Neuromodulation of the Cognitive Cerebellum

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CHAPTER 1

1.1 The cerebellum

"Just as we cannot see our faces with our own eyes, is it not reasonable to expect that we cannot mirror our complete mental structures in the symbols which carry them out?"- Douglas R. Hofstadter

When reading the sentence above, several brain areas work together in order to recognize letters, construct individual words and understand its meaning. We use our cognitive skills on a daily basis, which involves numerous higher order processes, such as working memory and language comprehension *(Kandel, Schwartz, & Jessell, 2013)*. Higher-order cognition is said to be located in the cerebral cortex as is evident in humans and non-human primates, but is also present in birds *(Kandel et al., 2013)*. Across species, the absolute number of neurons in the mammalian cerebral cortex, or in the bird pallium, positively correlates with cognitive capabilities, where great apes and corvids are among the highest performers *(Herculano-Houzel, 2017)*. Higher-order brain functions are, however, not reflected in the brain anatomy in an obvious manner. The cerebellum contains about 3.6 times more neurons compared to the cerebral cortex, a ratio that is present in many different mammalian species *(Herculano-Houzel, 2010)*, which suggest that the cerebellum is capable of powerful mechanisms for processing information. In this introduction we will first touch upon on the function of cerebellum and its traditional role in motor control and motor learning. After that, we will focus on the potential involvement of the cerebellum in cognition and investigate ways to modulate performance with non-invasive stimulation.

The cerebellum is a three-dimensional structure, and therefore can be viewed from different perspectives. In the medial-lateral view, the cerebellum has three different sub regions: the vermis, the intermediate part and the lateral zones. In the anterior-posterior view, the cerebellum is separated into two large components, namely the anterior and posterior lobes, divided by the primary fissure. It also holds a third, smaller lobe called the flocculonodular lobe, which is the oldest region *(Koziol & Budding, 2009)*. In its global connectivity, the cerebellum receives input from the cerebral cortex via the pontine nuclei and projects back to the cerebral cortex via the dentate and the thalamus with independent, reciprocal loops *(Kelly & Strick, 2003)*. Similar to the anatomical connections between the cerebellum and the primary motor cortex, a closed, reciprocal loop is present between the cerebellum and cognitive, prefrontal areas (Figure 1), suggesting that the cerebellum can hold a comparable function, independent of its input *(Kelly & Strick, 2003; Salmi et al., 2010; Steele et al., 2016)*.

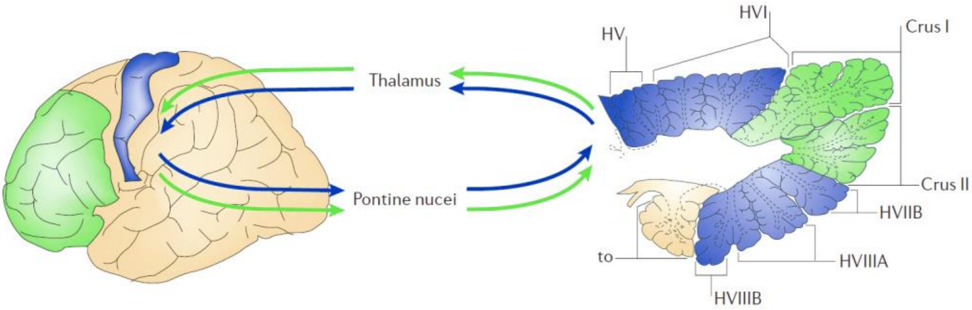

Figure 1. Schematic illustration of reciprocal loops between the cerebellum and the cerebral cortex.

Blue denotes the motor loop and green denotes the prefrontal loop. Modified from Ramnani 2006.

The cerebellar function has been examined for over a century. Early animal studies *(Luciani, 1891)* and clinical investigations *(Holmes, 1917)* have led to a view that the cerebellum is engaged in motor control and motor learning. Since then, and for many years, several functional aspects of the cerebellum have been interpreted within a motor perspective (D'Angelo et al., 2011). The role of the cerebellum in cognition, however, was largely overlooked until the mid-eighties of the 20th century, when Leiner and colleagues proposed a potential role of the cerebellum in mental skills *(Leiner, Leiner, & Dow, 1986)*. They suggested that, based on clinical observations and information-processing capabilities, the cerebellum could be involved in mental skills, however, compelling evidence was not available at that time. Masao Ito later expanded the view by Leiner and colleagues by stating that the cerebellum is a multipurpose learning machine which supports all kinds of neural control, autonomic, motor or mental due to the general anatomical structure *(Ito, 1993a, 2008)*. In addition, he describes a functional dichotomy between the cerebral cortex and the cerebellum, where explicit thought processes are based in the cerebral cortex and implicit thought processes occur in the cerebellum *(Ito, 2008)*. Since then, there have been colloquial speculations about the potential cerebellar role in cognition. Evidence from multiple domains suggests that the cerebellum is capable of processing information in a uniform manner, regardless of the site of origin *(Ramnani, 2006)*. However, the precise role of the cerebellum in cognition is still largely unknown. This thesis aims to further investigate the involvement of the cerebellum in cognition on the basis of Ito's implicit-explicit thought distinction.

1.1.1 Cerebellar role in motor learning and motor control

The cerebellum provides a powerful experimental paradigm for studying synaptic plasticity, which an important neurochemical basis of learning and memory *(Hebb, 1949)*. Traditionally, cerebellar function was investigated in animals with simple motor tasks, such as eye blink conditioning and ocular reflex paradigms. These tasks rely heavily on error-dependent motor learning mechanisms, which give rise to valuable information regarding the cerebellar working mechanisms at cell level. *(D'Angelo, 2005)*. The Purkinje cell is the fundamental information-processing unit of the cerebellum, as it is the sole output of the cerebellar cortex *(Ito, Yoshida, Obata, Kawai, & Udo, 1970)*. The entire cerebellar cortex is covered with numerous Purkinje cells which are buried in the molecular layer in a homogenous manner *(Bloedel, 1992)*. It integrates information from multiple excitatory mossy fibers, originating from the pontine nuclei, and a single excitatory climbing fiber, originating from the inferior olive. The inferior olive receives its inputs from the deep cerebellar nuclei, the mesodiencephalic junction and sensory systems *(De Zeeuw et al., 1998; Ramnani, 2006; Xue, Yang, & Yamamoto, 2008)*. The Purkinje cell is the largest neuron in the vertebrate central nervous system and terminates an inhibitory projection on the deep cerebellar nuclei *(Ito, Yoshida, & Obata, 1964)*. The foundations of cerebellar (motor) learning lie at the parallel fiber-Purkinje cell synapses and multiple sites of various interneurons *(Gao, van Beugen, & De Zeeuw, 2012)*. One of several phenomena underlying synaptic plasticity is long-term depression (LTD) and long-term potentiation (LTP). These activity-dependent processes modify the efficacy of neuronal synapses, which alter synaptic strength and thus mediate learning. LTD can be induced by co-activation of parallel fibers and climbing fibers, decreasing synaptic strength, whereas LTP can be induced by parallel fiber activity alone, enhancing synaptic strength *(Grasselli & Hansel, 2014)*.

The cerebellum holds more than 80% of the neurons in the human brain, *(Azevedo et al., 2009)* which suggests powerful mechanisms for processing information. Several theoretical models have been developed to explain the functional implication of the cerebellar anatomical microstructure and its interconnections on a network level. To date, *optimal control theory* is the predominate theory of motor learning *(Ito, 1993b)* and motor control *(Ramnani, 2006)*. It describes how a control system can generate smooth, goal-directed movements and simultaneously interact with the body and the environment *(Yang, Donaldson, Marshall, Shen, & Iacovitti, 2004)*. In this process, the cerebellum and the primary motor cortex have distinct functional roles; the cerebellum plays an important role during acquisition of motor adaptation tasks by updating motor commands during error-dependent learning *(Donchin et al., 2012)*, whereas the primary motor cortex is involved in the retention of motor learning *(Robertson, Pascual-Leone, & Miall, 2004)*.

According to optimal control theory, the basic structure of a motor control system consists of an instructor (premotor cortex), a controller (primary motor cortex), a controlled object (body part) and a sensory system (proprioceptive feedback). The instructor gives instructions to the controller, which in turn manipulates the controlled object. The sensory system mediates external feedback to the controller. However, to create a control system that is able to learn an *internal model* is required *(Ito, 2008; Kawato, 1999)*. An internal model is a representation of the external world acquired through learning that can simulate cortical processes, such as movements *(Ramnani, 2006)*. Internal models are said to be located in the cerebellum and have a great advantage over the slower, cortical processes that they simulate in terms of speed, accuracy and automaticity, because they make predictions about ideal states of the body and sensory feedback *(Doya, 1999; Ito, 2008; Miles, Cerminara, & Marple-Horvat, 2006; Shidara, Kawano, Gomi, & Kawato, 1993; Wolpert, Miall, & Kawato, 1998)(Asanuma, Thach, & Jones, 1983; Ramnani, 2006; Thach & Jones, 1979)*. These predictions can in turn be used to overcome time delays associated with feedback control *(Wolpert et al., 1998)*. Additionally, in order to learn, the internal model should continuously be updated by sensory feedback to maintain accuracy of the predictions *(Shadmehr, Smith, & Krakauer, 2010)* and adapt input-output relationships between motor commands and their effects *(Ramnani, 2006)*. As the predictions become more accurate over trials, the difference between the prediction made by the internal model and the actual movement becomes smaller, resulting in fluent, skillful movements *(Ito, 2008)*. Over time, the primary motor cortex can perform control using the internal model in the cerebellum without the need of external sensory feedback and ultimately without conscious attention *(Koziol et al., 2014)*. In line with assumptions of control theory and internal models, damage to the cerebellum results in motor and mental impairments. For example, patients with cerebellar lesions show irregular and slowed movements, reaching difficulties and reduced mental skills *(Grimaldi & Manto, 2012)*. Behavior of cerebellar patients is obviously affected, however, still present *(Koziol & Budding, 2009)*. Motor areas are still able to instruct and control movements, however internal models are unable to speed up or improve slow, cortical processes, resulting in irregular, disturbed movements. Challenging the pure motor view, Kawato (1999) argued for extension of control theory from a sensory-motor perspective to the cognitive domain, due to the amount evidence indicating cerebellar involvement in language and executive functioning *(Schmahmann & Sherman, 1998),* which was later supported by multiple researchers *(Ito, 2008; Ramnani, 2006)*.

1.1.2 Cerebellar role in cognitive processes

Control theory originates from engineering and provides systematic explanations how specific forms of information are processed. It imports a set of theoretical principles that consider behavior of a dynamical system. Theoretically, optimal control theory can be extended to the cognitive domain because it assumes similar neural information processing *(Kawato, 1999; Ramnani, 2006)*. The basic structure of a thought control system looks as follows: an instructor (anterior cingulate gyrus), a controller (prefrontal cortex), a controlled object (mental model) and a sensory system (temporo–parietal cortex). The internal model would be a “functional dummy” of the mental representation, mimicking the essential properties of the thought and eventually improving and speeding up the cortical process *(Ito, 2008; Ramnani, 2006)*. The thought process starts at the anterior cingulate gyrus as an instructor. This area gives instructions to the prefrontal cortex, which in turn manipulates the mental model in the temporo–parietal cortex. The mental model is then copied to the an internal model in the cerebellum, as a thought without conscious awareness, which is under control of the prefrontal cortex, *(Ito, 2008; Koziol et al., 2014)*.

Keeping in mind that a thought involves explicit and implicit processes, where explicit processes are executed in the cerebral cortex and implicit processes occur in the cerebellum *(Ito, 2008)*, let's consider a problem solving example. When we try to unravel a new problem, we first think about the problem consciously (explicit thought). If we fail to come up with a sufficient answer repeatedly, the thought will become less attentive until it eventually will be forgotten. During this less attentive phase, the thought process proceeds implicitly and unaware to the observer (implicit thought). If an adequate answer to this problem is generated, the information is fed back to the prefrontal cortex and the solution appears suddenly, without any conscious intention. This phenomena is also known as intuition *(Ito, 2008).*

Evidence for cerebellar involvement in cognition dates back to the 1800' from clinical observations, reporting intellectual, psychiatric and social-emotional dysfunction in patients with cerebellar degeneration", *(Schmahmann, 1991)*, however, it was not until the mid-eighties of the 20th century that a possible role of the cerebellum in cognition was first considered *(Leiner et al., 1986)*. To date, the involvement of the cerebellum in cognition has great support from multiple domains, such as lesion studies, neuroimaging data and neurophysiology research *(Grimaldi & Manto, 2012; Kelly & Strick, 2003; Ramnani, 2006)*. The function of the cerebellum (motor or mental) is evidently apparent in people with cerebellar pathology. Patients with cerebellar lesions show impaired neurological and mental function, suggesting that cerebellar damage results in widespread complications across multiple domains besides the motor aspect *(Hokkanen, Kauranen, Roine, Salonen, & Kotila, 2006; R.B. et al., 2001; Schmahmann, 1991; Schmahmann & Sherman, 1998)*. Moreover, people suffering from complete primary cerebellar agenesis show difficulties in motor, language and mental activities. For example, a living case of complete primary cerebellar agenesis was found in a 24-year-old female *(Yu, Jiang, Sun, & Zhang, 2015)*. The patient presented mild mental impairment and medium motor deficits, which is line with other reported cases *(Ashraf, Jabeen, Khan, & Shaheen, 2016; Gelal et al., 2016; Mormina et al., 2016; Nitsche, Schauenburg, et al., 2003; Sener, 1995; Timmann, Dimitrova, Hein-Kropp, Wilhelm, & Dorfler, 2003; Velioglu, Kuzeyli, & Zzmenoglu, 1998; Yoshida & Nakamura, 1982)*. Secondly, cerebellar activation during cognitive tasks further supported this hypothesis as shown by early imaging studies *(Kim, Uğurbil, & Strick, 1994; Petersen, Fox, Posner, Mintun, & Raichle, 1988)*, electrical stimulation – fMRI studies *(Sultan et al., 2012)* and (resting state) connectivity research *(Buckner, Krienen, Castellanos, Diaz, & Yeo, 2011; Krienen & Buckner, 2009)*, the later claiming to find a correlation between the lateral cerebellum and cerebral networks associated with cognitive control and the default network. At first, results from imaging studies were received with some scepticism. Critics argued that cerebellar activation, seen in fMRI research, was actually induced by hand or eye movements. However, this was later proven to be false by numerous researchers *(Balsters, Whelan, Robertson, & Ramnani, 2013; Hayter, Langdon, & Ramnani, 2007; Kirschen, Chen, & Desmond, 2010; Peterburs, Cheng, & Desmond, 2016).*

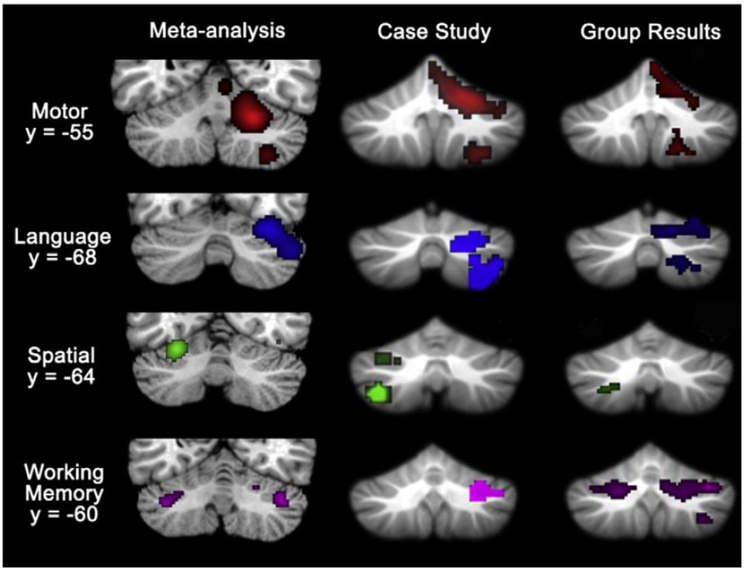

Figure 2. Localized cerebellar activations during motor (red), language (blue), spatial (green) and working memory (purple) paradigms.

Left is shown on the left. Modified from Stoodley et al. 2012.

Lastly, multiple tracer studies showed that the cerebellum has multiple reciprocal connections with prefrontal and limbic areas *(Bostan, Dum, & Strick, 2013; Kelly*

& Strick, 2003; Krienen & Buckner, 2009; X. Lu, Miyachi, Ito, Nambu, & Takada, 2007; X. Lu, Miyachi, & Takada, 2012), confirming the existence of neural correlates for control theory regarding mental activities *(Ito, 2008)*. As described earlier, the cerebellum is separated into two large components, namely the anterior and posterior lobes, divided by the primary fissure *(Koziol & Budding, 2009)*. This anatomical topography encompasses a distinct functional purpose. The anterior part of the cerebellum is associated with the motor cortex, whereas the posterior part of the cerebellum is linked to the prefrontal cortex. Specifically, the lateral posterior cerebellum (Crus I and Crus II) is assumed to be related to specific cognitive functions (Figure 2) *(Imamizu, Kuroda, Miyauchi, Yoshioka, & Kawato, 2003; Stoodley, Valera, & Schmahmann, 2012; Sultan et al., 2012)*. This notion is supported by other research showing that specific parts of the cerebellum (Crus I and Crus II) evolved in tandem with the prefrontal cortex *(Balsters et al., 2010; Weaver, 2005),* along with prefrontal inputs to the cerebellum *(Ramnani et al., 2006)* and cerebellar outputs to the prefrontal cortex *(Matano, 2001),* suggesting that these regions might contribute to the evolution of higher cognitive functions in humans *(Herculano-Houzel, 2012)*.

1.2 Transcranial Direct Current Stimulation

As described earlier, evidence from numerous domains suggest a potential role for the cerebellum in cognition. However, these studies do not investigate causal effects, as imaging research merely implies a correlation between two variables. Moreover, lesion studies come with confounding factors such as disperse brain injury and heterogeneity between subjects. A way to overcome problems in behavioural studies is by the use of non-invasive neuromodulation techniques, such as transcranial magnetic stimulation (TMS), theta burst stimulation (TBS) or transcranial direct current stimulation (tDCS). In this thesis we will focus on tDCS, which is the most practical non-invasive stimulation technique *(Grimaldi et al., 2016)*.

tDCS is a non-invasive neurostimulation technique where a weak current is applied through electrodes over the scalp, with approximately 45% of the stimulation reaching the brain *(Rampersad et al., 2014; Reinhart, Cosman, Fukuda, & Woodman, 2017; Yavari et al., 2016)*. It induces changes in neuronal excitability in a polarity and site-specific manner *(Nitsche et al., 2008),* meaning that the likelihood of a neuron firing will be enhanced near the positive (anodal) electrode and diminished near the negative (cathodal) electrode. The cathode electrode is usually placed over the cheek or arm to diminish interference of negative stimulation *(Reinhart et al., 2017)*. Current flows from the anodal to cathodal electrode with anodal tDCS increasing excitability and cathodal tDCS decreasing excitability, as measured by spike activity in rat cerebral cortex *(Bindman, Lippold, & T Redfearn, 1964)* and in motor-evoked potentials (MEP) in humans *(Nitsche et al., 2005)*.

Mechanisms underlying the effect of tDCS have predominantly been researched using electrophysiology studies in animals and pharmacology experiments in humans. Most studies have investigated the effect of tDCS over the cortex, however, recently researchers have expand their field of interest to the cerebellum *(Grimaldi et al., 2016)*. The exact mechanism through which tDCS works is still not fully understood. Current literature suggests that tDCS works on numerous mechanisms across multiple brain regions, such as intracellular plasticity mechanisms, neurotransmission and neuromodulators, but also presumably modulates brain oscillations *(Das, Holland, Frens, & Donchin, 2016)*. Evidence suggests that tDCS modulates cortical plasticity through NMDA, GABA, glutamate, BDNF and calcium-dependent mechanisms *(Antal et al., 2010; Cheeran et al., 2008; B Fritsch et al., 2010; Liebetanz, Nitsche, Tergau, & Paulus, 2002; Monte-Silva et al., 2013; Nitsche, Fricke, et al., 2003; Ottersen, 1993; Stagg et al., 2009)*, through somatic polarization of pyramidal neurons *(Radman, Ramos, Brumberg, & Bikson, 2009)* and axon terminal polarization of pyramidal neurons inputs *(Rahman et al., 2013)*. Moreover, researchers propose that anodal stimulation could induce LTP-like mechanisms, whereas cathodal stimulation could induce LTD-like mechanisms *(Das et al., 2017; Monte-Silva et al., 2013; Nitsche, Müller-Dahlhaus, Paulus, & Ziemann, 2012; Sun et al., 2016)*.

Similar to stimulation over the cortex, anodal tDCS increases cerebellar excitability, whereas cathodal tDCS decreases excitability in the cerebellum *(Galea, Jayaram, Ajagbe, & Celnik, 2009)*. Augmented excitability results in enhanced inhibition of the deep cerebellar nuclei DCN and reduced excitability results in disinhibition of the DCN *(Grimaldi et al., 2016)*. However, the cellular mechanism by which tDCS impacts cerebellar excitability is poorly understood. A recent study showed increased performance of VOR adaptation in wild type mice, however this facilitatory effect was disrupted in PP2B LTP-deficient mutants, suggesting that anodal cerebellar tDCS depends on PP2B-dependent Purkinje cell potentiation pathway *(Das et al., 2017)*. The modulation of Purkinje cell activity is dependent on synaptic processes involving calcium and sodium channels, GABA, and AMPA receptor modulation *(Shepherd, 2004)*, suggesting that cerebellar stimulation could work in a similar way to cortical tDCS. However, results from cortical research do not simply apply to the cerebellum. For example, synaptic plasticity mechanisms, such as LTD and LTD, which depend on calcium influx, have opposite effects in the cerebellum compared to other brain regions *(Lisman, 2006; Van Woerden et al., 2009)*. Several simulation studies have modeled the effect of tDCS on the human cerebellum. They showed that during stimulation the strongest electric field *(Rampersad et al., 2014)* and current density amplitudes *(Parazzini et al., 2014)* occur mainly in the cerebellum. Moreover, current spread to other structures outside the cerebellum is unlikely to produce functional effects *(Parazzini et al., 2014)*, indicating that cerebellar tDCS is a focal technique *(Galea, Vazquez, Pasricha, Orban De Xivry, & Celnik, 2011)*. However, it should be noted that neuronal modulation around the electrodes also induce changes in downstream structures *(Li, Uehara, & Hanakawa, 2015; Pope & Miall, 2012)*.

The downside of tDCS is its sensitivity to numerous parameters, which determine outcome efficacy *(Vannorsdall et al., 2016)*. For example, effects following tDCS are determined by subject's anatomical characteristics *(Das et al., 2016; Parazzini et al., 2014; Wurzman, Hamilton, Pascual-Leone, & Fox, 2016)* and methodology, as an increase in current intensity and stimulation duration can result in a weakening or reversion of the tDCS effects *(Hoy et al., 2013; Teo, Hoy, Daskalakis, & Fitzgerald, 2011)*. In addition, the effect of tDCS is also determined by the orientation of the neuron to current flow and morphology of the neuron *(Das et al., 2016; Radman et al., 2009; Rahman, Toshev, & Bikson, 2014a)*. Moreover, individual differences in the cortical folding pattern lead to changes in local current density *(Opitz, Paulus, Will, Antunes, & Thielscher, 2015)*, subsequently, multiple Purkinje cells will be more susceptible to hyperpolarize and depolarize. Lastly, subjects differ on a genetic and anatomical level, potentially confounding experimental results. As a result, high inter-subject variability is a vast problem in tDCS research *(Datta, Truong, Minhas, Parra, & Bikson, 2012; Li et al., 2015; Truong, Magerowski, Blackburn, Bikson, & Alonso-Alonso, 2013)*.

1.2.1 Effects of cerebellar tDCS on cognitive tasks

Stimulation studies can show a causal relationship between two variables, however, when it comes to the cerebellum, only a handful of studies have investigated the effect of tDCS on explicit cognitive learning and reported promising, yet mixed, effects in healthy humans. Two studies investigating verbal working memory showed impaired reaction time with anodal and cathodal tDCS over the cerebellum *(Ferrucci et al., 2008)* and reduced performance with cathodal tDCS only *(Boehringer, Macher, Dukart, Villringer, & Pleger, 2013)*. Another study found facilitation on verbal responses in a verb generation task and mental arithmetic task with cathodal tDCS. The authors argued that cerebellar stimulation can affect working memory differently depending on task difficulty. Moreover, they also suggest that when a task becomes more demanding the cerebellum is able to release cognitive resources *(Pope & Miall, 2012)*. However, a follow up study by another research group was not able to replicate that finding, showing increased variability in subjects' verbal response times a week following cathodal tDCS *(Spielmann et al., 2017)*. Finally, Miall and collegues (2016) found a decrease in subjects' response time advantage on after cathodal tDCS and an improvement of response time advantage after anodal tDCS in a linguistic prediciton task. However, effects were not significantly modulated by stimulation over time.

The studies described above show no robust effects on cognitive performance during cerebellar tDCS, indicating that this field of research is still far from understood. The abovementioned studies have investigated the effects of cerebellar tDCS in explicit learning tasks. However, we may provide more meaningful results using implicit learning tasks due to the substantial involvement of the cerebellum in implicit learning *(Ito, 2008)*. Moreover, previous research has shown modulatory effects of cerebellar tDCS on implicit learning in motor tasks *(Ferrucci et al., 2013; Galea et al., 2011)*.

1.3 Scope of this thesis

With this thesis, we aim to further explore the cerebellar function in motor and cognitive tasks with a non-invasive stimulation technique (tDCS). In Chapter 2 we will first test the assumption that tDCS over the cerebellum can modulate performance in a simple motor task. Moreover, in Chapter 3 we will investigate the effect of cerebellar tDCS during an explicit working memory task, exploring working memory load and polarity effects of cerebellar tDCS as suggested by previous research *(Pope & Miall, 2012)*. Finally, taking Ito's implicit thought theory into account, in Chapter 4 we will thoroughly examine the effect of tDCS in two implicit cognitive tasks with high-workload. Moreover, in Chapter 4, prefrontal tDCS will also be studied in order to explore effects of tDCS over the prefrontal cortex and replicate former positive findings.

Chapter 2. Neuromodulation of the cerebellum in (simple) motor tasks

(PICTURE BY REPUTATTIONS)

CHAPTER 2

In this chapter, we will discuss the effects of cerebellar tDCS on different motor learning paradigms. The cerebellum and the prefrontal cortex have distinct roles in various forms of motor learning. The cerebellum is involved in error-dependent motor learning, and as such, plays an important role in motor adaptation tasks *(Donchin et al., 2012)*. On the other hand, the primary motor cortex is involved in retention of motor learning *(Richardson et al., 2006)*. Different kinds of neuromodulation techniques, such as TMS and tDCS, have been used to study functions of the primary motor cortex and the cerebellum with various motor tasks *(Galea et al., 2011; Grimaldi et al., 2016; Tomlinson, Davis, & Bracewell, 2013)* finding pronounced effects following tDCS over the primary motor cortex on procedural motor learning tasks *(Monti et al., 2013)* and consolidation effects in a serial reaction time task (SRTT) *(Savic & Meier, 2016)*. However, effects after cerebellar tDCS are less prominent *(R. E. Shimizu, Wu, Samra, & Knowlton, 2017)*.

More distinct results were found in small sample sized studies, which demonstrate that anodal tDCS over the cerebellum enhances performance on a sequential visual isometric pinch task *(Cantarero et al., 2015)*, a synchronization-continuation task *(M. J. Wessel et al., 2016)*, a locomotor adaptation walking task *(Jayaram et al., 2012)* and a visuo-motor adaptation reaching task in healthy young adults *(Block & Celnik, 2013; Galea et al., 2011)* and older individuals *(Hardwick & Celnik, 2014)*. Finally, modulatory effects were also found in other studies investigating cerebellar-dependent learning in simple motor tasks. In a saccadic backward and forward adaptation tasks, cathodal tDCS tended to increase forward and backward adaptation, while anodal tDCS impaired forward adaptation *(Panouillères, Miall, & Jenkinson, 2015)*, supporting their previous findings with TMS *(Panouillères et al., 2012)*. Moreover, a study investigating classical eye blink conditioning found impairment after cerebellar continues Theta burst stimulation (cTBS), an inhibitory rTMS protocol. In addition, polarity dependent effects were found in a conditioned eye blink response task after cerebellar tDCS *(Zuchowski, Timmann, & Gerwig, 2014)*, however a follow up study was unable to replicate that finding *(Beyer, Batsikadze, Timmann, & Gerwig, 2017)*, highlighting the importance of replication.

In the next section we will investigate the effect of tDCS on a saccadic adaptation task *(Avila et al., 2015)*, similar to the study by Panouillères and collegues (2015) published in March and April 2015, respectively.

CHAPTER 2

2.1 Cerebellar transcranial direct current stimulation effects on saccade adaptation

Neural plasticity 2015: E. Avila, J. N. van der Geest, S. Kengne Kamga, M. C. Verhage, O. Donchin, M. A Frens.

Abstract

Saccade adaptation is a cerebellar-mediated type of motor learning in which the oculomotor system is exposed to repetitive errors. Different types of saccade adaptations are thought to involve distinct underlying cerebellar mechanisms. Transcranial direct current stimulation (tDCS) induces changes in neuronal excitability in a polarity-specific manner and offers a modulatory, non-invasive, functional insight into the learning aspects of different brain regions. We aimed to modulate the cerebellar influence on saccade gains during adaptation using tDCS. Subjects performed an inward (n=10) or outward (n=10) saccade adaptation experiment (25% intra-saccadic target step) while receiving 1.5 mA of anodal cerebellar tDCS delivered by a small contact electrode. Compared to sham stimulation, tDCS increased learning of saccadic inward adaptation, but did not affect learning of outward adaptation. This may imply that plasticity mechanisms in the cerebellum are different between inward and outward adaptation. TDCS could have influenced specific cerebellar areas that contribute to inward but not outward adaptation. We conclude that tDCS can be used as a neuromodulatory technique to alter cerebellar oculomotor output, arguably by engaging wider cerebellar areas and increasing the available resources for learning.

Introduction

Saccades are performed in order to foveate targets of interest. These fast and brief eye movements cannot rely on online (visual) feedback since visual delays are longer than the movement itself. This means that in order to maintain accurate eye movements, the motor commands for future saccades must be adjusted after each eye movement is completed. These plastic mechanisms are present to reduce or compensate motor errors due to either physiological or pathological behaviour *(Hopp & Fuchs, 2004; Pélisson, Alahyane, Panouillères, & Tilikete, 2010)*. Since McLaughlin (1967) described the "parametric adjustment", known today as short-term saccade adaptation, his paradigm has been used as a way to assess learning and plasticity in the oculomotor system. This is done by asking a subject to make a saccade to a new position and while the saccade is in-flight, the target moves (intra-saccadic step) causing a post-saccadic visual error *(McLaughlin, 1967; Seeberger, Noto, & Robinson, 2002; Wallman & Fuchs, 1998)*. When the subject is repeatedly exposed to the same error, the oculomotor system will gradually drive a change in the metrics of the eye movement over time, making the error smaller (Collins, Semroud, Orriols, & Doré-Mazars, 2008; Cotti et al., 2009; Deubel, Wolf, & Hauske, 1986; FitzGibbon, Goldberg, & Segraves, 1986; Frens & Opstal, 1994; Frens & Van Opstal, 1997; Herman, Blangero, Madelain, Khan, & Harwood, 2013; Robinson, Noto, & Bevans, 2003; Schultz & Busettini, 2012; Seeberger et al., 2002; Straube, Fuchs, Usher, & Robinson, 1997). The error can induce saccade shortening (gain-down), when the intra-saccade step of the target is in the direction of the starting point of the saccade (inward adaptation), or saccade lengthening (gain-up) when the step is away from the starting point (outward adaptation). Human subjects adapt faster in response to inward adaptation than to outward adaptation stimuli *(Hopp & Fuchs, 2004)*, which poses the hypothesis that these two types of adaptation involve different neural mechanisms (Ethier, Zee, & Shadmehr, 2008; Panouillères et al., 2009).

The cerebellum plays a crucial role in saccadic error detection *(Desmurget et al., 1998; Liem, Frens, Smits, & Van Der Geest, 2013; Van Broekhoven et al., 2009)*, and thus in saccade adaptation *(Pélisson et al., 2010)*. Evidence of the cerebellar involvement and its necessary integrity to oculomotor learning has been demonstrated as large lesions, focal inactivation or pathological conditions of different areas of the cerebellum impair the ability to adapt saccades *(Aschoff & Cohen, 1971; Barash et al., 1999; Golla et al., 2008; Optican & Robinson, 1980;*

Xu-Wilson, Chen-Harris, Zee, & Shadmehr, 2009). In addition, various loci in the cerebellum relate to inward and outward errors differently *(Liem et al., 2013)*. For instance, patients with vermal damage who are partially capable of inward adaptation but lack outward adaptation *(Golla et al., 2008)*. Also, MRI-guided TMS on lateral hemispheres potentiates the post-adaptation effects of outward adaptation and, in contrast, depresses gain-down adaptation (Panouillères et al., 2012).

Neuromodulatory techniques can be used to influence functional roles in various brain structures. Cerebellar output can be modulated with transcranial direct current stimulation (tDCS) with great specificity as shown by excitability changes after stimulation ranging from cognitive to motor skills (Boehringer, Macher, Dukart, Villringer, & Pleger, 2013; Ferrucci et al., 2008; Galea, Jayaram, Ajagbe, & Celnik, 2009; Jayaram et al., 2012). In this study, we used anodal tDCS as a tool to non-invasively modulate cerebellar output and provide functional insight into the learning aspects during saccade adaptation.

Materials and Methods

Participants

Thirteen healthy subjects (one author - E.A., 12 naive subjects to tDCS, mean age of 22.4, range 19-29 years, 6 females), right handed volunteers with no known history of neurological or psychiatric conditions, not taking chronic or acute medications or using drugs, with normal vision were recruited. They all gave informed consent to participate in the experiment, which was approved by the local medical ethics committee and adhered to the Declaration of Helsinki. Ten subjects participated in the inward saccade adaptation experiment and ten in the outward saccade adaptation experiment. Seven subjects participated in both experiments.

Setup

Subjects were seated in a completely darkened room at 84 cm in front of a 21 in. computer screen. The screen was covered with a red filter to eliminate light reflections of the monitor and after images. Eye movements were recorded binocularly at 250 Hz by means of video-oculography (SR Research EyeLink II, Ontario, Canada) *(Van Der Geest & Frens, 2002)*. Head movements were restrained by a chin rest and monitored throughout the measurements to ensure head stability.

Task

The inward and outward adaptation experiments were created using Experiment Builder (SR Research, Ontario, Canada). In both experiments, the subject was instructed to look at a red dot (0.5 degrees of visual angle) displayed on a black background. At the beginning of the trial, the dot was shown at 10 degrees to the left of the center of the screen (fixation position). After a random delay between 1.5 s and 2 s, the fixation point was switched off and the dot appeared at a position on the right of the center (target position), evoking a visually guided saccade. In the inward adaptation experiment, this target position was 10 degrees to the right of the center and in the outward adaptation experiment the target position was 5 degrees to the right of the center. In other words, in the inward adaptation experiment the target jump was 20 degrees and in the outward adaptation experiment it was 15 degrees. Both experiments consisted of three phases with 250 trials in total (Figure 1A, B):

1) 50 baseline trials, where the dot remained on the rightward position for 1.5 seconds until the end of the trial.
2) 150 adaptation trials, in which the initial target position was the same. At saccade detection, however, the target jumped toward the fixation point in the inward adaptation experiment (i.e., backward target jump) and away from it (i.e., forward target jump) in the outward adaptation experiment during the saccade towards it. The size of the intra-saccadic step was 5° in both experiments. The saccade was detected online using a velocity threshold of 50°/sec, and a boundary threshold of 7.5° to the right of the fixation position, to ensure that saccades were in the right direction. If no proper saccade was detected, the screen was blanked for 500 ms and the trial was presented again.
3) 50 ‘post-adaptation’ trials, identical to baseline trials.

tDCS

Anodal tDCS was delivered to the cerebellum through a constant current stimulator (NeuroConn, Ilmenau, Germany) through two annular sintered Ag/AgCl 12 mm diameter electrodes (MedCat, Erica, The Netherlands) with highly conductive gel (Signa Gel, Parker Laboratories, New Jersey, USA) *(Minhas et al., 2010)*. The anodal electrode was placed over the right cerebellum 3 cm to the right of the inion and the reference electrode (cathodal) was placed over left buccinator muscle. The total current density was 1.3 mA/cm^2, ramped up in 30 s to a constant 1.5 mA. Stimulation commenced 3 min before an experiment started and lasted for 15 minutes (i.e., during all baseline trials and adaptation trials). These criteria are well below the threshold for tissue damage (Boggio et al., 2006; Iyer et al., 2005; Nitsche et al., 2003).

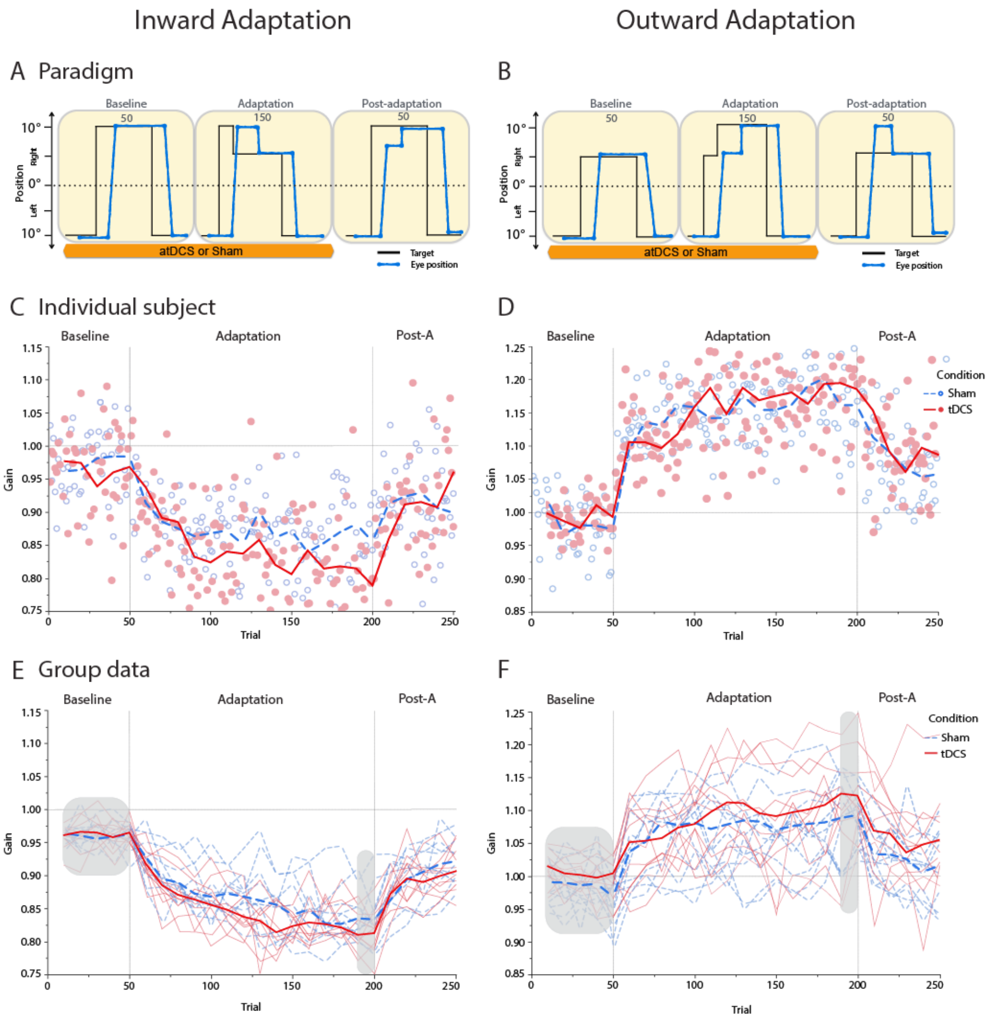

Figure 1. Experimental paradigms, single subject data and population data.

Panel A depicts inward adaptation where subjects performed an inward paradigm that consisted of 50 baseline trials of 20° saccades at intervals between 1.5 - 2 s, followed by 150 adaptation trials where the second target had an intra-saccadic step of 5°. Eye trace shows an overshoot at the beginning of the phase and the subject makes a corrective saccade to the target. Finally, 50 post-adaptation trials presented in the same way as baseline trials. Anodal tDCS was delivered for 15 min at the start of the experiment, or for 30 s in the sham condition. Panel B shows *outward adaptation consisting in the same trial structure as inward, but here the subjects experienced baseline trials of 15° saccades and a forward jump of 5° (in the direction of the saccade). The middle row shows examples of adaptation for a single subject in the inward adaptation (gain-decrease, panel C) and outward adaptation (gain-increase, panel D) experiment for the two tDCS conditions. Lines on top depict blocks composed of the median values of 10 trials. The bottom row shows group data for inward (panel E) and outward (panel F) adaptation. Thin, low-opacity lines show the course of adaptation for all subjects. Thick lines on top show the median value for all of the subjects for both paradigms in the two stimulation conditions. For the inward adaptation experiment no differences were observed in baseline or post-adaptation phases, but presented a significantly smaller gain under cerebellar tDCS condition (p = 0.02). In the outward adaptation experiment, subjects also presented a normal course of adaptation in which subjects in the sham condition present relatively smaller gains compared to tDCS condition observed since the baseline phase, though this was not significant in any of the three phases (see Results). Gray bars show the measures taken into account for the analysis in this study. (atDCS: anodal transcranial direct current stimulation Post-A: post-adaptation. Blue: Sham, Red: tDCS).*

Design

A subject participated twice in an experiment, once in a sham tDCS condition, and once in an anodal cerebellar tDCS condition. The order of the tDCS conditions was pseudo-randomized and counterbalanced across subjects, with three to seven days between recordings. In the anodal cerebellar condition, real stimulation was applied, while in the sham condition, the current was turned off after 30 s *(Gandiga, Hummel, & Cohen, 2006)*. Subjects and experimenter were blind to the tDCS condition (double blind design). At the end of each paradigm, subjects were asked to report perceived pain and fatigue using a verbal analog scale, (0 - no fatigue/pain to 5 - maximal fatigue/ pain), as well as the presence of headache, balance, nausea and discomfort. Recordings in subjects who participated in both the inward and outward adaptation experiments were separated by at least seven days to avoid carry-over effects.

Data Analysis

For each trial, the primary saccade from the left (fixation) to the right (target) was analyzed. Saccades were marked automatically using a velocity threshold of 50º/s and a duration threshold of 20 ms. Trials were excluded if 1) there was no fixation inside a 1.7º window around the fixation point or, 2) there was no saccadic movement from left to right. The amplitudes of the primary saccades were transformed into gain values, with gain being defined as the ratio between saccade amplitude and the distance between fixation and target position. A gain of 1 indicates a saccadic amplitude of 20° in the inward and 15° in the outward paradigm. The data was tested for normality using a Kolmogorov-Smirnov test. Median, mean and SD of the gains were calculated for individual subjects and pooled by paradigm and condition. Saccades that fell outside ± 1.96 SD from the mean of a subject were excluded separately for every phase. From the inward adaptation experiment 4.76 % of trials were excluded, and 3.56 % from the outward adaptation experiment. *Baseline gain* was defined as the median gain in all baseline trials, *adaptation gain* as the median gain of the last 10 saccades made in the adaptation phase, and *post-adaptation gain* as the median gain of the last 10 saccades in the post-adaptation phase. *Adaptation gain-change* was calculated as the difference between *adaptation gain* and *baseline gain*. *Retention* was calculated as the difference between the *post-adaptation gain* and *baseline gain*, giving a measure of how much learning was retained after the adaptation phase.

Table 1. Saccadic gains, kinematics, adaptation gain-change and retention. Gains, saccade kinematics (peak velocity and duration) measured during the three phases for inward and outward adaptation in the two conditions. Inward and outward adaptation gain-change for the two conditions shows the difference between pre-adaptation and adaptation phases. Adaptation phase values are the last ten trials (adaptation gain). Peak velocity are deg/s, and Duration in ms. Values are means ± SD. * p = 0.02, † p = 0.04.

Phase	**Inward**		**Outward**	
	Sham	**tDCS**	**Sham**	**tDCS**
Baseline				
Gain	0.95 ± 0.01	0.96 ± 0.01	0.98 ± 0.03	1 ± 0.02
Peak velocity (deg/s)	503.20 ± 79.02	534.70 ± 69.79	493.40 ± 104.81	479.15 ± 103.32
Duration (ms)	67.60 ± 8.93	68 ± 6.25	57.60 ± 4.69	60 ± 7.77
Adaptation				
Gain	0.83 ± 0.04*	0.81 ± 0.03*	1.08 ± 0.04	1.12 ± 0.07
Peak Velocity (deg/s)	450.85 ± 83.30	454.35 ± 87.42	492.35 ± 101.47	445.25 ± 104.76
Duration (ms)	67.20 ± 8.01	69 ± 16.68	65.60 ± 9.60	69.20 ± 15.52
Postadaptation				
Gain	0.92 ± 0.03	0.9 ± 0.03	1 ± 0.06	1.05 ± 0.07
Peak velocity (deg/s)	481.65 ± 128.86	517.90 ± 92.55	461.15 ± 131.26	490.50 ± 116.08
Duration (ms)	69 ± 8.70	65.80 ± 5.37	65 ± 10.55	61 ± 3.43
Adaptation gain-change	0.12 ± 0.04†	0.15 ± 0.03†	0.10 ± 0.04	0.12 ± 0.08
Retention	0.03 ± 0.03	0.05 ± 0.03	−0.02 ± 0.05	−0.04 ± 0.07

*Values are mean ± SD. * P = 0.02, † P = 0.04.*

Statistical analyses were performed using a custom script written in Matlab (The Mathworks, Natick, MA, USA), and SPSS (v. 20.0, IBM Corp., Armonk, NY, USA). We assessed the presence of adaptation for each subject by testing the difference between *baseline* and *adaptation* gain with a Student's t-test. For both the inward and outward experiment, gains and saccade kinematics (duration and peak velocity) were analyzed using repeated measures MANOVA with two within-subject factors: tDCS Condition (two levels: sham vs. cerebellar tDCS) and Phase (three levels: baseline, adaptation, post-adaptation). Post-hoc planned comparisons between the two stimulation conditions for each of the three phases were performed using paired t-tests on the saccadic gains. The effects of tDCS on *adaptation gain-change* and on *retention* were assessed using paired t-tests.

For each experiment, the difference in *adaptation gain-change* and the difference in *retention* between tDCS and sham stimulation were calculated. These differences were statistically compared between the inward and outward experiment using a Wilcoxon signed rank test using the 7 subjects that participated in both experiment.

Pain and fatigue were statistically assessed using a one way ANOVA with tDCS condition as within subject factor. Statistical significances were set at $p < 0.05$.

Results

All participants successfully completed the experiments and showed a significant change in gain during the adaptation phase. Example data of one subject and group data is shown in Figure 1C and D respectively. Table 1 summarizes the results obtained in each phase for inward and outward adaptation for the two tDCS conditions. Pain and fatigue scores were not different between the tDCS or sham conditions ($p > 0.5$).

Inward adaptation

A MANOVA on the gains for the inward adaptation experiment with sham and cerebellar tDCS and Phase as factors revealed an effect of tDCS Condition ($F(1,9) = 6.755$, $p = 0.02$, $\eta2=0.429$) and Phase ($F(2,8) = 49.801$, $p = <0.0001$, $\eta2 = 0.926$) as well as the interaction between tDCS Condition x Phase ($F(2,8) = 6.439$, $p = 0.02$, $\eta2 = 0.617$; Table 1).

Saccades during baseline trials tended to be slightly hypometric for both the sham and tDCS conditions (Table 1), which is normal for saccades above 10 degrees *(Albano & King, 1989; Bötzel, Rottach, & Büttner, 1993)*. The adaptation phase showed a gradual decrease in gain throughout the trials in the two tDCS conditions, in which smaller gains are present for the tDCS condition (Table 1). In the post-adaptation phase, we found that subjects in both groups did not present full recovery to baseline gains (Table 1).

Planned comparisons between the two conditions (sham and tDCS) showed no significant differences between the two stimulation conditions in baseline gains (0.95 ± 0.01 vs. 0.96 ± 0.02, t(9) = 0.88 p = 0.39, Figure 1E). The gain at the end of the adaptation phase was significantly smaller under cerebellar tDCS compared to sham stimulation (sham 0.83 ± 0.04, tDCS 0.81 ± 0.03, t(9) = -2.71, p = 0.02, Figure 1E). Post-adaptation phase did not exhibit differences between the two conditions (sham 0.92 ± 0.03, tDCS 0.90 ± 0.03, t(9) = -1.75, p = 0.11, Figure 1E).

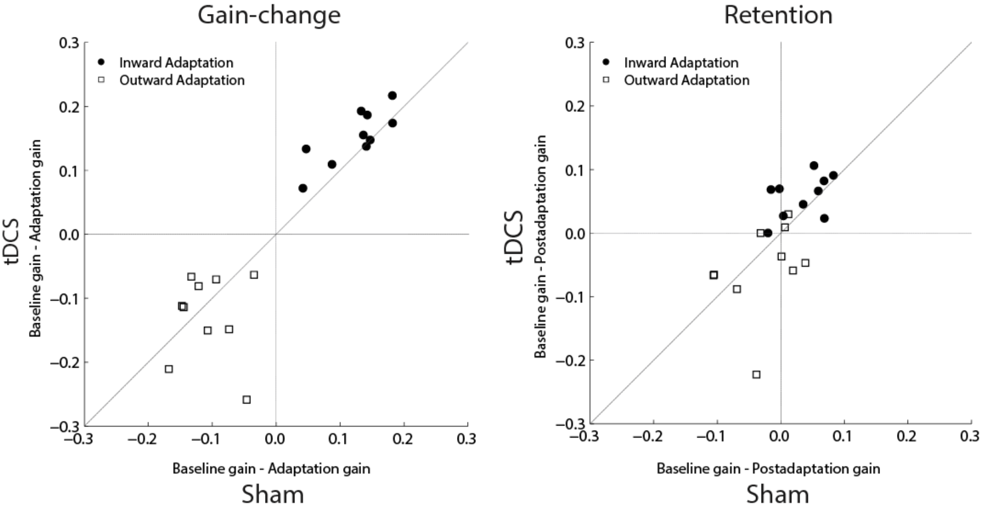

Figure 2. Adaptation gain-change and retention contrast between tDCS and sham condition.

Left, gain-change for inward and outward adaptation in which we can observe higher changes in gain (learning) for inward saccade adaptation with anodal cerebellar tDCS *compared to sham stimulation. No difference is observed in outward saccade adaptation gain-change between anodal cerebellar tDCS and sham stimulation. Right, Retention (difference between baseline and post-adaptation) for inward and outward adaptation in the two stimulation conditions.*

The optimal adaptation gain-change (difference between baseline and the last 10 adaptation trials) is of 25% (gain of 1 to 0.75). The observed adaptation gain-change was larger in the cerebellar tDCS condition than in the sham condition (0.15 ± 0.03 vs 0.12 ± 0.04, $t(9) = 2.26$, $p = 0.04$, Figure 2). Difference in retention, which reveals learning residual between the two conditions, was just not significant ($t(9)= 2.09$, $p = 0.06$, Figure 2).

We also assessed if differences in saccade kinematics were present. Repeated measures MANOVA analyses revealed an effect of Phase on peak velocities (baseline: 518 ± 21 deg/s, adaptation: 452 ± 22 deg/s, post-adaptation: 499 ± 29 deg/s, $F(2, 8) = 17.45$, $p = 0.001$, $\eta2 = 0.814$), but the effects of tDCS Condition ($F(1, 9) = 1.00$, $p = 0.34$, $\eta2 = 0.101$) or the interaction between tDCS Condition and Phase were not significant ($F(2, 8) = 1.24$, $p = 0.33$, $\eta2 = 0.23$; Figure 3A). No significant effects were found for saccade durations (Figure 3B).

Outward adaptation

Here, participants were subjected to an outward intra-saccadic jump of the target in the adaptation phase. As in inward adaptation, subjects received anodal stimulation during baseline and adaptation phases (Figure 1A, B). Figure 1D shows an example subject during the outward adaptation experiment in the two conditions. The resulting data from all subjects was approached in the same way as the previous experiment. The MANOVA analyses presented a main effect of Phase on saccadic gains ($F(2, 8) = 51.10$, $p = < 0.0001$, $\eta2 = 0.927$) and on the tDCS Condition ($F(1, 9) = 8.36$, $p = 0.01$, $\eta2= 0.482$), whereas the tDCS Condition and Phase interaction was not significant ($F(2, 8) = 0.658$, $p = 0.544$, $\eta2 = 0.141$; Table 1).

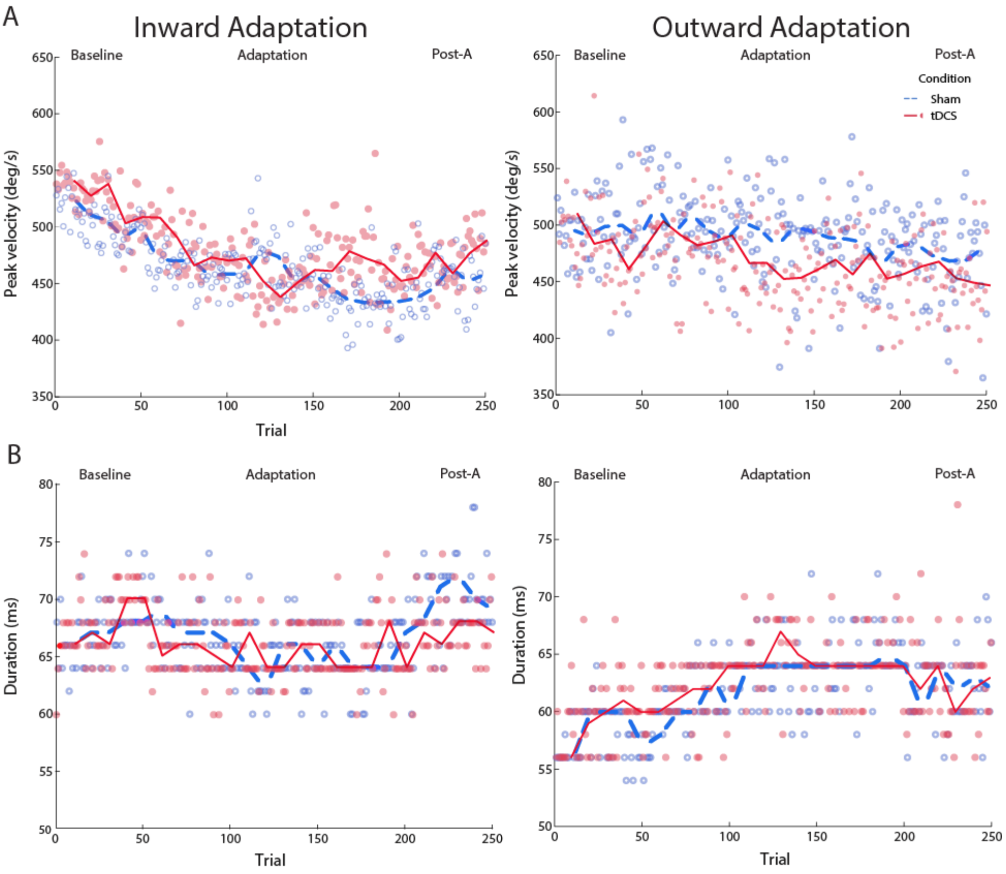

Figure 3. Saccade kinematics for sham and tDCS conditions.

Left Panel shows inward adaptation experiment and right outward adaptation experiment. A, presents peak velocity evolution throughout the trials as median values for all the subjects. Line on top depicts blocks composed of the median values of 10 trials. For inward adaptation (left) a clear reduction of the velocity is observed as gains become smaller, not present in the same way for the increasing gains in outward adaptation (right). B, shows saccade durations as median values for all subjects. Line on top depicts blocks composed of the median values of 10 trials. On the left, saccade durations become slightly smaller as gains become smaller. On the right, saccade durations increase as the task evolves as a result of saccade lengthening.

Planned comparisons did not show any statistical difference between the two tDCS conditions. When observing group data during baseline, subjects present relatively smaller gains in sham condition compared to tDCS (sham 0.98 ± 0.03, tDCS 1 ± 0.02, t(9) = 1.79, p = 0.10). Subjects also presented a normal course of adaptation throughout the trials increasing their gains (sham 1.08 ± 0.04, tDCS 1.12 ± 0.07, t(9) = 1.97, p = 0.08), to thereafter decrease them in the post-adaptation phase (sham 1 ± 0.06, tDCS 1.05 ± 0.07, t(9) = 2.23, p = 0.05), not reaching baseline again (Figure 1F).

Here we also assessed the amount of learning (gain-change) of each individual by comparing the baseline and the last 10 trials of the adaptation phase. The ideal amount of change in adaptation was 0.25, from 1 to 1.25. No significant differences were found between sham and tDCS conditions (t(9) = 0.79, p = 0.44) or for retention (t(9) = -1.21, p = 0.25, Figure 2).

Kinematic differences were assessed in the same way as gains. No effects of tDCS Condition or their interaction between Phase and tDCS Condition, except for an effect of Phase on saccade durations as a result of gain increase (baseline 58 ± 1 ms, adaptation 67 ± 4 ms, post-adaptation 63 ± 1 ms, F(2, 8) = 21.89, p = 0.001, η2 = 0.84; Figure 3).

Comparison between Inward and Outward adaptation

Inward and outward adaptation did not differ from each other in the seven subjects that participated in both experiments with respect to adaptation gain-change (0.16 ± 0.03 vs 0.11 ± 0.07, Wilcoxon Z = -1.35, p = 0.17) or retention (0.05 ± 0.03 vs. 0.06 ± 0.07, Wilcoxon Z = -0.33, p = 0.73).

Discussion

We observed that applying tDCS with a small contact electrode at 1.5 mA in an inward saccade adaptation experiment, with a 25% backward intra-saccadic step, induces a greater gain reduction when compared to sham condition. The effect of tDCS on gain-change is just not significant for outward adaptation, probably due to the low number of subjects.

Saccade adaptation is a widely used model for motor learning. When the eyes land on a location a target was displayed, the oculomotor system detects that an error has been made and updates its motor commands to adjust its amplitude on a trial-by-trial basis. We have explored the use of tDCS in a different type of motor learning in which previous results from other research groups have demonstrated the effect of this type of stimulation on cerebellar output. Galea et al. (2011) showed that anodal tDCS enhanced acquisition in a visuomotor transformation task by stimulating over the cerebellum and other experiments have also shown the effects of cerebellar tDCS in learning *(Jayaram et al., 2012)*, attention (Pope & Miall, 2012) or working memory (Boehringer et al., 2013; Ferrucci et al., 2008). The results of this study also show that tDCS exerts modulatory effects in behavior when applied to the cerebellum. The affirmation for the confined effects of the stimulation over the cerebellum is demonstrated mostly by previous reports of similar configurations which did not find any effects on brainstem or visual cortex, (Ferrucci et al., 2013; Galea et al., 2009; Zuchowski et al., 2014) and the use of modeling techniques, *(Miranda, Faria, & Hallett, 2009; Parazzini et al., 2014)* in which the current flow has been proven uniform *(Rahman, Toshev, & Bikson, 2014b)* with good sensitivity and response by Purkinje cells (PC) *(Chan & Nicholson, 1986).*

During the post-adaptation the subjects must de-adapt and any difference in this phase could indicate an effect on retention or a continuous effect of tDCS on (de-) adaptation. While we did not see any significant difference the groups here, our sense is that this does not necessarily reflect a real lack of effect. Our sample size and the degree of noise here make strong conclusions difficult. In any case, this is not the main issue that this research sought to address. This finding is consistent with the work of Galea et al. (2011), Jayaram et al. (2012), and Zuchowski et al. (2014) who observed differences in the speed of adaptation but found no post-stimulation effects in the extinction rate of the learned response in their tDCS group.

There are some possible explanations for the lack of differences between the two conditions in outward adaptation. The mechanisms for these two types of adaptation are not completely understood and are thought to involve different neural substrates. Diverse theories explore why this could be happening, such as a natural tendency of the system to be hypometric, and this way reducing gains will develop in a faster way than increasing them *(Hopp & Fuchs, 2004)*. A study by

Liem et al. (2013) using functional MRI, showed that forward and backward error target shifts elicited different cerebellar activation patterns. Also, different behavioral mechanisms might be in place for the two types of adaptation, namely a target remapping for outward adaptation *(Ethier et al., 2008)*.

Results by Panouillères, Miall, and Jenkinson (2015) on saccade adaptation showed that anodal stimulation tended to slow down adaptation in both directions, while cathodal enhanced outward adaptation. Differences could arise on account of different electrode size, position, current, and time of stimulation as with other studies where apparent opposite effects might be present.

On inward adaptation, significant differences were found in peak velocities due to the gain-decrease adaptation and on outward adaptation we only observed a significant increase in the duration of the saccades as a result of adaptation. This suggests that tDCS is exerting an effect in the stages or at a level where saccade kinematics are not coded yet. This supports the notion that tDCS actually affects adaptation and not the saccade generation per se *(Frens & Opstal, 1994)*.

Direct comparison between the two paradigms yielded no significant results on the tDCS effects. Despite the fact that the effect sizes are almost similar for the two experiments, outward adaption presents larger noise in the resulting data. We presume that this increased noise does not prevent tDCS from having an effect on performance or learning, but it may still cause that the effect of tDCS on outward adaptation failed to reach significance. The current inability to stimulate specific areas in the cerebellar cortex could also account for the apparent lack of response in outward adaptation. Another probable source is a difference in the mechanisms needed to elicit either type of adaptation. In other words, we think a preliminary hypothesis that the effect exists in both inward and outward adaptation is a good starting point for further exploration. Total cerebellectomies abolish complete means or adaptation *(Optican & Robinson, 1980)*, oculomotor vermis inactivation *(Jenkinson & Miall, 2010)* impair adaptation without affecting the production of saccades. Results from Kojima, Soetedjo, and Fuchs (2011) inactivated the same area with total incapacity for outward adaptation and a partial effect for inward adaptation. An MRI-guided TMS study *(Panouillères et al., 2012)* on Crus I had a dual effect on saccade adaptation, potentiating gain-up adaptation after-effects and depressing gain-down adaptation. We suggest that tDCS might have enhanced the cerebellar plastic mechanisms needed for a more prominent participation of the cerebellum in inward adaptation.

Being able to modulate cerebellar output earns particular interest as PC change their firing pattern in response to saccade adaptation. As observed by *(Catz, Dicke, & Thier, 2008)* while recording PC activity in primates performing an inward and outward adaptation task, they observed a change in the population burst throughout the course of adaptation. The population signal may have a modulatory role throughout the saccade, which could in turn be modulated or broadened by applying tDCS *(Scudder, 2003)*. This way, tDCS possibly elicits regional modifications to cerebellar output during saccade adaptation *(Galea et al., 2009)*. Extracellular recordings in primates have shown that inward adaptation increased PC complex spike activity *(Soetedjo, Kojima, & Fuchs, 2008)*. Consequently, PC activity may be enhanced and more ‘sensitive’ to error at the individual level; and at a regional level, tDCS might engage faster areas that are available for adaptation *(Jayaram et al., 2012)*. Assumptions of a local and regional cerebellar stimulation are further supported by modeling studies *(Rahman et al., 2013)* where somatic polarization together with axon terminal polarization seem to be key to the direct current response.

Another possible mechanism tDCS could possibly be influencing is by affecting short-term plasticity through brain-derived neurotrophic factor (BDNF). BDNF is involved in synaptic plasticity and its secretion affects motor learning in humans *(McHughen et al., 2010)*. TrkB, the receptor for BDNF, is located at the parallel fiber to PC synapse, where plasticity in the cerebellum takes place and might be regulating PC/parallel fiber mechanisms underlying short-term synaptic plasticity *(Carter, Chen, Schwartz, & Segal, 2002; Numakawa, Takei, Yamagishi, Sakai, & Hatanaka, 1999)*. Tests have shown that direct current stimulation plays a critical role in long-lasting synaptic potentiation in mouse slices *(Brita Fritsch et al., 2010)*. At this moment, only inferences can be made of how tDCS might be working at a cellular level and more studies are needed in this area to elucidate what are the actual effects of tDCS at the PC level.

In conclusion, we showed an effect of tDCS over the cerebellum in an inward saccade adaptation task displayed by a greater gain-reduction compared to sham stimulation. We could not demonstrate a similar effect in the outward adaptation task, although we also could not rule one out. Moreover, we contribute to the evidence that cerebellar tDCS may be used to enhance cerebellar (oculomotor) function. TDCS could help lead the way to a better understanding of motor learning and how the cerebellum is contributing to each of these processes; therefore, more studies are needed to clarify the extent and the mechanisms through which tDCS can modulate cerebellar functions.

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Chapter 3. Neuromodulation of the cerebellum in explicit cognitive tasks

(PICTURE BY REPUTATTIONS)

CHAPTER 3

In this chapter, we will briefly discuss the effect of cerebellar and prefrontal tDCS in explicit cognitive tasks. In the prefrontal cortex, tDCS had been shown to enhance cognitive skills *(Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016)*, with more prominent effects after anodal stimulation than cathodal stimulation *(Jacobson, Koslowsky, & Lavidor, 2012)*. Moreover, performance on high-demanding cognitive tasks, such as working memory *(Marshall, Mölle, Siebner, & Born, 2005)* and mental arithmetic *(Pope, Brenton, & Miall, 2015)*, are also improved by prefontal tDCS. Other forms of neurostimulation techniques besides tDCS are used to modulate cognitive performance, such as TMS or cTBS. Several studies have shown to modulate cognitive performance with TMS and cTBS over the cerebellum in language prediction *(Argyropoulos & Muggleton, 2013; Lesage, Morgan, Olson, Meyer, & Miall, 2012)*, verbal working memory *(Desmond, Chen, & Shieh, 2005)* and phonemic fluency *(Arasanz, Staines, Roy, & Schweizer, 2012)*. Moreover, as described in section 1.2.1., modulatory effects are found following cerebellar tDCS in numerous explicit cognitive tasks, yet, the results are mixed and hard to compare due to methodology differences. There is, however, consensus about the role of the cerebellum on cognitive performance in tDCS studies. Authors claim that the cerebellum is actively involved in working memory, as suggested by previous research *(Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005; Miller, Valsangkar-Smyth, Newman, Dumont, & Wolford, 2005)*, and stimulation of the cerebellum affects performance on high workload tasks. Nonetheless, these ideas have not been investigated thoroughly. Hence, in the next chapter, a study will be described investigating cerebellar tDCS in a classic working memory task, were different workloads are actively manipulated and polarity effects of cerebellar tDCS will be explored.

CHAPTER 3

3.1 Cerebellar tDCS does not affect performance in the N-back task

Journal of clinical and experimental neuropsychology 2016: B. W. van Wessel*, M.C. Verhage*, P. Holland, M. A. Frens, J. N. van der Geest.

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Abstract

The *N-back task* is widely used in cognitive research. Furthermore, the cerebellum’s role in cognitive processes is becoming more widely recognized. Studies using trancranial direct current stimulation (tDCS) have demonstrated effects of cerebellar stimulation on several cognitive tasks. Therefore, the aim of this study was to investigate the effects of cerebellar tDCS on cognitive performance by using the N-back task. The cerebellum of 12 participants was stimulated during the task. Moreover, the cognitive load was manipulated in N=2, N=3 and N=4. Every participant received three tDCS conditions (anodal, cathodal and sham) divided over three separated days. It was expected that anodal stimulation would improve performance on the task. Each participant performed 6 repetitions of every load in which correct responses, false alarms and reaction times were recorded. We found significant differences between the three levels of load in the rate of correct responses and false alarms, indicating subjects followed the expected pattern of performance for the N-back task. However, no significant differences between the three tDCS conditions were found. Therefore, it was concluded that in this study cognitive performance on the N-back task was not readily influenced by cerebellar tDCS and any true effects are likely to be small. We discuss several limitations in task design and suggest future experiments to address such issues.

Introduction

The N-back task is a widely used cognitive task that measures working memory capacity *(Gevins & Cutillo, 1993; Jaeggi, Buschkuehl, Perrig, & Meier, 2010; Jonides et al., 1997; Veltman, Rombouts, & Dolan, 2003)*. In its basic form, stimuli are sequentially presented, and the participant has to decide whether the currently presented stimulus is the same as the one presented one, two or more trials before. By increasing the number of trials between the current trial and the relevant trial before, referred to as N, the task becomes more difficult, which is known as increasing the cognitive load. Imaging studies have shown involvement of the left prefrontal cortex (PFC) in the N-back task *(D'Esposito et al., 1998; Owen, McMillan, Laird, & Bullmore, 2005)*. With increasing N, activity in this area increases as well *(Veltman et al., 2003)*. Moreover, stimulation of the PFC using Transcranial Magnetic Stimulation (TMS) has shown to modulate performance on the N-back task *(Mottaghy, 2006)*.

Transcranial Direct Current Stimulation (tDCS) is an emerging technique to investigate the relationship between specific brain areas and behavior (Nitsche & Paulus, 2011). Several studies on various cognitive tasks have observed modulatory effects of tDCS on task performance. Both anodal and cathodal stimulation on various brain areas have been found to have modulatory effects on various cognitive tasks *(Jacobson et al., 2012)*.

With respect to the N-back task, a few studies have observed improvements of performance after anodal stimulation of the left PFC in the N-back task on accuracy *(Fregni et al., 2005; Martin et al., 2013)* and reaction time *(Teo et al., 2011)*. tDCS changes cortical excitability by delivering a weak current (between 1 and 2 mA) through the scalp, which can have prolonged effects on task performance *(Dayan, Censor, Buch, Sandrini, & Cohen, 2013)*. For example, anodal stimulation over the primary motor cortex enhances performance on a motor task as reaction times decreased over time (Nitsche et al., 2003). tDCS on the PFC has been shown to affect performance in several cognitive tasks, including the N-back task *(Gladwin, den Uyl, Fregni, & Wiers, 2012; Martin et al., 2013)*, but also see the recent review by Horvath and colleagues (2015). Enhancement of performance in the N-back task using tDCS on the left PFC was observed with higher cognitive loads only, which may indicate the contribution of the PFC in complex cognitive and working memory tasks.

Over the past few decades, interest in the role of the cerebellum in cognition, in addition to its known importance in motor control, has increased *(Hayter et al., 2007; Schmahmann & Sherman, 1998)*. Anatomically, the cerebellum is reciprocally connected to various areas of the cortex, including the motor cortex and the prefrontal cortex via independent loops *(Kelly & Strick, 2003)*, which suggests that the cerebellum supports the motor and cognitive processes carried out by these cortical areas *(Ramnani, 2006)*. A cerebellar hemisphere is connected to the contralateral hemisphere of the cortex. Lesion studies confirm the idea of cerebellar involvement in cognition, by showing that right posterior damage to the cerebellum leads to cognitive deficits, in particular executive function, verbal working memory and attentional processes *(Timmann, 2007)*. Patients with cerebellar lesions have lower scores in attention and working memory tasks than healthy subjects *(Gottwald, 2004)*. In addition, children with cerebellar tumors show impairment of development of cognitive functions *(R.B. et al., 2001)*.

More evidence of cerebellar involvement in cognition comes from neuro-imaging studies. PET and functional MRI studies shows cerebellar activity in many tasks involving various cognitive processes like selective attention, visual and phonological working memory and semantic memory retrieval *(Cabeza & Nyberg, 1997; Stoodley, 2012)*. In a memory task increases in cognitive load are related to more cerebellar activation *(Kirschen et al., 2005)*. In that study, participants first had to memorize a set of stimuli of increasing load (two or six letters) and later had to decide which of two shown stimuli was present in the set they saw earlier.

Cerebellar activity during an auditory version and a visual version of the N-back task has also been reported in fMRI studies *(Hautzel, Mottaghy, Specht, Müller, & Krause, 2009; Salmi et al., 2010)*. In the visual version, participants performed a two-back task with both letters and abstract figures. In both tasks, left and right cerebellar activity was observed. In the auditory task, participants performed an N-back task with different pitched chords. Changing the task from a one-back task to a two-back task increased cognitive load. Significant load-dependent activations were observed in both the left and right cerebellum. More cerebellar activation, particularly on the right side, was observed with higher cognitive loads *(Jonides et al., 1997; Salmi et al., 2010)*. Finally, as with the PFC, TMS on the right superior cerebellum increases the reaction times (but not accuracy) of a working memory task *(Desmond et al., 2005)*.

The goal of the present study is to examine the effects of cerebellar tDCS on the N-back task. Similar to the previously observed effects of anodal left PFC stimulation *(Fregni et al., 2005; Teo et al., 2011)*, we hypothesized that anodal right cerebellar stimulation would improve performance as indicated by more hits, less false alarms and/or faster reaction times than for sham stimulation or cathodal stimulation. Cathodal stimulation might even be detrimental to performance, increasing, for instance, reaction times. We also expect a bigger effect of tDCS with a higher cognitive load. A within-subjects design is used to avoid confounds of individual differences.

Methods

Participants

Twelve healthy people (6 females) gave informed consent prior their participation in this study, which consisted of three experimental sessions. Ages ranged between 18 and 45 years (M = 29.9 years, SD = 11.0 years). All subjects came from the general population, (had) attended at least a high school, and were without any known neurological or psychiatric disturbances.

Participants did not receive a reward for their participation. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. The study took place at the Department of Neuroscience at the Erasmus Medical Centre in Rotterdam.

Task and Stimuli

The N-back task was implemented in MatLab (R2010a, version 7.10.0.499) based on the version used by Hoy et al. (2013) and by Thürling et al. (2012) and presented on a laptop (model Sony Vaio VPCEA3S1E, 14").

The experiment consisted of three sessions run on separate days. In a single session, 18 blocks of 48 trials each were presented. The participant started a block by pressing a key, allowing him or her to take a break between blocks. In a single trial, a single letter was presented for 500 ms in the center of the screen, followed by a blank screen for 1000 ms. The letter was an A, B, C, D or E. The participants were instructed to press a key on the keyboard of the laptop as fast as possible when they thought the letter was the same as N trials earlier. The participants had to respond within 1000 ms after onset of the trial. The value of N determined the load of the N-back task. Within each block, 25 trials required a key press to be denoted a correct trial—that is, in 25 trials the letter was the same as N trials before.

The load of the block (N) was given before each block of 48 trials. The load could be two, three or four. Each of the three loads was presented 6 times in each session (referred to as repetitions). The order of the different loads was pseudo-randomized across blocks so that no load was presented twice in a row. The order of loads was the same for all three sessions.

For each key press, it was determined if it was a correct response (hit), or an incorrect response (false alarm). The reaction time, relative to the onset of a trial was also determined.

Transcranial Direct Current Stimulation (tDCS)

In an experimental session, subjects received either anodal, cathodal or sham cerebellar tDCS. tDCS was delivered by a DC stimulator (Neuroconn GmbH, Ilmenau, Germany) connected to a pair of 12 mm sintered Ag/AgCl ring electrodes. The stimulation electrode was placed over the right cerebellar hemisphere (3 cm lateral to the inion), and the reference electrode was placed on the left buccinator muscle (similar to Verhage, Avila, Van Der Geest, Frens, & Donchin, 2014). Anodal or cathodal direct current at 2 mA intensity was started 3 min before the first block and lasted the whole session. When stimulation started, all participants felt the current under both electrodes as a mild itching sensation.

This sensation disappeared after a few seconds. In the sham condition, current was only applied for 30 seconds to give participants the same sensation without affecting brain processes *(Gandiga et al., 2006; Nitsche & Paulus, 2009).*

In all three groups, a gradual ramp up and ramp down of the current in 30 seconds reduced unpleasant side effects. Participants could not distinguish sham and real tDCS conditions. tDCS started three minutes prior to the task to in order for stimulation to be applied throughout performance of the task.

Design

Before performing the actual experiment, participants performed in 30 practice trials for each load. During these practice trials, feedback was provided. When a false alarm was detected, a red “X” was displayed in the center of the screen. When subjects missed a target, the word “miss” was displayed. When a correct response was made, nothing was displayed. After the practice session, the actual experimental session started and the tDCS stimulator was turned on. The stimulation was administered during the experiment for 20 min.

Each participant ran three experimental sessions. Across these sessions, they received three tDCS conditions (anodal, cathodal and sham), separated by at least five days between the sessions, to avoid carryover effects of the stimulation. The order of tDCS stimulation was randomized according to a Latin square design and counterbalanced across subjects.

Data analysis

For each block of 48 trials, the number of hits and false alarms was calculated, as well as the average reaction time of the correct responses. The reaction times represent the reaction times on the hits, not on the false alarms. Data were analyzed in SPSS 19 using repeated measures analyses of variance (ANOVAs) with three within-subjects factors: load (3 levels: N = 2, N = 3 or N = 4), repetition (6 levels) and tDCS condition (3 levels: anodal, cathodal or sham). In case of sphericity violations, we report corrected estimations of the degrees of freedom. Post hoc tests were done using Bonferroni correction. The three outcomes measures (hits, false alarms, and reaction times) were analyzed separately. All reported values are means ± standard deviations. The threshold of significance was set at 5% ($\alpha = .05$).

Results

Figure 1 shows the task performance over the six blocks per load (N) for the three conditions of tDCS stimulation. There were main effects of load on task performance (Table 1). On average, increasing the load reduced the number of hits, $F(1.13,12.41) = 51.95$, $p < .001$. $\eta_p^2 = .83$, and increased the number of false alarms, F(2,22) = 18.07, $p < .001$, $\eta_p^2 = .62$. There was no main effect of load on reaction times, F(2,22) = 0.106, $p = .80$, $\eta_p^2 = .01$.

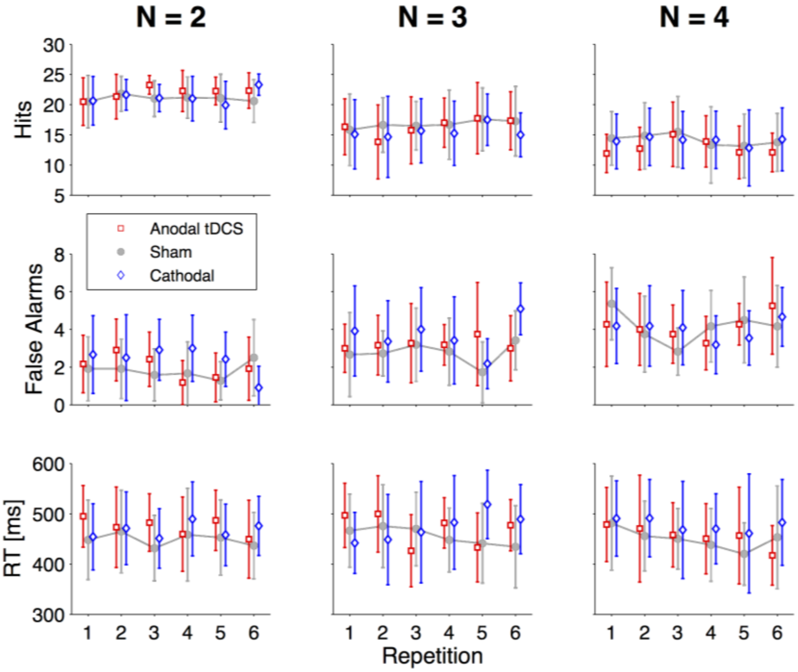

Figure 1. task performance over the six blocks per load (N) for the three conditions of tDCS stimulation.

N-back task performance over the 6 repetitions of a block with a specific load (N=2, N=3, or N=4), separated for the three cerebellar tDCS stimulation conditions (Anodal, Sham, Cathodal). Hits and False Alarms are scored per block of 48 trials. Each point shows the average of the 12 participants and error bars denote standard error of the means.

The main effects of tDCS stimulation on performance were not significant [hits: $F(2,22) = 0.17$, $p = .80$, $\eta_p^2 = .02$; false alarms, $F(2,22) = 1.12$, $p = .34$, $\eta_p^2 = .09$; reaction times, $F(2,22) = 1.13$, $p = .30$, $\eta_p^2 = .09$]. Furthermore, none of the interactions involving tDCS stimulation were significant (all $p > .30$ with effect sizes < 0.1). In addition, we found no effect of either stimulation condition when compared directly to sham using paired t tests (Table 2).

Table 1. Overall average performance per block for each of the three loads.

	Load (N)		
	N = 2	N = 3	N = 4
Performance	*Mean* ± *SD*	*Mean* ± *SD*	*Mean* ± *SD*
Hits**	21.43 ± 0.54	16.20 ± 0.86	13.71 ± 0.75
False Alarms**	2.07 ± 0.30	3.22 ± 0.43	4.08 ± 0.50
Reaction Times (ms)	463 ± 7	466 ± 7	461 ± 14

Note. 25 of the 48 trials in a block required a key press to be denoted as a hit.
** Effect of Load $p < 0.001$.

The main effects of repetition on performance were also not significant [hits: $F(5,55) = 1.56$, $p = .18$, $\eta_p^2 = .12$; false alarms: $F(5,55) = 1.82$, $p = .124$, $\eta_p^2 = .14$; reaction times $F(5,55) = 1.46$, $p = .20$, $\eta_p^2 = .12$], indicating that performance did not improve over blocks. The interaction between load and repetition was significant for hits, $F(10,110) = 22.77$, $p <.001$, $\eta_p^2 = .23$, and false alarms $F(2,22) = 18.07$, $p = < .01$, $\eta_p^2 = .62$, but not for reaction times, $F(10,110) = 0.68$, $p = .60$, $\eta_p^2 = .06$). The interactions were assessed comparing the effect of repetition for each load separately, yielding no effects of repetition on the hits for either load. For the lowest load (N=2), more false alarms were found in the first block compared to the five subsequent blocks of 48 trials, which was not observed in the other loads.

Discussion

The aim of this study was to examine the effects of tDCS over the cerebellum on performance in the N-back task. Previous research showed involvement of the cerebellum in this task (Hautzel et al., 2009; 2005; Owen et al., 2005), especially with higher loads *(Jonides et al., 1997; Kirschen et al., 2005)*. Based on the observations, improved performance after anodal left prefrontal tDCS, especially with higher cognitive loads *(Fregni et al., 2005; Teo et al., 2011),* we hypothesized that right anodal cerebellar tDCS would have similar effects.

As expected, increasing the load decreased performance: Participants in our study had fewer hits and made more false alarms. However, task performance was not significantly modulated by anodal or cathodal cerebellar tDCS. The statistical effect sizes of the direct comparison between anodal or cathodal stimulation and sham were small (between 0.02 and 0.24, see Table 2) and were also smaller than the effect sizes of other studies that did report an effect of tDCS stimulation *(Jacobson et al., 2012)*. Therefore, we conclude that in our study, tDCS over the right cerebellum does not critically influence performance in the N-back task.

Several previous studies using different cognitive tasks have observed performance changes with cerebellar tDCS. For instance, cerebellar tDCS has been shown to improve scores and reaction times on a Sternberg task, (Ferrucci et al., 2008) and on the Paced Auditory Serial Substraction Test (PASST; Pope & Miall, 2012) or to impair performance in the Digit Span Task *(Boehringer et al., 2013)*. However, a recent review meta-analysis suggests that the tDCS effects on cognitive processes may be not as prominent as proposed in the literature *(Horvath et al., 2015b)*. Therefore, an explanation for our results is that also cerebellar tDCS does not have modulating effects on cognitive processes.

Another explanation is that the cerebellum is not critically involved in learning the N-back task. It could be that this type of memory task relies much more upon processes in the prefrontal cortex as suggested by, for instance, imaging studies *(D'Esposito et al., 1998; Owen et al., 2005; Veltman et al., 2003)*. In the internal network model proposed by Ito (2002) the cerebellum and PFC are connected, but serve different memory processes: The PFC is involved in explicit memory and the cerebellum relates to implicit memory. One could argue that the N-back task is more explicit then implicit in nature and therefore the cerebellum is less involved. In turn, tDCS would then have little to no effect on performance. This can be tested by stimulating the PFC or the cerebellum in a within-subjects design allowing for a direct comparison between cerebellar and PFC stimulation effects.

Table 2. Performance per stimulation condition (Mean ± SD) and the statistics of the direct comparison between anodal or cathodal stimulation to sham.

	Stimulation condition			Anodal vs Sham			Cathodal vs Sham		
Performance	Sham (Mean ± SD)	Anodal (Mean ± SD)	Cathodal (Mean ± SD)	t	*p*	Cohen's *d*	t	*p*	Cohen's *d*
Hits	17.18 ±3.96	17.26 ±3.62	16.74 ±3.70	t(11)=0.11	*p* =.91	0.02	t(11)=0.39	*p* = .70	-0.11
False Alarms	3.03 ±1.04	3.30 ±0.97	3.56 ±1.42	t(11)=0.94	*p* = .38	0.04	t(11)=0.93	*p* = .37	0.07
Reaction Times (ms)	457 ±64	460 ±61	473 ±60	t(11)=0.21	*p* = .84	0.04	t(11)=1.14	*p* = .28	0.24

A within-subject design seems to be important in tDCS studies that investigate working memory. Studies using a between-subjects design often fail to observe an effect due to between-subject variability. For instance, Lally and colleagues observed that a group of subjects who received anodal tDCS stimulation on the prefrontal cortex in the N-back did not differ from a separate sham control group over time *(Lally, Nord, Walsh, & Roiser, 2013)*.

There are several limitations to our study. First, our sample size was small which may well have well contributed to the absence of statistical significance. Other studies on the effects of cortical tDCS on working memory did find effects with a small sample size (12 to 15 subjects; Horvath et al., 2015). However, in our study, the effect sizes of the analyses regarding tDCS stimulation were all small according to traditional metrics. This suggests that cerebellar tDCS does not seem to improve performance in the N-back memory task.

Another limitation could be the particular tDCS methodology we applied. Our present set-up using small electrodes of 1.13 cm^2 was based on a previous study in our lab in which we showed effects of cerebellar tDCS on saccadic eye movement learning *(Avila et al., 2015)*. Our protocol was also comparable with other cerebellar tDCS protocols. Other protocols do exist, and some of them are more commonly used than others. However, research on the effectiveness of various tDCS protocols is beyond the scope of this study *(Gandiga et al., 2006)*.

Future research should focus on optimizing tDCS effects for motor and cognitive tasks. tDCS shows an anodal-excitation and cathodal-inhibition effect for motor studies; however, for cognitive studies the polarity effect is not so distinct *(Jacobson et al., 2012)*. This diverse effect extends to cerebellar tDCS studies investigating cognitive tasks. Several studies have shown that anodal and cathodal tDCS can have similar, dissimilar or even no results in cognitive tasks *(Boehringer et al., 2013; Ferrucci et al., 2008, 2012; Pope & Miall, 2012)*

In conclusion, we found that cerebellar tDCS does not seem to improve performance in the N-back memory task. Since the number of subjects was rather small in our study, we cannot rule out the possibility that effects of cerebellar tDCS do exist. If these effects do exist, they are likely to be small. It could be worthwhile to compare prefrontal tDCS to cerebellar tDCS directly in a future study using more subjects.

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Chapter 4. Neuromodulation of the cerebellum in implicit cognitive tasks

(PICTURE BY REPUTATTIONS)

CHAPTER 4

As described before, results in cognitive tasks following cerebellar tDCS are mixed: varying from facilitation to inhibition, but also, show no effect at all. The reason for these diverse effects might rely in the origin of the tasks and the cerebellum's field of expertise.

TMS research has shown a dissociative role for the prefrontal cortex and the cerebellum when it comes to (motor) learning. The literature describes a more prominent role for the prefrontal cortex in observational learning and remembering, especially in the explicit part of the task. On the other hand, the cerebellum is assumed to be more involved in observational learning of the implicit part of the task *(Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004, 2007)*, which supports ideas by earlier lesion study research *(Gómez-Beldarrain, García-Moncó, Rubio, & Pascual-Leone, 1998; Molinari et al., 1997)*. These assumptions are in line with Ito's implicit thought theory, which states that explicit processes are based in the cerebral cortex and implicit processes occur in the cerebellum *(Ito, 2008)*. Building upon this notion, research with cerebellar tDCS have shown polarity-specific results in a procedural learning task: A serial reaction time task showed facilitatory effects on performance following anodal tDCS, whereas cathodal tDCS impaired performance *(Ferrucci et al., 2013; R. E. Shimizu et al., 2017)*, suggesting that more robust and polarity-specific effects can be found in implicit tasks following cerebellar stimulation.

Therefore, in the next chapter, two cognitive learning tasks will be investigated: a category learning task and a probabilistic classification task, which are both implicit by nature. Previous research has shown that tDCS and TMS over the cortex can modulate categorization performance *(Cho, Yoon, Lee, & Kim, 2012; Kincses, Antal, Nitsche, Bártfai, & Paulus, 2004; Perry & Lupyan, 2014)*, demonstrating that learning can be affected by stimulation in these kind of tasks. We expect that effects following cerebellar stimulation in an implicit cognition task will be more distinct compared to previous tDCS studies investigating cerebellar involvement in cognition using explicit tasks. Moreover, a pilot study and a replication study were included for more robust statistical inferences.

CHAPTER 4

4.1 Cerebellar tDCS does not enhance performance in an implicit categorization learning task

Frontiers in Psychology 2017: M. C. Verhage, E. Avila, M. A. Frens, O. Donchin, J. N. van der Geest

Abstract

Background: Transcranial Direct Current Stimulation (tDCS) is a form of non-invasive electrical stimulation that changes neuronal excitability in a polarity and site-specific manner. In cognitive tasks related to prefrontal and cerebellar learning, cortical tDCS arguably facilitates learning, but the few studies investigating cerebellar tDCS, however, are inconsistent.

Objective: We investigate the effect of cerebellar tDCS on performance of an implicit categorization learning task.

Methods: Forty participants performed a computerized version of an implicit categorization learning task where squares had to be sorted into two categories, according to an unknown but fixed rule that integrated both the size and luminance of the square. Participants did one round of categorization to familiarize themselves with the task and to provide a baseline of performance. After that, 20 participants received anodal tDCS (20 min. 1.5mA) over the right cerebellum, and 19 participants received sham stimulation and simultaneously started a second session of the categorization task using a new rule.

Results: As expected, subjects performed better in the second session than in the first, baseline session, showing increased accuracy scores, and reduced reaction times. Over trials, participants learned the categorization rule, improving their accuracy and reaction times. However, we observed no effect of anodal tDCS stimulation on overall performance or on learning, compared to sham stimulation.

Conclusions: These results suggest that cerebellar tDCS does not modulate performance and learning on an implicit categorization task.

Introduction

Over the past decades, transcranial Direct Current Stimulation (tDCS) has shown to be a promising tool for enhancing motor and cognitive learning in humans *(Ferrucci & Priori, 2014; Jacobson et al., 2012)*. While this has been shown for both cerebellar and supratentorial cortical tDCS in motor tasks, there have been inconsistent reports of enhanced cognitive learning following cerebellar stimulation.

Those studies that have examined cerebellar tDCS in cognitive tasks are focussed on explicit learning tasks, and this may be a partial explanation for the conflicting results. Explicit learning is a conscious process that involves predominantly the prefrontal cortex *(Ashby & Maddox, 2005)*. Implicit learning, on the other hand, is a subconscious process in which the cerebellum is more substantially involved *(Ito, 2008)*. With this dissociation in mind, we explored the cerebellar role in cognition by applying tDCS to the cerebellum in an implicit version of a learning task.

Transcranial Direct Current Stimulation over the cortex has shown different effects on cognitive learning. Compared to cathodal tDCS, the facilitating effect of anodal stimulation is more established *(Jacobson et al., 2012)*. Therefore, most brain stimulation research has investigated the effect of anodal tDCS over the prefrontal cortex and found facilitating effects on explicit problem solving, working memory and language tasks *(Brunoni & Vanderhasselt, 2014; Coffman, Clark, & Parasuraman, 2014; Monti et al., 2013)*. Also, several studies have investigated the effect of anodal tDCS on categorization; however, results are inconsistent. Stimulation over the left inferior frontal cortex improved performance on a simple, explicit categorization task *(Lupyan, Mirman, Hamilton, & Thompson-Schill, 2012)*. However, anodal (and cathodal) tDCS over the dorsolateral prefrontal cortex impaired categorization performance in a prototype distortion task *(Ambrus et al., 2011)*. Conflicting results were also found on a probabilistic classification task. A study found facilitating effects with anodal tDCS over the prefrontal cortex *(Kincses et al., 2004)*. However, a recent study was not able to reproduce this effect, highlighting the importance of replication *(Seyed Majidi et al., 2017)*.

The prefrontal cortex is primarily involved in cognitive processes; however, the role of the cerebellum in cognition is currently under debate. Imaging studies have consistently shown cerebellar activation in various cognitive tasks *(Balsters et al.,* *2013; Blackwood et al., 2004; Davis, Xue, Love, Preston, & Poldrack, 2014; E, Chen, Ho, & Desmond, 2014; Hayter et al., 2007; Helie, Roeder, & Ashby, 2010; Lam, Wächter, Globas, Karnath, & Luft, 2013; Stoodley, 2012; Tomasi, Chang, Caparelli, & Ernst, 2007)* including categorization tasks *(Milton, Wills, & Hodgson, 2009; Patalano, Smith, Jonides, & Koeppe, 2001)*. On the other hand, inconsistent results have been found in patients with cognitive impairment due to cerebellar lesions. Lesion studies have shown impaired categorization capabilities *(Bolcekova et al., 2012)* and abstract reasoning skills *(Schmahmann & Sherman, 1998)* in cerebellar patients, whereas other studies have not found any differences between lesion patients and healthy controls *(Ell & Ivry, 2008; Maddox, Aparicio, Marchant, & Ivry, 2005)*. These findings are in line with the notion of the cerebellum as an automating system. The prefrontal, or motor, cortex is essential for learning and the cerebellum automates these cognitive, or motor, processes; damage to the cerebellum results in impaired skilled performance and automaticity *(Balsters & Ramnani, 2008; Ramnani, 2006)*. Results of lesion studies should nonetheless be interpreted with care *(Timmann & Daum, 2010)*, especially since cause and location of cerebellar lesions vary widely between patients *(Gottwald, 2004)*.

This raises the question what the effect is of cerebellar tDCS on cognitive learning. A handful studies investigated the effect of cerebellar tDCS on explicit cognitive learning and reported promising effects. tDCS over the cerebellum impaired reaction time in a working memory task *(Ferrucci et al., 2008)* and impaired performance in a verbal working memory task *(Boehringer et al., 2013)*. Another study found facilitation on verbal responses in a verb generation task and addition task with cathodal tDCS *(Pope & Miall, 2012)*. The authors concluded that direct current stimulation over the right cerebellum affects working memory and attention differently depending on task difficulty and suggested that the cerebellum is capable of releasing cognitive resources when tasks become demanding. However, a recent cerebellar tDCS study investigating cognitive load in a working memory task was unable to confirm this hypothesis *(B. W. V. van Wessel, Claire Verhage, Holland, Frens, & van der Geest, 2016)*. Moreover, a small sample sized study investigating implicit cognitive learning in a probabilistic weather prediction task was unable to alter performance with cerebellar tDCS *(Seyed Majidi et al., 2017)*. The effect of cerebellar tDCS on various cognitive tasks have shown conflicting results. The majority of the abovementioned studies investigated the effects of cerebellar tDCS in explicit learning tasks. However, we believe results will be more consistent in an implicit learning task due to the substantial involvement of the cerebellum in implicit learning *(Ito, 2008)*. Moreover, previous research has shown modulatory effects of cerebellar tDCS on implicit learning in motor tasks *(Ferrucci et al., 2013; Galea et al., 2011)*.

We conducted a pilot study investigating anodal, cathodal and sham stimulation over the prefrontal cortex and cerebellum in a rule based (explicit) and information integration (implicit) categorization task. The pilot results in our implicit categorisation task showed improved accuracy scores in the cerebellar groups for anodal stimulation compared to cathodal and sham stimulation *(Verhage et al., 2014)*. The pilot study has a small sample size, and results should, therefore, be interpreted with care *(Slavin & Smith, 2009)*. We decided to partly replicate this pilot study in a larger sample. In this present study, based on the established effect of anodal tDCS *(Jacobson et al., 2012)* and our previous results *(Verhage et al., 2014)*, we investigate the effects of tDCS (anodal and sham) over the cerebellum in an information integration categorization task. We expect that anodal cerebellar tDCS will enhance performance during an implicit categorization task.

Materials and Methods

Subjects

Forty-one healthy right-handed subjects were recruited to participate in a single stimulation condition (age range: 20-31 years). The number of subjects was calculated according to the effect size observed in our pilot study (Cohen’s d = 0.56, alpha 5%, power 80%, yielding > 19 subjects per group). All subjects were college students and naïve to the experiment. Subjects were right handed, had no history of neurological deficits, no metal plate implanted in or near the head and no history of chronic drug abuse. In return for their participation subjects received course credit, and the highest scoring subjects received a small financial reward. The experiment took place at the Department of Neuroscience at the Erasmus MC in Rotterdam. The experiment was approved by the local medical ethics committee and adhered to the Declaration of Helsinki.

Experimental design

At arrival, participants signed the informed consent form and were seated before a laptop. The categorization task was explained by the experimenter and again on the computer screen. To determine basic categorization performance, subjects started with a baseline measurement without stimulation. After that, subjects executed an additional categorization task with tDCS (Figure 1). After every categorization task, subjects were asked to briefly describe how they categorized the grey squares, to see if they indeed used both dimensions (see task below) rather than one.

Subjects received anodal or sham stimulation over the right cerebellum. The study design had a randomized, single-blind, sham controlled between-subjects design. The entire experiment took approximately one hour.

Categorization task

The categorization task was performed on a 15-inch laptop computer. Stimulus presentation was done by custom-made software written in MATLAB (The Mathworks, Natick, MA, USA). The stimuli consisted of a square presented on a white background. Squares varied on two dimensions: size and luminance. A square could have one out of ten different sizes (side length ranged from 55 to 119 pixels) and one out of ten different luminances (black to nearly white), making up 100 different squares.

In a single trial, a fixation point was shown on the centre of the screen, followed by a square. The participant had to assign the square to category A by pressing the “Z”-key on the keyboard, or to category B by pressing the “M”-key, within 2 seconds. After pressing a key the subject received feedback for one second (Figure 1b). A short break was introduced every 50 trials. In total, 300 trials were presented in each stimulation condition (100 squares with three repetitions).

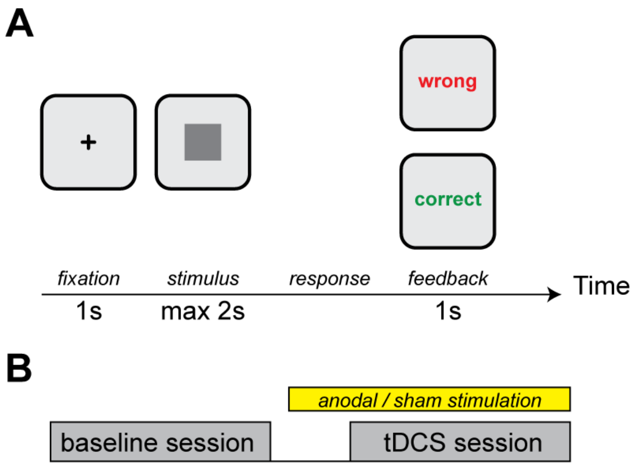

Figure 1. Experimental design.

(A) An example of a trial. Each trial started with a fixation cross followed by a square of a specific luminance and size. Participants received feedback on their categorization choice. (B) Design of the experiment. Subjects started with a baseline session, followed by a tDCS session during which participants received either anodal cerebellar or sham tDCS.

Subjects learned to categorize the squares without prior knowledge of how the categories were divided. During categorization, subjects aimed to employ an internal rule to classify the stimuli. Therefore, a large amount of simple, confusable stimuli were used to prevent subjects from remembering individual examples *(Ashby & Ell, 2001; Rouder & Ratcliff, 2006)*. The task used was an implicit category learning task; the goal is to combine information from two or more stimulus characteristics (information integration) to maximize accuracy, where the optimal rule is difficult or impossible to describe verbally *(Ashby & Maddox, 2005)*.

The rule dividing the categories was a combination of two stimuli dimensions (luminance and size). The categories were linearly separable. Subjects performed two implicit categorization tasks of the same complexity level; a baseline measurement without stimulation and an additional measurement with stimulation. In every categorization task, the same stimuli were used; however, the rule that divided the categories was different (Figure 2).

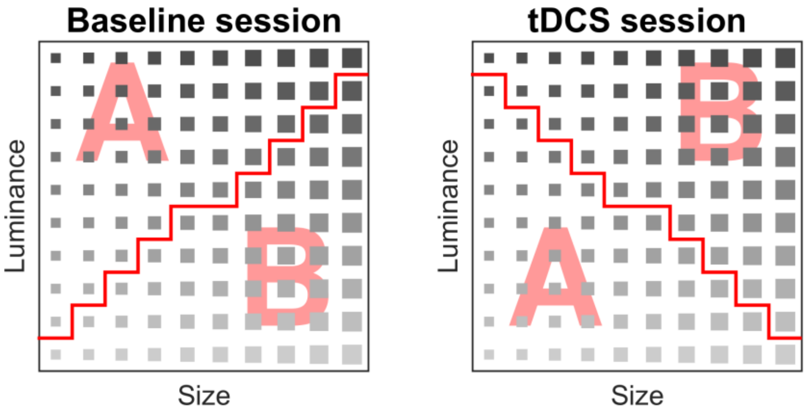

Figure 2. Stimulus matrix of the baseline and tDCS session.

A stimulus was assigned to category A or B. The red line denotes separation between category A and category B, i.e., the rule that participants implicitly learned during the task.

Transcranial Direct Current Stimulation (tDCS)

Transcranial Direct Current Stimulation (tDCS) stimulation was administered with a CE-certified constant current stimulator (NeuroConn, Ilmenau, Germany) through two annular sintered Ag/AgCl 12-mm diameter electrodes (MedCat, Erica, The Netherlands) with highly conductive gel (Signa Gel; Parker Laboratories, New Jersey, USA). The target electrode was placed over the right cerebellum 3 cm lateral to the inion, and the reference electrode was placed over the ipsilateral buccinator muscle. The tDCS was applied for 20 minutes with 1.5 mA (current density of 1.33 mA/cm^2). Sham stimulation was ramped up to 1.5 mA for 30 seconds and turned down after 60 seconds.

Data analysis

Participants who did not show a clear categorization strategy were removed from analysis. Data was analysed with SPSS (v20.0, IBM Corp., Armonk, NY, USA) and Matlab (The Mathworks, Natick, USA) Accuracy scores and reaction time were measured as dependent variables. For each session, we calculated the percentage of correct responses for each block of 25 trials (12 in total). We also calculated mean reaction time for each block. In addition, we calculated the percentage of correct responses, mean response times, and also the variance of the reaction times for the whole baseline session and for the whole stimulation session. Baseline performance was determined to assess potential group differences.

Statistical approach

The overall effect of tDCS was assessed by a mixed ANOVA with one between-participant factor stimulation condition (two levels: anodal and sham) and one within-participant factor session (two levels: baseline and tDCS). *T*-tests were used to investigate baseline performance and follow up comparisons. Additional analyses were performed comparing the first and last block. Analyses were performed for accuracy scores and response times separately. Performance was based on the average accuracy, reaction time and reaction time variance for every subject.

The effect of tDCS on learning was assessed by a mixed ANOVA with one between-participant factor stimulation condition (two levels: anodal and sham) and one within-participant factor block (12 levels: 12 blocks of 25 trials). In case of sphericity violations, we report corrected estimations of the degrees of freedom. All reported values are means ± standard deviations. The level of significance was set at $\alpha = .05$.

In addition to the frequentist method using Null Hypothesis Significance Testing (NHST), we also performed a Bayesian analysis to investigate tDCS effects on accuracy and reaction time (similar to Smittenaar, Prichard, FitzGerald, Diedrichsen, & Dolan, 2014). Inferences from Bayesian analyses are more informative than NHST, especially in the absence of experimental effects *(Kruschke, 2014)*. Here, we used it to statistically assess the observed data with a Bayesian model comparison analysis by fitting three models to our data: a null model, a main model and an extended model. The null model incorporates parameter block (to assess overall learning), the main model additionally incorporates the parameter stimulation condition (to assess the overall effect of tDCS), and the extended model further incorporates the interaction parameter block-by-stimulation condition (to assess the effect of tDCS on learning). To investigate our hypothesis that anodal cerebellar tDCS enhances implicit categorization learning task, we compared the null model to the main model, assessing overall effects of tDCS, and we compared the main model to the extended model, assessing an effect of tDCS on learning (similar to Smittenaar et al., 2014).

Results

Two subjects were removed from analysis because they did not show a clear categorization strategy based on two dimensions, leaving 39 subjects for analysis (13 males, 28 females; mean age ± SD: 22.8 years ± 2.3, age range: 20-31 years). Twenty participants formed the anodal tDCS group, and 19 participants belonged to the sham tDCS group.

Accuracy

Before stimulation, participants in both groups had similar accuracy scores (sham = 0.75 ± 0.10 %, anodal = 0.77 ± .08 %) in the baseline measurement (t (37) = 0.50, p = 0.62, d = 0.22). The ANOVA showed a significant effect of session (F (1, 37) = 10.50, $p < 0.005$, $\eta^2 = 0.22$). On average, participants performed better in the tDCS session compared to the baseline measurement (baseline = 0.74 ± 0.05 %, tDCS = 0.77 ± 0.05 %). The main effect of stimulation condition on accuracy was not significant (F (1, 37) = 2.11, p = 0.16, $\eta^2 = 0.06$). Moreover, the interaction effect between stimulation condition and session was not significant (F (1, 37) = 1.05, p = 0.31, $\eta^2 = 0.03$).

Subjects performed better over time (Figure 3). The ANOVA showed a significant effect of block (F (11, 407) = 4.33, $p < 0.001$, $\eta_p^2 = 0.11$). This was supported by the fact that participants performed better in the last block (0.80 ± 0.07 %) than in the first block (0.73 ± 0.09 %). The main effect of stimulation condition was not significant (F (1, 37) = 0.16, p = 0.69, $\eta_p^2 < 0.01$). The interaction between block and stimulation condition was significant (F (11, 407) = 1.95, p = 0.03, $\eta_p^2 = 0.05$), but *post hoc* comparisons per block showed no significant differences between sham and anodal stimulation.

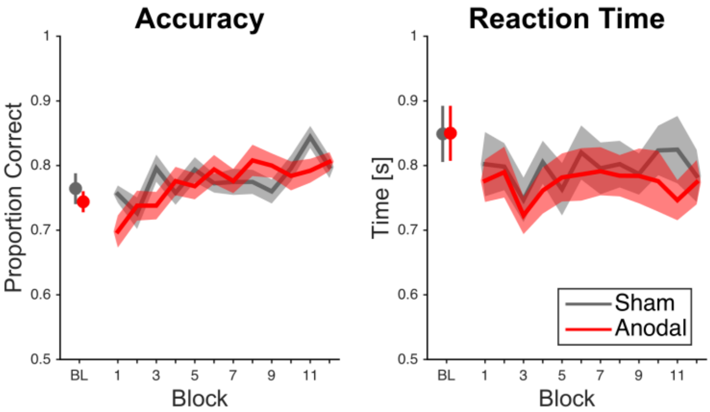

Figure 3. Raw mean accuracy scores and reaction times over time for cerebellar tDCS.

Data analysis on accuracy scores showed an interaction effect for Block and Stimulation Condition however significance did not survive Bonferroni correction. Error bars denote standard error of mean.

Reaction time

Before stimulation, participants had similar reaction times (sham = 0.87 ± 0.14 s, anodal = 0.88 ± 0.12 s) in the baseline measurement (t (37) = 0.23, $p = 0.82$, d = 0.15). The ANOVA showed a significant effect of session (F (1, 37) = 29.12, $p < 0.001$, $\eta^2 = 0.44$). On average, participants performed faster in the tDCS session (baseline = 0.87 ± 0.13 s, tDCS = 0.78 ± 0.13 s). The main effect of stimulation condition was not significant (F (1, 37) = 0.03, $p = 0.86$, $\eta^2 < 0.01$). Moreover, the interaction effect between stimulation condition and session was not significant (F (1, 37) = 1.04, $p = 0.32$, $\eta^2 = 0.03$).

The two groups had similar reaction time variance (both groups 0.03 ± 0.02 s) in the baseline measurement (t (37) = 0.74, $p = 0.46$). The ANOVA showed a significant effect for session (F (1, 37) = 14.14, $p < 0.005$, $\eta^2 = 0.28$). Subjects' responses were less variable in the tDCS session compared to baseline (baseline = 0.03 ± 0.02 s, tDCS = 0.01 ± 0.01 s). The main effect for stimulation condition was not significant (F (1, 37) = 0.39, $p = 0.54$, $\eta^2 = 0.01$). Moreover, the interaction effect between stimulation condition and session was not significant (F (1, 37) = 0.64, *p* = 0.43, $\eta^2 = 0.02$).

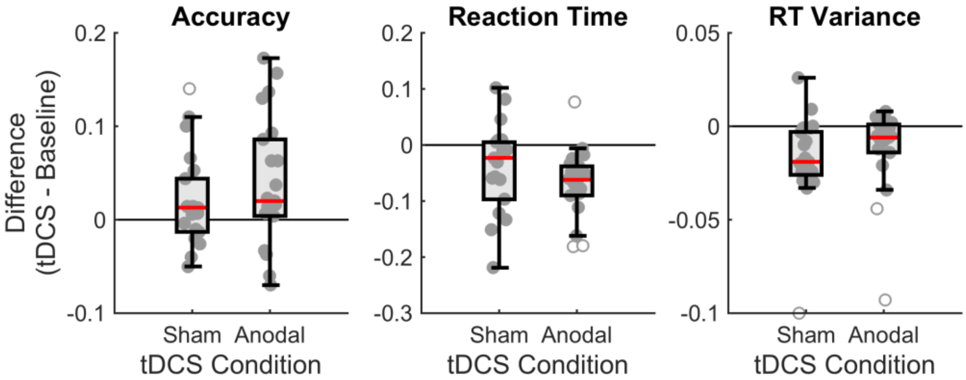

Figure 4. Boxplots of the differences between baseline and tDCS session for overall accuracy, reaction time and reaction time (RT) variance, for the two stimulation conditions (Sham and Anodal).

Each dot denotes an individual subject. The boxplot indicates the median (red line), the interquartile range (box) and the minimum and maximum (whiskers) after exclusion of outliers (open circles). For accuracy, positive scores indicate higher performance in the tDCS session. For reaction time, negative scores indicate faster performance in the tDCS session. For reaction time variance, negative scores indicate less variance in the tDCS session.

Subjects did not respond faster over time as the ANOVA did not show a significant effect of block (*F* (7.34, 271.46) = 0.91, *p* = 0.50, $\eta_p^2 = 0.02$ Greenhouse-Geisser corrected, ε = 0.67; Figure 3). This was further supported by the fact that participants did not perform faster in the last block (0.78 ± 0.16 s) than in the first block (0.79 ± 0.18 s; *t* (38) = 0.32, *p* = 0.75, *d* = 0.06). The main effect of stimulation condition was not significant (*F* (1, 37) = 0.32, *p* = 0.57, $\eta_p^2 < 0.01$). Moreover, the interaction between block and stimulation condition was not significant (*F* (7.34, 271.46) = 0.46, *p* = 0.87, $\eta_p^2 = 0.01$ Greenhouse-Geisser corrected, ε = 0.67).

Figure 4 summarizes our findings, showing that overall subjects performed better in the tDCS session (increases proportion correct, decreases reaction times and decreases variance of the reaction times) than in the baseline session, irrespective of the type of stimulation (anodal or sham).

Bayesian analysis

The lack of significant interaction effects obtained in the traditional null hypothesis testing, as presented above, only suggests absence of evidence for an effect of tDCS on implicit categorization learning, but, importantly, no actual evidence of absence *(Altman & Bland, 1995)*. The Bayesian analysis, however, did provide evidence against such interaction effects (see Table 1). We observed that the main model (including only the main effect of stimulation) is more plausible than the extended model (including the interaction between block and stimulation) for both response times and accuracy, as indicated by Bayes factors larger than 1 (6.36 and 2.26, respectively).

Table 1. Bayesian model comparison

Model	Response time		Accuracy	
	AIC	Bayes Factor	AIC	Bayes Factor
Null model	-344.65		-938.59	
Main model	-344.96	0.73	-937.12	4.37
Extended model (including interaction)	-343.11	6.36	-936.30	2.26

Bayesian results, showing the Akaike Information Criterion (AIC) for each of the three models, and the Bayes Factor comparing the Null model vs the Main model (BF>1 means the Null model is more plausible), and the Bayes Factor comparing the Main model vs the Extended model (BF>1 means the Main model is more plausible).

Discussion

In this study, we investigated the effects of anodal cerebellar tDCS in an implicit categorization task. Based on the reported effects of anodal tDCS *(Jacobson et al., 2012)* and results of our pilot study *(Verhage et al., 2014)*, we hypothesized that anodal cerebellar tDCS will enhance learning during an implicit categorization task. Compared to the baseline session, subjects improved on their overall performance in the second (tDCS) session, showing increased accuracy scores, reduced reaction times and reaction time variance, but this was independent of the type of stimulation (sham or anodal). As for learning, we did observe a small interaction effect, but *post hoc* comparisons failed to show any significant differences between anodal and sham stimulation over blocks. Additional Bayesian analysis provided evidence against an effect of anodal tDCS on learning over blocks. We, therefore, conclude that anodal cerebellar tDCS does not modulate performance and learning in an implicit categorization learning task.

The lack of tDCS effects on categorization performance is not that surprizing as its effects on cognition are still debated even for cortical tDCS *(Horvath, Forte, & Carter, 2015a)*. Recently, we also reported such absences of effect of cerebellar tDCS stimulation on the N-back memory task *(B. W. V. van Wessel et al., 2016)* and probabilistic categorization learning *(Seyed Majidi et al., 2017)*. This lack of a cerebellar tDCS effect on cognitive tasks could indicate that the cerebellum is not involved in cognition in general. This is, however, unlikely since several imaging studies *(Balsters et al., 2013; Blackwood et al., 2004; Davis et al., 2014; E et al., 2014; Hayter et al., 2007; Helie et al., 2010; Lam et al., 2013; Stoodley, 2012; Tomasi et al., 2007)* have shown cerebellar activity during cognitive tasks, including the task of categorization learning *(Milton et al., 2009; Patalano et al., 2001)*. Anatomical evidence also supports the idea that the cerebellum is involved in fine-tuning processes in the prefrontal cortex *(Balsters et al., 2010; Kelly & Strick, 2003)*. Moreover, anatomical connections between the cerebellum and the prefrontal cortex are likely to be cognitive in nature *(Ito, 2008; Ramnani, 2006)*. Another explanation for not finding an effect of stimulation is that cerebellar tDCS is unable to modulate cognitive functions. However, this does not explain the positive effects found on various other cognitive tasks in earlier cerebellar tDCS research *(Boehringer et al., 2013; Ferrucci et al., 2008; Pope & Miall, 2012)*.

Furthermore, the lack of tDCS effects in our study are somewhat surprising given the reported effects of cerebellar tDCS in several motor tasks in which the cerebellum plays an important role. It is reported that anodal tDCS on the cerebellum enhances, for instance, learning of hand movement control in visuo-motor adaptation *(Galea et al., 2011)* and force-field adaptation tasks *(Herzfeld et al., 2014)*. Cerebellar tDCS also seems to affect locomotor adaption *(Jayaram et al., 2012)*. Therefore, one could have expected that cerebellar tDCS has similar effects on cognitive learning given the uniform architecture and the overall capability to process both motor and cognitive information *(Ito, 2008; Kawato, 1999; Ramnani, 2006)*.

In our view, the most likely explanation for lack of cerebellar tDCS effect obtained here is that the cerebellum is not that critically involved in this type of cognitive learning. It is assumed that implicit learning in information integration tasks is dominated by an implicit procedural-learning-based system, which in turn is mediated by the caudate nucleus *(Ashby & Ell, 2001; Maddox & Ashby, 2004)* and the role of the cerebellum might be less prominent. Therefore, cerebellar tDCS could be less likely to modulate performance on our task. So, although it is widely acknowledged that the cerebellum is involved in cognition *(Koziol et al., 2014)*, it remains to be elucidated how the cerebellum contributes to specific cognitive processes.

A general problem in tDCS research, is the lack of standardized tDCS protocols. Furthermore, whether there is an effect of tDCS could depend on the level of task complexity (simple motor behaviour versus complex cognitive reasoning), stimulation intensity and/or the side of the stimulation. This could account for the conflicting outcomes reported in the tDCS literature. Yet, this does not explain the conflicting results of our pilot study, in which we observed an effect, and the current study, since identical tDCS protocols were used. This suggests that the positive effect of cerebellar tDCS found in the pilot study was observed by chance. Once more, this shows the importance of replication studies *(Vannorsdall et al., 2016)*. A limitation of the current study is the use of a between-subject design, which is not ideal for tDCS research because of high between-subject variability *(Li et al., 2015)*. Therefore, adopting a within-subjects design, where the test-retest effect is kept to a minimum, would be better for most tDCS studies. However, when it comes to study the effect of tDCS on categorization learning, a within-subject design is less feasible due to the inevitable changes in performance over sessions, irrespective of stimulation condition. In addition, randomizing the order of sham (or no) stimulation and anodal stimulation, might lead to problems with the assumed prolonged effects of tDCS when some subjects start with real stimulation.

Future research should focus on developing robust tDCS protocols. An earlier study investigating the effect of tDCS over the motor cortex on corticospinal excitability showed large variability in subject’s responsiveness to tDCS, which is in line with similar non-invasive brain stimulation studies. These results highlight the importance of robust tDCS protocols and the need to ascertain individual factors that determine tDCS responsiveness *(Wiethoff, Hamada, & Rothwell, 2014)*. Furthermore, future research should first aim to replicate promising effects of tDCS or related brain stimulation techniques (such as TMS or TBS; see Picazio, Oliveri, Koch, Caltagirone, & Petrosini, 2013) on cognitive processes before investigating new ground.

In conclusion, anodal tDCS applied over the cerebellum does not facilitated performance on an implicit categorization task and suggest that the cerebellum does not play a substantial role in implicit categorization based on the integration of information. Since we failed to replicate the positive results of our underpowered pilot study *(Verhage et al., 2014)*, the present outcome also highlights the importance of replication with sufficient power.

CHAPTER 4

4.2 Cerebellar tDCS does not improve performance in probabilistic classification learning

Experimental Brain Research 2016: N. S. Majidi*, M. C. Verhage*, O. Donchin, P. Holland, M. A. Frens, J. N. van der Geest.

*These authors contributed equally

Abstract

In this study the role of the cerebellum in a cognitive learning task using transcranial Direct Current stimulation (tDCS) was investigated. Using a weather prediction task, subjects had to learn the probabilistic associations between a stimulus (a combination of cards) and an outcome (sun or rain). This task is a variant of a probabilistic classification learning task, for which it has been reported that pre-frontal tDCS enhances performance. Using a between subject-design, all 30 subjects learned to improve their performance with increasing accuracies and shortened response times over a series of 500 trials. Subjects also become more confident in their prediction during the experiment. However, no differences in performance and learning were observed between subjects receiving sham stimulation (n=10) or anodal stimulation (2mA for 20min) over either the right cerebellum (n=10) or the left prefrontal cortex (n=10). This suggests that stimulating the brain with cerebellar tDCS does not readily influence probabilistic classification performances, probably due to the rather complex nature of this cognitive task.

Introduction

Probabilistic classification learning (PCL) tasks make use of cues that are variously predictive of class membership. For example, people can learn that certain cues are more often associated with category A than category B, despite the fact that no exclusive relationship exists between cue and category. Several neuroimaging studies have suggested involvement of the left prefrontal cortex in PCL tasks *(Aron, 2004; Flanery, 2005)*. Recently, an fMRI study showed that PCL tasks also induce activation in the right cerebellum as well as in the left orbitofrontal cortex, which increased as a function of the predictive value of stimuli *(Lam et al., 2013).*

The cerebellum is increasingly thought to be involved in both motor and non-motor functions *(Stoodley & Schmahmann, 2009; Timmann et al., 2010)*. Reciprocal cerebro-cerebellar connections connect the cerebellar hemispheres to various parts in the contralateral hemispheres of the cortex *(Ramnani, 2006; Strick, Dum, & Fiez, 2009)*. The general idea is that the anterior cerebellum, via the connections to the motor cortex, supports the cortex in learning new and modifying existing motor behavior *(Ito, 2000)*. The connections between the posterior cerebellum and pre-frontal cortex suggest that the cerebellum also plays a supportive role in learning cognitive behavior *(Balsters & Ramnani, 2011; Hayter et al., 2007)*. For both motor and cognitive learning tasks, it has been observed that stimulating the cerebellum non-invasively using transcranial direct current stimulation (tDCS) affects task performance and task learning *(Ferrucci & Priori, 2014; Jacobson et al., 2012).*

Here we studied the role of the cerebellum in cognitive learning, by assessing the effects of tDCS on performance in the weather prediction task. In this task, which is about learning probabilistic associations between cues and two weather categories, tDCS stimulation of the left prefrontal cortex (Fp3) allegedly improved task performance *(Kincses et al., 2004; Nitsche et al., 2007)*. We hypothesized that tDCS over the cerebellum also induces changes in performance in this PCL task. We predicted that stimulation of either the left prefrontal cortex or the right cerebellum would both result in improved learning in the weather prediction task, as indicated by a reduction of errors and a decrease of response times over the course of the experiment.

Material and methods

Participants

30 participants gave their informed consent to participate in this study, which was approved by the local ethical board. 13 of the participants were females and 17 were males. Ages ranged between 19 and 32 years (Mean (M) = 26.5 years, Standard Deviation (*SD)* = 3.4 years). All participants fulfilled the following inclusion and exclusion criteria: right handedness, normal or corrected-to-normal vision, no metallic implants in or near the head, no electronic implants, no history of neurological deficits and no history of chronic drug abuse. They were recruited through internet advertising; as a motivation the best performing participant received 30 euros.

Subjects were randomly assigned to one of the three groups of 10 participants each: the anodal cerebellar group (5 women, 5 men), the anodal prefrontal group (3 women, 7 men) and the sham group (5 women, 5 men). All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Task and Stimuli

Subjects performed a variation of the weather prediction task *(Gluck, 2002)* developed in Processing (version 2.0, available at http://www.processing.org). The experiment was performed on a laptop with a 15-in screen with full HD resolution (1920 x 1080 pixels) with cabled computer mouse.

In the experiment, participants were presented with 500 trials. In each trial, a visual stimulus was shown. For each stimulus, the participant had to indicate whether they thought the stimulus predicted sun or rain, based on their experiences in previous trials. They gave their response by clicking with the mouse on the corresponding symbol presented beneath the stimulus. After the response, the correct answer was presented.

The stimulus consisted of the combination of one, two or three distinct cards. In total there were four distinct cards containing four distinct geometric forms: a circle, a triangle, a square, and a diamond. Each card had a height of 200 pixels and a width of 200 pixels. The presence of each individual card was associated with one of both outcomes (sun or rain) with a fixed probability *(Gluck, 2002)*. The circle card was associated with rain in 75.6% of the trials in which a circle was part of the stimulus, for the diamond card this was 57.5%, for square 42.5%, and for triangle 24.4%. Over all trials, each of the four individual cards was used roughly the same number of times. This implied a specific probability for a stimulus, i.e., a combination of cards, to be associated with an outcome. The frequency of the stimuli and the probabilities of association with rain are shown in Table 1.

Transcranial Direct Current Stimulation (tDCS)

tDCS was delivered by a DC stimulator (NeuroConn GmbH, Ilmenau, Germany). The two 5x7 cm^2 electrodes were placed in synthetic sponges, which were soaked in a saline solution. Two electrode montages were used. In all participants of the prefrontal group, the anodal electrode was placed over the left prefrontal cortex (Fp3) and the reference (cathodal) electrode was placed over the right supraorbital region (on the forehead). In all participants of the cerebellar group, the anodal electrode was placed on the right cerebellar hemisphere (3 cm lateral to the inion) and the reference electrode was placed on the right buccinator muscle. In the sham group, each montage was used in half of the participants. In the prefrontal and cerebellar group, current was applied for 20 minutes with an intensity of 2.0 mA *(Ferrucci et al., 2008)*. In the sham group, current was only applied for 30 seconds to give participants the same tingling sensation as in the other groups. None of the subjects could distinguish the stimulation conditions. In all three groups, a gradual ramp up (fade in) and ramp down (fade out) of the current in 30 seconds reduced unpleasant side effects. Stimulation was started at the beginning of the experiment.

Questionnaire

After the test, participants had to rate the contribution of each of the four cards to the prediction of sun and rain on a scale from 1 (indicating a high contribution to sun) to 10 (indicating a high contribution to rain). They also had to rate their confidence in this rating on a scale from 1 (not sure at all) to 10 (very sure).

Table 1. Probability structure of the Weather Predication Task.

Type	Stimulus	n/100	Rain	Sun	P(Rain)	Optimal Response
A	▲	14	2	12	.143	Sun
B	■	8	3	5	.375	Sun
C	■ ▲	9	1	8	.111	Sun
D	◆	8	5	3	.625	Rain
E	◆ ▲	6	1	5	.167	Sun
F	◆ ■	6	3	3	.500	None
G	◆ ■ ▲	4	1	3	.250	Sun
H	●	14	12	2	.857	Rain
I	● ▲	6	3	3	.500	None
J	● ■	6	5	1	.833	Rain
K	● ■ ▲	3	1	2	.333	Sun
L	● ◆	9	8	1	.889	Rain
M	● ◆ ▲	3	2	1	.667	Rain
N	● ◆ ■	4	3	1	.750	Rain
Total		100	50	50		

Each of the 14 stimulus types (A-N) is a unique combination of 1 to 3 distinct cards from a set of four cards (circle, diamond, square, or triangle). For each trial the order of the cards within the combination was randomized. The column “n/100” denotes how many times a stimulus type occurred during a block of 100 trials. The columns labeled “Rain” and “Sun” denote how often the stimulus is combined with the outcome Rain or Sun, respectively. The column labelled “P (Rain)” is the probability that the weather outcome was “Rain” for a given stimulus type. The probability that the weather outcome was “Sun” is 1 minus P (Rain). For example, stimulus type G consisted of a diamond, a square and a triangle card, and was shown 4 times in a block of 100 trials; it was associated with an outcome “Rain” only once, so the optimal response for the participant would be to say that this stimulus predicted “Sun”.

Design and procedure

Participants were first informed on the safety of the tDCS procedure and the general procedure of the session. After giving consent, the tDCS montage was created and stimulation was applied. Five minutes after the beginning of tDCS stimulation, the experiment started. First, participants received the following literal on-screen instructions: “*Predict the weather: Rain or Sun? In this game you will learn to predict the weather by using the cards below: [pictures of the four cards were shown here]. In each round you will see a combination of these cards. You then decide if this combination predicts ‘rain’ or ‘sun’. First, your score is shown on the screen (maximum: 100, minimum: 0). Then you will see the cards and you will give your prediction by clicking on the ‘rain’ or ‘sun’ button. After clicking, the correct answer will turn green. Occasionally a pause screen will be shown so you can take a short break. Two more things: 1. The order of the cards does not matter, it’s only the presence of a card that matters. For example: ‘Circle Square Diamond’ is the same as ‘Square Circle Diamond’. 2. The best participant will receive 30 euro!*” [sic].

After the experimenter (NSM) made sure the instructions were understood the participant performed the task.

Before each trial, instead of a fixation cross a weighted score was presented for 0.5 seconds. This score was a weighted average of the participant’s performance on the last 10 trials and had a minimum value of 0 and a maximum value of 100. For each trial, this score was calculated with the following formula: $Score = 100 \cdot \sum (k=1:10)\ [\ (11-k) \cdot C_k\ ] / 55$, where k runs from 1 to 10, indicating the 10 previous trials. If a correct answer was given *k* trials before the current trial, C_k had a value of 1 (and a value of 0 if it was answered incorrectly). This score was used to keep the participant motivated. After an additional blank screen (presented for 0.3 seconds) a stimulus was presented together with a “sun” and “rain” symbol. The participant had a maximum time of 8 seconds to click on one of the symbols. Feedback was given for 1 second by removing the incorrect symbol and turning the correct symbol green. Finally the new weighted score was presented, indicating the beginning of the next trial (Figure 1).

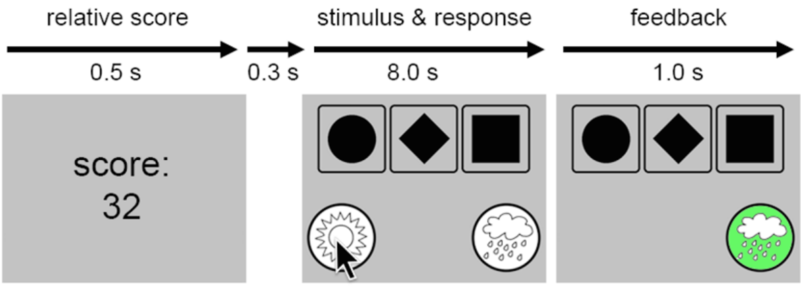

Figure 1. Trial example.

After the presentation of the weighted score (weighted average over the last ten trials) followed by a blank screen, a stimulus was presented (a given combination of the four cards, in this example, type N). After clicking with the mouse on one of the weather symbols, the correct answer was presented. In this example, the participant indicated that he believed that this stimulus corresponds to “sun”. In this particular trial this was not the case, although “sun” was indeed the optimal response for this stimulus, being the correct response in 3 out of 4 presentations (see table 1).

Subjects were presented with 10 blocks of 50 trials each. Pairs of two blocks contained all 100 trials as depicted in Table 1. Stimuli were divided as equally as possible over these two blocks, and we ensured that each block of 50 trials contained 25 stimuli that predicted rain. The order of stimuli was pseudo-randomized. All participants were presented the exact same order of stimuli.

Data analysis

Performance accuracy was based on the number of optimal responses given by a participant *(Gluck, 2002)*. The optimal response for a particular trial is that response that corresponds to the outcome (sun or rain) that is most probable for that stimulus (Table 1). Response times were measured by determining the time between the appearance of the stimulus and the mouse-click on the symbol of "sun" or "rain". Trials with stimulus types F or I were discarded, because they do not have an optimal outcome as they corresponded to Rain or Sun equally.

For each session we calculated the percentage of the given responses that were optimal for each block of 50 trials. We also calculated mean response time for each block. In addition we calculated the percentage of optimal responses and mean response times for the session as a whole.

To examine how participants performed on trials with different predictive values, separate accuracy scores were calculated for high informative and low informative trials. High informative trials consisted of stimulus types A, C, E, H, J, & L, because they had very high predictive values for either sun or rain (i.e., P (Rain) is close to either 0 or 1, see Table 1). Low informative trials consisted of stimulus types B, D, G, K, M, & N, because they had predictive values close to chance (0.5).

Statistical analysis

The effect of tDCS on learning was assessed by a mixed-design ANOVA with one between-participant factor tDCS condition (three levels: prefrontal, cerebellar, and sham) and one within-participant factor Block (10 levels: 10 blocks of 50 trials each). In case of sphericity violations, we report corrected estimations of the degrees of freedom. The overall effect of tDCS was assessed by a post-hoc one-way ANOVA with tDCS condition as a between-subject factor (three levels: prefrontal, cerebellar and sham stimulation). T-tests were used to compare the performance in high informative and low informative trials. Analyses were performed for accuracy scores and for response times separately. Additional analyses were performed comparing the first and last block.

To examine a relationship between speed and accuracy of responses, Pearson correlations between overall accuracy scores and overall mean response times were calculated for each tDCS stimulation condition separately, and for all participants combined.

Interaction effects of tDCS and questionnaire ratings were also assessed by a mixed-design ANOVA with one between-participant factor tDCS condition (three levels) and one within-participant factor Cards with four levels (one level per card). All reported values are means ± standard deviations. The threshold of significance was set at 5 percent ($\alpha = 0.05$).

Results

Overall accuracy

On average, participants gave more optimal response across all 500 trials than would be expected by chance (72 ± 9 %, range 55-89 %, t (29) = 13.0, $p < .001$, $d = 4.82$). The main effect of tDCS condition on accuracy was not significant (F (2, 27) = 2.30, $p = .12$) The post-hoc one-way ANOVA showed no significant differences in overall accuracy scores between right cerebellar (67 % ± 7 %), left prefrontal (75 % ± 11 %) and sham stimulation (73 % ± 8 %). Subjects preferred to report “sun” slightly more often than “rain” (52 % vs 48 % , resp.; sign test $Z = 2.23$, $p = .03$). The overall accuracy was higher in high informative trials (79 ± 11 %) than in low informative trials (57 ± 9 %, t (29) = 12.68, $p < .001$, $d = 2.34$).

Subjects performed better over time. Analysis showed a significant effect of Blocks, (F (4.55, 122.80) = 5.95, $p < .001$, $\eta_p^2 = .18$, Greenhouse-Geisser corrected, $\varepsilon = .51$). This was supported by the fact that, taken together, participants had higher scores in the last block (75 ± 15 %) than in the first block (64 ± 14 %, t (29) = 3.59, $p < .005$, $d = .66$).

The main effect of tDCS condition was not significant (F (2, 27) = 2.30, $p = .12$). Moreover, the interaction between Blocks and tDCS condition was also not significant (F (9.10, 122.80) = 5.95, $p = .85$), indicating that learning was not influenced by tDCS stimulation (Figure 2).

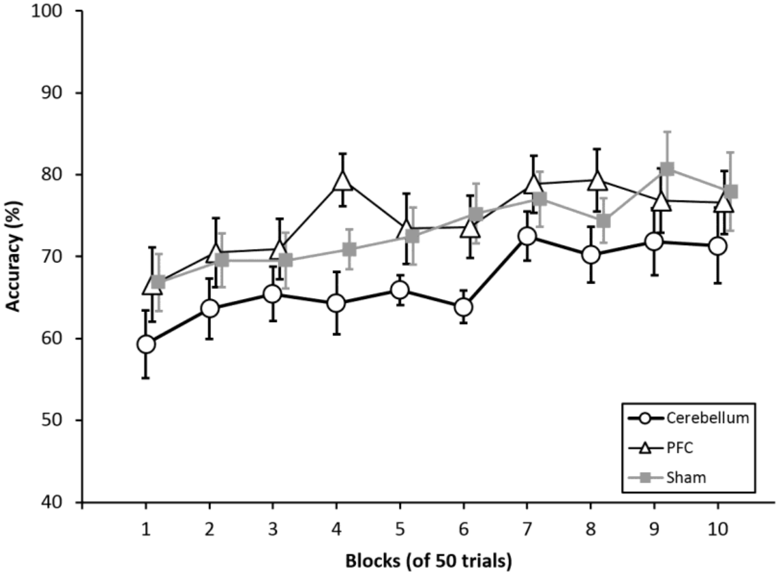

Figure 2. Accuracy.

The plot shows the average accuracy scores per block of 50 trials for each of the three stimulation conditions (right cerebellar, left prefrontal, or sham). The positive slope indicates that participants performed better over time. No interaction was found between stimulation condition and blocks. Error bars represent the standard error of the means.

Response times

Mean response times were obtained for each participant over the entire experiment (500 trials). Individual scores varied between .84 and 3.23 seconds (1.78 ± .50 s). Therefore, the total experiment time varied between 22 minutes and 42 minutes which is well within the time limits of 60 minutes for tDCS stimulation is thought to show an effect *(Monte-Silva et al., 2013; Nitsche & Paulus, 2001)*, 2012). The main effect of tDCS condition on response time was not significant (F (2, 27) = .78, *p* = .47). The post-hoc one-way ANOVA on overall mean response times showed no significant differences between right cerebellar (1.76 ± .54 s), left prefrontal (1.64 ± .58 s), and sham tDCS stimulation (1.93 ± .37 s). Response times for “sun” were the same as for “rain” (1.74 vs 1.79 s, resp.; sign test *Z* = 1.28, *p* =

.20). The overall mean response time was lower in high informative trials (1.67 ± .48 s) than in low informative trials (1.89 ± .53 s, t (29) = 7.13, $p < .001$, d = 1.36).

Subjects responded faster over time. The ANOVA showed a significant effect of Blocks, (F (4.72, 127.40) = 33.30, $p < .001$, $\eta_p^2 = .55$, Greenhouse-Geisser corrected, $\varepsilon = .52$). This was supported by the fact that participants had lower response times in the last block (1.44 ± .45 s) than in the first block (2.41 ± .63 s, t (29) = 9.58, $p < .001$, d = 1.83). The main effect of tDCS condition was not significant (F (2, 27) = .78, p = .47). Moreover, the interaction between Blocks and tDCS conditions was not significant (F (9.44, 127.40) = 1.78, p = .31), indicating that also the decrease in response times across the experiment were not influenced by stimulation (Figure 3).

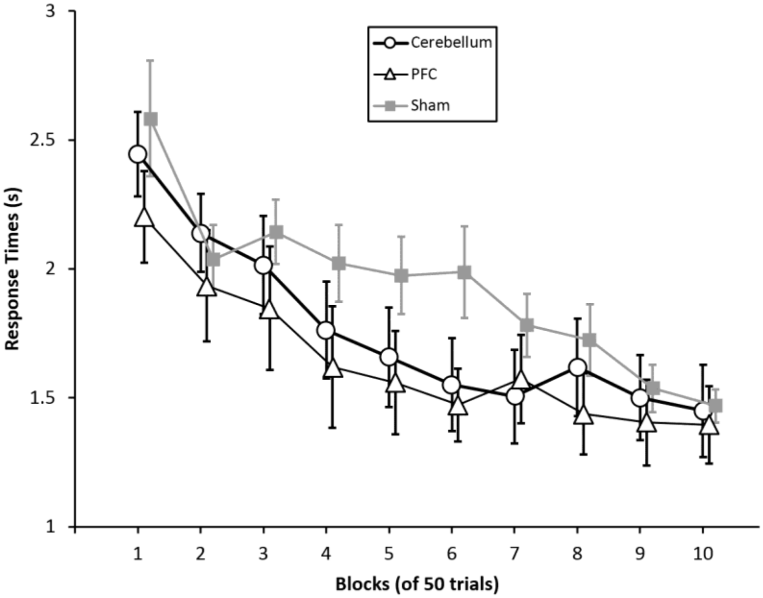

Figure 3. Response times.

The plot shows the average response times per block of 50 trials for each of the three stimulation conditions (right cerebellar, left prefrontal, or sham). The negative slope indicates that participants performed faster over time. No interaction was found between stimulation condition and blocks. Error bars represent the standard error of the means.

We performed an additional analysis including only the first and the last block in our ANOVA. This also showed a main effect of block for accuracy *(F*(1, 27) = 12.00, $p < .01$, $\eta_p^2 = .31$) and reaction time (*F* (1, 27) = 90.35, $p < .001$, $\eta_p^2 = .77$). Main effects of stimulation or interactions between stimulation and block were not significant (all p > .32).

No significant correlations between accuracy and response times were found in either of the tDCS conditions (sham: $r = -.59$, $p = .07$; right cerebellar: $r = .27$, $p = .45$; left prefrontal: $r = -.21$, $p = .56$). The correlation was also not seen when all 30 subjects were pooled ($r = -.14$, $p = .46$).

Questionnaire

Ratings of the contribution of each of the four cards to the prediction (Figure 4) were different for each card (*F* (2.68, 72.48) = 92.07, $p < .001$, $\eta_p^2 = .77$, Huyn-Feldt corrected, $\varepsilon = .76$). The main effect of stimulation condition and the interaction between card type and stimulation condition were not significant (both $p > .3$). Confidence ratings were also different per card type (*F* (2.93, 79.13) = 16.89, $p < .001$, $\eta_p^2 = .39$, Huyn-Feldt corrected, $\varepsilon = .83$), but again the main effect of stimulation condition and the interaction were not significant (both $p > .1$).

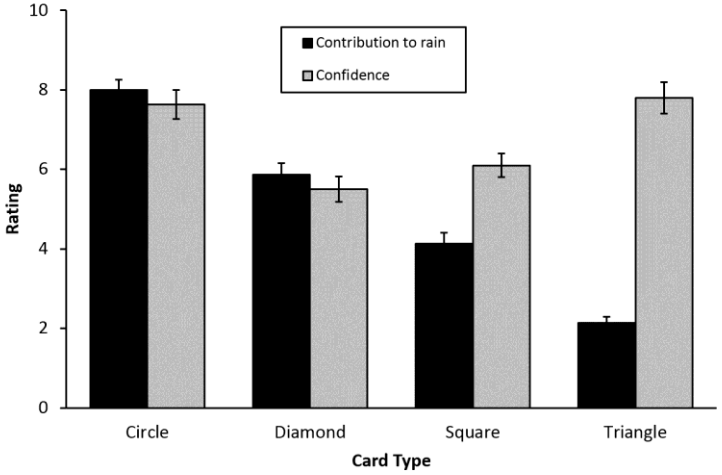

Figure 4 . Subjective card contribution and confidence ratings.

For each of the cards, participants were asked to rate their subjective contribution to the prediction of Rain, and the confidence they had in that subjective rating. Contribution ratings follow the same pattern as the actual card probabilities (Contributions to rain: circle 75.6%, diamond 57.5%, square 42.5%, triangle 24.4%), indicating that participants had gained knowledge about actual card contributions. Confidence ratings were higher for circle and triangle, probably due to the high predictive values of these cards. Error bars represent the standard error of the means.

Discussion

In this study, we investigated the role of the cerebellum in cognition by assessing the effects of cerebellar direct current stimulation (tDCS) on the weather prediction task, a type of probabilistic categorization learning (PCL). As expected, over the course of the experiment participants learned to improve their performance, showing increased accuracies and reduced response times while the experiment progressed. We only used anodal stimulation over the target brain areas as excitatory effects of anodal stimulation were more profound than the inhibitory effects of cathodal stimulation in a classification task *(Jacobson et al., 2012)*. However, we observed that anodal right cerebellar tDCS stimulation had no effects on either accuracy or response time. Post-hoc, we performed additional analyses to investigate the effect of tDCS on the first 50 trials separated in block of 10 trials, similar to previous studies *(Kincses et al., 2004; Nitsche et al., 2007)*. In line with those studies we did not find a significant interaction between stimulation and blocks. In our view, the lack of such an interaction effect suggests that tDCS had no effect on probabilistic categorization learning. The finding that individual blocks show differences between tDCS conditions in these previous studies does not provide compelling evidence that tDCS affects learning. Moreover, this difference for individual blocks was not replicated here.

Subjects had a preference for the outcome “sun”, which is in line with a previous study *(Lam et al., 2013)*. Contribution ratings followed similar patterns to the card probabilities, indicating participants had gained knowledge of the predictive values of the cards. This is supported by the higher confidence in the high predictive stimulus than in the low-predictive stimulus types. These two subjective measures of performance were also not influenced by tDCS stimulation.

The most likely explanation for our results is that the effects of tDCS are too small to have an effect on the performance in the weather prediction task. Moreover, observing such an effect would be hampered by the high variability in performance measures between participants. One way to reduce variability might be accomplished by some kind of normalization, which, on the other hand, also could distort the data. When we normalized our data by subtracting the data of the first block from the subsequent blocks, and performed our analyses again, we observed highly similar outcomes, suggesting again that tDCS is unlikely to have an effect on probabilistic categorization learning.

At first sight our findings seems to be in contrast with previous studies which did find an effect of anodal prefrontal tDCS (Fp3) using this type of task *(Kincses et al., 2004; Nitsche et al., 2007)*. However, these studies used a total of 50 trials and effects were only obtained using crude statistical analysis: both studies did not show, or even test for, interaction effects between blocks and tDCS, but did perform post-hoc tests to check for differences per tDCS condition for each block without corrections for multiple testing. Therefore, we think that these previous results do not provide clear evidence for tDCS effects on probabilistic categorization learning yet.

The variability between participants could be due to the fact that the task relies on multiple processes, e.g. working memory, strategy forming and strategy switching, assigning cue combinations to certain outcomes, and visual recognition. A task which relies on less cognitive processes could result in lower variance and would be better for revealing stimulation effects on implicit learning. An example of such a task is the probabilistic guessing task *(Hecht, Walsh, & Lavidor, 2010)*. Furthermore, this type of task is more suitable for a within-participant design for stimulation conditions. Here we choose to adopt a between-participant design because we wanted to avoid a test-retest effect due to the possibility that participants would become too familiar dealing with probabilistic rules in our task.

Another explanation for the lack of effect could be that the cerebellum and prefrontal cortex are not critically involved in probabilistic classification. However, the aforementioned neuroimaging studies reported activation of the left prefrontal cortex during probabilistic classification *(Kincses et al., 2004; Nitsche et al., 2007)*. Moreover, it is well established that the left prefrontal cortex is important in many cognitive learning processes. There is therefore a likely role of the left prefrontal cortex in probabilistic classification. Yet, the role of the cerebellum can be debated. Imaging studies suggest that the prefrontal cortex and the striatum are primarily involved with (correct) categorization in a weather prediction task *(Seger, 2008)*. This is confirmed by a study investigating PCL in patients with Parkinson’s disease and patients with cerebellar deficits. Results showed that Parkinson but not cerebellar patients are impaired on the weather prediction task, suggesting that successful PCL relies on intact basal ganglia but not on intact cerebellar structures *(Witt, Nuhsman, & Deuschl, 2002)*. Cerebellar activation increases on the right with increasing predictive values of card combinations *(Lam et al., 2013)*. Based on these observations, we analyzed the learning curves of the card combinations with a high predictive value alone, in order to increase the change of finding an influence of cerebellar tDCS. We, however, did not find such an effect.

Several adjustments to our experimental design can be suggested as various confounding factors like the exact electrode location, stimulation intensities and stimulus durations could influence the effect of tDCS *(Gluck, 2002)*. The tDCS settings used in our study were found to modulate behavior, albeit in different tasks *(Nitsche et al., 2007; Tomlinson et al., 2013)*. Nonetheless, in order to be able to claim that cerebellar tDCS does not influence probabilistic classification performances at all, future studies could look into the effect of these possible confounding factors, ideally adopting a within-subject design. On the other hand, negative findings with tDCS in cognitive tasks are not unexpected. There is increasing discussion about the robustness of tDCS effects and publication of negative findings is of importance *(Horvath et al., 2015a, 2015b)*.

Conclusion

We were not able to demonstrate the role of the cerebellum in probabilistic classification. Although we cannot rule out that such an effect is present, our data suggests that a tDCS effect on this task is likely to be quite small. Studies utilizing a different task or stimulation technique are needed to investigate cerebellar involvement in complex cognitive learning processes.

Acknowledgements

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Chapter 5. General Discussion

(PICTURE BY REPUTATTIONS)

CHAPTER 5

In this thesis, four experimental studies were described investigating the effect of non-invasive cerebellar stimulation on performance during a simple motor task and three types of cognitive tasks, addressing implicit and explicit reasoning. In this General Discussion I will go into the implications of the results and highlight difficulties with current tDCS research.

Results from our studies showed a modulatory effect following cerebellar tDCS on a saccade adaptation task in a small sample (n = 10). This result is in line with previous research by Panouillères and colleagues *(Panouilleres, Miall, & Jenkinson, 2015)*, who also found modulatory effects after stimulation, however with opposite effects. In addition, we were not able to modulate performance on multiple cognitive tasks with cerebellar tDCS in a robust manner. In a pilot study we found facilitating results in an implicit learning task, however, an attempt to replicate that effect in a larger sample failed. Moreover, we were not able to find evidence to support Ito's implicit thought theory with the aid of tDCS. Our results are not in line with previous research which showed modulatory, yet conflicting results following cerebellar tDCS in multiple, cognitive tasks *(Boehringer et al., 2013; Ferrucci et al., 2008; Pope & Miall, 2012)*.

5.1 Implications

The lack of effect on cognitive performance following cerebellar tDCS is not that surprizing as its effects on cognition are still debated even for cortical tDCS *(Horvath et al., 2015a)*. This could indicate that the cerebellum is not involved in cognition in general. This is, however, unlikely since several imaging studies have shown cerebellar activation during cognitive tasks, while controlling for eye movements *(Balsters et al., 2013; Hayter et al., 2007; Kirschen et al., 2010; Peterburs et al., 2016)*. Another explanation for our results is that the cerebellum is not that critically involved in these types of cognitive tasks. It is possible that the tasks performed rely much more upon working mechanisms in the prefrontal cortex *(Ashby & Ell, 2001; Owen et al., 2005; Seger, 2008)* or are supported by multiple brain networks *(Jacobson et al., 2012)*, which could compensate for up or down regulation of the cerebellum. This does, however, not explain our original positive result of cognitive performance following cerebellar tDCS in a pilot study *(Verhage et al., 2014)*, results by earlier tDCS research *(Boehringer et al., 2013; Ferrucci et al., 2008; Pope & Miall, 2012)*, other neuromodulation studies *(Arasanz et al., 2012; Argyropoulos & Muggleton, 2013; Desmond et al., 2005; Lesage et al., 2012)* and cognitive impairment following cerebellar lesion

(Hokkanen et al., 2006; R.B. et al., 2001; Schmahmann, 1991; Schmahmann & Sherman, 1998).

In our view, it is more likely that cerebellar tDCS in its current state, is unable to modulate cognitive functions in a robust manner. This is in line with recent replication studies, which show no modulatory effects after cerebellar tDCS in an eye blink conditioning task *(Beyer et al., 2017)*, a visuo-motor adaptation task *(Jalali, Miall, & Galea, 2017)*, a verb generating task *(Spielmann et al., 2017)* and a language prediction task *(Miall et al., 2016)*. An explanation for the lack of consistency in cerebellar tDCS research could lie in the sensitivity of tDCS to numerous parameters, which determine its effectiveness *(Vannorsdall et al., 2016)*. For instance: effects following tDCS are determined by methodology *(Buch et al., 2017)*, inter-subject variability *(Cooney Horvath, Vogrin, Carter, Cook, & Forte, 2016; Li et al., 2015; Wiethoff et al., 2014)* and subject’s anatomical characteristics *(Das et al., 2016; Parazzini et al., 2014; Wurzman et al., 2016)*. Hence, it is be expected that the reproducibility of tDCS effects has been weak in some behaviors *(Gladwin et al., 2012; Lally et al., 2013; Vannorsdall et al., 2016; Wiethoff et al., 2014)*. The inconsistent results found in cerebellar tDCS research are in line with cortical tDCS studies. In a recent meta-analysis, no evidence of cognitive effects was found after single session tDCS over the cortex in a healthy population *(Horvath et al., 2015b)*. This negative effect could be explained by individual responsiveness to tDCS, which could be lost in pooled data *(Minarik et al., 2016)*. However, a previous meta-analysis by the same group did find a robust effect following cerebral tDCS on MEP amplitude *(Horvath et al., 2015a)*, suggesting that individual responsiveness to tDCS is less important in simple, reflexive behavior. Evidently, tDCS effects are easier to detect in simple behavior because background noise and confounders are less involved *(Jacobson et al., 2012)*. At this time, reproducibility difficulties and inconsistent results among tDCS studies hinder application of tDCS in a clinical setting. It is therefore imperative to unravel the primary determinants for effective tDCS and improve the quality of tDCS research to obtain reliable and consistent results *(Jalali et al., 2017)*.

5.2.1 Potential genetic biases

A way to decrease subject variability and increase tDCS effectiveness could lie in the inclusion of genetic factors. This was recently implied by a cortical tDCS study on catechol-O-methyltransferase (COMT), an enzyme that regulates prefrontal dopaminergic neurotransmission, which is compromised in Val/Met polymorphism carriers. The study showed a polarity-specific and gene-dependent tDCS effect on a response inhibition task, indicating that genotype modulates tDCS outcomes on cognitive performance *(Nieratschker, Kiefer, Giel, Krüger, & Plewnia, 2015; Plewnia et al., 2013)*.

Another genetic component that could potentially play a substantial role in tDCS efficacy on learning is BDNF. BDNF is a neurotrophin growth factor, which is important for 1) regulation of synaptic plasticity 2) survival of existing neurons and 3) growth of new neurons and synapses *(Bath & Lee, 2006)*. It is highly expressed in CNS structures, including the neocortex and the cerebellum *(Huang & Reichardt, 2001; Yan et al., 1997)*, and elicits a wide range of effects *(B. Lu, Nagappan, & Lu, 2014)*. As described before, BDNF is one of many factors through which tDCS modulates cortical plasticity *(Brita Fritsch et al., 2010)*, and plays a significant role in motor learning *(Antal et al., 2010; Cheeran et al., 2008)*. In animal research, anodal tDCS has shown to enhance BDNF secretion in mouse M1 brain slices *(Brita Fritsch et al., 2010)* and in hippocampal slices *(Podda et al., 2016)*. Moreover, the latter study also showed increased hippocampal LTP, learning and memory suggesting that "tDCS may promote learning through BDNF release *(Das et al., 2017; Brita Fritsch et al., 2010)*". Interestingly, a common BDNF Val66Met polymorphism in humans (approx. 30% of the Caucasian population) impairs activity-dependent BDNF release *(E. Shimizu, Hashimoto, & Iyo, 2004)*, and is linked with reduced motor skill learning and cognitive performance *(Bath & Lee, 2006; Egan et al., 2003; Miyajima et al., 2008; Savitz, Solms, & Ramesar, 2006; van der Vliet, Ribbers, Vandermeeren, Frens, & Selles, 2017)*. Consequentially, comprised BDNF release in Val66Met carriers could influence tDCS efficacy, and therefore confound experimental results.

In contrast to animal research, the involvement of the BDNF genotype on tDCS efficacy in humans is less clear. A small sample study in BDNF Met carriers showed suppressed MEP peak amplitude following cathodal tDCS over the motor cortex in a metaplastic conditioning protocol, suggesting that genotype is a factor that can influence tDCS effectiveness *(Cheeran et al., 2008)*. This gene-dependent effect could, however, not be confirmed in stroke patients: A study investigating different tDCS protocols on motor skill learning showed reduced performance in BDNF Met carriers, irrespective of tDCS *(van der Vliet et al., 2017)*. On the other hand, anodal tDCS over the cerebellum showed enhanced learning in eye blink conditioning in BDNF Met non-carriers, suggesting, "that BDNF Val66Met determines to some extent the susceptibility to tDCS in healthy humans (REF Van Vliet in press)”. As for the current thesis, the BDNF Val66Met polymorphism is linked to impaired cognitive performance *(Egan et al., 2003; Miyajima et al., 2008)*, nonetheless, tDCS effects on cognitive functioning in BDNF Met carriers have not been investigated. It is imperative to investigate and control for potential genetic biases in tDCS research, as our knowledge of the underlying mechanisms is still limited *(Cheeran et al., 2008)*.

5.2.2 The replication problem

In addition to the unknown biological mechanisms, which determine tDCS effectiveness, a second and perhaps even more commanding issue is the problem with replication. The reproducibility of experiments is an essential component of the scientific method and an inability to replicate published experiments profoundly impacts on the quality of research and its inferences *(Button et al., 2013; Open Science Collaboration, 2015)*.

An important factor that plays a crucial role in the results obtained, is the size of the sample *(Button et al., 2013)*. Small sample sized studies can be valuable in the early stages of research, for instance in animal experiments *(Das et al., 2017; Slavin & Smith, 2009)* or they can be used in modern, statistical methods such as Bayesian statistics *(Rouder, Speckman, Sun, & Morey, 2009)*. On the other hand, small, underpowered studies show great variability in large, positive and negative, effect sizes *(Slavin & Smith, 2009)*. This entails that the magnitude of the experimental effect is over- or underestimated, which leads to incorrect estimations of the true effect size and sometimes to false conclusions *(Button et al., 2013; Loannidis, 2005; Minarik et al., 2016)*. This has recently been addressed by a large-scale replication study, demonstrating that replication results show half the effect size magnitude compared to the original study. This result was explained by the publication bias, a tendency which only affects the original study and not the replication *(Open Science Collaboration, 2015)*. Moreover, small, underpowered studies are also susceptible to incorrect inferences. This issue has been recently shown by a cognitive tDCS study using a resampling approach. An experiment by Minarik and colleagues (2016) investigating the effect of tDCS over the sensorimotor cortex on a choice reaction time task (CRT) showed that with sufficient large sample size (n = 75) determined a priori, a significant effect can be obtained with a moderate effect size (*d* = 0.45). However, when random samples of different sizes were drawn from the data pool (n = 75) and tested statistically, results varied dramatically. For instance, results could show reversed stimulation effects in small samples (n = 12), as previously predicted by multiple researchers *(Button et al., 2013; Loannidis, 2005)*. In addition, small samples that passed the threshold of significance obtained very high effect sizes, because they can only detect effects that happen to be large *(Button et al., 2013)*, whereas larger samples (n = 60) had small to moderate effect sizes, which is in accordance with previous research *(Slavin & Smith, 2009)*. Finally, power (80%) determined a priori dropped to an alarming 51% when 60 participants were included, a sample size that is considered large in tDCS literature *(Minarik et al., 2016)*.

Current tDCS literature is filled with numerous studies based on low power, small samples and reporting biases, which greatly undermines validity. Although limited, a meta-analysis could offer a solution *(Mancuso, Ilieva, Hamilton, & Farah, 2016)*, because it provides a more accurate estimate of the true effect size compared to individual studies *(Button et al., 2013)*. Strikingly, tDCS study designs generally include small samples, making them more susceptible to incorrect inferences and directly impact on the robustness of the meta-analysis. Hence, results on motor performance from tDCS meta-analyses are mixed, describing distinct polarity-dependent effects on numerous motor learning tasks following anodal and cathodal tDCS *(Jacobson et al., 2012)*, but also report little-to-no reliable neurophysiologic effects beyond motor cortex excitability modulation *(Horvath et al., 2015a)*. Similarly, results on cognition from various tDCS meta-analyses are equally diverse, obtaining small, positive results on numerous cognitive tasks following anodal tDCS *(Brunoni & Vanderhasselt, 2014; Dedoncker et al., 2016; Hill, Fitzgerald, & Hoy, 2016; Jacobson et al., 2012)*, but also report no effect at all after publication bias correction *(Horvath et al., 2015b; Mancuso et al., 2016)*.

5.2.3. Confounding motor responses and statistical inference

A final matter which needs to be addressed is the involvement of confounding motor responses in cognitive tasks. Inferences from tDCS research will be limited as a significant change in cognitive performance following tDCS can be attributed to a motor or non-motor task component *(Timmann, 2007)*. Ideally, an experimental design separates these factors *(Pope & Miall, 2012)*, for instance, by a motor control task which measures the effect of stimulation on motor performance in the absence of cognitive operations *(Desmond et al., 2005)*. Nonetheless, the studies described in this thesis have not assessed this distinct effect of motor related effects following tDCS.

Based on previous research, one can speculate about the effect of tDCS on motor and non-motor task components. Cortical tDCS studies show a robust polarity-dependent effect on multiple simple reaction time tasks in stroke patients *(Hummel et al., 2006)* and healthy humans *(Carlsen, Eagles, & MacKinnon, 2015; Drummond, Hayduk-Costa, Leguerrier, & Carlsen, 2017; Hupfeld, Ketcham, & Schneider, 2017; Minarik et al., 2016)*. However, results following cerebellar tDCS are more variable, showing faster reaction times following anodal tDCS on a serial reaction time task *(Ferrucci et al., 2013)*, improved reaction times after cathodal tDCS on a mental arithmetic task *(Pope & Miall, 2012)* and no effect at all on a linguistic prediction task *(Miall et al., 2016)*. A cerebellar TMS study by Desmond et al. (2005) incorporated a motor control task, showing increased reaction time on a verbal working memory task. Additional analyses displayed a greater impaired response for the cognitive component compared to the motor component, suggesting that the significant change in cognitive performance was not a result of motor response modulation, which is in line with results by Pope and Miall (2012). However, the combined effect of stimulation (TMS or tDCS) on motor responses and cognitive operations highlight the need for inclusion of motor control tasks, as the role of the cerebellum in motor functioning is coupled with its engagement in cognitive performance *(Oliveri et al., 2007)*.

Consequently, based on the current literature and the studies described in the current thesis, it is difficult to infer solid conclusions *(Mancuso et al., 2016)*. Results can be explained by 1) tDCS has no effect on learning and positive results obtained are probably due to sampling errors *(Chiolero, Paradis, Rich, & Hanley, 2013)* or 2) tDCS has a small to moderate effect on behaviour, however effects are difficult to replicate due to its sensitivity to unknown parameters (e.g. genetic factors). Either way, these explanations have complex implications for potential follow-up studies, since, a positive or negative result can be accounted for by both principles.

A way to overcome these issues is by incorporating Bayesian statistics to standard statistical approaches (as described in Chapter 4.1), as the traditional NSHT method comes with numerous limitations *(Hoenig & Heisey, 2001; Rouder et al., 2009; Smittenaar et al., 2014)*. For example, for every hypothesis test, there is a probability of making an type 1 or type 2 error, allowing for incorrect conclusions. These probabilities are even greater when potential biases are involved *(Loannidis, 2005)*. In addition, the threshold of significance (*p*-value) depends on the size of the sample, entailing that samples with sufficient large sizes will always show a statistically significant effect *(Sullivan & Feinn, 2012)*. Therefore, researchers often look at the effect size, presumably, because it is sample size-independent. However, in practice this is not always the case *(Minarik et al., 2016; Slavin & Smith, 2009)*. Even tough, traditional NSHT is the most practiced method for statistical inferences, it is currently under discussion in the scientific community *(Levine, Weber, Hullett, Park, & Lindsey, 2008)*". According to NSHT, a hypothesis test can only decide whether the existing evidence allow for a rejection of the null hypothesis or a failure to reject the null hypothesis *(Levine et al., 2008)*. It is, however, not possible to state evidence in favour of the null hypothesis *(Rouder et al., 2009)*, as absence of evidence does not mean evidence of absence *(Altman & Bland, 1995)*. This principle severely limits inferences when a null effect is found.

These limitations call for other ways of statistical inferences *(Hoenig & Heisey, 2001; Quinlan, 2013)*. Contrary to NHST, Bayesian statistics uses probabilities to assign meaning to specific hypotheses *(Rouder et al., 2009)* and to estimate parameter values in descriptive models *(Kruschke, 2014)*. Moreover, Bayesian statistics also uses prior knowledge to determine parameter values, creating informative models by combining prior knowledge with current data. Inferences from Bayesian analyses are more informative than NHST, especially in the absence of experimental effects *(Kruschke, 2014)*. In practise, this has been recently shown in a reproducibility project, showing higher replication rates in research using Bayesian statistics with strong support (Bayes factor >10). *(Etz & Vandekerckhove, 2016)*.

5.3 Recommendations for future research

The role of the cerebellum in cognitive processes has become increasingly popular, as evidenced by imaging studies *(Buckner, 2013; Stoodley, 2012)*, lesion research *(Schmahmann & Sherman, 1998)* and neuromodulation studies *(Grimaldi et al., 2014; Oliveri et al., 2007)*. However, critics argue that tDCS results are inconsistent and confounded by motor responses *(Timmann, 2007)*. Future research should take the following three recommendations into account to increase validity and improve reliability of tDCS research.

First, to reduce subject variability, genetic markers with common polymorphisms (such as BDNF and COMT) should be incorporated and controlled for in tDCS research. In that manner, the involvement of genetic factors on learning can be investigated on a behavioral level *(Cheeran et al., 2008; Nieratschker et al., 2015)*. Moreover, another way to minimize subject variability is by the use of a within-subjects design. tDCS studies using a between-subjects design often fail to observe an effect due to high between-subject variability *(Lally et al., 2013)*, however within-designs have lower random error, and consequently, higher power *(Howitt & Cramer, 2011)*.

Second, in order to confirm earlier, positive results of tDCS, replication studies are needed, using large samples to ensure the effects found by previous research are robust *(Vannorsdall et al., 2016)*. Moreover, adding a motor control task to the design would be of great benefit, as it assess the effect of tDCS on the motor task component in the absence of cognitive operations *(Desmond et al., 2005)*. In addition, when a ‘negative’ effect (or null result) following stimulation is found, researchers should aim to publish their findings to adjust for the publication bias *(Loannidis, 2005)*.

Third, the quality of tDCS research can be improved by the use of larger samples *(Slavin & Smith, 2009)*, determined a priori *(Hoenig & Heisey, 2001; Minarik et al., 2016)*, with high power *(Krzywinski & Altman, 2013)*. Moreover, absence of a relation between two variables cannot be proven according to null hypothesis significance testing (NHST) *(Altman & Bland, 1995)*, pinpointing the downside of this traditional approach *(Rouder et al., 2009)*. Therefore, additional statistical methods, like Bayesian statistics, can be of great value for future tDCS research *(Hoenig & Heisey, 2001; Quinlan, 2013; Smittenaar et al., 2014)*, as it assigns probabilities to specific hypotheses and allows for model selection *(Rouder et al., 2009; Smittenaar et al., 2014)*. Considering the recommendations mentioned above, future tDCS research can assess parameters of interest and develop robust protocols for optimal tDCS efficacy for patient groups and healthy humans.

5.4 Conclusion

Taken together, cortical and cerebellar tDCS studies investigating motor and cognitive learning show inconsistent effects and have replication difficulties. In this current thesis, four studies were described investigating the effect of non-invasive cerebellar stimulation on performance during a simple motor task and several cognitive tasks. In line with the literature, we found facilitating effects following tDCS in two small sampled studies, however replication failed.

Controlling for genetic factors (such as BDNF and COMT polymorphism) in tDCS research could decrease subject variability and potentially convey more robust effects. In addition, the quality of tDCS research can be improved by the use of larger samples, determined a priori, with high power*(Krzywinski & Altman, 2013)*. Moreover, adding modern statistical methods, like Bayesian statistics, would be of great value to accurately interpret statistical results. Therefore we conclude that cerebellar tDCS research in its current state is unable to modulate cognitive functions in a robust manner. The use of small samples and traditional statistical methods limit researchers to infer solid conclusion from the results.

CHAPTER 5

References

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Appendix

APPENDIX

Summary

In this thesis, the effect of non-invasive neurostimulation on motor and cognitive performance was investigated, taking an implicit-explicit notion into account. The main findings of this this are summarized below.

Chapter 2 describes modulation of oculomotor learning with the aid of cerebellar transcranial direct current stimulation in a small sample, showing increased learning of saccadic inward adaptation, but not outward adaptation. Oculomotor learning relies heavily on the cerebellum, implying that plasticity mechanisms in the cerebellum are different between inward and outward adaptation.

Chapter 3 investigates the effect of cerebellar transcranial direct current stimulation in an explicit working memory paradigm, showing no effect on performance following anodal or cathodal stimulation. This suggests that working memory is not readily influenced by cerebellar stimulation.

Chapter 4 studied the involvement of the cerebellum in two implicit cognitive tasks with transcranial direct current stimulation. Moreover, a replication was conducted to confirm positive findings by earlier studies with small samples. Results showed no effect of cerebellar stimulation on a category learning task or a probabilistic learning task. Moreover, replication of previous positive results failed, indicating that cerebellar stimulation does not modulate performance and learning on implicit categorization tasks.

Taken together, cerebellar stimulation studies investigating motor and cognitive learning show inconsistent effects and have replication difficulties. Therefore we conclude that cerebellar stimulation research in its current state is unable to modulate cognitive functions in a robust manner.

Samenvatting

In dit proefschrift is het effect van non-invasieve neurostimulatie op motorisch en cognitief leren onderzocht. Daarin is onderscheid gemaakt in impliciet en expliciet leren. De belangrijkste bevindingen zijn hieronder kort samengevat.

Hoofdstuk 2 beschrijft modulatie van oculo-motorisch leren met behulp van cerebellaire transcraniale gelijktijdige stimulatie in een kleine populatie. De resultaten laten zien dat saccadische binnenwaartse adaptatie, maar niet buitenwaartse adaptatie, wordt verbeterd met anodale stimulatie. Oculo-motorisch leren is afhankelijk van het cerebellum, hetgeen impliceert dat plasticiteitmechanismes in het cerebellum verschillen tussen binnen- en buitenwaartse adaptatie.

Hoofdstuk 3 onderzoekt het effect van cerebellaire transcraniale gelijktijdige stimulatie in een expliciet werkgeheugenparadigma. Leerprestatie werd niet beïnvloed anodale of cathodale stimulatie op het cerebellum. Dit suggereert dat werkgeheugen niet eenvoudig door cerebellaire stimulatie wordt beïnvloed.

Hoofdstuk 4 bestudeert de betrokkenheid van het cerebellum in twee impliciete, cognitieve taken met transcraniale gelijktijdige stimulatie. Daarbij wordt een replicatie onderzoek uitgevoerd om de positieve bevindingen van eerdere studies met kleine populaties te bevestigen. De resultaten lieten geen effect zien van cerebellaire stimulatie op een categorieleertaak of een probabilistische leertaak. Tenslotte liet de replicatie van eerdere positieve resultaten geen positieve effecten zien. Dit geeft aan dat cerebellaire stimulatie niet in staat is om impliciete, categorische leerprestaties te moduleren .

Samengevat, studies die motorisch en cognitief leren onderzoeken aan de hand van cerebellaire transcraniale gelijktijdige stimulatie, tonen inconsistente effecten en hebben problemen met replicatie. Derhalve concluderen wij dat cerebellair stimulatieonderzoek in zijn huidige toestand niet in staat is om cognitieve functies op een robuuste manier te moduleren.

APPENDIX

PhD Portfolio

Personalia

Family name:	Verhage	*Address:*	Westewagenstraat 10 3011 AS Rotterdam Nederland
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Education

April 2013 - January 2018:	**PhD Neuroscience** *Erasmus Medical Center*	Rotterdam, Netherlands
September 2010 - April 2013:	**Master Brain & Cognition** *Erasmus University Rotterdam (Part-time).*	Rotterdam, Netherlands
September 2004 - June 2010:	**Bachelor Psychology** *Erasmus University Rotterdam (Part-time).*	Rotterdam, Netherlands

Work experience and teaching

March 2015 - December 2016:	**Quantitative skills trainer** *Erasmus University College* 13 *ECTS*	Rotterdam, Netherlands
August 2013 - August 2015:	**Tutor Junior Med school** *Erasmus Medical Center* 3 *ECTS*	Rotterdam, Netherlands

Congresses, courses and skills

15 - 19 August 2016:	**Bayesian statistics course** *Introduction to Bayesian Statistics (NIHES)* *1.4 ECTS*	Rotterdam, Netherlands
3 - 7 November 2014:	**BROK course** *Basic course Regulation and Organization Clinical researchers* *1 ECTS*	Rotterdam, Netherlands
16 - 20 June 2014:	**Matlab course** *1 ECTS*	Rotterdam, Netherlands

4 - 5 June 2014:	**Helmholtz Retreat** *Poster presentation* 1.5 *ECTS*	Bergen, Netherlands
22 - 25 April 2014:	**NCM Annual Meeting** *Poster presentation* *0.5 ECTS*	Amsterdam, Netherlands
23 November 2013:	**NYC Neuromodulation** *tDCS course and poster presentation* 3 *ECTS*	New York, USA
13, 14 and 20 February 2013:	**Linear systems course** 2 *ECTS*	Rotterdam, Netherlands
April 2013 - now:	**Labmeetings and labtalks** *Presentations and discussions* *10 ECTS*	Rotterdam, Netherlands

** 1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours*

Publications and poster(presentations)

Verhage, M.C., Avila, E., van der Geest, J., Frens, M., & Donchin, O. (2014). Cerebellar involvement in categorisation: a bipolar tDCS study. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation,7*(2), e4.

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Seyed Majidi*, N., Verhage, M.C.*, Donchin, O., Holland, P., Frens, M. & van der Geest, J.N. (2016). Cerebellar tDCS does not improve performance in probalistic classification learning. *Manuscript submitted for publication in Experimental Brain Research. * shared first auteur.*

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APPENDIX

About the author

Claire Verhage was born as Marie Claire on September 13th 1985 in Capelle aan den IJssel. After graduation from the Emmaus college Rotterdam, she enrolled in psychology at the Erasmus University in Rotterdam. After attaining her first year of the bachelor program, she decided to finish psychology part-time to pursue her field hockey ambitions. For several years, she was part of the national field hockey team and national indoor hockey team, achieving multiple international prices. In 2010, she received her bachelor's degree and started a master in 'brain and cognition' psychology. During her master thesis, she was under the supervision of Prof.dr. Maarten Frens at the neuroscience department in the Erasmus Medical Center. The successful collaboration resulted in a PhD position, in which she studied the effect of cerebellar stimulation on cognitive performance, and later (oculo)motor learning. Throughout this time, she collaborated with Dr. Jeroen Dudink at the Sophia children's hospital to investigate the effects of stimulation in young children with motor disabilities. In the future, Claire will continue to work at the neuroscience department and collaborate with Rijndam rehabilitation.

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Dear Peter, thank you so much for all the code you’ve written for my projects. Your technical skills are admirable. I could always count on you if I had issues with anything computer related. Thank you for being so friendly and kind.

Lieve Brenda, jij was mijn eerste master student en het samenwerken met jou ging eigenlijk vanzelf. Een gedisciplineerde harde werker, ik kon het niet beter treffen. Bedankt voor al je inzet en je betrokkenheid!

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