of two sessions beginning with a practice session. A washout period of two weeks is needed to eliminate the prolonged effects of the intervention. The sessions include seven blocks with 36 trials each. The intervention is here defined as the real stimulation.*

Block 1 and 2 and block 6 and 7 were a combination of the BTT and the EEG measurements (***Table 1***). Whether the subject received sham or cbtDCS in block 3, 4 and 5 depended on a code installed on the cbtDCS device by an independent researcher and was randomised within each age group. Before and after a session, the brain activity of the subjects was measured with EEG for two minutes while they were at rest. Before each session, the subjects received a training session with all the combinations. In the training sessions, the line was repeated three times instead of six. The elderly received the training session twice because more difficulties were observed with the BTT than observed in the young people.

Table 1 *– The experiment starts with EEG recordings in rest, followed by the blocks with EEG recordings during the BTT and the three blocks with an intervention (sham or real), ending with the blocks with the combination of the BTT and EEG recordings and the EEG in rest. While in version one the in-phase mode (occurs four times and anti-phase three times, this changes in the second version where the in-phase mode occurs three times and the anti-phase four times. Every block consists of six combinations that are repeated six times, so 36 combinations in total. Fr: frequency, Bl: block, EEG: electroencephalography, and V: version.*

		EEG			**Sham (session A)** **Stimulation (session B)**			**EEG**		
			Bl 1	**Bl 2**	**Bl 3**	**Bl 4**	**Bl 5**	**Bl 6**	**Bl 7**	
Fr 1 (1:1) **Fr 2 (1:3)** **Fr 3 (3:1)**	**V1**	Rest	In-phase	Anti-phase	In-phase	Anti-phase	In-phase	Anti-phase	In-phase	Rest
	V2		Anti-phase	In-phase	Anti-phase	In-phase	Anti-phase	In-phase	Anti-phase	

5.2.3. Electroencephalography (EEG)

Electroencephalography (EEG) records electrical potentials, which are associated with the neuronal activity of the brain, between two recording sites (35). It records signals associated with the performance of motor, cognitive and affective tasks (35). The **BioSemi ActiveTwo system** was used as EEG recording system. This system includes a common mode sense (CMS) electrode and a driven right leg (DRL) electrode and gives cleaner EEG recordings by using the **common mode rejection**. Eye-related or muscle artefacts were removed by five **external electrodes** placed on the face on specific places (in the outer corner of the right and left eye, beneath the left eye, in the inner corner above the left eyebrow, and on the right cheek).

During the baseline condition (rest) and block 1 – 2 and 6 – 7, the brain activity was measured with 32 electrodes according to the **10-20 EEG-system**. This is a standardised system in which the electrodes are evenly distributed over the scalp.

5.2.4. Cerebellar transcranial direct current stimulation (cbtDCS)

cbtDCS is a technique that is able to enhance both motor and cognitive functions by modulating the excitability (23). The position of the electrodes determines the direction of the current flow and the orientation of the electric field (23). To target the whole cerebellum, an anode is used with a surface area of 5x7cm^2. The first electrode, the anode or active electrode, is centred horizontally on the median line over the whole cerebellum, approximately 1-2cm under the inion and with the lateral borders about 1cm medially to the bilateral mastoid apophysis (36). The second electrode, also with a surface area of 5x7cm^2, the cathode or indifferent electrode, is placed over the right deltoid muscle. The other safety concerns related to the use of cbtDCS are not mentioned here (***Appendix 10.2***). The device used was from the company neuroConn.

The **stimulation session** used a current intensity of 2mA with a ramp up of 30s and a ramp down of 30s and had a duration of 20 minutes. The impedance was monitored continuously throughout the stimulation. This current intensity was needed to establish an interaction with the cerebellar neurons and because of the amount of shunting accompanying the cerebellar cbtDCS setup (23). The current density was 0.057 mA/cm^2. In the **sham procedure**, the subject received a current density of 2mA for 1 minute with a ramp up of 30s and a ramp down of 30s. To effectively blind the subjects, the ramping up of the current intensity had to last at least 30s because the sensations of turning on the current, faded out in the first 30s; ramping down the stimulation did not elicit perceivable sensations (23).

5.2.5. Behavioural data

The outcome measures of the BTT or the behavioural data consisted of the **average trace deviation (Avg Trace Dev)** which was the average deviation from the pre-drawn trace, the **average target deviation (Avg Target Dev)** which was the average deviation from the target and **movement stability (MS)** which was calculated as the deviation from the regression line of the participant's trajectory. All the data from the BTT was collected in one document. Since the blocks of interest were block 1 – 2 (before intervention) and block 6 – 7 (after intervention),

the first trial of each task from these blocks were removed. With a script written in MATLAB, all the trials where the participant's data points were in the wrong quadrant for more than 40 percent, were isolated and shown. The conditions these trials had to meet to be preserved, included: the participant had to correct himself and if so, the end of the trajectory had to travel across half of the target's trajectory on the x- and y-axis. If the trial did not meet the conditions, it was removed from the analysis.

5.2.6. Excluded trials

If a lot of trials had to be removed from one participant, the percentage of the preserved trials was calculated; if less than 80% of the trials were preserved, the subject was not included in further analysis. The only participant excluded, was one elderly. So, 13 elderly remained in the analysis of the behavioural data, compared to 14 young people. From all the subjects from which trials were removed, the total errors per condition, before and after stimulation/sham and for each session are calculated; this will be reported in the results. The percentage of the errors per condition, before and after stimulation and per session in these blocks was calculated (***Equation 1***). Otherwise, the same was calculated for the sham/real stimulation (***Equation 2***).

Equation 1 *– The equations used to calculate the errors made during the BTT for each frequency ratio, block 1-2 and block 6-7, and per session.*

$$Frequency\ ratio = \frac{Errors\ made\ in\ frequency\ condition}{Total\ number\ of\ trials\ in\ that\ frequency\ condition}$$

$$Before\ and\ after = \frac{Errors\ made\ before\ or\ after\ stimulation}{Total\ number\ of\ trials\ before\ and\ after\ intervention}$$

$$Session\ = \frac{Errors\ made\ in\ session\ 1\ or\ 2}{Total\ number\ of\ trials\ block\ 1,2,6\ and\ 7\ in\ session\ 1\ or\ 2}$$

Equation 2 *– The equations used to calculate the errors made during the BTT for each frequency ratio, block 1-2 and block 6-7, and for sham/real stimulation.*

$$Frequency\ ratio = \frac{Errors\ made\ in\ frequency\ condition}{Total\ number\ of\ trials\ in\ that\ frequency\ condition}$$

$$Before\ and\ after = \frac{Errors\ made\ before\ or\ after\ stimulation}{Total\ number\ of\ trials\ before\ and\ after\ stimulation}$$

$$sham/real\ = \frac{Errors\ made\ in\ session\ 1\ or\ 2}{Total\ number\ of\ trials\ block\ 1,2,6\ and\ 7\ in\ sham\ or\ in\ real}$$

5.2.7. Linear mixed model

The average of the Avg trace dev, Avg target dev and MS was calculated for each participant, for each session, for block 1, 2, 6 and 7, for each quadrant combination (Q1 – Q3 and Q2 – Q4), and for each frequency ratio (1:1, 1:3, and 3:1). Next, the average of block 1 – 2 (before intervention) and 6 – 7 (after intervention) was calculated. This average was used for the **linear mixed model** which is a parametric linear model that quantifies the relationships between a continuous dependent variable (Avg Trace Dev, Avg Target Dev and MS) and

categorical independent variables (37). In this model, the equation accommodates multiple predictor variables (**Equation 3**).

Equation 3 – The general equation for the linear mixed model.

$$Y' = a + b_1X_1 + b_2X_2 + b_3X_3 + \cdots + b_kX_k + cZ$$

Y' is the predicted value for the dependent variable, *a* is a regression constant, b_1, b_2, b_3 through b_k are regression coefficients for each independent variable (cbtDCS, Session, Time and Age) and cZ is the random effect of the subjects (**Equation 3**)(38). The variable 'subject' was added as a random effect to account for the repeated measures within one subject. Once regression coefficients and a constant are obtained, the values of Y' can be predicted by substituting values for each independent variable in the equation (**Equation 3**). For categorial independent variables, the coefficients will be substituted by zero or one depending on the group of interest. Regression coefficients are interpreted as values that identify how much each variable contributes to the explanation of Y'. The model included both the individual coefficients, the two-way-interactions, the three-way-interactions and the four-way interactions.

A scatter plot was made for each dependent variable in which the predicted values are plotted on the x-axis against the residuals that were calculated in an earlier step on the y-axis. If the assumption of normality or homoscedasticity in the data was violated, the y-data was transformed using the 'Box-Cox transformation'. Next, the residuals were calculated, and a scatter plot of the transformed data was made and if the assumptions were met, the transformed data was used to build the model. A test of significance, the t-test, was performed on each regression coefficient. If the coefficient was not significant, the variable did not make a significant contribution to the prediction of the independent variable. The interactions or the coefficients with the highest p-value were first removed from the model. First the four-way interactions were evaluated, then the three-way, the two-way and at last, the coefficients. This was repeated for each insignificant interaction and coefficient, until the model could no longer be simplified. If a coefficient was found in a significant interaction, it remained in the model, even when it was not significant on its own. In addition, cbtDCS remained into the model, even when it was not significant because it was the main variable of interest. Again, a scatter plot was made from all the residuals. If the assumptions of normality or homoscedasticity in the data were met, a Tukey post-hoc test was performed for the significant interactions. This post-hoc test determines whether there is a difference between the mean of all possible pairs within a significant interaction using a studentised range distribution (38). The significance of the difference was obtained, the non-transformed data were plotted, and the final mixed model was established with the transformed dependent variables.

6. Results

The overall experiment consisted of two different parts. First, a meta-analysis was performed on fMRI studies with the ALE method to acquire more knowledge about the cerebellar activations and their cerebello-cerebral networks involved in bimanual coordination. This topic was divided into two separate ones: bimanual coordination and the contrast of bimanual coordination and unimanual coordination. Next, a BTT was performed with 14 young people and 14 elderly to evaluate their initial bimanual performance and the effect of cbtDCS on their performance of both age groups. A part of these results was only reported such as the excluded trials, while the other part, the behavioural data, was analysed with the linear mixed model.

6.1. Meta-analysis

The aim of the meta-analysis was to identify the cerebellar activations and their cerebello-cerebral networks related to bimanual coordination. The meta-analysis included fMRI studies that used either bimanual or unimanual coordination tasks performed with the right hand and contrasted against a control condition or a rest condition and excluded experiments with motor learning (***Appendix 9.4***). The analysis revealed the clusters that were most consistently activated across studies and the coordinates of the cluster's activation peak centre. In the tables the peak coordinates are given together with the area the peak is located in and the areas that are also included in the cluster.

6.1.1. Bimanual coordination

In the cerebrum, there were 12 clusters that were consistently activated across studies; however, because of the minimum cluster size of 100 m^3, only 7 clusters remained: the left precentral gyrus (BA4), the right and left globus pallidus externus, the medial dorsal nucleus of the thalamus, the right precentral gyrus (BA6), the left medial frontal gyrus and the right postcentral gyrus (***Figure 6*** and ***Table 2***). In the cerebellum, five clusters were identified, located in the right anterior cerebellum, the left anterior cerebellum (culmen), the left anterior cerebellum (dentate), and in the left posterior cerebellum (decline)(***Figure 7*** and ***Table 3***). No cut-off was set for the cerebellum. The clusters belong to the somatomotor network, dorsal and ventral attention and the executive network (***Table 2*** and ***Table 3***). Since, this study focuses on bimanual coordination, the sole results of the analysis of the unimanual data are not reported here (***Table 16***, ***Appendix 9.6***).

Table 2 *– The cluster that were consistently activated in fMRI studies with bimanual coordination for the cerebrum and are arranged according to size. The areas belonging to the cluster are reported below the peak coordinate area. A.= anterior, BA = Brodmann area, Ex.= externus, In.= internus, L.= left, P.=posterior, R.=right, VPM= ventral posteromedial, and VPL= ventral posterolateral.*

Bimanual coordination: Cerebrum					
Region of cluster	**Peak centre**			**Function**	**Confidence interval**
	X	**Y**	**Z**		
Precentral gyrus L. (BA4)	-37.7	-14.5	55.8	Somatomotor	
Postcentral gyrus 3					
Globus pallidus Ex. R.	25.5	-6.6	3		
Putamen					
Globus pallidus In.					
Globus pallidus Ex. L.	-22.3	-7.6	1.3		
Globus pallidus Ex.					
Putamen					
Globus pallidus In.					
Thalamus R. – medial dorsal nucleus	15	-19.9	6.4		
Mamillary body					
Pulvinar					
VPM nucleus					
VPL nucleus					
Lateral posterior nucleus					
Precentral gyrus R. (BA6)	-33.3	-11	61.6	Somatomotor	
Precentral gyrus (BA4)					
Medial frontal gyrus (BA6)					
Medial frontal gyrus L. (BA6)	-1.8	-8.8	54.8	Somatomotor	
Postcentral gyrus R. (BA3)	34.1	-29.2	59.2	Somatomotor	
Postcentral gyrus (BA40)					

Table 3 – *The cluster that were consistently activated in fMRI studies with bimanual coordination for the cerebellum and are arranged according to size. The areas belonging to the cluster are reported below the peak coordinate area. A.= anterior, BA = Brodmann area, Ex.= externus, In.= internus, L.= left, P.=posterior, R.=right, VPM= ventral posteromedial, and VPL= ventral posterolateral.*

Bimanual coordination: Cerebellum					
Region of cluster	**Peak centre**			**Function**	**Confidence interval**
	X	**Y**	**Z**		
Cerebellum R. A.	29.5	-60.6	-30.3	Executive	0.38247
Cerebellum L. A. Culmen (lobule V)	-17.7	-52.8	-22.8	Somatomotor	0.50963
Cerebellum L. A. Dentate	-20.8	-60.4	-29	Dorsal attention	0.05318
Cerebellum R. A.	24.3	-53.6	-25.7	Somatomotor	0.1509
Cerebellum L. P. Declive (lobule VI)	-2	-69	-18	Ventral attention	0.38664

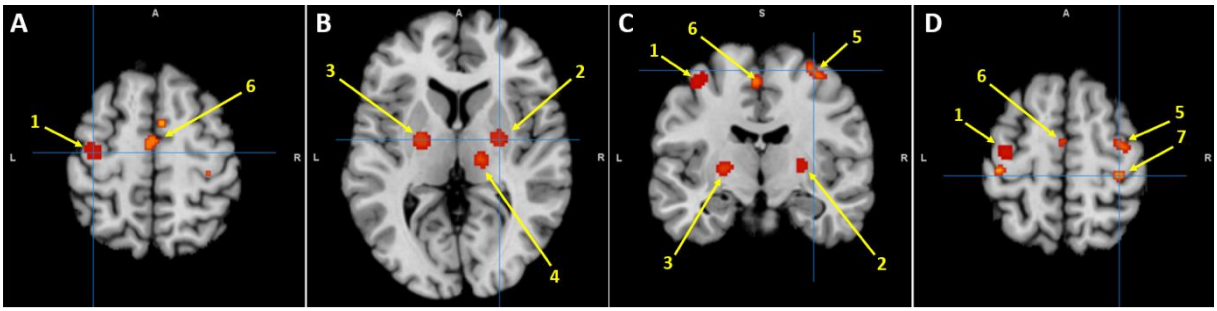

Figure 6 *– A figure with all the clusters of the analysis of the bimanual data. (A) axial slice (z =56), (B) axial slice (z = 3), (C) coronal slice (y = -11), and (D) axial slice (z = 59). The clusters are numbered according to their size: (1) Precentral gyrus (BA4 left), (2) Globus pallidus externus right, (3) Globus pallidus externus left, (4) Thalamus – medial dorsal nucleus, (5) Precentral gyrus (BA6 and 4) right, (6) Medial frontal gyrus (BA6), and (7) Postcentral gyrus (BA3) right.*

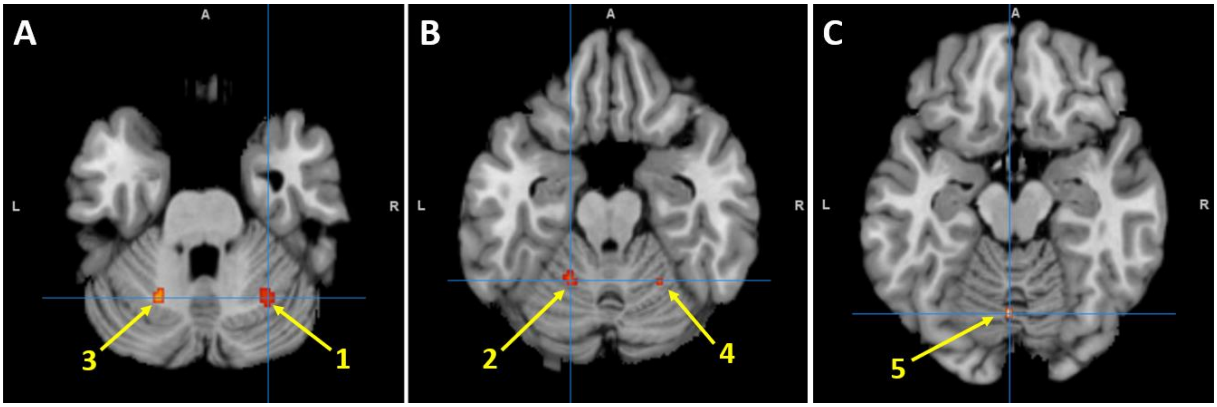

Figure 7 *– A figure with all the clusters in the cerebellum of the analysis of the bimanual data. (A) Axial slice (z = -30), (B) axial slice (z = -23), and (C) axial slice (z = -18). The clusters are numbered according to their size: (1) right anterior cerebellum, (2) left anterior cerebellum – culmen, (3) left anterior cerebellum – dentate nucleus, (4) right anterior cerebellum, and (5) left posterior cerebellum – declive.*

6.1.2. Bimanual coordination vs. Unimanual coordination

The contrast analysis yielded three kinds of data: bimanual vs. unimanual coordination, unimanual vs. bimanual coordination, and conjugated results which view the regions that exist in both datasets (***Appendix 10.5***). Since the focus of this study is bimanual coordination, only the results of the bimanual vs. unimanual coordination contrast are of interest (***Table 17*** and ***Table 18, Appendix 9.6).*** Even though the contrast analysis is unlikely to have enough statistical power to show a significant difference with less than approximately 15 experiments in each data set, the analysis revealed four clusters in the cerebrum and one cluster in the cerebellum that were more consistently activated in bimanual coordination compared to unimanual coordination (***Table 4***). The data of the bimanual and unimanual data viewed next to each other for comparison is not reported here (***Table 19*** and ***Appendix 9.6***).

In this contrast, the clusters that were most consistently activated in bimanual coordination in comparison with unimanual coordination include the right globus pallidus, the right precentral gyrus, the left putamen and the right thalamus; in the cerebellum, only the left anterior cerebellum was revealed (***Figure 8***, ***Figure 9*** and ***Table 4***). All these cluster belong to the somatomotor network.

Table 4 *– The clusters that were more consistently activated in bimanual coordination than in unimanual coordination and are arranged with the largest cluster first. The first part represents both the most consistently activated clusters in the cerebrum, while the second part only views those in the cerebellum. The areas belonging to the cluster are reported below the peak coordinate area. A.= anterior, BA = Brodmann area, Ex.= externus, In.= internus, L.= left, P.=posterior, R.=right, VPM= ventral posteromedial, and VPL= ventral posterolateral.*

Bimanual coordination vs. Unimanual coordination

Region of cluster	Peak centre			Function	Confidence interval
	X	Y	Z		
Globus pallidus Ex. R.	25.6	-7.2	3.5		
Putamen					
Thalamus – Ventral lateral nucleus					
Precentral gyrus R. (BA6)	34.6	-10.8	60.2	Somatomotor	
Precentral gyrus (BA4)					
Medial frontal gyrus (BA6)					
Putamen L.	-22.2	-7.4	2		
Globus pallidus Ex.					
Globus pallidus In.					
Thalamus – Ventral lateral nucleus					
Thalamus R.	15.2	-19.5	6.7		
Mamillary body					
Thalamus – VPM nucleus					
Thalamus – Medial dorsal nucleus					

	Pulvinar		
	Thalamus – VPL nucleus		
	Thalamus – lateral posterior nucleus		
	Thalamus – ventral lateral nucleus		

Cerebellum

Cerebellum L. A.	-14.3	-55.4	-22.1	Somatomotor	0.55219
Culmen (lobule V)					
Declive (lobule VI)					
Fastigum					
Dentate nucleus					
Declive (lobule VI)					
Cerebellar lingual (lobule I)					
Pyramis (lobule VIII)					
Nodule (lobule X)					

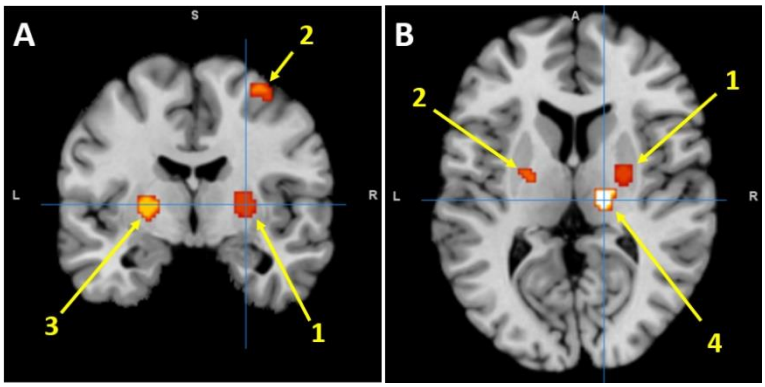

Figure 8 – *A figure with all the clusters of the clusters of the contrast of the bimanual data vs. unimanual data. (A) coronal slice (y = -7.2) and (B) axial slice (z = 6.7). The clusters are numbered according to their size: (1) right globus pallidus Externus, (2) right precentral gyrus (BA6), (3) left globus pallidus externus, and (4) right thalamus.*

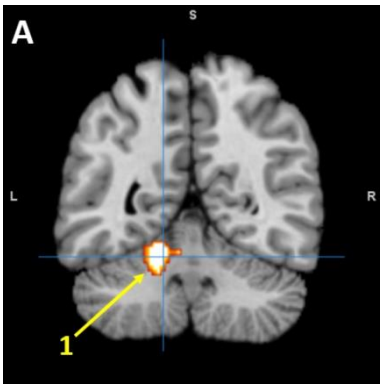

Figure 9 – *A figure of all the cerebellar clusters of the contrast analysis of the bimanual data vs. unimanual data. (A) coronal slice (y = -55.4). The clusters are numbered according to their size: (1) left anterior cerebellum.*

6.2. Bimanual tracking task (BTT)

The BTT was intended to evaluate the performance of the young people and the elderly. Both age groups had to complete the same task, only the elderly had four practice session instead of two like the younger age group. The aim was to identify a potential difference in the initial performance of young people and the elderly, to determine the effect of cbtDCS on the performance of both age groups and to detect the difference of the effect of cbtDCS between the younger age group and the elderly. The behavioural data obtained from the BTT was used to answer these aims by means of the linear mixed model. The results of the linear mixed model were divided into the three dependent variables: the Avg Trace Dev, the Avg Target Dev and the MS. In addition to these aims, the number of errors made during the BTT was reported.

6.2.1. Excluded trials

To report where most of the errors were made, the subjects that did not meet the given conditions were listed and by means of excel the percentage of errors within each frequency ratio, before and after stimulation and within each session was calculated and between sham/real stimulation. The subjects whom had trials excluded from the analysis belonged to both age groups with five young people and 12 elderly. In total, there were 16 subjects that had trials excluded. Since only block 1, 2, 6 and 7 are of interest, only the percentages of errors made in these four blocks are reported (***Table 5*** and ***Table 6***).

Although with a minimal dissimilarity, most of the errors were made in the 3:1 frequency ratio in session 1 after intervention. In session 2, on the other hand, most of them were made in the 1:3 frequency ratio. The percentage of errors decreased after stimulation with the lowest percentage of errors at the end of session 2. Finally, there was seen that the overall percentage of errors was higher in session 1 than in session 2. The detailed version of these results was not reported here (***Table 20, Table 21, Table 22*** *and* ***Table 23***, ***Appendix 10.6***). Together, it appeared that most of the errors were made in the first two blocks of session one in the 3:1 frequency ratio and less errors were made after the stimulation and in session 2.

Table 5 *– An overview of the percentage of errors made by the participants in block 1, 2, 6 and 7 in session 1. Fr. = frequency ratio.*

Session 1

		Before intervention (Block 1 and 2)			**After intervention (Block 6 and 7)**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	2.031%	1.562%	2.034%	0%	0.937%	2.031%
	Before/after	1.876%			0.990%		
	Session	1.433%					

Table 6 *– An overview of the percentage of errors made by the participants in block 1, 2, 6 and 7 in session 2. Fr. = frequency ratio.*

Session 2							
		Before intervention (Block 1 and 2)			**After intervention (Block 6 and 7)**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	0.625%	1.406%	1.25%	0.469%	0.626%	0.469%
	Before/after	1.093%			0.522%		
	Session	0.808%					

Otherwise, the percentages of the errors made per condition, before and after for sham and real tDCS were also calculated.

For the **sham**, in session 1, before intervention, the percentage of errors was highest in de 1:1 frequency ratio and the lowest in the 3:1 frequency ratio. However, the reverse was seen after the intervention. Within a session, the percentage of errors was declined after the intervention compared to before the intervention. Before the intervention in session 2, the same was seen as after intervention in session 1. Yet, after the intervention, most of the errors were made in the 1:3 frequency ratio, however, there was a rather small difference with the 1:1 frequency ratio and no errors were made in the 3:1 frequency ratio. Between sessions, the percentage of errors was decreased in session 2 compared to session 1 (***Table 7***).

Table 7 *– An overview of the percentage of errors made by the participants in block 1, 2, 6 and 7 for sham divided into the two sessions. Fr. = frequency ratio.*

Sham							
Session 1							
		Block 1 and 2			**Block 6 and 7**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	2.5%	2.187%	1.25%	0%	0.937%	1.875%
	Before/after	1.979%			0.937%		
	Session	1.458%					
Session 2							
		Block 1 and 2			**Block 6 and 7**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	1.25%	1.562%	2.5%	0.625%	0.631%	0%
	Before/after	1.771%			0.418%		
	Session	1.095%					
	Sham	1.277%					

In session 1 of the participants who received the **real stimulation**, frequency ratio 3:1 showed the highest percentage of error and the same was seen after the intervention. Within a session, the same was seen as for sham, with a decline or errors in session 2 compared to session 1. Before the intervention in session 2, no errors were made in the 1:1 and 3:1 frequency ratio, only in the 1:3 frequency ratio errors were made. After the stimulation, no errors were made in the 3:1 condition, and more errors seemed to be made in the 1:3 frequency ratio than in the 1:1 frequency ratio, however, the distinction was rather small. The percentage of errors was decreased in session 2 than in session 1 (***Table 8***).

Yet, the percentage of errors made in sham after intervention in session 2(0.937%) was higher than in the real stimulation after intervention in session 2 (0.418%) (***Table 7*** and ***Table 8***).

Because the effect of tDCS is part of the research aim, the difference in percentage of errors was calculated between after intervention in session 1 and before intervention in session 2. There was seen that a larger improvement is obtained by cbtDCS (-0.835%) than in sham (+0.84%)(*Table 7* and ***Table 8***).

Table 8 *– An overview of the percentage of errors made by the participants in block 1, 2, 6 and 7 for the real stimulation divided into the two sessions. Fr. = frequency ratio.*

cbtDCS

Session 1

		Block 1 and 2			**Block 6 and 7**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	1.562%	0.937%	2.821%	0.627%	0.937%	1.562%
	Before/after	1.772%			1.043%		
	Session	1.408%					

Session 2

		Block 1 and 2			**Block 6 and 7**		
		Fr. 1:1	**Fr. 1:3**	**Fr. 3:1**	**Fr. 1:1**	**Fr. 1:3**	**Fr. 3:1**
Total	**Condition**	0%	1.25%	0%	0.313%	0.625%	0%
	Before/after	0.208%			0.156%		
	Session	0.365%					
	cbtDCS	0.886%					

6.2.2. Linear Mixed Model

The linear mixed model was used to analyse the three different dependent variables, namely the Avg Trace Dev, Avg Target Dev and MS. These three variables will be described separately, starting with Avg Trace Dev.

The **Avg Trace Dev** is the average deviation of the subject from the trace. The final linear mixed model for the trans Avg Trace Dev included the four independent variables (cbtDCS, Time, Session and Age) and one interaction (Time x Session) (***Equation 4***).

$$Avg\ Trace\ Dev^{(\lambda)} = -6.66 + 0.0091\ [tDCS2] - 0.75\ [Time2] - 0.99\ [Session2] + 1.51\ [Age2] + 0.37\ [Time2{:}Session2]$$

Equation 4 *– The equation of the linear mixed model of the Avg Trace Dev where cbtDCS2 is the group receiving the real stimulation, Time2 are the results the group after the stimulation, Session2 is the session when the person came for the second time, Age2 are the elderly and Time2:Session2 is the interaction of those two groups.*

Table 9 *– The regression coefficients for each independent variable (b_z) and the value for the regression constant (a) together with their significance (p-value) for the Avg Trace Dev.*

Avg Trace Dev$^{(\lambda)}$	**Value (b_z)**	**p-value**
Intercept (a)	- 6.66	< 0.0001
cbtDCS	0.0091	0.9124
Time	- 0.75	< 0.0001
Session	- 0.99	< 0.0001
Age	1.51	0.0008
Time:Session	0.37	0.0238

The findings show that each independent variable had a significant contribution to the explanation of the predicted value for Avg Trace Dev, except cbtDCS (***Table 9***). Although cbtDCS was part of the research aim, his contribution seemed not to be significant (0.3873) (***Table 9***). A significant interaction between time and session was found (***Figure 10***).

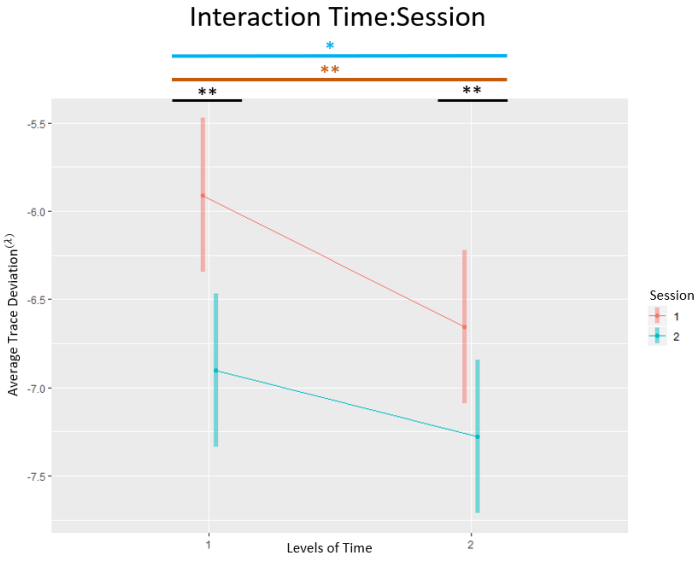

Figure 10 *– The interaction of Time and Session with the trans values of the Average Trace Deviation. The levels of time (before (1) and after intervention (2)) are shown on the x-axis, while session 1 is the orange line and session 2 is the blue line. The black line shows the significance for both session before and after stimulation (p = < 0.0001). While the orange line presents the significant difference between before and after stimulation in session 1(p = < 0.0001), the blue line shows the difference for session 2 (p = 0.0084).*

Before the intervention (1), a significant difference was seen between the two sessions (p-value = <0.0001; black bar, ***Figure 10***); the same was observed after the intervention (2)(p-value = <0.0001; black bar***, Figure 10***). This showed a significant improved Avg Trace Dev in session 2 compared to session 1 which suggested a learning effect after a consolidation period. In session 2, the Avg Trace Dev started higher before stimulation than after, however, it was not as high as at the end of session 1.

In addition, within a session, there was also a significant improvement of the Avg Trace Dev with a more significant difference in session 1 (p = <0.0001) than in session 2 (p = 0.0084), suggesting a larger improvement in session 1. The insignificant contribution of cbtDCS (p value = 0.3873) to the prediction of Avg Trace Dev suggested that this difference was due to a learning effect and not due to an effect of cbtDCS. The CIs showed the significance since the averages of each group were not included in the CIs of the other groups.

The equation of the **Avg Target Dev**, which is the average deviation from the target, did not show any interactions, only the independent variables (***Equation 5***).

$$Avg\ Target\ Dev^{(\lambda)} = -3.31 - 0.11\ [tDCS2] - 0.38\ [Time2] - 0.47\ [Session2] + 0.90\ [Age2]$$

Equation 5 *– The equation of the linear mixed model of the Avg Target Dev where cbtDCS2 is the group receiving the real stimulation, Time2 are the results the group after the stimulation, Session2 is the session when the person came for the second time, Age2 are the elderly and Time2:Session2 is the interaction of those two groups.*

Table 10 *– The regression coefficients for each independent variable (bz) and the value for the regression constant (a) together with their significance (p-value) for the Avg Target Dev.*

Avg Target Dev$^{(\lambda)}$	**Value (b_z)**	**p-value**
Intercept (a)	- 3.31	< 0.0001
cbtDCS	- 0.11	0.0408
Time	- 0.38	< 0.0001
Session	- 0.47	< 0.0001
Age	0.90	0.0053

The p-values suggested that each independent variable showed a significant effect on the predicted value of the dependent variable (***Table 10***). In contrast to the Avg Trace Dev, cbtDCS also had a significant contribution (p value = 0.0408) (***Table 10***).

The **MS** is a value for the average deviation from a line drawn through the line of the participant. The linear mixed model for this variable also included the independent variables only, without any interactions (***Equation 6***).

$$MS^{(\lambda)} = -5.64 + 0.01\ [tDCS2] - 0.16\ [Time2] - 0.22\ [Session2] + 0.72\ [Age2]$$

Equation 6 *– The equation of the linear mixed model of the Avg Target Dev where cbtDCS2 is the group receiving the real stimulation, Time2 are the results the group after the stimulation, Session2 is the session when the person came for the second time, Age2 are the elderly and Time2:Session2 is the interaction of those two groups.*

All the variables showed a significant contribution, expect for cbtDCS (p value = 0.5761) (***Table 11***).

Table 11 *– The regression coefficients for each independent variable (bz) and the value for the regression constant (a) together with their significance (p-value) for the Motion Stability.*

$MS^{(\lambda)}$	Value (b_z)	p-value
Intercept (a)	- 5.64	< 0.0001
cbtDCS	0.01	0.8137
Time	- 0.16	0.0050
Session	- 0.22	0.0001
Age	0.72	0.0004

To see what an effect of tDCS could be, the four-way interactions were plotted for the three dependent variables (***Figure 11***). Although these interactions did not show a significant contribution, it is showed because of the significant effect of cbtDCS in Avg Target Dev and because an effect of cbtDCS on consolidation was expected.

There could be seen that the participants who received the real stimulation (cbtDCS2) in the first session and sham in session 2, had a better performance and a larger improvement than the reverse situation, especially in MS and for the elderly in Avg Trace Dev (***Figure 11***). In the reverse situation, so sham in session 1 and the real stimulation is session 2, the trials showed approximately the same trend across the two sessions (***Figure 11***).

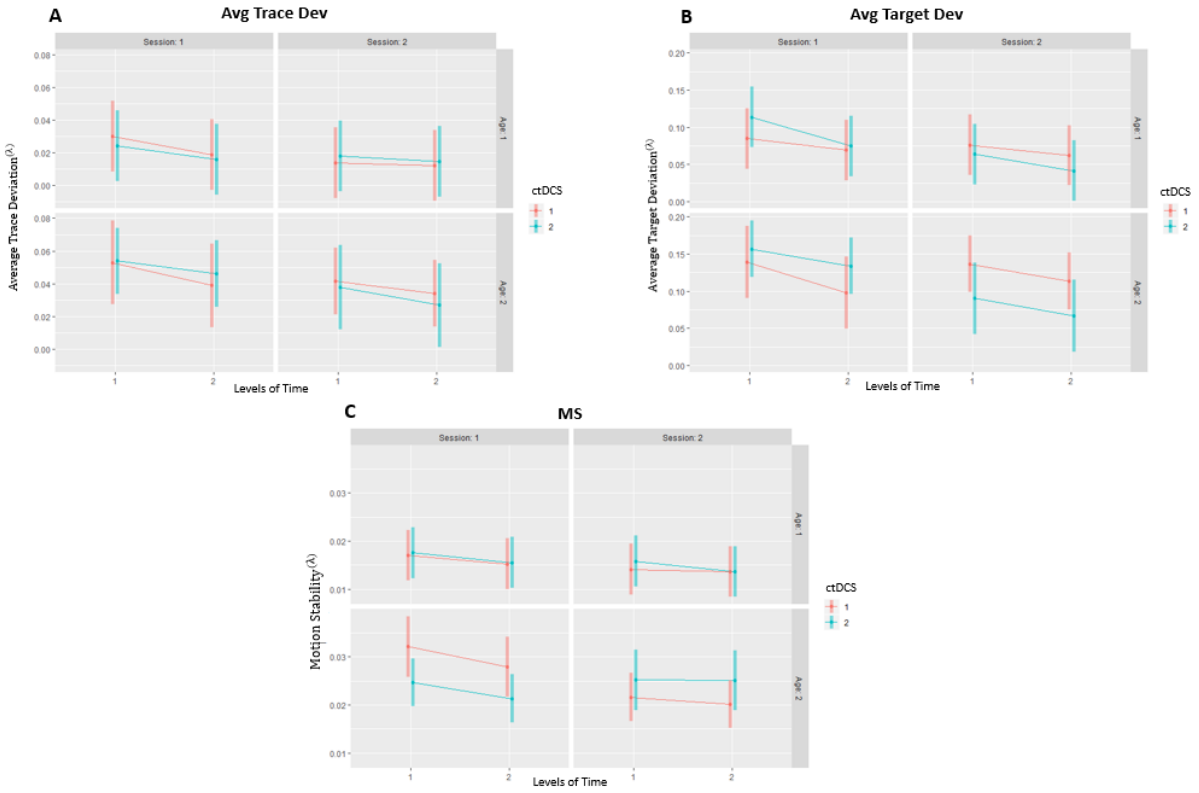

Figure 11 – *The three four-way interactions with before (1) or after intervention (2) on the x-axis, and on the y-axis the (A) Avg Trace Dev, (B) Avg Target Dev), and (C) MS. The x-axis is divided into the two sessions and the y-axis in the two age groups. Time level 1 is before the intervention and time level two is after the intervention. cbtDCS 1 is sham and cbtDCS 2 is the real stimulation. The transformed values of the variables are used.*

Only Avg Trace Dev shows an interaction (Time and Session) which suggested a learning effect within a session and between sessions. However, cbtDCS showed no significant contribution to the prediction of the value of the dependent variables with an exception for Avg Target Dev. Yet, all the other variables (Time, Session and Age) seemed to have an effect.

7. Discussion

7.1. Meta-analysis

The **cluster-level analysis** of the data from the fMRI studies about bimanual coordination reveals seven clusters that are consistently activated across the studies included. These clusters might be involved in bimanual coordination and are located in both cortical and subcortical structures: the left precentral gyrus (BA4), the left and right globus pallidus externus, the right thalamus, the right precentral gyrus (BA6), the left medial frontal gyrus and the right postcentral gyrus (BA3). In the **cerebellum** there were five cluster: the right anterior cerebellum, the left anterior cerebellum (culmen), the left anterior cerebellum (dentate), the right anterior cerebellum and the left posterior cerebellum (declive).

The **contrast analysis** revealed that the following clusters are specific for bimanual coordination as compared to right unimanual coordination: the right globus pallidus externus, the right precentral gyrus (BA6), the left putamen and the right thalamus. In the **cerebellum** there was only one cluster: the left anterior cerebellum.

In general, all the clusters were located in either the somatomotor network, the executive network, the ventral and dorsal network. The most important clusters and their role in bimanual coordination will be discussed below according to their network.

7.1.1. Bimanual coordination

The **precentral gyrus** (BA4) is the largest cluster of the analysis of the bimanual data and is located in the somatomotor network. It represents the primary motor cortex which provides precise control of movements (39). Together with the parietal regions, the area is required for the accurate performance of a sensorimotor task (40). While the precentral regions focus on a likely movement (motor intention), the parietal regions encompass a range of potential responses (motor preparation and motor inhibition)(40).

Also belonging to the somatomotor network is the medial frontal gyrus and the postcentral gyrus. The **medial frontal gyrus** is located rostral to the precentral gyrus and between the superior and the inferior frontal sulci (41). It is related to the ability to generate voluntary goal-directed behaviour which concerns controlling ongoing actions and performance outcomes, and subsequent adjusting behaviour and learning (42). It is located in the prefrontal cortex which plays an important role in cognitive control (43). The **postcentral gyrus** is part of the parietal lob and is located immediately posterior to the central sulcus and corresponds to the primary somatosensory cortex (44, 45). Damage to the gyrus may produce a loss of proprioception which is used to prepare for movement and to provide information regarding movement errors (39).

The precentral gyrus belongs to the **motor loop** that also consists of the premotor cortex, the putamen, the globus pallidus externus and internus and the motor areas of the thalamus. Since, the structures are involved in

the motor loop, they are directly related to movement execution. The motor loop manages the muscle contraction, muscle force, multi-joint movements, and sequencing of movements (39, 46).

The **globus pallidus** is one of the nuclei of the basal ganglia which had a crucial role in the control of voluntary movement and consist of two sections: the internus and the externus (39). The **globus pallidus internus (GPi)** is part of the **Go pathway** which disinhibits the motor thalamus and facilitates specific movements (39). In this pathway, the putamen inhibits the GPi which in turn, provides less inhibition to the motor thalamus that signals the motor areas in the cerebral cortex such as the precentral cortex, to activate specific corticospinal neurons (39). The **globus pallidus externus (GPe)**, however, is associated with the **No-Go pathway** which suppresses unwanted movements (39). The pathway also begins in the putamen, in different cells than the Go way (39). The putamen inhibits the GPe which provides less inhibition to the subthalamic nucleus that excites the GPi, leading to an increased inhibitory GPi output to the motor thalamus and less activity in motor areas of the cerebral cortex (39).

The **putamen** is involved in movement and learning (49). It may be associated with the initiation phase of bimanual movements and with the synchronisation of the specific contributions of the cortical motor area and prefrontal areas (49).

The basal ganglia, the cerebellum and almost all sensory systems transmit information to the **thalamus** which process the information and relays the selected information to specific sites of the cerebral cortex (39). The two nuclei of the thalamus that were most consistently activated across studies are the medial dorsal nucleus and the ventral posterolateral nucleus (VPL). The **VPL** mediates the spatiotemporal organisation of movement and transmits thalamus input to the M1 or the precentral gyrus, which monitors parameters of voluntary movements (46). The **mediodorsal nucleus** has a versatile role in higher cognitive functions together with the prefrontal cortex and other cortical and subcortical brain areas (47). It is required for the fast and accurate execution of cognitive tasks and temporally increases the efficiency of cortical networks involving the prefrontal cortex (47). The ventrolateral and ventromedial nuclear complex of the thalamus can be acknowledged as the main motor thalamic relay to the cerebral cortex (48). Via the motor thalamus, voluntary muscle activity is regulated by the motor loop (39).

The thalamus also belongs to the **cerebro-cerebello-cerebral loop** linking the cerebral cortex and the lateral cerebellar cortex which also includes the dentate nucleus of the cerebellum (39). The closed loop is part of the cerebrocerebellum which is a specialised region of the cerebellum coordinating precise, distal voluntary movements and functioning in the coordination of voluntary movements, planning of movements, timing and some cognitive functions (39). In conclusion, the thalamus has both a motor-coordinating function as an executive function since its involvement in the motor loop.

The clusters of the **cerebellum** belong either to the executive, the somatomotor or the dorsal and ventral attention network. While the *dorsal attention network* guides voluntary allocation of attention to locations of

features, the *ventral attention network* is involved in detecting unattended or unexpected stimuli and triggering shifts of attention (50). The activity of the ventral attention network increases when cues are presented which instruct where the attention should be directed to (50). The cerebellum is an important motor structure of the brain controlling both motor-related functions and cognition (51). However, there are no direct connections between the cerebellum and the motor neurons; it does not directly influence muscle activity (39). It influences muscle activity through connections with the motor cortex, premotor cortex and the brainstem (39). It represents a critical site for the control, organisation and execution of the bimanual task (1, 46). It also seems to have a role in motor timing and error correction, but it does not appear to be exclusively motor-related, since it is also activated in perceptual timing (1, 46). Indirect connections exist between the cerebellum and (pre)motor and associative areas via projections through the thalamus (46, 52). The cerebellum has two hemispheres consisting of the anterior (lobules I – V), posterior (lobules VI – IX), and flocculonodular (lobule X) lobes which are subdivided into ten lobules (I – X) (51). While the anterior lobe is generally engaged in motor control, the posterior lobe is associated with cognitive processes and includes the **declive** (51). Mediolaterally, the cerebellum can be divided into three functional regions, including the vermis, the paravermis and the lateral hemispheres (39). The cerebrocerebellum is the functional name for the lateral hemispheres which consists of most of the **dentate nucleus** (39, 51). While the anterior portion of the dentate nucleus is involved in motor control, the posterolateral portion is associated with motor planning, language production and cognitive processes (51). Alterations in the activity of the dentate nucleus precedes changes in activity in motor areas of the cerebral cortex (39). With respect to motor timing, the cerebellum integrates sensory input to time and correct the movement (52).

In **bimanual coordination**, the left precentral gyrus (BA4), the left and right globus pallidus externus, the right thalamus, the right precentral gyrus (BA6), the left medial frontal gyrus and the right postcentral gyrus (BA3), the right anterior cerebellum, the left anterior cerebellum (culmen), the left anterior cerebellum (dentate nucleus), the right anterior cerebellum and the left posterior cerebellum (declive) were consistently activated across studies. Koeneke et al (2004) also detected other clusters in their fMRI study including the intraparietal sulcus/superior parietal lobe, the inferior parietal lobe, the inferior temporal gyrus, the medial occipital gyrus, the precuneus, the superior frontal gyrus and the inferior frontal gyrus (3). However, the postcentral gyrus, the precentral gyrus, the medial frontal gyrus and the cerebellum did confirm (3). This may be explained by the difference in analysis; while this study did an analysis of multiple studies to determine the most consistently activated clusters, Koeneke et al (2004) analysed only their data (3).

7.1.2. Bimanual coordination vs. unimanual coordination

In the **contrast analysis**, the right GPe, the right precentral gyrus (BA6), the left putamen, the right thalamus, and the left anterior cerebellum were consistently more activated in bimanual coordination. This is in contrast with the study of Koeneke et al (2004). They did not detect any significant voxels in the bimanual condition as compared to the unimanual condition (3). The subjects had to perform a motor task with either two fingers of

one hand or with one finger of both hands (3). It is possible that their unimanual task was more complex than their bimanual task, resulting in more cognitive activations in the unimanual condition than in the bimanual condition.

Although the medial dorsal nucleus was not found in the contrast, the cluster was consistently activated in bimanual coordination. Yet, the **mediodorsal nucleus** of the thalamus is not detected in the results of the analysis of the unimanual coordination. This may be explained by the versatile role of the medial dorsal nucleus in higher cognitive functions which may be required by bimanual coordination (47).

7.1.3. Limitations

There are some limitations related to the first part of the experiment, the meta-analysis, because only the peak activation coordinates are entered into the analysis (31). The size of the clusters and the level of statistical significance of the clusters seen on the fMRI are not taken into account (31). However, the weakness is addressed by treating the foci as the centres of a probability distribution (31). In addition, most studies included healthy volunteers not older than an age of 30.

7.1.4. Summary

The results were as expected with activations in the motor and coordination regions of the cerebrum and the cerebellum, and with the recruitment of regions related to cognition. The executive network was only found in de cerebellar regions, as well as the dorsal and ventral attention network.

In the **cortical regions**, only the somatomotor network was detected. Seven cortical clusters were consistently activated in bimanual coordination and five cortical clusters were more activated in bimanual coordination compared to unimanual coordination. In the **cerebellum**, bimanual coordination activated five clusters, and one cerebellar cluster was more frequently activated in bimanual coordination compared to unimanual coordination.

Coupled bimanual coordination is mainly controlled from the dominant hemisphere and since almost all the studies of the meta-analysis included righthanded people, this corresponds to the largest cluster of bimanual coordination, the left precentral gyrus. This area is part of the motor loop, as well as the GPe, the largest cluster for bimanual coordination as compared to unimanual coordination. GPe is directly related to movement execution by suppressing unwanted movements. This is as expected since bimanual movements may require more coordination than unimanual actions.

7.2. Bimanual tracking task (BTT)

7.2.1. Excluded trials

The trials of subjects that did not meet the conditions to be included were removed from the analysis and documented. The frequency ratio of the trial was also documented, as well as the session and the moment (before or after stimulation). From this information, the percentage of errors was calculated with excel to report in which conditions the errors were made.

More errors were made by the elderly compared to the younger participants which may be due to the effects on aging on the movements, accuracy, and synchronicity (5); while only trials of 5 young people did not meet the conditions, there were trials of 12 elderly that were excluded.

While the 3:1 frequency ratio still belongs to the simple rhythms, it is more complex than the 1:1 frequency ratio and more errors were made in this frequency ratio than in the other frequency ratio in session 1 (2). Older adults may experience reduced interhemispheric inhibition which may induce the appearance of mirror movements that are also observed in young children where the CC is not fully formed until 6-8 years of age; since 1:1 frequency ratio is mostly a mirror movement, it may explain why less errors are made in this frequency ratio (53). Since all the participants were right-handed, the control of complex motor actions resides in the dominant left hemisphere (54). While the dominant hand, the right hand in this study, can perform movements more smoothly and is more resistant to perturbations, the left hand relies more on impedance mechanisms, that are less energetically efficient, to adapt a novel task dynamic (54). This may be due to a less effective contribution from the right hemisphere and more input has to be transferred from the left hemisphere through the carpus callosum which may be delayed due to age-related declines in CC size and integrity which both contribute to declines in bimanual performance (5, 7, 54). The size of the CC is also important in the young participants because it might predict the ability to perform and learn bimanual coordination patterns (6). Although, there might be interindividual variability across the younger participants, their CC is still intact; so, once they were familiarised with the task, less errors were made. In addition, they made initially less errors than the elderly.

Further, a decline in errors made was seen before and after stimulation and between sessions with more errors before stimulation than after stimulation and less errors in session 2 than in session 1. This may be due to a learning effect or due to an effect of cbtDCS which cannot be determined with the percentages calculated alone. Most of the errors were made in session 1 before stimulation where the participants executed the task for the first time after the practice sessions. After the stimulation, the percentage of errors dropped when the participants are familiarised to the task. In session two, the percentage is slightly higher than after the stimulation in session one, yet, not as high as in the beginning of the experiment. A reason could be that even though the participants know how to execute the task, they have to refresh their memory. After the stimulation in session two, the percentage is the lowest.

Because the reason of improvement between sessions cannot be determined from the percentages calculated alone, a separation was between sham and real stimulation. As expected, the percentage of errors was higher in the sham condition than when stimulated. In addition, a larger improvement seems to be seen if cbtDCS was applied in the first session than when it was applied in session 2. This is as expected since the cerebellum is especially active during the initial learning phase (13, 16).

7.2.2. Behavioural data

The behavioural data was used to assess the performance of the two age groups by the means of the Avg Trace Dev, the Avg Target Dev and MS. These variables were used to determine a potential difference between the young people and the elderly and whether cbtDCS can improve the performance. A linear mixed model was used to determine the influence of time (block 1-2 and block 6-7), age (young people and the elderly), cbtDCS (sham and real) and session (1 and 2) on the dependent variables.

The only interaction found was between time and session for the Avg Trace Dev with a poorer performance in session 1 than in session 2, as well as in the first two blocks compared to the last. All the independent variables had a significant effect on the three variables, except for cbtDCS which only showed an effect for Avg Target Dev.

The interaction of time and session for Avg Trace Dev showed a significant contribution to the prediction of the variable (p-value = 0.0230) because the improvement of performance was more robust in session one compared to session 2.Since cbtDCS had no significant contribution to Avg Trace Dev, these results imply a learning effect. Though no statistical analysis was performed on the data from the excluded trials, the results of the behavioural data corresponds to the data of the excluded trials.

cbtDCS seems to have no significant contribution to the linear mixed models, except for Avg Target Dev (p-value = 0.0408). However, since the variable was not found in a significant interaction, it is not clear what the effect is. The cerebellum is active in the initial phase of motor learning, so cbtDCS may show more effect in session 1 before the consolidation period (16). So, although not significant, cbtDCS may rather show an effect on the consolidation period, more specifically, on the rate of motor learning, than on the immediate effect on the performance. This is also seen in the four-way interaction that were plotted to see a possible effect of cbtDCS since it was significant for Avg Target Dev and in the reported percentages of errors for sham and the real stimulation. Another study has also seen the same effect of cbtDCS when they were testing the effect of cbtDCS on associative memory (55). They showed that cbtDCS led to an improved performance in older adults when tested after a delay, so after consolidation, yet, no immediate performance gains were seen (56). Although no distinction was made between the two age groups and it was only speculative, the percentage of errors between sham and cbtDCS and the visualisation of the four-way interactions support this with a larger improvement between the sessions if cbtDCS was applied in session 1.

Because of the strong connection of the cerebellum with the motor and associative regions of the cerebellum, an effect of cbtDCS was expected (1, 23, 24). Also, because the cerebellum is linked to error

correction, motor learning and complex movements (1, 23, 24). There was hypothesised that cbtDCS would enhance bimanual coordination skills, especially in the elderly because the cerebellum posed the strongest predictor of bimanual performance in individuals above the age of 60 (19, 57). Yet, no effect of cbtDCS was seen it this study. It was also expected that cbtDCS would have an influence on the performance of a frequency ratio, since the effects of aging may become more announced in more complex situations as the 3:1 frequency ratio (5). To see these influences, an interaction between cbtDCS and age was expected as well as between cbtDCS and condition.

The lack of significant interactions or an significant effect of cbtDCS could be due to insufficient statistical power due to the small sample size; because of the low sample size (27 participants) true differences between the two age groups or between sham and the real stimulation might be missed (38). It may also be due to external sources such as the learning effect which we did not control for (38). There were also social threats such as pressure(38). Another reason could be the ceiling effect which states that a patient who still functions well in basic activities of the daily life such as bimanual movements may show no improvement (38). Since only healthy individuals were included, this could explain the lack of effect of cbtDCS (57). In addition, the task may not be complex enough, since the effects of aging are more pronounced at higher levels of complexity and/or difficulty and the effects of cbtDCS vary with task demands (5, 6, 57). Next, the spatial orientation of neurons differs across persons and in the cerebellum compared to the cerebrum (23). These differences need to be taken into account in order to understand the effects of cbtDCS because the cerebellum has a complex neural distribution (23, 57). At last, it is speculated that the locus of neural coupling allowing bilateral interactions between the limbs depends on the structure of the movement task; while the CC is mostly involved in continuous tasks, the cerebellum is particularly involved in discrete bimanual actions and since the BTT belongs to continuous bimanual actions, which could be a reason no effect of cbtDCS is seen (58).

For these reasons, a larger sample size is needed to observe differences that are not visible in this set up. In addition, a more complex task is suggested to enlarge the differences between the two age groups. Together, this may make the effect of cbtDCS and the influence of age more distinguishable. Further, the addition of EEG measurements before, during and after the stimulation with cbtDCS may clarify the effect of cbtDCS since cbtDCS modulates brain activity and this can be measured with EEG which can also identify the specific brain responses to the stimulation (35). It can also make a distinction between the effect of cbtDCS and the learning effect. Nevertheless, there are other studies that failed to show significant effects of tDCS (23). Vancleef K. et al. applied tDCS over the left primary motor cortex and DLPFC and used nearly the same task as in this study (59). They found that the performance of the subjects improved, independent of tDCS (59). Another study who investigated the effects of tDCS on associative memory, also failed to provide evidence that tDCS have an effect on the performance of the elderly (55).

7.2.3. Limitations

Due to external delays, the pilot period was too short to solve the technical problems. The technical problems include complications with the EEG device which recorded a lot of noise because our lab was not equipped to filter out the activity of electrical devices in the building. Also, due to technical problems, the EEG data could not be included, and participants had to return, so less participants could be included.

7.2.4. Summary

The section of the excluded trial show that the results in session 1 are as expected with more errors made in the 3:1 frequency ratio because the performance may rely more on the communication from the right hemisphere through the CC which may encounter some delay (54). However, in session 2, more errors were made in the 1:3 frequency ratio which is the opposite of the literature. This may be due to a lack inhibition from the right hemisphere to the left hemisphere, so, the left hand follows the movements of the right hand or due to the small sample size. This is only a hypothesis, so, more research needs to be performed to support this. Also, the elderly made more errors than the younger people and less errors are made in session two which may be due to the learning process. At last, it is proposed that cbtDCS may rather have an influence of the initial motor learning and the rate of consolidation. Nonetheless, this is still speculative. More research needs to be performed to support these findings statistically.

The aim of the BTT was to reveal differences between the two age groups and the effect of cbtDCS on the performance. Except for Avg Target Dev, no effect of cbtDCS was seen. It seems that the improvement of both age groups was rather due to motor learning than due to stimulation of cbtDCS. Although, no proof could be provided that cbtDCS may have an effect on the consolidation, it is speculated that cbtDCS may not improve immediate performances but rather on performances after a delay.

7.3. Conclusion

The **meta-analysis** revealed the cerebral and cerebellar activations related to bimanual coordination and the functional network belonging to the area of activation. Activations were seen in motor and coordination regions of both the cerebrum and the cerebellum and also activations in regions related to cognition were shown. The largest cluster found in the bimanual coordination was the left precentral gyrus which is located in the dominant hemisphere which mainly controls bimanual coordination. The cluster represents the primary motor cortex providing precise control of movements and also belongs to the motor loop in which other clusters, such as the GPe, the putamen and the thalamus, participate. The involvement of the GPe which is directly related to movement execution by inhibition of unwanted movements was expected since bimanual coordination may require more control than unimanual actions.

The results of the **BTT** consisted of two different parts; one part reports the percentage of errors made by subjects from which trials were removed from the analysis and the other part assessed the performance of the young people and the elderly and the effect of cbtDCS. No significant effects of cbtDCS were seen, except for

Avg Target Dev, however, since it was not involved in an interaction, it is not clear which influence cbtDCS has. It is speculated that cbtDCS may rather have an influence on the consolidation than on the immediate performance. One significant interaction was found for Avg Trace Dev (Time:Session) which showed that a more robust improvement of performance was obtained in session 1 compared to session 2. This corresponds to the findings of the percentage of errors made. The percentage also seem to show that in the 3:1 frequency ratio, most of the errors were made in session 1.

In conclusion, it could not be demonstrated that modulation by cbtDCS could improve the performance in the elderly. However, this could be due to numerous reasons such as the sample size, the complexity and the structure of the movement task. It is proposed to repeat this experiment with a larger sample size, a more complex task and the addition of EEG measurements to make the difference between the two age groups more recognisable and to make a distinction between the effect of cbtDCS and the learning effect. It may also be interesting to perform the meta-analysis where the activations in the elderly are also considered because aging also influences interhemispheric interactions and the activity of neural recruitment (53).

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9. Appendix

9.1. Characteristics participants

Table 12 *– An overview of the characteristics with the numbers starting with "1" are the young people and the numbers starting with "2" are the elderly. Their age, gender and Edinburg score in percentage. The version the participants received per session are mentioned, as well as if they received sham or real tDCS.*

	Age (y)	**Gender**	**EHI (%)**	**Session 1**		**Session 2**	
				Version	**Sham/real tDCS**	**Version**	**Sham/real tDCS**
102	21	M	100	B	Real tDCS	A	Sham
103	28	M	70	A	Real tDCS	B	Sham
104	21	V	90	B	Sham	A	Real tDCS
105	21	V	100	A	Real tDCS	B	Sham
106	22	V	67	B	Sham	A	Real tDCS
107	18	V	100	A	Sham	B	Real tDCS
108	21	V	100	B	Sham	A	Real tDCS
109	21	V	100	A	Sham	B	Real tDCS
111	18	V	89	A	Real tDCS	B	Sham
112	22	M	80	B	Sham	A	Real tDCS
113	20	V	85	A	Real tDCS	B	Sham
114	22	V	75	B	Real tDCS	A	Sham
115	20	V	50	A	Sham	B	Real tDCS
116	19	M	71	B	Real tDCS	A	Sham
201	68	V	100	B	Real tDCS	A	Sham
202	67	M	80	A	Sham	B	Real tDCS
203	69	M	67	B	Sham	A	Real tDCS
204	70	V	100	A	Real tDCS	B	Sham
206	74	M	100	A	Sham	B	Real tDCS
207	67	M	100	B	Sham	A	Real tDCS
208	72	M	100	A	Real tDCS	B	Sham
209	70	V	100	B	Real tDCS	A	Sham
210	74	M	100	A	Real tDCS	B	Sham
211	75	V	100	B	Real tDCS	A	Sham
212	77	M	40	A	Real tDCS	B	Sham
213	67	V	100	B	Sham	A	Real tDCS
214	71	M	100	A	Real tDCS	B	Sham

9.2. tES Screening Questionnaire

Table 13 *– Screening questionnaire for transcranial electrical stimulation (tES) according to A. Antal (34).*

	Ja / *Yes*	Nee / *No*
Heeft u metalen (uitgezonderd titanium) of elektronische implantaten in de hersenen/schedel (bv. splinters, fragmenten, clips, cochleair implantaat, diepe hersenstimulatie, etc.)? Indien ja, specificeer het type metaal en de locatie: *Do you have metal (with an exception for titanium) or electronic implants in the brain/skull (for example, splinters, fragments, clips, cochlear implants, deep brain stimulation, etc.)? If yes, specify the type of metal and the location:*		
Heeft u metalen of elektronische implantaten in een ander deel van uw licaam (pacemaker, metalen fragmenten, etc.)? Indien ja, specificeer het apparaat en de locatie: *Do you have metal or electronic implants in another part of your body (pacemaker, metal fragments, etc.)? If yes, specify the device and the location:*		
Heeft u ooit chirurgische ingrepen gehad aan het hoofd of aan de ruggengraat? Indien ja, specificeer de locatie: *Have you ever had surgical procedures to the head or the spinal cord? If yes, specify the location:*		
Heeft u ooit een hoofdtrauma gehad waarna je het bewustzijn bent verloren? *Have you ever had a head trauma where you lost consciousness?*		
Heeft u huidproblemen zoals dermatitis, psoriasis of eczeem? Indien ja, specificeer de locatie: *Do you have skin problems such as dermatitis, psoriasis or eczema? If yes, specify the location:*		
Heeft u epilepsie of heeft u al stuiptrekkingen of een epileptisch insult gehad? *Do you have epilepsy, or have you ever had convulsions or an epileptic insult?*		
Heeft u last van appelflauwtes of syncopes? *Do you suffer from apple fainting or syncope?*		

Bent u zwanger of bestaat de kans dat u zwanger bent? *Are you pregnant, or is there a change that you are pregnant?*		
Neemt u medicatie? Indien ja, specificeer: *Do you take medication? If yes, specify:*		
Heeft u in het verleden al eens transcraniële magnetische of elektrische neurostimulatie gehad? Indien ja, had u toen ergens last van? Specificeer: *Have you had transcranial magnetic or electric neurostimulation in the past? If yes, did you suffer from something? Specify:*		

Een bevestigend antwoord op bovenstaande vragen is geen absolute contra-indicatie voor transcraniële neurostimulatie maar een herevaluatie van de risico's kan nodig zijn. Bij twijfel wordt er contact opgenomen met de arts-onderzoeker.

An affirmative answer to the questions above is not an absolute contra-indication to transcranial neurostimulation but a re-evaluation of the risks may be necessary. In case of doubt, the doctor-researcher is contacted.

Naam kandidaat: Handtekening en datum:

Name participant: *Signature and date:*

9.3. Recommendations regarding safety for application of cbtDCS in human

Table 14 *– Recommendations regarding the safety for application of cbtDCS in human according to Nitche et al., 2003 (60)*

Recommendations regarding safety for application of cbtDCS in human
Use of non-metallic, conductive rubber electrodes covered completely by saline-soaked sponges
Maximum current density of 0,02857 mA/cm^2
Maximum total charge of 0,022 C/cm^2
Wedge-shaped on and off-current switch
Avoiding electrode montages that might cause brainstem or heart nerve stimulation
Stimulation device delivering a constant current density
Caution for stimulation above foramina
Stimulation duration causing excitability changes >1h should be applied cautiously in healthy subjects
Long-term excitability changes should not be induced more than once a week

9.4. Articles used for the meta-analysis

Table 15 *– An overview of all the articles used for the meta-analysis.*

Author	Article title	Year	Task	Contrast	Number of subjects (range or mean age)
Bimanual coordination					
Y. Aramaki	Neural correlates of the spontaneous phase transition during bimanual coordination (1)	2005	Bimanual rhythmic finger-tapping task	Antiphase vs. rest Inphase vs. rest	15 (24y-31y)
N. Wenderoth	The role of anterior cingulate cortex and precuneus in the coordination of motor behaviour (2)	2005	Rhythmical line drawing and start drawing subtasks	Bimanual coordination vs. rest	10 (μ=25y)
N. Sadato	Role of the SMA and the right PMC in the coordination of bimanual finger movements (3)	1997	Sequential finger movement	Antiphase vs. rest Inphase vs. rest	12 (19y-25y)
			Bimanual abduction-adduction movements	Antiphase vs. rest Inphase vs. rest	9 (19y-25y)
S. Koeneke	Bimanual vs. unimanual coordination: what is the difference? (4)	2004	Motor coordination task	Bimanual coordination vs. rest	14 (μ=24.14y)
K. Müller	Perceptual influence on bimanual coordination: an fMRI study (5)	2009	Bimanual finger movement paradigm	Bimanual coordination vs. rest	11 (μ=35.5y)
F. Debaere	Changes in brain activation during the acquisition of a new bimanual coordination task (6)	2003	90° out-of-phase pattern	Bimanual coordination vs. rest	20 (21y-29y)
L. Jäncke	fMRI study of bimanual coordination (7)	2000	Bimanual rhythmic finger-tapping task	Bimanual coordination vs. rest	11 (22y-37y)

Unimanual coordination					
F. Debaere	Brain areas involved in interlimb coordination: a distributed network (8)	2001	Cyclical flexion-extension movement	Right wrist vs. rest	6 (21y-28y)
L. Jäncke	fMRI study of bimanual coordination (7)	2000	Rhythmic finger-tapping task	Right hand fast vs. rest	11 (22y-37y)
				Right hand slow vs. rest	
J.P. Kuhtz-buschbeck	Effector-independent representation of simple and complex imagined finger movements (9)	2003	Rhythmic finger-tapping task	Simple vs. rest	12 (21y-27y)
				Complex vs. rest	
S. Lehéricy	Motor control in basal ganglia circuits using fMRI and brain atlas approaches (10)	2006	Finger tapping sequence task	Simple vs. rest	12 (18y-33y)
				Scale vs. rest	
				Complex vs. rest	
E. Rounis	Frequency specific changes in regional cerebral blood flow and motor system connectivity following rTMS to the primary motor cortex (11)	2005	Finger tapping sequence task	Random sequence vs. rest	16 (20y-68y)
N. Sadato	Role of the SMA and the right PMC in the coordination of bimanual finger movements (3)	1997	Abduction-adduction movements	Unimanual coordination vs. rest	9 (22y-27y)
T. Aoki	The effect of tapping finger and mode differences on cortical and subcortical activities: a PET study (12)	2005	Single-finger tapping task	Index finger vs. rest	10 (20y-30y)
				Ring finger vs. rest	
			Double-finger tapping task	Index and ring finger vs. rest	
C. Calautti	Effects of age on brain activation during auditory-	2001	Thumb-to-index tapping task	Unimanual coordination vs.	10

	cued thumb-to-index opposition (13)			Rest in the elderly Unimanual coordination vs. Rest in young people	($\mu_{elderly}$ = 60.4y; μ_{young}= 24.4y)
M. Blinkenberg	Rate dependence of regional cerebral activation during performance of a repetitive motor task: a PET study (14)	1996	Finger tapping sequence task	Unimanual coordination vs. rest	8
M.J. Catalan	The functional neuroanatomy of simple and complex sequential finger movements: a PET study (15)	1998	Finger tapping sequence task	Repeated sequence vs. rest	13 (41y-64y)
R. Kawashima	Human cerebellum plays an important role in memory timed finger movement: an fMRI study (16)	2000	Finger tapping task	Memory timed finger movement vs. rest Visually cued finger movement vs. rest Silent articulation vs. rest	8 (19y-27y)
K. Lutz	Tapping movements according to regular and irregular visual timing signals investigated with fMRI (17)	2000	Finger tapping task	Regular visual stimulation Irregular visual stimulation	10 (21y-29y)
N. Sadato	Complexity affects cerebral blood flow change during sequential finger movements (18)	1996	Sequential finger movements	Unimanual coordination vs. rest	10 (20y-59y)
L. Jäncke	Cortical activation during paced finger-tapping	2000	Paced finger tapping task	Auditory synchronisation vs. rest	8 (20y-32y)

	applying visual and auditory pacing stimuli (19)			Visual synchronisation vs. rest	
C. Stoodley	Functional topography of the cerebellum for motor and cognitive tasks: an fMRI study (20)	2012	Finger tapping task	Unimanual coordination vs. rest	9 (μ=25.6y)
A. Riecker	Parametric analysis of rate-dependent hemodynamic response functions of cortical and subcortical brain strucures during auditorily cued finger tapping: an fMRI study (21)	2003	Auditory cued finger tapping task	Tapping 2Hz vs. rest	8
				Tapping 6Hz vs. rest	
T. Hanakawa	Motor planning, imagery and execution in the distributed motor network: a time-course study with fMRI (22)	2008	Finger tapping sequence task	IS-related activity vs. rest	13 (21y-48y)
				Imaginary-related activity vs. rest	
				Movement-related activity vs. rest	
R. Kawashima	A PET study of self-paced finger movements at different frequencies (23)	1999	Self-paced finger movements	Activation vs. rest	6 (18y-24y)
E. Gerardin	Partially overlapping neural networks for real and imagined hand movements (24)	2000	Extension/flexion fingers	Unimanual coordination vs. rest	8 (21y-35y)
H. Boecker	Role of the human rostral SMA and the basal ganglia in motor sequence control: investigations with PET (25)	1998	Finger tapping sequence task	Unimanual coordination vs. rest	7 (μ=32y)

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9.5. Buckner atlas

Colour codes of the functional networks

1	Visual network
2	Somatomotor network
3	Dorsal attention
4	Ventral attention
5	Limbic networks
6	Executive networks
7	Mentalizing/Default network

9.6. Unimanual, Bimanual vs. Unimanual, Unimanual vs. Bimanual and conjugated results

Table 16 *– The cluster that were most consistently activated in fMRI studies with unimanual coordination with subjects that are right-handed and are arranged with the largest cluster first. The first part represents both the most consistently activated clusters in the cerebrum and the cerebellum, while the second part only views those in the cerebellum. The results of the analysis of the unimanual data also includes multiple areas under one cluster that receives the name of the area belonging to the coordinates of the peak activation. The included areas are mentioned below the name of the area belonging to the coordinates of the peak activation. BA. = Brodmann Area, L.= left, R.=right, A.=anterior, and P.=posterior.*

Unimanual coordination

Cluster name	**Peak centre**			**Function**	**Confidence interval**
	X	**Y**	**Z**		
Postcentral gyrus (BA3/2)	-41.9	-20.2	55.7	Somatomotor	
Precentral gyrus (BA4)					
Inferior parietal lobule (BA40)					
Precentral gyrus (BA6)					
Postcentral gyrus (BA40)					
Postcentral gyrus (BA1)					
Postcentral gyrus (BA4)					
Precentral gyrus (BA4)					
Medial frontal gyrus (BA6)	-2.8	1.7	54.5	Ventral attention	
Cingulate gyrus (BA24)					
Paracentral lobule (BA31)					
Superior frontal gyrus (BA6)					
Cingulate gyrus (BA31)					
Medial frontal gyrus (BA32)					
Cingulate gyrus (BA32)					
Cerebellum R. A. Dentate	18	-54.2	-23	Somatomotor	0.53567
Culmen					
Declive					
Fastigum					
Nobule					
Claustrum	-35.7	-3.4	3.2		
Putamen					
Insula (BA13)					
Postcentral gyrus (BA40)	-60.8	-20.1	21	Somatomotor	

	Inferior parietal lobule (BA40)				
	Postcentral gyrus (BA43)				
	Postcentral gyrus (BA2)				
Thalamus L. – VPL nucleus		-14.2	-16.1	0	
	VPM nucleus				
	Subthalamic nucleus				
	Mammillary body				
	Ventral lateral nucleus				
	Substania nigra				
Inferior frontal gyrus (BA9)		-55.8	7.7	25.5	Dorsal attention
	Precentral gyrus (BA6)				
Cerebellum					
Cerebellum R. A. Dentate		17.4	-55.2	-22.3	Somatomotor
	Culmen				
	Declive				
	Cerebellar tonsils				
Cerebellum L. A. Culmen		-28	-58	-28.2	Executive
Cerebellum R. P. – Semi-lunar lobule		12	-65	-48	Somatomotor
	Cerebellar tonsils				

Table 17 *– The clusters that were most consistently activated in unimanual coordination than in bimanual coordination and are arranged with the largest cluster first. The first part represents both the most consistently activated clusters in the cerebrum and the cerebellum, while the second part only views those in the cerebellum. The results of the analysis of the unimanual data also includes multiple areas under one cluster that receives the name of the area belonging to the coordinates of the peak activation. The included areas are mentioned below the name of the area belonging to the coordinates of the peak activation. BA. = Brodmann Area, L.= left, R.=right, A.=anterior, and P.=posterior.*

Unimanual coordination vs. Bimanual coordination

Cluster name		Peak centre			Function	Confidence interval
Cingulate gyrus L. (BA24)		-11.5	4.1	52.3	Ventral attention	
	Medial frontal gyrus (BA6)					
	Sub-gyral (BA6)					
	Cingulate gyrus (BA32)					
	Medial frontal gyrus (BA32)					
Cerebellum						
No clusters found						

Table 18 *- The cluster that were most consistently activated in both unimanual and bimanual coordination and are arranged with the largest cluster first. The first part represents both the most consistently activated clusters in the cerebrum and the cerebellum, while the second part only views those in the cerebellum. The results of the analysis of the unimanual data also includes multiple areas under one cluster that receives the name of the area belonging to the coordinates of the peak activation. The included areas are mentioned below the name of the area belonging to the coordinates of the peak activation. A.= Anterior, BA= Brodmann area, L.= Left, R.= Right, P.= Posterior, VPL= Ventral posterolateral, and VPM= Ventral posteromedial.*

Bimanual coordination + Unimanual coordination

Cluster name	Peak centre			Function	Confidence interval
Precentral gyrus L. (BA4)	-37.7	-14.5	55.8	Somatomotor	
Precentral gyrus (BA4)					
Postcentral gyrus (BA3)					
Medial frontal gyrus L. (BA6)	-2	-8.7	54.7	Somatomotor	
Lentiform nucleus L.	-23.1	-9.2	0.4		
Putamen					
Globus pallidus In.					
Globus pallidus Ex.					
Cerebellum					
Cerebellum R. A.	22.1	-56.9	-25.9	Somatomotor	0.13058
Dentate					
Fastigum					
Declive					
Cerebellar tonsils					
Cerebellum R. A. Culmen	3.8	-60.6	-16.2	Somatomotor	0.29027
Declive					
Cerebellum L. A. Culmen	-23.5	-59.4	-27.7	Somatomotor	0.29027
Dentate					
Cerebellum L. A. Culmen	-26	-56	-26	Ventral attention	0.52669

Table 19 *– A comparison of the most consistently activated clusters across studies between bimanual and unimanual coordination. The function mentioned belong to the areas of the unimanual coordination. The results of the analysis of the unimanual data also includes multiple areas under one cluster that receives the name of the area belonging to the coordinates of the peak activation. The included areas are mentioned below the name of the area belonging to the coordinates of the peak activation. A.= Anterior, BA= Brodmann area, Ex.= Externus, L.= Left, P.= Posterior, R.= Right, VPL= Ventral posterolateral, and VPM= Ventral posteromedial.*

Bimanual coordination				**Unimanual coordination**				
Region of clusters	**Coordinates**			**Region of cluster**	**Coordinates**			**Function of region**
	X	Y	Z		X	Y	Z	
Globus pallidus R. Ex.	25.5	-6.5	2.9	Postcentral gyrus L. (BA3/2)	-41.9	-20.2	55.7	Somatomotor
Putamen				*Precentral gyrus (BA4)*				
				Inferior parietal lobule (BA40)				
				Precentral gyrus (BA6)				
				Postcentral gyrus (BA40)				
				Postcentral gyrus (BA1)				
				Postcentral gyrus (BA4)				
				Precentral gyrus (BA3)				
Globus pallidus L. Ex.	-22.3	-7.6	1.3	Medial frontal gyrus L. (BA6)	-2.8	1.7	54.5	Somatomotor
Putamen				*Cingulate gyrus L. (BA24)*				
				Medial frontal gyrus R. (BA6)				
				Paracentral lobule L. (BA31)				
				Superior frontal gyrus L. and R. (BA6)				
Globus pallidus In.				*Cingulate gyrus (BA31)*				
				Medial frontal gyrus (BA32)				
				Cingulate gyrus (BA32)				
Thalamus R. – medial dorsal nucleus	15	-19/9	6.4	Cerebellum R. A. Dentate	18	-54.2	-23	Executive
Mamillary body				*Culmen*				
Pulvinar				*Declive*				
VPM nucleus				*Fastigum*				
VPL nucleus				*Nobule*				
	-36.7	-14.5	56.8	Claustrum L.	-35.7	-3.4	3.2	

Precentral gyrus (BA4)				*Putamen*				
				Insula (BA13)				
Medial frontal gyrus (BA6)	-1.8	-8.7	54.6	Postcentral gyrus L. (BA40)	-60.8	-20.1	21	Somatomotor
				Inferior parietal lobule (BA40)				
				Postcentral gyrus (BA43)				
				Postcentral gyrus (BA2)				
Postcentral gyrus (BA3)	34.1	-29.9	59.2	Thalamus L. – VPL nucleus	-14.2	-16.1	0	
Postcentral gyrus (BA40)				*VPM nucleus*				
				Subthalamic nucleus				
				Mammillary body				
				Ventral lateral nucleus				
				Substania nigra				
				Inferior frontal gyrus L. (BA9)	-55.8	7.7	25.5	Dorsal attention
				Precentral gyrus (BA6)				

Cerebellum

Cerebellum R. A.	29.5	-60.6	-30.3	Cerebellum R. A. Dentate	17.4	-55.2	-22.3	Somatomotor
Culmen				*Culmen*				
Pyramis				*Declive*				
				Cerebellar tonsils				
Cerebellum L. A. Culmen	-17.7	-52.8	-22.8	Cerebellum L. A. Culmen	-28	-58	-28.2	Executive
Dentate								
Cerebellum L. A. Dentate	-20.8	-60.4	-29	Cerebellum R. P. – Semi-lunar lobule	12	-65	-48	Somatomotor
Culmen				*Cerebellar tonsils*				
Cerebellum R. A.	24.3	-53.6	-25.7					
Culmen								
Dentate								
Cerebellum L. P. Declive	-2	-69	-18					

9.7. Excluded trials

Table 20 *– An overview of the errors made by the subjects from which trials were excluded in session 1 showed with percentages.*

Session 1						
	Before intervention			**After intervention**		
	1:1 (%)	**1:3 (%)**	**3:1 (%)**	**1:1 (%)**	**1:3 (%)**	**3:1 (%)**
104	0	1.67	3.33	0	0	1.67
107	0	0	0	0	0	0
111	1.67	0	1.67	0	0	0
112	0	0	0	0	0	0
113	0	0	1.67	0	0	0
201	3.33	0	0	0	0	0
202	5	3.33	1.67	0	5	11.67
203	3.33	1.67	0	0	0	0
204	0	0	0	0	0	0
205	0	8.33	5	0	1.67	0
206	0	0	0	0	0	0
207	1.67	0	0	0	0	0
209	0	1.67	0	0	0	0
210	0	0	1.67	0	0	0
211	3.33	3.33	8.33	0	3.33	8.33
212	0	0	1.69	0	1.69	0
213	3.33	5	1.67	0	0	0

Table 21 *- An overview of the errors made by the subjects from which trials were excluded in session 1 showed with percentages.*

	Session 2					
	Before intervention			**After intervention**		
	1:1 (%)	**1:3 (%)**	**3:1 (%)**	**1:1 (%)**	**1:3 (%)**	**3:1 (%)**
104	0	0	0	0	0	0
107	0	1.67	0	0	0	0
111	0	0	0	0	0	0
112	0	0	1.67	0	0	0
113	0	0	0	0	0	0
201	0	5	0	0	0	0
202	0	0	0	0	3.33	0
203	0	0	0	0	0	0
204	1.67	5	0	0	0	0
205	10	0	11.67	0	13.33	10
206	0	0	0	0	0	0
207	0	0	0	0	0	0
209	0	0	0	0	0	0
210	0	5	0	0	0	0
211	0	3.33	11.67	3.33	3.33	5
212	5	0	0	0	0	0
213	0	0	0	1.67	0	0

Table 22 – *An overview of the errors made by the subjects from which trials were excluded in the sham condition showed with percentages.*

	Sham											
	Session 1						**Session 2**					
	1:1 (%)		**1:3 (%)**		**3:1 (%)**		**1:1 (%)**		**1:3 (%)**		**3:1 (%)**	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
104	0	0	1.67	0	3.33	1.67	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	1.67	0
113	0	0	0	0	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0
202	5	0	3.33	5	1.67	8.33	0	0	0	0	0	0
203	3.33	0	1.67	0	0	0	0	0	0	0	0	0
204	0	0	0	0	0	0	1.67	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	0
207	1.67	0	0	0	0	0	0	0	0	0	0	0
209	0	0	0	0	0	0	0	0	0	0	0	0
210	0	0	0	0	0	0	0	0	0	0	0	0
211	0	0	0	0	0	0	0	3.33	5	3.33	11.67	0
212	0	0	0	0	0	0	5	0	3.33	0	0	0
213	3.33	0	5	0	1.67	0	0	0	0	0	0	0

Table 23 – *An overview of the errors made by the subjects from which trials were excluded in the real stimulation showed with percentages.*

	Real stimulation (cbtDCS)											
	Session 1						**Session 2**					
	1:1 (%)		**1:3 (%)**		**3:1 (%)**		**1:1 (%)**		**1:3 (%)**		**3:1 (%)**	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
104	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	1.67	0	0	0
111	1.67	0	0	0	1.67	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0
113	0	0	0	0	1.67	0	0	0	0	0	0	0
201	3.33	0	0	0	0	0	0	0	0	0	0	0
202	0	3.33	0	0	0	0	0	0	5	3.33	0	0
203	0	0	0	0	0	0	0	0	0	0	0	0
204	0	0	0	0	0	0	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0	0	0	0	0	0
209	0	0	1.67	0	0	0	0	0	0	0	0	0
210	0	0	0	0	1.67	0	0	0	0	0	0	0
211	3.33	0	3.33	3.33	8.33	8.33	0	0	0	0	0	0
212	0	0	0	1.69	1.69	0	0	0	0	0	0	0
213	0	0	0	0	0	0	0	1.67	0	0	0	0