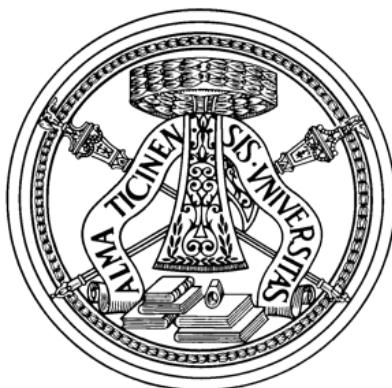


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CEREBELLUM IN COGNITION:
A BRAIN STIMULATION APPROACH

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"I carry a log...yes. Is it funny to you? It is not to me. Behind all things are reasons. Reasons can even explain the absurd. Do we have the time to learn the reasons behind the human being's varied behavior? I think not. Some take the time. Are they called detectives? Watch...and see what life teaches."

Twin Peaks. Prod. David Lynch and Mark Frost. American Broadcasting Company (ABC).
New York, 12 April. 1990. Television

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Abstract

A coprocessor is a processor that supports the operations performed by a primary processor, accelerating and/or optimizing the system performance. If we look at the central nervous system, the cerebellum can be identified as a coprocessor. Whenever we interact with the physical and social environment that surrounds us, the cerebellum contributes to guide the operations underpinning our behavior. The ubiquity of cerebellar activation has attracted the attention of researchers in different fields and disciplines that for decades deepened our knowledge on its contribution at a physiological and functional level.

The main research question behind this work concerns the role exerted by the cerebellum in cognitive- and emotive-related functions. The frame of the original studies illustrated in the dissertation is constituted by the Universal Cerebellar Transform (UCT) hypothesis. According to the latter, the cerebellum cooperates with the brain to produce harmonious motor, cognitive and emotive/autonomic behaviors. However, the broad question on "how" the cerebellum exerts its assisting functions is still partially unanswered. Along this line, the present work aims at further examining the contribution of the cerebellum to cognitive and emotive-related functions by means of brain stimulation techniques.

The purpose of the first chapter is to provide a general overview of the main evidence that cerebellar research has gathered through experimental investigations and clinical observations. The insights presented here on the computational scheme (how) used by the cerebellum to assist the brain in computing information of different nature (what) and on the anatomical and functional foundation of its contribution (where), together represent the theoretical and empirical basis on which the four studies, core of the present dissertation, have been built upon. The final part of this brief overview focuses on the specific contribution provided by brain stimulation studies.

Methodological aspects of two of the mainly applied brain stimulation techniques targeting the cerebellum are discussed in order to give aid in interpreting the original results of the studies illustrated in the following chapters.

To begin with, the experimental section of the thesis opens with a meta-analytic study on the effects of cerebellar transcranial direct current stimulation (cDCS) on behavioral performance (study 1). The aim of the meta-analysis was two-folded. Primarily, we were interested in assessing the efficacy and reliability of cDCS to induce behavioral effects. The second goal was to test the hypothesis that anodal cDCS improves and cathodal cDCS impairs performance, according to the anodal-enhancement and cathodal-impairment dichotomy observed in respect to cerebral stimulation. Results led support to the ability of cDCS to modulate behavior. However, we found no evidence of polarity-dependent effects of cDCS on behavioral performance. Interestingly, despite analyses showed that motor-related functions were significantly more affected by cDCS than cognitive functions, we provide evidence supporting the efficacy of cDCS in altering cognitive performance as well. This result converge with the growing consensus on the role played by the cerebellum outside the motor domain.

In the second study we used cathodal cDCS to examine the involvement of the cerebellum in error processing and performance monitoring (study 2). We expected cathodal stimulation to 1) interfere with the cognitive performance in a Erikson flanker task and 2) to alter Error-related Negativity (ERN) components, electrophysiological indexes associated with error-related processing. Analyses revealed an interesting dissociation in the effects of the stimulation on behavioral performance and electrophysiological measures. In fact, we observed a decrease in behavioral performance, whereas ERN signals were not affected by the stimulation. In light of the cerebello-thalamo-cortical anatomical and functional connectivity, we speculate that stimulation-perturbed signals of detection of errors coming from the cerebellum did reach the prefrontal cortex areas for conflict evaluation and response selection. However, operations of the cerebral cortex my have not been affected by this perturbation during response-conflict monitoring, which may explain why the output of

prefrontal cortex (i.e. the ERN potentials) remained unaltered. Nevertheless, these findings go in line with previous experimental evidence showing the involvement of the cerebellum in error processing.

In relation to error-related operations, the detection of sequences violations constitute the object of interest of the first of the two cerebellar transcranial magnetic stimulation (TMS) studies illustrated (study 3). In more details, in three experiments participants were asked to detect possible violations within sequences responding to different rules. Results showed that cerebellar TMS effectively modulated participants' accuracy in processing unfamiliar sequences (Experiment 1,2,3) – composed of geometrical varying in size or order of presentation and for which an internal template does not exist yet – but not highly familiar sequences (Experiment 2) – composed of strings of letters presented in a serial, predictable order. This finding converges with previous works suggesting that cerebellar activity increases during early learning and becomes less crucial after continued practice. Overall, our data add to previous neuroimaging findings showing cerebellar activity in the recognition of patterns of events and in the detection of violations of the relationship of these events in the cognitive-perceptual domain.

The goal of the last original contribution of the dissertation was to examine the role of the cerebellum in emotional appraisal (study 4). In a series of experiments, participants were presented with facial emotion expressions were the emotional cues were either relevant or irrelevant of the task at play. Analyses showed that cerebellar TMS impaired both explicit and implicit elaboration of the emotive-information, as indicated by decreased accuracy not only in a facial emotion expressions discrimination task, but also when the emotional cues were task-irrelevant (i.e. during a gender discrimination task). Moreover, the fact that the disruptive effect of cerebellar TMS was only observed during emotional cue recognition, helped us ruling out possible effects of the stimulation on visual encoding as well as detrimental effects of TMS on unspecific cerebellar operations.

Finally, the last chapter of the dissertation discusses and integrates the main findings of the studies illustrated in the experimental chapters.

Chapter 1

Introduction

CEREBELLAR OPERATIONAL MODE: HOW IS IT COMPUTED?

An increasing amount of research has been carried out in the last decade on the role that the cerebellum exerts within the nervous system. Experimental and clinical evidence indicate that the cerebellum is involved in a broad variety of motor and non-motor functions (Ito, 2005). Given the different nature of the tasks that imply to different extents a cerebellar assistance, a question has been raised by the researchers in the field about the computational scheme underpinning this contribution (D'Angelo & Casali, 2012).

The acquisition and the implementation of internal models has been pointed out as the computational basis throughout which the cerebellum would assist the brain at different operative levels, from low-level complexity processes to high-order cognitive functions (Ito, 1970, 1984). Initially applied to the study of movement control, the notion of internal models provides a solution to the problem of generating fast and accurate movements in lack of immediate sensory feedback (Miall & Wolpert, 1996). There are two types of internal models that generate motor commands. An inverse internal model generates a motor command necessary to achieve the desired state or movement in absence of a sensory feedback and taking into account the dynamics of the body. A forward internal model, based on the efferent copy of the to-be-implemented motor command, firstly generates a prediction about the consequences of the motor command itself. Then the cerebellum compares this prediction with the actual sensory inputs deriving from the implementation of the movement. In case a mismatch between prediction and sensory feedback is detected, a signal error is produced, that is later used to correct and guide the further implementation of the movement (Miall & Wolpert, 1996).

Forward modeling provides clear advantages when relying on feedback-based mechanisms of movement control and, therefore, when the temporal delays introduced by the transmission of afferent information would be too slow to generate a fast and accurate movement. Anticipatory changes in grip force in the presence of a predictable variation in weight load during object manipulation, reflects a classic example of

implementation of a forward model (Kawato et al., 2003). The fact that the compensation observed when applying a grip force occurs without temporal delays suggests that 1) no sensory feedback is used and 2) the nervous system is applying a state estimation of hands and arms to predict the variation in objects load and modulate the grip force, accordingly.

To summarize, a forward model generates a state prediction, consisting of an estimation of an internal feedback in replacement of the actual external feedback, while an inverse model provides a motor command to the motor apparatus to produce a desired movement. The idea that the cerebellum, thereby acquisition and storing of internal models, is active during motor control and motor learning has been thoroughly investigated and widely accepted (Wolpert, Miall, & Kawato, 1998). Whether the function of the cerebellum is specifically to act as a forward controller, predicting the sensory consequence of actions, or to generate inverse models, learning to acquire correct motor commands, or even to form both types of internal models, is still debated. Different theories have been proposed on the computational models of cerebellar function (see Wolpert et al., 1998 for a review). Among these, the hypothesis of inverse internal models (Kawato & Gomi, 1992) comprises the combination of a feed-forward control, exerted by the cerebellum, and feedback control mechanisms for error corrections computed in the motor cortex (Figure 1.). According to this hypothesis, the cerebellum conveys the efferent copy of the motor command with the inverse internal model of the state dynamics of the body to the motor apparatus to achieve the desired goal.

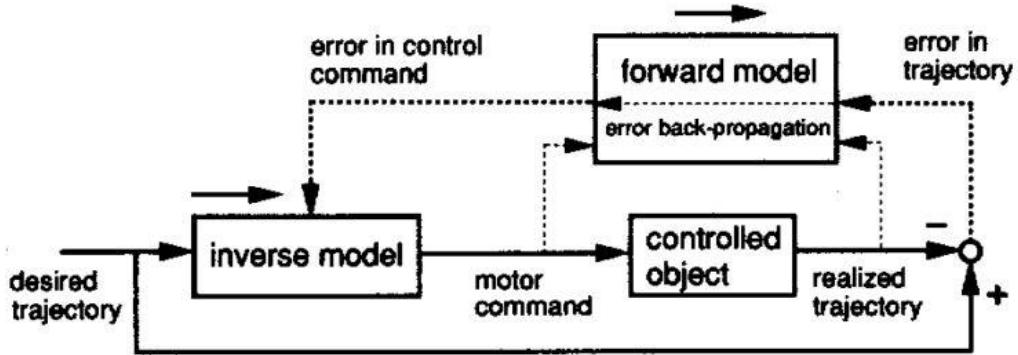


Figure 1. Schematic depiction of forward-inverse modeling in the cerebellum (from Kawato & Gomi, 1992). The forward model of the controlled object is learned by monitoring the input and the output of the controlled object. The desired movement trajectory is sent to the inverse model to calculate a feedforward motor command. The resulting error in the trajectory space is back propagated through the forward model to calculate the error in the motor command space, which is then used as an error signal for training the inverse model.

Based on the observation of different types of motor impairments, cerebellar damage has been more consistently linked to the disruption of movements requiring predictive (i.e. relative to mechanisms of feedforward modeling) rather than reactive control (i.e. relative to on-line error corrections of motor commands following peripheral feedback) (Bastian, 2006). In line with the forward internal models hypothesis, Izawa and colleagues (2012), showed that both cerebellar patients and healthy controls were able to successfully adapt their motor commands during a reaching task in the presence of a gradual perturbation schedule applied to the direction of the target. Nevertheless, when required to reach-out for the target and to additionally report the position of the hand, cerebellar patients showed impaired ability to associate the motor command with the newly acquired sensory consequences of the action. The apparent dissociation between prediction of sensory signals and implementation/adaptation of motor commands emerged in this study seems to suggest that the cerebellum is crucial in order to learn to predict sensory consequences of motor commands, whereas the adaptation and implementation of accurate motor commands might still take place despite the presence of cerebellar lesions.

Neuroimaging and neurophysiological observations that highlight the cerebellar-dependent adaptation according to a feedforward-based modeling, together point to

the cerebellum as a privileged candidate to host predictive mechanisms in the motor domain (Ishikawa, Tomatsu, Izawa, & Kakei, 2015). In the next paragraph, the concept of cerebellum as a forward controller for non-motor functions will be discussed.

MOTOR AND COGNITIVE/AFFECTIVE INFORMATION: WHAT IS COMPUTED?

Although it is still not clear whether the cerebellum would mainly act as a forward controller or generating inverse models, there is growing consensus on the fact that it might operate both types of models in combination. Ito (1993, 2005, 2008) proposed that the cerebellum might encode forward and inverse internal models not only reproducing sensory-motor inputs, but also reproducing internal representations of cognitive nature. According to the internal model hypothesis for mental activities, the cerebellum would be involved in systems of cognitive processing operating in an identical way as it would be during motor systems associated with motor coordination and control. In the latter system, the controlled object is the body part which is copied into an internal model – either a forward or an inverse model – where in the former, the controlled object is replaced with a mental model. In both scenarios, movement and thought are controlled by the same neuronal mechanism.

If the cerebellum assists the brain in carrying out basic computational operations, its contribution in tasks pertaining to different cognitive functions shouldn't surprise. Cerebellar involvement in timing (representing the temporal relationship between task-relevant events), sensory prediction (i.e. the ability to predict incoming information based on a combination of forward modeling and internally stored memory) and learning (referring to the cerebellar-dependent adaptation of motor and cognitive scripts), together constitute primitive properties that underpin more complex and high-order functions (D'Angelo & Casali, 2012).

A remarkable amount of evidence for cerebellum's role in cognitive/emotional processes derives from clinical investigations of cerebellar pathologies. Years of clinical observations linked cerebellar damage to impairments in executive functions (Peterburs

& Desmond, 2016; Ravizza et al., 2006), linguistic processing (Maria Giuseppa Leggio, Silveri, Petrosini, & Molinari, 2000; Peterburs, Bellebaum, Koch, Schwarz, & Daum, 2010), visuospatial processing (Battaglia et al., 2006; Molinari et al., 2008), sequence processing (Leggio et al., 2008), and autonomic regulation and emotion appraisal (Marien et al., 2009). Schmahmann (1998) first described the core features of executive, linguistic, spatial and emotional abnormalities following a range of heterogeneous lesions confined to the cerebellum, well-known under the name of Cerebellar Cognitive and Affective Syndrome (CCAS). In more details, this syndrome, alongside a general lowering of intellectual abilities, is characterized by 1) impairments in executive functions including deficits in planning, set-shifting and working memory 2) impairments in spatial cognition including visuospatial disorganization and impaired visuospatial memory 3) personality changes, emotional blunting, and disinhibited or inappropriate behavior and, lastly, 4) language deficits including dysprosodia, agrammatism and anomia. The damage of cerebellar components associated with different neural subsystems disrupts the production of harmonious cognitive, affective and autonomic behaviors, symptoms of a dysfunction called "cognitive dysmetria" or "dysmetria of thoughts" (Schmahmann, 1991; 1996).

In addition to the insights coming from the examination of the clinical population, a growing body of neuroimaging research in healthy participants confirmed the involvement of the cerebellum in cognitive and affective tasks. Cerebellar activation was reported in language processing (Marien et al., 2014), visuospatial processing (Mellet, Bricogne, Mazoyer, & Denis, 1997; Naito et al., 2002; Ryding, Decety, Sjöholm, Stenberg, & Ingvar, 1993; Vingerhoets, Lange, Vandemaele, Deblaere, & Achtert, 2002), probabilistic and deductive reasoning (Blackwood, Simmons, Bentall, Murray, & Howard, 2004; Osherson, Perani, Cappa, & Schnur, 1998), and executive functions (Hayter, Langdon, & Ramnani, 2007; Jahanshahi, Dirnberger, Fuller, & Frith, 2000; Lie, Specht, Marshall, & Fink, 2006; Priori et al., 2008). However, it is important to note that not all patterns of cerebellar activation are consistent with the Internal model hypothesis for mental activities introduced above. For example, in an fMRI study (Cheng, Desmond 2005), healthy participants performed a verbal working memory task composed of three

steps: encoding phase, rehearsal and retrieval phase. Different patterns of cerebellar activation emerged during distinct phases, with inferior regions being active during encoding, presumably reflecting the refreshment of contents of the phonological store, and superior regions being more active during retrieval, indicating that the latter regions are involved in the articulatory requirements of task. The authors suggested that superior cerebellar activation in the rehearsal phase may be partially related to motor preparation mechanisms, highlighting the link between the activation of specific cerebellar regions and functionally distinct roles in a given task. Nevertheless, depending on the specific requirements of a task, the authors also argued that motor preparation mechanisms performed by the cerebellum may explain to some extent the ubiquity of cerebellar activation found in neuroimaging studies investigating cognitive functions.

Regarding emotive-related functions, pattern of cerebellar activation has been reported by several neuroimaging studies during facial expression processing (George et al., 1993), viewing of emotional versus neutral stimuli (Lane, 1997), identification of emotional vocal intonation (Imaiizumi et al., 1997), emotion appraisal (Paradiso et al., 1999) and emotion regulation (Paradiso et al., 2003). In addition, functional and structural abnormalities of the cerebellum have been linked to several affective and mood disorders (Kyriakopoulos, Vyas, & Barker, 2008; Savitz & Drevets, 2009; Schmahmann & Sherman, 1998), strengthening the neuroimaging evidence that lend support to the contribution of the cerebellum to emotion processing.

Insights from the topographic organization of the cerebellum, briefly described in the following paragraph, can help to shed light on the anatomical substrates of cerebellar processing as well as disentangle its specific contribution to motor- and cognitive/emotive-related functions.

CEREBRO-CEREBELLAR LOOPS: WHERE IS IT COMPUTED?

According to the Universal Cerebellar Transform hypothesis (UCT), the cerebellum integrates internal representation with external inputs, providing automatic non-conscious responses in order to optimize the performance congruently with context demands (Koziol, Budding, Andreasen, et al., 2014). The term “universal” refers to the fact that the cerebellum modulates information of different nature in a supramodal way. Therefore, based on existing evidence, the debate on cerebellar involvement in non-motor functions should be focused on how the cerebellum contributes to both cognitive and affective processes as well as motor functions rather than questioning if it is involved at all. Although this question remains partially unanswered, investigating the anatomical and functional subsystems that requires an intervention of the cerebellum is a critical step to gain deeper understanding of the contribution of this structure to all domains of behavior.

To begin with, it is relevant to note that the cerebellum is connected to cortical and subcortical areas via afferent and efferent projections, which constitute the cerebro-cerebellar loops. Macroscopically, three distinct principal components can be observed, according to the origin of their afferent projections: the cerebellar cortex, the deep cerebellar nuclei (DCN) and the cerebellar peduncles.

On gross inspection, three lobes can be distinguished in the cerebellum. The cerebrocerebellum, or neocerebellum, is in evolutionary terms the youngest part of the cerebellum. It occupies the majority of the lateral cerebellar hemispheres and receives projections from the associative cortex. The connections from distinct regions of the cerebral cortex to the cerebellum through the thalamus, constitute separate cerebello-thalamo-cerebro-cortical loops (Manto, 2006). The parietal loop includes fibers connecting the Broadman area 7b of the inferior parietal lobe and the cerebellum (Clower, West, Lynch, & Strick, 2001). The prefrontal loop reciprocally connects the cerebellum to the medial prefrontal cortex (MPFC) (Watson, Jones, & Apps, 2009), the dorsolateral prefrontal cortex (DLPFC) (Kelly & Strick, 2003), and the anterior prefrontal cortex (APC) (Salmi, Pallesen, & Neuvonen, 2010). Patterns of connectivity between the

cerebellum and temporal areas – including the hippocampus and the amygdala – constitute the temporal loop (He, Zang, Jiang, Liang, & Gong, 2004). A second lobe, the spinocerebellum, also known as paleocerebellum, is positioned medially to the section called the cerebrocerebellum. It includes the anterior lobe, the vermis and the paravermis and receives projections directly from the spinal cord. Available evidence suggest that limbic-related structures are interconnected with the vermis via fastigial nuclei of the DCN, pointing at the connection between the medial parts of the cerebellum and subcortical regions, strongly implicated in emotion processing, as the anatomical substrate of cerebellar involvement in emotion. Lastly, the smallest region is the flocculomodular lobe, or vestibulocerebellum, which is mainly involved in the control of balance, posture and oculovestibular reflex. It receives projections from the vestibular system and the brain stem reticular formation. The connections between the cerebellum and the rest of the nervous system run via the cerebellar peduncles. The superior cerebellar peduncle is mostly composed of efferent fibers, projecting to the neurons of the motor cortex and the primary premotor cortex, that together constitute the anatomical substrate of the motor and somatosensory loops. The middle cerebellar peduncle consists of an afferent pathway, receiving projections from almost the totality of the areas of the cerebral cortex, contralaterally. Finally, the inferior cerebellar peduncle contains both efferent and afferent fibers which connect the cerebellum to the ventral tegmental area (VTA), the reticular formation and the spinal cord.

On the basis of anatomical, neuroimaging and clinical findings converging in pointing to a highly specific topographic organization of the cerebellum for what concerns motor and cognitive/affective processing (Figure 2) (Stoodley, Valera, & Schmahmann, 2012), Schmahmann and colleagues (Schmahmann & Vangel, 2009; Schmahmann, 1996) hypothesized the existence of a functional dichotomy between the anterior lobe, predominantly engaged in sensory-motor functions, and the posterior lobe, which would mainly contribute to cognitive functions.

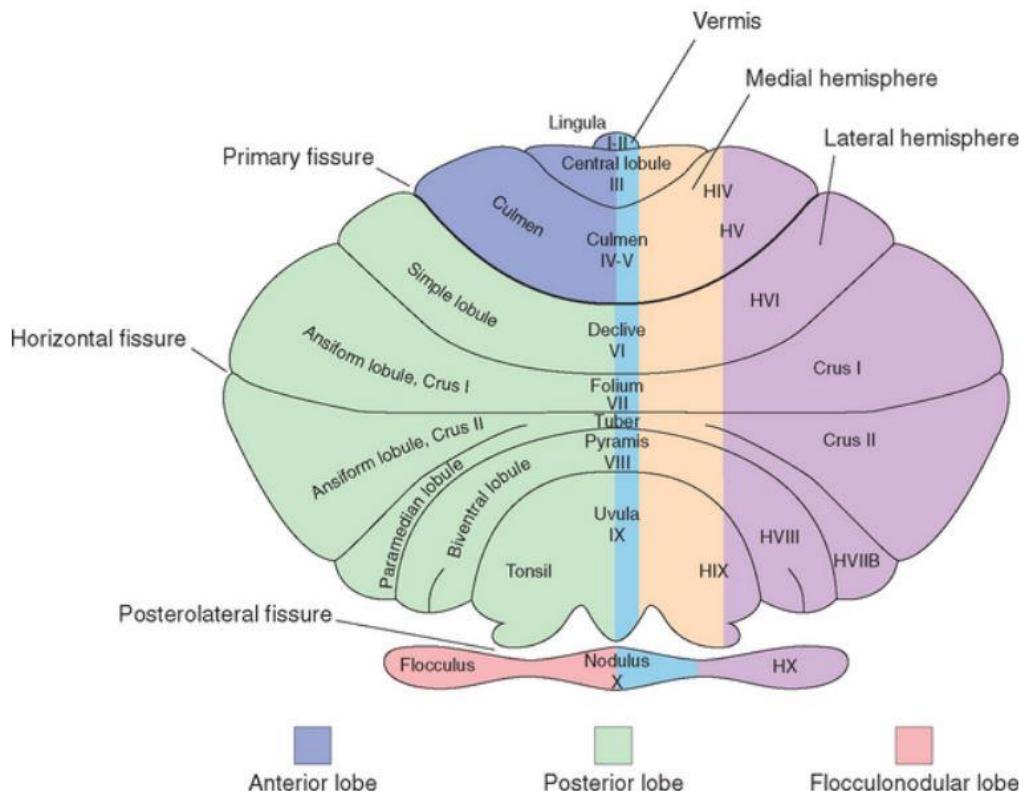


Figure 2. Large-scale anatomical subdivisions of the cerebellum. Schematic coronal view of the cerebellar lobules (I-X). Association area projections (prefrontal, parietal, temporal and cingulate areas) are localized to lobules VI and VII (posterior cerebellar lobe), while cortical areas concerned with sensory-motor processing are linked to lobules I, II, III, IV (anterior cerebellar lobe) and VII (Drawing modified from Larsell, 1951. Terminology based on Schmahmann et al., 1999).

At a microscopic level, the cerebellar cortex is composed of three layers of cortical sheet, with the most superficial one, the molecular layer, containing inhibitory neurons – the basket and stellate cells. Just below, there is the Purkinje layer, where Purkinje cells integrate the excitatory inputs coming from the granule cells, contained in the deeper granule layer, and the inhibitory ones coming from the molecular layer. Interestingly, the neurophysiological foundation of the key role of the cerebellum in representing internal models has been linked to the action of the parallel fibers, which intersect the Purkinje cells dendrites and convey together the elements required for the forward modeling: peripheral proprioceptive signals, efference copy of the motor command and sensory-motor inputs describing the current state of the motor apparatus (Wolpert et al., 1998).

Whether from a large-scale or a microscopic focus of observation, further investigation of the anatomical arrangement of neuronal cerebellar units and cerebro-cerebellar circuits, appears to be crucial to gain deeper knowledge of the organization of the cerebellum that is both functionally and clinically relevant.

The empirical evidence presented so far exclusively came from neuroimaging and clinical studies allowing the examination of the relationships between patterns of activations and functions, and the relationships between lesions and deficits respectively. Another strong contribution to cerebellar research derives from brain stimulation studies, that will be introduced and discussed below.

TARGETING THE CEREBELLUM: INSIGHTS INTO NON-INVASIVE BRAIN STIMULATION TECHNIQUES

In the last decade, non-invasive brain stimulation techniques have been increasingly applied to corroborate the findings of neuroimaging and lesion studies on the cerebellar involvement in motor and non-motor functions. Differently from the neuroimaging technology, brain stimulation techniques have the advantage of allowing researchers to test the causal relationship between neural activation and behavior. Along with basic research purposes, these techniques are promisingly tested in view of their possible application in clinical interventions for the treatment of numerous neurological, neuropsychological and psychiatric conditions (Ferrucci, Bocci, Cortese, Ruggiero, & Priori, 2016; Grimaldi et al., 2014). The scope of the present paragraph is to provide a brief overview of the major findings of the studies that have used transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) in the field of cerebellar research.

Initially applied in the study of the cerebral cortex, tDCS creates a continuous, low intensity electric current on the scalp which leads to an increase or decrease of the cortical excitability thereby depolarization or hyperpolarization of active neurons at a sub-threshold level (Paulus et al., 2013). Modulation of membrane potentials by means

of tDCS has been linked to both facilitation and impairment of behavior according to the polarity of the stimulation (Jacobson et al., 2012). Anodal stimulation increases the spontaneous firing frequency of cortical neurons and it is usually linked to the enhancement of the performance (Fregni et al., 2005; Javadi et al., 2012; Metuki et al., 2012; Straube et al., 2011; Wirth et al., 2011), whereas its impairment, due to decreasing spontaneous cells firing, is generally associated with cathodal stimulation (Boehringer et al., 2013; Pope and Miall, 2012; Straube et al., 2011). However, not all findings support the dichotomy between anodal-excitatory and cathodal-inhibitory effect (Jacobson, Koslowsky, & Lavidor, 2012). Whether the tDCS effect is excitatory or inhibitory seems to depend on various physiological factors, including the direction in which the electric field flows through different neural elements (Rahman et al., 2013). As a result, given the complex folding of the cerebellar cortex and the heterogeneous disposition of its neural structures, it is difficult to predict the outcome of cerebellar transcranial direct current stimulation (cDCS) on behavior (van Dun, Bodranghien, Mariën, & Manto, 2016).

Although there is no direct way to assess cortical excitability of the cerebellum in humans, cDCS has been proven able to modulate cerebellar functions by polarizing its cortical neurons. Moreover, available evidence indicate that this technique can have an effect on distant structures such as the cerebral cortex. This has been demonstrated by changes in TMS-induced motor evoked potentials applied to the primary motor cortex (M1) following the application of a low electrical current over the cerebellum (Popa, Russo, & Meunier, 2010; Ugawa et al., 1991). In this respect, it has been proposed that the stimulation affects the inhibition exerted by the Purkinje cells on the ongoing excitatory output from the M1 contralateral to the site of the stimulation (Ugawa, Rothwell, Day, Thompson, & Marsden, 1991). The latter is investigated as a measure of connectivity between the cerebellum and M1, termed as cerebellar brain inhibition (CBI). More recently, the application of cDCS has also proved effective in modulating behavioral performance. In more details it has been successfully deployed in the study of movement control, as emerged from studies that report changes in walking patterns (Fernandez et al., 2016; Jayaram et al., 2012), posture control (Inukai et al., 2016), visuomotor adaptation (Avila et al., 2015; Panico, Sagliano, Grossi, & Trojano, 2016;

Shah, Nguyen, & Madhavan, 2013; Yavari et al., 2016) and motor learning (Hardwick, Lesage, & Miall, 2014; Shimizu, Wu, Samra, & Knowlton, 2016; Taubert et al., 2015) following cerebellar stimulation.

Furthermore, tDCS is currently used to elucidate the contribution of cerebellar operations underpinning cognitive processing (Roberta Ferrucci & Priori, 2014). Stimulating the cerebellar cortex has been shown to modulate performances pertaining to different cognitive-related functions, such as working memory (Boehringer, Macher, Dukart, Villringer, & Pleger, 2013; Ferrucci et al., 2008; Pope & Miall, 2012), semantic processing (D'Mello, Turkeltaub, & Stoodley, 2017; Miall et al., 2016), speech timing (Lametti, Oostwoud Wijdenes, Bonaiuto, Bestmann, & Rothwell, 2016) and procedural learning (Roberta Ferrucci et al., 2013). Within the emotional-affective domain, a limited amount of research is available to date on the effect of cDCS in modulating emotion recognition (Ferrucci et al., 2012) and depressive symptoms (Ho et al., 2014). On a related note, a considerable amount of experiments did not find a significant influence of cerebellar stimulation on the behavioral performance under examination (Jalali, Miall, & Galea, 2017; Majidi et al., 2016; Spielmann et al., 2017; Steiner et al., 2016; van Dun et al., 2016). The heterogeneity of cDCS outcomes can be ascribed to methodological factors, from the stimulation setups, including positioning and time-window of application (Ferrucci, Cortese, & Priori, 2015), to the specific nature and complexity of the task. As discussed in the first paragraph, the view of the cerebellum as a controller of operations that are computed elsewhere in the brain should be taken into account when interpreting cDCS findings, as it is likely that the outcome of the stimulation may vary according to the cerebro-cerebellar interactions underlying a specific task.

Another way of gathering insights into the functions of the cerebellum may be provided by the TMS. This technique uses a strong current flowing through an electromagnetic coil to produce magnetic pulses that, administered upon the scalp, produce an electric field in the underlying cortical structures (Paulus, Peterchev, & Ridding, 2013). In this way TMS temporarily disrupts the function of a given cortical region, thereby creating what is referred to as a reversible or virtual lesion. Like tDCS, TMS has been used to assess the inhibitory tone that the cerebellum exerts over the

motor cortex. Similarly to what has been observed following the administration of an electric current (Ugawa et al., 1991), magnetic stimulation of the cerebellar cortex resulted in changes in electromyographic indexes elicited by TMS targeting the motor cortex (Yoshikazu Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1995). The inhibition exerted by cerebellar Purkinje cells over the contralateral M1 (i.e. CBI) can be probed using two magnetic pulses: a conditioning pulse over the cerebellum (conditioning stimulus (CS)) and a test pulse over the contralateral M1 (test stimulus (TS)). On a related note, CS has been found to reduce the size of electromyographic responses, recorded from the first dorsal interosseous muscle ipsilateral to the CS target, when it preceded the TS of 5.6 or 7 ms (Werhahn, Taylor, Ridding, Meyer, & Rothwell, 1996). In more details, the authors reported that a flat figure-of-eight coil induced CBI at more variable latency (5–9 ms) than a double-cone coil (5 ms) over the basal occiput. They proposed that the horizontal figure-of-eight, due to the fact that the lower wing of the coil overlaps with the posterior neck, may produce two types of motor cortical suppression: a cerebellar effect (5 ms) and late peripheral nerve effect (8 ms).

For what concerns the use of different types of coils to target the cerebellum, there is evidence that batwig and double-cone coils are more effective in delivering cerebellar stimulation to the motor cortex than the figure-of-eight one (Hardwick, Lesage, & Miall, 2014). Nevertheless, the authors argued that the distance from the scalp to the regions of the cerebellum where motor representations are stored (i.e. anterior lobe) may account for the higher effectiveness of batwig and double-cone coils in altering M1 activity. It follows that the figure-of-eight coil may be reasonably effective in modulating cognitive-related functions that are associated with the more phylogenetically recent posterior lobe of the cerebellum.

So far, only findings on the ability of cerebellar TMS to interfere with motor cortex activity have been reported. An intriguing finding has been published by Picazio and colleagues, showing an effect of cerebellar continuous theta-burst stimulation (cTBS) on cortical connectivity when motor evoked potentials (MEPs) were conditioned by stimulation of the inferior frontal gyrus but not through conditioning of the pre-supplementary motor area (Picazio, Ponzo, & Koch, 2016). This data suggests that the

cerebellum influences cortical inhibitory activity not only at the level of M1, but also through interactions with prefrontal regions.

Again similarly to what accounts for tDCS, changes in motor, cognitive and emotional-affective behaviors have been observed following the administration of TMS over the cerebellum (van Dun, Bodranghien, Manto, & Marien, 2017). Cerebellar TMS has shown to interfere with language (Argyropoulos, 2011; Argyropoulos & Mugleton, 2013; Lesage, Morgan, Olson, Meyer, & Miall, 2012), working memory (Desmond, Chen, & Shieh, 2005; Rami, Gironell, Kulisevsky, Garc, & Berthier, 2003), procedural learning (Torriero et al., 2010; Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004), timing (Bijsterbosch et al., 2011; Grube, Lee, Griffiths, Barker, & Woodruff, 2010; Koch et al., 2007) and spatial processing (Cattaneo et al., 2014; Oliver, Opavsky, Vyslouzil, Greenwood, & Rothwell, 2011; Picazio, Oliveri, Koch, Caltagirone, & Petrosini, 2013). Studies investigating emotion regulation and processing reported significant repeated TMS effect on mood regulation, as shown by an increase in negative mood in participants confronted with either neutral or aversive images (Schutter & van Honk, 2009), and facial expressions processing, as indicated by an increase in masked emotional responses to happy but not fearful or neutral facial expressions associated with magnetic stimulation targeting the vermis (Schutter & van Honk, 2006).

In sum, tDCS and TMS employment have proven to be promising tool in cerebellar research (van Dun et al., 2017). Despite differences in their physical principles of function, both techniques induce an electric current in the underlying cortical structures. While tDCS modulates the excitability of populations of neurons located in a relatively diffuse area, TMS is able to induce action potentials with a higher spatial focus resulting in the actual firing of neurons (Nitsche et al., 2008). Both cathodal-induced reduction in excitability and anodal-induced increase in cortical excitability can be elicited by distinct types of magnetic stimulation, with low-frequency repeated TMS and continuous theta-burst stimulation producing a transient reduction in cortical excitability (inhibitory effect) and high-frequency repeated TMS and intermittent theta-burst stimulation (iTBS) being associated with a reduction in inhibition (excitation effect). Although discrepancies concerning tDCS and TMS outcomes have emerged,

presumably due to differences in the specific physiological impact that they have on cerebellar neurons, the two stimulation techniques demonstrated their effectiveness in modulating cerebro-cerebellar functional connectivity (Tomlinson, Davis, & Bracewell, 2013). However, systematical and methodological research is still needed to clarify physiological mechanisms of action and the impact of the different paradigms and parameters of both techniques on cerebellar activity.

AIMS OF THE DISSERTATION

Together with neuroimaging correlations and lesion-deficit inferences, a brain stimulation approach can extend our knowledge of the functional and the topographic organization of the cerebellum. Among various brain stimulation techniques, tDCS and TMS are non-invasive tools deployed to test the relationship between the cerebellum and motor- and cognitive-related functions. The study presented in the following chapter (study 1), provides a quantitative review of the effects of tDCS over the cerebellum on behavioral performance. Indeed, given the growing number of cerebellar stimulation experiments that is currently being carried out, it may be relevant to analyze the effectiveness of one of the most commonly used neuromodulation technique to interfere with motor and cognitive performance. Despite the present dissertation focuses on the cognitive domain, we decided to include in the meta-analysis tDCS studies that modulated not only cognitive but also motor functions, due to the relatively small number of the published material on the topic. The studies presented in the following chapters aimed at examining the contribution of the cerebellum, previously highlighted by neuroimaging evidence and clinical observations, to distinct cognitive functions in healthy participants by means of tDCS (study 2) and TMS (study 3 and 4). Specifically, we concentrated on the so-called processing of primitives of the cerebellum, as error detection based on sensory prediction operations (study 2) and sequence processing (study 3), that have been suggested to sub-serve high-order cognitive and emotive processing (study 4).

Chapter 2

**STUDY 1: A meta-analysis on the effects of
cerebellar transcranial direct current stimulation
on behavioral performances**

Introduction

Growing attention has been directed in the last decades to the role of the cerebellum outside the motor domain. Along with the well-known involvement of the cerebellum in sensory-motor coordination, behavioral and physiological evidence suggest that its role of forward controller can be generalized and extended to cognition and emotion (Koziol, Budding, Andreasen, et al., 2014). In further support of this hypothesis, cerebellar lesions have been demonstrated to lead to impaired cognitive and emotional processing (Schmahmann & Sherman, 1998).

Non-invasive brain stimulation techniques have proved useful in examining cerebellar functions in healthy participants and promising in assessing therapeutic and rehabilitative interventions in the clinical population (Grimaldi et al., 2014; Tomlinson et al., 2013). Neurophysiological evidence for the ability to modulate cerebellar function using transcranial electric current comes from previous work that administered single high-voltage transcranial electric stimuli across the base of the skull and modulated activity in the dentate-thalamo-cortical pathway to the motor cortex (Ugawa et al., 1991). More recently, Galea and colleagues (2009) tested polarity-dependent effects of cerebellar transcranial direct current stimulation (cDCS) on the inhibitory tone exerted by the cerebellum to the primary motor cortex (M1), the so-called cerebellar-brain inhibition (CBI). They observed that cathodal stimulation over the cerebellum decreased the ability of the magnetic conditioning stimulus to elicit a reduction in M1 excitability, whereas anodal stimulation resulted in the opposite effect. Although there are evidence suggesting that cDCS is effective in dynamically modulating cerebellar excitability (Parazzini et al., 2014; Priori, Ciocca, Parazzini, Vergari, & Ferrucci, 2014), experiments conducted so far failed in observing clear polarity-specific effects. Discrepant results regarding changes in motor and cognitive/affective performance following either cathodal or anodal stimulation, may be due to differences in stimulation parameters, in the nature of the task and in its underpinning cerebellar neural substrates (van Dun et al., 2016). The discrepancy of findings found in the published literature indicate that further research is required to determine the exact impact of tDCS over the cerebellum to reliably predict its outcomes. To our knowledge, no meta-analytic study has tried to

systematically examine the efficacy of ctDCS on behavioral measurements. Therefore, the general aim of the present meta-analysis was to provide a quantitative synthesis of the published literature investigating the effects of tDCS over the cerebellum on motor and cognitive performances. Specifically, the aim of the study was two-folded: we wanted 1) to test cDCS efficacy in modulating behavior, and 2) test the hypothesis that anodal cDCS improves and cathodal cDCS impairs performances.

Methods

Study selection

A literature search was conducted using the scientific online database PubMed to identify potential studies for inclusion in the meta-analysis in the period between January 2000 and March 2017. Search criteria were “transcranial direct current stimulation” \wedge “cerebellum” and “tDCS” \wedge “cerebellum”. In addition, the reference lists of previous reviews [12,13] were screened to minimize the risk of overlooking potentially suitable studies for inclusion. Studies that met the following criteria were included: (i) Adult healthy volunteers; (ii) Sham controlled randomized experimental design; (iii) Administration of tDCS with at least one electrode placed over the cerebellum; (iv) Cognitive or motor performance index (i.e., accuracy or reaction times) as primary endpoint; (v) Article published in a peer-reviewed English-language journal; (vi) Study approved by a medical ethical committees or review board. In Figure 1 the flowchart of the selection procedure is presented.

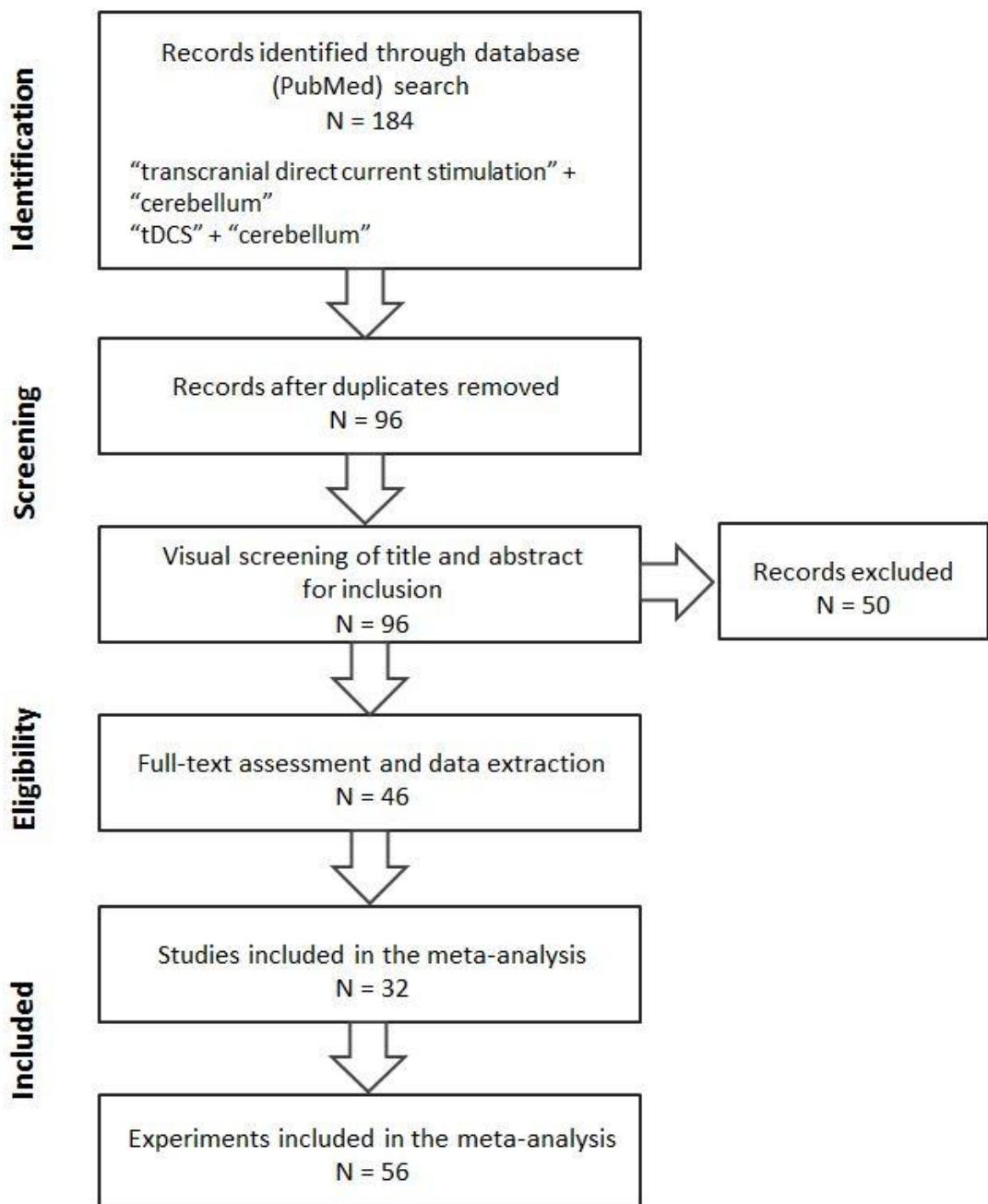


Figure 1. Flowchart study selection procedure.

The literature search identified a total of 184 articles. After the completion of studies retrieval and removal of duplicates, title and abstract of the ninety-six records remained were screened against inclusion/exclusion criteria. Sixty articles were selected to undergo full-text examination for eligibility and an additional eighteen studies were excluded. The remaining thirty-two studies were included in the present meta-analysis. Of these thirty-two studies, fifteen studies applied both anodal and cathodal stimulation (ID: 2, 4, 10, 14, 17, 19, 21, 22, 24, 27, 28, 29, 31 and 32) [14-27], fourteen anodal (ID 1, 5,

6, 7, 8, 11, 12, 13, 15, 16, 18, 20, 23, 30) [28–41] and three cathodal tDCS (ID: 3, 9, and 26) [42, 43, 45] (Table 1). In study 25 [44] two separate experiments were reported: in the first (i.e., experiment 25) both anodal and cathodal stimulation were applied, whereas only anodal stimulation was applied in the second experiment (i.e., experiment 22). In Table 1 an overview of the main study characteristics.

Table 1. Study characteristics

ID	Study	Exp.	Design	Timing	Stimulation type	Active electrode	Reference electrode	Electrodes size	Intensity	Duration	Sham
1	Avila et al., 2015	1	within	offline/online	anodal	right cerebellum	left buccinator muscle	1.2 cm ² - 1.2 cm ²	1.5 mA	15 min	30 s
2	Beyer et al., 2017	2	between	offline/online	anodal/cathodal	right cerebellum	right deltoid muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	40 s
3	Boehringher et al., 2013	3	between	offline/online	anodal/cathodal	right cerebellum	right buccinator muscle	36 cm ² - 35 cm ²	2.0 mA	20 min	40 s
4	Cantarero et al., 2015	4	between	offline	anodal/cathodal	right cerebellum	right buccinator muscle	37 cm ² - 35 cm ²	2.0 mA	20 min	40 s
5	Craig et al., 2017	5	within	offline/online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	30 s
6	D'Mello et al., 2017	6	between	offline	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	30 s
7	Doppelmayr et al., 2016	7	between	offline/online	anodal	vermis	right clavicle	50 cm ² - 25 cm ²	2.0 mA	20 min	/
8	Ehsani et al., 2016	8	between	offline/online	anodal	right cerebellum	right deltoid muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	30 s
9	Fernandez et al., 2016	9	between	offline	cathodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	/
10	Ferrucci et al., 2012	10	within	offline	anodal/cathodal	vermis	right deltoid muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	10-15 s
11	Ferrucci et al., 2013	11	within	offline	anodal	vermis	right deltoid muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	30 s
12	Galea et al., 2011	12	between	offline/online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	15 min	30 s
13	Hardwick et al., 2014	13	between	online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	15 min	30 s
14	Inukai et al., 2016	14	within	offline	anodal/cathodal	vermis	forehead	35 cm ² - 35 cm ²	2.0 mA	20 min	/
15	Jalali et al., 2017	15	between	offline/online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	10 s
16	Lametti et al., 2016	16	between	offline/online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	10 s
17	Macher et al., 2014	17	between	offline/online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	10 s
18	Majidi et al., 2016	18	between	online	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	10 s
19	Mhall et al., 2016	19	between	offline	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	25 min	10 s
20	Panico et al., 2015	20	between	offline	anodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	21 min	30 s

Table 1. (continued)

ID	Study	Exp.	Design	Timing	Stimulation type	Active electrode	Reference electrode	Electrodes size	Intensity	Duration	Sham
21	Picazio et al., 2015	29	within	offline	anodal/cathodal	left cerebellum	left deltoid muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	45 s
22	Pope & Miall, 2012	30	between	offline	anodal/cathodal	right cerebellum	right deltoid muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	110mA over 15 ms every 550 ms
		31	between	offline	anodal/cathodal	right cerebellum	right deltoid muscle	26 cm ² - 25 cm ²	2.0 mA	21 min	110mA over 15 ms every 550 ms
23	Sameai et al., 2017	32	between	offline/online	anodal	right cerebellum	right deltoid muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	
24	Shah et al., 2012	33	within	offline	anodal/cathodal	left cerebellum	left buccinator muscle	8 cm ² - 35 cm ²	1.0 mA	15 min	/
25	Shimizu et al., 2016	34	between	offline/online	anodal/cathodal	vermis	left or right buccinator muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	2.0 mA for 30 s, 1.0 mA for 20 min
		35	between	offline/online	anodal	vermis	left or right buccinator muscle	35 cm ² - 35 cm ²	2.0 mA	20 min	
26	Spielmann et al., 2017	36	within	offline	cathodal	right cerebellum	right deltoid muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	
27	Steiner et al., 2016	37	between	offline/online	anodal/cathodal	vermis	right and left buccinator muscle	35 cm ² - 25 cm ²	2.0 mA	10 min	30 s
28	Taubert et al., 2016	38	between	offline/online	anodal/cathodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	20 min	30 s
29	van Wessel et al., 2015	39	within	online	anodal/cathodal	right cerebellum	left buccinator muscle	1.2 cm ² - 1.2 cm ²	2.0 mA	20 min	30 s
30	Verhage et al., 2017	40	between	online	anodal	right cerebellum	right buccinator muscle	1.2 cm ² - 1.2 cm ²	1.5 mA	20 min	60 s
31	Yavari et al., 2016	41	between	offline	anodal/cathodal	right cerebellum	right buccinator muscle	25 cm ² - 25 cm ²	2.0 mA	15 min	30 s
32	Zuchowski et al., 2014	42	between	online	anodal/cathodal	right cerebellum	right buccinator muscle	35 cm ² - 35 cm ²	2.0 mA	42.9 min	30 s

Data synthesis and analysis

Performance accuracy was our primary dependent variable of interest. Reaction times were used in case no other behavioral indices were available. For performances that were evaluated across multiple time points, the aggregate mean $[(\mu_1 + \mu_2 + \dots + \mu_n^2) / k]$ and pooled SD $[\sqrt{(\text{SD}_1^2 + \text{SD}_2^2 + \dots + \text{SD}_n^2) / k}]$, where k = total number of data points, were calculated. The following descriptive data were taken from each study: sample size, mean and standard deviation (SD) of the outcome measure for the stimulation and the sham condition. In case standard errors of the mean (SE) were provided, SD were calculated by applying the following formula $\text{SD} = \text{SE} \times \sqrt{n}$. When data were presented graphically, mean and SD were estimated from the figures using free WebPlotDigitizer gms3.10 software (<http://arohatgi.info/WebPlotDigitize>). Corresponding authors were contacted in case the relevant numbers for the analysis could not be extracted from the paper.

The effect size metric Hedges' d was used which is a standardized mean difference that accounts for the fact that the sampling variance for 'active' and 'sham' conditions may not always be equal [36]. From these effect sizes the Hedges' d values were calculated to correct for a bias in effect size due to small group samples [46]. For the meta-analysis non-parametric variances were chosen to control for small sample sizes. Next, a weighted average was used to compute the cumulative effect size (\bar{E}) and the 95% confidence intervals (CI). \bar{E} represents the aggregated magnitude of the effect size of the included studies [46].

To address our first research question related to the efficacy and reliability of cDCS to induce behavioral effects, the absolute cumulative effect sizes ($|\bar{E}|$) were entered in a random effects model. Total heterogeneity of the effect sizes (Q_T) was calculated and tested against the χ^2 distribution with $(n - 1)$ degrees of freedom [47]. In addition, the I^2 $[(Q_T - df / Q_T) * 100]$ of heterogeneity was reported which is an index for the residual proportion of the observed variance if sampling error is zero [48].

A significant Q_T means that the variance of the effect sizes is greater than to be expected from sampling errors and suggests that the observed variance can be explained by other variables besides cDCS. To explore the robustness of the results to

the possibility of publication bias, fail-safe number of studies was computed to obtain an estimate of how many non-significant or missing studies would render the observed meta-analytical results non-significant (Rosenthal's method: $\alpha < 0.05$). To address our second research question concerning the assumed polarity-dependent effects of cDCS, data were analyzed in a similar way as was done in the first series of analyses, except that the signed effect sizes (\bar{E}) were used. All analyses were performed with MetaWin version 2 [49] and Wilson's macros for meta-analyses in SPSS [50].

Results

Unsigned cumulative effect size: The random effects model ($n=60$) between real and sham cDCS showed a significant $|\bar{E}|$ of 0.55, 95%CI = 0.38 – 0.73, $Z = 6.27$, $p < 0.001$. Total heterogeneity was not significant, $Q_T = 59.74$, $p = 0.45$, $I^2 = 1.23$.

Anodal ctDCS showed a significant $|\bar{E}|$ of 0.59, 95%CI = 0.34–0.83, $Z = 4.84$, $p < 0.001$ (Figure 2). Total heterogeneity was not significant, $Q_T = 35.94$, $p = 0.57$, $I^2 = 0$, and the Rosenthal's fail-safe number was 303.

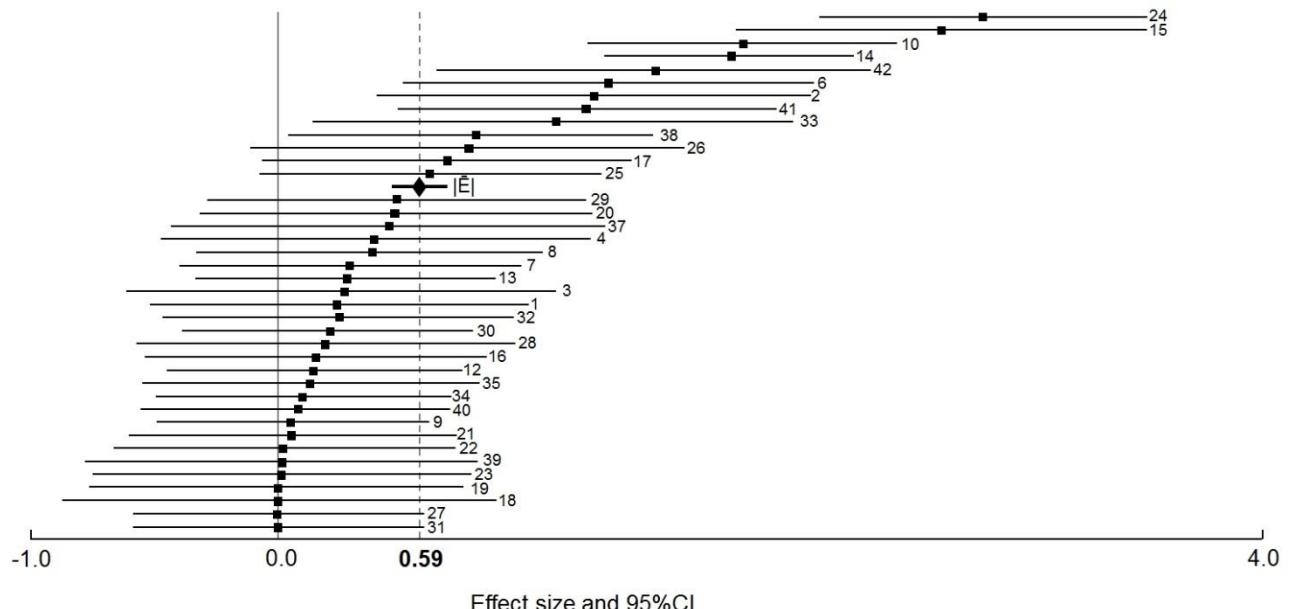


Figure 2 . Forest plot showing effect size estimates (Hedges' d) and 95% confidence interval of the experiments comparing anodal with sham cDCS in healthy volunteers.

Cathodal cDCS showed a significant $|\bar{E}|$ of 0.46, 95%CI = 0.23–0.69, $Z = 4.23$, $p < 0.001$ (Figure 3). Total heterogeneity was not significant, $Q_T = 23.65$, $p = 0.26$, $I^2 = 15.43$, and the Rosenthal's fail-safe number was 131.

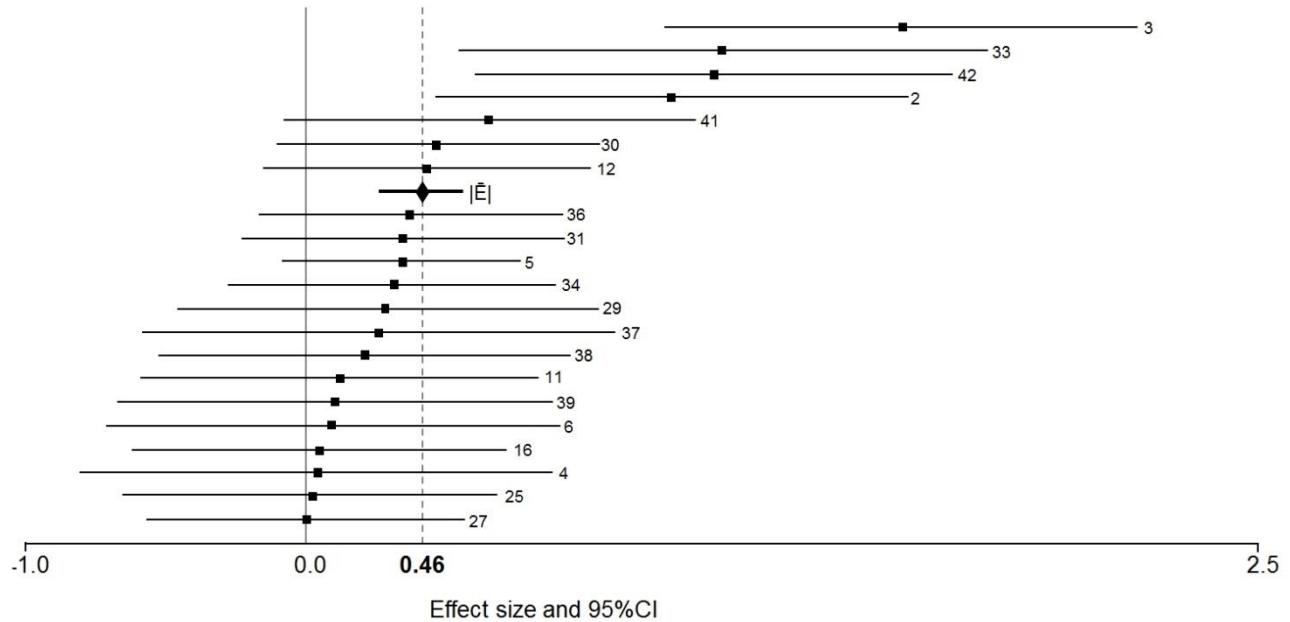


Figure 3. Forest plot showing absolute cumulative effect size estimates (Hedges' d) and 95% confidence interval of the experiments comparing cathodal with sham cDCS in healthy volunteers.

Even though effect sizes of anodal and cathodal cDCS were similar, $Q = 0.31$, $p = 0.58$, cDCS was more efficacious in modulating motor-, $|\bar{E}| = 0.71$, 95%CI = 0.49 – 0.92, than cognitive-related tasks, $|\bar{E}| = 0.32$, 95%CI = 0.06 – 0.58, $Q = 5.24$, $p = 0.02$. Whether performances were measured during cDCS (online), $|\bar{E}| = 0.70$, 95%CI = 0.32 – 1.07, after cDCS (offline), $|\bar{E}| = 0.61$, 95%CI = 0.33 – 0.88, or both during and after cDCS (on-offline), $|\bar{E}| = 0.42$, 95%CI = 0.14 – 0.69, did not influence the magnitude of the effect size, $Q = 1.67$, $p = 0.43$.

Signed cumulative effect size: The random effects model ($n=60$) between real and sham cDCS showed a non-significant $|\bar{E}|$ of 0.55, 95%CI = 0.38 – 0.73, $Z = 6.27$, $p < 0.001$. Total heterogeneity was not significant, $Q_T = 59.74$, $p = 0.45$, $I^2 = 1.23$.

Anodal cDCS showed a non-significant \bar{E} of 0.05, 95%CI = -0.22–0.31, $Z = 0.30$, $p = 0.77$. Total heterogeneity was not significant, $Q_T = 47.40$, $p = 0.14$, $I^2 = 19.83$.

Finally, the random effects model for cathodal versus sham cDCS was not significant, $\bar{E} = 0.15$, 95%CI = -0.15–0.46, $Z = 1.07$, $p = 0.29$. Total heterogeneity was also not significant, $Q_T = 24.86$, $p = 0.21$, $I^2 = 19.87$.

In sum, 1-2 mA cDCS is effective in modulating motor- and non-motor related performance, but there is no evidence for polarity dependent effects of anodal and cathodal cDCS on behavioral indices of cerebellar functioning in healthy volunteers. Table 2 shows the main statistical outcomes of the meta-analysis.

Random effects model	Functional domain	n	Effect size	95%CI		Z	p-value	Q_T	p-value	I^2	Fail-safe number
				lower	upper						
E	Total	60	0.55	0.38	0.73	6.27	< 0.001	59.74	0.45	1.23	844
	Motor	37	0.71	0.49	0.92	6.49	< 0.001	33.85	0.57	0	330
	Cognition	23	0.32	0.06	0.58	2.39	0.02	15.13	0.85	0	148
	Anodal	39	0.59	0.34	0.83	4.84	< 0.001	35.94	0.57	0	303
	Cathodal	21	0.46	0.23	0.69	4.23	< 0.001	23.65	0.26	15.42	131
\bar{E}	Total	60	-0.09	-0.31	0.13	0.78	0.44	63.61	0.32	7.81	x
	Anodal	39	0.05	-0.22	0.31	0.30	0.77	47.40	0.14	19.83	x
	Cathodal	21	0.15	-0.15	0.46	1.07	0.29	24.86	0.21	19.87	x

CI: Confidence interval, | E |: unsigned cumulative effect size, \bar{E} : signed cumulative effect size

Table 2. Main results.

Discussion

Transcranial direct current stimulation (tDCS) is increasingly used in cerebellar research. However, still little is known about how tDCS modulates cerebellar activity. A general agreement has risen on the need of systematic research aimed at investigating the impact of different stimulations setups on motor and non-motor functions, in order to test cerebellar transcranial direct current stimulation (cDCS) efficacy and its potential deployment in therapeutic protocols (Grimaldi et al., 2014).

The aim of the present meta-analysis was to investigate the effect of cDCS on behavioral performance in healthy participants. Effect size estimates of both anodal and cathodal experiments were not statistically significant. Despite the anodal-excitability

and cathodal-inhibition dichotomy known in the neuromodulation literature, a remarkable numbers of experiments included in the present work have reported polarity-independent effects. Using the unsigned effect size estimates allowed us to examine the influence of stimulation polarities regardless of their generally expected direction (i.e. facilitation following anodal and impairment following cathodal stimulation). Interestingly, a significant effect emerged from the analysis on the unsigned effect size estimates, suggesting that both anodal and cathodal stimulation effectively influenced participants performance as compared to sham stimulation. In more details, analysis on anodal experiments resulted in a medium effect size, whereas the one on cathodal effects resulted in a medium-low effect size – conventional values of “low”, “medium” and “high” effect size are $d = 0.2, 0.5$ and 0.8 respectively (Cohen, 1988). Overall, the present findings seem to suggest that cDCS is able to modulate motor and cognitive performance, although its effect appears to be polarity-independent.

The polarity-specificity of tDCS over the cerebellum has been observed in previous research that demonstrated decreased and increased motor cortical excitability following anodal and cathodal stimulation respectively (Galea et al., 2009). However, further behavioral evidence has been inconsistent with these findings. Studies that aimed at modulating behavior by means of cDCS failed in detecting clear anodal-facilitation and cathodal-inhibition effects on non-motor functions in healthy participants (Ferrucci et al., 2008; Ferrucci et al., 2012; Macher, Bohringer, Villringer, & Pleger, 2014; Panouillères, Miall, & Jenkinson, 2015; Shah, Nguyen, & Madhavan, 2013). The scarcity of polarity-specific effects of cDCS may be due to several factors. Firstly, recent evidence indicate that the impact of the stimulation depends on several physiological variables, as the spatial distribution of neurons in different cortical layers, the orientation of neuronal structures (parallel vs. perpendicular) relative to the electric field (with the current flowing from the soma to the dendrites or vice-versa) (Rahman et al., 2013), and on their previous physiological state (Thirugnanasambandam et al., 2011). Secondly, along with the well-established effects of different stimulation parameters, growing attention has been directed to the potential role of task characteristics in

modulating tDCS influence on behavior. Gill and colleagues (2015) pointed out that the outcomes of the stimulation are influenced by the nature of the cognitive activity elicited by the task applied. Given that tDCS does not directly stimulate action potential in neurons, whereas it modulates their spontaneous firing frequency, the authors argued that the extent to which tDCS is able to affect cortical excitability depends on the state of activation of the neural networks that are, in turn, activated accordingly to specific cognitive loads and demands. In other words, the interaction between task-dependent or load-dependent effects on the neuronal activation state and stimulation setups, might explain the heterogeneity of cDCS and cerebral tDCS results.

Relatively to the cerebellum as the target region of the stimulation, the interaction between the aforementioned factors should be considered in light of the multiple projections that connect the cerebellum to the brain. For instance, a task-specific facilitation effect has been observed in a between-subjects study that applied cathodal tDCS over the cerebellum prior to the completion of a working memory task with high or low cognitive loads (Pope & Miall, 2012). Results showed improved performance following cathodal stimulation as compared to sham condition but only in the high working memory load condition, hence when the task made high demands on cognitive resources. The authors suggested that the observed counter-intuitive facilitatory effect of cathodal stimulation may be owed to a stimulation-induced decrease in the inhibitory tone exerted by the cerebellum to the prefrontal cortex, resulting in a release of cerebral cognitive resources.

It is necessary to specify that, due to the relatively small number of articles published on the topic to date, we could not perform sub-analyses to explore questions such as under which circumstances polarity-dependent effects may still be applicable to cDCS and which types of function are particularly sensitive to cDCS. However, our data showed that motor performance was significantly more affected than cognitive performance. On one hand, this result is in line with the classic view of cerebellum as a structure predominantly involved in motor functions. On the other hand, analyses provided evidence supporting the efficacy of cDCS in modulating cognitive performance, indicating that the cerebellum operates outside the motor domain. On a

related note, it has been hypothesized that the several null effects of tDCS on cognition found in the literature may be due to the fact that when facing a cognitive task, broad and distributed neuronal networks are activated. It follows that modulating only one region within a network may be not sufficient to elicit or observe changes in behavior, whereas stimulation over motor areas is thought to be less vulnerable to external noise and, hence, more effective (Jacobson et al., 2012).

Another explanation for the heterogeneous findings of cDCS studies call into question the role of time-dependent factors. Several publications reported significant effects (p-Value) of cDCS on specific task time-points following or during the stimulation. However, outcomes entered in the present meta-analysis represent an average of multiple task time periods or blocks. If the cerebellum supports other cerebral cortex processes within a defined time-window, it could be conjectured that changes in performance could be more likely to occur only during a specific phase of the task, as suggested by previous research (Arasanz, Staines, Roy, & Schweizer, 2012; Oliver et al., 2011). Calculating an average value for each measurement from multiple task-points or blocks may explain why studies describing significant cDCS effects resulted in a non-significant or low effect size in the present meta-analysis.

In conclusion, the results presented here seem to suggest that tDCS applied over the cerebellum is overall able to influence behavior in healthy participants. We propose that the absence of a clear polarity-specificity may have led to non-significant effect size estimates in the first analysis, as suggested by results on the absolute effect size values indicating a medium effect size estimate for anodal and cathodal stimulation paradigms respectively.

Nevertheless, the present findings should be also interpreted in light of methodological limitations. First of all, due to the limited number of published data on the topic, we used both cognitive and motor measurements in each analysis even though, as discussed above, cDCS outcomes are likely to vary according to the domain, the nature of the task and how the cerebellum interacts within the brain network engaged in the latter. Furthermore, studies with different stimulation setups have been selected and compared with each other. Lastly, the results of the present meta-analysis

highlight the need of further research aiming to test cDCS efficacy in modulating behavior. In more details, the effects of factors like polarity, task type, timing and their interaction should be considered to optimally interpret future findings.

Chapter 3

STUDY 2: The role of the cerebellum in error processing and response inhibition: a tDCS/EEG study

Introduction

The fact that the cerebellum contains internal models which sub-serve the prediction and the processing of sensory events, the coordination and the fine-tuning of actions responses has put the cerebellum at the interface of motor- and cognition-related processes. Several researchers have proposed that the cerebellum functions as a generalized forward controller (D'Angelo & Casali, 2012), comparing internally stored templates – used as estimators of the expected feedback – with somatosensory feedback. The cerebellum is proposed to be involved in monitoring and guiding the process aimed at correcting errors, by mechanisms of detection of mismatch between expectation and actual feedback and updating of the internal template (Blakemore, Frith, & Wolpert, 2001; Ito, 2008a).

The contributions of the cerebellum in novelty/error-detection functions have been investigated in patients with cerebellar damage using the electroencephalogram (EEG). Specifically, event-related potentials (ERPs) have been used to examine the temporal dynamics of error-related processes and performance monitoring. Significant differences in ERPs components have been observed between cerebellar patients and healthy controls. The so-called Error Related Negativity (ERN) potential is an ERP component that arises approximately 100 milliseconds after the initiation of an erroneous response (Luu, Tucker, & Makeig, 2004), and has been found to play an important role in monitoring and inhibition of subsequent (erroneous) responses during conflict. In more details, the ERN signals the detection of a mismatch between the required and actual response (Christ, Falkenstein, Heuer, & Hohnsbein, 2000). Reduced ERN amplitude associated with error/processing and performance/monitoring have been reported in patients with cerebellar disorders (Peterburs et al., 2015).

Even though the anterior cingulate cortex (ACC) is the neural generator of the ERN (Averbeck, 2017; Dehaene, Posner, & Tucker, 1994), the ACC is part of a broader network involved in coding error-related processes. For example, medial prefrontal cortex (PFC) activation has been observed during error and conflict detection (Kiehl, Liddle, & Hopfinger, 2000). Accordingly, a group of participants with damage of the PFC failed to show the typical difference in ERN components between correct and erroneous

responses compared to healthy controls (Gehring & Knight, 2000). The authors argued that the observed ERN dysfunction may depend on disruptions of the information stream flowing between the PFC and the ACC. Importantly however, problems in action monitoring can arise without structural damage to the ACC (Rushworth, Hadland, Gaffan, & Passingham, 2003). For example, neuropsychological evaluation of patients with damage confined to the cerebellum showed deficits in ACC-like behaviors (Schmahmann & Sherman, 1998) and further strengthens the hypothesis of a contribution of the cerebellum in error-processing. Specifically, the cerebellum has been proposed to influence the activity of the ACC and lateral prefrontal cortical areas via the cerebello-thalamo-cortical projections (Ide & Li, 2011).

Transcranial direct current stimulation (tDCS) is increasingly used as a non-invasive stimulation technique to target the human cerebellum and examine cerebellar functioning (Oldrati & Schutter, 2017). By applying a weak electrical current in the vicinity of the target area, tDCS is able to modulate cortical neuronal excitability and produce changes in behavior. Interestingly, alterations in cortical activity following tDCS over the cerebellum have been observed in the execution of cognitive tasks. Mannarelli and colleagues (2016) applied cerebellar direct current stimulation (cDCS) in healthy participants performing a novelty task and observed reduced amplitude of N2 and P300 components – associated with the detection of conflict and novel target stimuli – following cathodal stimulation compared to pre-test. Similarly, another study observed decreased peak amplitude of mismatch negativity – an ERP arising in response to a deviating stimulus in a sequence of consistent stimuli – following cathodal cDCS compared to the control condition (Chen, Hammer, D’Ostilio, & Casula, 2014). Overall these studies show that tDCS is a validated method to study cerebellar functions *in vivo* and provide opportunities to further explore the role of the cerebellum in cognitive processes linked to error monitoring and response inhibition.

The aim of this study was to combine non-invasive transcranial direct current stimulation (tDCS) with EEG measurements to transiently interfere with cerebellar functions in order to test the hypothesis that action monitoring would be affected. On a behavioral level, we anticipated that cahtodal tDCS-based perturbation would lead to

an increase of the number of errors (i.e. failed response inhibition) and post-error slowing of reaction times (i.e. phenomenon consisting of longer reaction times following an erroneous response and considered a behavioral index of awareness) (Laming, 1979). On the electrophysiological level, we anticipated the decrease in behavioral performance could be explained by cerebellum-related reduction of the ERN amplitudes during cathodal as compared to sham tDCS over the cerebellum.

Methods

Participants

Twenty-six healthy volunteers (12 females, mean age=22.9 yrs, SD=3.2, range 18–30) took part in the study. All participants were right-handed as measured by the Edinburgh inventory of handedness (Oldfield, 1971) and had normal or correct-to-normal vision. Prior to the experiment all participant filled out a questionnaire to evaluate their suitability for tDCS. None of the volunteers had a history of neurological disorders or brain trauma, or a family history of epilepsy. Participants were excluded if they had metallic objects in their head, had any type of stimulator in their body, used medication (except oral contraceptives) or recreational drugs less than 48 hours before the experiment, or suffered from a skin disease or skin allergy. Participants were naïve to the aim of the study and paid for participation. Participants provided written informed consent. The study was approved by the medical ethical committee of the Radboud University Medical Centre in Nijmegen and carried out in accordance with the standards set by the Declaration of Helsinki.

Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) was delivered by a battery-driven constant DC current stimulator (Eldith DC Stimulator (CE 0118), Ilmenau), using one 5*7 cm electrode (35 cm²) – current density: 0.05 mA/cm² – and one 5*5 electrode (25 cm²) – current density: 0.08 mA/cm² – in saline-soaked synthetic sponge at a low continuous current intensity of 2 mA. In both stimulation conditions, the active electrode was

placed over the medial cerebellum – 2 cm below the inion with electrode's lateral borders 1 cm medially to the mastoid apophysis – whereas the reference electrode was placed over the right buccinator muscle (Figure 1). Both real and sham tDCS involved a ramp up period of 15s in which intensity was gradually increased to the established maximum. In the active cathodal condition, the stimulation was ramped down (15s) after 20 minutes of 2 mA cDCS. In the sham condition, the ramp up period was followed by 30s of real stimulation after which the intensity was ramped down to 0 mA (15s), thus participants felt the characteristic tingling sensations in the vicinity of the electrodes for a brief period of time, which enhanced the plausibility of the control condition.

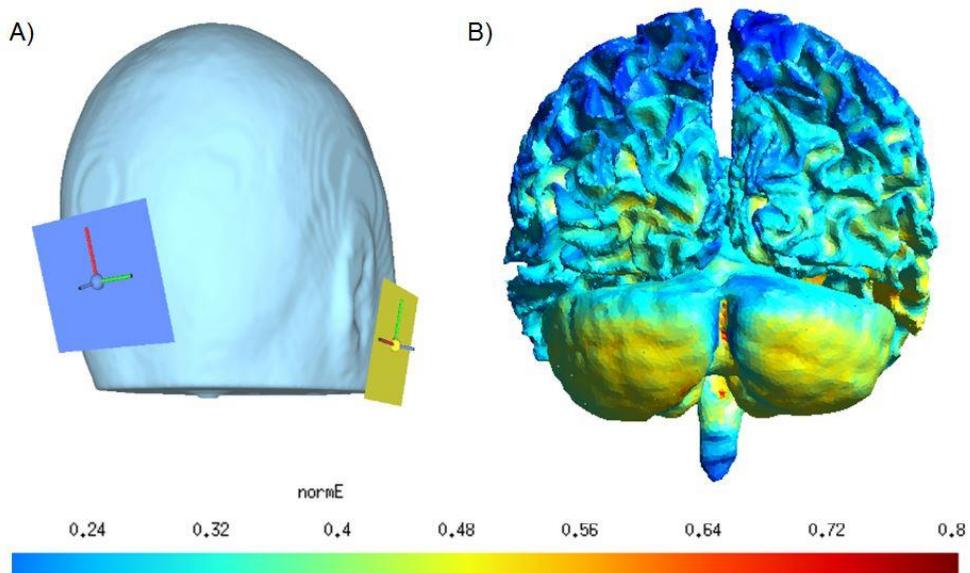


Figure 1. A) View of electrodes positioning; B) Model of current density amplitude field distributions over the cerebellum according to the stimulation setup (created with SimNIBS 2.0 - Thielscher, Antunes, & Saturnino, 2015).

Electroencephalogram

To monitor changes in electric activity, EEG was recorded from 32 scalp locations according to the International 10-20 EEG System using Ag–AgCl tipped electrodes (sampling rate: 256 Hz) during tDCS stimulation. Electro-oculogram (EOG) was recorded from a bipolar montages from the sub- and supra-orbital regions (VEOG1 and VEOG2) of the right eye and the outer canthi of the eyes (HEOG1 and HEOG2). EEG recordings were made with the BrainAmp system (Brain Products GmbH, Gilching, Germany) with

passive electrodes that are compatible with magnetic and electrical brain stimulation (www.brainproducts.com).

Eriksen flanker task

The Eriksen flanker tasks is a validated cognitive task that assesses performance monitoring (Eriksen & Eriksen, 1974). This task is routinely used to measure ERN evoked potentials and behavior in the context of error monitoring and response inhibition. On a computer screen, a central target stimulus flanks by two non-target stimuli on each side of the target stimulus for each trial. Participants were asked to indicate whether the non-target stimuli were either congruent or incongruent with the target stimulus. The task was composed of a total of 480 trials. Half of the trials consists of congruent strings – ‘BBBBB’ (120 trials) and ‘88888’ (120 trials) - whereas the other half of incongruent strings - ‘BB8BB’ (120 trials) and ‘88B88’ (120 trials). After the participants provided their response, a feedback indicating correct (“right”) or incorrect response (“wrong”) was presented for 500 ms on the screen. Inter-trial interval varied randomly between 1400-1600 ms. Response time limit was set at 500 ms following stimulus offset. Participants had to respond by left/right mouse pressing using their left/right thumbs as fast as possible. Response button assignment was counterbalanced across participants (e.g. congruent trials were associated for half of the sample with the left response button, for the other half with the right response button). Participants were presented with eight example trials to allow them to familiarize with the task before the beginning of the actual experiment. Figure 2 depicts an example of experimental trial.

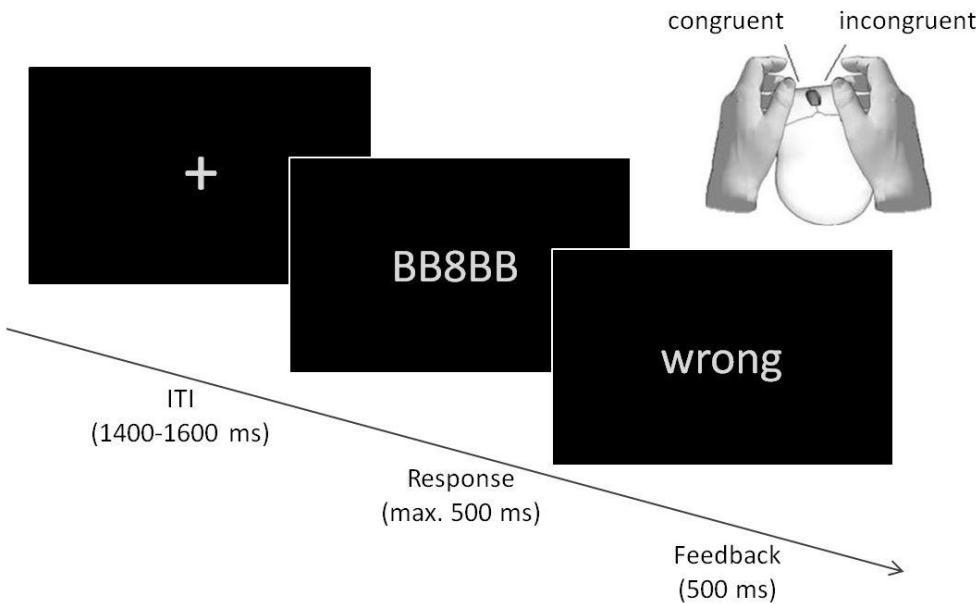


Figure 2. Example of an experimental trial. Participants were presented with congruent strings ("BBBBB" or "88888") or incongruent strings ("BB8BB", "88B88") and were asked to indicate whether the non-target stimuli at the center of the string were either congruent or incongruent with the target stimulus. After participants provided their response, a feedback (right or wrong) was presented.

Procedure

In this single-blind, sham-controlled cross-over design, a university participant database was used to recruit healthy adult volunteers. The experiment consisted of two sessions of approximately 1 hour each, taking place one week apart. Furthermore, participants were tested at the same time of the day in both sessions. Participants that received cathodal stimulation in the first session, were administered with sham stimulation in the second session and vice versa. Order of stimulation condition was randomized and counterbalanced.

Before the experiment started, volunteers filled out a safety screening and handedness form. After the placement of EEG, participants received 20 minutes tDCS while performing the Eriksen flanker task. At the end of the experiment, participants were asked if they had experienced any side effects (e.g. itching or burning feeling) during tDCS.

Sample size was calculated based on moderate effect size of 0.5 (Cohen, 1988) and a statistical power of 0.8. Parameters for sample size calculation were based on a recent meta-analysis (Oldrati & Schutter, 2017). A random effects model based on

Hedges' g effect sizes showed a significant cumulative effect size ($|E|$) of 0.46, bootstrap confidence interval = 0.23–0.69, indicating that cathodal as compared to sham tDCS is effective for establishing behavioral effects.

Data reduction and statistical analysis

Behavior

Percentage errors were computed for all participants in the active and sham cDCS. Post-error slowing of reaction times was computed by subtracting the reaction times of correct response following an incorrect response on the previous trial from reaction times of correct response following a correct response (Cavanagh, Cohen, & Allen, 2009).

Two separate repeated measures analysis of variance (ANOVA) were conducted with "stimulation" (active versus sham cDCS) as within-subject factor for the percentage errors and post-error slowing of reaction time. For all comparisons, the level of significance was set to $\alpha = 0.05$ (two-tailed) and all mean values are presented \pm SEM.

EEG recordings

Raw EEG data were recorded and stored for offline analysis using BrainVision Analyzer 2.0 (Brain Products GmbH, München, Germany). EEG recordings were offline band-pass filtered between 1 and 30 Hz (48dB/Octave) and re-referenced to an average reference. Ocular artifacts were removed using the Gratton and Coles algorithm (Gratton, Coles, & Donc0hin, 1983). Average response locked event-related potentials was computed for correct and incorrect trials by selecting the time window between -100 – 1000 ms. The 100 ms prior to response has been used as the baseline for the 1000 ms post-response period. Epochs containing amplitudes larger than minus/plus 50 μ V were excluded from further analysis. Ne/ERN amplitude was scored from the central midline (Cz) electrode as the peak negative amplitude between 0 and 100 ms after button press for the incorrect, correct and correct-after-incorrect trials.

A separate 2x2 repeated measures analysis of variance (ANOVA) was conducted with factors "stimulation" (active versus sham cDCS) and "response" (correct versus incorrect) for the ERN component.

Results

Brain stimulation was well tolerated and no adverse events occurred. Three out of twenty-six participants (11.5%) reported an itching sensation at beginning of the actual stimulation, which quickly disappeared, while three participants reported an unpleasant sensation in the vicinity of the electrodes during both real and sham stimulation. Finally, fifteen of twenty-six participants (57%) correctly guessed, at the end of both sessions, when they had received either real or sham stimulation.

Behavioral performance

Two separate repeated measure ANOVA were performed to detect differences in the percentage of error and in the post-error slowing reaction time between cDCS conditions. The analysis on the percentage error showed a significant difference between cathodal and sham stimulation ($F_{25}=4.17$, $p=0.05$, $\eta_p^2=0.14$; cathodal stimulation: mean=15.29, SEM=1.33; sham stimulation: mean=13.89, SEM=1.09). The data indicate that participants produced more errors during cathodal than during sham cDCS (Figure 3).

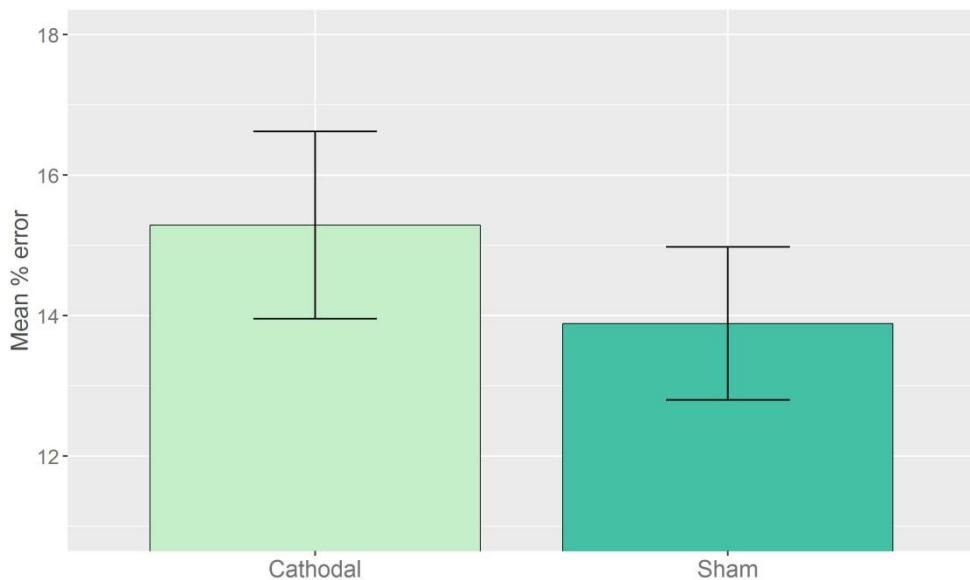


Figure 3. Mean percentage of errors among conditions. Error bars represent ± 1 SEM.

Although mean comparisons showed that in both experimental conditions participants took longer in providing a response following erroneous trials as compared to correct trials (i.e., post-error slowing effect) (cathodal stimulation: $t_{25}=1.99$, $p=0.06$; sham stimulation: $t_{25}=2.68$, $p=0.01$), the result of the repeated measure ANOVA the on post-error slowing reaction time between cathodal and sham stimulation was not significant ($p=0.55$).

Error-related Negativity

We conducted a 2x2 repeated measure ANOVA with factors stimulation (real vs. sham tDCS) and response type (correct vs incorrect trials) for ERP components. Results showed a significant main effect of response type on ERP mean peak amplitude ($F_{25}=66.24$, $p=0.00$, $\eta_p^2=0.73$), confirming the presence of a strong mid-frontal negative deflection (i.e. ERN) after erroneous responses compared to correct ones (cathodal/correct response: mean=0.60, SEM=0.28; sham/correct response: mean=0.57, SEM=0.29; cathodal/incorrect response: mean=-2.74 ; SEM=0.4; sham/incorrect response: mean=-2.57, SEM=0.46).

The main effect of stimulation was not significant ($p=0.73$) (Figure 4), nor the interaction between stimulation and response type ($p=0.5$) (Figure 5). These data indicate that the cathodal stimulation did not alter ERN amplitude.

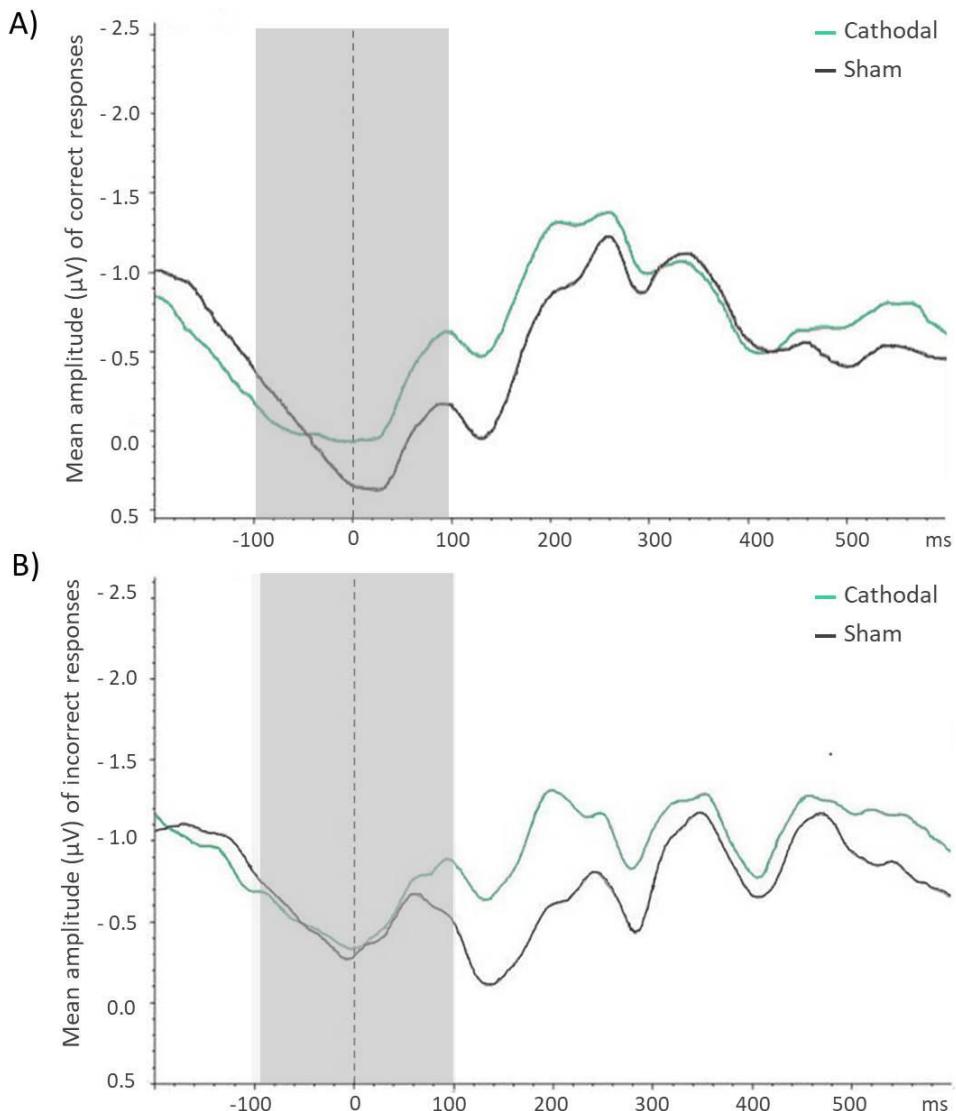


Figure 4. Difference waveforms at electrodes Cz of correct (A) and incorrect responses (B) for cathodal and sham stimulation, time-locked to the response onset, and time windows for ERN analyses.

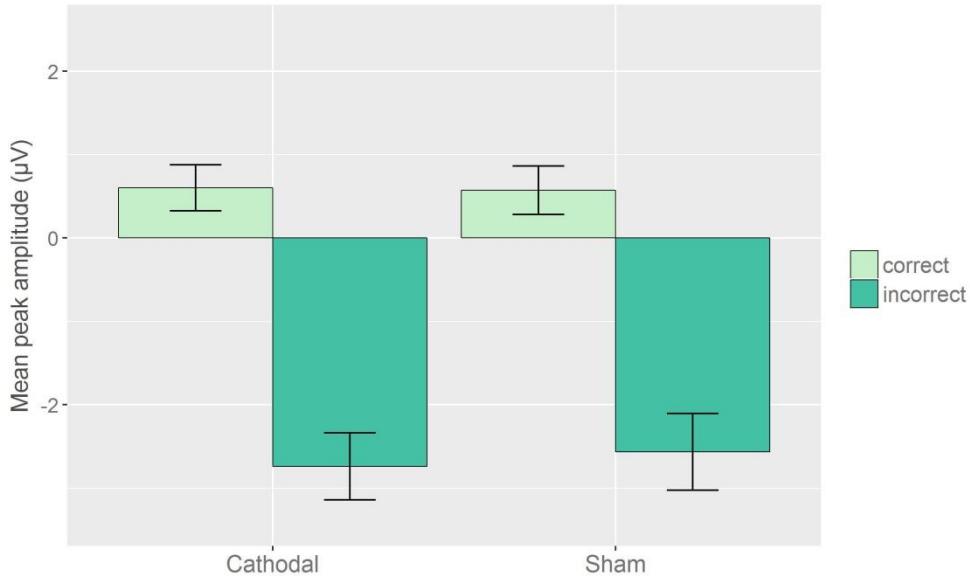


Figure 5. Mean peak amplitude of ERN for correct and incorrect responses among conditions. Error bars represent ± 1 SEM.

Discussion

The present study investigated the behavioral and electrophysiological effects of tDCS over the cerebellum on error processing and response inhibition in healthy participants. In line with our hypothesis, we found a disruptive effect of cathodal cDCS on performance as participants produced more erroneous responses in the active stimulation condition as compared to sham condition. Unlike, post-error slowing reaction time was no affected by the stimulation. In contrast to our expectations, we did not find evidence that cathodal cDCS reduced ERN amplitude. In fact, no significant difference was found in ERN amplitude between active and sham stimulation.

Interestingly, we found a dissociation between unchanged electrophysiological signals and impaired performance. The increased number of errors induced by cathodal stimulation goes in line with previous findings showing the ability of cathodal cDCS to modulate cognitive performances. We suppose the cathodal stimulation interfered with error-related processes ongoing in the cerebellum. However this paradigm doesn't allow us to disentangle the specific role of the cerebellum during the different levels and aspects of error processing, such as error detection, error monitoring, response selection and inhibition. On the other hand, the fact that no effect of stimulation was found on the post-error slowing reaction time, which is considered an index of

awareness, seems to suggest that cDCS may have affected specifically pre-attentive components. This results can be put in relation with the computational mode of the cerebellum, which is thought to be mainly unconscious and automatic (Courchesne & Allen, 1997). In line with this view, neuro-functional models propose that cerebellum is involved in pre-attentive, short-range, event-based temporal processing (Buhusi & Meck, 2005; Lewis & Miall, 2006). Accordingly, cerebellar damage has been demonstrated to alter ERP components specifically linked to pre-attentive detection of somatosensory oddball stimuli (Restuccia, Marca, Valeriani, Leggio, & Molinari, 2007). Despite not all published findings point to a clear polarity-specific effects of cDCS on behavior (for a review, see van Dun, Bodranghien, Manto, & Marien, 2017), our results are consistent with previous research linking cathodal stimulation with behavioral impairment (Boehringer, Macher, Dukart, Villringer, & Pleger, 2013b; Macher et al., 2014; Mannarelli et al., 2016; Panico et al., 2016). Notably opposite findings, showing that cathodal stimulation is associated with improvement in performance (Panouillères et al., 2015; Pope & Miall, 2012), have been reported. In this respect it's worth noting that the heterogeneity of behavioral outcomes of cDCS seems to depend on numerous factors (see discussion of study 1), including stimulation parameters, the nature (motor or cognitive) and the complexity of the task, and methodological issues as the timing of the stimulation (Grimaldi et al., 2014; van Dun et al., 2016).

A possible explanation for the lack of effect of cDCS on the electrophysiological measurements is that the anatomical origin of these components may not be affected by the tDCS-induced perturbation occurring at the level of cerebellar operations. A broad network of cortical and subcortical regions – including the ACC, ventrolateral (VLPFC) and dorsolateral (DLPFC) prefrontal areas, motor areas, the basal ganglia, pontine regions, the thalamus and the cerebellum – is involved in distinct conflict and error-related cognitive control mechanisms (Iannaccone et al., 2015; Simmonds, Pekar, & Mostofsky, 2008). A ventral/dorsal dissociation within the prefrontal cortex has been suggested, with ventral regions engaging in the maintenance of task-relevant information and dorsal regions being mainly involved in the manipulation of information and in the representation of task set and instructions under condition of

high cognitive demands (Postle, Berger, & D'Esposito, 1999). Functions of integration and evaluation of conflicting information – coming from the prefrontal areas – has been linked to the activation of the ACC, whereas pre-supplementary motor area (pre-SMA) activity has been recently proposed to reflect the inhibition of competing motor responses (Iannaccone et al., 2015). Results of a neuroimaging study provide supporting evidence to cerebellar involvement in error processing, suggesting that the cerebellum mediated error-related processes in prefrontal regions, as indicated by correlated activations of the cerebellum and the VLPFC during post error slowing in a stop signal task (Ide & Li, 2011). The authors also found error-related activations of the thalamus and the SMA to correlate with VLPFC activity, highlighting patterns of functional connectivity that are consistent with the knowledge of the cerebello-thalamo-cortical circuits. In line with this finding, reduced ERN and impaired error processing and performance monitoring in cerebellar patients have been associated with damaged cerebellar information flow received by the ACC via the thalamus (Peterburs et al., 2015; Seifert, von Cramon, Imperati, Tittgemeyer, & Ullsperger, 2011).

Given that in the present experiment cDCS influenced behavior but did not affect electrophysiological responses, we speculate that tDCS modulated operations occurring at a subcortical level, without influencing cortical processing and thus resulting in a lack of stimulation effect on the ERN component. Medial and lateral prefrontal cortical regions involved in error-related and monitoring functions have been identified as target areas of the basal ganglia output signaling (Middleton & Strick, 1994; Middleton & Strick, 2000). Moreover, patterns of functional connectivity between the basal ganglia, the associative cortex (included the ACC) and cerebellum indicate that these structures together participate in a network for inhibitory control of motor actions (Li, Peisi, Sinha, & Lee, 2008) and error processing (Falkenstein et al., 2001; Li et al., 2008). We conjecture that the tDCS-perturbed signal of detection of mismatch coming from the cerebellum and the basal ganglia did reach the ACC presumably for conflict evaluation. However, unaltered ERN may indicate that, despite the tDCS perturbation of incoming signals, ACC operations were not affected by the this perturbation during response-conflict monitoring.

Alternatively, it can be argued that the stimulation was not effective in modulating cerebellar activity. However, the behavioral impairment emerged from the analysis suggests that tDCS interfered to some extent with cerebellar operations. In accord with this, the model of current density amplitude field distributions (Figure 1, in method) shows a main focus of the electric field on the cerebellum. Even though we cannot exclude that the stimulation spread on other cortical regions, the effect on these regions are likely to be negligible. It is also conceivable to hypothesize that the severity of cerebellar damages is able to produce neural alterations thereby compromising both cerebello-thalamo-cortical functional connectivity and compensatory processes (Peterburs et al., 2015), that are on the contrary well preserved in healthy participants despite the administration of cDCS.

Furthermore, to exclude that the impairment of performance could be explained by an effect of cDCS on motor execution, we performed additional ad hoc analyses on the lateralized readiness potential (LRP). This component, associated with response processes, directly reflects hand-related motor activity (Coles, 1989). Widely used in the investigation of response activation in cognitive conflict tasks, an LRP positive deflection in response of incongruent trials indicates motor activation of the incorrect response hand. In case of a behavioral adjustment, the positive deflection is later replaced by a negative one, reflecting the selection of the correct response hand (Valt, Stürmer, Sommer, & Boehm, 2017). Given the involvement of the cerebellum in motor preparation (Battaglia et al., 2006; Deiber, Ibañez, Sadato, & Hallett, 1996), thus it cannot be excluded that interfere with cerebellar operations by means of tDCS may affect electrophysiological indexes of motor activity in response execution and response inhibition. However, our data showed that the stimulation did not alter LRPs. We suggest that unaltered LRPs may indicate a lack of effect of stimulation on motor preparation due to the confinement of tDCS interference at the subcortical level. Accordingly, cerebellar damage seems to be mostly associated with impairment in motor learning, rather than motor planning and execution (Molinari et al., 1997a). In conclusion, the present data support the involvement of the cerebellum in error processing. An important point to consider when interpreting this experiment is that the

cerebellum assists brain operations. In other words, it contributes to different neural operations without being directly responsible for their computation (Bower, 1997). It follows that the involvement of the cerebellum in both motor and cognitive functions needs to be considered in light of its interaction with the cortical and subcortical structures forming the cerebello-cerebral pathways. The explanation for which unaltered ERPs may depend on the ACC not being aware of cerebellar operations is purely speculative. Nevertheless, it is in accordance with the behavioral effect found here.

Chapter 4

**STUDY 3: Ordered sequences processing impairment by means
of transcranial magnetic stimulation of the cerebellum**

Introduction

Predicting and anticipating events that occur in the external environment is crucial to successfully drive our behaviors. The brain constantly predicts and anticipates future events base on the recognition of patterns and sequences of elements gathered through the experience. The brain compares those predictions with the external outcome to detect possible errors or violations and to act accordingly. Making estimations and comparing estimations with the actual peripheral outcome represents an highly adaptive mechanism that has been suggested to reflect the implementation of internal models (Miall & Wolpert, 1996). In last decades, researchers have pointed at the cerebellum as an optimal candidate in the brain able for producing and storing internal models (Ito, 2008b; Kawato et al., 2003; Moberget, Gullesen, Andersson, Ivry, & Endestad, 2014; Wolpert et al., 1998).

According to the sequence detection hypothesis (Leggio, Chiricozzi, Clausi, Tedesco, & Molinari, 2011), the cerebellum is implicated in sequencing incoming patterns, regardless of the nature – motor or cognitive – of the stimuli forming the latter. As a comparator, the cerebellum is thought to compare internal templates with incoming information to detect possible discrepancies. When template and incoming information corresponds, the cerebellar output is minimal. On the contrary, when a mismatch is detected between the two, cerebellar activity increases. Congruently, the contribution of the cerebellum differs according to distinct phases of sensory-motor based sequence processing, being predominant during sequence acquisition rather than sequence execution (Manto et al., 2012). Flament and colleagues (1996) reported higher cerebellar activity during the early stages of a visual-motor learning task and observed that, commensurate with an improvement in the motor performance after practice, was a decrease of cerebellar activity. This and similar pieces of evidence support the hypothesis that the cerebellum is active during learning of motor sequences, thereby identifying event sequences, as indicated by high cerebellar activation associated with learning sequence of key-pressing (Jenkins, Brooks, Frackowiak, & Passingham, 1994) and learning to point to sequences of visually presented dots (Decety, Kawashima, Gulyás, & Roland, 1992).

Clinical studies further support the evidence in favor of the crucial involvement of the cerebellum in sequence processing, showing patients with cerebellar damage to be impaired in procedural learning assessed by serial reaction time test (Molinari et al., 1997b; Pascual-Leone et al., 1993), independently on the type and the localization of the lesion (Leggio et al., 2008). Interestingly, the authors reported a lack of correlation between the severity of procedural deficits and the degree of motor impairment, suggesting that cerebellar circuitry are active in the recognition of patterns of events rather than in their planning and execution (Molinari et al., 1997b).

For what concerns non-motor functions, Schubotz and von Cramon (2001) observed the activation on lateral portions of the cerebellum in participants that were asked to encode the ordinal information of a sequence of visual stimuli and to detect possible violations. Similarly, pattern of cerebellar activation emerged in a more recent neuroimaging study, showing strong activity of lateral portions of the posterior cerebellum during the detection of size-based violations occurring within ordered sequences of geometrical shapes (Bubic, von Cramon, Jacobsen, Schröger, & Schubotz, 2009). Bubic and colleagues (2009) provide an explanation in line with the sequence detection hypothesis, arguing that the increased activity of cerebellar components triggered by the detection of sequential deviants, may reflect the updating of the internal sequence representation (i.e. forward model) based on the detection and elaboration of the deviant element.

In light of the empirical evidence in favor of crucial involvement of the cerebellum in the detection of both motor and cognitive sequential patterns, the aim of the present study was to address the causal role of this structure in perceptual sequences processing by means of transcranial magnetic stimulation (TMS). TMS was delivered on the cerebellar hemispheres during the execution of a series of tasks that required participants to attend and recognize irregularities in unfamiliar sequences – “new sequences” – composed of geometrical shapes varying in size and/or order of presentation (Experiment 1, 2 and 3) and in familiar sequences – “overlearned sequences” – composed of ordered series of letters and numbers (Experiment 2).

Experiment 1

Method

Participants

Eighteen Italian students (7 males, mean age=22.4 years, SD=2.1) participated in the experiment. All participants were right handed as assessed by a standard test (Oldfield, 1971). Prior to the TMS experiment, each participant filled in a questionnaire (Rossi, Hallett, Rossini, & Pascual-Leone, 2009) to evaluate compatibility with TMS. None of the participants reported neurological problems and history of seizures. None was taking medications that could interfere with neuronal excitability. Written informed consent was obtained from all participants before the experiment. The protocol was approved by the local ethical committee and participants were treated in accordance with the Declaration of Helsinki.

Stimuli and Procedure

Participants were seated comfortably at a distance of 57 cm from a 17" (1024 × 768 pixels resolution) TFT-LCD computer monitor and wore earplugs to minimize TMS click sound interference. Stimuli consisted of 3 visual shapes (circles, triangles, squares), each appearing in three different sizes throughout the experiment: small (approx. 2*2 deg of visual angle), medium (approx. 4*4 deg) and large (approx. 8*8 deg).

The timeline of an experiment trial is presented in Figure 1. Each trial consisted of a learning sequence and of a test sequence. Circles were only used in learning sequences, triangles and squares were only used in test sequences. The use of different shapes in the test compared to the learning sequences aimed at avoiding any possible visual adaptation effect. The learning sequence consisted in the consecutive presentation of three circles varying in size (e.g., small-large-medium; small-small-large). Each learning sequence started with a central fixation cross (presented for 1000 ms), followed by the first item of the series (500 ms), a blank screen (200 ms), the second item (500 ms) till the sequence ended. The learning sequence was immediately followed by the test sequence. In the test sequence either 3 triangles or 3 squares were

presented. Stimuli duration was the same as in the learning sequence except for the latest stimulus of the sequence that was visible until response. The first two shapes of the test sequence were always identical in size to those of the learning sequence, whereas the size of the last shape could be the same as that used in the learning sequence (i.e., learning and test were identical in terms of size-sequence) or different (violation of the learnt sequence). Three shapes of the same size were never presented in the same sequence; furthermore, sequences organized in entirely ascending or descending order (i.e., small-medium-large; large-medium-small) were not used. Participants were instructed to indicate as fast as possible whether the test size-sequence was identical to the learning one by left/right key pressing using their right hand (with response keys counterbalanced among participants). TMS was given before the onset of the last stimulus of the test sequence (where possible violations occurred) (see below for TMS details). The experiment consisted of four experimental blocks, one for each TMS condition (left cerebellum, right cerebellum, left sham and right sham, see below). Each block consisted of 72 trials (half in which the test sequence was identical to the learning one, and half in which it was different). Within each experimental block, trials were presented in random order. A short practice session consisting of 10 trials was presented at the beginning of the experiment in order to allow participants to familiarize with the stimuli used. The order of TMS sites was counterbalanced across participants.

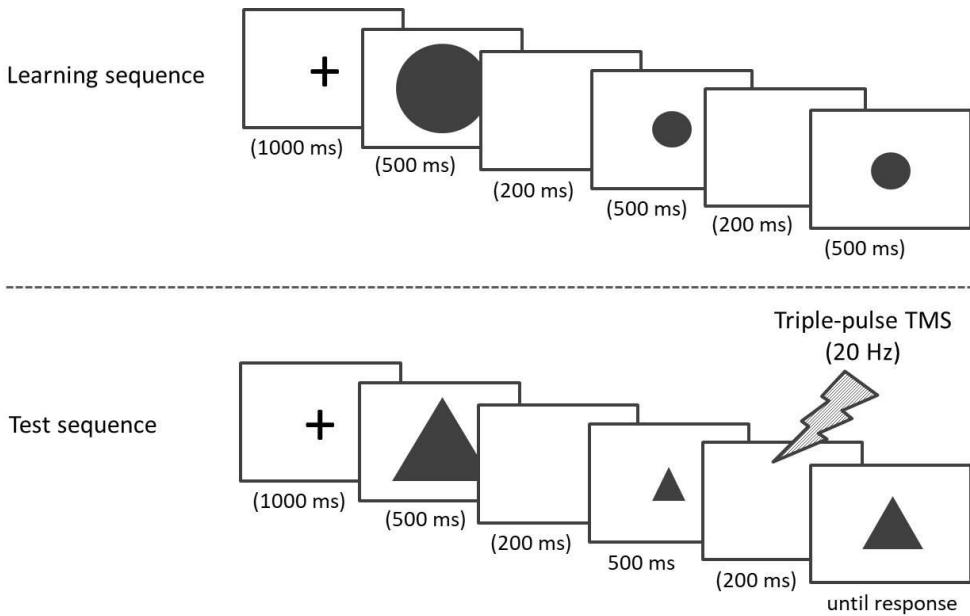


Figure 1. Example of experimental trial. The last item of the test sequence (medium-size triangle) constitutes a deviant element. In fact, a small-size triangle would fit correctly the sequential order shown in the preceding learning sequence ("big-small-small" order).

Transcranial Magnetic Stimulation (TMS)

Online neuronavigated TMS was performed with a Magstim Rapid2 stimulator (Magstim Co., Ltd, Whitland, UK) connected to a 70-mm butterfly coil. At the beginning of each session single pulse TMS was applied at increasing intensities to determine individual motor threshold. Motor threshold (MT) was defined as the lowest TMS intensity capable of evoking a muscle twitch in the contralateral hand in 5/10 consecutive trials (Hanajima et al., 2007; Koch et al., 2007). During the experiment, participants were stimulated at 100% of their MT (mean intensity: 50.8%, SD=3.4%).

Triple-pulse 20 Hz TMS was delivered 150 ms before the presentation of the last stimulus of the test sequence (so that the last TMS pulse was given 50 ms before the onset of the stimulus), with similar parameters of stimulation used in previous TMS studies targeting the cerebellum (Cattaneo et al., 2014; Gamond, Ferrari, La Rocca, & Cattaneo, 2017; Koch et al., 2007). TMS (real and sham, see below) was delivered over the cerebellum, in the two hemispheres. Left and right cerebellum were localized by means of stereotaxic navigation on individual estimated magnetic resonance images (MRI) obtained through a 3D warping procedure fitting a high-resolution MRI template with the participant's scalp model and craniometric points (Softaxic, EMS, Bologna,

Italy). This procedure has been proven to ensure a global localization accuracy of about 5 mm, a level of precision closer to that obtained using individual MRI scans (Carducci & Brusco, 2012), and has been successfully used in many prior studies (Cattaneo, Mattavelli, Papagno, Herbert, & Silvanto, 2011; Mattavelli, Cattaneo, & Papagno, 2011). Anatomical Talairach coordinates (Talairach & Tournoux, 1988) used for neuronavigation were taken from a previous neuroimaging study investigating neural correlates of sequences violation (Bubic et al., 2009), and were $x=-20$, $y=-75$, $z=-18$ for the left cerebellum, and $x=22$, $y=-75$, $z=-21$ for the right cerebellum (Figure 2). The coil was placed tangentially to the scalp with the handle pointing superiorly. In sham blocks, TMS was delivered over the same sites as real TMS and at the same intensity, but the coil was flipped 90 degrees leftward or rightward (flip direction counterbalanced within participants) so that the magnetic pulses did not reach the scalp, in line with previous studies (Lisanby, Gutman, Luber, Schroeder, & Sackeim, 2001).

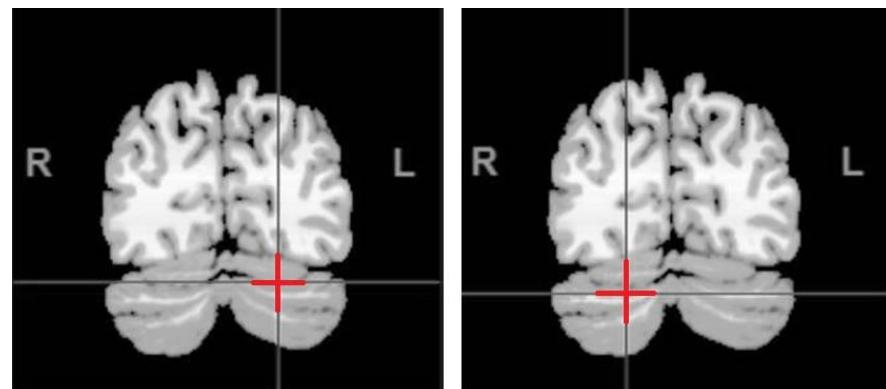


Figure 2. Anatomical Talairach coordinates of the targeted sites in Experiment 1,2 and 3: on the left side, left cerebellum ($x=-20$, $y=-75$, $z=-18$); on the right side, right cerebellum ($x=22$, $y=-75$, $z=-21$).

Results

Mean accuracy rates and mean reaction times (RT) for correct responses were computed for each participant in each TMS condition, and are shown in Figure 3. Accuracy and RT were analyzed via repeated-measures ANOVAs with TMS (real vs. sham) and cerebellar hemisphere (left vs. right) as within-subjects variables.

The ANOVA on mean accuracy scores revealed a significant main effect of TMS ($F_{1,17}=6.45$, $p=0.021$, $\eta_p^2=0.28$), indicating that accuracy rates were lower during real compared to sham stimulation. Neither the main effect of hemisphere ($F_{1,17}=1.65$, $p=0.22$) nor the interaction TMS by hemisphere were significant ($F_{1,17}<1$, $p=0.56$) (Figure 3).

The ANOVA on mean correct RT revealed no significant main effects for either TMS ($F_{1,17}<1$, $p=0.70$) or hemisphere ($F_{1,17}<1$, $p=0.50$). The interaction TMS by hemisphere was not significant ($F_{1,17}<1$, $p=0.60$).

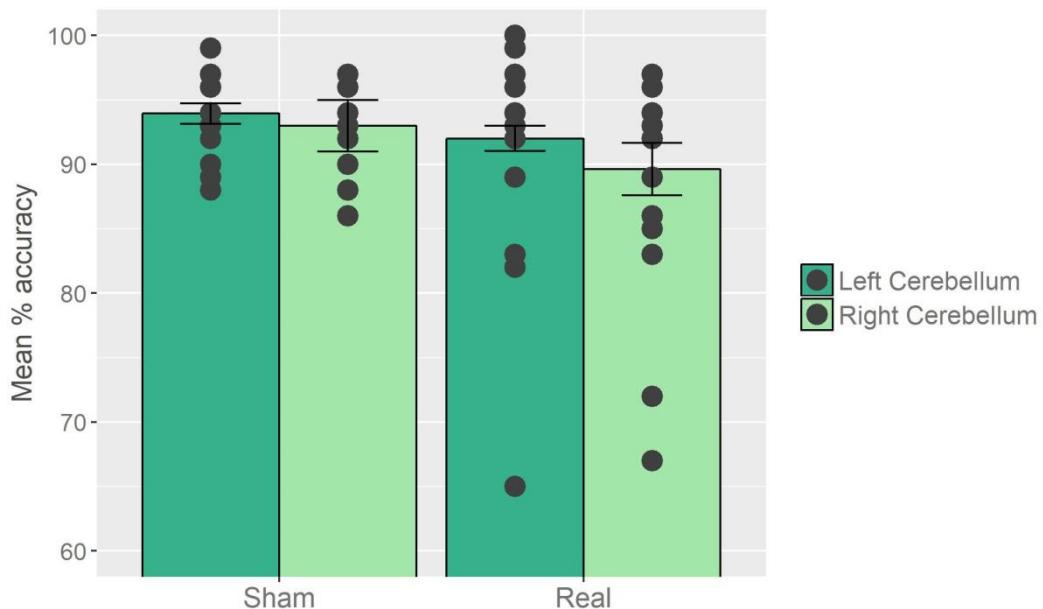


Figure 3. Mean percentage accuracy scores as a function of TMS condition (Left Sham, Right Sham, Left Real and Right Real) in Experiment 1. Real TMS over both right and left cerebellum lowered participants' accuracy compared to sham stimulation. Error bars represent ± 1 SEM.

Experiment 2

Experiment 1 showed that TMS delivered over the cerebellar hemispheres impaired participants' ability to recognize geometrical stimuli that were immediately learned before the test phase. This result is consistent with previous evidence showing higher cerebellar activity during motor learning phase and decrease cerebellar activity after practice and, presumably, consolidation in memory of the motor pattern (Doyon et al., 2002; Flament et al., 1996). To test whether this applies also for perceptual learning, in Experiment 2 we asked participants to attend and detect possible violations within to-be-learned new sequences (identical to the ones presented in Experiment 1) and overlearned sequences (i.e. strings of letters and numbers). Cerebellar TMS was expected to interfere with the processing of novel sequences, for which learning is still occurring, but not with familiar sequences, that are thought to be already stored in memory. In Experiment 2, the vertex and the early visual cortex were targeted as active control sites. Stimulating the latter is a critical control condition to rule out the possibility that TMS effect may be due to an interference with early visual processing of the stimuli (Renzi, Vecchi, D' Angelo, Silvanto, & Cattaneo, 2014). Moreover, TMS pulses over the early visual cortex did not overlap with visual stimuli presentation. Finally, we targeted only the right cerebellar hemisphere, as results in Experiment 1 showed both left and right cerebellar stimulation to lead to the same effects.

Method

Participants

Eighteen Italian students (3 males, mean age=21.7 ys, SD=1.5) participated in the experiment. None of them had participated in Experiment 1. Inclusion criteria were the same as for Experiment 1.

Stimuli and Procedure

In Experiment 2 the task was identical to that of Experiment 1 (detect possible irregularities between sequences) but consisted of two experimental conditions: in the

"new sequences" condition, the sequences used and the paradigm were identical to Experiment 1 (there were in total 72 trials, half in which the sequence was violated). In the "overlearned sequences" condition, test sequences were presented as in Experiment 1 but there were no learning sequences (since sequences were already overlearned). Overlearned sequences consisted of either 4 letters or 4 digits (in the 1-9 range), presented in black ink, 16-point Calibri font and measured approx. 4*4 deg of visual angle. The first three items of the sequence were always presented in correct consecutive order, that is ascending for digits (e.g., 3-4-5) and alphabetic for letters (e.g., L-M-N). The fourth latest item could be either the correct consecutive item of the sequence (e.g., 3 4 5 6) or not (e.g., 3 4 5 1): in the latter case, in half cases it was an item preceding in the series (as in the example above) and in half following in the series. The overlearned sequences condition consisted of 40 trials presented in random order, half in which the correct sequence order was respected, and half in which it was violated. A fixation cross (1000 ms) was presented during the intertrial interval. Participants had to detect possible violations in overlearned sequences responding as in Experiment 1. Each condition was consecutively presented three times, one for each TMS site (see below). Order of TMS site and task condition was counterbalanced across participants. The experiment was preceded by a short practice session to let participants to familiarize with the task.

TMS

TMS was delivered as in Experiment 1 (same parameters and timing) over the right cerebellum, early visual cortex and the vertex. The right cerebellum and the early visual cortex were localized by means of stereotaxic navigation (see Experiment 1 for details). Anatomical Talairach coordinates for the right cerebellum were identical to those used in Experiment 1. Early visual cortex coordinates were $x=-2$, $y=-81.4$, $z=1.4$ (Alink, Schwiedrzik, Kohler, Singer, & Muckli, 2010). The vertex was localized as the point falling half the distance between the nasion and the inion on the same midline. The coil was placed tangentially to the scalp and held parallel to the midsagittal line, with the handle pointing backward in the vertex and with the handle pointing superiorly in the

cerebellum and early visual cortex TMS stimulation. TMS intensity was equal to 100% of each participant's MT computed as in Experiment 1 (mean TMS intensity delivered: 56.1%, of the maximum stimulator output SD = 2.9%). No participants reported phosphene perception during the experiment.

Results

Analyses were performed as in Experiment 1 on mean accuracy scores and mean correct RT (Figure 4). Data were submitted to repeated-measures ANOVAs with task condition (new vs. overlearned sequences) and TMS site (Vertex, Right Cerebellum and Early visual cortex) as within-subjects variables.

The ANOVA on mean accuracy scores revealed a significant main effect of task condition ($F_{1,17}=19.06$, $p<0.001$, $\eta_p^2=0.53$) but not of TMS site ($F_{2,34}<1$, $p=0.42$). Critically, the interaction task condition by TMS was significant ($F_{2,34}=7.53$, $p=0.002$, $\eta_p^2=0.31$). The significant two-way interaction was investigated by looking at the simple main effect of TMS within each task condition. TMS significantly affected detection of irregularities of new sequences ($F_{2,17}=4.69$, $p=0.016$, $\eta_p^2=0.22$) supporting findings of Experiment 1. In particular, post-hoc comparisons showed that cerebellar TMS impaired participants' accuracy compared to both early visual cortex TMS ($t_{17}=2.63$, $p=0.018$) (with Bonferroni-Holm correction, $p=0.054$) and vertex TMS ($t_{17}=2.34$, $p=0.032$) (with Bonferroni-Holm correction, $p=0.064$). The effects of TMS over the early visual cortex and vertex was comparable ($p=0.70$). In turn, TMS did not affect detection of irregularities of overlearned sequences ($F_{2,17}=1.38$, $p=0.26$).

The ANOVA on mean correct RT revealed a significant main effect of task condition ($F_{1,17}=22.95$, $p<0.001$, $\eta_p^2=0.57$) indicating that participants were faster (mean correct RT = 445 ms, SD = 47) in overlearned compared to new sequences (497 ms, SD = 56). Neither the main effect of TMS, $F_{2,34}=1.87$, $p=0.17$, nor the interaction task condition by TMS, $F_{2,34}<1$, $p=0.67$, reached significance.

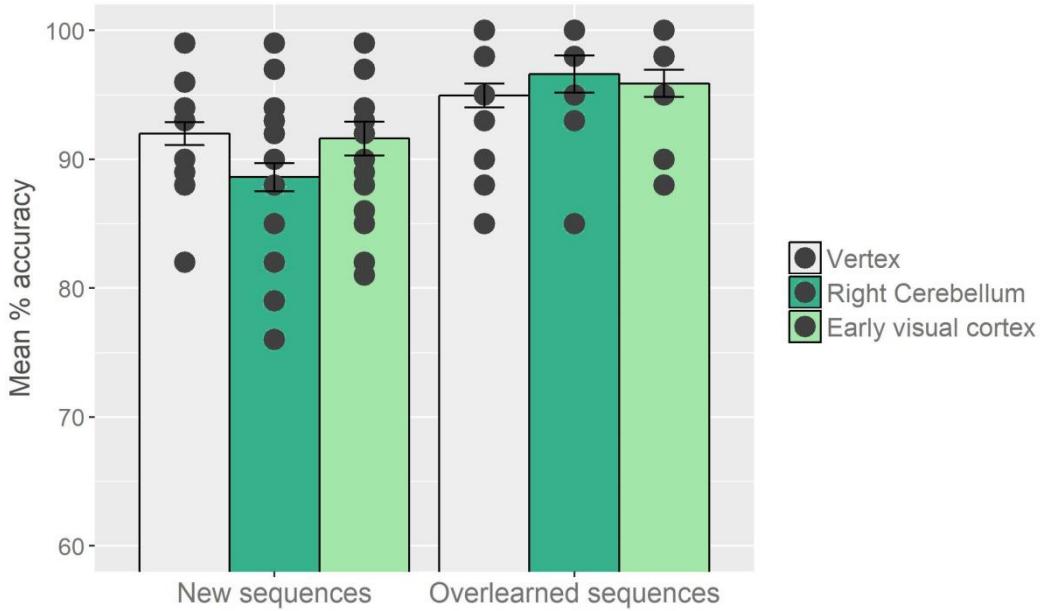


Figure 4. Mean percentage accuracy scores as a function of TMS condition (Vertex, Right Cerebellum and Early visual cortex) and task type (New vs. Overlearned sequences) in Experiment 2. TMS over the cerebellum selectively impaired participants' accuracy compared to Early visual cortex and Vertex stimulation in processing new but not overlearned sequences. Error bars represent ± 1 SEM.

Experiment 3

Experiment 2 confirmed the results of the first experiment, showing TMS over the cerebellum to affect processing of unfamiliar sequences but not of highly familiar ones. These data are in line with the previous evidence showing cerebellar activity to be commensurate with cerebellar contribution during learning phase of novel material rather than consolidation following practice and exposure (Flament et al., 1996).

However, in both experiments violations exclusively consist in variations in the last item of the sequence, making arguable that processing of serial events was required in order to perform the task and suggesting TMS-induced impairment of cerebellum contribution to short-term memory of a single item as an alternative explanation for our previous results. To clarify the actual process underpinning previous results, a third experiment (Experiment 3) was conducted, where a new group of participants were again presented with sequences of geometrical shapes. Differently from the other two experiments, here participants were asked to indicate the order of appearance of an item presented within a three-items sequence. In order to successfully perform the task,

participants had to pay attention to all the elements of the sequence since they could all be potential targets. If cerebellum is involved in processing the temporal relation between events, cerebellar TMS should not modulate participants' performance.

Methods

Participants

Eighteen Italian students (6 males, mean age=23.1 ys, SD=2.3) participated in the experiment. None of them had participated in Experiment 1 or 2. Inclusion criteria were the same as for previous experiments.

Material and Procedure

The experimental setting was the same as in Experiment 1 and 2. Stimuli consisted of the same visual shapes used in Experiment 1 but only the medium size (4*4 deg) was used. Each trial started with a central fixation cross (2500 ms) followed by the presentation of a sequence of three shapes. Each shape lasted for 500 ms and was separated by the consecutive one by a blank screen (200 ms). The last shape was followed by a blank screen (200 ms) and then by the presentation of the target shape (i.e., one of the elements of the sequence). Below each target shape an ordinal number (1^{\wedge} , 2^{\wedge} or 3^{\wedge}) appeared. The number indicated in which order position the target element displayed above was presented in the just presented triplet (e.g., 2^{\wedge} to indicate the second element of the sequence). In half of the trials, the number indicated the correct order position of the target element, whereas in the other half the number indicated an incorrect position. Importantly, the target element depicted with equal frequency all the order positions (first, second and last) of the sequence (Figure 5). In each trial, participants had to indicate by left/right key pressing with their right hand whether the number corresponded to the position in which the target element was presented in that sequence. Response keys assignment was counterbalanced among participants. The target shape remained on the screen until participants' response. Participants were instructed to be as fast as possible. Each block consisted of 108 trials and each participant performed the same block three times (once for each TMS site, see

below). A practice session consisting of 16 trials was presented prior to the beginning of the experiment.

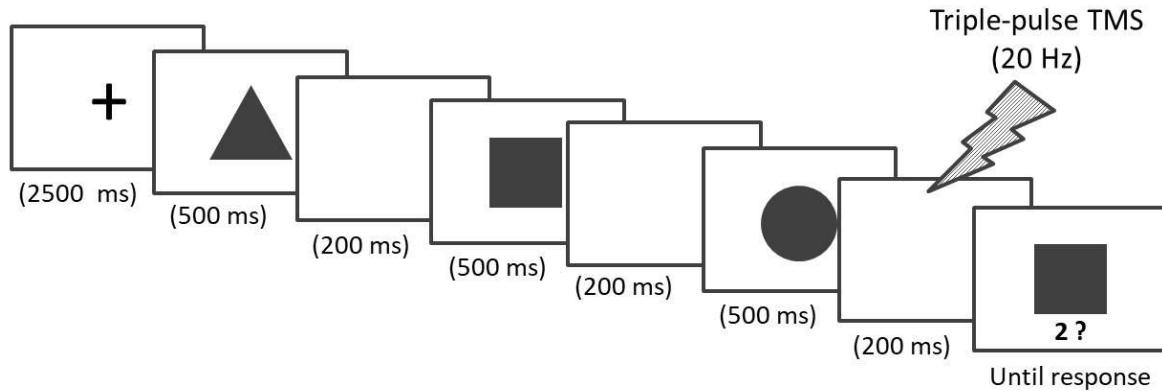


Figure 5. Example of experimental trial (Experiment 3). Participants were asked to indicate whether the number illustrated below the target element corresponded to the position in which the target element was previously presented in the sequence.

TMS

TMS parameters and TMS sites were identical to Experiment 2. Triple-pulse 20 Hz TMS was delivered 150 ms before the presentation of the target element (so that the last TMS pulse was given 50 ms before the onset of the stimulus). Mean TMS intensity used was: 53.7% (SD =4.0%). No participants reported phosphene perception during the experiment.

Results

Mean accuracy rates and mean RT for correct responses were computed for each participant in each TMS condition. Accuracy and RT were analyzed via repeated-measures ANOVAs with TMS site (Vertex , Right Cerebellum and Early visual cortex) as within-subject variable.

The ANOVA on mean accuracy scores revealed a significant main effect of TMS ($F_{2,34}=4.12$, $p=0.025$, $\eta_p^2=0.20$) indicating that TMS impaired participants' performance (Figure 5). Post-hoc comparisons showed that accuracy rates were lower in the cerebellar TMS condition compared to both TMS over the early visual cortex ($t_{17}=2.64$, $p=0.017$) (with Bonferroni-Holm correction, $p=0.051$) and over the vertex ($t_{17}=2.08$,

$p=0.053$) (with Bonferroni-Holm correction, $p=0.11$). The effect of TMS over early visual cortex and vertex was comparable ($t_{17}<1$, $p=0.72$) (Figure 6).

The ANOVA on mean correct RT revealed a non-significant main effect of TMS ($F_{2,34}<1$, $p=0.18$).

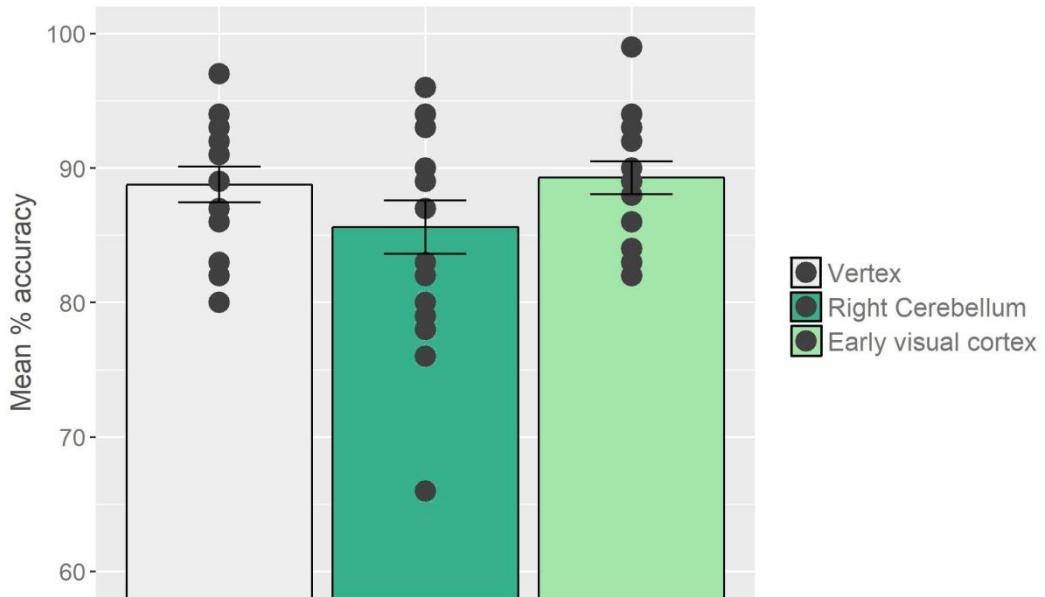


Figure 6. Mean percentage accuracy scores as a function of TMS condition (Vertex, Right Cerebellum and Early visual cortex) in Experiment 3. TMS over the cerebellum decreased participants' accuracy rates compared to Early visual cortex and Vertex stimulation. Error bars represent ± 1 SEM.

Discussion

The present data show that TMS delivered on the cerebellar hemispheres effectively modulated participants' accuracy in processing unfamiliar – or "new" – sequences (Experiment 1,2,3) but not highly familiar – or "overlearned" – sequences (Experiment 2). It is worth to specify that this effect cannot be ascribed to a spread of the magnetic pulses to the early visual cortex, resulting in a possible detrimental effect of the stimulation on visual encoding, as demonstrated by preserved performance following occipital TMS, delivered as a control condition (Experiment 2 and 3).

Our findings led support to previous neuroimaging evidence that report cerebellar activation in the recognition of patterns of events and in the detection of violations of spatial and temporal relationship of events in the motor (Doyon et al.,

2002; Jenkins et al., 1994) and cognitive-perceptual domains (Brown et al., 2013; Bubic & Cramon, 2009; Schubotz, 2007).

Experiment 3 rules out the possibility that the detrimental effect of TMS reflected an unspecific impairment in the short-term retention of the last item of the sequence, rather than in encoding of a sequential order, as both in experiments 1 and 2 only the last element of the sequence could vary between the learning and test sequences. In fact, the paradigm used in the first two experiments potentially allowed participants to focus only on the last item to successfully perform the task. Thus, in Experiment 3 participants were asked to indicate in which ordinal position one of the three items was presented within the sequence, forcing in this way the participants to process the whole sequence. Interestingly, the disruptive effect of cerebellar TMS on participants' performance seems to confirm that the stimulation interfered with operations of sequential encoding exerted by the cerebellum, rather than with unspecific short-term memory-related processes. This finding is in line with similar research that point at the cerebellum as a relevant part of a broader neural network active in serial order processing (Attout, Fias, Salmon, & Majerus, 2014; Majerus et al., 2007).

Interestingly, the detrimental effect of the stimulation over the cerebellum was only evident in response to unfamiliar sequences. Unlike highly familiar sequences, composed of strings of letters (in alphabetical order) and numbers (in consecutive order) to which participants have been extensively exposed through experience, the items forming the geometrical sequences constituted novel material. Therefore, the ordinal relationship between these items required to be recognized for the first time during the learning phase. The result presented here is consistent with prior findings showing significant cerebellar activation during serial ordering of alphabetical and numerical items, in which the order had to be learned for the first time when attending at the sequence, and lowered activation during the processing of those sequences where the items were presented in a progressive, predictable order (Attout et al., 2014).

Cerebellar disruption affecting unfamiliar sequences may be also interpreted as a proxy of the role of the cerebellum in assisting the brain when facing tasks that require high cognitive demands and attentional resources (Pope & Miall, 2012). Accordingly,

neuroimaging studies reported a positive correlation between cerebellar activity and increased cognitive load of a sequential finger movements task (Boecker et al., 2002) and a working memory task (Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005). The correlation between practice-related improvement in behavior and increased cerebellar activation is consistent with the view of the cerebellum as co-processor for operations computed elsewhere in the brain, due to known patterns of anatomical and functional connectivity (Chen & Desmond, 2005).

We targeted the lateral portions of the cerebellum, guided by prior evidence indicating activation of both left and right cerebellar hemispheres in the detection of perceptual violations during a sequence processing task (Bubic & Cramon, 2009). In accordance with this study, we found TMS to equally disrupt performance when delivered both over the left and right hemispheres (Experiment 1). A similar result has been reported in an experiment showing detrimental effects on procedural motor learning following TMS over left and right hemispheres (Torriero et al., 2004).

In sum, our study extends prior literature showing that the cerebellum plays a causal role in processing unfamiliar sequences. Our findings are relevant for future work aiming to clarify the contribution of the cerebellum not only in extracting regularities in incoming sensory information, but also in using this information to generate predictions about future events in both the motor and sensory domains.

Chapter 5

**STUDY 4: Effects of transcranial magnetic stimulation
over the cerebellum on explicit and incidental
emotional cue recognition**

Introduction

The subsidiary role of the cerebellum to motor and cognitive functions has been recognized to extent to emotion and affective processing. In light of the role of general controller exerted by the cerebellum towards operations carried out elsewhere in the brain, whether this structure constitutes a critical node in the circuitry involved in emotion processing or to which extent it functions as an unspecific relay station for the optimization of information processing, is still an open question.

The neuroanatomical foundation of the involvement of the cerebellum in processing emotional information consists in the intimate afferent and efferent projections linking this structure to the brainstem and the limbic system. Meta-analytic studies on functional neuroimaging findings delineated distinct regions of the so-called "limbic" cerebellum, pointing at medial (vermis and para-vermis) and posterior lateral cerebellar portions (Crus I and Crus II) as relevant parts active in emotion processing (Fusar-Poli et al., 2009; Keren-Happuch, Chen, Ho, & Desmond, 2014; Stoodley et al., 2012). Interestingly, cerebellar operations are recruited not only in experience and regulation of mood and emotion, but also in the elaboration of the cognitive aspects of emotive information processing, required to provide appropriate behavioral responses (Adamaszek et al., 2017).

In addition to functional neuroimaging evidence, clinical observations provide further support to the link between cerebellum and emotion. The well-known cerebellar cognitive-affective syndrome, described for the first time by Schmahmann and Sherman (1998), among a series of cognitive deficits includes behavioral disinhibition and emotional blunting as symptoms of cerebellar damage. Accordingly, these symptoms and signs of dysregulation of affects have been observed in patients with cerebellar abnormalities in later studies (Duggal, 2005; Levisohn, Cronin-Golomb, & Schmahmann, 2000; J D Schmahmann et al., 2009). For what concerns the cognitive, high-order processing of emotional cues, converging clinical evidence showed impaired facial expression processing in cerebellar patients, whereas the processing of non-emotional facial features appeared to be preserved (Adamaszek et al., 2015). Furthermore, both structural and functional alterations in fronto-temporal-thalamo-cerebellar networks has

been associated with uncoordinated affective processing and depressive symptoms in a broad variety of psychiatric disorders (Shakiba, 2014).

A possible way to gather deeper understanding of the functional role of the cerebellum in emotion processing may be provided by non-invasive brain stimulation techniques. For instance, Ferrucci and colleagues (2012) demonstrated that transcranial direct current stimulation (tDCS) applied to the medial cerebellum was able to interfere with the recognition of negative but not positive or neutral facial expressions in healthy volunteers. In another study, twenty minutes of cerebellar high-frequency repetitive transcranial magnetic stimulation (TMS) altered participants' performance in a masked emotional faces response task, as indicated by enhanced implicit processing of happy but not fearful or neutral facial expressions (Schutter, Enter, & Hoppenbrouwers, 2009). Results of two experiments combining neuro-stimulation and electrophysiological measurements provided support to the modulatory role of the cerebellum on emotion processing, showing cerebellar repetitive TMS to alter prefrontal cortical asymmetry in the gamma frequency band – which is associated with emotive information processing – alongside elevations in self-reported mood (Schutter, Van Honk, D'Alfonso, Peper, & Panksepp, 2003), and stimulation-induced increase in theta activity – neural oscillatory pattern strongly linked to emotional information processes – following cerebellar as compared to vertex and occipital TMS (Schutter & van Honk, 2006).

Despite the flourishing interest on the topic, what the specific role of the cerebellum is in emotion processing is still an open question. Neuroimaging and lesion evidence point at discrete neural networks supporting emotional perception – all including the cerebellum – that operate at an explicit or implicit level (Critchley et al., 2000; Gorno-Tempini et al., 2001; Wright et al., 2008). Whether cerebellar operations sub-serve predominantly conscious (explicit) or un-conscious (implicit) processes, is still debated. Patterns of cerebellar activation have been observed in participants matching emotional faces as a function of their emotional expression but not as a function of their gender, suggesting that the cerebellum might be critically engaged in the elaboration of emotive information requiring explicit rather than implicit processing (Scheuerecker et al., 2007). Accordingly, evidence of cerebellar activity during explicit

evaluation of pleasant and unpleasant images but not during frequency estimation of the same images (Wright et al., 2008), strengthen the hypothesis that the cerebellum would exert a top-down modulation on emotive information processing. An interesting study (Clausi et al., 2015) observed that cerebellar patients were able to anticipate regret to maximize expected values in a gambling study, as indicated by increased level of skin conductance, which is considered a physiological proxy for negative emotion evaluation. However, when asked to self-report their affective state, cerebellar patients failed in recognize and express the negative emotion experienced during the task. This datum is in line with observations that demonstrate explicit/implicit differences in cerebellar emotion processing. Nevertheless, contrasting findings associated cerebellar activity with implicit processing of emotional faces (Habel et al., 2007; Schutter, Enter, & Hoppenbrouwers, 2009).

The goal of the present study was to examine the role of the cerebellum in facial emotional expression recognition by means of TMS. Specifically, the primary endpoint was to disentangle its contribution to explicit and implicit facial emotion recognition. To do so, we administered cerebellar TMS to participants during the execution tasks presenting facial emotional expressions that 1) had to be explicitly discriminated or 2) were incidental for the task at play (i.e., gender discrimination).

Experiment 1

Method

Participants

Thirty-six Italian volunteers (9 males, mean age=22.5 years, SD=2.2) took part in the experiment. All participants were right-handed and had normal or corrected-to-normal vision. Prior to the TMS experiment, each participant filled in a questionnaire to evaluate compatibility with TMS (translated from Rossi, Hallett, Rossini, & Pascual-Leone, 2011). The protocol was approved by the local ethical committee and participants were treated in accordance with the Declaration of Helsinki.

Stimuli

Stimuli were images selected from the Radboud database (Langner et al., 2010) depicting 14 male and 14 female different young faces (each covering approximately 23×14 degrees of visual angle) showing happy or angry expressions.

Procedure

Participants seated in front of a 19' screen at an approximate distance of 80 cm. Figure 1 shows an example of the experimental trial. Each trial started with a black fixation cross (2500 ms), followed by a first face (visible for 150 ms), a blank screen (150 ms), and a second face (150 ms). The second face was followed by a blank screen until participants' response. Participants were randomly assigned to two different task conditions. In the Emotion Discrimination task, participants indicated whether the two faces expressed the same or a different emotion; in the Gender Discrimination task participants indicated whether the two faces belonged to the same gender. Participants had to respond by left/right key pressing using their right hand as fast as possible. Response key assignment was counterbalanced across participants. In both experiments, the same face pairs were used. In particular, 28 face pairs were created for each of the four possible gender/emotion combinations (i.e., same emotion/same gender; same emotion/different gender; different emotion/same gender; different emotion/different gender), for a total of 112 trials in each experimental block. After a short training session consisting of 8 trials, participants performed three experimental blocks, one for each TMS site (see below). Order of site stimulation was counterbalanced across participants. The software E-prime 2.0 (Psychology Software Tools, Pittsburgh, PA) was used for stimulus presentation, data collection and TMS triggering.

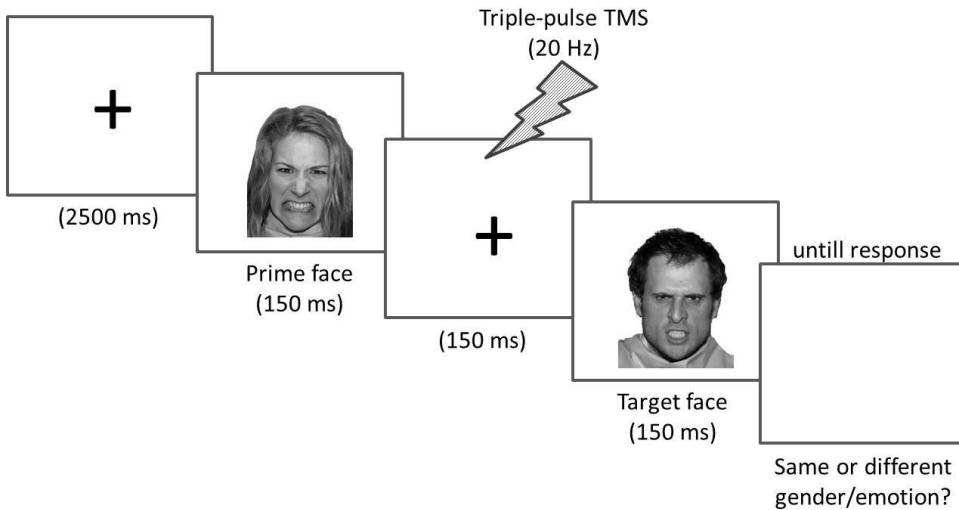


Figure 1. The timeline of an experimental trial. Each trial started with a fixation cross (2500 ms), followed by the first face (150 ms), a blank screen (150 ms), and then by the second face (150 ms). Depending on the task they were assigned to (between-groups design), participants had to indicate either 1) whether the two faces expressed the same or a different emotion (Emotion discrimination task); or 2) whether the two faces belonged to the same gender (Gender discrimination task). Triple-pulse TMS (20 Hz) was delivered between the offset of the first face and the onset of the second face.

Transcranial magnetic stimulation (TMS)

Online neuronavigated TMS was performed with a Magstim Rapid2 stimulator (Magstim Co., Ltd, Whitland, UK) connected to a 70-mm butterfly coil. At the beginning of each session, single pulse TMS was applied over the left M1 at increasing intensities to determine each individual motor threshold (MT). MT was defined as the lowest TMS intensity capable of evoking a muscle twitch in the contralateral hand in 5 out of 10 consecutive trials (Hanajima et al., 2007; Koch et al., 2007). During the experiment, participants were stimulated at 100% of their MT (mean intensity: 53.2%, SD = 3.9). Triple-pulse 20 Hz TMS was delivered 150 ms before the presentation of the second face, with similar parameters of stimulation leading to transient disruption of the undergoing neural activity in the stimulated area (e.g., Cattaneo et al., 2014). TMS was delivered over the left cerebellum, early visual cortex and the vertex as control sites. The early visual cortex was chosen as additional control area since prior evidence suggests that cerebellar stimulation may spread to primary visual cortex (Renzi et al., 2014); it is thus important to rule out the possibility that cerebellar TMS effects depend on indirect

stimulation of early visual cortex. The timing of stimulation was chosen so not to affect the processing of face information by the early visual cortex, information about facial features already reaching face-selective regions in extra-striate visual cortex 60-100 ms from face onset (see Pitcher et al., 2011). The left cerebellum and the early visual cortex were localized by means of stereotaxic navigation on individual estimated magnetic resonance images (MRI) obtained through a 3D warping procedure fitting a high-resolution MRI template with the participant's scalp model and craniometric points (Softaxic, EMS, Bologna, Italy). This procedure has been proven to ensure a global localization accuracy of about 5 mm, a level of precision closer to that obtained using individual MRI scans (Carducci & Brusco, 2012), and it has been successfully used in different studies (e.g., Cattaneo, 2010; Makris & Urgesi, 2014; Mattavelli et al., 2011). The anatomical Talairach coordinates (Talairach & Tournoux, 1988) of the left cerebellum were $x=-15$, $y=-82$, $z=-32$ (corresponding to cerebellar loci of activation during emotional processing reported in prior neuroimaging work, see Schraa-Tam et al., 2012) (Figure 2), and Talairach coordinates of early visual cortex were $x=-2$, $y=-75$, $z=32$ (Anderson et al., 2011). The coil was placed tangentially to the scalp and held parallel to the midsagittal line. The handle pointed backward in the vertex stimulation condition, and superiorly in the cerebellum and in the early visual cortex stimulation conditions.

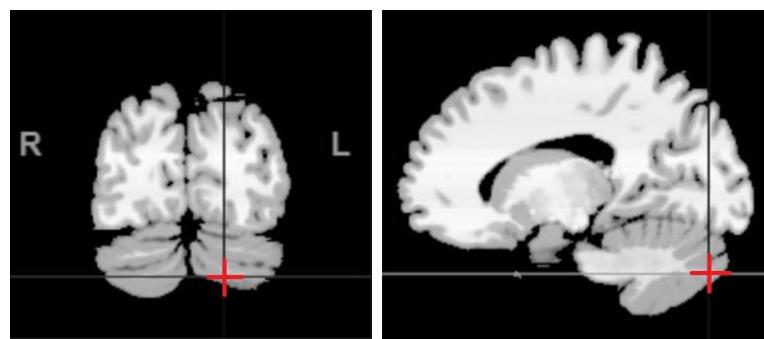


Figure 2. Anatomical Talairach coordinates of the targeted sites (Experiment 1, 2 and 3) on left cerebellum: $x=-15$, $y=-82$, $z=-32$.

Results

The dependent variables were mean accuracy rates, and mean correct response latencies (RT). A repeated-measures ANOVA with TMS site (cerebellum, early visual cortex and vertex) as within-subjects variable and Task (Emotion Discrimination vs. Gender Discrimination) as between-subjects factor was carried out on both dependent variables. Bonferroni-Holm correction was applied to post-hoc multiple comparisons. Trials in which participants' RT were ± 3 SD compared to their block mean were excluded from the analyses (following this criterion 1.4% of trials were excluded).

The ANOVA on mean accuracy scores revealed a significant main effect of Task ($F_{1,34}=9.88$, $p=0.003$, $\eta_p^2=0.23$) with accuracy being overall higher in the Gender Discrimination task (mean=92.6%, $SD=4.4$) than in the Emotion Discrimination task (mean=87.3%, $SD=5.6$). The main effect of TMS was also significant ($F_{2,68}=6.94$, $p=.002$, $\eta_p^2=0.17$) indicating that accuracy was lower during TMS over the cerebellum compared to vertex stimulation ($t_{35}=3.06$, $p=0.012$) and to early visual cortex stimulation ($t_{35}=2.58$, $p=0.028$) (Figure 3). Accuracy was similar when TMS was delivered over early visual cortex and vertex ($t_{35}=1.28$, $p=0.21$). The interaction Task by TMS did not reach significance ($F_{2,68}<1$, $p=0.65$).

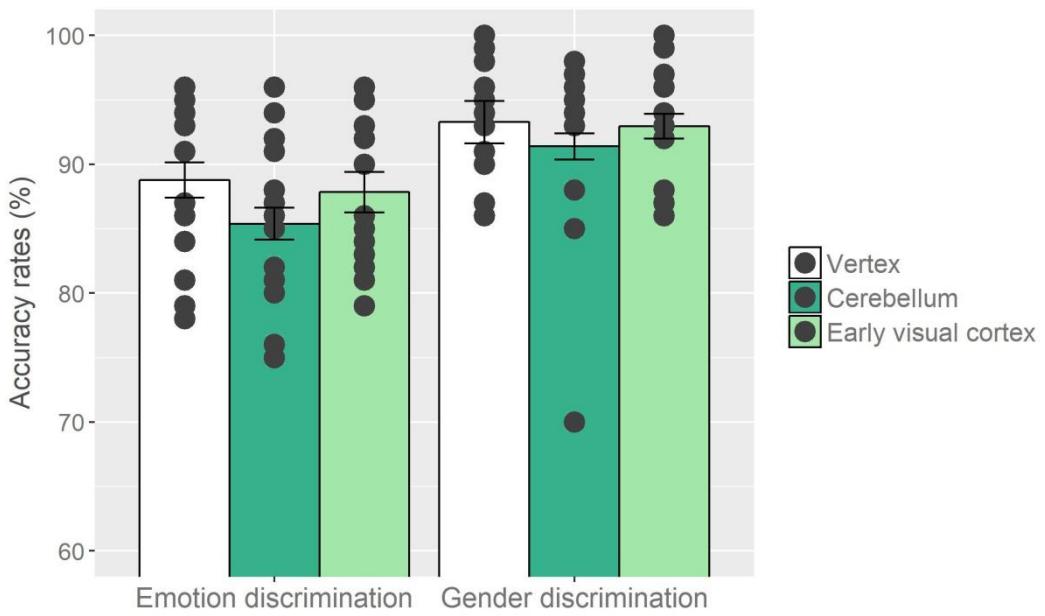


Figure 3. Mean percentage accuracy scores as a function of TMS condition (Vertex, Cerebellum and Early visual cortex) and Task (Emotion Discrimination vs. Gender Discrimination) in Experiment 1.

The ANOVA on mean correct RT revealed neither a significant main effect of TMS ($F_{2,68}<1$ $p=0.47$) nor of Task ($F_{1,34}=1.20$, $p=0.28$). The interaction TMS by Task did not reach significance ($F_{2,68}=1.29$, $p=0.28$).

Experiment 2

In Experiment 1 we found that applying TMS over the left cerebellum affected participants' performance in both the explicit emotional task and in the gender discrimination task where the emotional cues were irrelevant for the task. These results suggest that the cerebellum is likely to automatically encode the emotional content of a stimulus. Given the importance of replicability when testing new hypotheses, we carried out Experiment 2 to ensure that the findings of Experiment 1 could be replicated within-subjects in a new sample of participants (within-subjects designs also allowing for more powerful tests of effects than between-subjects designs, reducing variability due to individual differences among subjects, see Pollatsek & Well, 1995). Procedure was identical to Experiment 1 but face stimuli and their duration were slightly modified in order to increase the complexity of gender discrimination so to make the two tasks

equally difficult. This because in Experiment 1 participants performed overall better in gender discrimination than in emotion discrimination, possibly due to the use of natural faces with also hair visible (for similar effects, see Gorno-Tempini et al., 2001). Previous fMRI studies indicated that cerebellar responses may vary as a function of task complexity (e.g., Boecker et al., 2002; Schubotz & von Cramon, 2002) and response uncertainty (Volz et al., 2003): although in Experiment 1 cerebellar stimulation affected both tasks, regardless their different difficulty, we considered it as important to balance task performances when repeating the experiment to better control for possible confounding effects.

Method

Participants

Eighteen Italian volunteers (6 males, mean age= 23.6 years, SD=3.4) took part in the experiment. None of them had participated in Experiment 1. Inclusion criteria were the same as for Experiment 1.

Stimuli and procedure

Stimuli and procedure were similar to Experiment 1, but this time the same participants performed both the Emotion Discrimination and the Gender Discrimination task (in two different blocks). Moreover, to make gender discrimination harder (so to reach a similar level of accuracy in the two tasks), face images used in both tasks were cropped so that hair was not visible. With the same purpose, faces in the Gender discrimination block were presented for 100 ms (rather than for 150 ms as in the Emotion discrimination task). TMS parameters were similar to Experiment 1, but only two sites were targeted (to ensure a proper duration of the experiment, within safety-limits guidelines): the left cerebellum and early visual cortex (control site). Mean stimulation intensity - decided for each participant as in the previous experiment - was 53.1% (SD =2.6).

Results

Data were analyzed similarly to Experiment 1. A repeated-measures ANOVA with Task (Emotion Discrimination vs. Gender Discrimination) and TMS site (cerebellum vs. early visual cortex) as within-subjects variables was conducted on accuracy rates and mean correct RT. Trials in which participants' RT were ± 3 SD compared to their block mean were excluded from the analyses (following this criterion 1.4% of trials were excluded).

The ANOVA on mean accuracy scores revealed a significant main effect of TMS, ($F_{1,17}=6.21$, $p=0.023$, $\eta_p^2=0.27$) indicating that participants performed worse when TMS was delivered on the cerebellum compared to early visual cortex (Figure 4). Neither the main effect of Task ($F_{1,17}<1$, $p=0.86$) nor the interaction TMS by Task reached significance ($F_{1,17}<1$, $p=0.50$).

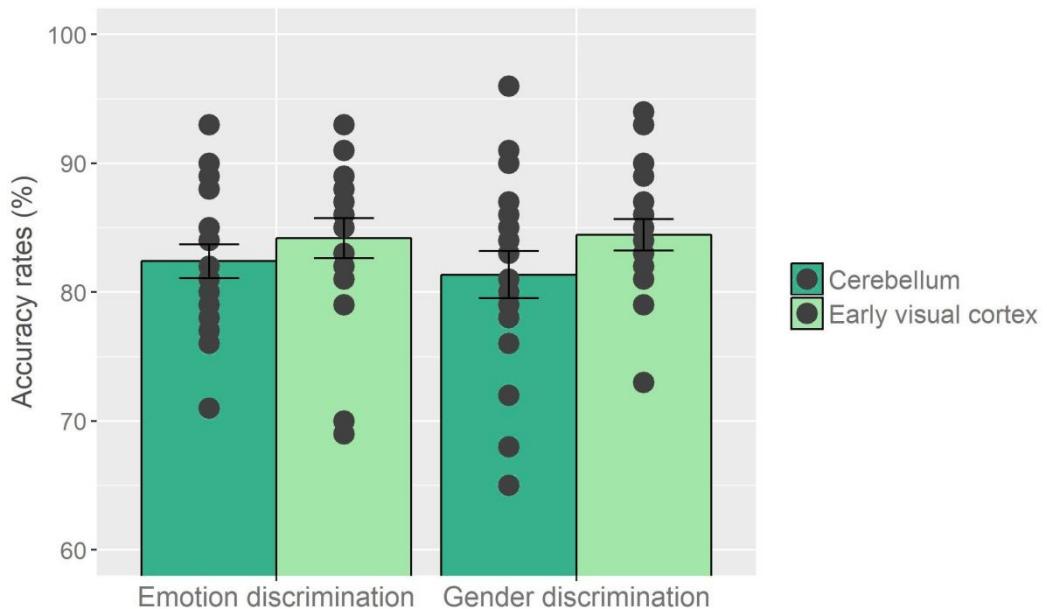


Figure 4. Mean percentage accuracy scores as a function of TMS condition (Cerebellum and Early visual cortex) and Task (Emotion Discrimination vs. Gender Discrimination) in Experiment 2.

The ANOVA on mean correct RT revealed a significant main effect of Task ($F_{1,17}=5.07$, $p=0.038$), indicating that overall participants performed slightly slower in the Gender Discrimination task (mean=564 ms, $SD=133$) compared to the Emotional Discrimination task (mean=525 ms, $SD=177$). Note that the Task effect we reported here

shows that cropping the face stimuli and reducing their presentation in the Gender task were effective manipulations in making gender discrimination harder compared to Experiment 1. Neither the main effect of TMS ($F_{1,17}<1$, $p=0.51$), nor the interaction Task by TMS reached significance ($F_{1,17}<1$, $p=0.08$).

Experiment 3

Experiment 2 replicated the results of Experiment 1 by showing (in a within-subjects design) that interfering with activity in the left cerebellum impaired participants' ability to process emotional facial expressions both when these had to be explicitly discriminated and when they were incidental for the task. However, a possible objection to both Experiment 1 and 2 is that the observed impairment following cerebellar stimulation may have depended on unspecific effects of cerebellar TMS in evaluating facial characteristics or, more in general, in decision making per se (regardless of the stimuli used). In order to rule out this possibility, in Experiment 3 we presented a new group of participants with the same paradigm used in Experiment 2 but in which neutral faces, rather than emotional faces, were used in the Gender discrimination task. If the effects we reported in Experiment 1 and 2 reflect the specific contribution of the cerebellum in processing emotional cues, cerebellar TMS should not affect Gender discrimination when neutral faces are used.

Methods

Participants

Eighteen Italian volunteers (3 males, mean age=23.6 years, SD=2.6) took part in the experiment. None of them had participated in either Experiment 1 or 2. Inclusion criteria were the same as for the previous experiments.

Stimuli and procedure

As in Experiment 2, participants performed both the Emotion discrimination task and the Gender discrimination task. Stimuli of the Emotion discrimination task were the same used in Experiment 2. In the Gender discrimination task though, the emotional

faces used in the prior experiments were replaced by corresponding male and female faces showing a neutral expression (stimuli from the Radboud database, (Langner et al., 2010). Neutral faces were cropped so that hair was not visible (i.e. to make gender not too obvious), but duration of face presentation in the Gender task was set back to 150 ms (as in Experiment 1). Each experimental block consisted of 36 trials (repeated twice for a total of 72 trials). TMS sites and parameters were identical to Experiment 2. Mean stimulation intensity - decided for each participant as in the previous experiments - was 53.5% (SD =3.0). Order of Task and TMS targeted sites was counterbalanced among participants.

Results

Accuracy rates and mean correct RT were analyzed via a repeated-measures ANOVA with Task (Emotion Discrimination vs. Gender Discrimination task) and TMS site (cerebellum vs. early visual cortex) as within-subjects variables. Trials in which participants' RT were ± 3 SD compared to their mean were excluded from the analyses (following this criterion 1.3% of trials were excluded). One participant was excluded because of extremely long RT (2 SD above the group mean).

The ANOVA on mean accuracy scores revealed neither a significant main effect of Task ($F_{1,16}=2.71$, $p=0.12$) nor of TMS ($F_{1,16}1$, $p=0.94$). In turn, the interaction TMS by Task reached significance ($F_{1,16}=5.15$, $p=0.037$, $\eta_p^2=0.24$). TMS over the cerebellum lowered accuracy rates compared to TMS over early visual cortex in the Emotion Discrimination task ($t_{16}=2.26$, $p=0.038$), but not in the Gender Discrimination task ($t_{16}=1.61$, $p=0.13$) (Figure 5).

The ANOVA on mean RTs revealed an almost significant main effect of Task ($F_{1,16}=4.26$, $p=0.06$), indicating that overall participants performed slightly faster in the Gender Discrimination task (mean=509 ms, SD=135) compared to the Emotional Discrimination task (mean=584 ms, SD=167). Neither the main effect of TMS ($F_{1,16}=1.09$, $p=0.31$) nor the interaction Task by TMS reached significance ($F_{1,16}<1$, $p=0.85$).

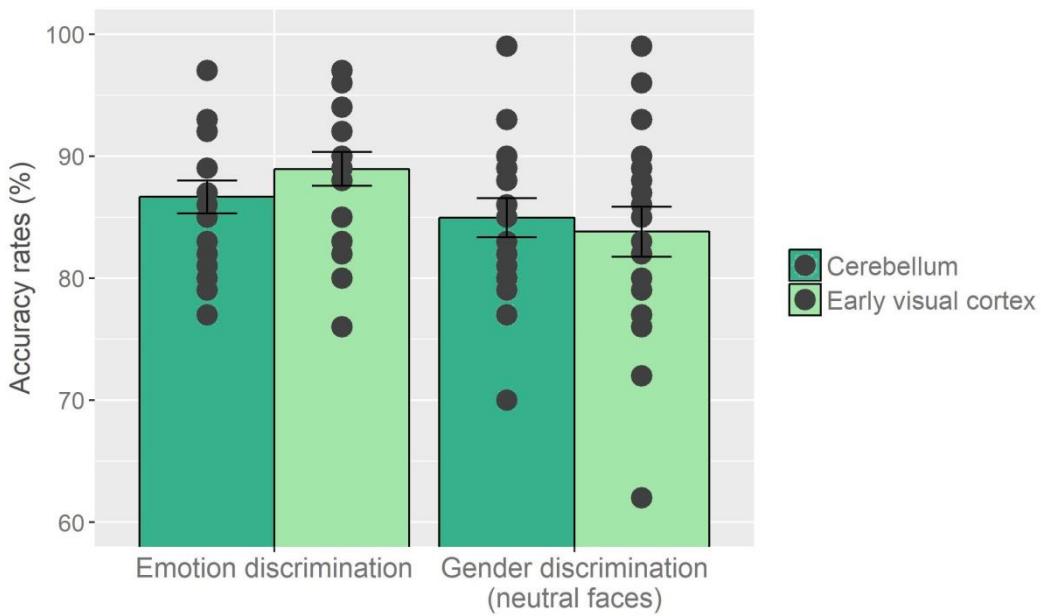


Figure 5. Mean percentage accuracy scores as a function of TMS condition (Vertex, Cerebellum and Early visual cortex) and Task (Emotion Discrimination vs. Gender Discrimination – neutral faces) in Experiment 3.

Discussion

The goal of the present study was to use TMS to interfere with cortical activity of the left cerebellar hemisphere during facial emotion processing. In order to examine the specific contribution of the cerebellum to explicit/implicit processing of emotional cues, participants were presented with facial emotional expressions that had to be explicitly discriminated (emotion discrimination task) or were incidental for the task at play (gender discrimination task). Results showed that cerebellar TMS impaired both explicit and implicit elaboration of emotive cues, as indicated by decreased accuracy not only in the emotion discrimination task, but also when the emotive cues were irrelevant for the correct execution of the task.

This finding is line with previous neuroimaging evidence that point at the cerebellum as part of the cortico-limbic networks sub-serving emotion processing. Specifically, the lateral portions of the cerebellum – forming what is referred to as the “lateral limbic cerebellum” – have been suggested to contribute to the cognitive aspects of emotion (Habas et al., 2009). This hypothesis is consistent with clinical observations reporting cognitive deficits following hemispheric lesions and affective disturbances

occurring with damage or structural abnormalities of the medial cerebellum (Levisohn et al., 2000; Okugawa, Sedvall, & Agartz, 2003). Nevertheless, there is no full consensus to date about the distinction between the functional role of the medial and the lateral cerebellum in emotion processing (Adamaszek et al., 2017). Interestingly, the left lateral cerebellum (specifically of Crus I and II of lobule VII) have been proposed as a key cerebellar hub for the convergence of cognitive and emotional information, presumably due to the projections linking these regions with associative cortices, in particular the contralateral prefrontal cortex (Han, Chen, Jeong, & Glover, 2016). Activation of the left cerebellum has been associated with observation and congruent facial reactions in response to facial expressions exhibited by others (Likowski et al., 2012; Schraa-Tam et al., 2011), suggesting that the cerebellum may contribute to facial expression recognition thereby mechanisms which involve mimicking the other person's facial expression. Consistent with this hypothesis, mirror neurons (i.e., active during action observation and execution) have been identified in the cerebellum (Gazzola & Keysers, 2009). Furthermore, a recent voxel-bases morphometry study reported a correlation between the white matter volume of the Crus II of the left cerebellum with the ability to recognize facial expressions (Uono et al., 2017). Indeed, our findings support the role of cerebellar Crus I and II in emotive-information processing, in accordance with the hypothesis that neurons of lobule VII, structure including Crus I and II and target site of the stimulation in the present study, contribute to both emotional perception and evaluation (Adamaszek et al., 2017).

The contribution of the cerebellum to emotion processing is not confined to facial emotion recognition and affection regulation, but seems to extent to the experience of moral compassion (Moll et al., 2007), pain perception (Moulton, Schmahmann, Becerra, & Borsook, 2010) and emotion learning (Utz et al., 2015). All these findings can be put in relation with the computational mode of the cerebellum, as proposed by the Universal Cerebellar Transform (UCT) hypothesis (Koziol et al., 2014; see also General introduction). According to this hypothesis, the cerebellum integrates internally stored representations with external stimuli in order to optimize performance according to the context demands. The term "universal" refers to the heterogeneity of

the information processed, underlying a broad range of functional domains. It follows that the cerebellum would contribute to produce harmonious motor, cognitive as well as emotive/affective responses. A growing consensus is being built on “how” the cerebellum assists brain processes underpinning motor and non-motor functions, i.e. thereby implementation, storage and update of internal models of multi-modal representations. Within the emotion domain, the observation of other agent’s actions and emotional expressions is processed by the cerebellum as an internal forward model, in which perceptual inputs are elaborated and transformed into a simulated motor pattern which facilitates action and emotion comprehension (Gazzola & Keysers, 2009). In accord with this, we argue that cerebellar TMS perturbation might have altered these sensory-motor integration mechanisms, impairing emotional processing even when the emotional cues were task-irrelevant. However, whether the general mechanisms of the cerebellum are sufficient to explain its contribution to emotion remains still an open question. These mechanisms, which have been suggested to include error detection or differentiation between positive or negative emotions, can be considered primitive properties of the cerebellum (see D’Angelo & Casali, 2012). It follows that a disruption of the so-called cerebellar primitive functions would affect and desynchronize streams of information processing of different nature, arising the question on the specificity of cerebellar computations. It is worth noting in this regard that our results are unlikely to reflect an unspecific interference on general cerebellar computational operations, as TMS did not alter the performance at the gender discrimination task. On a related note, we also argue that the time-window of TMS in our experiments reduces the risk of interfere with the processing of face information exerted by the early visual cortex, as perceptual information about facial features have been demonstrated to reach face-selective regions in extra-striate visual cortex 60-100 ms from face onset presentation (Pitcher, Walsh, & Duchaine, 2011). It follows that the lack of occipital TMS effect in our experiments represents an important control to claim for cerebellar-selective stimulation effects (see Renzi, Vecchi, D’Angelo, Silvanto, & Cattaneo, 2014), given the anatomical proximity of the cerebellum to the occipital lobe.

In sum, our data add to previous evidence showing the involvement of the cerebellum in facial emotion expression processing. Specifically, the TMS-induced impairment emerged in the present study suggests that the left cerebellar hemisphere is involved in emotion discrimination, even when the emotional cues are irrelevant for the task at play, reflecting emotive-information processing at an automatic, implicit level. Further research is needed to clarify the role of the cerebellum to emotion. Moreover, a better understanding of the neural mechanisms sub-serving the contribution of the cerebellum to emotion processing may have critical clinical implications, as cerebellar dysfunctions have been causally linked to various emotional disorders (Shakiba, 2014).

Chapter 6

General discussion and Conclusions

The present dissertation focuses on the contribution of the cerebellum to behavioral and cognitive performance. The topic has been approached through different methodologies, gathering insights from behavioral performances, electrophysiological measurements and modulation of the former outputs by means of brain stimulation techniques.

The ubiquitous activity of the cerebellum is changing the view of this structure as strictly confined to motor control. Indeed, a consensus is growing on the role played by the cerebellum in assisting brain functions pertaining not only to the motor domain, but also to cognition and emotion. Despite that the re-evaluation of cerebellar functioning is still not widely accepted by the scientific community, consistent and multidisciplinary lines of research are trying to answer the question on "how", rather than "if", the cerebellum exerts a contribution to cognitive and emotional processes. The work presented here follows this broad research question.

Overall, the findings of this work add to a growing body of literature that put the cerebellum at the interface of cognitive- and emotive-related functions. Even though the methodology applied did not allow us to draw any conclusion on cerebellar mechanisms, we made inferences on the contribution of the cerebellum to the investigated cognitive and emotive functions based on the observation of the behavioral outcomes. The detrimental effect of stimulation emerged in several experiments illustrated in the previous chapter can be put in relation with the view of the cerebellum as a generalized forward-controller not only for motor but also for cognitive functions (D'Angelo & Casali, 2012; Ito, 2008). The acquisition and the implementation of internal models (inverse and forward) has been pointed out as the computational basis throughout which the cerebellum exerts its assisting functions. Specifically, a forward model generates a prediction, consisting of an estimation of an internal feedback in replacement of the actual external feedback. The model then compares this prediction with the actual peripheral sensory inputs. In case of detection of a mismatch between prediction and sensory feedback, a signal error is produced, that is later used to correct and successfully guide behavior (Ito, 2008; Miall & Wolpert, 1996). Hence, as a "supervised learning machine", the cerebellum is thought to learn to

modify the model itself in presence of persistent deviations from the internal prediction. In line with the hypothesis on the operational mode of the cerebellum involving the updating of internal models, we propose that the impairment in behavior observed in our studies may reflect a stimulation-induced perturbation of the cerebellar computational primitives, which include error detection, sequence processing and sensory-perceptual integration mechanisms. It is relevant to highlight that the modulation of performance is unlikely to reflect an effect of the stimulation on cerebellar motor functions or on perceptual encoding of stimuli. Taken together, the data illustrated in the present dissertation go in the same direction of the perspective proposed by the Universal Cerebellum Transform (UCT) hypothesis (Koziol, Budding, D'Arrigo, et al., 2014). According to this hypothesis, the contribution of the cerebellum to different anatomical and functional pathways permits the production of harmonious motor, cognitive and affective behaviors. The cerebellum is thought to receive inputs of different nature from multiple projections linking it to various cerebral regions, and to elaborate these inputs with same computations. Hence, the multi-modal computational mode of the cerebellum applied to different stream of information processing has been suggested to be at the base of the cerebellar contribution to a wide range of functions. The experiments, core of this work, provide supporting evidence to this perspective.

Given that we used a brain stimulation approach to the study of the functional role of the cerebellum, the present dissertation opens with a review on the effects of one of the most commonly used brain stimulation techniques applied in the field of cerebellar research. In more details, the meta-analysis (study 1) has been conducted with the purpose of providing a quantitative review on the effects of cerebellar transcranial direct current stimulation (cDCS) on behavioral performance in healthy participants. Firstly, given the existing skepticism on the reliability of tDCS outcomes, the study aimed at testing the efficacy of this technique applied to the cerebellum in modulating behavioral performance. Results showed that cDCS is effective in modulating both motor-and non-motor-related performance. However, there is no evidence for polarity-dependent effects of anodal and cathodal cDCS on behavioral indices of cerebellar functioning. Modeling studies suggest that the observed effects

can be attributed to changes in cerebellar function. Estimations of electric field properties of bipolar DC stimulation using an ion-based cerebellum-buccinator muscle montage show that, due to the volume of the cerebellum and the homogeneity of its neural structures, the electric field distributions are more focused as compared to other montages targeting the cerebral cortex (Parazzini et al., 2014). In addition, it is worth mentioning that the motor performance was significantly more affected than the cognitive performance. In line with the classical view of the cerebellum, this result suggest that this structure may be predominantly involved in motor-related functions. On the other hand, analyses also showed that cDCS was effective in modulating cognitive performance, indicating that the cerebellum is not exclusively related to the motor function. Furthermore, still little is known regarding the degree to which the cerebellum is engaged in a particular motor task or cognitive function. This may explain why neither anodal nor cathodal cDCS were predictive of improvement or impairment of behavior, respectively. In sum, even though results showed that a low electrical current applied in correspondence of the cerebellar cortex is able to produce behavioral effects, it is difficult to exactly predict the direction of these effects depending on current polarity.

The second study (study 2) relies on the efficacy of the cDCS in altering cognitive performance. We found that cathodal cDCS impaired the cognitive performance during the Eriksen flanker task but did not alter error-related negativity (ERN) components, associated with error processing and monitoring functions. The dissociation between behavioral and electrophysiological effects can be interpreted in light of the anatomical and functional pathways that connect the cerebellum to the brain regions that are jointly involved in the complex set of functions underlying error processing and performance monitoring, including error detection, response selection and response inhibition. We argue that the stimulation-perturbed signal of detection of mismatch coming from the cerebellum and other subcortical regions may have reached the prefrontal cortex areas for conflict evaluation and response selection. This speculation in accord with the observed impairment in behavior. At the same time though, regardless the interference of incoming signals produced by the stimulation, we

conjecture that operations of the cerebral cortex may have not been affected by this perturbation during response-conflict monitoring. As a result, the output of prefrontal cortex (i.e. the ERN potentials) remained unaltered. Interestingly, the impairment of behavior is unlikely to reflect a detrimental effect of cathodal stimulation on motor execution, as indicated by unaltered lateralized readiness potentials (LRP), which are considered an index of hand-related motor activity associated with response processes. In conclusion, these data provides evidence supporting an involvement of the cerebellum in error-related mechanisms. Moreover, results are in line with previous works reporting cathodal-DC to impair behavioral performance. Nevertheless, repeating the experiment applying anodal stimulation would help exclude that the impairment observed here reflects a detrimental artifact effect of the stimulation.

The second study focused on error-related processes, such as response selection and response inhibition. Nonetheless, error processing and performance monitoring together comprise a broad set of sub-processes. Among these, sensory prediction mechanisms and detection of errors are required for the updating of motor and cognitive scripts and for successful guidance of behavior. The detection of a mismatch between internal representations and external feedback is considered a computational mode of cerebellar operations (Wolpert, Miall, & Kawato, 1998). This computational scheme has been proposed to be the basis for the brain's predictive functioning. Indeed, the brain constantly predicts and anticipates future events base on the recognition of patterns and sequences of elements gathered through the experience. The third study (study 3) aimed at investigating the contribution of the cerebellum to sequence processing and violations detection by means of transcranial direct current stimulation (TMS). Findings showed that cerebellar TMS interfered with processing of unfamiliar – or “new” – sequences (Experiment 1,2,3) but not of highly familiar – or “overlearned” – sequences (Experiment 2). The present result can be interpreted in light of a previous neuroimaging evidence that reported an experience-dependent shift of activation of the cerebellum during motor learning, with increased cerebellar activation during early learning and decreased activation following extended practice (Doyon et al., 2002). Accordingly, high cerebellar activity has been observed during serial ordering

of elements embedded in patterns presented for the first time, i.e. for which a corresponding internally stored template does not exist, as compared to processing of probes presented in a progressive, predictable order (Attout, Fias, Salmon, & Majerus, 2014). The latter findings and ours are in favor of the hypothesis that the cerebellum is mainly involved in early learning, presumably reflecting operations of adaptation of motor and cognitive scripts. More in general, these data are in line with the view of the cerebellum as a coprocessor, assisting operations that are computed elsewhere in the brain in presence of tasks that require high cognitive demands and attentional resources (Pope & Miall, 2012).

As already discussed, the cerebellum is thought to implement, store and update internal models referring to multi-modal representations. This notion has been further extended from the motor and cognitive-perceptual domain to the processing of emotive information. It has been suggested that other people's actions and facial expressions may be processed by the cerebellum as an internal forward model. In more details, perceptual and emotive-information deriving from the observation of other agents would be elaborated and transformed into a simulated pattern to facilitate action and emotion comprehension (Gazzola & Keysers, 2009). In the last study (study 4), the detrimental effect of cerebellar TMS on both implicit and explicit facial emotion processing seems to converge with the abovementioned hypothesis. We argue that the TMS-induced perturbation might have interfered with sensory-motor integration mechanisms, impairing emotional processing even when emotional cues were irrelevant for the task at hand (i.e. during a gender discrimination task). However, the fact that disruption of the so-called cerebellar primitive functions – including sensory-perceptual integration mechanisms – is expected to desynchronize streams of multi-modal information processing, arises the question on the specificity of cerebellar computations. In other words, the fact that the stimulation might have interfered with the unspecific encoding of perceptual features should be carefully considered to not misinterpret the data. Nevertheless, it may be relevant to highlight that our results are unlikely to reflect an unspecific interference of general cerebellar operations, as cerebellar TMS did impair not impair gender recognition. Moreover, using occipital TMS

as a control condition helped us to exclude that the impairment in emotion recognition was due to detrimental artifact effects of the stimulation on face information processing.

In conclusion, given the role of the cerebellum as a coprocessor, it is important to bear in mind that cerebellar stimulation usually modulates cognitive functions in a subtle manner. As a result, alongside the consideration of cerebro-cerebellar anatomical and functional connectivity, methodological factors concerning stimulation parameters and task characteristics – such as task sensitivity and complexity – are crucial to observe and interpret the effects of cerebellar stimulation on non-motor functions.

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