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CONTRIBUTION OF BASAL GANGLIA, CEREBELLUM AND PARIETAL OPERCULUM TO ANTICIPATORY POSTURAL ADJUSTMENTS

SSD BIO-09

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SUMMARY

SCIENTIFIC PRODUCTION

This thesis collects the scientific works performed as PhD student from October 2016 to September 2019.
During this period, I have been working at: Brain Connectivity Center, "C. Mondino", National Neurological Institute, Pavia, Italy (from October 2016 to December 2017); Human motor control Laboratory, Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; CIAMS Laboratory, UFR STAPS, University Paris-Sud, Orsay, France (from December 2018 to February 2019).

The research outcomes led to four full length papers:

Bolzoni F, Esposti R, Marchese SM, Pozzi NG, Ramirez-Pasos UE, Isaias IU, Cavallari P (2018) Disrupt of Intra-Limb APA Pattern in Parkinsonian Patients Performing Index-Finger Flexion. *Frontiers in Physiology* 9: 1745, doi 10.3389/fphys.2018.01745. **Paper 1**

Farinelli V, Palmisano C, Marchese SM, Strano CMM, D'Arrigo S, Pantaleoni C, Ardissone A, Nardocci N, Esposti R, Cavallari P. Postural control in children with Congenital Cerebellar Ataxia. Ready to be submitted to *Applied Sciences*. **Paper 2**

Marchese SM, Palesi F, Savini G, Vitali P, Germani G, Gandini Wheeler-Kingshott CAM, Cavallari P, De Vita E, D'Angelo E. Characterization and reproducibility of cerebellar metabolites in Crus I-II using MRS. Ready to be submitted. **Paper 3**

Marchese SM, Esposti R, Bolzoni F, Cavallari P (2019). Transcranial Direct Current Stimulation on Parietal Operculum Contralateral to the Moving Limb does not affect the Programming of Intra-limb Anticipatory Postural Adjustments. *Frontiers in Physiology* 10: 1159, doi 10.3389/fphys.2019.01159. **Paper 4**

As well as to 9 contributions to congresses:

School of Brain Cells & Circuits "Camillo Golgi": The cerebellum inside out: cells, circuits and functions. Erice, Italy. 2016.
Marchese SM, Palesi F, De Vita E, De Rinaldis A, Vitali P, Germani G, Wheeler-Kingshott CA, D'angelo E. Characterization of cerebellar metabolites in white and gray matter using 3T magnetic resonance spectroscopy. School Abstract, published in **Front. Cell. Neurosci**. (2017) pp. 67-73. ISSN 1662-453X. doi: 10.3389/conf.fncel.2017.37.000031

IX Congresso AIRMM, Italian Association of Magnetic Resonance in Medicine. Italian Chapter di ISMRM. Padova, Italy. 2018.
Marchese SM, Palesi F, Savini G, Vitali P, Germani G, Gandini Wheeler-Kingshott CAM, Cavallari P, De Vita E, D'Angelo E. Characterization and reproducibility of cerebellar metabolites in Crus I-II using MRS. Poster session. http://www.ismrm.it/it/.

69th National Congress of the Italian Physiological Society. Firenze, Italy. 2018.
Bolzoni F, Esposti R, Marchese SM, Pozzi NG, Ramirez-Pasos UE, Isaias IU, Cavallari P. Disruption of intra-limb anticipatory postural adjustments in parkinsonian patient. Published in **SIF 2018**: Programme & Abstracts, pp. 62-62. ISBN 9788894010596.

69th National Congress of the Italian Physiological Society. Firenze, Italy. 2018.
Marchese SM, Bolzoni F, Esposti R, Cavallari R. Anodal tDCS on parietal operculum does not affect the programming of intra-limb anticipatory postural adjustments. Published in **SIF 2018**: Programme & Abstracts, pp. 236-236. ISBN 9788894010596.

Progress in Motor Control XII: Movement Improvement. Amsterdam, Netherlands. 2019.
Cavallari P, Bolzoni F, Esposti R, Marchese SM, Pozzi N, Ramirez-Pasos U, Isaias I. The pattern of intra-limb APAs stabilising the arm during index-finger flexion is disrupted in parkinsonian patients. Poster session. https://pmc2019.org.

FEPS 2019 Federation of European Physiological Societies and SIF the Italian Physiological Society. Bologna, Italy. 2019.
Marchese SM, Bolzoni F, Esposti R, Cavallari P. Cathodal or Anodal tDCS on parietal operculum do not affect intra-limb Anticipatory Postural Adjustments. Published in **ACTA physiologica**, special issue September 2019. https://www.feps-sif2019.com.

FEPS 2019 Federation of European Physiological Societies and SIF the Italian Physiological Society. Bologna, Italy. 2019.
Farinelli V, Marchese SM, Strano C, D'Arrigo S, Ardissone A, Nardocci N, Bolzoni F, Esposti R, Cavallari P. Postural Control during Gait Initiation in Children with Cerebellar Ataxia. Published in **ACTA physiologica**, special issue September 2019. https://www.feps-sif2019.com.

ESMAC 2019 Annual Meeting of the European Society for Movement Analysis in Adults and Children. Amsterdam, Netherlands. 2019.
Farinelli V, Palmisano C, Marchese SM, Esposti R, Bolzoni F, Strano CMM, D'Arrigo S, Frigo C, Cavallari P. Gait initiation in children with cerebellar ataxia. Published in **Gait & Posture Journal**, special issue. http://www.esmac2019.org

SIAMOC 2019. 20th Annual Congress of the Italian Company for analysis of movement in the clinic. Bologna, Italy. 2019.
Farinelli V, Palmisano C, Marchese SM, Strano CMM, D'Arrigo S, Ardissone A, Nardocci N, Frigo C, Bolzoni F, Esposti R, Cavallari P. Gait initiation in children with Joubert syndrome. Published in **Gait & Posture Journal**, special issue. https://www.siamoc.it.

Forewords

During my PhD studies, I deepened several aspects of the Anticipatory Postural Adjustments (APAs) and the neural network involved in their control. APAs are a crucial aspect of the voluntary movement organization, being fundamental in stabilizing both the whole-body (inter-limb APAs) and its segments (intra-limb APAs). At the present time, only a few works investigated the cortical and subcortical structures involved in APAs programming. Some of these works correlated neurological diseases with APAs changes, elucidating not only the knowledge regarding these pathologies, but also the relationships between these structures and the APA command.

To enrich the knowledge about the neural network generating and influencing APAs, I moved my attention on two aspects.

First, I investigated the role of two subcortical structure, basal ganglia and cerebellum, in APAs organization. In particular, I analyzed the intra-limb APAs stabilizing the arm and the forearm before a brisk flexion of the index-finger, in patients with basal ganglia dysfunction, as well as the inter-limb APAs stabilizing gait initiation, in children with cerebellar pathologies.

The second aspect dealt with in the present thesis was the possible influence of cognitive processes on APA control. To achieve this goal, a cerebellar area with a well-known cognitive role, Crus I-II, was examined. In this context, we developed a reproducible protocol of Magnetic Resonance Spectroscopy, to characterize and quantify metabolites in a cerebellar area of interest. The protocol was validated in healthy subjects and could be used to investigate metabolic changes in neurological diseases, also affecting other

brain areas. Finally, the cognitive approach to APA control led me to focus on the Parietal Operculum (PO), a cortical sensory-motor integration center involved in a multimodal network. So, intra-limb APAs associated to index-finger flexion were analyzed in healthy subjects before, during and after modulating the contralateral PO excitability by anodal and cathodal transcranial Direct Current Stimulation.

The present thesis is articulated in six main sections: i) the *Introduction* describes the state-of-the-art literature on motor control and anticipatory postural adjustments; ii) the *Research hypothesis* explains the aim of this thesis and how it has been articulated in the ensuing research papers; iii) the *Experimental design* details how data were collected and analyzed; iv) the *Results* summarizes the outcomes of each paper included in this thesis; v) the *Article section* reports the original papers, two already published and two to be submitted; vi) the *Discussion* reviews the interpretation of the results obtained in each paper and highlights new possible research pathways.

INTRODUCTION

MOTOR CONTROL

Several cerebral structures are involved in motor control, i.e. the process which allows the nervous system to produce the most disparate movements. The contraction and relaxation of muscle groups make the various segments moving one in respect to the others upkeeping the body posture and allowing the regulation of vital functions (such as breathing, heartbeat and peristalsis of the digestive system) according to external world inputs.

Functional hierarchy

The motor system has a functional hierarchical organization, which can be divided into three levels of control (Figure 1) from the lowest one (less complex) to the highest one (more complex): the spinal cord, brain stem and forebrain (Fentress, 2001).

The spinal cord, the lowest level of the hierarchy, is the primary interaction between the peripheral nervous system and the muscle fibers translating nerve signals into mechanical actions. The spinal cord receives descending inputs from the brain; it is able to control the actions of the upper and lower body and it is responsible also for simple reflexive movements. Spinal cord activity is influenced by inputs from the brainstem, the posterior

part of the brain, which contains the cranial nerves essential for reflexes like breathing, eye movements, eating and facial expressions. The brainstem includes the midbrain, the pons and the medulla and it is influenced, in turn, by the cerebellum and the basal ganglia.

Cerebellum receives information not only from several association areas of the cortex, but also from somatosensory, visual, vestibular, auditory streams. These inputs are direct to the cerebellar cortex which sends in turn information to deep nuclei at cerebellar core. From these nuclei originate all cerebellar outputs involved in motor system.

As regards the basal ganglia, the input station is located in the caudate and in the putamen, the two nuclei forming the striatum. The output streams come mostly from globus pallidus and part of substantia nigra and terminate in the thalamus, which in turn sends projects to motor and frontal regions of the cerebral cortex.

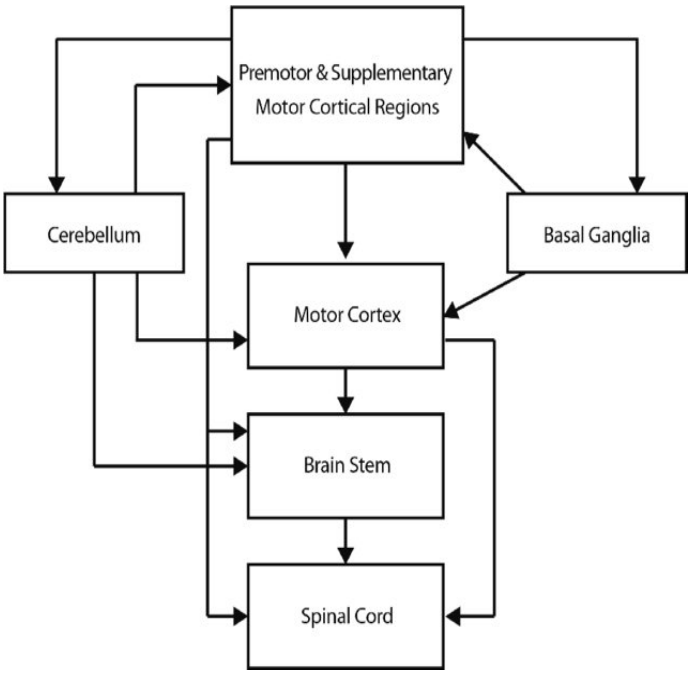

Figure 1. Functional hierarchy organization of motor control. The highest control level is represented by Premotor and Supplementary motor cortical areas aimed to planning motor actions. Motor cortex, influenced by cerebellum and basal ganglia inputs, sends motor projections to the lowest level of the hierarchy: spinal cord. This is, in turn, influenced by inputs derived from the brainstem. *Anema, 2013*.

Premotor and supplementary motor cortex regions are at the highest level and their activity is focused on planning actions based on sensory information. Thanks to this functional architecture, the motor cortex becomes able to translate action goal into movement.

In addition to the above, there are other association areas of the cortex which are involved in motor functions, such as the insula, the posterior inferior frontal gyrus and the posterior parietal cortex. The first two have a role in the production of speech movements, while the latter in the planning and control of actions.

Voluntary movement

Movement is the change of position of an object, measured by an observer, as a function of time. The observer must be aware of the initial and final position of the object with respect to spatial coordinates which can be global or local, depending on whether the object moves in space or in relation to something, respectively. The motor system generates the forces necessary to change over time the relationships between the body and the external world or the relationships between the different body segments producing movement. The movements can be very simple when they involve a single segment, or complex if they involve the entire body, such as maintaining stability during walking. They can be induced from the outside (reflex movements), generated by our will (voluntary movements) or generated by a pacemaker (automatic movements).

When a motor task is performed intentionally, the movement is defined as *voluntary*. To achieve this goal, it is necessary to coordinate the

mobilization and immobilization of body segments with multiple parallel commands depending on the complexity of movement (Bouisset and Do, 2008).

The actions we perform are able to create smooth and elegant movements which are the result of a continuous improvement of their quality thanks to processes of coordination and learning control systems. These actions, simple or complex, share several aspects that concern *degrees of freedom*, *timing and sequential order*, *integration with sense systems* and *learning ability*.

To perform an action, there are numerous possibilities and different strategies given by the *degrees of freedom* which represent an opportunity to be able to choose the best action in a specific situation. Therefore, a control system chooses a unique movement strategy for a particular situation among the infinite possibilities that the system has at its disposal. Depending on the strategy chosen there are different temporal consequences as the time needed to reach the goal will be different. The degrees of freedom can be decreased by reducing the complexity of the choice, or by using the so called *synergies* which are pre-established interactions among muscles. Synergies do not eliminate degrees of freedom of movement, but promote the spontaneity with which the central nervous system performs one or the other action, in fact they act on the probability of moving better in one way or another. The fact that the movement performed is the most efficient one, also in energy terms, explains the primary choice that the brain applies with respect to the various possibilities offered by the degrees of freedom.

Moreover, when we execute a movement involving several segments, the individual movements must be ordered from a point of *temporal* view and with a precise *sequential order*. The latter is certainly essential to obtain a

successful outcome. Indeed, to grasp an object correctly it is necessary that the displacements of the proximal and distal joints occur according to a precise and predetermined plan that must take into account times and ways.

The link between *sensation-perception* and movement are certainly of vital importance. Some movements, called ballistics, are rapid and explosive and they cannot be corrected during their trajectory. If the ballistic movement is poorly calculated, an incorrect trajectory and an error at the destination point will be obtained. However, this error is processed and it will be useful to calibrate better the next movement. Other movements are instead controlled step by step and their trajectory can also be changed during the implementation phase. This needs direct communication between the sensory system and the motor system and vice versa. The *feed-back control* systems (positive or negative) are based on the ability of sensor system to monitor the movement in progress and to send information to a control centre that modulates motor output (negatively or positively) in accordance with those signals. On the contrary, the so-called *feed-forward contro*l is based on a predictive aspect and this action is obtained without retroactive control.

To improve the characteristics of the movement, it is necessary to "*learn by doing*" or by actively exploring the effects of movement, based on the perceptive consequences of movement itself, in fact learning an action involves sensory-motor integration. Various experiments show that people who use a particular part of the body develop the cortical areas responsible for controlling that part, this is the reason why training to do something improves performances.

The voluntary movement could be theoretically divided into three phases: ideation, planning and execution of motor action. The specification of the motor command should occur during the Reaction Time (RT) before

movement. The RT measures the delay between a stimulus and the onset of a motor response. During this period, which lasts on average about 200 ms, sensory and motor processes take place in different brain areas. This allows us to perceive the surrounding environment, identify an object, determine the action to be taken with respect to that object and generate a command motor to perform the desired action.

Taking into consideration that the control of movements is based on making decisions (Wolpert and Landy, 2012), Wong et al. (2015) have identified six critical processes (Figure 2) involved in movement generation: three "what" processes that establish the motor goal, and three "how" processes that describe the movement to achieve that goal (motor planning).

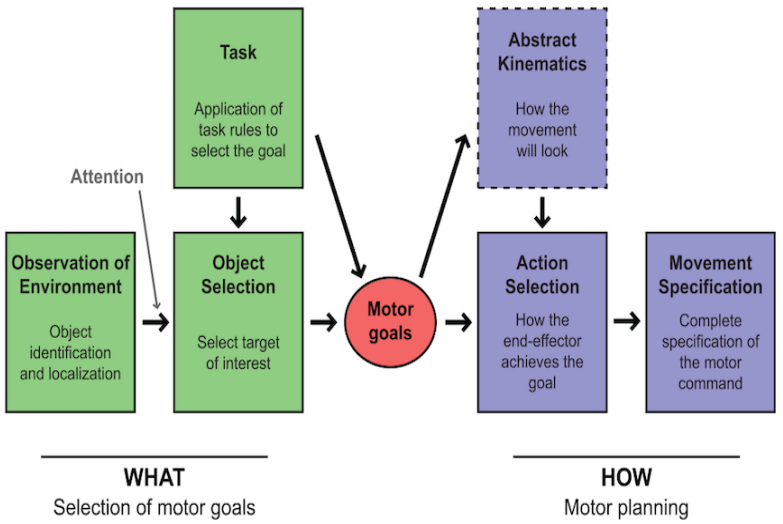

Figure 2. Graphic representation of movement generation. Six processes crucial along the pathway from perception to movement. The first three steps, on the left (green), represent the "what" processes, aimed to achieve motor goals. The three steps, on the right (in purple) represent the "how" processes, which stabilize the motor planning. *Wong et al. 2015*.

First, the generation of a movement involves a set of processes which identify and localize an appropriate object in the environment, through attention, and of task rules to apply for that object. These processes can be described as decision-making steps and consume the majority of the RT. In this way, the motor goal is defined. The other three processes are related to the abstract kinematic representation, the action selection and the movement specification. They are defined as motor planning and determine how the motor goal will be achieved. The abstract kinematics are the descriptions of how the movement will look. The understanding of how the effector muscles work, will define the selection of the action. Finally, in the last step, the complete set of the muscle activations is required to execute the movement output.

The genesis of the motor goal

The identification of a motor goal, or definition of "what", involves the perception, the choice of an object of interest and the definition of what must be done to this object. This process includes the localization of the object in the environment, the application of rules to identify the goal, the choice to start or not the action. These perceptual decisions are necessary to produce a motor response, but they are not strictly motor as they define the goal rather than the action.

As mentioned above, the use of attention is a fundamental prerequisite for motor action planning. Attention facilitates the generation of priority maps that describe objects of interest in the environment promoting the selection of motor tasks.

In simple tasks the object of interest is the goal of the movement. More complex tasks may instead require a decision-making task to choose which of many goals to achieve. When the stimulus is difficult to identify, it is necessary a careful observation of the environment before carrying out the action. The decision-making processes are formalized in the *drift-diffusion model*. In this model, the signs in favor of a particular goal mature step by step, until they exceed a threshold beyond which the goal is selected and the most appropriate movement is generated (Figure 3). When multiple solutions are possible or a counter-order occurs, signs are accumulated in favor of each of the different alternatives, they add up until one of them reaches the threshold to which the movement is directed. The signs that accumulates later, sometimes involve a decision change that directs the movement in a different direction.

Experiments on the existence of *drift-diffusion models* have been carried out on saccadic eye movements of monkeys. Using a motor discrimination task, some monkeys have been instructed to detect the direction of movement through a random-point kinematogram and perform a saccade in that direction. During the experiment the Frontal Eye Fields (FEFs) stimulated a saccadic movement whose direction depended strictly on the discrimination of the points in the kinematogram (Rorie et al., 2010).

The activity of the FEF seems to reflect a decision on a motor goal which guides the action, so it seems there is no separate perceptive decision-making process and that the motor goal reflects motor planning and that the FEF is analogous to the primary motor cortex (M1).

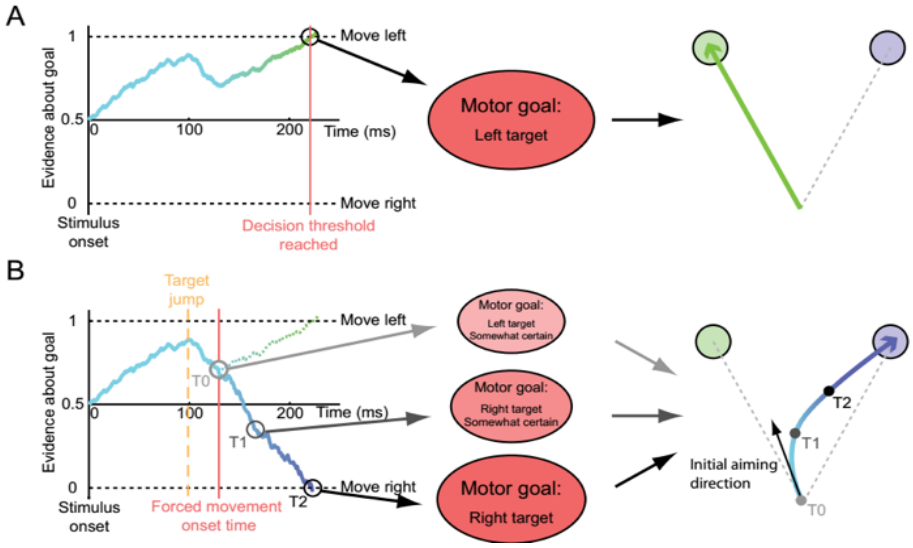

Figure 3. The drift-diffusion model. When a sign between two (or more) goals (A) is required, careful observation of the environment provides signs, in favor of each alternative, that are accumulated over time until a threshold is reached. Crossing the threshold involves putting in place a specific motor strategy to reach the goal. When the position of the target suddenly changes (B), the signs accumulate in favor of an alternative movement. Under normal circumstances, this leads to a lengthening of the reaction time. If the subjects are forced to move before having formulated a decision on the motor act to be taken, they are inclined to choose a movement that is in line with the certainty about the position of the target at the beginning of the movement. At zero time the subject moves to the left, but as the action evolves and signs of movement towards the right mature, the subject corrects the motor act by changing its trajectory online.

However, the frontal field of the eyes appears to be unnecessary for the generation of all saccades suggesting it is associated more with the goal of the movement than with its planning. Among all the regions of the brain exhibiting accumulation activity, for example the Lateral Intraparietal cortex involved also in the saccades, only the frontal field of the eyes is involved in the decision-making processes that concern both the stimulus and the motor act. It is precisely in this area, therefore, that the information regarding the

object selected through the attention process is transformed into the goal towards which the motor act must be directed.

Cognitive influences

To achieve a motor goal it is necessary to apply appropriate rules encoded by the Prefrontal Cortex (PFC), which not only include any decision-making act but also define how it is translated into an appropriate motor response. In the PFC, the association between a specific signal and the motor goal occurs. The selection of motor goals is fundamentally a decision-making process indicated by the fact that cognitive processes can influence the outcome of such choices. Target selection can also be influenced by multiple abstract representations. So the selection of a motor goal is the result of a computationally intensive non-motor decision-making process.

These decision-making processes take into account the relative difficulty of performing possible movements, so that the motor targets are preferentially selected when the movement required is easier from the biomechanical point of view. These decisions don't need the simulation of motor commands for each of the potential targets, but the choice occurs only through a general knowledge of the easiest directions to reach.

Realization of the motor goal

Motor planning is the definition of a motor goal, the "how", and it is necessary to start the movement useful to achieve the goal. It translates the abstract concept of movement towards a concrete action. In this phase, the

neural activity in the motor cortex increases only when a visual stimulus becomes significant to plan an adequate movement. It has been observed that when stimuli provide partial information about the goal, the motor cortex responds only when a key stimulus occurs.

The “how” is reached through three processes, one of which may be optional depending on the complexity of the requested action. The optional high-level process involves decisions on the shape of the trajectory, independently of the effectors used *(abstract kinematics*). The other two processes are necessary for motion planning. The first is the *selection of the action*, which involves the choice and description of the activity of the end effectors. The selection of the end effector is probably separated from the *determination of the movement*, that is the moment in which the voluntary motor command and the relative postural adjustments are specified. The distinction between *selection of action* and *determination of movement* is supported by comparing the neural activity of the Pre-Motor Ventral area (PMV) with that observed in the Pre-Motor Dorsal area (PMD) and in the motor primary (M1). For movements in which the end effector action is identical but the posture changes, postural activity is modulated only in the PMD and in the primary motor, while the activity of the PMV seems to reflect a more abstract and independent strategy from the posture of the limb.

So, the *selection of the action* and the *determination of the movement* convert the motor goal into voluntary movement. This would seem to be possible by producing a flexible feedback control which determines the trajectory from the initial state of a limb, the end point and the distance between the effector and the end point. If during the movement there is an unexpected perturbation which requires rapid compensatory movements, the subjects do not make corrections that bring them back to the original trajectory

but follow a new trajectory to reach directly the target. These control modes can also be stored to allow rapid recycling when they need to be applied flexibly to specify movements directed to different goals.

Motor planning

Movement planning seems to use only a small fraction of the reaction time. It has been proposed that point-to-point movements are generated by selecting and activating an appropriate control mechanism in an existing repertoire as a reflex action, so as to avoid to prepare a new motor command for each movement. Consequently, a movement could start with a reaction time of the order of 160 ms, 90 ms shorter than that necessary for an action that instead requires cognitive decisions. Furthermore, identical kinematically movements to voluntary movements may require low times if evoked through a *startle response*, an unconscious defensive response. The duration of these reaction times are on the same order of latencies with which transcortical reflexes occur which can initiate corrections to feedback of decided movements. Such reflex arcs could serve as mechanisms through which feedback controls are implemented and stored. Thus, motor planning occupies only a small part of the reaction time and it could simply represent the choice of one of the many movements or modes of control from an existing repertoire.

As mentioned before, in some circumstances, an additional planning process is necessary to perform a more complex motor act or the trajectory *abstract kinematic*. It is inherent in the notion of motor equivalence or in the ability to achieve a goal in different ways. For example in writing, the characteristic shape and style of a particular letter or number don’t change

with respect to the effector used. This phenomenon occurs thanks to the abstract representation of the trajectory needed to produce the desired movement and from the trajectory choice itself. Abstract kinematics is not necessary for point-to-point movement, but it is used to solve an indecision of motor planning. This process, challenging from a cognitive point of view, occupies a significant fraction of the reaction time but it is able to resolve the decision on how to reach the goal and allow the generation of the desired movement. The representation of abstract trajectories seems to reside in the posterior parietal cortex near the areas involved in attention. In these regions, the activity is not only correlated with the desired motor act but also with the effective pathway of the limb. For example, patients with ideomotor apraxia who often have a lesion in the left parietal lobe, show no difficulty in point-to-point movements, but they have both difficulties in producing complex gestures following a verbal command and inability to select an alternative tool to achieve the same aim. Probably, these patients with apraxia have difficulty in planning the abstract trajectories necessary to obtain the desired result with a limb or a tool.

Cortical organization of voluntary movement

By recording activity from different cortical structures, it has been possible to observe that each of them is dedicated to a particular type of task. In some areas, however, neurons receive multisensory afferents and project them to neuronal systems (non-specific cortical areas) with or without motor output. In the field of motor control, some structures are involved in the processing of low-level signals rather than higher- level signals processed by

areas involved in more complex aspects. The cortical areas which generate low-level signals and control the muscles for movement are: primary motor cortex (M1), premotor areas (which project directly to M1) and parietal cortex. Each of these cortical areas accesses to the spinal structures through direct or indirect corticospinal projections. Motor commands can therefore derive from multiple motor areas and each of these areas makes a specialized contribution to the planning, execution, or control of voluntary movement.

Subcortical organization of voluntary movement

In addition to cortical structures, subcortical areas play also a key role in the motor organization. The basal ganglia and the cerebellum can be considered collateral circuits of the motor hierarchy, but not secondary ones. Their activity takes part in the elaboration of the motor act by modulating the output of the descending pathways without causing movement in a direct way. The motor cortex sends information to both structures which after processing them, send it back to them through the thalamus. The cerebellum also processes sensory signals produced by movement. The output of the cerebellum is excitatory, while the nuclei of the base are inhibitors. The balance between these two systems makes it possible to regulate and coordinate movement. However, both systems have important extra-motor functions.

Basal ganglia do not constitute a separate motor pathway but they are involved in the initiation of voluntary movement, in the enabling of motor acts modulating the programs stored in the motor cortex and in other structures of the hierarchy of voluntary movement.

As mentioned before, a number of elementary motor programs is stored in cortex and activated following a precise temporal order to perform a complex motor act. Since the main output from the nuclei is an inhibitory connection that goes from the globus pallidus to the thalamus, they could be normally activated in suppressing inappropriate motor programs. The basal ganglia are also involved in the modulation of associative and limbic functions and in implicit memory tasks. As in the case of movement, many types of cognitive tasks require repeated tests and often unconscious learning.

As regards the cerebellum, it has always been considered a motor structure, in fact a cerebellar damage leads to impairments of motor control and posture and most of the cerebellum output are directed to motor structures. Moreover, the cerebellum regulates the direct commands to the spinal motor neurons to compensate for variations in the position of the body through the elaboration of vestibular and proprioceptive signals. Unlike basal ganglia, the cerebellum receives a large amount of peripheral sensory signals, so that it may have a function of sensitive integration, rather than a motor function. Indeed, the cerebellar hemispheres are involved in the acquisition and discrimination of sensory information related to movement. Moreover, the cerebellum has a role as error detector because it is able to establish the differences between the planning of the cortical movement and the result of the movement itself, identifying whether this was performed correctly or not and informing the cortex. Another important function of the cerebellum is to coordinate the timing of muscle activation. It has been shown that this structure regulates the temporal distribution of the activation and deactivation of antagonist muscles determining the order of recruitment of the other muscle groups involved in complex movements. For this reason, cerebellum is the *time machine* of the movement allowing the fluidity of the execution of the

gesture and the locomotion. Finally, the cerebellum is important for motor learning in the management and adaptation of precision movements through "trial and error" processes. The cerebellum, as basal ganglia, regulates cognitive functions such as language and music to construct temporal dynamics which process vocal and musical sounds (Callan et al., 2007).

An interface between the cerebellum and basal ganglia

Cerebellum and basal ganglia constitute distinct systems involved in different aspects of motor and cognitive behaviours. The circuit that connects the cerebral cortex to the cerebellum is anatomically distinct from the one which connects the same cortex to the basal ganglia (Figure 4). The information processed by the two subcortical structures is transmitted through different thalamic nuclei to reach the cerebral cortex again, which acts as an interface between the two systems (Mori et al., 2016). However, through the technique of trans-neuronal transport of rabies virus in monkeys, two disynaptic pathways, connecting cerebellum and basal ganglia, have been observed (Bostan and Strick, 2012): the first one starts from the dentate nucleus of cerebellum and reaches the striatum through the thalamic nuclei; the second one starts from the subthalamic nucleus and reaches the cerebellar cortex through the pontine nuclei. The definition of these two pathways has allowed us to deepen the role of the cerebellum and basal ganglia in movement disorders.

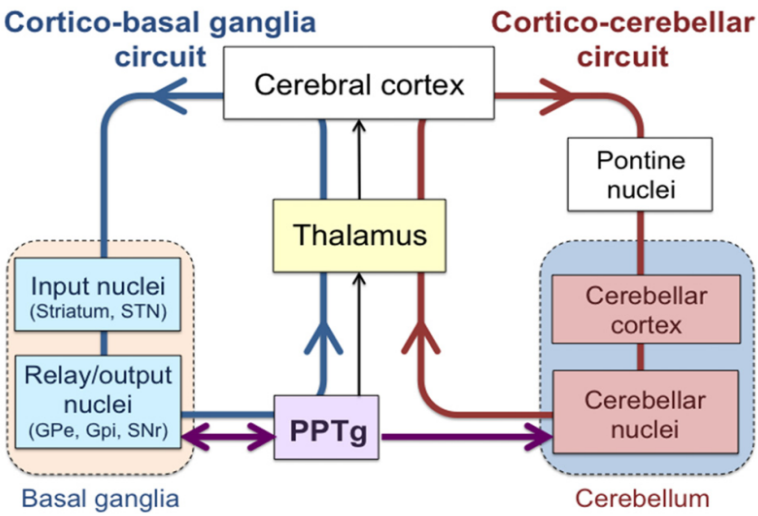

Figure 4. Circuit among cerebral cortex, basal ganglia, cerebellum e nucleus of the pontine peduncle (PPTg).
The circuit that connects the cerebral cortex with the basal ganglia is represented in blue; (cortico-strio-pallido / nigro-thalamic-cortical). The circuit that connects the cerebral cortex with the cerebellum is in represented in red (cortico-cerebello-thalamic cortical). The nucleus of the pontine peduncle is connected both to the nuclei of the basal ganglia and to the cerebellar nuclei. *Mori et al., 2016.*

As it is known, Parkinson's disease is a neurodegenerative disease related to dopamine neuron death in the ventral tegmental area or *substantia nigra*, specifically on *pars compacta* area of the *substantia nigra*. This causes a dysfunction of the dopaminergic system of the basal ganglia, but in parkinsonian patients, physiopathological changes have also been observed in other regions of the brain, including the cerebellum. It has been shown that surgical lesions of the dentate nucleus are able to reduce the dyskinesias, involuntary parasitic movements. An increase in the oscillatory activity of the subthalamic nucleus together with hyper-activation of the cerebellum was also

demonstrated in patients with Parkinson's disease (Mirdamadi, 2019). Furthermore, Deep Brain Stimulation (DBS) of the subthalamic nucleus appears to have a normalizing action on cerebellar activity and reduces motor disorders, probably through the subthalamic-cerebellar pathway (Crenna et al., 2006).

Various nuclei of the human brain stem, such as the nucleus of the pontine peduncle, show an increase in activity when a participant, subjected to fMRI, is asked to imagine walking. Since the low-threshold electrical stimulation of the pontine peduncle nucleus induces locomotion in the animal, it may represent a relay between basal ganglia and spinal cord controlling particularly the tone of the postural muscles.

The nucleus of the pontine peduncle and the nucleus of the laterodorsal tegmentum form the Reticular Activator System (RAS), which connects the brain stem to the cortex and controls the cognitive processes such as attention, learning, memory, wakefulness and sleep-wake. Even the basal ganglia are involved in the regulation of the sleep-wake cycle, in attentional processes, in phenomena related to reward and learning; so, the reciprocal relationship with the pontine peduncle nucleus is fundamental for these functions. It is therefore possible to hypothesize that the nucleus of the pontine peduncle and basal ganglia play a common role as a limbic-motor interface.

In addition to its connections with the basal ganglia, the pontine peduncle nucleus is also connected to the cerebellum. Projections from the nucleus of the pontine peduncle have been anatomically identified in the deep cerebellar nuclei of the rat and radio-imaging studies have identified the same connections in humans. Recently, the functional connectivity between the nucleus of the pontine peduncle and the cerebellum has been studied with microstimulations which evoke a short latency activation of the dentate and

to a lesser extent of the fastigial and interposed nuclei (direct excitatory projections) (Vitale et al., 2016). This suggests that the pontine peduncle nucleus acts as an interface between the cerebellum and the basal ganglia to influence motor control and cognitive functions.

Classical studies postulate that the cerebellum plays a fundamental role in controlling adaptive behavior. In particular it seems to be involved in the modulation of the gain of feedback circuits, through an intermittent control. It is interesting to note that the intermittent control of the gain of feedback circuits can be established by learning the phenomenon of reinforcement with simple reward. The nucleus of the pontine peduncle could forward information on reward learning and reinforcement from the basal ganglia to the cerebellum, generating and regulating postural tone and stabilizing posture. In parkinsonian patients, dysfunction of the basal ganglia could therefore be the cause of postural instability.

Recent studies in the treatment of Parkinson's disease, through deep stimulation of the pontine peduncle nucleus, confirm its direct involvement in the coordination between basal ganglia activity and cerebellar activity (Tykocki et al., 2011). The dysfunction of the pontine peduncle nucleus leads to various motor disabilities similar to those observed in Parkinson's disease. The cholinergic and glutamatergic excitatory projections of the pontine peduncle nucleus regulate the activity of the dopaminergic neurons of the pars compacta of the substantia nigra and of the ventral tegmental area (French and Muthusamy, 2018). An inhibition of the neurons of the pontine peduncle nucleus in the primate delays the start of the movement and slows down the acceleration, the deceleration and the quantity of movements produced. Furthermore, unilateral lesions of the pontine peduncle nucleus cause hemiparkinsonism in the contralateral hemisoma. Several symptoms of

Parkinson's disease are associated to an alteration of the dopaminergic system but many studies have shown that freezing of the step and postural instability, generally in the advanced stages of disease, are resistant to dopaminergic drugs. Several studies carried out in humans, have shown that the severity of walking and posture disorders are directly related to the loss of acetyl-cholinergic neurons in the pontine peduncle nucleus and that they are relieved by deep stimulation (Hirsch et al., 1987; Karachi et al., 2010).

In addition to disorders of axial movement, patients with Parkinson's also show disabilities in the control of eye movements, particularly saccadic movements. Indeed, in the monkey the activity of the neurons of the pontine peduncle nucleus is modulated in parallel with the activity of the basal ganglia neurons during the execution of saccades providing for a reward; while on the occasion of fixational saccades these neurons are active together with neurons of the cerebellar nuclei (Mori et al., 2016). This supports the idea that the nucleus of the pontine peduncle could be the interface between the basal ganglia and the cerebellum.

POSTURAL CONTROL

The neuromuscular system controls muscular segments linked together by flexible joints, which create body posture. The central organization of posture involves interactions between external forces, such as gravity, and internal forces, such as the mechanical properties of the body and the neuromuscular forces (Massion, 1994). The vision, proprioceptive, somatosensorial system and the vestibular system play an important role and work together with the neuromuscular system to guarantee posture control.

Posture can be defined according to the relative position of the several parts of the body with respect to one another (egocentric coordinate system), in the relation to the environment (exocentric coordinate system) and in the relation to the gravitational field (geocentric coordinate system). Regulation of posture with respect to gravity is essential to maintain postural balance.

Postural stabilization occurs when the Center of Mass (CoM), point of application of the gravity force vector in which mass body is concentrated, remains above the Base of Support (BoS). In the standing posture, the BoS is the area that comprises all points of contact that the feet create with the supporting surface. To maintain the body equilibrium, postural muscles (such as hip, knee and ankle) must counteract the effect of gravity and of internal forces. The Centre of Pressure (CoP) corresponds to the barycentre of the ground reaction forces and moves within the base of support, leading CoM projection to stay confined in the BoS.

Gait initiation, the period of transition between upright standing posture and locomotion, is particularly studied in the control of balance. In order to walk, the subject must shift the weight toward the *stance leg*, propel the body forward and stabilize balance when the body is sustained by one leg

(swing phase) through anticipatory postural adjustments. The CoP, before swing foot rises, moves briefly backward toward the swing foot and behind the vertical projection of CoM. This latter acquires forward momentum essential to start walking. In this case, the base of support width is reduced and the CoM is not relocated above the new base of support and this creates a mediolateral gap between the CoM and the CoP (Figure 5).

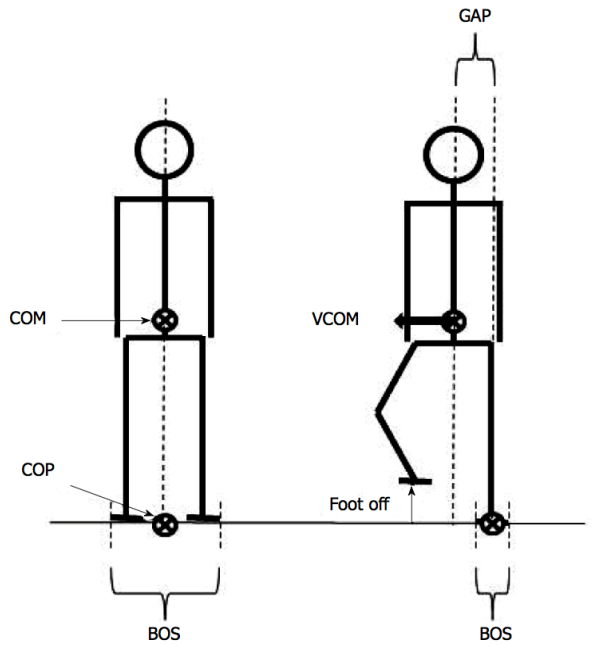

Figure 5. Representation of biomechanical parameters for balance analysis. In the left figure, the quite standing posture, the vertical projection on the ground of the Center of Mass (CoM) falls on the Center of Pressure (CoP) within the Base of Support (BoS). In the right figure, the subject lifts his foot to gait initiation and the size of BoS decrease leading a gap between the CoP and the CoM. VCoM is the velocity of the CoM represented in the figure. *Yiou and Caderby, 2017*.

This gap leads the body to fall towards the *swing leg* side. The amplitude of this mediolateral fall can be estimated calculating the centre of mass displacement and the velocity at the time of swing foot contact. These perturbations have to be counterbalanced, somehow, to avoid the body disequilibrium and fall (Yiou and Caderby, 2017).

From a biomechanical point of view, human body can be considered as a combination of anatomical rigid structures, which interact and work together in an "*articulated chain"*. But given that these anatomical segments are closely linked to each other, every motor action will be discharged to the adjacent articulated segment (Bouisset and Do, 2008). Consequently, there will be specific preprogrammed anticipatory postural movements, able to counterbalance the perturbations caused by a forthcoming voluntary movement on adjacent segments, and then discharged on all segments constituting the postural chain (Bouisset and Zattara, 1981).

Anticipatory Postural Adjustments

Anticipatory Postural Adjustments (APAs) are described as unconscious muscular activities, which counterbalance the perturbations induced by a voluntary movement (Bouisset and Do, 2008; Massion, 1992). Their aim is to stabilize body segments and to ensure the whole-body balance, such as in initiating the displacement of the body center of mass during gait initiation (Breniere, 1987).

Depending on the aim, APAs can create one or more fixation chains which spread over several muscles of the same limb or of different limbs.

When the voluntary motor action is independent from an external go signal, APAs usually precede the EMG onset of prime mover by about 50-90 milliseconds (Horak et al., 1984).

In many voluntary movements involving large masses, like a shoulder flexion or an arm push-pull, APAs spread over several muscles of different limbs creating one or more *inter-limb* APA fixation chains. The latter APAs have been showed to stabilize the whole-body postural equilibrium.

APAs however may develop also in the same limb in which one of the distal segments is moved, e.g. in muscles acting on the shoulder and elbow when flexing/extending the wrist or even just the index-finger. These activities are named *intra-limb* APAs and, considering that the moving mass is quite tiny, such APAs have been supposed to optimize movement performance by stabilizing the limb's proximal segments (Caronni and Cavallari, 2009b).

Classification of APA chains

Inter-limb APAs

There are several studies regarding *inter-lim*b APAs chains which precede a voluntary movement, such us a shoulder flexion and extension (Belen'kii et al., 1967; Bouisset and Zattara, 1987) elbow flexion (Friedli et al., 1984), shoulder lateral abduction (Aruin and Latash, 1995) and also movements which involve the lower limbs, hips and trunk and gait initiation (Crenna and Frigo, 1991; Honeine et al., 2016). The majority of the literature regarding APAs has analyzed the postural chain developing in the lower

limbs, hips and trunk when performing a shoulder flexion. As we mentioned above, since this movement involves a relatively large mass, the perturbation induced by the primary movement may displace the projection on the ground of the whole-body CoM (Bouisset and Zattara, 1987) and cause a whole-body equilibrium disturbance (Bouisset and Do, 2008; Bouisset and Zattara, 1981). Therefore, the prime mover activation is preceded by *inter-limb* APAs. When we consider a pointing movement, the recruitment of the prime mover, the Anterior Deltoid, would provoke an upper limb flexion but, contemporarily, a backward displacement of the shoulder, and thus could make the subject to miss the target or even to fall down. Recruitment of Anterior Deltoid is thus preceded by a specific pattern of EMG activities developing in the lower limbs and trunk, the APA chain, inducing a forward displacement able to counteract the backward perturbation caused by the primum mover segment.

The APA chain starts with an inhibition of the tonically active ipsilateral Soleus, between 50 to 100 ms prior to the prime mover onset. Then, there is a sequence of excitatory and inhibitory EMG activities, beginning with activation of the contralateral Tensor Fasciae Latae and Rectus Femoris.

This APA pattern starts with the contralateral lower limb and hip, the ipsilateral ones, and, ending with the ipsilateral shoulder, follows a bottom-up progression, as the postural segment accelerations follow a "posture-focal gradient", starting from the support base (ground, seat, etc...), proceeding through the postural chain and then terminating on the prime mover (Bouisset and Do, 2008). APAs are efficient in counteracting the perturbation induced by the voluntary movement only if the APA chain encounters resistance originating from the environment, usually a physical support (fixation point/s), which offers the appropriate reaction to the forces generated by APAs.

The timing and magnitude of APAs are rapidly tailored according to the characteristics of the prime mover contraction, i.e. the expected intensity of the perturbation induced by the primary movement. Indeed, postural adjustments developing at an inappropriate time or with incorrect amplitude, may be a source of destabilization and thus be considered as a perturbation. Therefore, to ensure the effectiveness of these postural actions, the central nervous system requires information on the motor task to be performed and on the likely interaction of the single body segments (Mille and Mouchnino, 1998). The correct analysis of these information within the CNS predisposes a correct direction, timing and magnitude of postural adjustments, so that they precede prime mover activation and therefore permit the correct execution of the voluntary movement (Colombo et al., 2002; Frank and Earl, 1990).

An example of *inter-limb* APAs chain is the one involved in a bimanual task, called the *barman task* (Figure 6). During this task, the participants must hold an object in one hand and with the other hand they must lift the object voluntarily (active lifting) or the object must be lifted by an experimenter (passive lifting). During the active lifting, in the hand with the object, an inhibitory APA chain is synchronous to recruitment of the prime mover and precedes the lifting. While, when the object is lifted by the experimenter, the BB inhibition starts about 50 ms after the unloading. Moreover the unexpected removal of the object leads to an impaired balance of the arm, which can be compensated by a sensory feedback, a postural reflex. During the active lifting, instead, a feedforward command is generated to develop APAs in BB. This mechanism allows greater stabilization of balance, in fact, the degrees of elbow rotation are lower in the subject who unloads the forearm with his other hand. In this way, an uncontrolled flexion

of the elbow is avoided and the upper limb posture is maintained (Hugon et al., 1982).

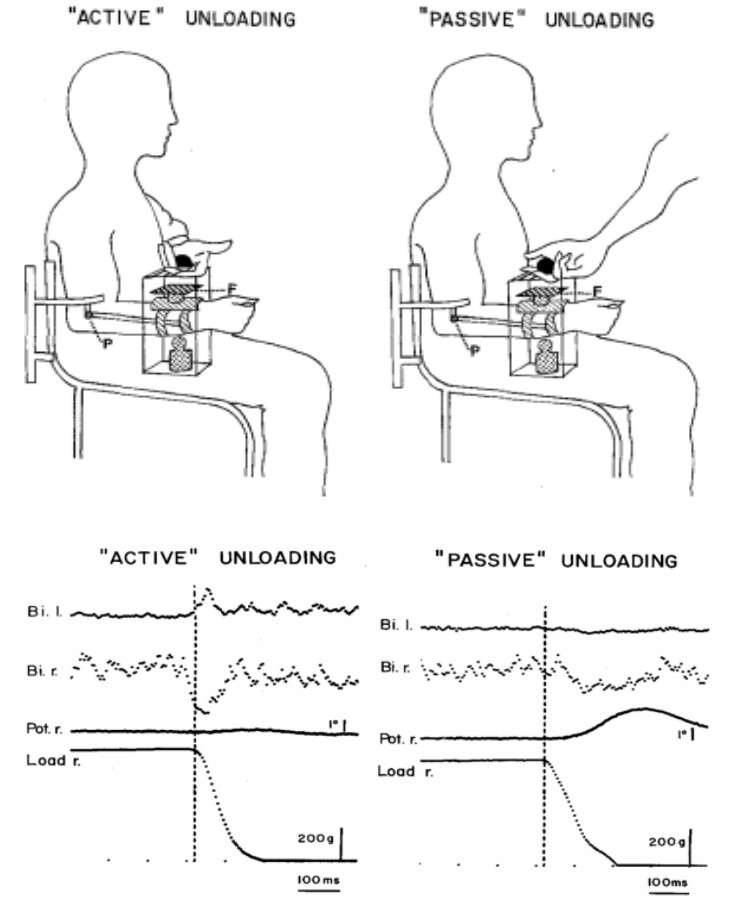

Figure 6. Inter-limb APAs during barman task. The subject is sitting on a chair while maintains its right forearm with a weight applied at the wrist. During "active" unloading, on the left, the object is lift by his forearm (self-unloaded). During the "passive" unloading, the weight is lift by the experimenter, on the right. When the subject lifts the weight (represented by the dotted line) applied at his wrist, an inhibitory APA is developed in the right biceps brachii (Bi r), synchronous to recruitment of the prime mover (left biceps brachii, Bi l). When the experimenter removes the weight, Bi r inhibition starts 50 ms after the removal of object. The angular displacement of the elbow (Pot.r.) indicates that the displacement of the forearm is less in the unload "active". *Hugon et al, 1982*.

From this bimanual load-lifting experiment, it follows that even a movement not concerning the whole body imbalance is preceded by *inter-limb* APAs to stabilize a body segment and these APAs can develop also in muscles that are not usually considered as postural muscles.

In another work on *inter-limb* APAs, it has been demonstrated that the anticipatory forces are directed in the opposite direction to the reaction forces associated with movement performance reducing the postural disturbances caused by the movement (Figure 7). The subjects were instructed to perform bilateral shoulder movements in three different directions, from forward to backwards with increments of 30 degrees (Aruin and Latash, 1995). Bilateral shoulder movements gradually changed the magnitude of postural perturbations in the sagittal plane. APAs were recorded from trunk muscles (Erector Spinae, ES and Rectus Abdominis, RA). When shoulder is flexed an excitatory APA in the ES occurs without any activation in the RA; on the contrary, shoulder extension is preceded by RA activation and no APA in ES. The reversal of APA sign is due to the inversion of the movement direction. Finally, no APAs are found in the EMG traces when bilateral shoulder abduction are performed. The patterns in proximal and distal posture muscles showed the largest anticipatory increase in background activity during movements in one of the two opposite directions (forward or backward). These changes frequently disappeared during movements in the intermediate directions. So, a change in the direction of voluntary bilateral shoulder movements leads to changes in anticipatory EMG activity of both proximal and distal muscles of the dorsal and frontal parts of the trunk and legs.

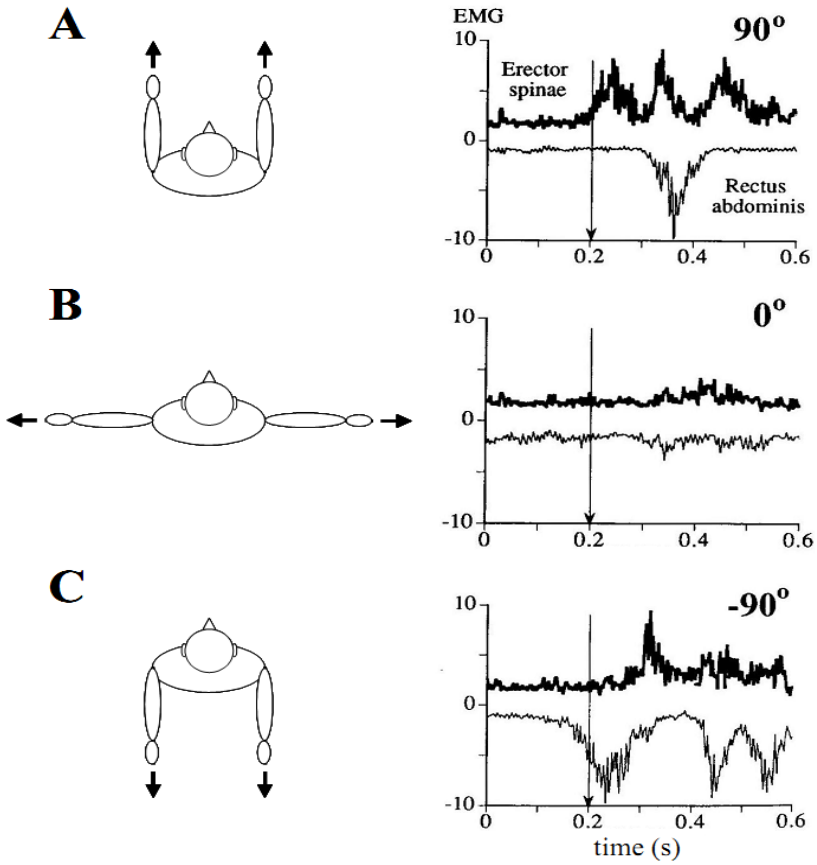

Figure 7. Inter-limb APAs during bilateral shoulder movements. Participants performed bilateral shoulder movements in three different directions (flexion, A; abduction, B; extension, C). Graphs on the right show APAs recorded in the erector spinae (ES) and rectus abdominis (RA), antagonist trunk muscles. Shoulder flexion (A) is accompanied by an excitatory APA in ES, while no APA was recorded in RA; on the contrary, shoulder extension is preceded by RA activation and no APA in ES. No APA can be seen in the EMG traces when bilateral shoulder abduction are performed. *Aruin and Latash, 1995*.

Intra-limb APAs

As mentioned above, *intra-limb* APAs are distributed on muscles of the same limb in which the movement occurs. Examples of intra-limb APAs are reported for shoulder and elbow movements (Almeida et al., 1995; Gribble and Ostry, 1999; Hopf and Hufschmidt H. J., 1963) and wrist flexions (Aoki, 1991). Caronni and Cavallari (2009) reported the flexion of index-finger, in which an APA chain develops in several upper-limb muscles to stabilize the segmental equilibrium of the arm. Indeed, these authors described that with the prone hand a brisk finger flexion (Figure 8) was preceded by an excitatory burst in Extensor Carpi Radialis (ECR), Triceps Brachii (TB) and Superior Trapezius (ST) and by a concomitant inhibitory burst in Flexor Carpi Radialis (FCR), Biceps Brachii (BB) and Anterior Deltoid (AD). The coupled activities of ECR-FCR contrasted the wrist flexion torque due to the voluntary contraction of the index-finger flexors. At the same time, the coupled activities of TB-BB and ST-AD contrasted the elbow and shoulder flexion torques produced by the perturbation of the index-finger flexion discharged on the metacarpophalangeal (MP) joint.

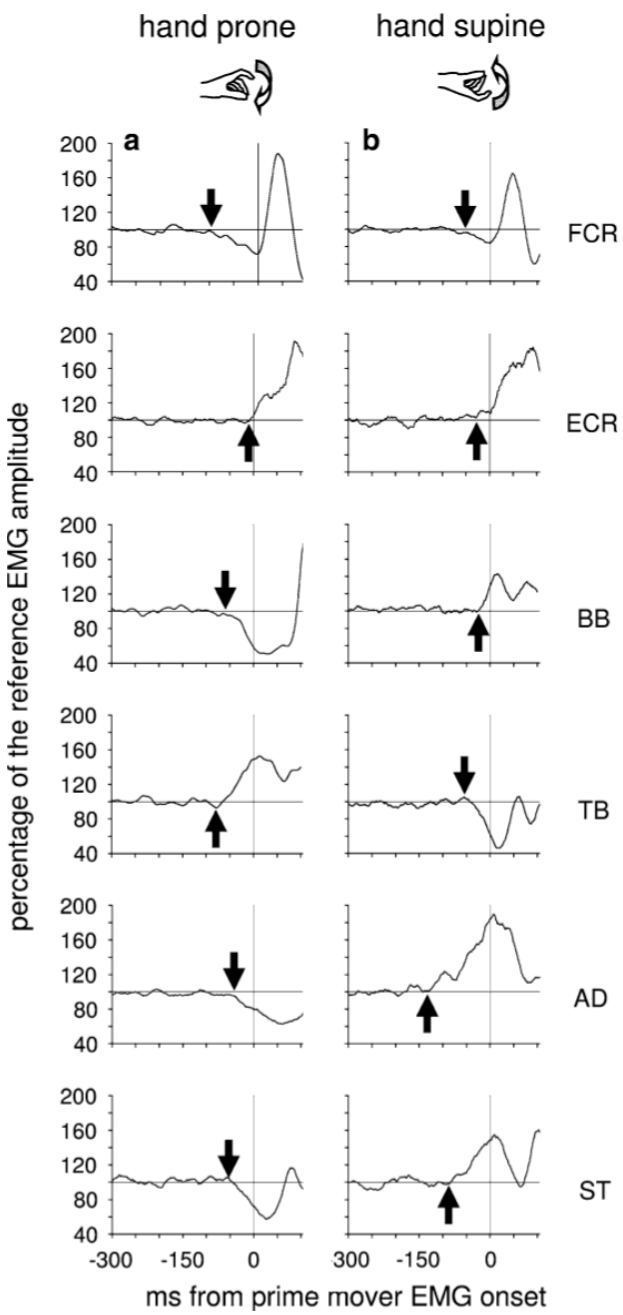

Figure 8. Intra-limb APAs in upper-limb in an index-finger task with the prone or supine hand. The graphs show the APA pattern on the tonic EMG recorded from postural muscles, of a representative participant, with the prone (a) or with the supine (b) hand. Postural muscles recorded are Flexor Carpi Radialis (FCR), Extensor Carpi Radialis (ECR), Biceps Brachii (BB), Triceps Brachii (TB), Anterior Deltoid (AD), Superior Trapezius (ST). The APA onset is marked with black arrow. The vertical line at 0°ms shows the onset of the prime mover activity. The graphs display that APAs in the muscles of elbow, shoulder and trunk, revert in sign when the hand changes from prone to supine. EMG is rectified, integrated and averaged with 75 trials and its size expressed in percentage of the mean EMG level recorded 1s before the go signal. *Caronni and Cavallari, 2009*.

When the hand posture was changed from prone to supine, this APA pattern reverted in sign in the elbow and shoulder muscles but not in ECR and FCR. Consequently, BB and AD were excited and TB inhibited. So, this study shows the versatility of APAs to adapt themselves depending on the postural context.

Such *intra-limb* APAs not only would guarantee the maintenance of the arm posture, but should also be very important in controlling the trajectory and the final position of the moving segment, i.e. metria (Figure 9).

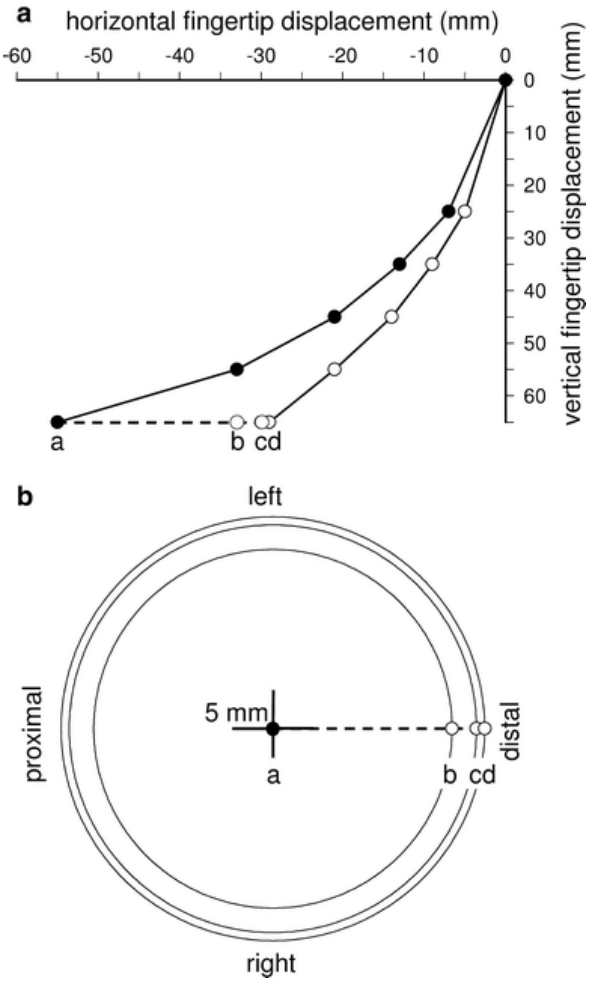

Figure 9. APA and dysmetria. Model simulation of the horizontal fingertip displacement, as function of different strokes, when the proximal segments are immobilized (**a**, fictive APAs, *filled circle*), and when they are free to rotate (**a**, *empty circle*). Note that for a vertical displacement of 65 mm, the fingertip hits the table surface (*dashed line*) more proximally with APAs (*a*) then when no-APAs are involved (*d*). Dots b and c mark the hitting position when the APAs concern the sole shoulder or shoulder plus elbow, respectively. In the planar graph (**b**), in which also lateral segment displacements are considered, the *filled circle* is the univocal target position resulting from a fully correct APA control, a disturbance of the APAs chain would necessarily produce the impact of the fingertip in any other point. *Caronni and Cavallari 2009*.

Actually, when simulating an index-finger flexion using a four-joint software mechanical model of the arm in which only the prime mover was recruited, a clear disturbance of both focal movement and upper-limb posture was observed, with relevant changes at wrist and elbow level. This would affect the final position of the intentional finger movement. In the model, the only way to prevent these effects was to block all segments except the finger, preventing the proximal joints from rotating (fictive *intra-limb* APAs). Since this observation derived from a very simplified system, Caronni and Cavallari (2009) also looked for a more realistic situation: a finger tap was electrically evoked in a real arm by stimulating the median nerve; such an experiment showed recordings comparable in sign and size to those predicted by the software mechanical model including the dysmetric motor output (Figure 10). However, both the software simulation and the electrically evoked tap paradigms did not faithfully represent the "natural" dysmetric behavior, since in the two cases no voluntary command is modelled or generated, respectively.

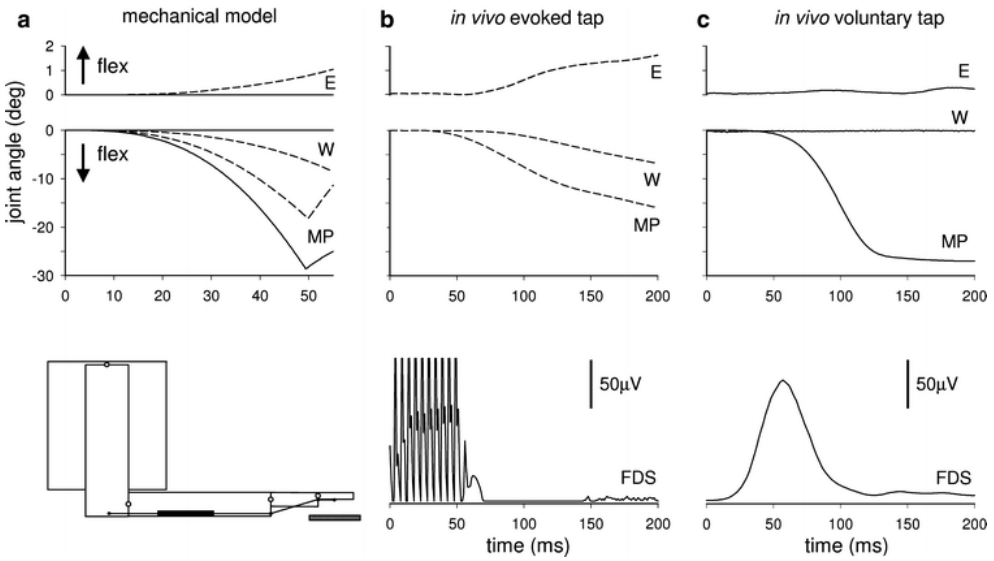

Figure 10. Angular displacements at metacarpo-phalangeal, wrist and elbow joints during simulated, evoked and voluntary index-finger tap. Time course of a simulated finger flexion at the MP joint and the related changes at wrist (W) and elbow (E) level, all in degree of angular rotation, measured when segments were free to rotate (a, dashed lines) or immobilized (a, fictive APA, solid lines). The modeled arm is sketched in the bottom left inset. Angular displacements of the three joints were also recorded when an index-finger tap was passively evoked in vivo by the median nerve electrical stimulation (b) and when it was voluntary performed (c). Rectified FDS activity in the two lower graphs. Note that the mechanical model well predicts the displacements of the proximal joints both during passive (solid lines) and during voluntary (dashed lines) index-finger tap. *Caronni and Cavallari 2009*.

Kinematic aspects

In spite of the different classification about *intra-limb* and *inter-limb* APAs, they share several behavioral characteristics, in addition to work on several muscles preventing the effects of the interaction torques generated by primary motors. *Intra* and *inter-limb* APAs, as mentioned above, revert in sign when movement direction is reverted (Aruin and Latash, 1995; Caronni and Cavallari, 2009b), moreover, they adapt to changes in the postural context, in

movement speed within few trials of movement repetition (Bruttini et al., 2014; Esposti et al., 2015; Hall et al., 2010) and they have a link with the movement precision (Bruttini et al., 2016; Caronni et al., 2013). Furthermore, APAs are influenced by the availability of visual information (Esposti et al., 2017) and this effect is also important on the maintenance of the whole-body balance (Krishnan et al., 2013).

Indeed, postural context affects APAs a lot and its characteristics depend on both the physical properties of the support base and the interface between the body and the support. The first depends on the characteristics of the environment in which we are moving: properties and geometrical parameters of the available fixation points, such as flatness or curvature, inclination and stability of the support bases. The second depends on our own body posture, i.e. depend on whether we are firmly standing on two feet or in an unstable posture on only one. Indeed, the amplitude of the anticipatory postural adjustments is affected by the whole body stability at the time of the movement execution. APAs are reduced in size when performing a movement in a stable postural context. A view that agrees with the arm-pull experiment in standing subjects by Cordo and Nashner (1982) in which the Soleus APAs were strongly reduced when adding a fixation point to the trunk. Furthermore, the length of the APA chain depends on the position of the fixing point to which it is anchored. In this experiment the fixation point is located in proximity of the moving segment, thus shortening the fixation chain length. If the condition in which the subject is unstable, i.e. when reducing the support base area, APAs are usually reduced in amplitude. Indeed, since the APA themselves determine movement (Bouisset and Do, 2008), when the support base is small the APA themselves could cause a CoM displacement,

potentially threatening the whole body balance. The importance of an adequate support base to ensure a reliable fixation point for the APA chain is suggested by Dietz & Colombo (1996), who showed that no APAs in lower limbs could be observed when performing push/pull movements when the body fully immersed in water. It is thus apparent that moving without any fixation point is not an adequate condition for the APA chain to develop.

Moreover, APAs are known to have a link with movement precision. In a study of Caronni et al. (2013) on *inter-limb* APAs during an upper limb pointing movement wearing or not with prismatic lenses, APAs were modified to obtain an accurate voluntary movement. As regards *intra-limb* APAs, Bruttini et al (2016) have studied the role of APAs in movement precision comparing pointing movements performed with the preferred vs. non-preferred hand. When moving the non-preferred side, APAs are delayed. This delay was associated to an impaired stability of the elbow joint during the wrist pointing movement and the perturbation of voluntary movement caused an increased elbow excursion in the non-preferred hand, eventually leading to the diminished movement precision on that side. Furthermore, also training leads improvement the performance of APAs chain associated to prime mover recruitment (Kanekar and Aruin, 2014). In fact, in some motor behaviors, an optimal stabilization is needed to achieve an effective performance. As in the case of professional pianists which seek to play using less muscular activity and take greater advantage on shoulder joint rotation (Furuya and Altenmüller, 2013). APAs are not the only determinant of movement precision. Considering that the parietal cortices play a critical role in integrating visual and somatic inputs for building up this sensorimotor transformation, an error in reaching the target can be due not only to APAs

modified, but also to an incorrect sensorimotor transformation, from the visual representation of the target to the kinematics representation of the planned trajectory (Soechting and Flanders, 1989). Thus, the central nervous system seems to use the same organization of the motor command for controlling both the segmental and the whole-body posture.

As regards the influence of the visual information availability on APAs, when exposing subjects to an external perturbation induced by an aluminum pendulum attached to the ceiling with or without holding onto a walker, no significant differences in the APAs activity were found when a full vision was available. It has also been illustrated the importance of visual acuity in a correct tailoring of APAs (Mohapatra et al., 2012). Indeed, the anticipatory postural control changes when asking the subject to wear eye-glasses with negative or positive powered lenses. The above described literature suggests that the CNS rely upon vision for tailoring a correct APA pattern when it has to counteract an external perturbation or when it has to deal with a voluntary movement oriented to a target, thus interacting with external objects. According to literature, in a well-lit environment with a firm base of support, healthy individuals, which are asked to maintain a bipedal up-right stance, rely on a combination of somatosensory (70%), vestibular (20%) and only 10 % of visual feed-back (Peterka, 2002). For what regard APAs, it has been shown that cutaneous inputs provide sufficient information to plan the anticipatory postural adjustments for gait initiation (Mouchnino and Blouin, 2013). These observation suggest that proprioceptive inputs are sufficient to modulate the APA pattern that precede gait initiation.

Neural organization of APA

A shared command for postural and prime mover muscles

From classical literature, it is known that the prime mover activity and the related postural adjustments (Anticipatory Postural Adjustments, APAs; Synchronous Postural Adjustments, SPAs and Consecutive Postural Adjustments, CPAs) of the muscles generating the postural chain, come from two separate central commands (Hess, 1943). In this regard, Babinsky (1899) has described the motor system with two kinds of organization, one referring to movement and the other one to posture. The focal component, therefore, concerned the body segments involved in performing motor action, while the postural one was referred to the stabilizing reactions of the whole body.

However, at least for what regards APAs, this *dual command hypothesis* has been increasingly replaced by the idea that postural and prime mover muscles are both controlled by a *shared motor program* (Aruin and Latash, 1995; Caronni and Cavallari, 2009b). In this regard, a key observation has been carried out by Bruttini et al. (2014), who showed that even when the focal movement does not occur because the prime mover innervation is blocked by local ischemia or anaesthesia, overt APAs are nevertheless produced along the fixation chain whenever the subject tries to move. Thus, APAs are produced each time the voluntary motor command is delivered, even if no perturbation occurs because the prime mover is paralysed, strongly supporting the oneness of the postural and voluntary motor commands.

Further support to this view comes from the observation that when correcting an ongoing arm pointing movement the CNS is able to create a predictive mode of postural control adapting consistently the postural muscle

activities before modifying the prime mover recruitment (Gritsenko et al., 2009; Leonard et al., 2011). In these studies, the postural corrections are described as parts of the voluntary movement, rather than aimed to ensure the maintenance of equilibrium of body. The performance of voluntary movements of standing human subjects is always accompanied by adjustments in their posture. In most cases, the muscles responsible for those postural changes anticipate the ones acting as prime movers, indicating that a feedforward type of neural control is involved also in these cases.

Neural structures governing APAs

Inter-limb APAs and *intra-limb* APAs don't share only several kinematic aspects but also several neural structures involved in their programming.

The involvement of *Primary Motor Cortex* was studied through the Transcranial Magnetic Stimulation (TMS) to induce a silent period in either the left or the right M1 while the subject abducted his left arm, a movement preceded by APAs in the contralateral *Latissimus Dorsi muscle* (Palmer et al., 1994). The stimulation of the left motor cortex, ipsilateral to the prime mover and contralateral to the postural muscle, produced a delay of the APA onset, while the prime mover timing was unmodified. When the stimulation was carried out on right M1, the prime mover activation was delayed and APAs were slightly modified. So, in this case, the motor cortex showed to play an important role for both APA organization and prime mover generation.

Moreover, it has been shown for both *inter*- (Petersen et al., 2009) and *intra-limb* APAs (Caronni and Cavallari, 2009a) that when adding a support point that relieves a muscle from the fixation chain, even if the excitability of

its spinal motoneurons is not modulated during the period of motor preparation, the cortical excitability in the M1 representation of that muscle actually changes, depicting the time course of the overt APA produced when the muscle is involved in the fixation chain. This actually proves that both the postural and the voluntary motor commands pass through M1.

Studies about anatomical structure linkage with APAs suggest that *Supplementary Motor Area* (SMA) has an important role. Indeed, APA impairments were observed in patients with a lesion of the SMA during the *barman task* (Massion et al., 1999).When the load was held with the forearm contralateral to the lesioned SMA, APAs were damaged compared to patients with normal SMA but with impairment in a callosal section. More recently, a study carried out with the Transcranial Direct Current Stimulation (tDCS) over the SMA has shown a the involvement of SMA in modulating APAs amplitude without influencing the primary movement (Bolzoni et al., 2015). In the *shared motor command* perspective (see previous paragraph) this means that the two originally united command flows toward postural and prime mover muscles bifurcate before SMA.

Schmitz et. al (2005) reported the involvement also of *Sensorimotor areas*, using functional Magnetic Resonance Imaging (fMRI), while Schepens (2004) suggested the role of *Pontomedullary Reticular Formation* (PMRF) in the coordination of posture and movement. PMRF, site of integration of signals from both cortical and subcortical structures, is able to mediate APAs in time and magnitude to optimize motor control of posture and movement.

Other neural structures involved in APA organization and on which we wanted to focus attention are b*asal ganglia* and *cerebellum*.

A study on neuromagnetic brain activity has shown that during the bimanual load-lifting task, APA inhibitory activities in BB of the load-bearing

arm, were associated to the cerebral activity of the precentral gyrus, basal ganglia, SMA and thalamus, contralateral to the arm holding weight (Ng et al., 2013). These areas are important component of the *basal ganglia-thalamo-cortical motor network*, implicated in well-learned finger movements (Boecker et al., 2008). As a result, this network and the neural structures associated are necessary for generation of APAs and also for voluntary motor command confirming the hypothesis of a oneness of the motor command for both posture and primary movement.

It is worth noting that the cerebellum is involved in motor coordination and postural control. So, a cerebellar lesion disrupts the coordination between voluntary movement and equilibrium stabilization (Babinski, 1899) as such structure is effectively used to overcome time delays associated with feedback control (Imamizu et al., 2000; Wolpert and Kawato, 1998). Several studies suggest that the cerebellum contains forward internal models that could predict the consequences of an action. Cerebellar impairments are associated with a normal pattern of inter-limb APAs, but with abnormalities in their timing (Diener et al., 1992); the latter results was also confirmed in intra-limb APAs by Bruttini et al. (2015), who demonstrated that when performing a brisk index-finger flexion, cerebellar subjects showed a timing-disruption of intra-limb APAs, while their pattern was unmodified.

RESEARCH HYPOTHESIS

Taking into account the role of anticipatory postural adjustments in optimizing the voluntary movement performance, this thesis aims to further clarify and deepen some aspects of the Anticipatory Postural Adjustments (APAs) organization and the neural structures generating them. As mentioned above, at the present time there are only a few studies on neural structures involved in APAs, such as the primary motor cortex, the supplementary motor area, the sensory motor areas and the pontomedullary reticular formation. Some of these works correlated some neurological disease with APAs changes elucidating not only the knowledge regarding these pathologies, but also the relationships between the structures and the APA command.

In this context, subcortical structures which have an important role in refining the voluntary movement are the basal ganglia. Actually, the pathologies associated to their dysfunction are known to be correlated with an impairment in *inter-limbs* APA control (Lee et al., 1995; Viallet et al., 1987). The first goal upon which we wanted to focus our attention is the lack of studies about *intra-limb* APA linked to basal ganglia impairment. So, *intra-limb* APAs stabilizing the arm and the forearm before index-finger flexion (Caronni and Cavallari, 2009b) were recorded in patients suffering Parkinson's disease (as model of basal ganglia dysfunction) and in healthy subjects. From this experiment, we would have expected, as we have found, that the impairment of basal ganglia lead to a change in the pattern of APAs, compromising the kinematics parameters of voluntary movement.

Secondly, the investigation about the structures contributing to APAs also concerned the cerebellum. As reported in the last paragraph of the Introduction, adult cerebellar patients showed similar timing-disruption in both inter- and intra-limb APAs (Bruttini et al., 2015; Diener et al., 1992). To what extent does the development of such structure affects the APA control? To answer this question, the research was directed to compare postural and gait initiation parameters in children affected by Congenital Cerebellar Ataxia, CCA) to those obtained in healthy subjects of comparable age. CCA group was further classified into those affected by the non-progressive Joubert Syndrome (NonP) and those with an unknown or slowly progressive diagnosis (SlowP). Moreover, an electromyographic analysis was carried out from lower limb and trunk muscles involved in APA stabilization. Surprisingly enough, although clinical evaluation of motor impairments was similar in NonP and SlowP, only the latter showed a clearly worse postural behavior than H. Indeed SlowP were less stable in standing and showed more severe timing disruption in lower limb and back muscles activities when starting gait, paralleled by a shorter and slower first step. Such different behavior between SlowP and NonP children is probably linked to a different consolidation of compensatory strategies.

As mentioned above, the cerebellum has a fundamental role in movement coordination but recently it has been considered as involved also in emotion and cognition (D'Angelo and Casali, 2012), two aspects which may affect the movement performance which, in turn, has been shown to depend also on the postural control (Bruttini et al., 2016). In fact, it is known that postural control is an adaptive process affected by many aspects of human behavior. By the way, there are several studies which showed the influence both of emotional and cognitive aspects in balance control and during a

movement performance (Doumas et al., 2018; Gélat et al., 2011; Zaback et al., 2019). Therefore, it is interesting to investigate the cognitive areas which may affect the postural control. Recent studies have confirmed the influence of cerebellum in cerebral processes mediated by the cerebro-cerebellar loop. Most of the loop streamlines involves cerebral associative areas and their cerebellar cognitive counterpart. Many cerebellar lesions lead to clinical motor disorders, as in motor ataxic syndromes, but they are also the neural basis of cognitive disorders which involve impairments in executive, visual-spatial and linguistic abilities. To investigate the cerebellar areas involved in cognitive processes, we focused our attention on the lateral Crus I-II, known cerebellar cognitive areas (Stoodley et al., 2012), and investigated their activity level by assessing the production of cerebellar metabolites by Magnetic Resonance Spectroscopy (MRS). Firstly, we wanted to create a standard range of concentrations in lateral Crus I-II of healthy subjects. In order to avoid that the metabolites quantification was contaminated by tissue composition, such effect was corrected by means of segmentation of T1-weighted brain images. Moreover, we tested the reproducibility of the acquisition protocol in order to estimate the total variability and validate the MRS protocol for investigating metabolic changes in neurological diseases affecting cerebellum. At the moment, the application of such protocol on cerebellar patients is on stand-by, searching for a more easy available MRS facility.

In parallel however, the “cognitive” approach to APA control led us to focus on a cortical sensory-motor integration centre within a multimodal network: the Parietal Operculum (PO). Recent studies, indeed, suggested that the PO acts as a “hub” in which converge visual, somatosensory and auditory functional streams and, in turn, PO is connected to motor and premotor areas

(Sepulcre et al., 2012). Considering that *intra-limb* APAs are influenced by primary movement kinematics adapting to postural context (Bruttini et al., 2014) and by the availability of visual information (Esposti et al., 2017), it seemed of interest to investigate the eventual involvement of PO in APAs organization by perturbing this area with a direct current stimulation, applied transcranially. The *intra-limb* APAs associated to index-finger flexion and recorded in healthy subjects before, during and after *anodal* and *cathodal* transcranial Direct Current Stimulation (tDCS) on contralateral PO (coPO), were compared to those obtained in a *sham* session. Since, despite the high power of the statistical design, no significant changes were found in the APA pattern and the focal movement kinematics, we excluded the coPO as a relay of the neural network controlling both APAs and prime mover recruitment.

EXPERIMENTAL DESIGN

The experimental procedures of the two works (**Paper 1,4**) regarding the recordings of *intra-limb* APA before index-finger flexion, share similar methodological features.

Subjects were sitting on a chair with the elbow flexed at 90° and the prone hand in axis with the forearm and they were asked to flex their index-finger at the metacarpophalangeal joint after an acoustic signal. The electromyographical activity of postural muscles (Biceps Brachii, BB; Triceps Brachii, TB and Anterior Deltoid, AD) and prime mover (Flexor Digitorum Superficialis, FDS) were recorded with couples of pre-gelled surface electrodes (H124SG, Kendall ARBO, Tyco Healthcare, Neustadt/Donau, Germany). EMG was AC amplified (IP511, Grass Technologies®, West Warwick, Rhode Island, USA; gain 1–20 k) and band-pass filtered (30–1000 Hz, to minimize both movement artefacts and high frequency noise). Movements of the index-finger at the metacarpophalangeal joint were recorded by a strain-gauge goniometer (mod. F35, Biometrics Ltd®, Newport, UK). Angular signal was DC amplified (P122, Grass Technologies®, West Warwick, Rhode Island, USA) and gain was calibrated before each experiment. Goniometric and EMG signals were then sampled at 1 kHz (in **Paper 4** protocol) or 2 (in **Paper 1** protocol) with 12-bit resolution (A/D converted PCI-6024E, National Instruments®, Austin, Texas, USA).

In **Paper 1**, three sessions of 15 trials recorded in parkinsonian patients were compared to those recorded in healthy subjects. All the EMG traces were digitally rectified and integrated (time constant: 10 ms). The onsets of FDS

EMG were extracted by running a 1-s mobile-window algorithm over the recordings, searching for those positions in which the samples in the 50 ms following the window were all above the mean value +2 SD of the samples within the window. When this criterion was met, the end of the window was considered an onset. In each muscle, all the 45 EMG recordings were then time aligned to the FDS onset and averaged. The onset of index-finger flexion was identified on the averaged goniometric trace by applying the same mobile-window algorithms user for FDS onset, but searching for the window position in which all samples were all below the mean value −2 SD of the samples within the window. Movement amplitude and duration were measured as the amplitude and timing difference between peak index-finger flexion and movement onset, respectively. The onset of an excitatory or inhibitory APA in each postural muscle was searched for on the averaged trace by applying the same moving-window algorithm used for FDS onset; if the samples in the 50 ms following the window were all above the main value +2 SD of those within the window, the APA was recognized as excitatory, while if the samples in the 50 ms were all below the mean value −2 SD, the APA was recognized as inhibitory. If the above criteria failed to identify any onset, the APA was lacking for that muscle.

In **Paper 4**, on the contrary, several sessions of 15 trials were recorded before, during and after an *anodal* or *cathodal* tDCS comparing with each other. All the EMG traces were digitally rectified, then the traces collected while moving the index-finger were expressed in % of the highest average EMG value recorded for 1 s during the subject's MVC monitoring. For each EMG and goniometric variable, the 30 traces recorded in two sequences before the stimulation were time-aligned to the point (trigger) in which finger flexion reached 15° with respect to its resting position and averaged. So, it

was verified that at 15° flexion the index-finger was moving at more than 50% of its peak velocity. The resulting averaged trace extended from 2 s before to 0.3 s after trigger. The same procedure was applied for the traces obtained during and after stimulation. As regards the onset of index-finger movement, the mean level of the signal recorded from 1 to 0.5 s prior to the trigger was subtracted from the averaged trace, then a software algorithm searched the first time point in which the trace fell below –2 SD of the signal in the reference period and remained below that level for at least 50 ms. When the criterion was met, the algorithm searched backward the point in which the trace started to deviate from the mean reference value. Movement amplitude and duration were measured, as the amplitude and timing difference between the peak flexion of index-finger and the onset of its movement, respectively. For each average EMG trace, the period from 1 to 0.5 s before movement onset was assumed as reference. The trace was integrated (time constant= 11 ms) and the mean reference level was subtracted from it; then the onset of an excitatory or inhibitory EMG change was identified by the above-described software algorithm, setting the threshold at +2 SD or –2 SD of the reference signal, respectively. As regards the stimulation, tDCS of 20 min at 2 mA was applied by using a neuroConn® DC-Stimulator Plus (model 0021) connected to two sponge electrodes, soaked with conductive gel. The active electrode (3.16 x 3.16 cm) was positioned on the scalp point closest to the PO identified by means of the Talairach coordinates through a neuronavigation system (Softaxic Optic 2.0). The reference electrode (8 x 12 cm) was positioned on the forehead over the contralateral supraorbital area. Anodal and cathodal tDCS started with a 60 s fade-in period, followed by 20 min DC at 2 mA and a 30 s fade-out. In sham configuration, instead, the 60 s fade-in was immediately followed by the 30 s fade-out. The resulting current density (2

A/m^2) was much lower than the safety limit (25.46 A/m^2) reported on humans (Bikson et al., 2009). Throughout the experiment, it was checked that scalp impedance was constant and never exceeded 5 kΩ (range 1.2-4.2 kΩ).

For the work regarding cerebellum involved in *inter-limb* APAs (**Paper 2**), participants were asked to stand quietly on a force plate for 30 seconds, then start walking after a vocal prompt at their usual speed, self-selecting the leading limb. The dynamometric force plate (sampling frequency of 960 Hz, KISTLER, Winterthur, Switzerland) was used to measure the Center Of Pressure (CoP) displacements. Kinematic data were optoelectronically recorded by means of a motion analysis system (SMART-E, BTS, Italy) with six-cameras (sampling frequency of 60 Hz) using a full body 29 marker set. Electromyographic (EMG) analysis were carried out through wireless probes (sampling frequency of 1000 Hz, FREEEMG 1000, BTS, Italy) used to bilaterally record the muscular activity of Tibialis Anterior (TA), Soleus (SOL), Erector Spinae (ES), Biceps Femoris (BF) and Rectus Femoris (RF). The raw EMG data were high-pass filtered (fcut=50 Hz) with a zero-phase shift, 6th-order elliptic filter to remove movements artifacts; then the signals were rectified. To extract postural parameters during quiet standing, the statokinesigram (the projection onto a 2-dimensional space of the trajectory of the CoP) was used. The main variables calculated were the ellipse area and its eccentricity, the total CoP length, the CoP velocity and the anterior-posterior and medial-lateral CoP displacements. To investigate the gait initiation motor program, two phases (APA phase and the stepping phase) were identified. The APA phase was evaluated measuring the duration of imbalance and unloading phases, the CoP length, the anterior-posterior (AP) and medial-lateral (ML) shift of the CoP. The stepping phase was evaluated measuring the first step length and the first step velocity. As regards the

muscular activity at gait initiation, the onset of muscles activation was considered as the instant at which the EMG signal deviated more than the mean ±2 SDs of the baseline activity in the temporal window from 200 ms before to 1300 ms after the backward CoP displacement (the movement onset, time 0). For each subject, all variables were averaged over the trials.

For the cerebellar study on cognitive approach (**Paper 3**), Magnetic Resonance Spectroscopy (MRS) was used to identify and to quantify the cerebellar metabolites in Crus I-II region of a cohort of healthy subjects. MRI data were acquired on a 3T Skyra scanner (Siemens, Erlangen, Germany) with the manufacturer's 32-channel head-coil for signal reception. Structural information was obtained with an MPRAGE T1-weighted 3D sequence with the following parameters: TR/TE/TI = 2300/2.95/900 ms, flip angle = 9°, 176 sagittal slices, acquisition matrix = 256 x 256, in-plane resolution = 1.05 x 1.05 mm, slice thickness = 1.2 mm. The acquisitions of MRS were carried out using a single-voxel (SVS) point-resolved spectroscopy (PRESS) sequence on three different Voxel Of Interests (VOIs): one on cerebellar Gray Matter (GM), Crus I-II, one on cerebellar White Matter (WM) and the last one on WM of the cerebral hemisphere, as reference area. MRS parameters used for this protocol were: TR/TE = 3000/30 ms, 256 spectral points, spectral bandwidth = 1200 Hz; 4 measurements for cerebral VOI (4 spectra; voxel of 2x2x2 cm^3) for a total acquisition time about 5 minutes and 8 measurements (8 spectra; voxel of 1.5x1.5x1.5 cm^3) for the two cerebellar VOIs for a total acquisition time about 20 minutes. To determine tissue composition of each VOI, the structural T1-weighted images were segmented into GM, WM and cerebrospinal fluid (CSF) using the statistical parametric mapping (SPM12) toolbox Segment (http://www.fil.ion.ucl.ac.uk/spm/). Using in-house written software, MATLAB R2017b, VOI geometry information was used to create

corresponding binary masks in the same space of each individual's segmented T1-weighted image. Spectroscopy data were analyzed using two freely available software packages: JMRUI 5.2 and TARQUIN 4.3.10.

RESULTS

In **Paper 1**, the comparison of *intra-limb* APA pattern and latencies and focal movement kinematics between healthy subjects and parkinsonian patients showed significant differences.

Indeed, index-finger movement was smaller and more delayed in subjects with Parkinson disease than in healthy participants. As regards postural muscles, the APA pattern recorded in healthy subjects was in agreement with the literature (Caronni and Cavallari, 2009b): the FDS onset was preceded by an inhibitory APA in BB and in AD, and simultaneously by an excitatory APA in TB. In parkinsonian subjects, four of them didn't show any BB *intra-limb* APAs and five of them developed an APA excitation in BB instead of an APA inhibition. So, the *intra-limb* APA pattern in parkinsonian patients seems to be disrupted. This observation reinforces the hypothesis that basal ganglia may not only contribute to shaping the focal movement, but also play a key role in *intra-limb* APA organization.

As regards *inter-limb* APAs work (**Paper 2**), the analysis of static posturography showed some differences between ataxic and healthy children. Indeed, the pathological group showed an ellipse area greater than the controls due to the greater CoP displacement in medio-lateral direction, however this difference was statistically significant only in SlowP subjects. The spatial and temporal parameters in the imbalance and unloading phases were not significantly different between the groups; while, differences were found on step execution with a reduction of first step length and velocity in SlowP. The

electromyographic analysis revealed a muscular pattern characterized by an inhibitory postural chain involving Erector Spinae, Biceps Femoris and Soleus, followed by an excitatory chain in Rectus Femoris and Tibialis Anterior, similar in all subjects. However, SlowP showed more severe timing disruption in lower limb and back muscles activities when starting gait, confirming alterations in the timing of APAs in cerebellar patients (Bruttini et al., 2015).

As regards MRS study (**Paper 3**), we have created the boxplots of metabolites concentration in specific cerebellar areas to have a standard range of concentration on this cohort of subjects enrolled. The mean values of raw and corrected metabolites concentration obtained segmenting the volumetric T1-weighted images and correcting for cerebrospinal fluid contamination and relaxation, were significantly different, while their coefficients of variance resulted comparable for each VOI. To investigate the reproducibility of MRS protocol, the Interclass Correlation Coefficient (ICC) for each metabolite, for each VOI, was calculated using data obtained with the test-retest protocol. ICC values that estimate the fraction of the total variability due to biological differences between subjects, range from 0 to 1. ICC values smaller than 0.5 suggest that the variability due to measurement errors is bigger than that of biological differences, while values greater than 0.5 suggest that biological variability in the considered cohort of subjects has the greatest role (Grussu et al., 2015). The ICC values suggested that the total variability of protocol was equally due to both the biological variation and the measurement errors. Moreover, the comparison between raw and corrected ICC data did not show any different overall. From these results would emerge that the correction for

the composition tissue contamination did not significantly improve metabolites quantification.

In work on role of PO in APAs control (**Paper 4**), it has been demonstrated that *intra-limb* APA pattern (Caronni and Cavallari, 2009b) recorded before, during and post tDCS on coPO didn't show any significant differences not only in the *sham* group but also in *anodal* and *cathodal* polarity. Results obtained with *anodal* and *cathodal* tDCS were comparable to those recorded in *sham* condition suggesting that stimulation of either polarity didn't influence APAs amplitude or latency, as well as the amplitude of prime mover recruitment and index-finger kinematics. Indeed, the present results seem to exclude the hypothesis of coPO involvement in *intra-limb* APA associated to index-finger flexion.

ARTICLE SECTION

PAPER 1

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ORIGINAL RESEARCH
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Disrupt of Intra-Limb APA Pattern in Parkinsonian Patients Performing Index-Finger Flexion

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Voluntary movements induce postural perturbations which are counteracted by anticipatory postural adjustments (APAs). These actions are known to build up long fixation chains toward available support points (*inter-limb* APAs), so as to grant whole body equilibrium. Moreover, recent studies highlighted that APAs also build-up short fixation chains, within the same limb where a distal segment is moved (*intra-limb* APAs), aimed at stabilizing the proximal segments. The neural structures generating intra-limb APAs still need investigations; the present study aims to compare focal movement kinematics and intra-limb APA latencies and pattern between healthy subjects and parkinsonian patients, assuming the latter as a model of basal ganglia dysfunction. Intra-limb APAs that stabilize the arm when the index-finger is briskly flexed were recorded in 13 parkinsonian patients and in 10 age-matched healthy subjects. Index-finger movement was smaller in parkinsonian patients vs. healthy subjects ($p = 0.01$) and more delayed with respect to the onset of the prime mover flexor digitorum superficialis (FDS, $p < 0.0001$). In agreement with the literature, in all healthy subjects the FDS activation was preceded by an inhibitory intra-limb APA in biceps brachii (BB) and anterior deltoid (AD), and almost simultaneous to an excitatory intra-limb APA in triceps brachii (TB). In parkinsonian patients, no significant differences were found for TB and AD intra-limb APA timings, however only four patients showed an inhibitory intra-limb APA in BB, while other four did not show any BB intra-limb APAs and five actually developed a BB excitation. The frequency of occurrence of *normal sign*, *lacking*, and *inverted* BB APAs was different in healthy vs. parkinsonian participants ($p = 0.0016$). The observed alterations in index-finger kinematics and intra-limb APA pattern in parkinsonian patients suggest that basal ganglia, in addition to shaping the focal movement, may also contribute to intra-limb APA control.

Keywords: intra-limb anticipatory postural adjustments, Parkinson disease, basal ganglia, motor control, human

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INTRODUCTION

Anticipatory postural adjustments (APAs) represent a crucial aspect of the voluntary movement organization. Throughout their feed-forward control, APAs are able to limit the displacement of the center of mass (CoM), caused by the interaction forces induced by the voluntary movement. Indeed, such activities build up fixation chains toward the available support point, where to discharge

the interaction forces produced by the voluntary movement, in this way granting the whole body equilibrium (Massion, 1992; Bouisset and Do, 2008). Since these activities usually involve several trunk and limb muscles, they may also be referred to as *inter-limb* APAs (see Cavallari et al., 2016 for a review). However, it has been demonstrated that also movements involving very tiny masses, like an index-finger flexion, are accompanied by APAs (Caronni and Cavallari, 2009). In this case, indeed, specific APAs were observed in arm and shoulder muscles that stabilize the *segmental equilibrium* of the upper limb and optimize the movement performance (Bruttini et al., 2016). Because of their localization with respect to the moving segment, these postural activities were named *intra-limb* APAs (see also Aoki, 1991; Caronni and Cavallari, 2009).

Intra- and inter-limb APAs share not only their principal behavioral features, like the flexibility to adapt to the available support points (Cordo and Nashner, 1982; Bruttini et al., 2014) as well as to the direction and speed of the focal movement (Horak et al., 1984; Aruin and Latash, 1995; Caronni and Cavallari, 2009; Esposti et al., 2015), but also many of the neural structures involved in their control, including primary motor cortex, supplementary motor area, sensorimotor areas (Viallet et al., 1992; Schmitz et al., 2005; Petersen et al., 2009; Ng et al., 2013; Bolzoni et al., 2015). In this regard, some studies correlated neurological diseases with APAs modifications. These experiments not only deepened the knowledge of these pathologies, but also elucidated the structures involved in APAs control. So far, the majority of those studies investigated the effects of pathologies of the central nervous system, like stroke and cerebellar lesions, on inter-limb APAs and on whole-body postural control (Diener et al., 1992; Rajachandrakumar et al., 2016), but the effects of cerebellar lesions was also documented on intra-limb APAs by Bruttini et al. (2015) who reported a disruption of the temporal organization of such postural adjustments. Another subcortical structure that plays a role in movement control is composed of the basal ganglia, and also in this case some studies showed that basal ganglia pathologies correlate with impairments in inter-limb APA control (Viallet et al., 1987; Lee et al., 1995). Since a linkage between basal ganglia and *intra-limb* APAs is still missing, the present study aims to compare the kinematics parameters of the index-finger flexion and the intra-limb APA latencies and pattern between healthy subjects and patients affected by Parkinson disease (PD), assuming the latter as a model of basal ganglia dysfunction.

Considering the well-known role of basal ganglia in shaping the pattern of motor activities driving voluntary movement, one would mainly expect a pattern disruption (i.e., changes in intra-limb APAs sign, excitatory or inhibitory), possibly even associated to a timing alteration.

MATERIALS AND METHODS

Thirteen patients affected by PD (PARKINSON group, mean age 60.8 years ± 9.3 SD, four females) and 10 age-matched healthy subjects (HEALTHY group, mean age 61.4 years ± 6.7 SD, six females) were enrolled in this study. Healthy subjects had no history of orthopedic or neurological disorders.

Individual demographic and clinical parameters of PD patients are reported in **Table 1**. They had no history of orthopedic disorders and followed pharmacological treatments. However, at the time of the experiment, they were in pharmacological wash-out from at least 36 h.

All participants gave written consent to the procedure, after being informed about the nature of the experiment. The experiments were conducted in conformance with the policies and principles contained in the Declaration of Helsinki and were approved by the Ethical Committee of the University of Milan (counsel 5/16 – 15.02.16).

Experimental Design

Participants were tested on the dominant limb; the assessment of the handedness was performed according to Oldfield (1971). Participants were sitting and explicitly asked to keep their back supported, the upper-limb still and both feet on the ground. The non-dominant arm was supported by an armrest while the dominant arm was kept along the body, with the elbow flexed at 90°. The hand was prone, in axis with the forearm, with the index-finger pointing forward (i.e., 180° at the metacarpophalangeal joint) while all the other fingers were hanging freely. Subjects kept the back leaning against the seatback and the feet on the ground. The body position was visually checked by the investigator throughout the experiment.

After an acoustic signal, delivered every 7 s, subjects had to perform a self-paced brisk flexion of the index-finger at the metacarpophalangeal joint. Subjects were specifically instructed to perform the movement at will, so as to exclude any reaction-time effect. Each subject performed 45 movements, divided in three sessions of 15 movements with 5–7 min interval in between, in order to avoid fatigue.

Movement and EMG Recordings

The excursion of the metacarpophalangeal joint was recorded by a strain-gauge goniometer (model F35, Biometrics Ltd®, Newport, United Kingdom), fixed with surgical tape. Angular displacement was amplified by a bridge amplifier (model P122, Grass Technologies®, West Warwick, RI, United States), which gain was calibrated before each experiment.

Electromyographic (EMG) signals were recorded from the prime mover flexor digitorum superficialis (FDS) and from the biceps brachii (BB), triceps brachii (TB), and anterior deltoid (AD) muscles, involved in the upper-limb postural stabilization (Caronni and Cavallari, 2009). After scrubbing the skin with cotton and alcohol, two pre-gelled surface electrodes (model H124SG, Kendall ARBO, Tyco Healthcare, Neustadt/Donau, Germany) were placed on each muscle, 24 mm apart. Electrode placement for BB, TB, and AD muscles followed the SENIAM guidelines (Freriks and Hermens, 1999). For FDS, SENIAM did not provide specific guidelines; however, the same general approach was adopted: the subject kept the arm and forearm in the experimental position and was asked to repeatedly strongly flex one finger at a time, at the metacarpophalangeal joint. Meanwhile, the experimenter palpated his forearm, so as to

TABLE 1 | Demographic and clinical parameters of PD patients.

PARKINSON patient	Age (years)	Disease duration (years)	LEDD (mg)	UPDRS-III total (units)	UPDRS-III upper-limb (units)
1	64	10	610	28	7
2	73	8	560	30	5
3	53	4	640	13	5
4	54	4	555	26	5
5	73	8	1245	11	3
6	76	9	1180	17	2
7	62	9	772	16	5
8	49	11	910	28	7
9	53	4	560	19	5
10	62	12	994	31	6
11	57	6	455	14	5
12	49	3	540	7	2
13	66	5	340	11	2
mean ± SD	60.8 ± 9.3	7.1 ± 3.0	720 ± 280	19.3 ± 8.3	4.5 ± 1.8

The Levodopa Equivalent Daily Dose of the pharmacological treatment (LEDD) was determined according to Tomlinson et al. (2010). Patients' evaluation with the Unified Parkinson Disease Rating Scale motor part (UPDRS-III, cfr. Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003) is also provided, both as total score and as the dominant upper limb sub-score.

isolate the belly of the FDS from that of the surrounding muscles. Electrodes were then placed on the FDS belly, at about 1/3 of the distance of the wrist from the cubital fossa. The selectivity of the EMG recordings was verified by checking that activity from the recorded muscle, during its phasic contraction, was not contaminated by signals from other sources. The EMG signals were amplified (gain 2–10 k) and band-pass filtered (30–1,000 Hz, to minimize both movement artifacts and high-frequency noise) by four differential amplifiers (model IP511, Grass Technologies®, West Warwick, RI, United States).

Conditioned goniometric and EMG analog signals were then sampled at 2 kHz with 12-bit resolution by an A/D board (model PCI-6024E, National Instruments®, Austin, TX, United States), visualized online and stored for further analysis.

Data Analysis

Each EMG recording was digitally rectified and integrated (time constant: 10 ms). The onsets of FDS EMG were extracted by running a 1-s mobile-window algorithm over the recording, searching for those positions in which the samples in the 50 ms following the window were all above the mean value +2 SD of the samples within the window. Whenever this criterion was met, the end of the window was considered an onset; all onsets were visually validated. In each muscle, all the 45 EMG recordings were then time aligned to the FDS onset and averaged, so as to obtain an average trace extending from −2000 to +300 ms from the FDS onset, which was then considered time 0; the same was done for the 45 goniometric traces. All subsequent measurements were taken on the averaged traces.

The onset of index-finger flexion was identified on the averaged goniometric trace by applying the same mobile-window algorithm used for FDS onset, but searching for the window position in which all samples in the 50 ms following the window were all below the mean value −2 SD of the samples within the window. Movement amplitude and duration were then measured, respectively, as the amplitude and timing difference between peak index-finger flexion and movement onset. The mean values and variability of the movement latency, amplitude and duration were compared between PARKINSON and HEALTHY groups by means of unpaired *t*-tests and Levene's tests, respectively. Whenever Levene's test was significant, the *t*-test for the corresponding variable was corrected for unequal variances estimates.

The onset of an excitatory or inhibitory APA in each postural muscle was searched for on the averaged trace by applying the same moving-window algorithm used for FDS onset; however, the search was stopped at the movement onset, in order to avoid any effect due to re-afferentation triggered by the focal movement. In case an onset was found, if the samples in the 50 ms following the window were all above the mean value + 2 SD of those within the window, the APA was recognized as excitatory, while if the samples in the 50 ms were all below the mean value −2 SD the APA was recognized as inhibitory. If the above criteria failed to identify any onset, it was concluded that the APA was lacking for that muscle. The mean values and variability of the APA latencies, for each postural muscle, were compared between PARKINSON and HEALTHY groups by means of unpaired *t*-tests and Levene's tests, respectively. Data from patients in which the APA was lacking or had an inverted sign (e.g., excitatory instead of inhibitory) with respect to that observed in healthy subjects (in which APAs always have the same sign, see Results), were excluded from the comparisons.

The pattern of APAs for each postural muscle was assessed in each group by counting the number of participants that showed an inhibitory, excitatory, or lacking intra-limb APA. The frequency of occurrence of the three above outcomes was then compared in the PARKINSON vs. HEALTHY group by the Freeman-Halton extension (2 groups × 3 categories) of the non-parametric Fisher Exact test.

For the sake of completeness, as secondary measurements, linear correlations were tested between the intra-limb APA latencies in the PARKINSON group and each of the following demographic and clinical parameters: patient's age, disease duration, Levodopa Equivalent Daily Dose of the pharmacological treatment (Tomlinson et al., 2010) and Unified PD Rating Scale motor part (UPDRS-III, cfr. Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease, 2003; both total score and upper-limb sub-score). Data from patients in which APA was lacking or inverted were excluded also from these analyses. Non-parametric Spearman's R correlation was evaluated between the sign of intra-limb APAs (−1 when inhibitory, +1 when excitatory, and 0 when lacking) and those same parameters.

For all tests, statistical significance was set at $p < 0.05$. All relevant data for the statistical analyses drawn in this study are included in the manuscript, either in **Figure 2** or in **Tables 1**, **2**.

RESULTS

Figure 1 illustrates the EMG and kinematics recordings obtained in one representative healthy participant (HEALTHY) and two PD patients (PARKINSON A and B), who were representative of a normal APA pattern in the three recorded postural muscles and of an altered pattern in BB, respectively. Taking the onset of prime mover FDS EMG as time reference, the index-finger flexion occurred with a lower delay in the healthy participant (∼25 ms) than in both patients (∼70 ms in A and ∼40 in B). With regard to postural muscles, in the healthy participant the activation of FDS was preceded by an inhibitory intra-limb APA in BB and AD, whose activity was reduced with respect to the mean reference level, and almost simultaneous to an excitatory intra-limb APA in TB. Postural activities of similar sign, even if slightly delayed, could be observed also in the PD patient A, while patient B showed a change in pattern, as the intra-limb APA in BB muscle was reversed (excitatory instead of inhibitory).

Kinematics Parameters

The individual latencies of index-finger flexion in HEALTHY and PARKINSON participants are illustrated in the upper panels of **Figure 2**, while inferential statistics are plotted in the lowermost panel. Compared to healthy participants, the latency of movement onset was larger in PD patients and showed a greater between-subjects variability. Statistical analysis confirmed such results, both with regard to mean values (*t*-test $t_{16.27} = 2.911$, $p = 0.010$) and to variability (Levene's $F_{1,21} = 8.677$, $p = 0.0077$). Movement amplitude and duration are reported in **Table 2**, both as individual values and as mean ± SE. Also mean amplitude was significantly lower in PARKINSON vs. HEALTHY participants ($t_{21} = 5.030$, $p < 0.0001$), but with no significant differences in between-subjects variability ($F_{1,21} = 3.118$, $p = 0.0919$). Movement duration, instead, was comparable both in mean value ($t_{21} = 0.686$, $p < 0.5004$) and variability ($F_{1,21} = 1.326$, $p = 0.2624$).

Intra-Limb APA Latency

The individual latencies of intra-limb APAs in HEALTHY and PARKINSON subjects are illustrated in the central panels of **Figure 2**. The fact that in all participants the identified APAs had a lower latency with respect to the index-finger movement witnesses the anticipatory nature of the postural muscles recruitment. Inferential statistics are plotted in the lowermost panel, showing that the latencies of intra-limb APAs in TB and AD muscles were comparable, both in mean value and variability, between the PARKINSON and HEALTHY groups. This was statistically confirmed by *t*-tests (for TB, $t_{20} = 0.510$, $p = 0.6154$; for AD, $t_{19} = 0.372$, $p = 0.7137$) and Levene's tests (for TB, $F_{1,20} = 1.036$, $p = 0.3208$; for AD, $F_{1,19} = 2.857$, $p = 0.1073$). Note that the lowermost panel and the related statistical comparisons

TABLE 2 | Individual values of movement amplitude and duration in the HEALTHY and PARKINSON groups, together with the corresponding mean value ± SE.

HEALTHY subject	Movement amplitude (°)	Movement duration (ms)	PARKINSON patient	Movement amplitude (°)	Movement duration (ms)
1	81.8	222	1	31.5	64
2	87.9	220	2	50.2	96
3	83.7	177	3	49.3	115
4	69.4	220	4	34.8	284
5	42.7	144	5	38.6	177
6	68.2	208	6	36.1	253
7	86.7	216	7	67.2	196
8	90.2	209	8	52.2	255
9	53.5	63	9	47.0	174
10	58.5	196	10	55.4	138
			11	33.4	115
			12	38.1	150
			13	34.3	196
mean ± SE	72.2* ± 5.2	187.5 ± 15.8	mean ± SE	43.7* ± 3.0	170.2 ± 18.4

*Significant differences between the two groups are marked by *.*

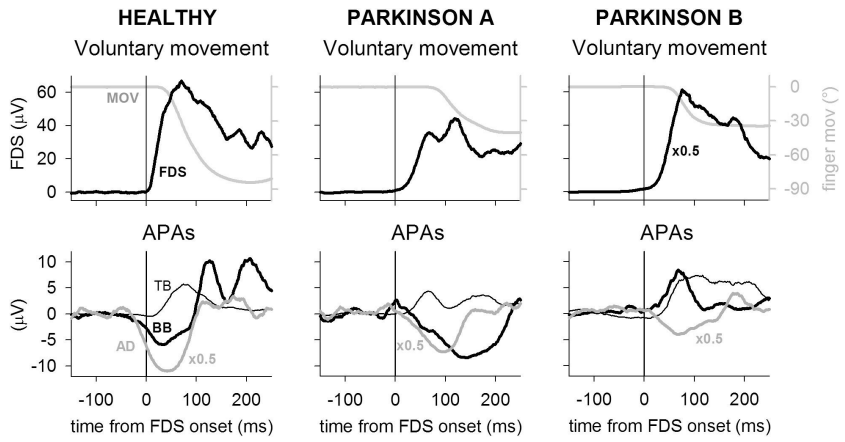

FIGURE 1 | Recordings from one representative participant of the healthy group (HEALTHY) are compared to recordings of two PD patients (PARKINSON A and B). Each trace is the average of 45 recordings, time aligned on the onset of the prime mover flexor digitorum superficialis (FDS). In each participant, top panel shows the FDS activation, matched to the ensuing finger flexion (MOV); bottom panel illustrates the intra-limb APAs in TB, BB, and AD. For illustration purpose, the mean reference signal level, recorded from 750 to 250 ms before FDS onset, has been subtracted from each EMG trace. FDS amplitude has been down-scaled by a factor 2 in patient B and AD amplitude has been down-scaled by a factor 2 in all participants. Note that in the healthy participant the FDS activation was shortly followed by index-finger flexion but preceded by inhibitory intra-limb APAs in BB and AD and almost synchronous with an excitatory intra-limb APA in TB. In both PD patients, finger flexion delay was larger than in the healthy participant, while APAs timing actually showed a delay. However, while patient A produced the same intra-limb APA pattern observed in healthy individuals, patient B had an inverted intra-limb APA in BB, which underwent excitation instead of inhibition.

regarded data from all HEALTHY subjects (gray solid bars) vs. data from those PARKINSON patients which had an intra-limb APA of the same sign normally observed in HEALTHY subjects (black solid bars). Data from those patients in which the intra-limb APA was lacking (marked by an "X" in the central panels) or inverted (white bars) were excluded from the analyses. Because only four PD patients had an intra-limb APA of normal sign in BB, thus resulting in a very insufficient sample size, latency data from that muscle were excluded from statistics. For the same reason, it was not feasible to subdivide the latency comparison into different subgroups.

Inter-Limb APA Pattern

The central panels of **Figure 2** also illustrate that while all HEALTHY subjects presented intra-limb APAs of the same sign (excitatory in TB and inhibitory in BB and AD, gray bars), in some PD patients the intra-limb APA was lacking ("X") or had the opposite sign with respect to what normally observed in HEALTHY subjects (white bars). While this seldom occurred for TB and AD (one patient lacked APAs in both muscles while another had an inverted APA in AD), it was not the case for BB. For this muscle, indeed, only 4 out of 13 patients had an inhibitory intra-limb APA, while 5 had an inverted APA and in 4 the APA was lacking. The Freeman-Halton extension of the non-parametric Fisher Exact test proved that the frequency of occurrence of *normal sign*, *inverted sign*, and *lacking* intra-limb BB APAs was significantly different ($p = 0.0016$) in the PARKINSON group (4, 5, and 4, respectively) vs. the HEALTHY one (10, 0, and 0).

Secondary Measurements

For the sake of completeness, linear correlations were drawn between the *latency* of intra-limb APAs in TB or AD and the demographic and clinical parameters of PD patients, which are illustrated in **Table 1**. Non-parametric correlations were also drawn between the sign of intra-limb APAs in BB and those same parameters. Such correlations never reached significance (in all cases $p > 0.28$).

DISCUSSION

Present results show that the pattern of intra-limb APAs, that stabilize the arm when briskly flexing the index-finger (excitatory APA in TB and inhibitory in BB and AD), may be disrupted in PD patients, indirectly suggesting that basal ganglia could participate also in intra-limb postural control.

The pattern disruption, in particular the presence of an inverted intra-limb APA, mainly regarded the BB muscle, with sporadic occurrence in AD. One possible explanation is that the PD patients enrolled in this study, despite having received the first diagnosis from 3 to 12 years (**Table 2**), were in an initial stage of the disease, as witnessed by the moderate UPDRS-III scores. This could also justify the lack of significant correlations we observed between the intra-limb APA latencies or sign and the demographic and clinical parameters of PD patients. Another possible reason for the APA sign reversal occurring more frequently in BB than in TB and AD, could be the fact that if one approximates the whole arm as a rigid body, the reactive torque induced by the index-finger flexion

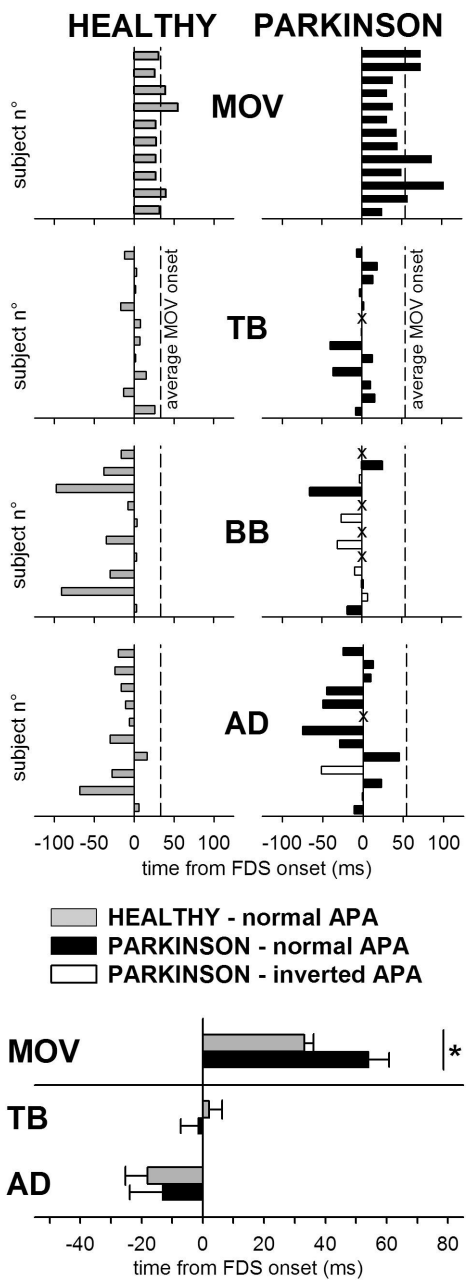

FIGURE 2 | Comparison of the intra-limb APA chain in healthy subjects (HEALTHY) and PD patients (PARKINSON). Latencies of finger flexion (MOV) and intra-limb APAs in TB, BB, and AD are plotted with respect to onset of FDS. Each single participant is represented in the upper panels, where dashed lines mark the average movement latency for either group of participants.
(Continued)

FIGURE 2 | Continued
For PD patients, latencies of intra-limb APAs which had the same sign observed in all healthy individuals (excitatory in TB and inhibitory in BB and AD) are plotted by black bars, latencies of intra-limb APAs of inverted sign are plotted by white bars and lack of intra-limb APAs is marked by "X." Note that in several patients intra-limb APAs in BB were lacking or even inverted. The lowermost panel shows, for both groups, the mean latency (±SE) of the onset of finger flexion and of the intra-limb APAs in TB and AD, excluding data from those PD patients in which the APA was lacking or inverted. The asterisk marks the significant difference. The BB muscle was excluded from the latency comparison because only four patients had an APA of the same sign normally observed in healthy subjects.

should be the same on the elbow and the shoulder, because the lever-arm between the metacarpophalangeal joint and each of those two joints was identical (recall that the upper arm was vertical and hand prone in axis with the horizontal forearm). However, much more mass should be moved in order to flex the arm at the shoulder rather than at the elbow, so that it could be argued that the *TB-BB co-contraction* strategy adopted by some of the patients mainly aimed at increasing the elbow stiffness so as to discharge the perturbation on a larger sprung mass and, consequently, attenuate the unwanted displacement at the shoulder level. Moreover, with regard to focal movement kinematics, present data confirmed that PD patients were slower than age-matched healthy subjects, not only for what regards average speed but also in terms of prime mover recruitment, as witnessed by the longer delay between FDS activation and movement onset. However, such result should not have biased the observed intra-limb APA alteration because a previous study (Esposti et al., 2015) demonstrated that (i) intra-limb APAs are affected by the *intended* movement speed, not the actual one, and in this regard both healthy subjects and PD patients had to move at their fastest speed; (ii) even when moving at 50% of their fastest speed, healthy subjects did never show any reversal of the APA sign. Finally, the control experiments involved a cohort of healthy subjects of comparable age. This was chosen considering that APAs programming is affected by age both in self-initiated movements (Man'kovskii et al., 1980; Inglin and Woollacott, 1988; Rogers et al., 1992; Woollacott and Manchester, 1993) and when APAs are produced in order to respond to an external postural perturbation (Kanekar and Aruin, 2014).

The finding that the pattern of intra-limb APAs may be disrupted in some PD patients adds to the observations carried out in ataxic patients (Bruttini et al., 2015), which showed an altered intra-limb APA timing in absence of significant pattern disruptions. These results suggest a pathophysiological frame that well fits with the known roles of basal ganglia and cerebellum in selecting the correct motor program and temporizing the motor output, respectively (Grillner et al., 2005; Diedrichsen et al., 2007). In this regards, two recent results are also worth noting: first, literature reports proofs that basal ganglia and cerebellum are reciprocally interconnected through the pedunculopontine tegmental nucleus (see Wu and Hallett, 2013 for a review; Mori et al., 2016). The information exchange through these connections could justify the partial overlap observed between the symptomatic framework of cerebellum and

basal-ganglia pathologies (Bostan and Strick, 2018). This has also been observed in intra-limb APAs, indeed Bruttini et al. (2015) reported cases of lacking intra-limb APAs in cerebellar ataxic patients, while signs of altered intra-limb APAs timing in parkinsonian patients are reported in the present paper (see **Figure 1**). Second, it has been reported that Parkinson's disease, especially in its later phase, may also affect the cerebellum (Wu and Hallett, 2013). The same review paper also indicate that the cerebellum activation is abnormally high in PD patients performing various upper limb movements and hypothesize that at the initial stage of the disease the cerebello-thalamo-cortical loop may act so as to compensate for the progressive impairment of the striato-thalamo-cortical circuit (see also Blesa et al., 2007). Consequently, once the parkinsonian degeneration had affected the cerebellum, its compensation would fade-out, leading to a quicker development of the motor impairments.

While many observations confirmed that *intra-* and *inter-limb* APAs share so many behavioral properties that they are seemingly parts of the same phenomenon (Cavallari et al., 2016), it should be noted that our results only partially fit with data on APAs during gait initiation in PD patients. In the latter framework, indeed, some studies reported an altered APA pattern (e.g., Crenna and Frigo, 1991) while other studies reported delayed APAs in the absence of pattern disruptions (Delval et al., 2014). Such discrepancy could stem from the different mechanical context characterizing the two cases: (i) when the voluntary movement is limited to only part of the body, e.g., one or both arms, APAs *counteract the interaction forces* so as to grant that the rest of the body stands still; (ii) when the voluntary movement involves the whole body, like in gait, what are commonly called APAs are instead those actions that *produce the de-stabilizing forces* leading to the movement of the CoM (Jian et al., 1993; Elble et al., 1994; Lepers and Brenière, 1995; see Yiou et al., 2017 for a review). In particular, many studies about gait initiation considered APAs those co-ordinated activities in muscles acting on both ankles (tibialis anterior and gastrocnemius/soleus) that preceded the heel-off, taking the latter as the onset of the focal movement (Crenna et al., 2006; Honeine et al., 2016). However, those actions actually moved the Center of Pressure backward and toward the "future" leading foot, *directly producing* a shift of the CoM forward and toward the "future" trailing foot. Therefore, it might be even proposed that the forward shift of the CoM "*is*" the correct onset of gait, so that APAs should be searched for not before heel-off but before CoM displacement. If so, situations (i) and (ii), described above, appear to be conceptually different, so that a direct comparison of what is classically called APA in the two cases is not feasible.

AUTHOR CONTRIBUTIONS

PC and II conceived the study. II, NP, and UR-P recruited the patients and provided their clinical evaluation. FB, RE, and SM conducted the experiments and analyzed the results. PC, FB, and RE drafted the paper. All authors contributed to and approved the final version.

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PAPER 2

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Postural control in children with Congenital Cerebellar Ataxia

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Abstract: Controlling posture, i.e. governing the ensemble of involuntary muscular activities preserving body equilibrium, represents a demanding function in which the cerebellum plays a key role. Postural activities are particularly important in gait initiation, when passing from quiet standing to locomotion. Indeed such motor task has been repeatedly used for evaluating pathological conditions, including cerebellar diseases.

The linkage between cerebellum maturation and postural control development received less attention. Therefore we evaluated postural control during gait initiation in children congenitally affected by either a slow progressive generalized cerebellar atrophy (SlowP) or a non-progressive vermian hypoplasia (Joubert syndrome, NonP), age-matched with healthy children (H).

Despite the clinical evaluation of motor impairments was similar in NonP and SlowP, only the latter showed a clearly worse postural behaviour than H. Indeed, SlowP were less stable in standing, while in starting gait they showed more severe timing disruption in lower limb and back muscles activities, with a shorter and slower first step.

Such differences might stem from the extent of cerebellar damage, but during childhood the neural plasticity of intact brain areas could compensate even for cerebellar agenesis; we thus propose that the difference may stem from disease progression, which contrasts the consolidation of compensatory strategies.

Keywords: children, gait initiation, postural control, generalized cerebellar atrophy, cerebellar vermis hypoplasia, progressive ataxia, compensatory strategies

1. Introduction

Postural adjustments are involuntary muscular activities, finalized to build-up fixation chains that stabilize the body posture. Such chains are aimed at discharging the mechanical perturbations, which act on the body, down to the available support points and/or at producing a movement counterbalancing the primary voluntary action. Whenever the mechanical perturbations may be estimated beforehand, like when programming a voluntary movement, appropriate postural actions are usually produced in advance of the perturbations themselves, witnessing that such Anticipatory Postural Adjustments (APAs) are programmed in a feed-forward way [1–4].

Postural actions are particularly evident before gait initiation, in which they maintain the body's dynamic balance and create the propulsive forces to move the center of mass forward. Several studies demonstrated the critical role of such actions in postural transitions, showing alterations in those neurological diseases characterized by poor motor control, as Rett syndrome [5] and Parkinson disease [6]. This is of particular interest when examining pathological conditions affecting the cerebellum, which has a well-known role not only in voluntary movement and locomotion, but also in controlling postural actions, where it contributes to modulate sensori-motor interactions and integrate feed-forward and feed-back modes [7].

In this context, the role of cerebellum in postural control was investigated in different works, taking cerebellar ataxia as an experimental model [8–12]. In particular, it is worth to recall the involvement of cerebellum in building up the temporal pattern of APAs. Indeed, patients with cerebellar lesions failed to show a normal anticipatory adjustment in grip force when lifting or moving objects [13,14] and delayed hindlimb APAs have been described in transgenic spinocerebellar ataxic mice [15]. The same applies in humans to upper limb APAs accompanying fast flexions of the index-finger [16].

Less attention has been devoted to the linkage between the development of postural control and the maturation of cerebellum. Aiming at elucidating this topic, we explored gait initiation in children affected by Congenital Cerebellar Ataxia (CCA) vs. a healthy control group of comparable age. CCAs are a heterogeneous group of cerebellar developmental disorders characterized by dysfunctional motor coordination and very early cerebellar symptoms. First clinical signs are a marked hypotonia, wobbling gait, dysmetria, dysarthria and a significant developmental delay. Most children show also marked speech, cognitive and intellectual deficiency. In some cases, the cerebellum degenerates with time, but so slowly that it becomes difficult to classify the disorder as progressive or not [17]. In this framework, we studied a group of children with a generalized cerebellar athrophy and an ascertained clinical and/or radiological diagnosis of slow progression (SlowP). In other cases, the disease has a proven non-progressive course as in Joubert syndrome, which is characterized by a cerebellar hypoplasia limited to the vermis and peduncoli [18]. A second group of patients (NonP) was thus extracted from this kind of pathology.

Gait initiation was chosen as motor task since it is already established at the school-age and the related APAs are well described in adults, both in healthy subjects [19–23] and in patients affected by cerebellar ataxia [24,25]. Should a difference be found in gait initiation parameters and related APAs in CCAs *vs.* age-matched healthy children, this would be fruitful not only to assess the role of cerebellum in the development of postural control, but also in tailoring rehabilitation for these pathologies. Moreover, a different behaviour of SlowP vs. NonP would suggest possible compensation mechanisms; in particular, a better motor behaviour in SlowP vs. NonP could suggest

the involvement of extracerebellar regions, while a better behaviour in NonP could as well stem from a compensatory involvement of the cerebellar hemispheres, which are left unaffected in Joubert syndrome.

2. Materials and Methods

2.1. Participants

CCA participants were recruited at the Istituto Neurologico "Carlo Besta" of Milan: 7 of them had radiographic signs of generalized cerebellar athrophy and an ascertained clinical diagnosis of slow progression (SlowP, mean age: 12±3 years) while the remaining 6 suffered from Joubert Syndrome, i.e. a proven non-progressive pathology (NonP, mean age: 12±3 years). All of them underwent clinical evaluation, including the administration of the Scale for the Assessment and Rating of Ataxia (SARA, [26]), an MRI scan for ascertaining the cerebellar malformation (atrophy and/or hypoplasia) and a genetic screening. In particular, all Joubert Syndrome patients showed a unique cerebellar and brainstem malformation known as the "molar tooth sign" [18]. Demographic and clinical data of each patient are shown in Table 1.

Eight healthy children, free from neurological or psychological pathologies were enrolled as control from the primary school "FAES" in Milan (H, 4 males and 3 females, mean age: 10±3 years).

The experimental procedure was carried out in accordance with the standards of the Declaration of Helsinki. The Ethical Committee of the "Università degli Studi di Milano" approved the study and the written consent procedure (counsel 5/16), which was signed by each participant's parents.

Patient	Age	Gender	Molecular diagnosis	SARA
SlowP_01	9	M	mutation in a candidate gene	8
SlowP_02	8	M	mutation in a candidate gene	14
SlowP_03	12	M	EX0SC3: c.572G>A	15
SlowP_04	13	F	KCNC3: c.1268G>A	17
SlowP_05	13	F	to be evaluated	13
SlowP_06	16	F	ADCK3: c547C>T; c1042C>T	13
SlowP_07	17	M	to be evaluated	18
NonP_01	10	M	NPHP1: c.1358G>T; c.1438-4C>T	12
NonP_02	12	F	to be evaluated	15
NonP_03	18	M	to be evaluated	14
NonP_04	9	M	AHI1: c.1829G>C; c.2671C>T	15
NonP_05	12	F	SUFU: c.1217T>C	11
NonP_06	9	M	SUFU: c.1217T>C	15,5

Table 1. Demographic and clinical characteristics.

2.2. Experimental protocol

Subjects were asked to repeatedly perform a gait initiation task. They had to stand quietly on a force plate for 30 seconds, then they were allowed to walk at their natural speed after a vocal prompt, self-selecting the leading limb [27]. After three to five steps, subjects stopped and returned to the initial position for another trial. The width of the base of support was self-selected by each subject in the first trial, then kept fixed for all further trials. At least three valid trials were collected from each subject, allowing 5 min rest in between. When asked, no participant complained about fatigue.

2.3. Recordings

Body kinematics were recorded by means of a six-cameras optoelectronic system (SMART-E, BTS, Italy) using a full body marker set [28]. A dynamometric force plate (9286AA, KISTLER, Winterthur, Switzerland) was used to measure the Center of Pressure (CoP). Wireless probes (FREEEMG 1000, BTS, Italy) were employed bilaterally to record the surface electromyographic (EMG) activity of Tibialis Anterior (TA), Soleus (SOL), Rectus Femoris (RF), Biceps Femoris (BF) and Erector Spinae (ES). Electrodes were placed according to the Surface Electromyography for the non-invasive Assessment of Muscles (SENIAM) guidelines [29]. Synchronous data acquisition was accomplished by the SMART-E workstation; sampling rate being 60 Hz for optoelectronic cameras, 960 Hz for dynamometric signals and 1000 Hz for EMG.

2.4. Data processing

During the 30 s quiet standing period, the statokinesigram, i.e. the trajectory of the CoP in the horizontal plane, was used to extract the static postural parameters: the area and the eccentricity of the ellipse containing 95% of CoP positions, the total length of CoP trajectory (CoP length), the average CoP velocity and the peak-to-peak medial-lateral and anterior-posterior CoP displacements (ML and AP ranges, respectively). In particular, ellipse area (A) and eccentricity (e) were calculated according to the following formulae:

$$\mathrm{A} = a * b * \pi \quad (1)$$

$$e = \frac{\sqrt{|\, a^2 \, - b^2 \,|}}{a} \quad (2)$$

in which a and b were respectively the semimajor axis and the semiminor axis of the ellipse.

Gait initiation was subdivided into three phases [30]: the *imbalance phase*, in which CoP moves backward and toward the future swing foot; the *unloading phase*, in which CoP moves laterally toward the stance foot and the *first swing*, in which CoP moves forward along the stance foot, from toe-off to heel-strike of the swing foot. The temporal markers delimiting each phase were determined by visual inspection of CoP trajectory; in particular, the onset of the CoP backward shift was considered to represent the APA onset [30]. For the imbalance and unloading phases, separately, we measured the phase duration, the length of CoP trajectory and the maximum AP and ML CoP shifts. The first swing phase was evaluated by measuring the length of the first step, normalized to the lower limb length (LL), and its velocity (v), expressed in Froude number ($\mathrm{Fr}=\frac{v}{\sqrt{g*LL}}$, g being gravity acceleration [31]).

For each subject, the kinematic and dynamometric variables were averaged over the recorded trials. Data normality was evaluated by means of Shapiro-Wilk test. Considering that data were not

normally distributed, the differences among SlowP, NonP and H groups were analyzed non-parametrically by using Kruskal-Wallis tests followed by Dunn post-hoc. Level of significance was set to 0.05 and the reported *p*-values reflect Bonferroni adjustment.

The analysis of EMG recordings regarded the timing of muscles activation or inhibition, surrounding the APA onset in gait initiation. Raw EMG data were high-pass filtered (f_{cut}=50 Hz) with a zero-phase shift, 6th-order elliptic filter, to remove movements artifacts, then the signals were rectified. For each muscle, the traces of the recorded trials were time-aligned to the APA onset and averaged. For each average EMG trace, the period from 1 s to 0.5 s before the APA onset (where no EMG changes were observed) was assumed as reference. The trace was integrated (time constant = 11 ms) and the mean level in the reference period was subtracted; then the onset of an excitatory or inhibitory EMG change was identified by a software algorithm which searched the first time point in which the trace fell above or below 2 SD of the reference signal (excitation or inhibition, respectively) and remained there for at least 50 ms. Whenever the criterion was met, the algorithm searched backward the point in which the trace started to deviate from the mean reference value [32]. No statistical analysis was performed on EMG timings because of the many cases in which no clear inhibitory or excitatory changes could be identified.

3. Results

3.1 Postural Parameters

The analysis of quiet stance (static posturography) highlighted alterations of postural control in cerebellar subjects. In fact, SlowP and NonP groups showed an ellipse area greater than H subjects, mainly due to a greater CoP displacement in medial-lateral direction, however this difference was statistically significant only in SlowP subjects (Table 2). In particular, the latter group revealed an inversion of the normal ellipse configuration with medial-lateral oscillation as preferred direction (Figure 1), which leaded to a reduction of the ellipse eccentricity.

	SlowP	**NonP**	**H**
CoP length (mm)	1924 (1222.73÷2983.37)	1481.507 (893.99÷2911.39)	1594.91 (895.93÷2309.69)
Average CoP velocity (mm/s)	64.13 (40.76÷99.44)	49.92 (29.80÷110.43)	53.16 (29.86÷76.99)
ML range (mm)	37.15* (24.40÷97.47)	29.72 (13.89÷85.06)	19.05* (11.24÷33.82)
AP range (mm)	39.77 (28.36÷57.52)	31.01 (18.39÷77.96)	26.58 (15.74÷60.30)
Ellipse area (mm^2)	564.89* (354.94÷2857.05)	447.25 (123.88÷2075.47)	208.51* (54.36÷609.06)
Ellipse eccentricity	0.68 (0.48÷0.87)	0.73 (0.65÷0.93)	0.79 (0.51÷0.83)

Table 2. Postural parameters during quiet stance. Median values (ranges); *p<0.05, Dunn test.

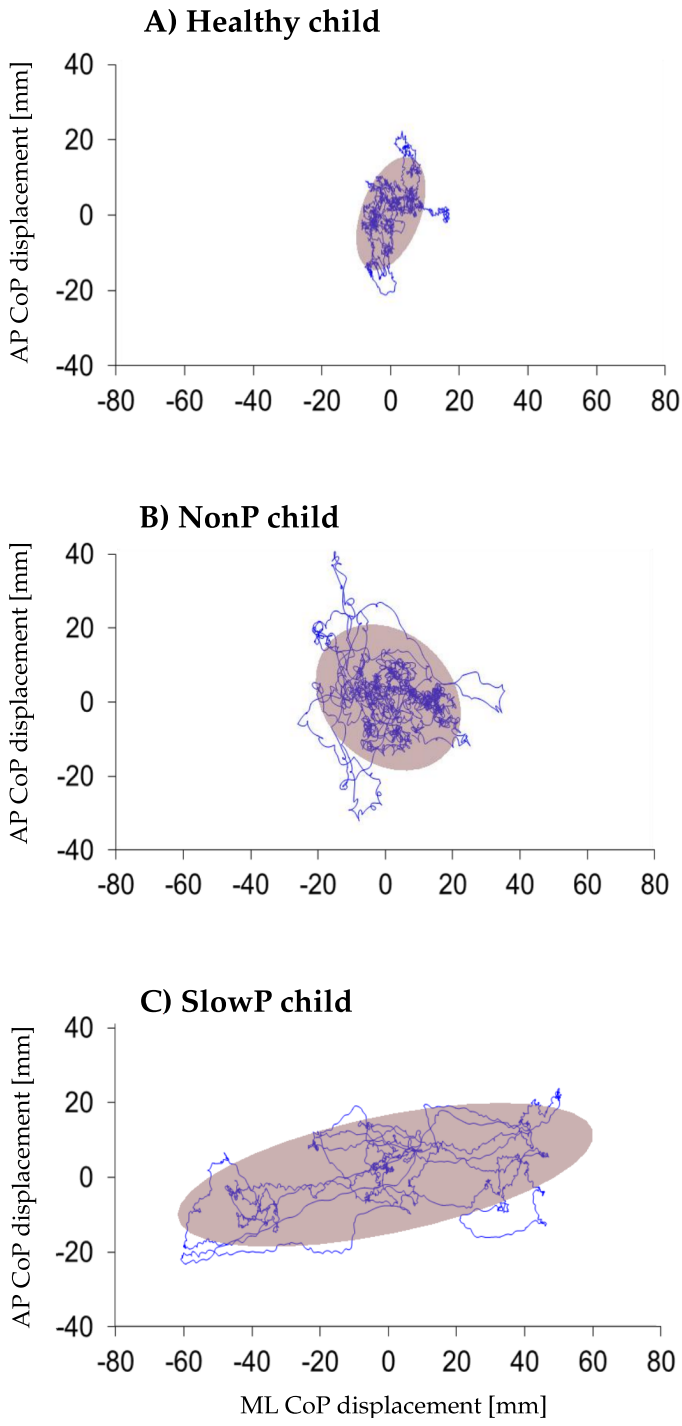


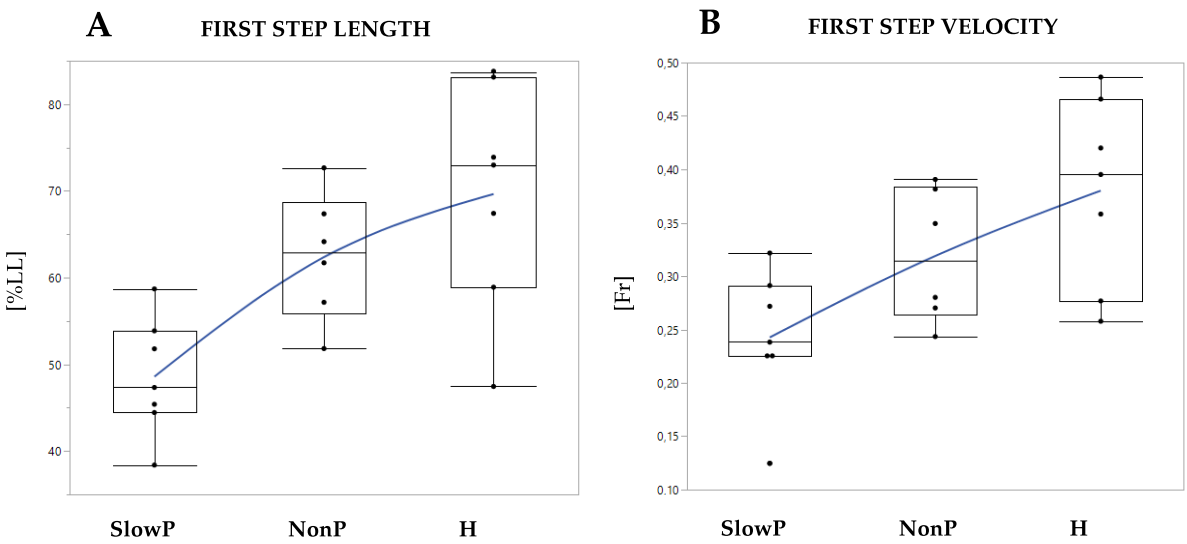
Figure 1. Statokinesigram, with 95% confidence ellipse, for representative subjects of the three groups.

3.2. Gait initiation parameters

The spatial and temporal parameters in the imbalance and unloading phases were not significantly different among the groups (Table 3). On the other hand, SlowP subjects showed a reduced first step length and velocity (Figure 2 and Table 4).

	SlowP	**NonP**	**H**
Imbalance phase duration (s)	0.41 (0.25÷1.03)	0.42 (0.31÷1.90)	0.31 (0.25÷0.53)
Imbalance CoP length (mm)	34.05 (24.98÷100.72)	70.42 (33.24÷134.58)	42.60 (17.84÷68.94)
Imbalance ML CoP shift (mm)	15.99 (13.53÷28.42)	39.80 (-6.5÷67.96)	25.95 (8.54÷49.87)
Imbalance AP CoP shift (mm)	-18.3 (-43.86÷-5.05)	-25.38 (-43.97 ÷-11.86)	-14 (-41.05÷ -8.71)
Unloading phase duration (s)	0.46 (0.21÷1.53)	0.33 (0.23÷1.20)	0.41 (0.21÷0.56)
Unloading CoP length (mm)	172.7 (112.12÷202.63)	134.99 (62.21÷175.17)	143.03 (90.21÷172.16)
Unloading ML CoP shift (mm)	-104.75 (-147.00÷-48.37)	-127.23 (-168.77÷-9.92)	-121.31 (-152.05÷-76.91)
Unloading AP CoP shift (mm)	9.7 (-12.94÷28.91)	-1.53 (-26.78÷17.37)	-9.19 (-59.01÷9.61)

Table 3. Postural parameters during the imbalance and unloading phases. Median values (ranges); AP shift is positive when forward, ML shift is positive toward the swing foot.



171 **Figure 2.** First step parameters: length and velocity in the panels (A) and (B), respectively. Boxplots
172 (median, interquartile range and extreme values) and trend lines (blue). LL: lower limb length; Fr:
173 Froude number.

	SlowP	NonP	H
First step length (%LL)	47.34*	62.93	72.99*
	(38.39-58.70)	(51.83-72.67)	(47.46-83.61)
First step velocity (Fr)	0.24*	0.31	0.39*
	(0.12-0.32)	(0.24-0.39)	(0.26-0.49)

174 **Table 4.** First step parameters. Median values (ranges); LL: lower limb length; Fr: Froude number;
175 * $p<0.05$, Dunn test.

3.3. EMG

Postural EMG changes accompanying APA onset were not detected in all recorded muscles and for all subjects. A descriptive analysis of electromyographic recordings allowed to appreciate the development of an inhibitory postural chain involving Erector Spinae, Biceps Femoris and Soleus, followed by an excitatory chain in Rectus Femoris and Tibialis Anterior. Such general pattern was observed in both the stance and swing sides, irrespectively from the healthy or pathological status. Nevertheless, a different timing distribution of the chains was found in the three groups. In the stance limb side, healthy subjects showed a clear cranio-caudal progression, for both the inhibitory and excitatory chains. Such a progression was lost in both NonP and SlowP patients; moreover, in SlowP, the recruitment of the excitatory chain was delayed. On the contralateral side (swing limb), both chains had a caudo-cranial progression in the healthy group, while NonP children displayed a disrupted progression of the inhibitory chain, followed by an almost synchronous activation of RF and TA. Instead, in SlowP patients the inhibitory chain still maintained a caudo-cranial progression, but was overall delayed. Also in this group, the following excitatory actions in RF and TA were synchronous.

		STANCE limb					**SWING limb**				
		ES	**BF**	**SOL**	**RF**	**TA**	**ES**	**BF**	**SOL**	**RF**	**TA**
		inhib	**inhib**	**inhib**	**exc**	**exc**	**inhib**	**inhib**	**inhib**	**exc**	**exc**
H	**median**	**-102**	**-100**	**-72**	**-72**	**-31**	**-90**	**-94**	**-121**	**68**	**-61**
	min	-115	-187	-146	-110	-49	-263	-199	-259	-80	-101
	max	-80	119	-25	57	108	100	70	-111	113	6
NonP	**median**	**-79**	**-68**	**-98**	**-35**	**-41**	**-111**	**-136**	**-53**	**-11**	**6**
	min	-109	-84	-130	-112	-49	-127	-160	-100	-83	-67
	max	-49	-64	-63	22	26	-95	-113	-24	162	45
SlowP	**median**	**-68**	**-70**	**-72**	**15**	**31**	**-55**	**-71**	**-98**	**3**	**-2**
	min	-85	-70	-199	-32	-52	-99	-90	-184	-40	-51
	max	-52	-70	63	141	66	2	-39	-35	24	145

Table 5. Latencies (ms) of postural EMG changes with respect to the APA onset (time 0). Median, minimum and maximum values for healthy (H), non-progressive (NonP) and slow progressive (SlowP) children. ES=Erector Spinae, BF=Biceps Femoris, SOL=Soleus, RF=Rectus Femoris, TA=Tibialis Anterior; exc=excitation, inhib=inhibition.

Of note, in the control group the stance leg SOL started to be inhibited about 40 ms prior to TA excitation. While this timing was overall preserved in NonP group (about 60 ms), it was effectively increased in SlowP patients (about 100 ms, Figure 3). Similar changes were detected also for the swing leg.

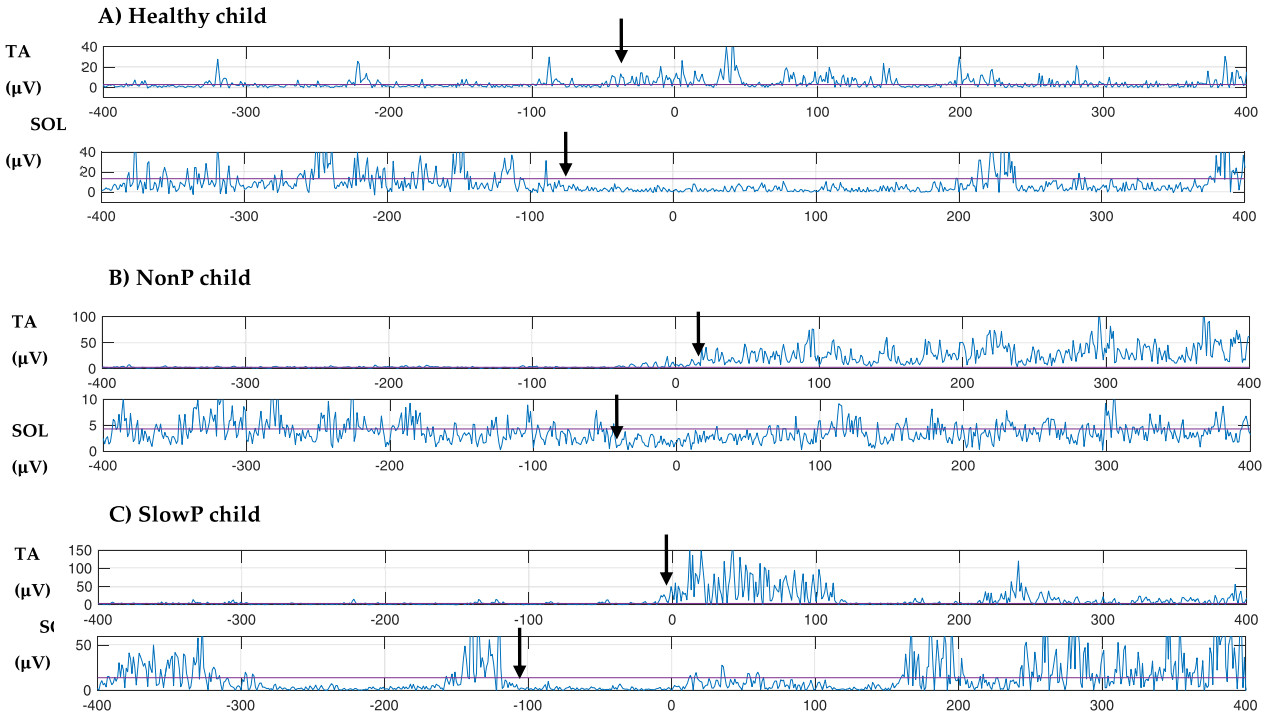


Figure 3. EMG comparison among Healthy (A), NonP (B) and SlowP (C) children, one representative subject for each group. Time 0: APA onset, defined as the first backward shift of the CoP. Black arrows show SOL inhibition and the following TA excitation. Note that the time delay between these two reciprocal actions gradually increases in NonP and SlowP with respect to healthy subject.

4. Discussion

The aim of this study was to describe the postural control adopted by children with CCA during gait initiation, in order to extract considerations on the role of the cerebellum in the development of postural control. As a main result we observed that in slow progressing CCA patients, i.e. SlowP, both static and dynamic components of postural control were significantly disturbed, while the postural behavior of non-progressive patients, i.e. NonP, was much similar to that of healthy children.

During the maintenance of upright posture, SlowP patients showed a large ellipse area, mainly due to large medial-lateral oscillations of the CoP. Considering CoP oscillations in anterior-posterior direction too, this resulted in a general reduction of the ellipse eccentricity, outlining an omnidirectional decrease of SlowP stability. This finding is in agreement with results repeatedly described in adults with cerebellar lesions [12]. No statistical posturographic differences were instead found between NonP and Healthy participants.

Gait initiation parameters in the imbalance and unloading phases remained substantially unchanged in both SlowP and NonP children compared to controls. Also this observation fits with previous results obtained in adults with cerebellar ataxia [25,33]. First step length and velocity showed instead a marked reduction in SlowP patients with respect to healthy controls, possibly reflecting a compensatory strategy for their poor balance control, consistently with what previously described in adults [25].

Electromyographic data, despite the roughness of the descriptive approach, showed that NonP and SlowP patients suffered more alterations in temporal (when) than in spatial distribution (to what muscle) and sign of activity (how, i.e. excitation or inhibition). This aspect once more witnesses the general view that considers the cerebellum as a "timing-machine" [34–38], leaving the pattern selection to other brain structures, like basal ganglia. Such perspective has been confirmed also for what regards APAs in adults [16,39].

A short comment also deserves TA and SOL reciprocal activation: all CCA patients, as well as healthy children, displayed in the stance limb the classical anticipatory postural pattern characterized by SOL inhibition followed by TA activation. However, the latency between SOL and TA activity of the healthy group (about 40 ms) was consistent with what reported by Isaias et al. (2014) [5] and much lower than that found in adults (about 100 ms, [19]). This finding supports the choice of devoting a paper to gait initiation in children and, at the same time, confirms that the present healthy group well represents the underlying population. On the contrary, alterations in the timing of the muscular activity were observed in CCA patients, being slightly increased to about 60 ms in NonP children and attaining about 100 ms in SlowP, underlying abnormal feed-forward muscle synergies [10,25].

4.1 Disease progression and postural behaviour

When looking to the present results as a whole, SlowP subjects showed a clearly worse postural behavior with respect to both NonP and Healthy children, a result which is unlikely related to the severity of the pathology, since all patients had an homogeneous SARA score, indicating comparable motor deficits in clinical terms. Therefore, the difference might stem either from the kind of cerebellar lesion (generalized atrophy vs. vermian hypoplasia) or from the progressive or non-progressive nature of the pathology. In this regard, SlowP children suffered from a generalized cerebellar atrophy, which represents macroscopic neuronal death, and received an ascertained clinical and/or

radiological diagnosis of slow progression. On the contrary, Joubert's syndrome, affecting NonP children, is a congenital malformation that causes an anomalous organogenesis of both the cerebellar vermis and peduncoli and has an intrinsically stable nature along the growth of the subject. In fact, once the organogenesis is completed the vermian hypoplasia remains stable throughout the patient's lifetime.

It could be argued that our observation of worse postural control in SlowP subjects may be related to the larger extent of their cerebellar compromission (generalized atrophy vs. vermian hypoplasia). However, literature reports an emblematic case which contrasts with this interpretation. In fact, Titomanlio et al. [41] published a case report in which a 17-year-old subject with complete cerebellar agenesis showed only a mild ataxia with slight dysmetria at cerebellar tests, accompanied by moderate mental retardation, but no difficulty in attaining very complex motor tasks. Other examples showing no direct correlation between the extent of the lesion and the ensuing motor impairments may be found in Vining et al. [42], reporting 58 cases of hemispherectomy in children who, in the majority of cases, were able to recover lower limb function during walking and running, as well as almost normal phonation after resection of the left hemisphere. In parallel, Liu et al. [43] described how children may recover visual processing abilities after large resections of the ventral occipito-temporal cortex. Such evident functional compensations could be explained only through the plasticity of the remaining brain areas, which had to cope with a stable lesion since childhood, which in the case described by Titomanlio et al. [41] starts from embryogenesis. These observations suggest to restrict the hypothesis to the progressive nature of the pathology.

Returning to the framework of the present study, we envisage that NonP subjects could use the plasticity of their intact brain areas, which may include the cerebellar hemispheres, to effectively compensate for their stable lesion, leading to an almost normal psychomotor development. On the contrary, SlowP subjects suffer from a continuous, although slow, cerebellar degeneration, who conflicts with the consolidation of a compensatory functional strategy. This perspective not only fits with the gradual worsening of postural deficits we documented here when passing from healthy to NonP to SlowP children, but would also explain why patients with adult-onset cerebellar lesions show even more pronounced postural deficits [16]. Indeed, neural plasticity gradually but consistently decreases over the lifespan [44].

4.2 Putative compensatory network

Finally, it remains to figure out which neural substrate is involved in the functional compensation. In this regard, it is interesting to highlight recent evidences showing subcortical bidirectional connections between the basal ganglia and the cerebellum [45–47]. In particular, both the subthalamic nucleus and the dentate nucleus have disynaptic projection addressed to, respectively, cerebellar cortex and striatum. It is thus evident the existence of an integrated network among basal ganglia, cerebellum and cerebral cortex, which may explain how abnormal activity at one of these nodes may elicit network-wide effects [48].

The functional role of the basal ganglia to cerebellum connections has been deeply investigated. Indeed, it has been observed that patients with Parkinson's disease (PD) show abnormal functioning also in the cerebellum [49]. A SPECT study in patients with PD confirms an increased cerebellar activity when the effect of the anti-parkinsonian drug extinguishes [50]. Further evidence comes from animal studies: the loss of dopaminergic neurons of the substantia nigra was shown to be correlated

with a persistent hyper-excitation of Purkinje cells, indicating an alteration of both the basal ganglia-thalamic and cerebello-thalamic pathways [51]. Indeed, after a pharmacologically induced dopaminergic degeneration, the thalamic projections from these two areas showed a reduction in the metabolic activity and a decrease in the firing rate [52,53]. Considering the reciprocal connectivity, it is of interest that functional MRI showed increased putamen-cerebellar activity in PD patients performing simple motor tasks, and that a greater putamen-cerebellar connectivity is significantly correlated with better motor performance [54,55]. On the contrary, the administration of levodopa reduced this connectivity, relieving the cerebellum from its compensatory task [56]. It was also observed that the compensatory role of the cerebellum contributes to prevent the full manifestation of the typical motor symptoms during the initial stage of PD; this compensatory ability saturates with time, leading PD patients to develop cerebellar symptoms too [57].

In conclusion, although the compensatory role of the cerebellum in basal ganglia pathology has strong evidence not only in Parkinson's disease but also in Tourette's syndrome, obsessive-compulsive disorder and dystonia [48], the putative compensatory role of the basal ganglia on cerebellar diseases is still to be demonstrated. Should this be proved, it could be a straightforward explanation for the graded postural impairments we found in children affected by CCA, as well as for those reported in adults [16]. Evidences in this regard might of course come from functional MRI and diffusion tensor imaging techniques.

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PAPER 3

Characterization and reproducibility of cerebellar metabolites in Crus I-II using MRS

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Abbreviations: MRS= Magnetic resonance spectroscopy; GM= Gray Matter; WM= White Matter; CSF= cerebrospinal fluid; NAA= N-acetylaspartate; CHO= Choline; CR= Creatine; GLX= Glutamate+Glutamine; INS= Myo-inositol; SVS= Single-Voxel Spectroscopy; CV= Coefficient of Variation; ICC= Interclass Correlation Coefficient.

ABSTRACT

Magnetic resonance spectroscopy (MRS) is an analytical technique that enables the identification and the quantification of metabolites. Several reproducibility studies on different brain areas have been performed in healthy subjects but few of them have focused on the cerebellum, particularly on the vermis. It is known that the cerebellum plays a key role in motor control and learning but recent evidence suggests its involvement also in cognition and emotion. Thus, the aim of this work was to characterize cerebellar metabolites in cognitive areas, such as CrusI-II and to examine the effect of tissue composition on metabolites quantification. The reproducibility of the final acquisition protocol was also investigated. The study was carried out on 11 healthy subjects. On 5 of these 11 the acquisition was repeated twice to assess protocol reproducibility. The study was performed on a 3T scanner and MRS data were acquired using a single-voxel PRESS sequence on three different voxels of interest: one in the CrusI-II, one in the cerebellar white matter and one in the cerebral white matter, as standard reference. Boxplots of metabolites concentration were created providing a standard range of concentrations in the cerebellar areas studied in this cohort of subjects. The volumetric T1-weighted images were segmented to correct the metabolites concentration for cerebrospinal fluid contamination and relaxation. The mean values of raw and corrected metabolites concentration were significantly different ($p<0.0001$), while their coefficient of variance resulted comparable. About the reproducibility, the interclass correlation coefficient (ICC) for each metabolite was calculated to estimate if the fraction of the total variability was due to biological variation (>0.5) or to measurement errors (<0.5). The 95% confidence interval of ICC was 0.33 to 0.51, suggesting that the variability is due to the contribution of both factors. Furthermore, the corrected ICC values were similar or slightly higher to the raw ones, showing that the correction for the tissues contamination could improve slightly the reproducibility results. Overall, the characterization of cerebellar metabolites in Crus I-II could be a useful clinical and research tool to study cerebellar pathophysiology involved in the cognitive functions.

INTRODUCTION

Magnetic Resonance Spectroscopy (MRS) is an analytical technique that allows the identification and the quantification of metabolites in specific areas of interest. In the last decades, MRS has been further developed to cover larger brain areas and to improve its accuracy and applicability in clinical settings. Indeed, MRS was proven to be valuable to diagnose and characterize cerebral tumors and to assess the cerebral damage due to post-stroke lesions (Saunders, 2000; Zhang et al., 2016). Other studies have used MRS to explore changes due to neurodegenerative diseases, such as Parkinson's (Clarke and Lowry, 2000) and Alzheimer's disease (Jones and Waldman, 2004). Nevertheless, MRS can be also used to investigate the biochemistry of healthy brains (Terpstra et al., 2015; Zhang et al., 2018). It is worth noting that a MRS protocol should be used for clinical purpose only if its efficacy had been previously assessed and validated. In this context, several reproducibility studies on different brain areas have been performed in healthy subjects (Bednařík et al., 2015) in order to check for MRS scan accuracy and reliability. Nevertheless, only few studies have used MRS to investigate chemical properties of the cerebellum and many of these have been acquired on cerebellar vermis (Currie et al., 2013; Terpstra et al., 2015) (Table 1).

Author, year	Scanner	Healthy subjects (n)	VOI
Minati, 2010	1.5T	28	Frontal brain white matter Parietal brain white matter Medial Temporal Lobe white matter **Cerebellar white matter**
Benito-Leon, 2016	3T	n=14	**Cerebellar white matter** **Cerebellar vermis gray matter** Midparietal gray matter
Long, 2015	3T	n=10	**Cerebellar white matter** **Dentate cerebellar gray matter**
Deelchand, 2015	3T	n=24-33	**Vermis gray matter**
Terpstra, 2015	3T - 7T	n=6	Posterior cingulate cortex **Cerebellar vermis**
Currie, 2013		n=55	**Superior cerebellar vermis**

	Deep cerebellar white matter

Table 1. Literature about MRS cerebellar studies. Works investigating chemical cerebellar properties in different areas and most of them have been acquired on cerebellar vermis.

It is known that the cerebellum plays a key role in movement coordination and motor learning but also its involvement in cognition and emotion is increasingly recognized (D'Angelo and Casali, 2012). Recent investigations have supported the hypothesis that cerebellar influence in cerebral processes is mediated by the cerebro-cerebellar loop (Palesi et al., 2014, 2017), which is composed of two main pathways: an efferent cerebellar tract, i.e. the cerebello-thalamo-cortical tract, and an afferent cerebellar one, i.e. the cortico-ponto-cerebellar tract. In particular, most of the cerebello-thalamo-cortical and cortico-ponto-cerebellar streamlines involves cerebral associative areas and their cerebellar cognitive counterpart (Palesi et al., 2014, 2017). Studies using functional connectivity have confirmed these evidences (Bernard et al., 2012; Castellazzi et al., 2017), further supporting the importance of investigating cerebellar features in those areas involved in cognitive processes, like Crus I-II (Stoodley et al., 2012). Moreover, several studies have focused on structural and functional characterization of the cerebellum in neurological disorders revealing that it is affected in neurodegeneration, such as dementia (Castellazzi et al., 2014; Palesi et al., 2018) and Parkinson (Wu and Hallett, 2013), as well as psychiatric diseases (Phillips et al., 2015), such as autistic spectrum disorder (Scott et al., 2009). Many diseases and several lesions that involve the cerebellum do not always manifest only motor disorders, such as ataxic motor syndromes, but also cognitive disorders, as the Cerebellar Cognitive Affective Syndrome (CCAS) (Schmahmann, 2004) that involves impairment in executive, visual-spatial, linguistic abilities as well as personality changes (Schmahmann and Sherman, 1998).

In this context, a better chemical characterization of the cerebellar structures is highly valuable. Hence, to provide a reproducible MRS protocol for characterizing metabolites in cerebellar cognitive regions, we have focused our attention on specific cerebellar areas of cognitive functions such as the lateral Crus I-II. We have evaluated the reproducibility of MRS-3T protocol on healthy subjects, positioning MRS Volumes Of Interest (VOIs) in the Gray Matter (GM) and the White Matter (WM) of the left cerebellar hemisphere. Furthermore, an additional VOI in the WM of the right periventricular cerebral hemisphere has been acquired as standard reference. The metabolites taken into consideration were: N-acetylaspartate (Naa), Choline (Cho), Creatine (Cr), Glutamate+Glutamine (Glx) and Myo-inositol (Ins) (Soares and Law, 2009).

The positioning of VOIs in a small and specific region, as in the cerebellum, could lead to large variability in spectral quality because of the effect of composition tissue contamination, i.e. partial volume effect (Bednařík et al., 2015). To mitigate this effect, the segmentation of T1-weighted brain images was performed.

The aim of this work was to characterize cerebellar metabolites in cognitive areas, e.g. Crus I-II, and to examine the effect of tissue composition on metabolites quantification of each considered voxel. The reproducibility of the final acquisition protocol was also investigated in order to provide guidelines for a MRS protocol that could be applied to study metabolic changes in neurological diseases affecting the cerebellum.

METHODS

Participants

Eleven healthy subjects (9 females, 2 males, mean age 27 ± 5 years) were enrolled for this study. On 5 of these 11, the acquisition was repeated twice (test-retest) to assess protocol reproducibility. This study was carried out in accordance with the Declaration of Helsinki with written informed consent from all subjects. The protocol was approved by the local ethic committee of the IRCCS Mondino Foundation.

MRI acquisition

MRI data were acquired on a 3T Skyra scanner (Siemens, Erlangen, Germany) with the manufacturer's 32-channel head-coil for signal reception. Structural information was obtained with an MPRAGE T1-weighted 3D sequence with the following parameters: TR/TE/TI = 2300/2.95/900 ms, flip angle = 9°, 176 sagittal slices, acquisition matrix = 256 x 256, in-plane resolution = 1.05 x 1.05 mm, slice thickness = 1.2 mm. MRS data were acquired using a single-voxel (SVS) point-resolved spectroscopy (PRESS) sequence on three VOIs, exemplified in Figure 1: the first was placed in the left cerebellar GM of the Crus I-II, the second in the left cerebellar WM, and the third in the WM of the right cerebral hemisphere, which was used as reference region. The cerebral WM VOI was located on the contralateral side to the position of cerebellar VOI because of the cerebro-cerebellar streams which are contralateral. Common main parameters were: TR/TE = 3000/30 ms, 256 spectral points, spectral bandwidth = 1200 Hz. Other parameters were: for cerebral VOI 4 (4x16=64) measurements, i.e. 4 spectra, and a voxel of 2x2x2 cm_3 were used, for a total acquisition time about 5 minutes, while for the two cerebellar VOIs 8 measurements (8x16=128), i.e. 8 spectra,

and a voxel of 1.5x1.5x1.5 cm3 were used, (8x16=128) for a total acquisition time about 20 minutes. After automated shimming, manual shimming was performed to further minimize B_0 inhomogeneity over each VOI using the *Manual Adjustments* command. Spectra were acquired with and without water suppression in order to provide absolute quantification of metabolites.

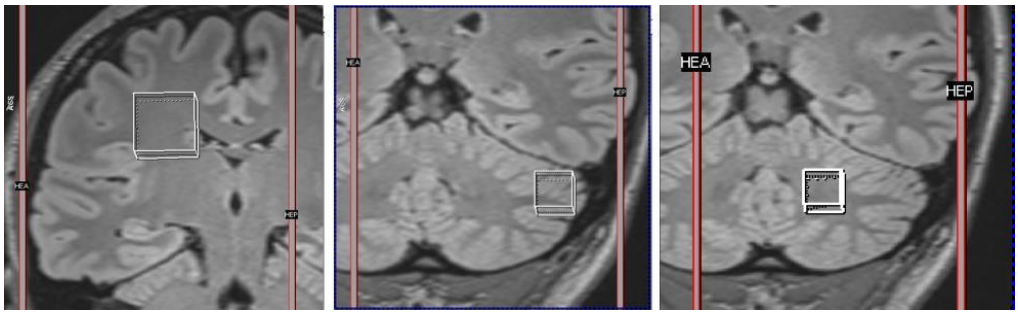

Figure 1: Localization of MRS volumes of interest (VOIs). Coronal sections of T1-weighted images from a representative subject for VOIs localization. From left to right: VOI in the WM of the right cerebral hemisphere (2x2x2 cm^3, used as reference region), VOI in the left cerebellar GM (Crus I-II), and VOI in the left cerebellar WM (both 1.5x1.5x1.5 cm^3).

Quality control

To check the reliability of data obtained, quality control was performed. Each spectrum was analyzed and the signal to noise ratio (SNR) was controlled. It was necessary to check the full width at half-maximum (FWHM). This value had to be less than 20 Hz and between 15-20 Hz.

T1 segmentation and MRS data analysis

To determine tissue composition of each VOI, the structural T1-weighted images were segmented into GM, WM and cerebrospinal fluid (CSF) using the statistical parametric mapping (SPM12) toolbox *Segment* (http://www.fil.ion.ucl.ac.uk/spm/). Using in-house written software, MATLAB R2017b, VOI geometry information was used to create corresponding binary masks in the same space of each individual's segmented T1-weighted image. Each voxel within the VOI was then assigned a single tissue class (out of GM, WM, CSF), corresponding to the class with higher partial volume fraction. The total percentage composition of GM, WM and CSF was then calculated for each VOI.

Spectroscopy data were analyzed using two freely available software packages: JMRUI 5.2 (Stefan et al., 2009) and TARQUIN 4.3.10 (Reynolds et al., 2006; Wilson et al., 2011). For each VOI, all acquired spectra were pre-processed using JMRUI: first, all spectra were aligned in the frequency

domain using the pre-processing option of *frequency shift* and then averaged using the pre-processing option of *averages selected signals*. The resulting mean spectrum was analyzed using TARQUIN. Absolute metabolites quantification in mM was performed using both the suppressed and unsuppressed water signal of each VOI. Although TARQUIN is almost completely automatic, preprocessing/fitting parameters were optimized to guarantee robust metabolites quantification. In particular, correction for eddy-currents and correction of dynamic frequency were used, while max dref, the maximum variability with which the spectrum can shift respect to the reference value, was set at 0.2 units; line-broadening was set at 0.5 Hz and the start point of acquired signal was set at 1 as these settings were observed to result in flatter baseline reduced residuals.

Metabolites concentrations were calculated both without and with correction for contamination of other tissue fractions on each VOI. To correct metabolites concentration, the following equation was used (Long et al., 2015):

$$Mcor_k = (Mraw_k) \times \frac{43300 \times fGM + 35880 \times fWM + 55556 \times fCSF}{35880} \times \frac{1}{1-fCSF} \times \mathrm{VISw} \times \frac{\exp\left(-\frac{TE}{T2\,water}\right)}{\exp\left(-\frac{TE}{T2mk}\right)}$$

where k indicates the metabolite, *Mcor* is the corrected value, *Mraw* is the uncorrected value and VISw is a correcting factor for MR water visibility (0.65) (Long et al., 2015). The default TARQUIN water concentration in mM for WM, GM and CSF are respectively 35880, 43300 and 55556; fractions of GM, WM and CSF within the voxel are identified with *fGM*, *fWM* and *fCSF*. *T2mk* is the T2 relaxation time of metabolite k. *T2m* of tNaa, tCho, tCr, Glx, Ins and water were chosen to be 308, 239, 166, 204, 188 and 95 ms, respectively following references 30 and 31 (Ganji et al., 2012; Kirov et al., 2008).

The coefficient of variation (CV) – defined as the degree of variation between two groups -, for each metabolite and for each VOI, was calculated for all subjects before and after the metabolites concentration correction, in order to compare the effect of tissue composition correction.

Protocol reproducibility

A test-retest approach was used to study the reproducibility of the protocol: 5 of the 11 subjects were scanned twice with the same protocol on the same scanner, in different days. The reproducibility was estimated by calculating the Interclass Correlation Coefficient (ICC) for each metabolite on data without and with correction for partial volume effects. The ICC was evaluated as:

$$\mathrm{ICC} = \frac{\sigma_B^2}{\sigma_{TOT}^2}$$

where σ_B^2 was the between-subject variability, while $\sigma_{TOT}^2$was the total variability calculated as the sum of σ_B^2 and σ_W^2 (the within-subject variability) (Grussu et al., 2015).

The ICC was calculated in order to assess the fraction of variability due to measurement errors (intra-subject) and biological differences (inter-subjects) in healthy subjects.

Statistical analysis

Statistical analyses were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Mean values of metabolites concentration for each VOI were calculated on data without (raw) and with correction for partial volume effect. The effects of such correction in each VOI was assessed by General Linear Model approach in which metabolites concentration were entered as repeated measurement dependent variables and classified according to two factors: correction (raw vs corrected) and VOI (cerebral periventricular WM, Crus I-II and cerebellar WM); actually configuring at two-way repeated measurement MANOVA; The CVs values of raw and corrected concentration of metabolites were also compared using a paired t-test to assess the presence of significant differences. $P < 0.05$ was considered to indicate significance.

RESULTS

MRS data quantification

Figure 2, 3 and 4 show spectra analyzed by TARQUIN for each VOI (Crus I-II, cerebellar WM and cerebral WM, respectively) of representative subject.

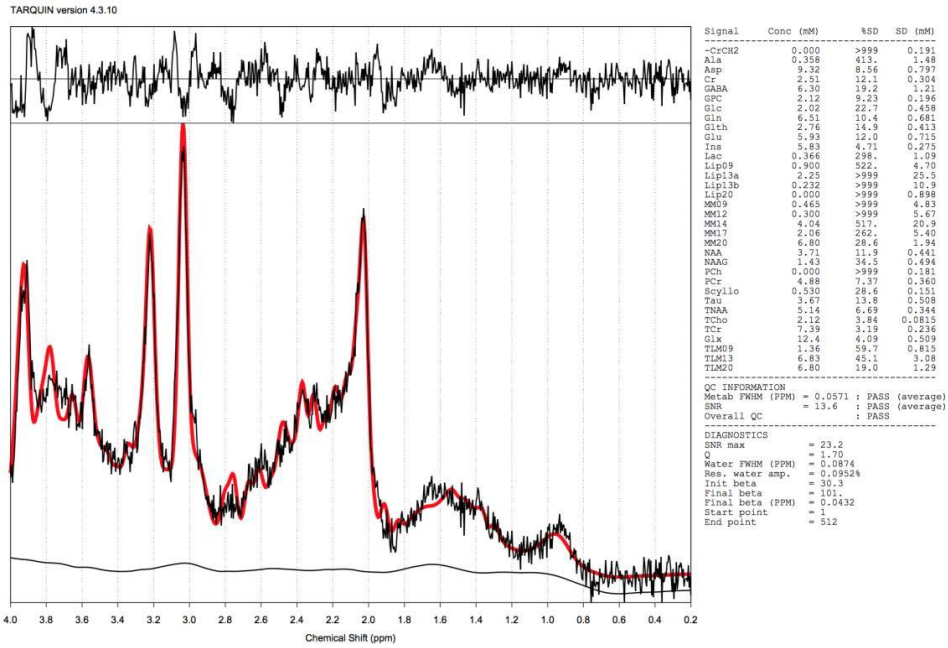

Figure 2. Representative spectrum of VOI in the cerebellar GM (Crus I-II) of representative subject. Analysis of metabolites absolute quantification and their standard deviations.

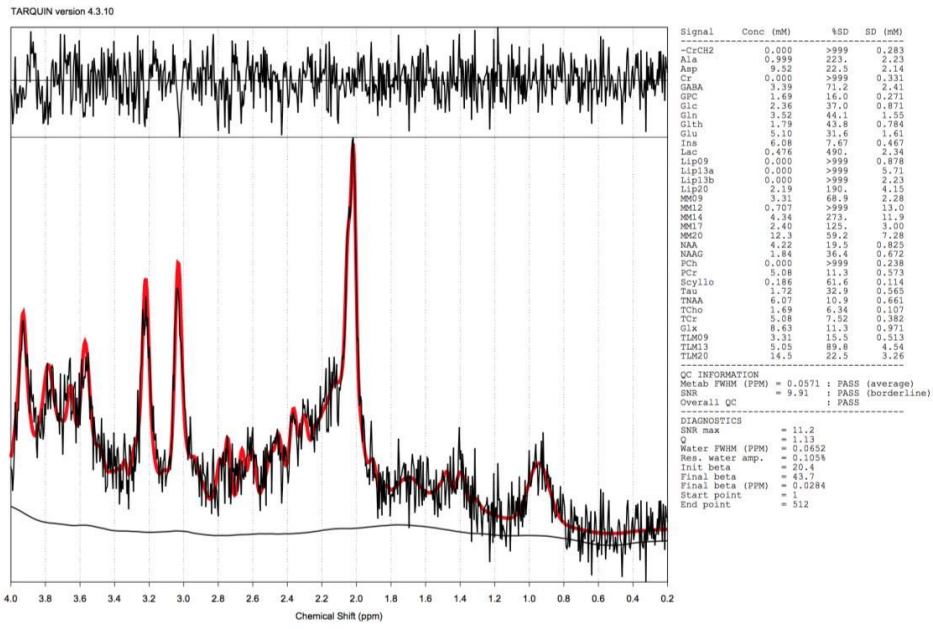

Figure 3. Representative spectrum of VOI in the cerebellar WM of representative subject. Analysis of metabolites absolute quantification and their standard deviations.

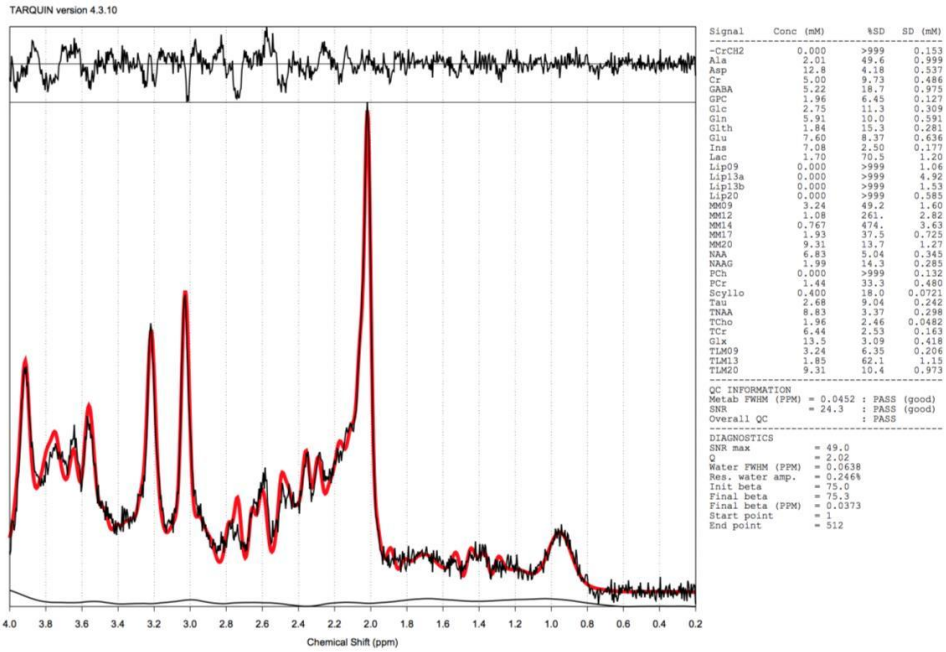

Figure 4. Representative spectrum of VOI in the cerebral periventricular WM of representative subject. Analysis of metabolites absolute quantification and their standard deviations.

Table 2 illustrates mean and standard deviation values of raw and corrected metabolites concentration for cerebral (right periventricular WM) and cerebellar (left CrusI-II and left WM) VOIs. Mean values of corrected metabolites concentration are roughly halved compared to uncorrected concentrations, not only for the partial volume correction but also for the relaxation correction. Repeated measurement MANOVA found a very significant effect of correction ($F_{(5,6)}$ = 428.7; $p<0.0001$), in absence of significant effects of VOI ($F_{(10,1)}$ =28.35; $p=0.145$) and of interaction ($F_{(10,1)}$= 111.83; $p=0.073$). Tukey post-hoc confirmed that correction had a significant effect on each single metabolite ($p<0.0002$).

Metabolite	**Cerebral WM**		**Cerebellar Crus I-II**		**Cerebellar WM**	
	Raw	**Corrected**	**Raw**	**Corrected**	**Raw**	**Corrected**
tNaa	9.0±0.4	4.9±0.3	5.6±0.8	3.7±0.5	7.4±1.0	3.9±0.5
tCho	1.8±0.2	1.0±0.1	1.7±0.4	1.2±0.3	1.8±0.2	1.0±0.1
tCr	6.1±0.3	3.6±0.2	7.7±1.4	5.5±1.0	5.5±0.6	3.2±0.4
Glx	13.3±0.9	7.7±0.5	10.4±3.3	7.3±2.3	10.4±2.0	5.8±1.1
Ins	6.3±0.8	3.6±0.4	4.7±1.7	3.3±1.2	6.3±1.1	3.5±0.6

Table 2: Raw and corrected metabolites concentration for cerebral and cerebellar VOIs. Mean metabolites concentration across all subjects are reported in mM and expressed as (mean ± SD).

Figure 5 reports boxplots of raw and corrected metabolites concentration in mM for all considered VOIs to provide standard range of metabolites concentration in the cerebellar investigated areas.

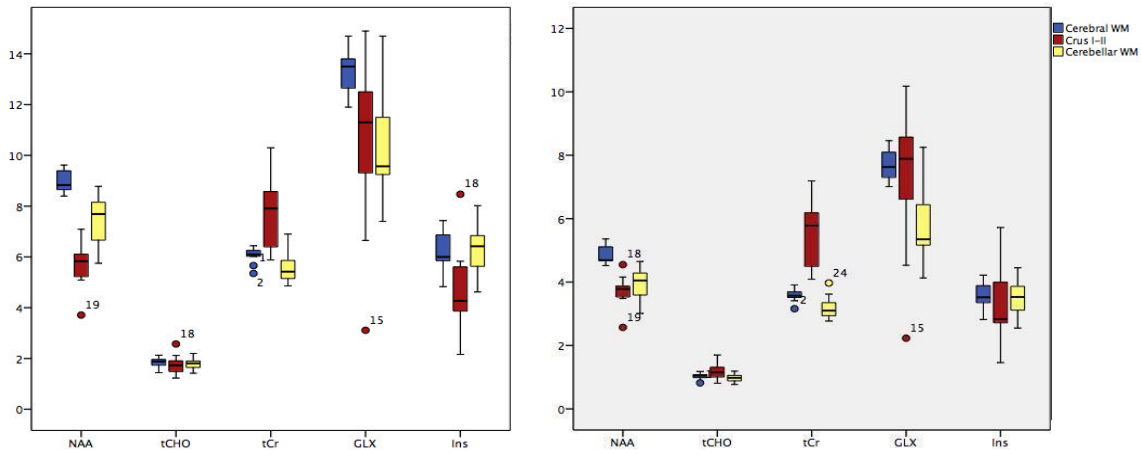

Figure 5: Boxplots of raw (left) and corrected (right) metabolites concentration for each VOI. Crus I-II VOI (red), cerebellar WM VOI (yellow) and cerebral VOI (blue) are reported.

Table 3 shows the CVs values of uncorrected and corrected metabolites concentrations for cerebral (right periventricular WM) and cerebellar (left CrusI-II and left WM) VOIs. Corrected and uncorrected CVs values were not significantly different (p>0.2) for any VOI for any of the metabolites analyzed.

CV	Cerebral WM		Cerebellar Crus I-II		Cerebellar WM	
	Raw	Corrected	Raw	Corrected	Raw	Corrected
tNaa	0.05	0.06	0.16	0.13	0.14	0.14
tCho	0.11	0.10	0.22	0.22	0.14	0.14
tCr	0.05	0.05	0.19	0.18	0.11	0.12
Glx	0.07	0.07	0.31	0.31	0.20	0.20

Ins	0.13	0.12	0.36	0.36	0.17	0.17

Table 3: **Coefficients of variation of raw and partial volume corrected metabolites concentration for each VOI.** Coefficients of variation (CVs) are expressed as ratio of mean metabolite concentration across all subjects and relative standard deviation.

Protocol reproducibility

ICCs values calculated using data obtained with the test-retest protocol are reported in Table 4. ICCs values range between 0.30 to 0.66 and it is to note that ICCs of corrected data were very similar to ICCs for uncorrected data, generally very slightly higher (in 8 out of 15 cases).

		ICC raw	**ICC corr**
Cerebral WM	tNaa	0.16	0.45
	tCho	0.47	0.56
	tCr	0.32	0.34
	Glx	0.50	0.49
	Ins	0.45	0.48
Crus I-II	tNaa	0.46	0.48
	tCho	0.65	0.66
	tCr	0.61	0.65
	Glx	0.30	0.32
	Ins	0.51	0.47
Cerebellar WM	tNaa	0.08	0.07
	tCho	0.63	0.63
	tCr	0.30	0.30
	Glx	0.41	0.41
	Ins	0.50	0.50

Table 4: **Interclass correlation coefficients (ICC) of metabolites concentration for each VOI.** All ICCs values reported in cerebral VOI and in cerebellar WM VOIs. Pooling values from all VOIs and metabolites, the 95% confidence interval resulted from 0.33 to 0.51 for the raw ICC, while from 0.37 to 0.54 for the corrected ICC.

DISCUSSION

The present work aims to characterize cerebellar areas dedicated to high-level cognitive processes, such as Crus I-II, in terms of metabolites concentration. In particular, a clinically usable

SVS-MRS protocol was utilized to measure metabolites concentration in cerebellar GM (Crus I-II) and WM. The present investigation assessed the reproducibility of the proposed SVS-MRS protocol using a test-retest approach to demonstrate its intra and inter-subject stability.

Quantitative data obtained from spectra analyzed by TARQUIN seem to be comparable with respect to ones obtained from literature. For example, the mean concentration of tNaa in WM brain VOI obtained in this work is 9.0, while in one of the works from literature, the mean concentration of tNaa in white matter areas of brain is 9.9 (Pradhan et al., 2015). The same comparison can be carried out between the others metabolites of cerebellar WM and GM VOIs (Benito-Leòn et al., 2016). So, the comparison of quantitative results seems about similar, considering that the areas analyzed are slightly different and the spectra analysis were carried out using a different software (LC model). As expected, tCho and tCr concentration were more accurate and stable, i.e. they had smaller standard deviations, than those of the other metabolites, such as Ins and Glx. Furthermore, metabolites concentration in Crus I-II and in cerebellar WM region showed more variability than in the cerebral region, maybe because the VOI positioning is more challenging in cerebellar than in cerebral areas. This result is in agreement with previous studies that have reported how variability in cerebral metabolites quantification showed standard deviation values smaller than the ones in the cerebellum (Baker et al., 2008; Hong et al., 2011). To improve metabolites concentration and provide range of values that might be used as reference in future works, T1-weighted brain images were segmented and estimated tissue partial volume fractions were used to correct metabolites concentrations for the effects of relaxation and of contamination due to the presence of more than one tissue in each VOI. This approach has been supported by previous works focused on different cerebral regions (Minati et al., 2010), which have reported a better metabolites quantification when data were corrected for partial volume effect. Furthermore, our findings supported previous work (Long et al., 2015) showing that mean values of metabolites concentration corrected for partial volume effect were significantly reduced compared to uncorrected concentrations. Indeed, our corrected values were approximately halved with respect to those without correction. Repeated measurement MANOVA showed a significant difference between raw and corrected values of metabolites quantification supporting the importance of the correction for partial volume effect when a range for acceptable values of metabolites concentration must be identified. Nevertheless, the CVs values of raw and corrected metabolites concentration resulted comparable meaning that the effect of tissues contamination does not significantly affect protocol reproducibility.

Although the CVs values of corrected data did not significantly improve compared to the uncorrected data, we suggest it is appropriate to perform this correction using the segmentation of T1-weighted brain images for correcting raw metabolites concentration.

The test-retest protocol aimed at estimating how biological variation between individuals (inter-subjects) and measurement errors (intra-subjects) in this cohort of subjects contributed to the total variability of metabolites concentration. ICC values that estimate the fraction of the total variability due to biological differences between subjects, range from 0 to 1. ICC values smaller than 0.5 suggest that the variability due to measurement errors is bigger than that of biological differences, while values greater than 0.5 suggest that biological variability in the considered cohort of subjects has the greatest role (Grussu et al., 2015). In our data most of ICC values for cerebellar ROIs ranged from 0.30 to 0.66 (except for tNaa that had an ICC value of 0.08 in cerebellar WM VOI), with similar values for the periventricular WM region. This means that the total variability seems to be influenced both by measurement features and by intra-subject variability. Some ICC values which are greater than 0.5 suggest that the variability of these values is more influenced by the contribution of biological differences than that of measurement errors, such as the reproducibility of tCho and tCr in the Crus I-II and of tCho in the cerebellar WM (ICC values of 0.65, 0.61 and 0.63 respectively). This is not unexpected given our cohort of subjects was homogeneous in terms of age, likely resulting in relatively small biological variability. Probably, this might depend on the fact that the quantification of these two metabolites is more accurate than the others, hence the variability due to measurement errors tends to decrease with respect to the variability due to biological differences. As regards the ICC value of tNaa in cerebellar WM, the measurement errors would seem to contribute more to total variability (ICC value of 0.08). This result might be due to a harder quantification of tNaa which would lead to increase measurement errors with respect to the inter-subjects variability. Comparable results were obtained when ICC values of metabolites concentration were calculated on data corrected for partial volume effect, which were similar or slightly higher to those calculated on raw data. For example, Glx of the cerebellar WM showed an ICC value of 0.41 when it was calculated both on data with and without correction for tissue contamination, while cerebral tNaa had ICCs values of 0.16 and 0.45 if calculated without or with correction, respectively. Even though the correction for the contamination of CSF and other tissues did not absolutely improve metabolites quantification and CVs results, it seems to improve the reproducibility results slightly.

The results obtained from this study should be interpreted within the context of few limitations. First, it was difficult to apply the segmentation of T1-weighted brain images, above all regarding the voxels located in cerebellar areas because of their sizes. Moreover, the sample examined size was relatively small. In a prospective study, it could be interesting to increase a sample size to raise the reliability of work.

CONCLUSIONS

This work focused on characterization of cerebellar metabolites in regions commonly involved in cognitive processes. Our findings revealed that correct for the effect of tissues contamination does not appreciably improve protocol reproducibility in cerebellar VOIs. Nevertheless, correction for partial volume effect is important when a range of acceptable values of metabolites concentration must be identified. Hence, in the present work standard range of metabolites concentration was suggested for both WM and GM cerebellar VOIs. Despite the MRS is widely used in clinics, few studies have used it to correlate neurological disorders with the concentration of cerebellar metabolites. In particularly, since the present work focused on characterization of cerebellar metabolites in regions commonly involved in cognitive processes, future studies could include the proposed protocol as a clinical and research tool to investigate cerebellar pathophysiology in pathologies affecting cognitive and motor aspects of cerebellar circuit, such as cerebellar ataxia.

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PAPER 4

frontiers in Physiology

BRIEF RESEARCH REPORT
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Transcranial Direct Current Stimulation on Parietal Operculum Contralateral to the Moving Limb Does Not Affect the Programming of Intra-Limb Anticipatory Postural Adjustments

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Recent data suggest that the parietal operculum acts as an integration center within a multimodal network, originating from different primary sensory and motor cortices and projecting to frontal, parietal and temporal cortical hubs, which in turn govern cognitive and motor functions. Thus, parietal operculum might also play a crucial role in the integrated control of voluntary movement and posture. As a first step to test this hypothesis, the Anticipatory Postural Adjustments (APAs) stabilizing the arm when the index-finger is briskly flexed were recorded, on the preferred side, in three groups of 10 healthy subjects, before, during and after *CATHODAL* or *ANODAL* transcranial Direct Current Stimulation (tDCS, 20 min at 2 mA) applied over the contralateral Parietal Operculum (coPO). Results were compared to those obtained in a *SHAM* group. In agreement with literature, in the *SHAM* group the activation of the prime mover Flexor Digitorum Superficialis was preceded by an inhibitory APA in Biceps Brachii and Anterior Deltoid, and almost simultaneous to an excitatory APA in Triceps Brachii. The same pattern was observed in both the *CATHODAL* and *ANODAL* groups, with no significant tDCS effects on APAs amplitude and timing. Index-finger kinematics were also unchanged. These negative results suggest that the coPO does not disturb the key network governing APAs in index-finger flexion. Since it has been well documented that such APAs share many features with those observed in trunk and limb muscles when performing several other movements, we suggest that coPO may not be crucial to the general APA control.

Keywords: tDCS, parietal operculum, intra-limb APAs, integration of voluntary movement and posture, human

INTRODUCTION

Voluntary movements induce postural perturbations, which are usually counteracted by muscular activities involving muscles other than the prime mover. Some of them, the Anticipatory Postural Adjustments (APAs), develop well before the onset of the focal movement itself, and such anticipation witnesses that they are programmed in a feed-forward way (Belen'kii et al., 1967;

Massion, 1998). APAs are tailored to the kinematical aspects of the primary movement and usually spread over different muscles, creating one or more fixation chains toward the available support points. Several studies described the APA chains that precede movements involving large masses, such as a shoulder flexion that produce a so large postural perturbation to threaten the whole-body equilibrium (Bouisset and Zattara, 1987). In these conditions, where it is important to avoid falling (Dakin and Bolton, 2018), APAs usually spread over different limbs so that they are referred to as *inter-limb* APAs. It has also been demonstrated that similar adjustments develop in the same limb when moving one of its distal segments, e.g., APAs in the arm when flexing/extending the hand (Aoki, 1991) and even when moving a very tiny mass as when flexing the index-finger at the metacarpophalangeal joint (Caronni and Cavallari, 2009). Such postural actions are referred to as *intra-limb* APAs. Considering that the perturbation produced when moving very tiny masses is irrelevant with respect to whole body equilibrium, it has been suggested that *intra-limb* APAs contribute to attain an higher precision of the focal movement (Bruttini et al., 2016).

The present study belongs to a broad line of research oriented to investigate how the APA control is organized. In fact, several studies showed the role of sensory and motor areas, including the primary and supplementary motor cortices, as well as subcortical structures like basal ganglia, cerebellum and spinal cord (Viallet et al., 1992; Palmer et al., 1994; Petersen et al., 2009; Bolzoni et al., 2012, 2015, 2018; Ng et al., 2013; Bruttini et al., 2015). Although it is still not well established whether the command for recruiting the prime mover muscles and that governing the postural muscles are separately processed or have a common origin, we provided evidence that a functionally unique motor command should drive both the prime mover and the muscles of the *intra-limb* APAs chain (Bruttini et al., 2014). Moreover, we have also found that the command splits before reaching the SMA (Bolzoni et al., 2015), since interfering with the excitability of this area affected the APAs but not the prime mover recruitment. In this context, it was of interest to move the investigation toward an higher-level integration center.

Considering that APAs are tuned depending on primary movement kinematics and that they adapt to the postural context, we chose a neural structure deeply involved in the integration of sensory-motor information. Recent studies highlighted the parietal operculum (PO) as a "hub," in which converge several sensory-motor streams originating from different cerebral areas. The PO is "the cortical flap that covers the dorsocaudal part of the Sylvian fissure," which may be divided into four cytoarchitectonical areas (OP1–OP4) (Eickhoff et al., 2006b; Cattaneo et al., 2015). Several studies focused on the role of PO in secondary sensory processes, highlighting its involvement in the integration of proprioceptive and tactile information within the framework of motor control (Milner et al., 2007; Sepulcre, 2014). Sepulcre et al. (2012) revealed how the connectivity of sensory and motor systems converge in a network that seems involved in linking external and internal information. PO is the crucial part, in this multimodal network, where visual, somatosensory and auditory functional streams converge; in turn, PO is connected to motor and premotor areas (Felleman and Van Essen, 1991). In this regard, it is also interesting to note that APAs are influenced by the availability of visual information (Esposti et al., 2017), which may indirectly point out a PO involvement. On these premises, we tested whether the PO contralateral to the moving limb contralateral Parietal Operculum (coPO) is involved in the control of *intra-limb* APAs associated to index-finger flexion movements.

In this aim, we modulated coPO excitability by using anodal and cathodal transcranial Direct Current Stimulation (tDCS), a technique which has been proved to selectively interfere with the excitability of many cortical structures involved in motor and cognitive processes (for a review see Brunoni et al., 2013), including PO (Fujimoto et al., 2017). Notably, the applied currents are usually sufficiently low to grant a focal stimulation but nevertheless they produced long-lasting effects in many cases (Brunoni et al., 2013).

Thus, by analyzing the effects of tDCS, it would be possible to test the hypothesis that coPO is involved in processing the *intra-limb* APAs associated to index-finger flexion, as well as whether in this area the motor commands to the prime mover and to postural muscles are split or still processed as a single functional stream.

MATERIALS AND METHODS

A total of 30 healthy volunteers (mean age ± SD: 27.5 ± 2.9 years, 20 males) were enrolled in this study. Oldfield questionnaire was used to ascertain handedness, resulting in only one left-handed participant. No subject had any history of neurological or orthopedic diseases, as well as of intake of drugs acting on the Central Nervous System. Participants provided their informed consent, but were kept completely unaware of the stimulation condition. The experimental protocol complied with the policies and principles contained in the Declaration of Helsinki and were approved by the Ethical Committee of the University of Milan (counsel 6/19).

Subjects were randomly assigned and equally distributed to one out of the three tDCS conditions (*ANODAL*, *CATHODAL*, and *SHAM*, see second-next paragraph). This between-subjects approach was chosen so as to exclude a carry-over effect due to multiple stimulations performed in the same subject on the same day. Subjects were tested on their preferred side. They were sitting on a chair with the non-preferred arm lying on an armrest, while the preferred upper arm was along the body, elbow flexed at 90° and the hand prone, lined-up with the forearm. The index-finger was kept extended and aligned with the hand, while all other fingers were hanging. During the experiment, subjects had to keep their back supported and both feet on the ground (**Figure 1A**). The experimenters adapted the set-up to each subject's body size and supervised the subject's position during the whole experimental session.

Motor Task

At the beginning of the procedure, one of the experimenters held the preferred upper limb of the subject, who was instructed to exert a Maximal Voluntary Contraction (MVC) of each of the

recorded muscles (see second-next paragraph), one at a time, for about 6–10 s, while the experimenter was monitoring the EMG. Then, after resting for about 10 min, the subject had to perform several sequences of 15 brisk flexion movements of the index-finger, at the metacarpophalangeal joint: two sequences, with about 30 s of rest in between, were performed just before applying tDCS (*Pre*), two at about half of the tDCS period (*Dur 10'*), two in the last minutes of full-current tDCS (*Dur 20'*) and two at 5, 10, and 20 min after tDCS end (*Post 5'*, *Post 10'*, *Post 20'*). Each movement was self-paced, after a beep (go signal, repeated every 7 s), so as to avoid any reaction time. In fact, the time between the go signal and the movement onset changed according to the subject's will. No subject complained about fatigue.

Neuronavigation and tDCS

Transcranial direct-current stimulation was applied by using a neuroConn® DC-Stimulator Plus (model 0021) connected to two sponge electrodes, soaked with conductive gel. The active electrode (3.16 × 3.16 cm) was positioned on the scalp point closest to the PO of the non-preferred side (**Figure 1C**), as Mălîia et al. (2018) reported motor effects only when applying direct electrical stimulation on this side. The electrode positioning was guided by a neuronavigation system (SofTaxic Optic 2.0, see **Figure 1B**). In this aim, the coPO was identified by means of the average Talairach coordinates of its sub-areas PO1 and PO4, which are the closest to the subdural space (on the left: −52, −18.5, 22; on the right: 52, −18.5, 22.5; values obtained from MNI coordinates in Eickhoff et al. (2006a) and converted to Talairach according to Lacadie et al. (2008). The scalp position closest to coPO was then identified using the neuronavigation pointing stylus (**Figure 1B**). A much larger reference electrode (8 × 12 cm, so as to be functionally inefficient) was instead placed on the forehead over the contralateral supraorbital area (**Figure 1D**). Both electrodes were fixed by elastic bands.

Ten subjects underwent *ANODAL* tDCS, ten underwent *CATHODAL* and ten *SHAM*. Anodal and cathodal tDCS started with a 60 s fade-in period, followed by 20 min DC at 2 mA and a 30 s fade-out. In sham configuration, instead, the 60 s fade-in was immediately followed by the 30 s fade-out. The resulting current density (2 A/m^2) was much lower than the safety limit (25.46 A/m^2) reported on humans by Bikson et al. (2009) and even smaller than the minimal current density (142.9 A/m^2, Liebetanz et al., 2009) that might induce brain lesion in the rat.

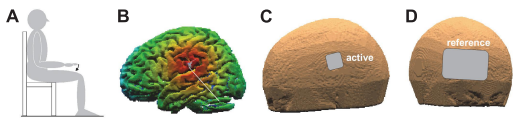

FIGURE 1 | (A) Position of the experimental subject. The arrow indicates index-finger flexion with the preferred hand prone. **(B)** False-color map of the distance between the tip of the neuronavigator pointing stylus, positioned on the scalp, and the reconstructed brain surface. The white line points to the coPO, identified by means of its Talairach coordinates. **(C,D)** Position of the active and reference electrodes (3.16 × 3.16 cm and 8 × 12 cm, respectively).

We actually applied a similar setup in one of our previous studies (Bolzoni et al., 2017) and none of the subjects reported unpleasant sensations or could recognize the DC polarity. Throughout the experiment, it was checked that scalp impedance was constant and never exceeded 5 kΩ (range 1.2–4.2 kΩ).

Movement and EMG Recordings

Flexion-extension of the index-finger at the metacarpophalangeal joint was recorded on the preferred side by a strain-gauge goniometer (mod. F35, Biometrics Ltd®, Newport, United Kingdom) stuck on the skin with hypoallergenic tape. Angular signal was DC amplified (P122, Grass Technologies®, West Warwick, RI, United States) and gain was calibrated before each experiment.

EMG signals were recorded by pairs of pre-gelled surface electrodes (H124SG, Kendall ARBO, Tyco Healthcare, Neustadt/Donau, Germany) placed on the prime mover Flexor Digitorum Superficialis (FDS) of the preferred upper limb and from some of the ipsilateral muscles involved in stabilizing the arm (Caronni and Cavallari, 2009): Biceps Brachii, Triceps Brachii, and Anterior Deltoid (BB, TB, and AD). The inter-electrode distance was 24 mm and electrode placement for BB, TB, and AD followed the SENIAM guidelines (Hermens and Freriks, 1999). The same general approach was adopted for FDS, for which muscle no specific SENIAM guidelines are available: the subject kept his preferred arm and forearm in the experimental position while repeatedly flexing one finger at a time. Meanwhile, the experimenter palpated the forearm, so as to feel the contraction of the FDS belly, on which the electrodes were placed. Recordings selectivity was verified by checking that activity from the recorded muscle, during its phasic contraction, was not contaminated by other muscular sources. EMG signals were amplified (IP511, Grass Technologies®, West Warwick, RI, United States) with a 1–20 k gain and a band-pass filter at 30–1000 Hz, so as to minimize movement artifacts and high frequency noise.

Conditioned goniometric and EMG analog signals were then sampled at 1 kHz, with an anti-aliasing low-pass filter at 500 Hz and a 12-bit resolution (A/D board model PCI-6024E, National Instruments®, Austin, TX, United States).

Data Analysis

All the EMG traces were digitally rectified, then the traces collected while moving the index-finger were expressed in % of the highest average EMG value recorded for 1 s during the subject's MVC monitoring.

For each EMG and goniometric variable, the 30 traces recorded in the two sequences *Pre* tDCS were time-aligned to the point (*trigger*) in which finger flexion reached 15° with respect to its resting position (mean value from 1 to 0.1 s before the go signal), and averaged. Such trigger choice actually granted the time-alignment precision, as it was verified that at 15° flexion the index-finger was moving at more than 50% of its peak velocity. The resulting averaged trace extended from 2 s before to 0.3 s after the trigger. The same procedure was applied for the 30 traces obtained in *Dur 10'*, *Dur 20'*, *Post 5'*, *Post 10'*, and *Post 20'*. All

subsequent measurements were taken on the averaged traces, and visually validated.

The first measurement regarded the onset of index-finger movement. The mean level of the signal recorded from 1 to 0.5 s prior to the *trigger* (reference period) was subtracted from the averaged trace, then a software algorithm searched the first time point in which the trace fell below −2 SD of the signal in the reference period and remained below that level for at least 50 ms. When the criterion was met, the algorithm searched backward the point in which the trace started to deviate from the mean reference value. Movement amplitude and duration were measured, respectively, as the amplitude and timing difference between the peak flexion of index-finger and the onset of its movement.

For each average EMG trace, the period from 1 to 0.5 s before movement onset (where no voluntary activity in FDS nor EMG changes in postural muscles occurred) was assumed as reference. The trace was integrated (time constant = 11 ms) and the mean reference level was subtracted from it; then the onset of an excitatory or inhibitory EMG change was identified by the above-described software algorithm, setting the threshold at +2 SD or −2 SD of the reference signal, respectively. The search was stopped at the onset of index-finger movement, so as to avoid any effect due to re-afferentation triggered by the focal movement. All timings were expressed as latencies with respect to FDS onset, with negative values representing time-advances. Finally, the amplitude of the EMG changes were measured as the mean level in the time-window from the onset of the EMG change to the onset of index-finger movement.

For each measured variable, a two-way ANOVA was applied to test for the effects of tDCS *polarity* (*SHAM* vs. *ANODAL* vs. *CATHODAL*) and *time* (*Pre* vs. *Dur 10'* vs. *Dur 20'* vs. *Post 5'* vs. *Post 10'* vs. *Post 20'*; repeated measurements factor), as well as their *interaction*. For all tests, statistical significance was set at $p < 0.05$ and the effect size was calculated by the partial eta square (η^2_p).

With this statistical design, the meaningful effect to be searched for is whether the within-subjects changes in *time* (*Pre* vs. *Post 20'*) are different among the three *polarities*, which is the *interaction* effect. Power analysis showed that the present design has 87% power to detect an interaction effect as low as $\eta^2_p = 0.13$. Such value is half the effect size of the minimum significant difference that we found in a similar experiment in which tDCS modulated SMA excitability (Bolzoni et al., 2015).

RESULTS

The upper part of **Figure 2** illustrates the mean integrated EMG and kinematics traces obtained from a representative subject who underwent *SHAM* tDCS. Traces are averages of 30 movement trials, recorded immediately before tDCS application (*Pre*), in the last minutes of "virtual" full-current tDCS (*Dur 20'*), and after 5 and 20 min of "virtual" recovery (*Post 5'* and *Post 20'*). In full agreement with the literature (Caronni and Cavallari, 2009), in *Pre* the FDS onset (solid vertical line) was accompanied by an inhibition in BB and AD, and by an excitation in TB. Such EMG changes always occurred before movement onset (dashed vertical line) and acted so as to stabilize the arm against the perturbation due to finger flexion, thus being classified as APAs. It is also apparent that the traces recorded in *Dur 20'*, *Post 5'*, and *Post 20'* were at all comparable to those recorded in *Pre*. The lower part of **Figure 2** reports the mean values of APAs amplitude and latency obtained in the whole population. Note that the APAs recorded during and after *SHAM* tDCS were at all comparable to those recorded in *Pre*; this confirms that repeating the motor task had no effect, actually excluding any contribution of fatigue.

Results obtained with *ANODAL* and *CATHODAL* tDCS were at all comparable to those recorded in *SHAM* condition (**Figures 3**, **4**), indicating that applying current of either polarity had no effect on APAs amplitude or latency. Such finding was also supported by statistics: two-way ANOVAs failed to highlight any significant *interaction* (in all muscles, $F_{10,135} \leq 1.604$, $p \geq 0.11$, $\eta^2_p \leq 0.106$). The same was true for the main effects of *time* ($F_{5,135} \leq 1.326$, $p \geq 0.26$, $\eta^2_p \leq 0.047$) and *polarity* ($F_{2,27} \leq 1.641$, $p \geq 0.21$, $\eta^2_p \leq 0.108$). Finally, statistics witnessed that tDCS had no effect on amplitude of FDS recruitment and index-finger kinematics too, as the two-way ANOVAs failed to find any significant effect (*interaction* $F_{10,135} \leq 1.392$, $p \geq 0.19$, $\eta^2_p \leq 0.093$; *time* $F_{5,135} \leq 1.577$, $p \geq 0.17$, $\eta^2_p \leq 0.055$; *polarity* $F_{2,27} \leq 1.159$, $p \geq 0.33$, $\eta^2_p \leq 0.079$).

DISCUSSION

According to our results, tDCS of either polarity applied over the coPO does not affect amplitude or latency of *intra-limb* APAs associated to index-finger flexion. Before concluding that coPO is not involved in the control of such APAs, some considerations are worthy. First, it could be argued that tDCS duration and current intensity or density were insufficient for modulating PO excitability. This could be reasonably excluded, as Fujimoto et al. (2017) obtained significant differences in tactile orientation discrimination when applying tDCS over PO, with the same current intensity but a 2.5 times smaller current density and for a duration 5-minute shorter than in our work. Moreover, if one takes into account the electric field simulations published by Fujimoto in the same paper, it is apparent that 2 mA tDCS is more than sufficient to alter the electric potential over the area of interest. Second, problems in locating our active electrode over the target area should be excluded. Indeed, despite it is impossible to check having found the right scalp position by, e.g., eliciting overt motor responses using transcranial magnetic stimulation, we feel confident that our neuronavigation system granted a reasonably good positioning. Third, it could be objected that more subjects are needed to highlight tDCS effects. However, in a similar study (Bolzoni et al., 2015), we applied tDCS over SMA and gathered evident results with a comparable number of subjects, while changes observed in the present study were inconsistent (**Figures 3**, **4**). Finally, from a purely speculative perspective, it may be hypothesized that a significant difference would have been observed if stimulating the PO ipsilateral to the moving finger, or both POs. This hypothesis would contrast with the general scheme of motor pattern generation, which classically

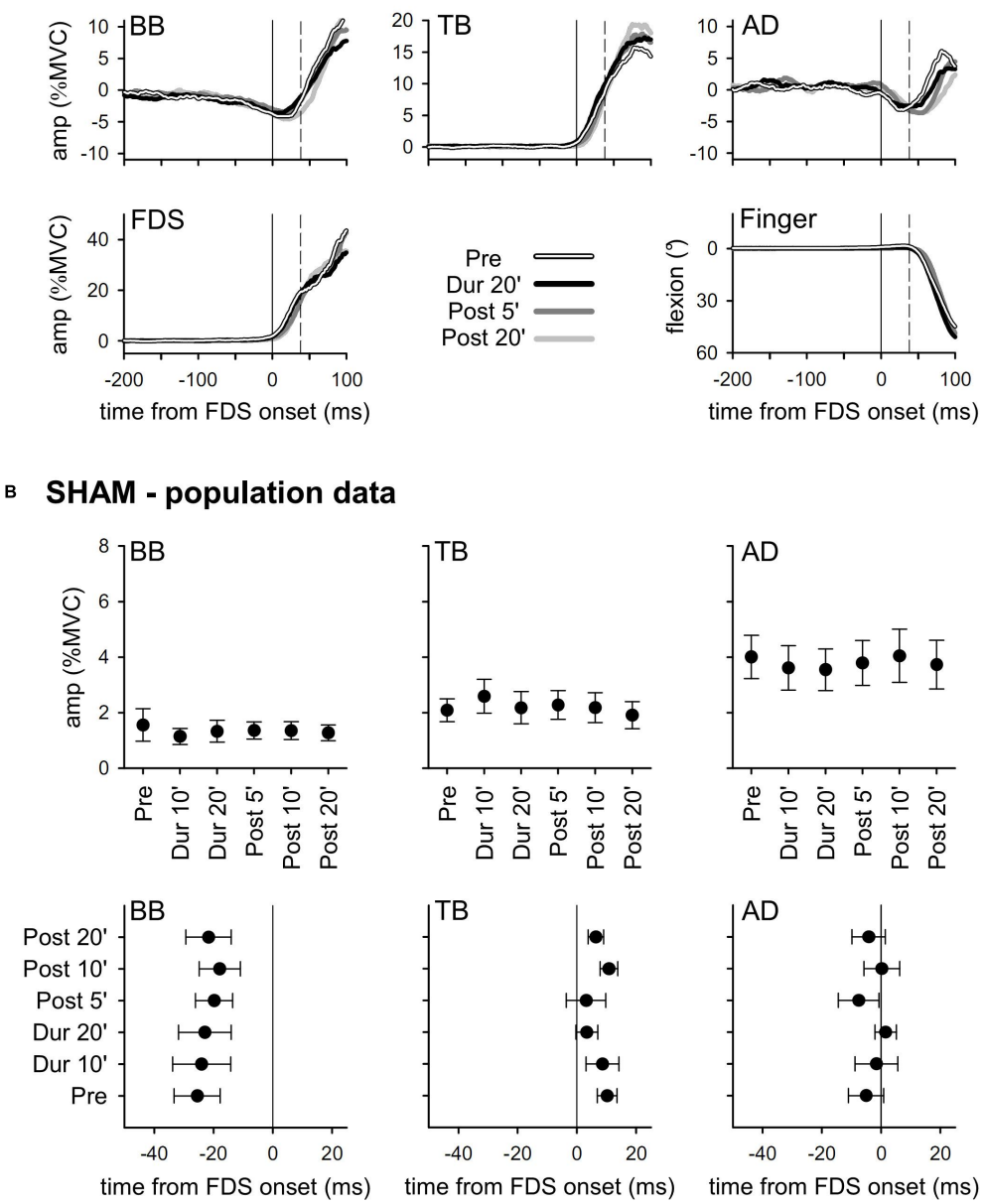

FIGURE 2 | In the **(A)**, rectified EMG and kinematics traces from a representative subject, who underwent *SHAM* tDCS (shades of black). Averages of 30 movement trials, recorded immediately before tDCS (*Pre*), in the last minutes of the "virtual" full-current period (*Dur 20'*), and at 5 and 20 min after it (*Post 5'* and *Post 20'*). At all the time-points, the onset of activity (solid vertical line) in the prime mover Flexor Digitorum Superficialis (FDS) was accompanied by inhibitory APAs in Biceps Brachii (BB) and Anterior Deltoid (AD), and by an excitatory APA in Triceps Brachii (TB), which always preceded movement onset (dashed vertical line). Note how at each time point the traces are at all comparable, indicating that repeating the motor task had no effect on APAs, prime mover recruitment and focal movement kinematics. In the **(B)**, mean (±SE) amplitude and latencies of APAs recorded in the BB, TB and AD muscles of all subjects of the *SHAM* group. No significant changes occurred among the different time-points (*Pre* vs. *Dur 10'* vs. *Dur 20'* vs. *Post 5'* vs. *Post 10'* vs. *Post 20'*), confirming the stability of APAs.

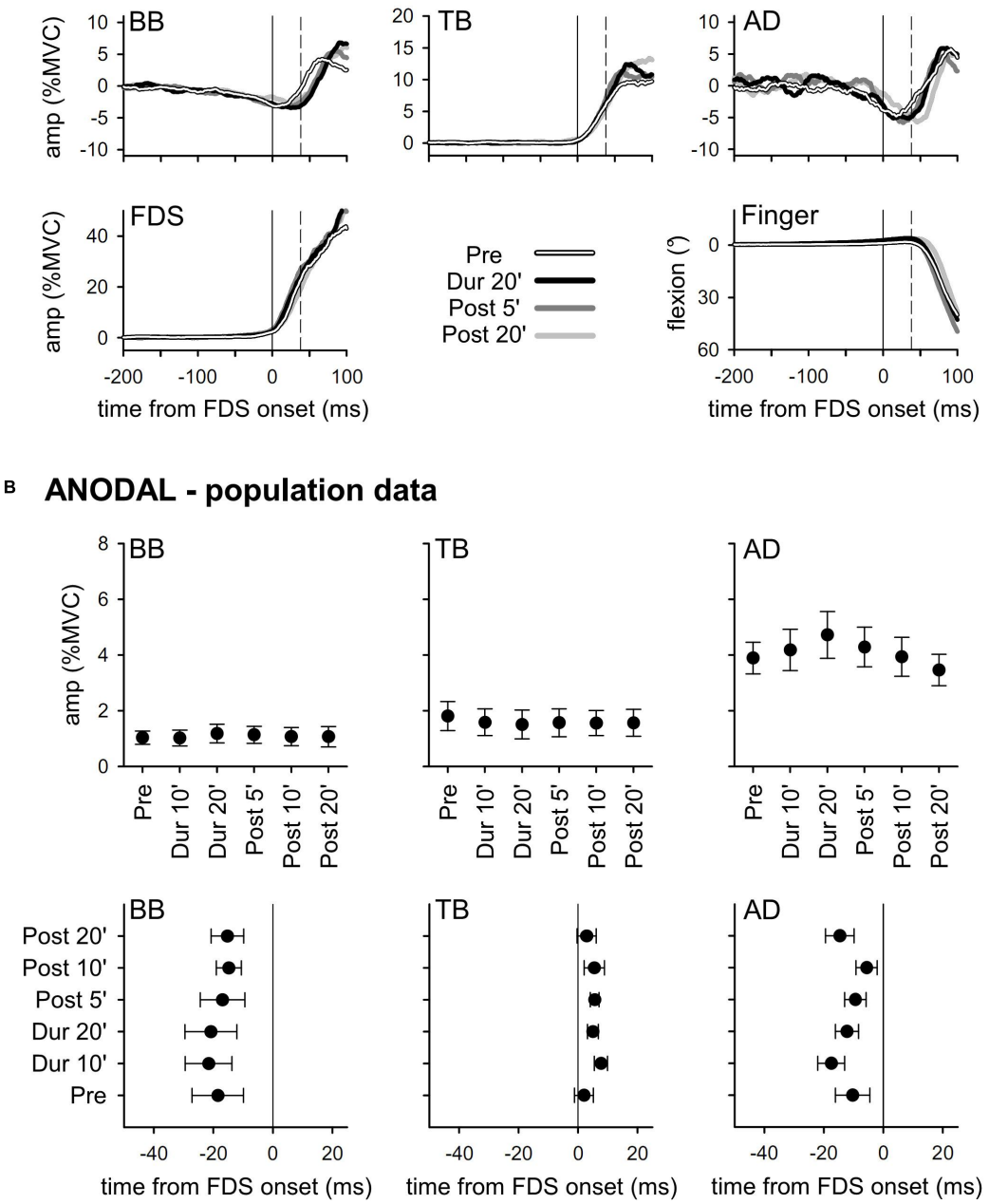

FIGURE 3 | Traces from a representative subject **(A)**, as well as mean (±SE) amplitude and latencies of APAs recorded in the BB, TB, and AD muscles of all subjects **(B)** who underwent *ANODAL* tDCS. Same layout as in **Figure 2**. It is apparent that tDCS had no effect on APAs, as no significant changes occurred among the different time-points (*Pre* vs. *Dur 10'* vs. . . . vs. *Post 20'*).

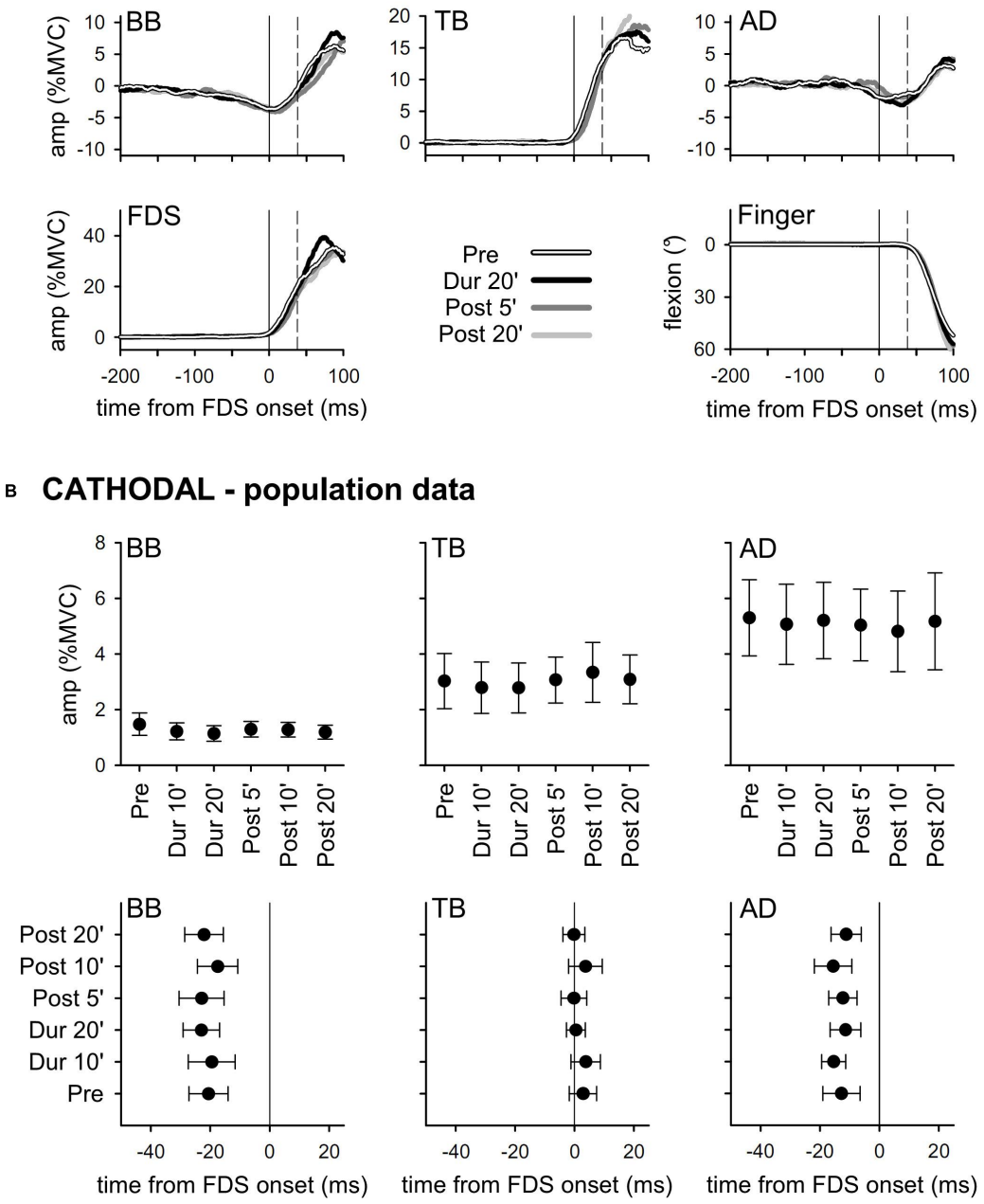

FIGURE 4 | Traces from a representative subject **(A)**, as well as mean (±SE) amplitude and latencies of APAs recorded in the BB, TB, and AD muscles of all subjects **(B)** who underwent *CATHODAL* tDCS. Same layout as in **Figure 2**. Also with this polarity, tDCS had no effect on APAs (no significant changes *Pre* vs. *Dur 10'* vs. . . . vs. *Post 20'*).

involves the cerebral hemisphere contralateral to the moving limb and the cerebellar hemisphere ipsilateral to it. In this regard, it should be also recalled that direct electrical stimulation of PO evoked motor effects only when applied to the left side, and that motor and sensory effects were mainly (90%) on the right side (Mălîia et al., 2018). In any case, it cannot be *a priori* excluded that the earlier phases of the motor act processing might involve both hemispheres. Clearly, a definite answer could be obtained only by direct testing. Should also these last possibilities fail, our search for an area in which the motor command to prime mover and postural muscles are still functionally unique (as defined in the Introduction) will have to address other structures.

On the other side, the conclusion that coPO stimulation does not disturb the control of APA associated to index-finger flexion does not contrast with the principal role that literature assigns to such structure. Indeed, the PO seemingly exerts its influence in the earlier strategic phase of selecting the motor goal, rather than in the planning of the motor act, where the motor program for the prime mover and the related APA chains are defined (Tunik et al., 2008; Woods et al., 2014; Valyear and Frey, 2016). For this reason, PO may not directly affect the integration of voluntary movement and posture, thus leaving these APAs unchanged. The absence of any significant effect on prime mover recruitment and index-finger kinematics is at all consistent with the fact that in our experiments the motor goal is intrinsically defined by the experimental task and thus its selection had already occurred well-before tDCS application.

Several studies suggested that PO has an important role in working memory and tactile learning (Jäncke et al., 2001), indeed, this neural structure seems to contain haptic memory information and it might be more important for object-directed motor behavior (Maule et al., 2015) rather than in planning the motor act, as it has been demonstrated for its neighbor frontal operculum (Tunik et al., 2008). Moreover, the PO network seems to modulate auditory-sensorimotor control, by mediating multimodal integration (Tanaka and Kirino, 2018), as well as orofacial muscles movements (Grabski et al., 2012), probably for phonation purposes. So, it may be argued that the contribution of PO concerns more specific motor actions and learning-memory rather than the motor planning. Our observation that no alteration occur in APAs associated to index-finger flexion when modulating coPO excitability is consistent with the above reasoning.

Lastly, since it is well documented that the index-finger APAs and those preceding other limb movements (*intra-* and *inter-limb* APAs, for a review see Cavallari et al., 2016) share not only their principal behavioral features but also their neural control structures, it may be advisedly suggested that coPO may not be crucial to the APA control in general.

CONCLUSION

The well-known role of PO in sensory-motor integration processing led us to inquire its possible involvement in postural control during index-finger flexion, a task which notably adapts to the perceived postural context, as it has been shown to occur for more general APAs. However, the present results seem to exclude such an hypothesis. Indirectly, this supports literature data that place PO within the sensorimotor integration network for selecting the motor goal. In order to definitely exclude the role of PO in APA control, future experiments should apply tDCS over the PO ipsilateral to the moving upper limb and/or bilaterally over the two POs. If also those trials will fail, the search for an area in which the motor command to prime mover and postural muscles are still functionally unique will have to move to other structures.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the University of Milan (counsel 6/19). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PC conceived the study. SM, RE, and FB conducted the experiments and analyzed the results. All authors contributed to drafting the manuscript and approved the final version.

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DISCUSSION

This section is a general discussion on the logical connections between the experimental studies presented in the previous chapter and on the implications that the ensuing results have in the context of the current knowledge on anticipatory postural control. Therefore, the specific aspects of the discussion of each study are left in the corresponding paper section.

Since the anticipatory postural adjustments are a crucial aspect of the voluntary movement organization, as they are fundamental in stabilizing both the whole-body (inter-limb APAs) and its segments (intra-limb APAs), this thesis aimed to deepen the knowledge about the control of these anticipatory activities and, in particular, the role of the neural structures involved in their programming and organization. Several studies have already investigated the involvement of different cortical and subcortical areas in modulating APAs. In this framework, my attention moved to the subcortical structures, focusing on the basal ganglia and cerebellum.

Basal ganglia and intra-limb APAs

Basal ganglia are involved in refining the voluntary movement and enabling motor acts, playing an important role in inter-limb APA control, as detailed in the Introduction. Results obtained from the comparison of *intra-limb* APAs between patients with Parkinson's Disease (PD) and healthy subjects (**Paper 1**), suggest that basal ganglia could participate also in *intra-*

limb postural control. In fact, the normal pattern of *intra-limb* APAs seems to be disrupted in PD patients, as some of them didn't show any BB *intra-limb* APAs and others developed a BB excitation, i.e., the opposite of what has been documented in healthy individuals. As regards focal movement kinematics, these data confirmed that PD patients were slower than age-matched healthy subjects, not only for what regards movement speed but also in terms of prime mover recruitment, as witnessed by their longer delay between FDS activation and movement onset. Note however that such result should not influence the observed *intra-limb* APA alterations, because a recent study (Esposti et al., 2015) showed that *intra-limb* APAs are affected by the intended movement speed and not the actual one, and in this regard both healthy subjects and PD patients had to move at their fastest speed. Moreover, the same paper showed that even when healthy subjects moved at 50% of their fastest, they did not show any reversal of the APA sign.

It is currently well established that basal ganglia and cerebellum play interconnected roles in motor control. In this regard, the involvement of the latter structure in *intra-limb* APA control has been recently demonstrated by observing altered APA timings, in absence of significant pattern disruptions, on patients with cerebellar dysfunction (Bruttini et al., 2015). This underlines that basal ganglia select the correct motor program and cerebellum temporizes the motor output for both *inter-* (Diedrichsen et al., 2007; Saitoh et al., 2005) and *intra-limb* APAs (**Paper 1** and Bruttini et al., 2015).

Moreover, recent studies have showed that basal ganglia and cerebellum are reciprocally interconnected through the pedunculopontine tegmental nucleus (Mori et al., 2016; Wu and Hallett, 2013). These connections could justify the partial overlap observed between the symptomatic framework of cerebellum and basal ganglia pathologies (Bostan

and Strick, 2012). This has also been observed in *intra-limb* APAs, as Bruttini et al. (2015) reported cases of lacking anticipatory actions in cerebellar ataxic patients, while signs of altered *intra-limb* APAs timing in Parkinsonian patients are reported in the present thesis. Literature also reports that Parkinson's disease, especially in its later phase, may also affect the cerebellum (Wu and Hallett, 2013) and that the activation of the latter is abnormally high in PD patients performing various upper limb movements. This fact led to the hypothesis that at the initial stage of the disease the cerebello-thalamo-cortical loop may act so as to compensate for the progressive impairment of the striato-thalamo-cortical circuit, as also proposed by Blesa et al (2007). Consequently, once the parkinsonian degeneration had affected the cerebellum, its compensation would fade-out, leading to a faster development of the motor injuries. So, these works confirm the close link between basal ganglia and cerebellum, justified by the reciprocal connections created throughout the pedunculopontine tegmental nucleus, as discussed in **Paper 2**.

The role of cerebellar ontogenesis in APA control

As already described, adult cerebellar patients showed similar timing-disruption in both inter- and intra-limb APAs (Diener et al., 1992; Bruttini et al. 2015), which in turn share several behavioural features. On the contrary, APA literature is quite scarce regarding the role of cerebellum in children. Thus, it was of interest to explore the role of cerebellar ontogenesis in APA control, by drawing a comparison of postural and gait initiation parameters in children affected by Congenital Cerebellar Ataxia (CCA) vs. a group of

healthy subjects of comparable age (**Paper 2**). CCA group was further classified into those affected by the non-progressive Joubert Syndrome (NonP) and those with an unknown or slowly progressive diagnosis (SlowP). We observed that in SlowP, both static and dynamic components of postural control were significantly disturbed, while the postural behavior of NonP was much similar to that of healthy children (H).

During the maintenance of upright posture, only SlowP and NonP patients showed an ellipse area greater than H, mainly due to large medial-lateral oscillations of the CoP, outlining an omnidirectional decrease of stability. However, statistical posturographic differences were found only between SlowP and Healthy participants.

Gait initiation parameters in the imbalance and unloading phases remained substantially unchanged in CCA children compared to controls. First step length and velocity showed instead a marked reduction in SlowP patients with respect to healthy controls, possibly reflecting a compensatory strategy for their poor balance control.

The electromyographic analysis revealed more severe timing alteration in lower limb and back muscles activities in SlowP group. This data confirmed the idea that the cerebellum is involved in setting the temporal organization of APAs while not affecting the APA pattern. A surprising finding has been that SlowP subjects showed a clearly worse postural behavior with respect to both NonP and healthy children. Interestingly enough, such difference between SlowP and NonP children was not reflected by a significantly more severe clinical score of symptoms (SARA) in the former group, maybe suggesting a poor sensitivity of such score. A possible explanation for the different behavior of SlowP and NonP may be linked to the specific nature of these pathologies. In fact, while the former is

characterized by a slow and mild progression of cerebellar degeneration, Joubert syndrome is characterized by a cerebellar hypoplasia with an intrinsically stable nature along the patient's lifetime. Therefore, while the neural connections of patients with slowly progressive non-JS congenital ataxia gradually change over time because of disease progression, preventing the consolidation of functional strategies, NonP patients could functionally compensate the cerebellar deficits since the early childhood, thanks to the plasticity of the remaining brain. In this perspective, it is interesting to note that even more pronounced alterations in APA timing has been reported in patients with adult-onset cerebellar syndromes (Bruttini et al., 2015), a finding at all consistent with the well-known gradual but consistent decrease over the lifespan in CNS ability to functionally compensate for developmental and degenerative disorders (Lu et al., 2017).

The neural substrates involved in this functional compensation remain still an open case, even if bidirectional connections between the basal ganglia and the cerebellum have been found (Bostan et al., 2010). The subthalamic nucleus in the basal ganglia and the dentate nucleus in the cerebellum have disynaptic projections addressed to, respectively, cerebellar cortex and striatum, suggesting the existence of an integrated network. An abnormal activity at one node could have network-wide effects (Bostan and Strick, 2018); in fact it has already been showed that PD patients have dysfunctions both at the level of the basal ganglia and the cerebellum (Wu and Hallett, 2013). Moreover, the level of loss of dopaminergic neurons of the substantia nigra has been shown to correlate with a persistent hyper-excitation of Purkinjie cells, indicating an alteration of both the basal ganglia-thalamic and cerebello-thalamic pathways (Heman et al., 2012). A SPECT study reported similar condition in patients with PD, in which an increased cerebellar activity

was detected when the effect of the anti-parkinsonian drug is extinguished (Rascol et al., 1997). A greater putamen-cerebellar connectivity was significantly correlated with better motor performance. The administration of levodopa reduced this connectivity, a finding consistent with the supposed relief of the cerebellar compensatory mechanism (Simioni et al., 2016). Given the existence of this connection between the two subcortical structures and the cerebellar functional compensation in basal-ganglia dysfunction, it could be hypothesized (**Paper 2**) that the basal ganglia could perform a specular compensatory activity in cerebellar diseases, as in cerebellar ataxia. So, in future studies, it would be interesting to investigate the basal ganglia activity during a motor task performed by patients with cerebellar dysfunctions.

Anticipatory postural control and cognition

As detailed in the Research Hypothesis section, it is not uncommon for cerebellar patients to suffer not only from clinical motor disorders but also from cognitive/emotional impairments, involving executive, visual-spatial and linguistic abilities, a cognitive-affective syndrome called CCAS (Schmahmann and Sherman, 1998). Indeed, most of the cerebro-cerebellar loop streamlines involve both cerebral associative and cerebellar cognitive areas. Clearly, the cognitive and emotional aspects may as well affect movement performance, which in turn has been shown to depend also on the postural control (Bruttini et al., 2016). Another indirect suggestion that cerebral cognitive areas may affect APAs came from Bolzoni et al. (2015) who showed altered APAs when influencing the excitability of the Supplementary Motor Area by tDCS. Indeed, such area has been repeatedly

shown to be involved in cognitive processing (for reviews, see Cona and Semenza, 2017; Nachev et al., 2008). These observations suggested to explore also cognitive areas for their possible involvement in the APA network (**Paper 3 and 4**).

Evaluating the involvement of cerebellar cognitive areas

In this regard, the role of cerebellum in cognitive functions as well as in emotional processes is increasingly recognized (D'Angelo and Casali, 2012). To investigate the activation of cerebellar areas involved in cognitive processes, my collaboration with prof. D'Angelo's group (**Paper 3**) aimed at developing a reproducible protocol of Magnetic Resonance Spectroscopy (MRS), able to characterize and quantify metabolites in selected cerebellar Volume of Interests (VOI). In particular the lateral Crus I-II, notably involved in cognition (Stoodley et al., 2012). Raw values seemed to agree to those obtained in the literature (Pradhan et al., 2015; Study et al., 2016). However other studies (such as Minati et al., 2010) reported that a better metabolites quantification could be obtained if raw data were corrected for partial volume effect, i.e., the concomitant presence of more than one tissue type (white matter, gray matter and cerebrospinal fluid) in each VOI. In this aim, T1-weighted brain images were segmented and the resulting tissue partial volume fractions were used to correct raw concentrations, so as to compensate for the effects of relaxation and of contamination due to such partial volume effect. In average, corrected metabolites concentrations resulted to be significantly lower than the raw ones, a finding in agreement with previous works, such as Long et al. (2015). Nevertheless, we have to take into account that it is quite difficult to apply the segmentation of T1-weighted brain images, especially

for voxels within the cerebellar areas, because of their small size. Moreover, the examined sample was relatively small. Therefore, in perspective, it could be useful to increase the sample size, so as to better estimate the reliability of the MRS protocol.

Even though this work would seem to be thematically far from the main topic of my thesis, actually, it establishes the experimental basis for future works regarding also cognitive dysfunctions. In fact, taking into account that some of the motor pathologies, such as cerebellar ataxia, show both motor and cognitive impairments, it would be useful and interesting to investigate a specific disease from many aspects. Despite MRS is widely used in clinics, few studies have applied it to correlate neurological disorders with the concentration of cerebellar metabolites in Crus I-II. The relative insensibility of MRS to tissue depth also suggests to apply it on deep nuclei like the basal ganglia, which have known implications in cognitive processes. In this way, it would be of extreme interest to obtain standard metabolites concentration in cerebellum and basal ganglia cognitive areas, to explore their involvement in APA control. Unfortunately, these experiments are on stand-by, searching for a more easy available MRS facility.

Parietal operculum does not influence APA control

In parallel, the investigation of APA control using the cognitive approach targeted the parietal operculum, a well-known cortical sensory-motor integration center (**Paper 4**). So, the *intra-limb* APAs stabilizing the arm when the index-finger is flexed were recorded in a cohort of healthy subjects, before, during and after modulating the excitability of the contralateral Parietal Operculum (coPO) by cathodal or anodal tDCS. Should

the coPO be involved in APA control, it would be expected to observe alterations in APA timing and/or amplitude, like it was reported by Bolzoni et al. (2015) when applying tDCS on the Supplementary Motor Area. Our results showed instead that tDCS of either polarity did not affect amplitude or latency of *intra-limb* APAs, witnessing that coPO does not influence the control of such postural actions. This negative finding does not contrast with the principal role that literature assigns to PO. Indeed, such area seemingly exerts its influence in the earlier strategic phase of selecting the motor goal, rather than in the planning of the motor act, where the motor program for the prime mover and the related APA chains are defined (Tunik et al., 2008; Valyear and Frey, 2016; Woods et al., 2014). The observed absence of any significant effect of tDCS on prime mover recruitment and index-finger kinematics is absolutely consistent with the fact that in our experiments the motor goal is intrinsically defined by the experimental task, thus its selection had already occurred well-before tDCS application. If so, no wonder that coPO excitability modulation did not affect APAs. Considering the well documented fact that the APAs preceding index-finger movements and those preceding other limb movements (*intra-* and *inter-limb* APAs, for a review see Cavallari et al., 2016) share not only their principal behavioural features but also their neural control structures, it may be suggested that coPO may not be crucial to the APA control in general and that the *shared motor program* that details the recruitment of postural and prime mover muscles (see Introduction paragraph) is assembled at a lower hierarchical level along the motor processing streamline.

Anyway, in order to definitely exclude the role of PO in APA control, future experiments should apply tDCS over the PO ipsilateral to the moving upper limb and/or bilaterally over the two POs. Indeed, it cannot be a-priori

excluded that the earlier phases of the motor act processing might involve both hemispheres. If also those control experiments will fail, our search for cognitive areas involved in postural control, in which the command flows to postural and prime mover muscles could still be unique, will have to address other structures.

CONCLUSION

Despite the quite large literature body already available, the research work carried out during this Ph.D. actually deepened the knowledge of the subcortical structures, basal ganglia and cerebellum, involved in anticipatory postural control and their reciprocal connections, also allowing to identify new research pathways.

Since the postural control is also influenced by cognitive and emotional aspects, we wanted to include in our works the investigation of cognitive structures, cerebellar CrusI-II and parietal operculum, to have a greater understanding about postural control.

To achieve our targets, we have used methods and technologies different from each other. Indeed, this thesis has been the result of combining a relatively simple but well standardized and reproducible experimental paradigm (the index-finger flexion protocol) with a highly multidisciplinary approach, spanning from EMG and posturography to neuronavigation-guided brain stimulation and imaging-based spectroscopy.

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