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Neural Mechanisms Underlying Prim Adaptation

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Neural Mechanisms Underlying Prism Adaptation

Abstract

Prism Adaptation (PA) is a non-invasive tool to stimulate short-term visuomotor plasticity that provides the opportunity to experimentally study the consequences of a misalignment of visual and proprioceptive-motor maps and the realignment ability of the human sensori-motor system. In the rehabilitation context it can represent a promising technique to durably ameliorate symptoms of spatial neglect. The present thesis presents five different experiments aimed at clarifying the neural correlates of PA by means of functional brain stimulation. Several PA procedures (Single-step PA, Multiple-Step PA, Reversing PA) and different protocols of transcranial Direct Current Stimulation (tDCS) are combined to assess the role of distinct brain regions (Cerebellum, Posterior Parietal Cortex, Primary Motor Cortex) and their relative connections (Cerebellar-Parietal circuitry) during PA. Results from the present experiments support the theoretical distinction in PA between a strategic mechanism of error correction and a deep process of adaptation. The process of adaptation to prism would be achieved by fast cerebellar involvement during PA, since a very early phase of the pointing performance, and would crucially rely on the connections of this structure with the Parietal Cortex. The continuous information flow between the cerebellum and the PPC would permit full error compensation during PA and cerebellar functioning would allow after-effect development following PA. The after-effect, that can be considered as a kind of motor memory, would be stored in the Primary Motor Cortex and it can be reactivated by means of delayed functional stimulation. The theoretical impact of these findings and possible clinical applications are discussed.

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Chapter 1
Prism Adaptation: a tool to investigate plasticity of visuo-motor coordination and a neuropsychological rehabilitation method

Brief history of Prism Adaptation

The use of prism glasses in the scientific field can be traced back to the 17th century, when Renè Descartes and Isac Newton argued on the wave-form versus the corpuscular-nature of light, making some preliminary observations using prism lenses (Newton, 1672; Sabra, 1981). Later, in the 19th century, the psychologist George Stratton started a series of experiments on visual perception aimed at investigating the ability of the perceptual system to adapt to artificial and stable changing of the retinal images (Stratton 1896, 1897a, 1897b). For his experimentation, Stratton built the first reversing spectacles that consisted in optical lenses to invert retinal image both upside-down and left-right using a system of mirrors mounted in a frame. Although these preliminary observations in the field of physics and perception, the first Prism Adaptation (PA) applications date back to the mid-1800s, when von Helmoholtz published his influential work on optics and used prism spectacles as supportive evidence to the Perceptual Learning Theory (Helmoholtz, 1962). Later Held (1965) demonstrated that adaptation to wedge prisms depended on the interaction between the visual and motor systems and that such interaction normally induces a plastic change in the brain. These applications represented the birth of the first experimental tradition in PA concerning the possibility to study the relationship between visual perception and motor planning and the ability of the brain to flexibly compensate for a mismatch between these two levels. This line of research continued with the comprehension of the hidden mechanisms underlying this ability and research on the best ways to get and test adaptation attracted the attention of several researchers such as Welch (1974a, 1974b), who proposed one of the first models of PA, and Redding and Wallace, that deeply studied the adaptive processes, methodology and modelling of PA (Redding & Wallace, 1988; 1990;1997a).

The paper published in 1998 by Rossetti (Rossetti et al., 1998) represented the beginning of the clinical tradition in PA for its promising applications in neuropsychological rehabilitation. As a consequence, research moved from healthy participants of the previous studies to the clinical population. Rossetti et al. (1998) found that PA could at least transiently rehab spatial neglect, a neurological deficit following right brain damage in perceiving, attending, representing and acting toward the contralesional space (Bisiach, 1999), and shaded light on the close relationship between sensori-motor and cognitive systems. In the following twenty years the number of studies on PA have rapidly increased and supramodal, generalized (Rode et al., 2001) and long-lasting (Frassinetti et al., 2002; Serino et al., 2006; Serino et al., 2009) ameliorations have been reported for left neglect patients, proposing PA as a new rehabilitative tool for spatial neglect (Mattingley, 2002; Jacquin-Courtois et al., 2013). Very recent papers also report possible applications of PA in the treatment of other neurological conditions such as the Complex Regional Pain Syndrome, an invalidating chronic condition subsequent to peripheral lesions, that has been shown to durably improve after few session of PA (Christophe et al, 2016).

Current research is focused on the mechanisms underlying PA and their brain correlates, with the aim to understand the relationship between the visuo-motor and cognitive levels and to identify the factors that make some patients more or less suitable for this application. In fact, although an increasing literature shows that patients can benefit from PA, some patients seem to not respond to this rehabilitation tool (Rousseaux et al., 2006; Sarri et al., 2008). As a consequence, the need to improve this technique has motivated the development of a recent line of research (Jacquin-Courtois et al., 2013). In this respect research has progressively moved back to healthy participants to study the mechanisms of such a complex phenomenon in controlled experimental conditions. In this way the

two traditions in PA, one more related to perception and visuo-motor coordination and the other one closer to neuropsychological rehabilitation, have started to thickly flow one into the other.

Procedure and methodology of Prism Adaptation

A possible definition of PA is a non-invasive procedure representing both a valid tool to study short-term visuomotor plasticity and a promising rehabilitation method to treat spatial neglect. In the field of visuomotor flexibility it provides the opportunity to experimentally study the misalignment of visuomotor maps and the realignment ability of human motor system, while in the rehabilitative contest it represents a bottom-up technique to automatically and durably ameliorate the various symptoms of spatial neglect.

PA consists in the exposition of a participant (a healthy subject or o patient when is used for rehabilitation purposes) to a left or right shift of the visual field by means of prismatic goggles. Depending on the different experimental procedures, the subject is asked to point at a visual target in the space (pointing task) or to grasp it (grasping task). As a consequence of the prismatic shift, the actual position of the target is shifted in the same direction of the prismatic deviation toward a virtual position, and participants classically fail to reach the object in its real position stopping at the displaced/virtual one. This kind of error, that is the direct effect of the prismatic deviation, is called **terminal error** and it is quickly compensated when it is visually noticed by the motor system and/or when successive trials are performed. It represents an offline signal that participants can use to change the movement plan in a direction opposite to the prism shift to reach the object in its actual position. Once glasses are removed, depending on whether participants had been

allowed to perform the pointing task for only a few or several trials, an error in the opposite direction of the prism-induced deviation can be observed. This error is named **after-effect** and, as the terminal error, is quickly compensated as the visual information on the discrepancy between the motor intent and the reached position is computed.

In order to assess the several aspects mentioned above, a classical procedure of PA involves at least three steps: Pre-Exposure, Exposure and Post-Exposure. In **Pre-Exposure** phase a baseline measurement of pointing performance is collected before wearing prisms glasses. During **Exposure** phase participants are actively exposed to prismatic goggles and perform fast movements toward a visual target. In **Post-Exposure** phase the same tasks completed during Pre-Exposure are performed once again to quantify PA after-effects. According to Jacquin-Courtois et al. (2013) it should be emphasized that either the demonstration and quantification of PA are obtained through the measurement of after-effects, i.e. by the comparison of participants' performance in Pre and Post Exposure tests. In fact, *errors' compensation* observed during Exposure does not mean that participants developed *adaptation*. To achieve a consistent adaptation, it is necessary not only to compensate for the initial pointing error, which usually happens quickly in a few trials, but it is necessary that the task is repeated for several trials. In order to measure adaptation and its different aspects, several tasks have been used during Pre-Exposure and Post-Exposure phases. The measure of midsagittal judgment based on different sensory information has been commonly employed. In the **Visual Straight-Ahead (V-SA)** participants are asked to indicate when a moving (left-to-right and right-to-left) visual target is straight ahead in front of their eyes; in the **Motor/Proprioceptive Straight-Ahead (M/P-SA)** participants are asked to make straight ahead pointing movements without vision; the **Open Loop Ponting (OLP)** consists of pointing movements towards a visual non-exposed target with no vision of the movement and

represents a global measure of after-effects combining visual, motor and proprioceptive information (Redding & Wallace 1997; 2005).

During Exposure phase specific experimental procedures can be used also affecting adaptation (Facchin et al., 2013b). In **concurrent exposure** there is total visibility of the arm that performs the pointing movement in a way that both the movement path and its outcome are visible, while in **terminal exposure** the proximal and distal parts of the limb are not in sight and only the hand and the outcome of the movement are visible. Ladavas et al. (2011) compared the concurrent and terminal exposure procedures demonstrating that the latter is able to produce more substantial rehabilitative effects than the first one and that error reduction is faster in the first case than the second one. Moreover, the full or partial visibility of the limb and the speed of the pointing movements during Exposure phase are two important variables to control in a PA experiment. As an example, Redding et al. (2005) noted that when adaptation to prisms is made by performing very slow movements and with complete vision of the limb along its trajectory, the terminal error and the following adaptation can be null since the first movement.

The experimental procedures during Exposure phase can be distinguished also on the basis of the glasses used during Exposure phase. Indeed, it is possible to use the same pair of glasses with the same power during the whole Exposure phase or several glasses with different powers that are actively changed in a progressive or random order. In the traditional **Single-step PA procedure** (the most used in previous studies), participants are exposed to a full prismatic shift in one time experiencing a strong and visible change from a no shift condition to the shifted one. In the **Multiple-step PA procedure**, the full optical deviation is achieved by means of progressive stepwise increases from a no-shift condition to the complete prism displacement, thus participants remain unaware of the prism deviation (Michel et al., 2007). In the **Reversing PA procedure**, the power and the

base of prisms are frequently changed in a random order to make participants unable to develop full adaptation (Clower et al., 1996).

Cognitive effects of Prism Adaptation

Several studies on neglect patients and healthy participants show that the after-effects of PA are not restricted to the sensorimotor level, i.e. sensorimotor realignment assessed by the above mentioned measures, but extend to spatial cognition, i.e. to cognitive functions assessed by several test of spatial attention. On neglect patients Rossetti et al. (1998) demonstrated the benefit of rightward adaptation on various test of spatial exploration like line bisection, line cancellation and drawing. Rode et al. (2001) also reported an effect of PA on tasks that do not require a manual response such as the spatial exploration of a mental image. Authors found that the mental evocation of left-sided information from an internal image of the map of France was fully recovered following PA to the right (see also Jacquin-Courtois, 2013). Similarly, in healthy participants several studies report that PA can simulate neglect-like symptoms. Following leftward PA, rightward neglect-like biases were observed on manual and perceptual line bisection tasks that required healthy participants to estimate the midpoint of line segments (Colent et al., 2000; Michel et al., 2003; Schintu et al., 2014). Many studies have replicated these results and extended the after-effects of PA to numerous cognitive functions (Girardi et al., 2004; Nicholls et al., 2008; Bultitude et al., 2010). These results demonstrate that although PA operates at a low-level sensorimotor coordination, it can affect high-level spatial representations also leading to **cognitive after-effect** and clearly demonstrate the existence of a strong link between the sensorimotor plasticity and cognitive functions.

Adaptive mechanisms and neural correlates of Prism Adaptation

The finding of significant effects of PA both on the level of sensory-motor coordination (low-level) and on the level of cognitive processing (high-level) has led to a tangled discussion on the mechanisms and the brain areas that, starting from low-level functions, are able to affect higher levels of cognition.

Two mechanisms seem to contribute to error correction during exposure to prim glasses (Redding et al., 1997; 2002; 2005). The process of **recalibration** is an *ordinary* adaptive response needed to modify motor commands when reaching objects within the space. It represents an *immediate reaction* to the prism-induced deviation by means of a *strategic-cognitive* modification of the motor plan to quickly reduce the terminal error. The subject would use information resulting from the outcome of his first movement to plan an updated movement that takes into account the prism visual-shift. This is why recalibration process is at least partly a "conscious" and “voluntary” phenomenon.

When the spatial relationship between visuomotor and proprioceptive-motor reference frames is changed, as it is when prisms displace the visual-motor reference frame, a process of re-alignment is necessary to align again the two reference frames. **Spatial realignment** can be defined as a *slow and automatic* process that re-aligns the spatial maps that have been perturbed by the prism shift leading to an indirect correction of motor plan. Only when a kind of misalignment occurs, e.g. when the prism experimental manipulation is used, the realignment process becomes apparent. As a consequence, this latter process can be seen as an *extraordinary* alignment process of visuomotor and proprioceptive reference frames.

This conceptualization seems to be supported by several neuroimaging studies investigating functional activity during PA (Clower et al., 1996; Danckert et al., 2008; Luautè et al. 2009; Chapman et al., 2010; Kuper et al., 2014).

The first study assessing neural correlates during PA by Clower et al. (1996), used Positron Emission Tomography (PET) to record changes in regional cerebral blood flow in participants who performed a pointing task using reversing prism spectacles. Authors showed that "adaptation" was correlated to the activation of the posterior parietal cortex (PPC) contralateral to exposed arm. However, in the task used for this experiment the optical deviation was reversed (left to right) every few trials (n=5) to keep participants in a state of ongoing compensation of errors. As a consequence, the pattern of activation described in Clower et al., (1996) is likely to be associated with the fast process of strategic recalibration of the visuomotor perturbation due to prisms, which occurs mainly in the first few trials of exposure, then with proper adaptation, i.e. the slow process of spatial realignment, which develops slowly over more trials (at least 50 trials; see Redding et al., 2005).

The same task and the same observations characterize the event-related functional Magnetic Resonance (MRI) study from Danckert et al. (2008), who demonstrated changes in the activity of anterior cingulate, anterior intraparietal region and medial region of right cerebellum while participants performed pointing movements (n=10) wearing prism glasses.

More recent studies used a higher number of trials but still not enough to properly study the slow process of spatial realignment. For example, Luautè et al. (2009) investigated dynamic brain changes during PA (24 pointing movements to visual targets) comparing errors during early (n= 12) and late (n=12) trials of adaptation. Results revealed that the earliest phase of prism exposure was primarily characterized by an activation of anterior

intraparietal sulcus and parieto-occipital sulcus supposed to be implicated respectively in error detection and error correction, and a later progressive increase of cerebellar activity that authors considered as in accordance with a key role in spatial realignment. A bilateral activation of the superior temporal sulcus associated with sustained prism exposure when pointing errors were fully compensated was also reported, suggesting a role for superior temporal sulcus in the slow realignment process and longer-lasting changes induced by prisms, such as the ones that might underlie changes in spatial cognition.

Chapman et al. (2010) aimed at studying the neural correlates of recalibration and spatial realignment by means of event-related fMRI while participants had to make pointing movements (n=18) with a manipulandum to back-projected stimuli. Authors found an activation in the inferior parietal lobe contralateral to the adapting hand and in the ipsilateral posterior cerebellum that was interpreted as associated to, respectively, the recalibration process and spatial realignment. However, it has to be noted again that the small number of pointing movements performed during exposure to prisms made this experiment not suitable to make any inference on the development of spatial realignment.

A recent neuroimaging study on neural correlates of PA has been performed by Kuper et al. (2014) that closely focused on cerebellar cortex and deep cerebellar nuclei while participants performed pointing movements toward visual targets (3 blocks of 20 trials each). Results by Kuper et al.'s report both an early and late activation of the cerebellum and dentate nuclei, confirming an involvement of the cerebellum in spatial realignment, in line with the previous studies, and proposing cerebellar involvement in strategic recalibration as well.

Open questions and aim of this work

Results from neuroimaging studies converge in identifying the PPC contralateral and ipsilateral to the adapted arm and the ipsilateral cerebellum as target regions in PA. These data are also in line with classical patient studies showing that the ability to adapt to prismatic displacement remains with intact cerebellum and damaged PPC (Pisella et al., 2004), while adaptation to prisms is lost with damaged cerebellum and intact PPC (Martin et al., 1996; Weiner et al., 1983). Although areas implicated during PA have been widely described by neuroimaging and patients' studies, these studies do not converge in the functional specialization of these areas in the mechanisms of recalibration and spatial realignment. For example, Luautè et al.'s study and Kuper et al.'s study do not converge on the role of the cerebellum in PA with the first supporting a prominent and exclusive role of the cerebellum in spatial realignment and the latter proposing a cerebellar involvement in strategic motor control responses, i.e. in recalibration too.

Two major problems related to neuroimaging studies in PA prevent to make direct links between brain correlates and specific adaptive mechanisms. The first problem deals with the small number of pointing movements used in the previous neuroimaging studies that do not allow to make any inference on the mechanism of spatial realignment. The second problem is referred to the nature itself of these techniques that permit observing brain activity during the execution of a target task in a correlational perspective but do not allow studying the causal relationship between brain and behavior.

Non-invasive brain stimulation techniques (Woods et al., 2016) provide an almost unique opportunity to modulate activity of target brain areas by means of facilitatory or inhibitory procedures. The rationale underlying these methods is that interfering or facilitating the activity of a target area would affect the behavior/process supposed to be related to that

area as well, providing the opportunity to study the direct link between human brain and behavior.

Among non-invasive brain stimulation techniques, transcranial Direct Current Stimulation (tDCS) offers a good compromise between the need to ensure focal stimulation and the need to easily combine stimulation with the experimental setting required to perform PA. tDCS has been recently used to study behavioral effects of simulated cerebral (Fertonani et al., 2010; Moos et al., 2012; reviews: Ferrucci et al., 2015; Van Dun et al., 2016, 2017) or cerebellar lesions (Pope & Miall, 2012; Hardwick and Celnik, 2014; Ferrucci et al., 2015).

The combination of tDCS and PA could provide stringent cues to understand the neural correlates of PA. In the next chapters several experiments will be presented aimed at identifying the correspondences between functional mechanisms in PA and their relative brain substrates. The role of the cerebellum, the PPC and primary motor cortex (M1) will be evaluated and discussed in three lines of experiments. The first series of experiments uses different protocols of PA aimed at isolating the functional mechanisms of PA and clarifying the role of the cerebellum in the processes of recalibration and spatial realignment (Chapter 2). The idea of a clear-cut separation of brain areas subserving the two mechanisms is then challenged proposing an interpretation in terms of interrelated neural circuits (Chapter 3). Finally, the possibility to boost and reactivate the adaptive circuitry elicited by PA is tested and possible clinical applications are discussed (Chapter 4 and Chapter 5).

Chapter 2
Cerebellar contribution to the fast adaptive mechanisms of Prism Adaptation

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Introduction

When we move in the in the world we are able to modify our motor programs to perform accurate movements and successful target-directed actions combining several spatial representations in a dynamic way (Iversen et al., 2014). PA provides an almost unique opportunity to experimentally study the consequences of a misalignment of visual and proprioceptive-motor maps and the realignment ability of the human sensori-motor system. To quickly recall what has been described in the previous chapter, it is needed to remind that when wearing prism glasses during a pointing task, individuals typically miss objects making errors towards their virtual location (terminal error). Errors tend to disappear as the task goes on, since computation of the terminal error is used to correct motor planning (adaptation). Immediately after the removal of glasses, individuals' movements will be transiently shifted in the opposite direction of prism deviation (after-effect). The processes of recalibration and spatial realignment have been proposed to contribute to error correction and after-effect during PA (Redding and Wallace 2002; 2006): recalibration would ensure strategic correction of movements to quickly reduce the terminal error and it would occur mainly in the first stage of prism exposure acting on very early movements; spatial realignment, would require more practice to fully develop and would be responsible of the genesis of the after-effect (Redding et al., 2005). Recent neuroimaging studies report conflicting evidence on the brain correlates of these two mechanisms: Luauté et al. (2009) supported the idea that recalibration is mainly related to the activity of the PPC and spatial realignment depends on the cerebellum, whereas Kuper et al. (2014) proposed cerebellar involvement in strategic motor control responses during very early trials of prism exposure. It is thus possible that the cerebellum plays a role across all stages of PA, i.e. that it is implied since early trials, consistent with theories

postulating that the cerebellum participates into online monitoring of motor commands and ensures precise ongoing adjustment of actions (Manto et al., 2012).

The Experiments presented in the present chapter aimed at understanding the contribution of the cerebellum in PA combining functional stimulation and different procedures of PA (Single-step PA, Multiple-Step PA, Reversing PA). Indeed, to provide new insight on the role of the cerebellum during visuomotor adaptation it is interesting to assess cerebellar contribution to PA by interfering with its functioning directly using transcranial Direct Current Stimulation (tDCS). Until now, only one stimulation study has been conducted by Galea et al. (2011) on visuo-motor coordination. This study showed that anodal tDCS on the cerebellum determined faster adaptation to a 30° counterclockwise rotation and that anodal tDCS over the primary motor cortex increased the retention of the newly learnt visuomotor transformation. Although, this study provided evidence that it is possible to use tDCS to affect the acquisition and retention of a visuomotor transformation, no stimulation study has been performed to study the neural correlates of PA and to assessed the contribution of the cerebellum throughout different phases of visuomotor adaptation.

Experiment 1

Aim

To first address the issue that the cerebellum plays a role across all stages of PA, Experiment 1 evaluated whether cerebellar tDCS could modulate error correction and after-effect during PA by applying online cathodal stimulation. In line with the above theories on cerebellar functions and with recent neuroimaging evidence (Küper et al., 2014), it can be hypothesized that cerebellar tDCS is able to affect both error compensation, with incremented errors in early pointing movements on horizontal axis, and after-effect, with an alteration of pointing movements on horizontal axis after prisms

removal. On the vertical axis, a dimension not affected by the prism shift, no specific previsions can be made.

Materials and Method

Participants and experimental design

Twenty-six (16 females) students of University of Campania Luigi Vanvitelli (average age = 21.57, SD = 2.33) voluntary participated to Experiment 1.

All participants were naïve to the purpose of the study, had normal or corrected-to-normal vision, were right-handed and had no contraindications to tDCS. They were informed to be assigned to one of two stimulation conditions and that the tDCS was used to evaluate the involvement of the brain during a target task, then they gave their written informed consent to take part in the experiment. All procedures were in agreement with 1975 Helsinki Declaration and were approved by the Local Ethic Committee.

Participants were randomly divided in two groups of stimulation: 13 participants (8 females; average age = 21.61, SD = 2.53) were assigned to cathodal stimulation (ctDCS Group), while 13 participants (8 females; average age = 21.53, SD = 2.21) were assigned to the sham stimulation (Sham Group).

tDCS protocol

Stimulation was delivered by a battery-driven, constant current stimulator (BrainSTIM, EMS Medical, Italy) using two surface saline-soaked sponge electrodes (area=25cm^2). The intensity of stimulation was set at 2.0 mA. tDCS was turn-on 5 minutes before participants started PA and it was kept on during the whole task. Stimulation could last for a maximum of 21 minutes and it was planned to stop it as soon as participants completed the task or to exclude data from analysis if subjects did not complete the PA

procedure within the remaining stimulation time (16 minutes), to comply with safety guidelines (Nietzsche et al., 2003; Iyer et al., 2005). Following Pope and Miall's study (2012), cathodal electrode was placed on the right cerebellum, 1 cm below and 4 cm right to the inion, whereas anodal electrode was placed on the right deltoid muscle; this site slightly differed from that adopted by Galea et al. (2011), to ensure selective stimulation of the right cerebellum. Stimulation was delivered over the right cerebellum, since participants had to use their right hand to perform the task (Schlerf et al., 2014). Sham stimulation was performed in the same way as active stimulation but the stimulator was turned-off after 30s. This procedure ensured that participants felt the same itching sensation at the beginning of tDCS like participants assigned to the experimental group, and were thus blinded for the stimulation condition they had been assigned to (Gandiga et al., 2006).

PA Procedure

Both groups (ctDCS Group and Sham Group) performed a pointing task on a touch-sensitive screen before exposure to the prism goggles (Pre), while wearing goggles (Exposure), immediately after the removal of the prisms (Post 1) and 10 minutes after prisms removal (Post 2; Figure 1). The participant sat in front of a 17-inch screen at arm's reach distance. Participants were told to make pointing movements as fast and accurately as possible from a given starting position (the right hand placed on the desk) and then to return to the same position as soon as they had touched the target (Redding et al., 2002).

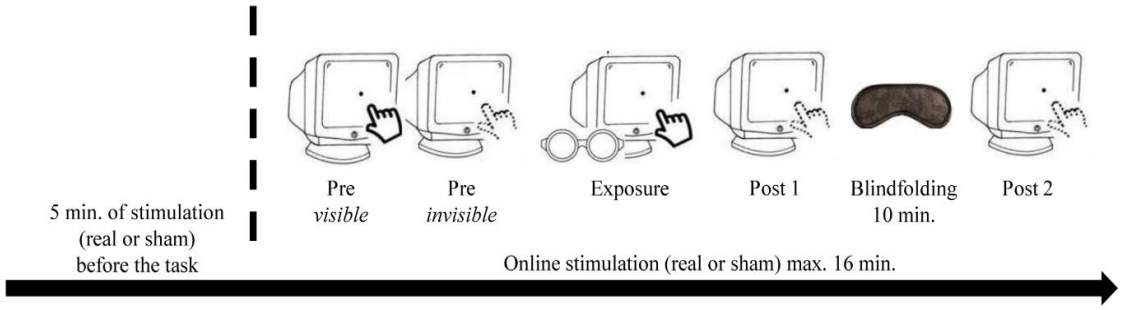

Figure 1. Participants underwent 5 minutes of stimulation (real or sham) before the execution of the pointing task; then received online stimulation during the whole task (16 minutes at most). The pointing task consisted to point at a target before wearing prism goggles (Pre visible and invisible), wearing prism glasses (Exposure), soon after (Post 1) and 10 minutes after (Post 2) their removal.

During the Pre phase participants were asked to point, with their right index finger, at 27 dots randomly presented, one at a time, in three positions on the screen (center, right, left). A custom-built wooden open box (28x52x28 cm), combined with a black cloth cape, was used to hide the proximal part of the arm, leaving individuals' hand and index finger on sight ('visible pointing' trials, first 9 trials), or to hide the whole arm during pointing movements ('invisible pointing' trials, remaining 18 trials). In both 'visible' and 'invisible' pointing movements the dot was always visible to participants, but in 'invisible pointing' movements participants were required to point to the dot under the top face of the wooden box (Figure 2).

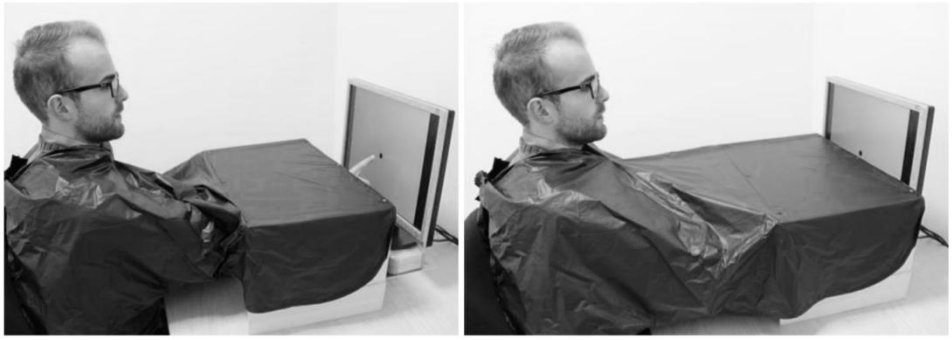

Figure 2. Illustration for visible pointing movements (on the left) and invisible pointing movements (on the right). During visible pointing movements participants could see their pointing finger, whereas in invisible pointing movements they could not see the movement's outcome.

During Exposure phase, 20 diopters left-based prismatic goggles were put on participants' eyes displacing the visual field about 11.3° to the right in one time (Single-step PA) and participants were asked to perform 90 'visible pointing' movements to allow full deployment of adaptation.

In Post 1 and Post 2 phases, after removal of prism glasses, participants performed 18 'invisible pointing' movements. During the 10 minutes between the two Post phases, participants were asked to stay sit and to keep the arm in the starting position, wearing a blindfold.

No feedback was provided to participants during invisible trials, so participants had no knowledge about the outcome of their pointing movements.

Errors were computed as the distance (in pixel; in Experiment 1, 34 pixels = 1 cm) between the point touched by the participant and the actual position of the target on the horizontal and vertical axes. Negative values (-) indicated leftward or downward deviations.

Data analysis: horizontal displacement

Error rates were computed for each task phase separately. To assess the time-course of the effect of cerebellar stimulation, all phases were divided in three bins of 30 trials each, and mean errors for the first, middle and last bin of each phase were computed.

Pointing movements performed with or without prism glasses were separately analyzed by means of two repeated measure analyses of variance (ANOVAs). To evaluate participants' ability to quickly adapt to the prism shift, a 3X2 ANOVA was performed on mean deviation on the horizontal axis from the target during Exposure phase (when participants wore prisms), considering the Time (First, Middle and Last trials) as a within-subject factor and the Group (ctDCS Group and Sham Group) as a between-subject factor. To evaluate development and duration of after-effect, a 3X3X2 ANOVA was carried out on mean deviation from the target on the horizontal axis, considering the Phase (Pre, Post 1 and Post 2) and the Time (First, Middle and Last trials) as within-subject factors and the Group (ctDCS Group and Sham Group) as a between-subject factor. Post hoc comparisons were performed by Bonferroni-corrected tests, with level of significance set at $p < 0.05$.

Data analysis: vertical displacement

To evaluate whether pointing accuracy changed on a dimension (y axis) not affected by any experimental manipulation, the same analyses presented above were performed on the mean vertical displacement as dependent variable.

Results

All participants completed the task within 16 minutes, so no subject was excluded from analyses.

Horizontal axis

Trial-by-trial errors in the two groups on horizontal axis are depicted in Figure 3A.

The 3X2 ANOVA on horizontal errors during Exposure phase revealed a significant main effect of Time [$F(2, 48)= 30.97$, $p< .001$, $\eta^2p= .56$], as errors were larger in First trials (M= 21.37, SE= 3.43) compared to Middle (M= 3.76, SE= 1.55) and Last trials (M= 2.55, SE= 1.62; all $p< .001$). This finding shows that participants progressively corrected the terminal error through successive pointing movements. A significant main effect of Group [$F(1, 24)= 18.99$, $p< .001$, $\eta^2p= .44$] was also found, as the ctDCS Group (M= 17.04, SE= 2.54) showed a greater rightward error compared to the Sham Group (M= 1.41, SE= 2.54; $p< .001$). Moreover, we found a significant interaction between Group and Time [$F(2, 48)= 4,41$, $p= .02$, $\eta^2p= .15$]. Bonferroni-corrected post hoc contrasts showed that the ctDCS Group made larger errors while wearing prisms in the First ($p= .01$), Middle ($p< .001$) and Last trials ($p= .01$) compared to the Sham Group (Figure 3B), demonstrating that the ctDCS Group experienced more difficulty in quickly compensate for the terminal error. Moreover, the ctDCS Group made significantly larger errors in First trials than in Middle and Last trials (all $p> .001$), while errors did not differ between Middle and Last trials ($p> .05$); in the Sham Group there was a significant difference in pointing accuracy only between First and Middle trials ($p= .04$), whereas difference between First and Last trials ($p= .09$) and between Middle and Last trials ($p> .05$) was not significant.

The 3X3X2 ANOVA on the horizontal errors in the three phases (Pre, Post 1, Post 2) indicated a significant main effect of Phase [$F(1, 24)= 105.75$, $p= .001$, $\eta^2p= .81$], as

participants showed significant leftward errors during Post 1 (M= -114.35, SE= 7.45) and Post 2 (M= -61.81, SE= 7.16) with respect to Pre (M= 23.82, SE= 4.51). This data shows that participants developed the prism after-effect, and that it was maintained following 10 minutes. A significant main effect of Time [F(1, 24)= 12.82, p= .01, η^2p= .35] was also revealed, as participants were more accurate during Last trials (M= -39.87, SE= 5.54) than in Middle trials (M= -52.96, SE= 4.72; p< .001) and in First trials (M= -59.50, SE= 5.17; p= .002; comparison Middle vs. First trials: p> .05). In addition, a significant main effect of Group [F(1, 24)= 6.94, p= .02, η^2p= .22] was found, as the ctDCS Group (M= -62.62, SE= 6.36) showed a greater leftward error compared to the Sham Group (M= -38.93, SE= 6.36; p= .02). Results also revealed a significant interaction between Phase and Group [F(1, 24)= 5.68, p= .03, η^2p= .19]. Bonferroni post hoc comparisons showed a greater leftward error in the ctDCS Group during Post 2 compared to the Sham Group (p= .01; Figure 3B). This finding reveals that cathodal stimulated participants were less able to compensate the after-effect and to re-adapt to the new condition of the visual field.

Interactions between Group and Time [F(2, 48)= .21, p= .81, η^2p= .01], between Phase and Time [F(4, 96)= .24, p= .92, η^2p= .01] and between Phase, Group and Time [F(4, 96)= .48, p=.75, η^2p= .02] were not significant.

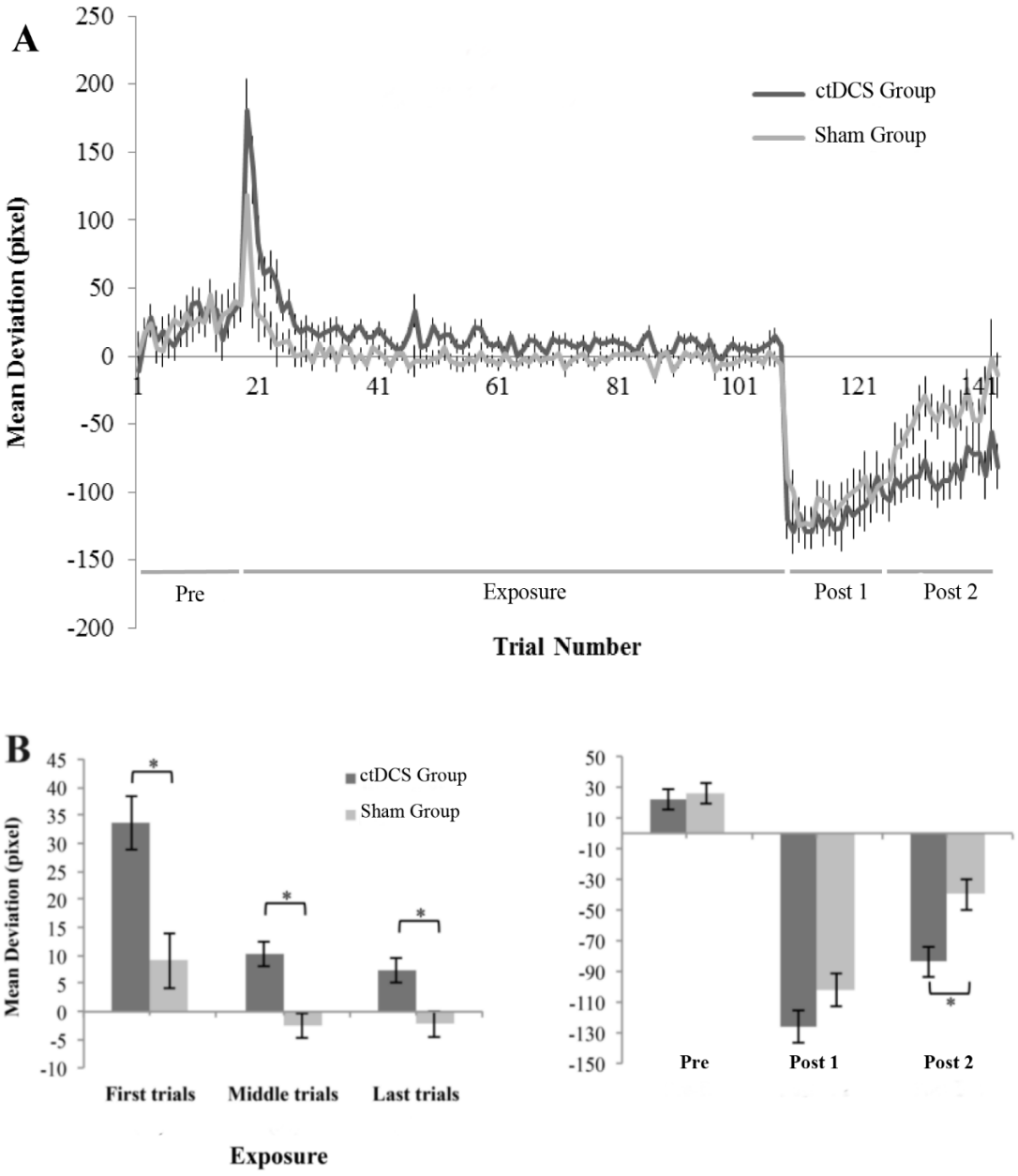

Figure 3. Panel A shows mean trial-by-trial errors on horizontal axis (in pixel) in the two groups during the task. Panel B: left, mean error on horizontal axis (in pixel) in the two subject groups during First, Middle and Last trials of the Exposure phase; right, mean error on horizontal axis (in pixel) in the two subject groups during Pre, Post 1 and Post 2 phases. *significant at $p < .05$.

Vertical axis

Mean trial-by-trial errors in the two groups on vertical axis are depicted in Figure 4A.

The 3X2 ANOVA on mean vertical deviation from the target during the Exposure phase revealed a significant main effect of Time [F(2, 48)= 8.04, p= .001, η^2p= .25], as participants pointed lower from the target position in the First trials (M= -28.27, SE= 2.5) compared to the Last trials (M= -21.15, SE= 2.07, p< .01), whereas performance on Middle (M= -22.78, SE= 1.82) and Last trials did not differed (p> .05). Main effect of Group [F(1, 24)= 3.18, p> .05, η^2p= .12] and the interaction between Time and Group [F(2, 48)= .35, p> .05, η^2p= .01] were not significant (Figure 4B). This pattern of results reflected the shift from the Pre, in which participants were required to point to the dot under the top face of the wooden box, to the Exposure phase, in which they were required to point strictly on the dot.

The 3X3X2 ANOVA on the vertical deviation from the target in the three phases (Pre, Post 1, Post 2) indicated a significant main effect of Phase [F(2, 48)= 7.41, p< .01, η^2p= .24], as participants deviated downward more in Pre (M= -228.29, SE= 17.24) than in Post 2 (M= -197.4, SE= 18.64; p=.01), while differences between Pre and Post 1 (M= -209.5, SE= 15.44) and between Post 1 and Post 2 were not significant (all p> .05). A significant main effect of Time [F(2, 48)= 32.19, p< .001, η^2p= .57] was also observed, as participants pointed closer to the target position on vertical axis in the First pointing movements compared to the Middle and Last ones (respectively: M= -185.1, SE= 14.47; M= -215.73, SE= 17.01; M= -234.3, SE= 18.91; all p<.01). The main effect of Group was not significant [F(1, 24)= .44, p>.05, η^2p= .03].

A significant interaction between Phase and Time [F(4, 96)= 9.27, p<.001, η^2p= .28] was also found, since accuracy on the vertical axis deteriorated from the First to the Last movements of each phase but improved across the different phases of the task. Bonferroni post hoc comparisons revealed that in the Pre phase accuracy was higher in the First (M= -197.95, SE= 16.62) than in the Middle (M= -232.89, SE= 17.56) and in the Last pointing

movements (M= -253.80, SE= 19.71), and the same pattern was observed for Post 1 phase (First trials: M= -170.72, SE= 12.15; Middle trials: M= -211.62, SE= 16.55; Last trials: M= -246, SE= 19.99; all p< .05). A similar pattern was also observed for the Post 2 phase, but in this case a significant difference was observed between the First (M= -186.61, SE= 17.20) and Middle trials (M= -202.68, SE= 19.34; p< .05) and between First and Last trials (M= -202.92, SE= 19.96; p< .05), but not between the Middle and Last trials (p> .05). Moreover, Bonferroni post hoc contrasts revealed that: in the First trials there was no significant difference between Pre, Post 1 and Post 2 phases (all p> .05); in the Middle trials accuracy was lower in the Pre than in the Post 2 (p< .05), without other significant differences between phases (p> .5); in the Last trials accuracy was significantly lower in Pre with respect to Post 2 and in Post 1 with respect to Post 2 (both p< .05).

We also found a significant interaction between Time and Group [F(2,48)= 4.62, p< .05, η^2p= .16]. Bonferroni post hoc comparisons showed that in the ctDCS Group accuracy on the vertical axis progressively decreased from the First pointing movements (M= -188.89, SE= 20.46) to the Middle (M= -228.57, SE= 24.04) and to the Last ones (M= -256.92, SE= 26.75; p< .01 for all comparisons), whereas participants in the Sham Group were more accurate in the First trials (M= -181.30, SE= 20.46), than in the Middle trials (M= -202.88, SE= 24.04) and in the Last trials (M= -211.67, SE= 26.75; all p< .05), without significant differences between the Middle and Last trials (p> .05). No significant difference was found between the ctDCS Group and the Sham Group in any bin (all p> .05).

The interactions between phase and group [F(2,48)= .36, p> .05, η^2p= .01; Figure 4B] and between Phase, Time and Group [F(4,96)= 1.41, p> .05, η^2p= .06] were not significant.

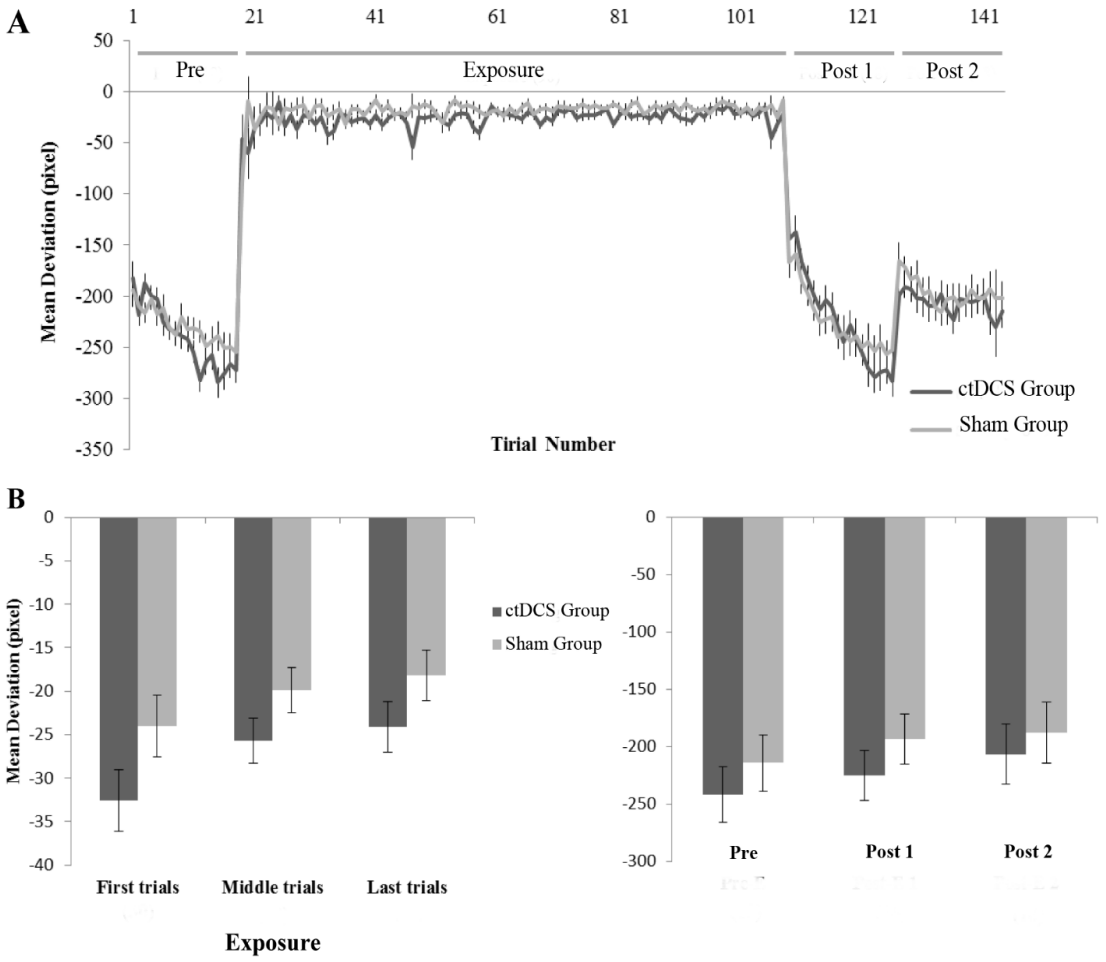

Figure 4. Panel A shows mean trial-by-trial deviations on vertical axis (in pixel) in the two groups during the task; number of trials for each phase is specified in parenthesis. Panel B: left, mean error on vertical axis (in pixel) in the two subject groups during First, Middle and Last trials of the Exposure phase; right, mean error on vertical axis (in pixel) in the two subject groups during Pre, Post 1 and Post 2 phases.

Comments

Experiment 1 was designed to assess the role of the cerebellum in the ability to adapt to prism lenses and in the development of after-effect by means of online cathodal cerebellar stimulation. On the horizontal dimension, real stimulated participants showed significant larger errors (to the right) during the First, Middle and Last trials of Exposure phase and significant larger errors (to the left) after prisms removal. On the vertical axis a different pattern of results was found, since in both groups accuracy on the vertical axis improved

through the different phases of the task, likely for a learning process, but deteriorated in successive movements within each task phase, likely due to fatigue. The effect related to cerebellar stimulation was detected in Post 2 phase only, whereas errors in Post 1 were comparably high in both real and sham stimulation groups. These findings can be accounted assuming that both ctDCS Group and Sham Group were comparable affected by the prisms, as suggested by the fact that both groups finally modified their motor programs, whereas the stimulated group compensated for this drift only more slowly than the Sham Group (i.e., they showed higher errors in the Post 2 phase).

Although the present tDCS experiment allows to demonstrate that the cerebellum plays a key role during all stages of PA, i.e. from Exposure to Post Exposure phases, it cannot elucidate the specific role of the cerebellum in recalibration and realignment because the experimental procedure implied here used a temporal criterion to disentangle these two processes. Experiment 2 and Experiment 3 combine cerebellar cathodal tDCS with specific procedures of PA aimed at experimentally isolating the contribution of recalibration and realignment to the pointing performance.

Experiment 2

Aim

Consistent with Kuper et al.'s findings (2014), Experiment 1 allowed to observe that interfering with cerebellar activity during PA can impair healthy participants' performance during all phases of the experimental procedure. These findings converge in suggesting that the cerebellum is activated since early trials of PA. However, the meaning of early cerebellar activation during PA remains to be clarified. In fact, it is possible to interpret this activation as the result of the involvement of the cerebellum in recalibration, or to hypostasize that realignment (thought to be directly related to cerebellar function)

takes place since early trials of exposure to prims. It is indeed possible that both recalibration and realignment, although different in nature, initiate their development during early trials of prism exposure. The traditional Single-step PA used in Experiment1, where participants are exposed to a full prismatic shift in one shot, does not allow to test these two alternative hypothesis because in this case recalibration and spatial realignment are only distinguished on the basis of time: recalibration is ascribed to early trials and spatial realignment to later trials of adaptation. To untangle the knot, it would be necessary to assess the effect of interference over the cerebellum in experimental conditions where only one of the two processes takes place and the other is completely eluded. The Multiple-step PA procedure (Michel et al., 2007) keeps participants unaware of the optical deviation by means of progressive stepwise increases from a no-shift condition to the full prism displacement. Since participants are not aware of the progressive displacement of the visual field, they are not in the position of using strategic processes for error correction (i.e., recalibration), and can only rely on the slow automatic process of spatial realignment. As a consequence, this procedure allows to isolate the process of spatial realignment from the process of recalibration and paradoxically in surface, it leads to stronger after-effects than during the single-step exposure (Michel et al., 2007).

Experiment 2 therefore was designed to ascertain the direct link between cerebellar activity and spatial realignment and to test whether the unconscious and automatic process of spatial realignment starts form the first trials of prism exposure. To this purpose tDCS was delivered during Multiple-step PA. We reasoned that if spatial realignment starts in an early phase of adaptation, inhibitory functional stimulation of the cerebellum should interfere with error compensation since the first trials of prism exposure and with

the magnitude of after-effect, that is the direct outcome of successful realignment (Redding et al., 2005).

Materials and Method

Participants and experimental design

Thirty-two right-handed students from University of Naples Luigi Vanvitelli (average age = 21.92, SD = 2.48, 20 females) voluntarily participated to this study. Participants had normal or corrected-to-normal vision and no contraindications to tDCS. They were naïve to the purposes of the study and they were included only if they had not previously participated to PA experiments and had no knowledge on PA.

Participants were then randomly divided in two stimulation groups: 16 participants were assigned to the ctDCS Group, while 16 participants were assigned to the Sham Group. Participants were informed that tDCS was used to evaluate the role of specific brain regions during a visuo-motor task, and gave their written informed consent to take part in the experiment. All the procedures of this experiment were in agreement with 1975 Helsinki Declaration and were approved by the Local Ethic Committee.

transcranial Direct Current Stimulation

Stimulation methodology and procedure were the same than in Experiment 1, with the only difference that in Experiment 2 tDCS was delivered exclusively during the Exposure phase.

Experimental Procedure

The experimental procedure of this study is depicted in Figure 5.

As in Experiment 1 a pointing task was performed on a 17-inc touch-sensitive screen before wearing prisms (Pre), during multiple-step PA (Exposure), and differently to Experiment 1, three times after exposure (Post 1; Deadaptation; Post 2). Participants were asked to point at rounded targets located to the left or to the right of the screen for Exposure and Deadaptation phases and to the center during Pre, Post 1 and Post 2 phases. Experiment 2 indeed used an unexposed target for after-effect evaluation. A wooden panel (25x50x20 cm) combined with a black cloth cape allowed to perform visible pointing movements (in Exposure and Deadaptation phases) or invisible pointing movements (all other phases). The whole procedure was performed in dim light and both the background of the touch screen and the wall were totally black to prevent the use of any cues which may lead to cognitive effects.

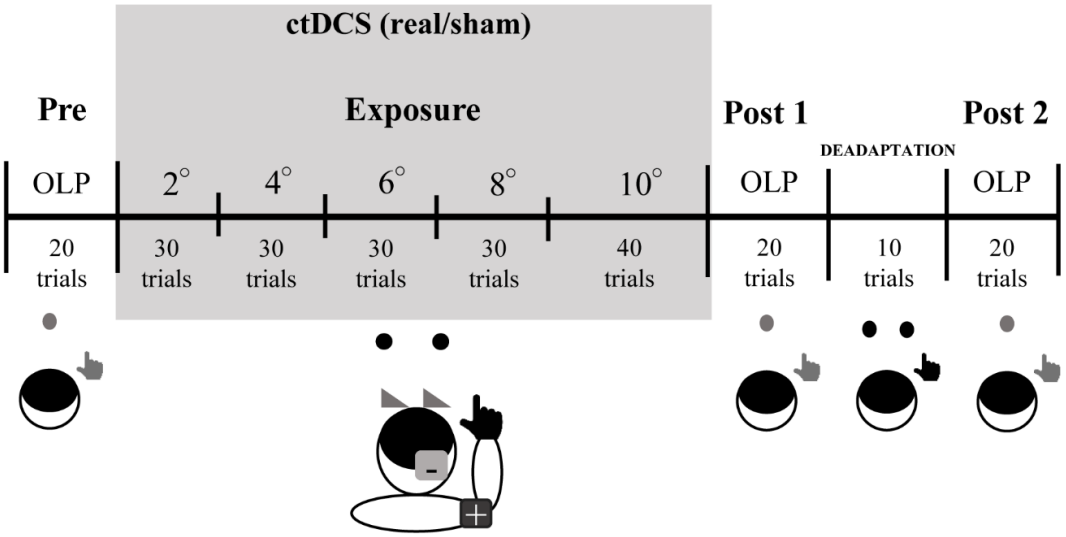

Figure 5. During Pre, Post 1 and Post 2 participants pointed at rounded target in central position (in grey), while during Exposure and Deadaptation phases they pointed at left or right located target (in black) in a random order. Cathodal or sham tDCS over the cerebellum was delivered during Exposure when participants wore rightward shifting prisms. Number of trials is indicated for each phase of the task. Gray hand indicates that participants could not see the pointing finger and the outcome of their movement, while black hand indicates availability of visual feedback.

Multiple-step PA

Multiple-step PA was performed using prisms that produced a progressive visual shift of 2°, 4°, 6°, 8°, and 10° (Michel et al., 2007). The wedge prisms were fitted into Cebe glacier goggles (optiquepeter.com, France) to induce a right displacement of the visual field. Black leather components on temporal and nasal portions of the goggles ensured that participants could not see any unshifted portion of the lateral visual field. The weight of the five pairs of goggles with different visual shifts was made identical by small pieces of lead on the goggles temple in order to reduce cognitive cues about changes in the prisms related, for example, to their weight. The pointing task involved a total of 160 pointing trials during Exposure phase. The subjects wore 2° deviating prisms until trial 30, 4° deviating prisms until trial 60, 6° deviating prisms until trial 90, 8° deviating prisms until trial 120, and 10° deviating prisms until trial 160 (the last stage of adaptation included more trials compared to all others to avoid that participants could implicitly anticipate a further progressive shift at the end of the series). Short breaks were made to change the goggles and during the change participants were asked to close their eyes and to not move the adapting arm. Participants were told to make random visible pointing movements to the left or right target as fast and accurately as possible from a given starting position (the right index placed on a felt pad stuck on the desk) and then to return to that position as soon as they had touched the target (Redding et al., 2002).

In the Pre, Post 1 and Post 2 participants performed an OLP task (20 trials) on the touch-sensitive screen. The OLP task consisted of invisible pointing movements from the starting position toward the unexposed central target, without visual feedback of the arm trajectory and of the outcome of the movement.

During active Deadaptation phase participants were asked to perform 10 random visible pointing movements to the right or left target without any visual distortion.

At the end of the experiment participants were asked to describe their experience about the glasses, such as what the glasses were used for, if they differed among each other and in which way they differed. Participants that became aware of the gradual prism deviation were then excluded, since the main requirement in this experiment was that participants were completely unaware of the prism shift and could not adopt a strategic process of error correction.

As in Experiment 1, errors were computed as the distance (in pixel) between the point touched by the participant and the actual position of the target on the horizontal axis (in Experiment 2: 22 pixels= 1 cm) with negative values (-) indicating leftward deviations.

Data analysis

Error rates on the horizontal axis for each task phase were computed separately. To directly compare errors as soon as the visual perturbation was induced, increased or removed, the first and last trials of each phase (3 trials each) were isolated. In fact, if spatial realignment is a precocious process, then the effect of its modulation should be evident in very early trials of the pointing behavior and quite slight in last trials due to the fact that healthy individual, in spite of the stimulation, are nevertheless able to develop adaptive processes of error correction. Data on vertical axis were not analyzed because the lack of any effect of cerebellar ctDCS on the unshifted axis from Experiment 1.

A preliminary 2X2 ANOVA was performed on errors during Pre phase considering Group (ctDCS Group and Sham Group) as a between-subject factor and Time (First and Last trials) as within-subject factors in order to exclude any difference in the two groups at the baseline.

A 5X2X2 ANOVA was performed on mean errors during Exposure phase (when participants wore progressive prisms), considering adaptation Stage (2°, 4°, 6°, 8°, and

10°) and Time (First and Last trials) as within-subject factors, and Group (ctDCS Group and Sham Group) as a between-subject factor.

To evaluate development and duration of after-effect, a 2X2X2 ANOVA was carried out on mean deviation from the target on the horizontal axis, considering Phase (Post 1 and Post 2) and Time (First and Last trials) as within-subject factors and Group (ctDCS Group and Sham Group) as a between-subject factor.

Differences in the Deadaptation process were tested by means of a 2X2 ANOVA on mean error with Time (First and Last trials) as a within-subject factor and Group (ctDCS Group and Sham Group) as a between-subject factor.

Post hoc comparisons were performed by Bonferroni-corrected tests, with level of significance set at $p < 0.05$.

Results

All participants completed Exposure phase within the stimulation time, so no one was excluded from analyses on this basis. Three participants in the ctDCS Group and 3 participants in the Sham Group noticed some changes in their visual field caused by prism glasses and were therefore excluded from the data analysis (e.g. "it seems that the glasses shift the visual field" or "my impression was that there was a distortion of my vision"). Thus the final sample for the analyses included 13 participants for the ctDCS Group (8 females; average age = 22.54, SD = 3.38) and 13 participants for the Sham Group (9 females; average age = 21.31, SD = .75).

ANOVA on errors during the Pre revealed no difference at the baseline between the two groups [Time: $F(1, 24) = .63$, $\eta^2p = .03$, $p = .43$; Group: $F(1, 24) = .02$, $\eta^2p = .001$, $p = .89$; Time and Group interaction: $F(1, 24) = .09$, $\eta^2p = .001$, $p = .76$; Figure 6].

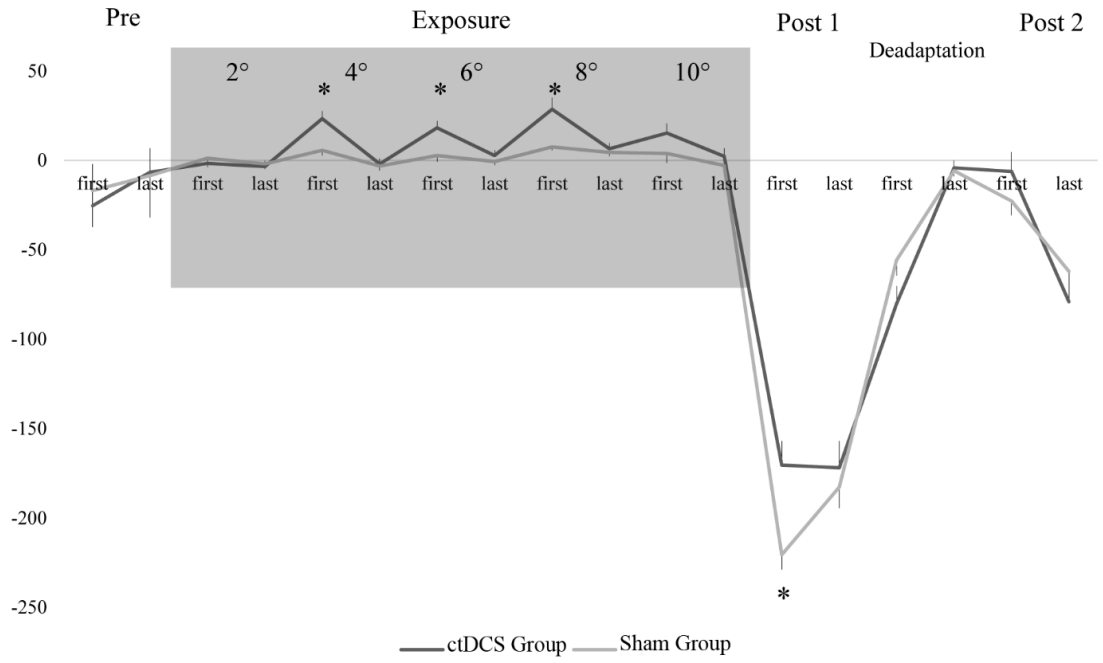

Figure 6. Mean error differences (in pixel) in the two subject groups during the several phases and stages of the task for the first and last trials. *significant at p< .05.

Trial-by-trial errors for the two groups during Exposure phase are depicted in Figure 7. Results from the ANOVA on mean errors in this phase revealed a significant main effect of Stage [F(4, 96)= 9.48, p<.001, η^2p=.28], because of a smaller prism rightward error during the first stage of adaptation compared to all others (all p< .05, Table 1), and of a smaller errors for 6° than for 8° stages (p< .01, Table 1). A significant main effect of Time [F(1, 24)= 39.57, p<.001, η^2p=.62], due to larger rightward error in the First trials compared to the Last trials (M= 10.46, SE= 1.83; M= .19, SE= 1.24) and a significant main effect of Group [F(1, 24)= 7.47, p=.012, η^2p=.24], with larger errors in the ctDCS Group compared to the Sham Group (M= 8.97, SE= 1.59; M= 1.67, SE= 1.09), were also found.

More interestingly, we observed a significant interaction between Time and Group [F(1, 24)= 10.17, p=.004, η^2p=.29], and a significant interaction between Stage and Group [F(4,

96)= 3.16, p=.017, η^2p=.12]. Bonferroni post-hoc contrasts revealed that although both the ctDCS Group and the Sham Group exhibited larger error in the First trials compared to the Last trials (p< 0.01, p= .038), the ctDCS Group showed an overall larger error compared to the Sham Group (p< .001) in the First trials with no significant difference in the Last trials (p= .41; Table 2). Crucially, post-hoc comparisons (Table 1) revealed that mean error was larger during the 4°, 6° and 8° adaptation stages in the ctDCS Group compared to the Sham Group (all p<.01, Figure 6). In the ctDCS Group the error in the first stage of adaptation (2°) was significantly lower than in the other stages (all p< .001), and in 6° with respect to 8° stage (p= .03), whereas in the Sham Group there was no difference across adaptation stages (all p>.05; Table 1).

Table 1. Exposure Phase

	Total (n= 26)		**ctDCS (n= 13)**		**Sham (n= 13)**	
	M	**SE**	**M**	**SE**	**M**	**SE**
2°	-1,47	2,59	-2,57	2,862	-0,37	2,86
4°	5,98	3,03	10,71	3,56	1,25	2,06
6°	5,74	2,61	10,43	2,86	1,05	2,01
8°	11,75	3,31	17,53	4,19	5,97	1,43
10°	4,61	3,57	8,76	3,492	0,46	3,49

Table 2. First and Last trials during Exposure Phase

	First trials		**Last trials**	
	M	**SE**	**M**	**SE**
ctDCS	16,72	2,46	1,24	1,52
Sham	4,21	1,73	-0,86	1,28

The interactions between Stage and Time [F(4, 96)= 1.87, p= .12, η^2p=.07] and between Stage, Time and Group [F(2, 48)= 1.14, p= .34, η^2p=.05] were not significant.

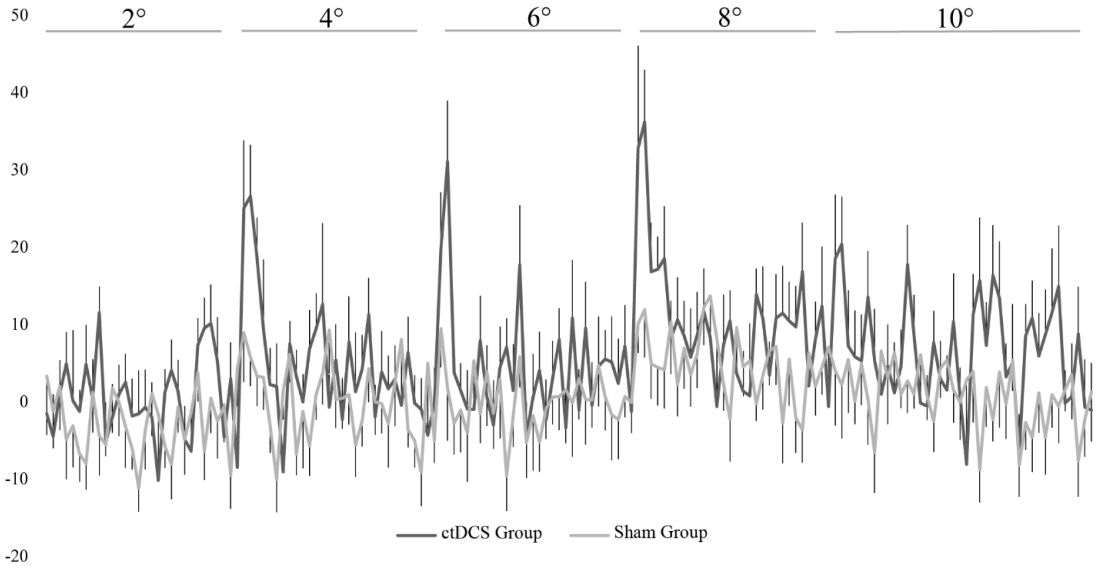

Figure 7. Mean trial-by-trial deviations (in pixel) for the two groups during the several stages of the multiple-step prism exposure.

The ANOVA on the after-effect revealed a significant main effect of Phase [F(1, 24)= 495.97, p< .001, η^2p=.95], as participants showed a larger leftward deviation (opposite to the prism shift) during Post 1 (M= -186.46, SE= 6.64) compared to Post 2 (M= -42,52, SE= 7.08), and a significant main effect of Time [F(1, 24)= 6.16, p=.02, η^2p=.20] as participants showed smaller after-effect in the First trials (M= -105.03, SE= 5.67) compared to the Last trials (M= -123.95, SE= 8.79).

A significant interaction between Phase and Time [F(1, 24)= 40.22, p< .001, η^2p=.63] was also found. Post-hoc contrasts showed that both in the First and Last trials the after-effect was larger during Post 1 (first: M= -195,59, SE= 7,93; last: M= -177,33, SE= 9,52) compared to Post 2 (first: M= -14,47, SE= 6,77; last: M= -70,56, SE= 9,78; all p<.001) and that in Post 2 the after-effect was significantly smaller in the First trials compared to the Last trials (p<.001; same comparison in Post 1: p= .09). Crucially significant interactions between Phase and Group [F(1, 24)= 5.66, p=.03, η^2p=.19] and between Time and Group [F(1, 24)= 5.71, p= .03, η^2p=.19] were found. Post-hoc contrasts (Table

3) revealed smaller after-effect in ctDCS Group compared to Sham Group during Post 1 (p= .04; Figure 2), and larger after-effect in Post 1 compared to Post 2 (all p<.001) in both groups. Post-hoc contrast for Time and Group interaction (Table 3) showed that the ctDCS exhibited a smaller after-effect in the First trials compared to the Sham Group (p<0.1).

Main effect of Group [F(1, 24)= 1.41, p= .25, η^2p=.05] and interaction between Phase, Time and Group [F(1, 24)= 55.05, p= .06, η^2p=.003] were not significant.

Table 3. After-effects

	Post 1		**Post 2**		**First trials**		**Last trials**	
	M	**SE**	**M**	**SE**	**M**	**SE**	**M**	**SE**
ctDCS	-171,24	12,27	-42,68	11,36	-88,39	18,49	-125,53	14,33
Sham	-201,68	7,21	-42,36	8,58	-121,67	20,59	-122,37	14,32

The ANOVA on errors during the Deadaptation phase only revealed a significant effect of Time [F(1, 24)= 92.86, p< .001, η^2p=.79], as participants exhibited a larger leftward deviation in the First trials (M= -68.06, SE= 6.88) compared to the Last trials (M= -4.97, SE= 2.56). The main effect of Group [F(1, 24)= 2.27, p= .14, η^2p=.08] and the interaction between Time and Group [F(1, 24)= 3.95, p= .058, η^2p=.14] were not significant.

For a detailed description of the error curves, Figure 8 depicts trial-by-trial errors in the two groups during Post 1, Deadaptation and Post 2.

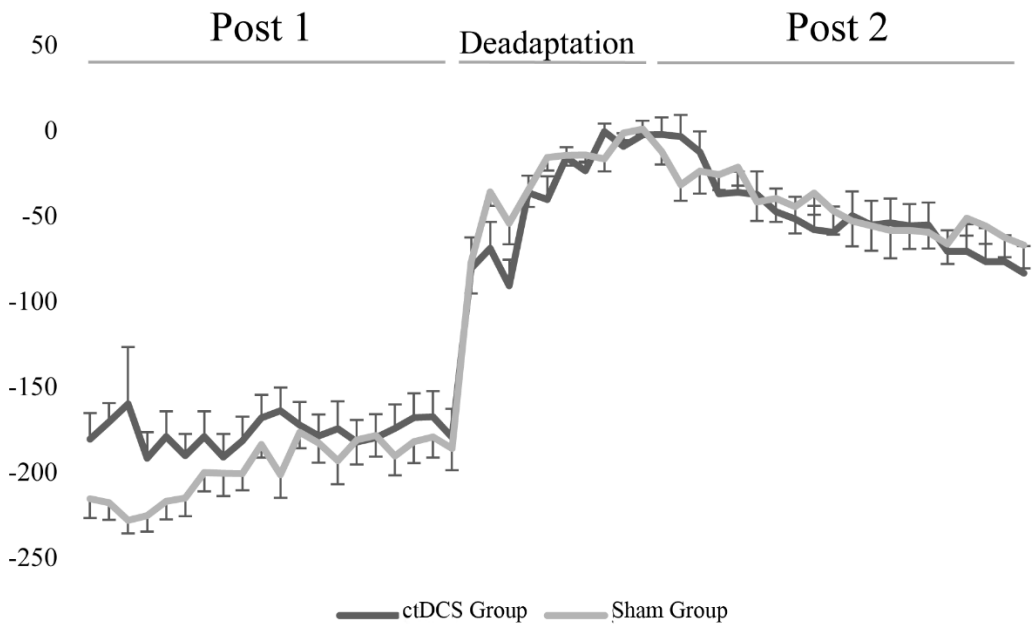

Figure 8. Mean trial-by-trial deviations (in pixel) for the two groups during Post 1, Deadaptation and Post 2.

Comments

Experiment 2 aimed at studying the effect of inhibitory brain stimulation on the cerebellum in a PA paradigm (Multiple-step Pa; Michel et al., 2007) where only spatial realignment was deployed. The results showed that the Sham Group exhibited negligible errors during the several stages of Exposure, a significant after-effect in Post 1, a significant reduction of errors from the first to the last trials in Deadaptation for the presence of true visual feedback, and a late reappearance of the after-effect at the end of Post 2 (when hands were not on sight) showing the robustness of adaptation. This pattern of results is in line with findings from Michel et al. (2007) showing that, notwithstanding the lack of prismatic errors during Exposure, the multiple-step exposure to wedge prisms leads to a significant after-effect even higher than that observed following single-step PA.

With respect to the sham group, the ctDCS Group showed a similar pattern of results during Deadaptation and Post 2, but it crucially exhibited a significant larger rightward deviation during the central stages of Exposure phase (4°, 6° and 8°), and a reduced after-effect during Post 1. The larger rightward deviation was more evident in the central stages of Exposure Phase probably due to the fact that: i) when participants were exposed to a 2° deviation of the visual field, the induced shift was too small to detect any difference compared to a no-shift condition, and ii) when participants were exposed to a 10° deviation, in the latest stage of adaptation, the visuo-motor system had become progressively able to correct for the prism shift. Anyway the inspection of the two curves depicted in Figure 7 reveals that trial by trial errors were generally larger in the ctDCS Group compared to the Sham Group during all stages of adaptation.

The finding of significant larger errors during the First trials of adaptation would suggest a role of the cerebellum in an automatic process of error detection needed to provide a signal to the motor system to develop error correction. This correction, as stated before, can be achieved both by means of strategic calibration and spatial realignment (leading to true adaptation). However, the task used in this experiment allowed to study spatial realignment alone, isolated from any conscious process of strategic calibration. As a consequence, any effect of cerebellar stimulation during Exposure Phase and in Post evaluations is likely to be related to the role of the cerebellum in spatial realignment.

Although the present study demonstrated very early development of realignment during PA, it was not designed to test whether the cerebellum is also involved in the mechanism of strategic recalibration, that was purposefully eluded by the experimental paradigm adopted here. To address this issue, it would be necessary to modulate cerebellar activity in conditions where the process of recalibration is kept active during PA. Reversing PA procedure (Clower et al, 1996), in which the optical deviation is reversed or removed after

a few trials, thus keeping participants in a constant state of ongoing recalibration without triggering adaptation, could provide evidence about the possible role of the cerebellum in this mechanism and represents the aim of Experiment 3.

Experiment 3

Aim

The aim of Experiment 3 was to assess possible cerebellar involvement in recalibration. To this purpose cerebellar stimulation was delivered while participants performed rapid pointing movements toward visual targets wearing prism goggles with different prism power and directional shift (-12°, -8°, 0°, +8°, +12°). This procedure, i.e. the Reversing PA procedure (Clower et al., 1996), keeps participants in an ongoing process of error compensation and prevents full adaptation, allowing to study the process of recalibration isolated from spatial realignment. To further assess cerebellar contribution to recalibration, kinematic recordings were obtained.

The study of recalibration isolated from spatial realignment by means of the Reversing PA task, would be confirmed by a complete lack of after-effect following exposure to prism. On the adaptive mechanisms, if the cerebellum is involved in the process of recalibration, functional cathodal transcranial Direct Current Stimulation should affect participants' pointing behavior during exposure to prim glasses with an overall larger deviation compared to not stimulated participants.

Materials and Method

Participants and experimental design

Eighteen healthy subjects from University Claude Bernard (Lyon, France) were recruited to participate to this experiment. Participants were selected from the university network and received money for their participation. All of them were naïve to the purpose of the study, had normal or corrected-to-normal vision, were right-handed and had no contraindications to the use of tDCS. Before inclusion they gave their written informed consent. All procedures were in agreement with 1975 Helsinki Declaration and approved by the Local Ethic Committee. Participants were then randomly divided in two groups: the ctDCS Group (10 participants) received real cathodal stimulation of the right cerebellum while the Sham Group (8 participants) received sham stimulation of the cerebellum.

Reversing PA task

The pointing task was performed on a pointing table before wearing prisms (Pre), during Reversing PA (Exposure) and immediately after Exposure phase (Post). Participants were asked to point at two targets located to the left or to the right of the table during Exposure phase and to a central target during Pre and Post phases. The target distance was 57 cm from eye-level. Participants had their head position fixed by a chinrest and their right index finger in the starting position, pressing a switch located to the base of the chinrest and aligned to their body axis. The chinrest also allowed to occlude the starting hand position and to prevent vision of the early part of participants' pointing movement. The trial started when participants left the starting position releasing the switch, and ended when participants touched on the table.

During Pre and Post phases, OLP movements (n=10) and SA measures (n=10) were collected. OLP involved pointing movements to the central target with a comfortable speed and participants could only view the target at the start of each trial but then vision was occluded during the reach. To prevent vision shutter goggles were used during the OLP task that occluded vision as soon as participants released the switch to perform the movement. SA measures consisted in straight-ahead pointing movements without vision. This task was performed in total darkness with vision occluded by the shutter goggles. Participants were asked to point straight ahead in front of them touching on the table.

During Exposure phase participants performed the Reversing PA task using prism glasses producing a visual shift of different magnitude (-12°, -8°, 0°, +8°, +12°) to the left or to the right. The weight of the goggles was made identical by small pieces of lead on the goggles temple to prevent cognitive cues in participants. The pointing task involved a total of 60 closed loop pointing trials during Exposure phase. The five pairs of glasses were randomly changed after blocks of 3, 4 or 5 trials. Specifically, participants performed 12 trials wearing each pair of glasses with one block of 3 trials, one block of 4 trials and one block of 5 trials for each pair of glasses. Short breaks were made to change the goggles and participants were asked to close their eyes and to not move the adapting arm during the change. Participants were told to make random visible pointing movements to the left or right target as fast and accurately as possible from the starting position (right index pressing the switch), to stay one second on the touched position and then to return to the starting position.

transcranial Direct Current Stimulation

Cerebellar stimulation was delivered exclusively during Exposure phase using the same protocol of stimulation described for Experiment 1 and Experiment 2.

Kinematic recordings

Reach kinematics were recorded on each trial of the experiment using an ultrasound emitter attached to the index finger (Motion Analysis, 100Hz, United States). Finger position signals were low-pass filtered at 6 Hz with a second-order Butterworth dual-pass filter. Movement velocity was computed from the filtered position signal using a least squares second-order polynomial method (5 point moving window). The same method was used to compute the acceleration of the finger from the velocity signal. The onset and endpoint of each reaching movement on each trial was computed automatically (using in-house custom software written in Matlab). Movements were detected using the following thresholds: onset was defined as the point at which hand velocity and acceleration exceeded 80 mm/s and 150mm/s2, respectively, while offset was defined as the time-point at which hand velocity and acceleration dropped below the respective thresholds. After automatic detection, all trials were cross-checked visually, and movement onset and offset points were adjusted manually where necessary. For the sake of clarity, the small curvature observed with respect to the vertical (z) axis was omitted from the present analyses, and only the projections of the hand trajectories in the X–Y pointing plane were considered.

Data analysis

Kinematic parameters and errors were analyzed to test for changes as a function of the stimulation groups by means of repeated measures analyses of variance (ANOVAs).

Analysis of closed loop pointing movements during Exposure phase focused on characterizing the possible change in endpoint errors and kinematic measures that occurred while wearing the different goggles during Exposure phase. To this purpose two separate 5X2 ANOVA on errors and the kinematic parameters were performed

considering the Prism (-12°, -8°, 0°, +8°, +12°) as within group factor and the Group (tDCS Group vs Sham Group) as between group factor.

2X2 ANOVAs on main errors before and after Exposure phase was performed considering the Phase (Pre vs Post) as a within group factor and the Group (tDCS Group vs Sham Group) as between group factor on the SA and the OLP measures. The comparison of these measures before and after Exposure phase was used to assess after-effect.

Planned comparisons with level of significance set at $p< 0.05$ were performed to address specific questions on the presence of any difference in the two groups of stimulation.

Results

Analyses on errors during Exposure phase revealed no difference on the terminal error in the two groups of participants. Indeed, the 5X2 ANOVA on the terminal error during Exposure phase revealed a significant main effect of the Prism [$F(4, 64)=151.01$, $p<.001$, $\eta^2p=.9$] as participants exhibited different errors in response to the several prism powers (all comparisons: $p<.001$; Table 4), while no main effect of Group [$F(1, 16)=.06$, $p=.81$, $\eta^2p=.004$] and PrismXGroup interaction [$F(4, 64)=.54$, $p<.71$, $\eta^2p=.03$] were found.

Table 4. Main errors in response to the several prism glasses.

	Error (n= 18)	
	M	**SE**
-12°	-40.95	5.01
-8°	-17.76	3.36
0°	10.01	3.91
+8°	59.67	7.72
+12°	77.46	7.69

Analyses on the kinematic parameters supported the lack of any difference in the two groups of participants during Exposure Phase and are reported in Table 5.

Table 5. Statistical analyses on kinematic measures.

	Prism		Group		PrismXGroup	
	F	**p value**	**F**	**p value**	**F**	**p value**
MT	F(4,64)=11.82	p<.001	F(1,16)=.23	p=.64	F(4,64)=.81	p=.53
PA	F(4,64)=4.26	p=.004	F(1,16)=1.52	p=.24	F(4,64)=.45	p=.77
PV	F(4,64)=7.66	p<.001	F(1,16)=.92	p=.35	F(4,64)=.54	p=.71
PD	F(4,64)=2.26	p=.07	F(1,16)=.16	p=.69	F(4,64)=.88	p=.48
TPA	F(4,64)=2.77	p=.04	F(1,16)=.23	p=.64	F(4,64)=.70	p=.59
TPV	F(4,64)=9.27	p<.001	F(1,16)=1.26	p=.28	F(4,64)=.30	p=.87
TPD	F(4,64)=14.85	p<.001	F(1,16)=.19	p=.67	F(4,64)=.26	p=.90

MT, movement time (ms); **PA**, peak acceleration (mm/ms^2); **PV**, peak velocity (mm/ms^2); **PD**, peak deceleration (mm/ms^2); **TPA**, time to PA (ms); **TPV**, time to PV (ms); **TPD**, time to PD (ms).

Analyses on after-effect showed that participants did not develop after-effect following Reversing PA task. Indeed, the ANOVA on errors in the SA task revealed no main effect of Phase [F(1, 16)=2.53, p=.13, η^2p=.14], no main effect of Group [F(1, 16)= .19, p=.66, η^2p=.01] and no interaction between Phase and Group [F(1, 16)=.19, p=.68, η^2p=.01]. The ANOVA on the OLP task showed converging results with a lack of any Phase [F(1, 16)=2.32, p=.15, η^2p=.13], Group [F(1, 16)= .76, p=.39, η^2p=.05] or PhaseXGroup [F(1, 16)=.01, p=.93, η^2p=.00] effects.

Comments

The aim of Experiment 3 was to ascertain the contribution of the cerebellum in the process of recalibration. To this purpose we combined inhibitory functional stimulation with a PA task (Reversing PA) that kept participants in an ongoing process of recalibration of motor commands thus avoiding the development of the adaptive process of spatial realignment. Results from Experiment 3 reasonably showed that participants exhibited different error

magnitudes in response to the different shift of the visual field used during Reversing PA and, as expected, they did not develop any after-effect. This latter finding was due to the frequent change of the visual displacement during Exposure phase after a few trials that, as expected, made participants unable to develop full adaptation. Moreover, no significant difference was found in Pre and Post measures in the two groups of participants and, crucially to the purpose of the present experiment, no significant difference between the tDCS Group and the Sham Group was found during Exposure Phase. As a consequence, these findings did not support the involvement of the cerebellum in the process of recalibration. The findings on kinematic measures contribute to corroborate this evidence.

General Discussion and Conclusions

The present chapter described three tDCS experiments focusing on the contribution of the cerebellum in the adaptive processes developed during PA in order to clarify the contrasting evidence provided by neuroimaging studies (Luauté et al., 2009; Kuper et al., 2014).

Experiment 1 used a temporal criterion to distinguish recalibration and spatial realignment while Experiment 2 and Experiment 3 used specific tasks to isolate these two processes. The rationale of Experiment 2 and Experiment 3 was to avoid the development of one of the two target processes and to permit the sole employment of the other.

Results from Experiment 1 demonstrated that online cathodal tDCS was effective in modulating cerebellar functions and affected participants' performance during all stages of Single-step PA. Specifically, this experiment confirmed the involvement of cerebellum in early prism exposure, as shown by larger errors since the first trials of Exposure phase and confirm an effect of cerebellar cathodal stimulation on later stages of adaptation, as

shown by larger errors during the middle and last part of Exposure phase and in the Post. Results from Experiment 1 also complement Galea et al.'s findings (2011) by showing that: i) it is possible to decrease, and not only to enhance, cerebellar functioning using tDCS, and ii) the cerebellum is implied not only in learning but also in remodeling newly acquired sensorimotor transformations. In fact, while Galea et al. (2011) reported that the cerebellum was exclusively implied in the acquisition of a visuomotor transformation, data from Experiment 1 show that the cerebellum is implicated also in the retention of the newly acquired motor correction. The differences between the experimental design employed could account for the different result on the role of the cerebellum in retention. In their study, Gelea et al. (2011) delivered tDCS during the Pre Exposure and Adaptation phase with Post Exposure evaluations performed after the end of stimulation, whereas in Experiment 1 stimulation was applied during the entire task. Consequently, it has been possible to detect the direct effect of cerebellar stimulation in the Post Exposure too, whereas the absence of stimulation during Post Exposure evaluations (Galea at al., 2011) probably hindered the effect of cerebellar stimulation in the late phase of the task.

Experiment 2 studied spatial realignment isolated from recalibration using Multiple-step PA. It provided causal demonstration that the cerebellum is implied in spatial realignment and that the mechanism of spatial realignment, traditionally thought to be a later process, develops in very early stage of PA. Findings from Experiment 2 represent the first causal demonstration of the link between the functioning of the cerebellum and genuine realignment in PA, and evidence that spatial realignment is initiated from the earliest stage of prism exposure and is not confined to later adaptation phase. Moreover, results from Experiment 2 are in keeping with previous observations from Experiment 1 and Kuper et al. (2014) suggesting that the contribution of the cerebellum during PA may not

be restricted to late adaptation phases, clarifying the controversial correlation between the activity of the cerebellum and the ongoing adaptive processes of visuo-motor adaptation. Experiment 3 allowed to ascertain whether the process of recalibration relies on cerebellar functioning using a reversing PA task. The process of spatial realignment was indeed eluded in favor of strategic calibration. Measures of terminal error and kinematic parameters were jointly used to ascertain cerebellar contribution to recalibration. Results from Experiment 3 converge in suggesting the lack of any effect of cerebellar stimulation on the pointing performance during PA. In fact, no significant effects were found in the investigated measures.

Taken together evidence provided in these experiments suggest that the cerebellum is implicated during all stages of PA and that the meaning of early cerebellar activation during PA is due to very early development of spatial realignment. These results extend the classical models of PA (Redding et al., 2005) showing that the process of spatial realignment, that was supposed to develop later during prims adaptation, is active from a very early stage and affects pointing performance since early trials of adaptation.

The findings on cerebellar stimulation reported in Experiment 1 and Experiment 2 are in line with previous neuropsychological studies reporting a cerebellar contribution in motor adjustment (Martin et al., 1996; Werner et al., 2010; Norris et al., 2011). For instance, Norris et al. (2011) reported a transient greater deviation of reaching movements to a target in the direction of prismatic displacement after lidocaine injections in cerebellar cortex of rhesus monkeys. The role of the cerebellum in visuomotor adaptation and after-effect development has been also demonstrated in humans with ischemic lesions of the superior cerebellar artery who showed larger errors than controls in a visuomotor adaptation task, requiring reaching movements in conditions of 60° rotation of visual field (Werner et al., 2010). Similarly, Martin et al. (1996) reported that patients with damage

of the cerebellum, or of connected areas, showed an impaired adaptation ability and, in some cases, a missing after-effect in a task in which they had to launch balls to a visual target while wearing prism goggles. In Experiment 1 participants from the ctDCS group developed a wider after-effect with respect to the participants of the sham group. These data apparently diverge from those reported by Martin et al. (1996). However, as suggested by Frassinetti et al. (2002), a complete lack of after-effect might be expected when full adaptation is not deployed at all. In Experiment 1, adaptation was not heavily impaired but only slowed down, and this would likely explain why an after-effect was found in conditions of interfered adaptation. It is important to underline that in Experiment 1 online cerebellar stimulation during all phases of the experimental procedure resulted in a larger error during the whole Exposure phase and then a larger after-effect with respect to the sham stimulation. In Experiment 2, using cerebellar stimulation during the multiple-step exposure only, a *smaller* after-effect in the group receiving active stimulation than in the control group was observed. These contrasting findings can be possibly explained by the fact that in the Experiment 2 stimulation was specifically delivered during Exposure to prims and after-effect was evaluated after the stimulation ended. However, it seems likely that the different adaptation procedures (Single-step vs Multiple-step) allowed to tap different aspects of the role of the cerebellum during PA: in single-step PA of Experiment 1 the task permitted to pick the role of the cerebellum in achieving flexible motor adjustments in response to sudden and consciously noticed changes in the visual environment, whereas in Experiment 2 multiple-step PA allowed to explore the automatic mechanism of spatial realignment – or true adaptation - and its development.

Experiments reported in the present chapter all targeted a single region in the brain, i.e. the right cerebellum. These experiments did not consider the contribution of the PPC and

especially the contribution of the crossed circuitry between the cerebellum and the PPC. Anatomical studied report tick connections between such structures that would be provided by cortico-ponto-cerebellar pathways (Brodal and Bjaalie, 1997) that link motor and premotor areas, associative prefrontal areas, and associative posterior parietal areas with the cerebellum via pontine nuclei. By these connections, the cerebellum participates to the multiple mechanisms that allow online motor adjustments (Manto et al., 2012) and the same connections could be responsible of error correction and after-effect development during PA. The study of the circuitry that links the cerebellum and the PPC is the object of the experiment described in the following chapter.

Chapter 3
Posterior-parietal and cerebellar circuitry underlying error correction during Prism Adaptation

Introduction

Previous models on the mechanisms of PA (Redding et al., 1997; 2002; 2005) as well as the research flowing from this conceptualization (e.g. Luautè et al., 2009; Kuper et al., 2014; and the experimental series presented in Chapter 2) are based on the idea that these processes rely on distinct and isolated areas in the brain. In the attempt to look at the neural correlates of PA it has to be also explored the possibility that the mechanisms of error correction and adaptation are ensured by the activity of a brain circuit or a network of regions that all together, as one, contribute to all behavioral and cognitive manifestations of PA rather than isolated areas. In this light, from a theoretical level, the firm distinction between recalibration, that achieves error correction, and spatial realignment, that allows the development of after-effect, would lose its meaning and would leave the place to a dynamic view of interconnected areas that affect behavior and cognition by means of a fast, thick and continuous information flow. From a functional level as well, the rigid correspondence that links the PPC to the mechanism of recalibration and the cerebellum to the process of spatial realignment will not be supported anymore. Data from healthy participants presented in the previous chapter could be interpreted as a possible evidence of a brain circuitry in which the constitutive brain areas are involved at the same time in the same processes with no functional specialization. On the other hand, classical data on patients reporting that the ability to adapt to prisms remains with intact cerebellum and damaged PPC (Pisella et al., 2004), while adaptation to prisms is lost with damaged cerebellum and intact PPC (Martin et al., 1996; Weiner et al., 1983), could be interpreted as the consequence of an impairment of the circuits that underlie PA instead of the consequence of impaired functioning of a single brain structure.

Experiment 4: aim

Aim of Experiment 4 was to ascertain whether error correction during PA is achieved by means of a circuitry connecting the PPC and the cerebellum rather than isolated functioning of these two areas. To test this hypothesis bi-cephalic tDCS was delivered simultaneously on the left PPC and the right cerebellum while healthy participants performed Single-step PA using their right arm. Three groups of stimulation were tested to assess this hypothesis: active stimulation of the PPC combined to inhibitory stimulation of the cerebellum (aPPC_cCb Group), inhibitory stimulation of the PPC combined to active stimulation of the cerebellum (cPPC_aCb Group), sham stimulation (Sham Group). Two alternative predictions can be made on the results. If error correction is achieved by means of a circuit that links the PPC and the cerebellum with no functional specialization of these single structures, it is possible to expect the same pattern of results in the two stimulations groups (i.e. no difference at all in the aPPC_cCb Group and the cPPC_aCb Group in error compensation that would both differ from the Sham Group), whereas if there is a functional specialization of these areas a characteristic pattern of results can be expected in the stimulated groups, e.g. error correction could be impaired only in the cPPC_aCb Group, that would differ from both the aPPC_cCb Group and the Sham Group.

Materials and Method

Participants and experimental design

Forty-five (30 females) students of University of Campania Luigi Vanvitelli (average age = 22, SD = 2.3) voluntary participated to this Experiment.

All had normal or corrected-to-normal vision, were right-handed and had no contraindication to tDCS. They were naïve to the purpose of the study, and they were only informed to be assigned to one of three stimulation conditions aimed at evaluating

the involvement of different brain regions during a pointing task. Participants gave their written informed consent to take part in the experiment and all procedures were in agreement with 1975 Helsinki Declaration and approved by the Local Ethic Committee. Participants were finally divided in three groups of stimulation on a random basis: 15 (10 females) participants were assigned to the aPPC_cCb Group, 15 (10 females) participants were assigned to the cPPC_aCb and 15 (10 females) participants were assigned to the Sham Group.

tDCS protocol

Stimulation methodology, parameters and procedure were overall the same as in Experiment 1 described in Chapter 2. The only difference was related to the stimulation montage and to electrodes placement to target the PPC. In this experiment a bi-cephalic stimulation was used instead of the mono-cephalic stimulation of the previously described experiments. The electrode to target the PPC was located on P3 of the extended International 10-20 system for EEG electrode placement, while cerebellar electrode was placed 1 cm below and 4 cm right to the inion (like in the previous experiments).

PA procedure

The Single-step PA procedure and setting were similar to those followed in Experiment 1 of Chapter 1. In this experiment, three groups of participants (aPPC_cCb Group, cPPC_aCb Group and Sham Group) performed a pointing task on a 17-inch touch-sensitive screen before prism exposure (Pre), during exposure to 10° rightward deviating glasses (Exposure), immediately after and 10 minutes after their removal (Post 1 and Post 2). Participants had their chin on a chinrest and were asked to make invisible pointing movements (n= 18) from a given starting position (on the bottom of the chinrest) to a

central not exposed target during Pre, Post 1 and Post 2, and to make fast and accurate pointing movements (n=90) to a left or right target during Exposure.

Errors were computed as the distance in pixel (22 pixels= 1 cm) between the point touched by participants and the actual position of the target on the horizontal axis with negative values (-) indicating leftward deviations.

Data analysis

Errors rates on the horizontal axis for each task phase were computed separately. To compare errors as soon as participants took on or took off the glasses the first and last trials (n=5) of each phase were isolated.

A 2X3 ANOVA was performed on mean errors during Exposure phase considering the Time (First and Last trials) as within-subject factor and the Group (aPPC_cCb Group, cPPC_aCb Group and Sham Group) as a between-subject factor to detect possible differences in error compensation.

A 3X2X3 ANOVA was performed on errors considering the Phase (Pre, Post 1 and Post 2) and the Time (First trials, Last trials) as within-subject factors and the Group (aPPC_cCb Group, cPPC_aCb Group and Sham Group) as between-subject factor to exclude any difference in the baseline and to assess possible differences in after-effect.

Post hoc comparisons were performed by Bonferroni-corrected tests, with level of significance set at $p < 0.05$.

Results

Two participants (1 form the aPPC_cCb Group and 1 from the Sham Group) were removed from analyses due to a problem in the data acquisition.

The 2X3 ANOVA on mean error during Exposure phase revealed a significant main effect of Time [$F(1, 40)= 56.87$, $p<.001$, $\eta^2p=.6$], a significant main effect of Group [$F(2, 40)= 7.02$, $p<.01$, $\eta^2p=.26$] and a significant interaction between Time and Group [$F(2, 40)= 6.97$, $p<.01$, $\eta^2p=.26$]. Post hoc contrast revealed larger rightward errors in the First trials compared to the Last trials (First: M=63.03, SE=8.32; Last: M=5.96, SE=3.88; p<.001), larger rightward errors in the cPPC_aCb Group (M=55.8, SE= 8.92) compared to the Sham Group (M=8.3, SE= 9.24; p<.01) and a tendency to larger errors in the aPPC_cCb Group (M=39.39, SE=9.24) compared to the Sham Group (p=.067). Crucially post hoc contrast revealed larger errors in the First trials both in the aPPC_cCb Group (M=74.07, SE=14.57) and in the cPPC_aCb Group (M=97.73, SE=13.87) compared to the Sham Group (M=17.28, SE= 14.57; p=.026, p<.01) with no difference between the two real stimulation groups (p=.75). No difference was found in the three groups in the Last trials (aPPC_cCb Group: M=4.71, SE=6.80; cPPC_aCb Group: M=13.87, SE= 6.57; Sham Group: M=-.67; SE= 6.81; all p>.05). Moreover, while the main error in the First and Last trial in the aPPC_Cb Group and the cPPC_aCb Group significantly differed (both p<.001), in the Sham Group there was no significant difference in the errors during the First compared to the Last trials (p=.18).

The 3X2X3 ANOVA on main errors in the other phases of the task revealed a significant main effect of Phase with larger leftward errors during Post 1 (M=-187.11, SE=7.34; p<.001) and Post 2 (M=-158.57, SE= 8.63, p<.001) compared to Pre (M=-28-07, SE=9.96) and a larger leftward error in Post 1 compared to Post 2 (p<.001), confirming the development of the after-effect following PA and its decrease with time going from Post 1 to Post 2.

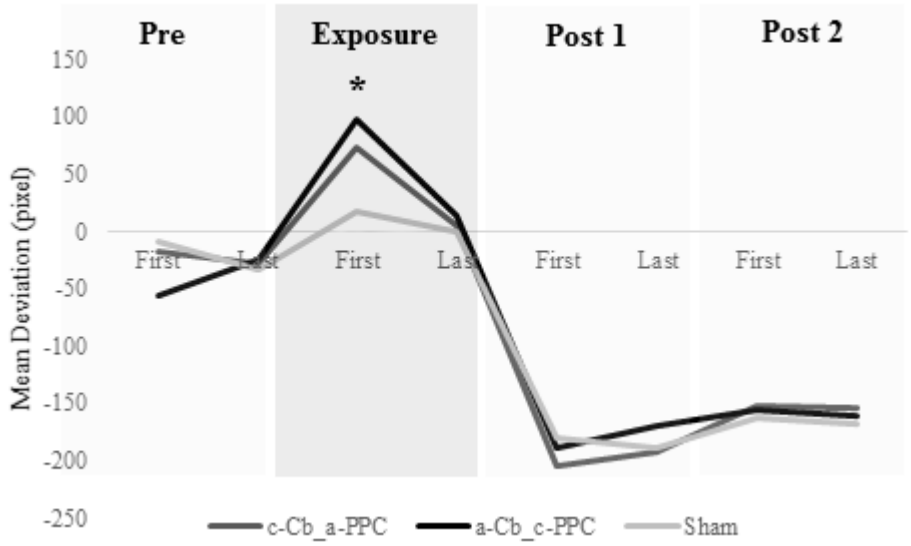

Figure 1. Mean error differences (in pixel) in the three groups of participants during the several phases of the task for the First and Last trials. *significant at p< .05.

Main effect of Time [F(1, 40)= .01, p=.92], Group [F(2, 40)=.01, p<.98, η^2p=.001], interactions between Phase and Group [F(4, 80)= .89, p=.48, η^2p=.04], Time and Group [F(2, 40)= 1.21, p=.31, η^2p=.06], Phase and Time [F(2, 80)= .51, p=.6, η^2p=.01] and Phase, Time and Group [F(4, 80)= 1.04, p=.39, η^2p=.05] were not significant.

Conclusions

Aim of the present study was to explore the possibility that the adaptive processes developed during PA are the manifestation of the involvement of a brain circuit connecting the target regions previously showed to be implicated in PA. The contrasting evidence from neuroimaging studies (for example Luauté et al., 2009 vs Kuper et al., 2014) could be explained hypothesizing that the mechanisms of recalibration and spatial realignment rely on a thick flow of information within several areas in the brain. This hypothesis would contrast the traditional view of a functional specialization of the adaptive processes of PA within distinct brain areas.

For this purpose, we implied functional bi-cephalic stimulation of the cerebellum and the PPC during PA comparing two complementary protocols of stimulation (aPPC_cCb Group and cPPC_aCb Group) and a group of sham stimulation (Sham Group). Results from our experiment showed that the three groups of participants manifested i) no difference in the baseline pointing performance (Pre), ii) a rightward deviation during exposure to wedge prisms (Exposure) and iii) a decreasing leftward error from the first and second after-effect measurement (Post 1 and Post 2). Most interestingly we found a significant group difference. Indeed, results showed that both groups of real stimulation (aPPC_cCb Group, cPPC_aCb Group) manifested an identical pointing performance wearing prisms, i.e. a larger rightward error in response to the prismatic displacement during the first pointing trials, compared to the control participants (Sham Group). No group difference was instead observed in the last trials of Exposure Phase and in Post Exposure measurements of the after-effect.

The finding of larger errors specifically in the first trials of Exposure phase in the two stimulation groups would suggest that the circuitry between the cerebellum and the PPC mainly reflect the process of recalibration and the consequent error compensation, while the lack of any effect of tDCS on the last trials of Exposure phase and after-effect measures cannot permit to extend the same conclusion to the process of spatial realignment. As a consequence, this experiment would suggest that although some mechanisms of PA can rely on a complex process of interaction between the PPC and the cerebellum, the process of recalibration and spatial realignment remain two distinct processes in their functional aspects. In other words, the present results can support the presence of a circuitry that links the PPC and the cerebellum to compensate for errors during PA but still support the theoretical distinction between recalibration and spatial realignment. To better understand weather the circuitry hypothesis can be extended to the

mechanism of spatial realignment, it would be necessary to use specific paradigms of PA such as the Multiple-step PA or to study clinical populations. In fact, the high level of functioning that characterize healthy participants can also limit the possibility to affect basic processes of motor function and flexibility.

The findings reported in the present study are compatible with some theoretical explanations to account for several evidence on patients with parietal and cerebellar lesions made by Pisella et al. (2005; 2006) and Newport et al. (2006). The hypothesis of a complex cerebro-cerebellar network has been previously claimed by Pisella et al. (2005) that studied a patient with a lesion to the cerebellum who showed adaptation to be limited to a rightward (not leftward) prism deviation, independent of the hand used during exposure. Since connections between the cerebellum and the cerebral cortex are crossed authors hypothesized the presence of a consistent cerebro-cerebellar lateralized network for the computation and integration of directional visual error in PA. The implication of such a lateralized network has been also hypothesized to explain the functional anatomy of the therapeutic effects of PA on neglect by Luauté et al. (2006) in which authors suggested that the clinical effect of PA was mediated by the modulation of cerebral areas in the left hemisphere via a bottom-up signal generated by the cerebellum. Also Newport et al. (2006) claimed a disconnectionist account for their findings in a patient with bilateral lesions to the PPC that was not able to adapt to a visual perturbation induced by the optical prisms with either hand within four times the number of trials required by healthy adult subjects. Authors interpreted the impairment in correcting the visual shift and the missing after-effect as the effect of a disconnection between the damaged PPC and the cerebellum that did not allow an updating of spatial coordinates. These interpretations are compatible with the anatomical organization of the brain. In fact, it has been previously demonstrated (Middleton et al., 2000; Clower et al., 2001; Dum et al.,

2003) that the temporal cortex, the frontal cortex and the PPC are target areas of the outputs from the cerebellum through a neuronal loop also implicating the dentate nucleus and subcortical structures, such as the thalamus and the globus pallidus.

In conclusion, the preliminary findings reported in the present study are compatible with the idea that at least some mechanisms (error compensation) developed during PA are the result of a continuous flow of information between the cerebellum and the PPC. The possibility to replicate these findings and to extend the circuitry hypothesis to the other adaptive processes of PA (such as after-effect development by spatial realignment) would imply an important updating of classical models of PA.

Chapter 4
Primary Motor Cortex contribution on after-effect reactivation and retention

The content of this chapter has been published as:

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Introduction

Adaptation to prism glasses represents a kind of motor learning in which participants learn to modify their motor programs in order to incorporate a displacement of the visual field. In this experimental task the learning process would be represented by full adaptation, e.g. the absence of errors during the last trials of exposure to prisms, and after-effect measures, i.e. the compensatory errors that participants classically show following prim exposure.

Several studies have demonstrated the possibility to ameliorate motor skill learning and motor adaptation in both healthy individuals and brain damaged patients. As it has been revised in Reis et al. (2012), tDCS has shown preliminary success in improving motor performance and motor learning in healthy individuals, and it has interestingly promoted restitution of motor deficits in stroke patients. For instance, there is strong evidence that timely co-application of (hand/arm) training and anodal tDCS to the contralateral M1 can improve motor learning and it has been reported by Galea et al. (2011) that anodal facilitatory tDCS of M1 is able to consolidate the retention of a newly acquired visuo-motor transformation (Galea et al., 2011). In the PA literature specifically, O'Shea et al. (2013) have recently shown that tDCS combined with PA is able to consolidate the prisms after-effect over a time-scale of several days.

Although very recent evidence has suggested the possibility to affect the acquisition and retention of a new visuo-motor transformation, no study has assessed the possibility to use neuromodulation to reactivate the adaptive mechanisms implied during PA and the consequent after-effect, that is the most accurate prove that adaptation occurred and that seems to be related to neglect amelioration (Rossetti et al., 1998).

Experiment 5: aim

Aim of the current study on healthy subjects is to ascertain the possibility to rebound the prism after-effect by means of tDCS delivered 24 hours after a combined session involving both PA and tDCS, and to test for retention 24 hours later. Given the above mentioned evidence related to the possibility to improve motor learning and retention in a visuo-motor task trough neuromodulation (Reis et al., 2011; Galea et al., 2011) and to enhance consolidation of prism after-effect (O'Shea et al., 2013), the purpose of this experiment is to test whether anodal facilitatory tDCS on M1 is able to reactivate the prisms' after-effect 24 hours later PA and to check for its retention 24 hours later.

Materials and methods

Participants and experimental design

Twenty healthy subjects from the Hospital Henry Gabrielle and University Claude Bernard (Lyon, France) randomly divided in two groups (Re-Activate Group, 10 participants; Control Group, 10 participants) were tested. All participants were naïve to the purpose of the study, had normal or corrected-to-normal vision, were right-handed and had no current or previous history of neurological or psychiatric diseases. All procedures were in agreement with 1975 Helsinki Declaration and approved by the Local Ethic Committee.

On Day1 and Day2 pre-test and post-test were used to measure after-effects of an intervention. On Day1 the intervention consisted in tDCS during PA. On Day2 participant received tDCS alone (no PA) to ascertain the possibility to reactivate the prism after-effect using neuromodulation (real tDCS in the Re-Activate Group and sham tDCS in the Control Group). To further assess the potential long lasting nature of the reactivated after-effect, a follow-up test was carried out after 24 hours (Day3; see Figure 1).

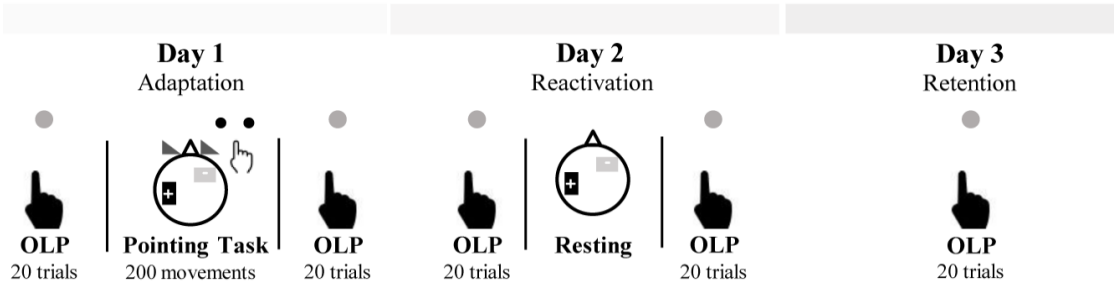

Figure 1. On Day1, participants performed a pointing task wearing prism glasses during real stimulation. Before and after the stimulation they performed open loop pointing (OLP) to assess adaptation. On Day2, participants received real (Re-Activate Group) or sham stimulation (Control Group) while wearing prism glasses. Before and after the stimulation both groups performed an OLP to assess the reactivation of the after-effect. On Day 3, participants performed only the OLP in order to assess retention. The black hand indicates that participant could see neither their hand nor the outcome of their movement during the OLP, while the white hand indicates that they could see both the terminal part and the outcome of the pointing movement during prism exposure.

tDCS protocol

tDCS was delivered by a battery-driven, constant current stimulator (Neuroconn GmH) using two surface saline-soaked sponge electrodes (area=35cm^2). A constant current of 1.0 mA intensity was used, so a current of 0.1 mA/cm^2 was delivered, which is considered to be safe (Iyer et al., 2005) and below the threshold for tissue damage (Liebetanz et al., 2009). Anodal electrode was placed over the left M1, 5 cm ventro-lateral to the vertex, while cathodal electrode was placed on the skin over the right orbitofrontal region (Nitsche et al., 2003). Stimulation was delivered over the left M1, since participants had to use their right hand to perform the pointing task during PA.

tDCS started right-before PA, it was turned on during the pointing task and automatically stopped at the end of the task. Time of stimulation was set at 20 minutes that was sufficient to allow participant performing the pointing task required for PA. Sham

stimulation was performed in the same way as active stimulation but the stimulator turned off after 30s automatically.

Prism Adaptation task

PA was performed by wearing a pair of glasses producing a 10° rightward optical deviation of the visual field (OptiquePeter.com). The prismatic lenses were fitted with a "glacier" frame containing lateral protectors used to avoid access to non-shifted vision. Both groups of participants (Day 1) had to execute 200 rapid pointing movements with the right index toward two different visual target (a blue or a yellow dot) located 10 degrees to the left or to the right of the middle of their body in a random order. Participants were comfortably seated in front of a table with their head positioned on a chinrest and were asked to point as fast and accurate to the target in a one shot movement, starting from a given starting position and then to return to that position (Redding et al., 2002). A wooden panel allowed to hide the proximal part of the arm used during adaptation, preventing the sight of its trajectory but allowing subjects to notice the terminal error. Overall, PA lasted 20 minutes and was completely covered by stimulation. Pointing was measured using a contractor attached to a thimble that participant wore on the right index finger, on a wooden table covered with a isoresistant carbon paper on which two tension electrodes were applied. A current was generated between the electrodes and when the finger touched the surface of the table, tension between the thimble contact point and the reference electrode was recorded.

These tension measurements were then converted in angular position by means of a mathematical formula and then into degrees allowing to record the terminal error.

After-effect measurement

Prisms after-effect was evaluated by means of OLP in the direction of a visual target with no hand sight. Participants were seated in front of the same setting but in total darkness and a luminescent red diode was aligned with their sagittal axis. A wooden panel allowed to hide both the terminal and proximal part of the arm in a way that did not allow participants to see their hand and, consequently, to receive a feedback from their movement. The instruction was to place the right pointing index immediately below the red light touching on the table, moving from the starting position and then to go back. Twenty OLP movements were performed before and after the PA-tDCS session in Day 1 (Day 1 Pre, Day 1 Post), before and after tDCS alone in Day 2 (Day 2 Pre, Day 2 Post) and in Day 3 (Day 3), in which participants performed the OLP alone. All OLP measurements were performed with no ongoing stimulation.

Data analysis

The difference between the mean deviation in the OLP movements in Day1Pre-Day1Post and Day2Pre-Day2Post provided measures of the prisms after-effect. In addition, 20 follow-up OLP movements were performed on Day 3 and retention was measured as the difference between Day 3 and Day 2 Pre. Negative values indicate a deviation to the left and positive values indicate a deviation to the right.

These measures were then compared to the zero value (meaning no after-effect) by means of Student t-tests. Inferential statistics about the differential behavior of the two groups were provided by a repeated measures ANOVA (Group: Re-Activate Group vs Control Group; Day: Day1 vs Day2) on after-effects. Retention was evaluated by an additional univariate ANOVA.

It was further tested whether tDCS alone may be sufficient to produce a significant after-effect, independent from PA. Therefore, 20 OLP measures before and after tDCS (no PA)

from 10 healthy subjects were compared by a repeated measure ANOVA (Pre_tDCS vs Post_tDCS).

Results

On Day 1 after-effect was present in both the Re-Activate Group [M=-6.32, SE=1.15; t(9)=-5.47, p<.001] and in the Control Group [M=-5.28, SE=.88; t(9)=-5.94, p<.001], showing that both groups properly adapted to prims (Figure 2 left). On Day 2 the two groups were no longer shifted in the direction of prism after-effects in pre-test condition (Re-Activate Group: M=.53, SE=1.4; Control Group: M=-.81, SE=1.2), which means that there was no 24h retention.

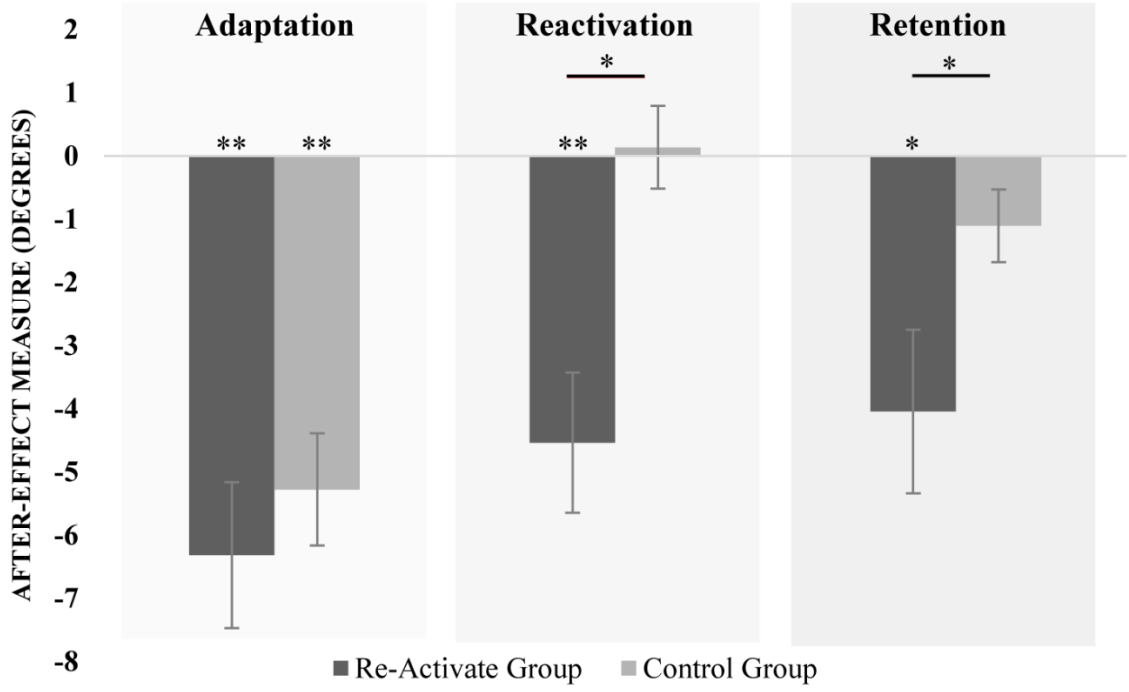

Figure 2. After-effect measures in the Re-Activate Group and Control Group in Day1 (which assessed adaptation), Day2 (which assessed the reactivation of after-effect) and Day3 (assessing retention). The horizontal bars report the results from ANOVAs; ** different from 0 at p<.01; *different from 0 at p< .05

Following tDCS (Figure 2 middle), a significant after-effect reappeared in the Re-Activate Group [M=-4.54, SE=1.1; t(9)=-4.10, p=.003], but not in the Control Group [M=.13, SE=.65; t(9)=.2, p=.84]. The 2x2 repeated-measure ANOVA on after-effect revealed a significant main effect of Day [F(1, 18)=18.43, p<.001, η^2p=.5; Day1: M=-5.8, SE=.73; Day2: M=-2.2, SE=.64], and a significant main effect of Group [F(1, 18)=6.87, p=.2, η^2p=.28; Re-Activate Group: M=-5.43, SE=.77; Control Group=-2.57, SE=.77] and crucially a significant effect of interaction between Day and Group [F(1, 18)=4.7 , p=.04, η^2p=.21]. Bonferroni post-hoc contrasts revealed that there was no significant difference in the after-effect between the two groups on Day 1 (Reactivate Group: M=-6.32, SE=1.03; Control Group: M=-5.28, SE=1.03; p=.49) while there was a significant after-effect reactivation on Day 2 in the Re-Activate Group (M=-4.54, SE=.9) compared to the Control Group (M=.13, SE=.91; p=.002). Moreover, while in the Re-Activate Group there was no difference in the after-effect between the two days (p=.15), the Control Group showed a significant after-effect in Day 1 and not on Day 2 (p<.001). The follow-up assessment on Day 3 revealed that the after-effect retention was significantly different from zero in the Re-Activate Group [M=-4.05; SE=1.29; t(9)=-3.13, p=.01] and not in the Control Group. In addition the univariate ANOVA on the retention measure reached p=.05 [F(1, 18)=4.3, η^2p= .2], confirming that the reactivation produced on Day 2 lasted for at least 24h (Figure 2 right).

The control experiment on tDCS alone showed no significant difference [F(1, 9)=1.47, p=.26,] between the OLP measures performed before (Pre_tDCS: M=-2.6, SE=1.08) and after stimulation (Post_tDCS: M=-1.7; SE=1.16).

Conclusions

Findings from Experiment 5 show that both groups of subjects normally adapted to prisms and that real anodal tDCS on M1 alone was able to re-activate the prism after-effect 24 hours later. In addition, retention on Day 3 was significantly different from zero in the Re-Activate Group. Such reactivation and retention were not found in the Control Group. Moreover, control data showed that after-effect reactivation could not be achieved by means of stimulation alone with no PA.

The data from Experiment 5 provide the first striking demonstration that is possible to reactivate learning through neuromodulation alone. This suggests that some latent activity related to adaptation persists for 24 hours, and it must be boosted by tDCS reactivation to generate a measureable after-effect. As the combination of tDCS and PA was used on Day 1 to increase adaptation activity (O'Shea et al., 2013), further studies should explore whether PA alone is sufficient to produce durable effects to be boosted 24h later, the duration of the sensitive window for reactivation, and the physiological mechanisms at work.

At the methodological level these original results provide a new tool to uncover latent activity in adaptation circuits.

Chapter 5
General Discussion

Summary of the empirical evidence presented in this thesis

In the previous chapters several experiments have been described aimed at understanding the role of distinct brain regions in PA. These experiments implied functional brain stimulation (tDCS) in a facilitatory or inhibitory modality and targeted several brain regions (Cerebellum, PPC, M1) or their relative connections (Cerebellar-PPC circuitry). The evidence provided in the present thesis allows to answer three general questions related to i) the neural correlates of adaptive processes developed during PA, ii) the possible contribution to PA from the neural circuitry that links these regions and iii) the possibility to actively boost and modulate the mechanisms of PA.

Experiment 1 employed cathodal tDCS of the cerebellum during Single-step PA, where participants are directly exposed to the full prismatic shift in one time, revealing a general cerebellar contribution to all stages of PA, from exposure to prism to after-effect evaluation following prisms' removal.

Experiment 2 and Experiment 3 used specific protocols of PA to isolate experimentally the mechanisms of spatial realignment and recalibration to individuals' performance during PA. Indeed, Experiment 2 combined cathodal tDCS of the cerebellum during Multiple-step PA, where the full 10° prismatic shift was achieved thought multiple progressive steps of 2°, allowing to isolate the adaptive process of spatial realignment. Results from Experiment 2 allowed to causally demonstrate the contribution of the cerebellum to the process of spatial realignment and extended classical models of PA showing that the process of spatial realignment, classically attributed to late adaptation stages, develops very early to affect participants' performance.

Experiment 3 delivered cathodal tDCS on the cerebellum during Reversing PA, an experimental procedure in which the prism deviation and displacement are iteratively

modified after a few trials to prevent adaptation, allowing to keep participants in a constant process of recalibration of the motor commands. Both data on terminal error and kinematic parameters collected in Experiment 3 converged in suggesting that the cerebellum does not participate to the process of recalibration.

Experiment 4 assessed the involvement of a circuitry that links the cerebellum with the PPC in individuals' pointing performance during PA. In contrast with the previous experiments, that used monocephalic stimulation, Experiment 4 implied bi-cephalic stimulation given that the aim of this experiment was to affect the neural circuit between the cerebellum and the PPC. Results from Experiment 4 support the theoretical distinction between recalibration and spatial realignment and crucially highlight that error correction during PA relies on a tick flow of information between the cerebellum and the PPC rather than on isolated brain areas.

The last experiment presented in the present thesis (Experiment 5) aimed at assessing the possibility to affect the neural circuit sustaining PA by mean of neuromodulation. Indeed, Experiment 5 used anodal functional stimulation on M1 to study the storage of the learning achieved by PA and the possibility to reactivate the neural correlates of this kind of learning. Results from Experiment 5 demonstrated the possibility to reactivate the prism after-effect 24 hours following stimulation by means of an induced plasticity of the same circuits sustaining learning during PA and that the reactivated after-effect was long lasting.

To sum-up the above-presented evidence supports the theoretical distinction in PA between the strategic mechanism of error correction and the process of adaptation. The process of adaptation to prism would be achieved by fast cerebellar involvement during PA, since a very early phase of the pointing performance, and would crucially rely on the

connection of this structure with the PPC. The continuous information flow between the cerebellum and the PPC would permit full error compensation during PA and cerebellar functioning would allow after-effect development following PA. The after-effect, that can be considered as a kind of motor memory, would be stored in M1 and it can be reactivated by mean of functional stimulation.

Future clinical research

The evidence provided in the present thesis could have important clinical applications in the field of neuropsychological rehabilitation. As it has been discussed in the previous chapters, PA has been used for rehabilitation of spatial neglect, a neurological deficit following right brain damage in perceiving, attending, representing and acting toward the contralesional space (Bisiach, 1999). Rossetti et al. (1998) first found that a single session of PA could ameliorate spatial neglect for several hours. Other studies confirmed supramodal, generalized (Rode et al., 2001) and long-lasting (Frassinetti et al., 2002; Serino et al., 2006; Serino et al., 2009) improvements in left neglect patients following a training implying several sessions of PA. A key aspect related to PA is that this tool does not require awareness of patients' symptoms, classically impaired in neglect patients, thus representing a bottom-up rehabilitation tool. For these reasons, to the present day PA is considered one of the most promising rehabilitative tools to treat spatial neglect (Mattingley, 2002; Jacquin-Courtois et al., 2013).

Several models have been proposed to explain PA functioning in the healthy and damaged brain and to explain cognitive effects of PA in neglect patients (Redding et al., 2006; Serino et al., 2006; Angeli et al., 2004; Pisella et al., 2006; Luautè et al., 2006; Saj et al., 2013; Clarke et al., 2016). Redding et al. (2006) proposed neglect as a dysfunction in the

selection of the region of the space appropriate for a certain task. Normally, a task-work space is strategically sized and positioned around the task-relevant objects. Neglect patients would present a pathological reduction in size and allocation of the task-work space. PA would ameliorate dysfunctional positioning of the task relevant space but not sizing, bringing at least part of the neglected hemispace in the still reduced in size task-work space. Serino et al. (2006) proposed that the low order visuo-motor reorganization induced by PA promotes a resetting of the oculo-motor system in favor of the neglected hemispace. The resetting of the oculo-motor system would then lead to an improvement in high order visuo-spatial representation explaining neglect amelioration (see also Angeli et al., 2004). Pisella et al. (2006) proposed an explanation of neglect amelioration following PA based on the principle of interhemispheric balance (Kinsboune 1994). According to the interhemispheric balance framework, the deficit of patients with neglect would be explained by a hyper-activation of the left hemisphere compared to the right damaged hemisphere. PA would act at the cerebellar level and would indirectly interfere with the left hemisphere such that the balance is improved or restored. The hypothesis that PA modulates inter-hemispheric balance has been explored by neuroimaging studies. Saj et al. (2013) studied the neural substrates underlying the therapeutic benefits of PA providing data consistent with recent proposals that neglect may reflect lateralized deficits induced by bilateral hemispheric dysfunction. Indeed, Authors investigated the neural mechanisms underlying prism effects on visuo-spatial processing in neglect patients by means of functional magnetic resonance imaging (fMRI). Following PA, fMRI data showed increased activation in bilateral parietal, frontal, and occipital cortex during bisection and visual search demonstrating that visuo-motor adaptation induced by prism exposure can restore activation in bilateral brain networks controlling spatial attention and awareness. Moreover, in the same framework of hemispheric balance,

Clarke et al. (2016) demonstrated that rightward prismatic adaptation is able to shift visual field representation from right to left inferior parietal lobule, thus changing hemispheric dominance within the ventral attentional system. This change would redirect visual input to the dorsal attentional system and re-install balance between its left and right hemispheric components in neglect (Clarke et al., 2016).

Although an increasing number of papers report PA as an effective rehabilitative tool for spatial neglect and although several models provide relevant explanations for PA functioning in the brain, there is still no general consensus on the exact processes underlying neglect amelioration. Moreover, in the literature on PA in neglect patients, negative results have been also reported. Indeed, Morris et al. (2004) and Rosseaux et al. (2006) did not confirm the efficacy of PA in rehabilitation of spatial neglect. Several aspects such as the extension and location of the brain lesions or the time elapsed from the brain lesion could account for these controversial results. It is also possible that PA may affect some spatial tasks more than others (Rousseaux et al., 2006; Sarri et al., 2011; Serino et al., 2006; Striemer and Danckert, 2010).

Taking into account the limitations related to use of PA in neglect patients several studies aim at boosting the efficacy of PA, developing alternative rehabilitation tools or merging them in combined rehabilitation protocols. The latter possibility, i.e. the combination of several approaches, represents an encouraging perspective in order to: i) boost efficacy of these techniques (Ladavas et al., 2015) in terms of amelioration amplitude and lasting; ii) provide valid alternatives to patients that do not respond to these treatments used alone (O'Shea et al., 2013); iii) extend the theoretical knowledge about PA and neglect.

In this framework, among the alternative rehabilitation techniques used in neglect recovery, transcranial Direct Current Stimulation (tDCS) has been also used to treat

spatial neglect (Ko et al., 2008; Sparing et al., 2009; Sunwoo et al., 2013; Brem et al. 2014). Also tDCS applications in neglect rehabilitation are mainly related to the concept of interhemispheric rivalry (Kinsboune 1994; 1997), i.e. the idea that in neglect patients there would be a hyper-activation of the contralesional hemisphere following the brain damage due to a decrease of the inhibiting influence of the injured hemisphere. The rationale for using tDCS in neglect rehabilitation would be to restore the balance between the hemispheres through neuromodulation. However, in this case, only preliminary findings on non-ecological tests are available, that have to be transposed into clinically-relevant effects (Jacquin-Courtois, 2015).

A more interesting aspect to be evaluated is related to the possibility to combine PA and tDCS to treat chronic treatment-resistant patients. Findings reported in the previous chapters of the present thesis could prompt the development of combined PA-tDCS rehabilitation protocols in neglect rehabilitation. Indeed, Ladavas et al. (2015) showed that anodal tDCS of the ispilesional parietal cortex was able to boost the effect of PA in neglect patients and O'Shea et al. (2017) recently showed that facilitatory stimulation delivered during PA is able to promote recovery also in chronic treatment-resistant patients in which PA alone was not associated to significant improvement. In the latter study anodal tDCS of the left motor cortex enhanced the consolidation of PA, stabilizing both sensorimotor and cognitive prism after-effects. Indeed, 20 minutes of combined stimulation-adaptation caused persistent cognitive after-effects that lasted until 18 and 46 days after the training. Since adaptation without stimulation was ineffective O'Shea et al. (2017) suggested that stimulation reversed treatment resistance in chronic visual neglect. These findings are very interesting also from a theoretical perspective because they challenge the consensus on the idea that left hemisphere in neglect is pathologically over-excited and it has to be suppressed to restore brain balance.

The results reported in the present thesis open a new possible research field on tDCS in neglect rehabilitation.

Experiment 1 and Experiment 2 demonstrated that is possible to modulate individuals' behavior during PA affecting the mechanism of spatial realignment. Crucially, Experiment 2 combined tDCS to a PA task (multiple-step PA) that induced spatial realignment in a condition where participants were not aware of the progressive shift of the visual field, and of the consequent progressive modification of their visuo-motor coordination. Several studies reported that healthy individuals are usually aware of the prism shift during single-step PA and this induces a short-lasting (Welch et al., 1974) and humble after-effect (Efstathiou, 1969). In contrast, clinical observations suggest that neglect patients are classically not aware of the visual distortion during PA, and exhibit large and long-lasting negative after-effects (Rossetti et al., 1998; Pisella et al., 2002; Rossetti et al. 2015). These observations suggest that patients' recovery largely relies on spatial realignment mechanisms subtended by the cerebellum, often unimpaired in these patients, although this link may be indirect (e.g. Pisella et al. 2002; Jacquin-Courtois et al. 2013). Our findings confirmed that inhibiting the cerebellum can interfere with spatial realignment during PA, and open the possibility of using facilitatory, anodal functional stimulation of the cerebellum in the attempt to boost spatial realignment and possibly neglect recovery. Additional clinical applications are suggested by Experiment 5 that describes the possibility to durably reactivate prism after-effect through neuromodulation 24 hours following PA. Intermingling PA sessions with tDCS alone sessions could considerably simplify rehabilitation training. Indeed, compared to daily PA sessions, that require active presence of a therapist assisting the patient during the pointing task, tDCS sessions are less time consuming and do not require active effort from either the therapist or the patient. The first step in this direction would be to replicate Experiment 5 in neglect

patients to ascertain whether the same kind of reactivation can be achieved in brain damaged participants too. Then specific rehabilitative training could be designed to ascertain: i) the possibility to evoke prism after-effect in neglect patients through neuromodulation, ii) the duration of the sensitive window for after-effect reactivation, and iii) the efficacy of intermingled PA-tDCS sessions on spatial cognition and neglect recovery.

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